0031-6997/00/5203-0415$03.00/0
PHARMACOLOGICAL REVIEWS
U.S. Government work not protected by U.S. copyright
Pharmacol Rev 52:415–472, 2000

Vol. 52, No. 3
34/848966
Printed in U.S.A

International Union of Pharmacology. XXIII. The Angiotensin II Receptors

M. DE GASPARO,[1] K. J. CATT, T. INAGAMI, J. W. WRIGHT, AND TH. UNGER

Novartis Pharma AG, Metabolic & Cardiovascular Diseases, Basel, Switzerland (M.d.G.); Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland (K.J.C.); Department of Biochemistry, Vanderbilt University, School of Medicine, Nashville, Tennessee (T.I.); Department of Psychology, Washington State University, Pullman, Washington (J.W.W.); Institute of Pharmacology, Christian-Albrechts-University of Kiel Hospitalstrasse 4, Kiel, Germany (Th.U.)

This paper is available online at http://www.pharmrev.org

[1] Address for correspondence: Marc de Gasparo, Novartis Pharma AG, Metabolic & Cardiovascular Diseases, WKL 121-210, P.O. Box 4200, Basel, Switzerland. E-mail: marc.de_gasparo@pharma.Novartis.com and m.de_gasparo@bluewin.ch (after October 1, 2000)

Abstract——**The cardiovascular and other actions of angiotensin II (Ang II) are mediated by AT_1 and AT_2 receptors, which are seven transmembrane glycoproteins with 30% sequence similarity. Most species express a single autosomal AT_1 gene, but two related AT_{1A} and AT_{1B} receptor genes are expressed in rodents. AT_1 receptors are predominantly coupled to $G_{q/11}$, and signal through phospholipases A, C, D, inositol phosphates, calcium channels, and a variety of serine/threonine and tyrosine kinases. Many AT_1-induced growth responses are mediated by transactivation of growth factor receptors. The receptor binding sites for agonist and nonpeptide antagonist ligands have been defined. The latter compounds are as effective as angiotensin converting enzyme inhibitors in cardiovascular diseases but are better tolerated. The AT_2 receptor is expressed at high density during fetal development. It is much less abundant in adult tissues and is up-regulated in pathological conditions. Its signaling pathways include serine and tyrosine phosphatases, phospholipase A_2, nitric oxide, and cyclic guanosine monophosphate. The AT_2 receptor counteracts several of the growth responses initiated by the AT_1 and growth factor receptors. The AT_4 receptor specifically binds Ang IV (Ang 3–8), and is located in brain and kidney. Its signaling mechanisms are unknown, but it influences local blood flow and is associated with cognitive processes and sensory and motor functions. Although AT_1 receptors mediate most of the known actions of Ang II, the AT_2 receptor contributes to the regulation of blood pressure and renal function. The development of specific nonpeptide receptor antagonists has led to major advances in the physiology, pharmacology, and therapy of the renin-angiotensin system.**

I. Introduction

A. Historical Background

Blood pressure was measured for the first time in 1733 by Stephen Hales, in a dramatic experiment on a horse, by inserting a brass pipe into the carotid artery. The technique of modern blood pressure measurement was introduced in 1905 by Nicolai Korotkov using the stethoscope invented by Laennec in 1815 and the relatively recently devised wraparound inflatable rubber cuff. The latter was first described by Riva-Rocci in 1896 and was improved by von Recklinghausen in 1901 (Freis, 1995).

The first insight into the regulation of blood pressure came from the discovery of a pressor principle by Tigerstedt and Bergman in 1897. They called this factor "renin" because it was extracted from the kidney. This pioneering work led to the description of reno-vascular hypertension in animals and in humans (Goldblatt et al., 1934). However, it was not until 1940 (Braun-Menendez et al., 1940) that a vasoconstrictor substance was isolated from renal venous blood from the ischemic kidney of a Goldblatt hypertensive dog. A similar finding was made simultaneously and independently by Page and Helmer (1940) after the injection of renin into an intact animal. This group also isolated a so-called "renin activator" that later proved to be angiotensinogen. The pressor substance was named "hypertensin" in Argentina and "angiotonin" in the United States and was later isolated and shown to be an octapeptide (Skeggs et al., 1956; Bumpus et al., 1957; Elliott and Peart, 1957). There were differences between laboratories concerning interpretations and nomenclature but in fact hypertensin and angiotonin were the same substance. In 1958, Braun-Menéndez and Page agreed on the hybrid term angiotensin for the highly potent pressor octapeptide. This proved to be an appropriate choice, given the later recognition of angiotensin's numerous actions in addition to its hypertensive effects. The sequence of angiotensin II is Asp-Arg-Val-Tyr-Ile-His-Pro-Phe in the human, horse, and pig. In bovine angiotensin II, the isoleucine residue in position 5 is replaced by valine.

Following this major discovery, the various components of the cascade leading to the formation of angiotensin II were characterized, including angiotensinogen, angiotensin converting enzyme (ACE),[2] and angiotensins I, II, and III (Table 1). The synthesis of the peptide angiotensin II by Bumpus et al. (1957) and by Rittel et al. (1957) was followed by a continuing series of investigations into the structure-activity relationship of angiotensin analogs, mainly in the hope of finding a peptide antagonist.

In 1987, a committee of the International Society for Hypertension, The American Heart Association, and the World Health Organization proposed abbreviating angiotensin to Ang using the decapeptide angiotensin I as the reference for numbering the amino acids of all angiotensin peptides (Dzau et al., 1987).

Angiotensin II plays a key role in the regulation of cardiovascular homeostasis. Acting on both the "content" and the "container", Ang II regulates blood volume and vascular resistance. The wide spectrum of Ang II target tissues includes the adrenals, kidney, brain, pituitary gland, vascular smooth muscle, and the sympathetic nervous system. Angiotensin is not only a blood-borne hormone that is produced and acts in the circulation but is also formed in many tissues such as brain, kidney, heart, and blood vessels. This has led to the suggestion that Ang II may also function as a paracrine and autocrine hormone, which induces cell growth and proliferation and controls extracellular matrix formation (Dzau and Gibbons, 1987; Griffin et al., 1991; Weber et al., 1995a,b). Other angiotensin-derived metabolites such as angiotensin 2–8 (Ang III), angiotensin 1–7, or angiotensin 3–8 (Ang IV) have all been shown to have biological activities (Table 1) (Peach, 1977; Schiavone et al., 1990; Ferrario et al., 1991; Ferrario and Iyer, 1998; Wright et al., 1995).

As for other peptide hormones, Ang II was postulated to act on a receptor located on the plasma membrane of its target cells. This receptor should possess the dual functions of specific recognition of the ligand and stimulation of the characteristic cellular response. Comparison of changes in steroidogenesis in the adrenal cortex, adrenal catecholamine release, and developed tension in aortic strips in response to Ang I, Ang II, and Ang III clearly indicated different affinities of these target organs for the three peptides (Peach, 1977; Devynck and Meyer, 1978). These pharmacological experiments showed that effector organs responded to Ang I, II, and

[2] Abbreviations: ACE, angiotensin converting enzyme; NC-IUPHAR, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification; GPCR, G protein-coupled receptor; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; PKC, protein kinase C; kb, kilobase(s); bp, base pair(s); RT-PCR, reverse transcriptase-polymerase chain reaction; IGF-1, insulin-like growth factor 1; NO, nitric oxide; NOS, NO synthase; VSMC, vascular smooth muscle cell(s); MAPK, mitogen-activated protein kinase; EPO, erythropoietin; CHO, Chinese hamster ovary; TMD, transmembrane domain; PKB, protein kinase B; EGF, epidermal growth factor; GAP, GTPase-activating protein; PDGF, platelet-derived growth factor; JNK, c-Jun N-terminal kinase; PAK, p21-activated kinase; PLC, phospholipase; SFO, subfornical organ; OVLT, organum vasculosum lamina terminales; ACTH, adrenocorticotropin; APA, aminopeptidase A; APN, aminopeptidase N; GnRH, gonadotropin-releasing hormone; DTT, dithiothreitol; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; MKP-1, MAPK-phosphatase-1; PTP, phosphotyrosine phosphatase; PGE_2, prostaglandin E_2; IL, interleukin; IRS, insulin response sequence; IRF, interferon regulatory factor; NGF, nerve growth factor; NBC, Na^+/HCO^- symporter system; NHE, Na^+/H^+-exchanger; PAI, plasminogen activator inhibitor; Ang, angiotensin; L-NAME, N^ω-nitro-L-arginine; AP-1, activator protein-1; ERK, extracellular signal-regulated kinase; TMD, transmembrane domain; JAK, Janus cytosolic protein kinase; STAT, signal transducers and activators of transcription.

TABLE 1
Amino acid sequences of Ang II precursors and metabolites

	1 2 3 4 5 6 7 8 9 10 11 12 13 14
Angiotensinogen	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser
Ang I	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
Ang II	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Ang III	Arg-Val-Tyr-Ile-His-Pro-Phe
Ang IV	Val-Tyr-Ile-His-Pro-Phe
Angiotensin 1–7	Asp-Arg-Val-Tyr-Ile-His-Pro

III with 2 to 3 log differences in potency from tissue to tissue. Based on these studies, Ang II receptor selectivity for the agonists was proposed to be structure-activity related. Comparison of Ang II and a large number of synthetic agonists and antagonists formed by substituting various amino acids of Ang II indicated marked dissimilarities between the analogs in each of the preparations, suggesting differences in the structure of the receptor sites (Khosla et al., 1974; Papadimitriou and Worcel, 1974; Peach and Levens, 1980).

Early binding studies detected sites with binding characteristics that differed between the various target tissues (Peach and Levens, 1980). Also, receptor density was up- or down-regulated in different tissues following either Ang II infusion or Na^+ restriction (Aguilera and Catt, 1978). The characterization of receptor types in rat liver and kidney cortex (Gunther, 1984; Douglas, 1987; Bouscarel et al., 1988b) suggested further Ang II receptor heterogeneity. An early classification proposed for Ang II receptor types was based on studies in only a few tissues or species (Levens et al., 1980; Peach and Levens, 1980; Ferrario et al., 1991). It was not until the end of the 1980s that tools became available to demonstrate the existence of at least two receptor types in many tissues for which the conventional peptide analogs such as saralasin have high affinity but little or no selectivity. These included the nonpeptide antagonists losartan (or Ex89 or DuP 753) and PD123177, and a new generation of peptide ligands such as CGP42112 and *p*-aminophenylalanine Ang II (Chiu et al., 1989a; Whitebread et al., 1989; Speth and Kim, 1990). This new development was made simultaneously and independently in three different laboratories, and the initial nomenclature was confusing: the receptor sensitive to losartan was called 1, B, or α, and that with no affinity for losartan was termed 2, A, or β. The High Blood Pressure Research Council in 1990 and the International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) in 1992 therefore appointed a subcommittee[3] to address the problem, and a classification was proposed in 1991 and updated in 1995 (Bumpus et al., 1991; de Gasparo et al., 1995).

[3] Members of the NC-IUPHAR Subcommittee on Angiotensin Receptors: R. Wayne Alexander, Kenneth E. Bernstein, Andrew T. Chiu, Theodore Goodfriend, Joseph W. Harding, Ahsan Hussain, Pieter B. M. W. M. Timmermans.

B. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification

Criteria for Classification

To obtain a "fingerprint" capable of identifying distinct receptors, three main criteria have been proposed: operational, transductional, and structural (Humphrey et al., 1994). The operational criteria include the drug-related characteristics of the receptor, such as ligand binding affinities, and selective agonists and antagonists. The receptor-effector coupling events constitute the transductional criteria, and the receptor sequence and gene cloning represent the structural criteria. It is clear that all of these criteria are not necessarily achieved simultaneously and at an early stage. The coupling mechanism may not have a major influence on receptor pharmacology but it helps in differentiating receptor types. Also, receptors with diverse structures may respond to the same endogenous ligands. Finally, receptors may be cloned without having a known pharmacology. The combination of the three criteria should clearly help in defining true receptor types.

Any such classification will essentially evolve as our knowledge increases. Nevertheless, there is a need for an official scheme that will help to avoid confusion among investigators. Two Ang II receptor types fulfill the three classification criteria, and are termed AT_1 and AT_2 receptors. According to the NC-IUPHAR recommendation, the AT_1 and AT_2 receptors have an IUPHAR Receptor Code of 2.1.Ang.01.000.00.00 and 2.1 Ang.02.000.00.00 (Humphrey and Barnard, 1998). The first two numbers indicated the structural class: they are seven transmembrane domain, G protein-coupled receptor (GPCR) member of the rhodopsin subclass (2.1). The receptor family is abbreviated Ang. The types indicated as 01 and 02 for AT_1 and AT_2. The following series of null are reserved for splice variants chronologically numbered according to identification within species.

Two other receptors (AT_3 and AT_4) have been proposed, based on operational criteria, but their transduction mechanisms are unknown and they have not yet been cloned. The name AT_3 was initially given to a binding site described in the Neuro-2a mouse neuroblastoma cell line that was not blocked by either AT_1-specific losartan, or AT_2-specific PD123319 and was not affected by GTP analogs (Chaki and Inagami, 1992). This AT_3 binding site, which has a low affinity for Ang III, should

be called a non-AT_1-non-AT_2 site until more information about its nature has been obtained. The endogenous ligand for the AT_4 receptor is Ang 3–8 or Ang IV. Its binding properties and physiological characteristics, described in more detail in another section, are sufficiently different from those of the AT_1 and AT_2 receptor to warrant keeping the name AT_4 for this putative Ang IV-selective receptor until the binding protein is cloned and further characterized.

C. Current Nomenclature

The present angiotensin receptor identification is based on six principles. 1) The receptor is abbreviated to AT followed by a numerical subscript. 2) Further subdivisions are indicated by subscript letters that are in upper case for pharmacologically defined receptor subtypes (e.g., AT_{1B}). 3) The species is identified by a lowercase prefix preceding AT (e.g., r AT_1, h AT_2). There is a space between the species and the receptor name. 4) Mutant receptors should be designated with specification of the position of the amino acid substitution in bracket (e.g., [L112P]AT_{1A} when leucine at position 112 has been changed to proline. 5) The human gene is written in upper case and preferably but not essentially in italics (e.g., *AGTR1* and *AGTR2*). In mouse and rats, it would be Agtr1a, Agtr1b and Agtr2 in lowercase.

D. Structural Analysis

The strategy of expression cloning was successfully applied to the AT_1 receptors of rat smooth muscle and bovine adrenal gland, and subsequently the corresponding receptors of mouse, rabbit, human, pig, dog, turkey, and frog angiotensin receptors were cloned and sequenced. The nonmammalian receptors have 60% identity with the mammalian receptor and are pharmacologically distinct in their ligand binding properties. Hydropathy analysis indicated that both AT_1 and AT_2 receptors contain seven hydrophobic transmembrane segments forming α helices in the lipid bilayer of the cell membrane. The structural information for the AT_1 receptor is coded as follows: h 359 aa, P30556, chr.3. This indicates that the human AT_1 receptor contains 359 amino acids, with the sequence reported in the SwissProt file under the number 30556 and the gene coding for the receptor (abbreviated *AGTR1*) is located on chromosome 3 q. Similarly, the structural coding for rat and mouse AT_1 receptor is r 359 aa, P29089, P25095 and m 359 aa, P29754, P29755 as there are two subtypes A and B in rat and mouse located on chromosomes 17 and 2 and 13 and 3, respectively. The AT_2 receptor is only 34% identical with its AT_1 counterpart (Fig. 1). The structural information is coded h 363 aa, P50052, chr.X

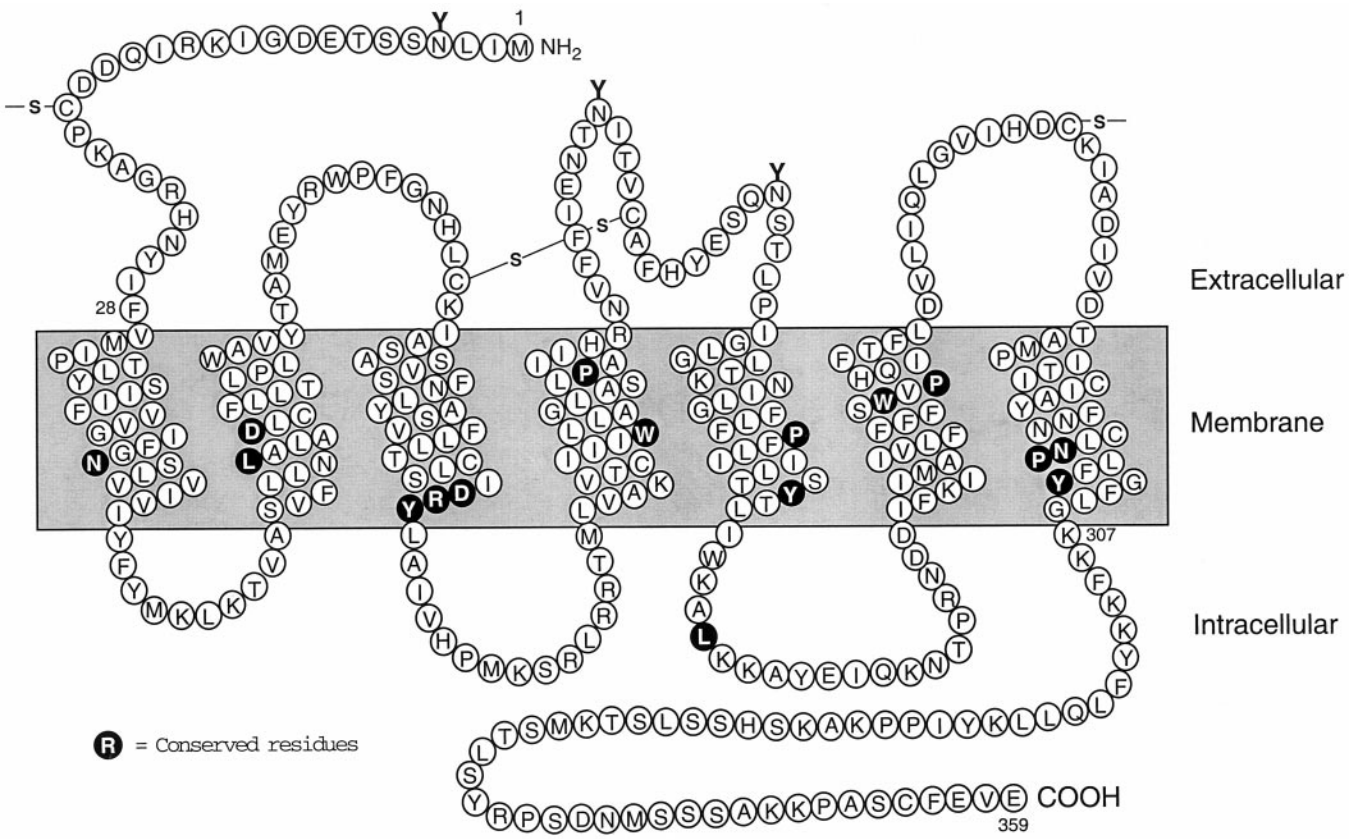


FIG. 1. Secondary structure and consensus sequence of the mammalian angiotensin AT_1 receptor. The amino acid sequence shown is based on the derived sequences of five individual cloned mammalian AT_1 receptors. The amino acid residues that are highly conserved among G protein-coupled receptors are indicated by bold letters. The positions of the three extracellular carbohydrate chains, and of the two extracellular disulfide bonds, are also indicated.

q22-q23 as the gene *AGTR2* is located on human chromosome X with the cytogenetic location q23-q24. For rat and mouse, the respective information is r 363 aa, P35351 and m 363 aa, P35374. As in human, the AT_2 receptor in rodents is also located on chromosome X.

An evolutionary analysis based on the alignment of cloned AT_1 receptor sequences, using the CLUSTAL algorithm of PC/gene, has suggested that rat and mouse AT_1 receptors coevolved. (Sandberg, 1994). Amphibian and avian receptors diverged early during evolution. So far, gene duplication has been observed only in rats and mice (see following section). Two isoforms of the AT_1 receptor derived by alternative splicing of the same gene have been reported in man (Curnow et al., 1995). They have similar binding and functional properties. A receptor with as much as 97% identity to the AT_1 receptor has been cloned from human placenta (Konishi et al., 1994). It differs in its C-terminal amino acid sequence, tissue distribution, and pharmacological properties. The gene has not been cloned and it may well be a splice variant of the AT_1 receptor.

II. The Type 1 (AT_1) Angiotensin Receptor

The angiotensin AT_1 receptor mediates virtually all of the known physiological actions of angiotensin II (Ang II) in cardiovascular, renal, neuronal, endocrine, hepatic, and other target cells. These actions include the regulation of arterial blood pressure, electrolyte and water balance, thirst, hormone secretion, and renal function. The AT_1 receptor belongs to the G protein-coupled receptor (GPCR) superfamily and is primarily coupled through pertussis toxin-insensitive G proteins to the activation of phospholipase C and calcium signaling. The AT_1 receptors of several species have been cloned and their amino acid sequences determined from the respective cDNAs. Ang II binding to the AT_1 receptor induces a conformational change in the receptor molecule that promotes its interaction with the G protein(s), which in turn mediate signal transduction via several plasma membrane effector systems. These include enzymes, such as phospholipase C, phospholipase D, phospholipase A_2, and adenylyl cyclase, and ion channels, such as L-type and T-type voltage-sensitive calcium channels. In addition to activating several intracellular signaling pathways that mediate agonist-induced phenotypic responses in a wide variety of Ang II target cells, the agonist-occupied AT_1 receptor undergoes desensitization and internalization in the same manner as many other GPCRs.

The cellular responses to AT_1 receptor signaling include smooth muscle contraction, adrenal steroidogenesis and aldosterone secretion, neuronal activation, neurosecretion, ion transport, and cell growth and proliferation. The AT_1 receptor is coupled not only to the well recognized G_q-mediated calcium and protein kinase C signaling pathways, but also to intracellular signaling cascades that extend into the nucleus. These pathways regulate gene transcription and the expression of proteins that control growth responses and cell proliferation in several Ang II target tissues. Some of the latter consequences of AT_1 receptor activation are counteracted by the structurally dissimilar AT_2 receptor, which antagonizes the effects of AT_1-mediated growth responses in several cell types, in particular endothelial cells, cardiomyocytes, and ovarian granulosa cells. These actions of the AT_2 receptor are described in more detail below. This account of the AT_1 receptor will address its gene expression, ligand binding, activation and signal transduction pathways, and physiological roles in the regulation of the activity and growth of its major target cells in cardiovascular, neuronal, and endocrine tissues.

A. Angiotensin II Receptors: Early Studies

The angiotensin receptor was identified as a functional entity by Lin and Goodfriend (1970), who first described the binding of radioiodinated Ang II to its receptor sites in the adrenal gland. These sites were subsequently shown to be located in the plasma membrane (Glossmann et al., 1974a), and the binding reaction was found to be influenced by the ambient Na^+ concentration and guanyl nucleotides (Glossmann et al., 1974b,c). The G proteins had not been discovered at that time, but this finding indicated that the binding activity of a noncyclic AMP-coupled receptor is regulated by guanine nucleotides. Subsequent studies showed that the AT_1 receptor is coupled to both G_q and G_i proteins in the adrenal glomerulosa zone and several other tissues in the rat.

Many of the properties of the angiotensin II receptor were first identified in studies on the adrenal gland and liver, both of which are abundant sources of receptors that are coupled to well defined physiological responses (Saltman et al., 1975; Campanile et al., 1982). As in the rat adrenal gland, guanine nucleotides reduced agonist binding of ^{125}I-Ang II to hepatic receptors, largely by increasing its dissociation rate constant. Guanine nucleotides also decreased the number of high-affinity binding sites for Ang II, but not those for the peptide antagonist, [Sar^1,Ala^8]Ang II. These changes were accompanied by inhibition of adenylyl cyclase activity in hepatic membranes, and of cyclic AMP production in intact hepatocytes (Crane et al., 1982). The high-affinity Ang II receptors in the liver were found to be inactivated by dithiothreitol, with a concomitant loss of Ang II-induced stimulation of glycogen phosphorylase in isolated hepatocytes (Gunther, 1984). These and related studies also presaged the existence of angiotensin II receptor types with distinct biochemical properties and intracellular mechanisms of action. Differential effects of guanine nucleotides on receptor binding of Ang II agonist and antagonist ligands were also observed in the bovine adrenal gland. This effect was evident for both membrane-bound and solubilized receptors. Concerning the latter, the association of the agonist-occu-

pied receptor with a putative G protein was suggested by its larger size on steric exclusion HPLC (De Lean et al., 1984).

The ability of Ang II to inhibit glucagon-stimulated cyclic AMP production in hepatocytes, and adenylyl cyclase activity in hepatic membranes, was consistent with its coupling to an inhibitory G protein, now termed G_i. This was confirmed by the ability of pertussis toxin to prevent the inhibitory action of Ang II on adenylyl cyclase. The ability of GTPγS to further reduce receptor binding affinity when all G_i molecules were ADP-ribosylated by the toxin indicated that Ang II receptors are also coupled to other G protein(s) that could mediate actions of Ang II on additional signaling pathways (Pobiner et al., 1985). Subsequent studies on cultured hepatocytes revealed a single population of Ang II binding sites and demonstrated that agonist and antagonist analogs had parallel actions on cytosolic calcium and phosphorylase activity, as did treatment with dithiothreitol to inactivate the receptors (Bouscarel et al., 1988a). Reconstitution studies in hepatocyte membranes showed that G_{i3} is the major form of G_i in these cells and is responsible for coupling the Ang II receptor to agonist-induced inhibition of adenylyl cyclase (Pobiner et al., 1991). One of the few physiological actions of Ang II that is mediated by G_i, rather than $G_{q/11}$, is the AT_1 receptor-dependent stimulation of angiotensinogen production in the rat liver (Klett et al., 1993).

B. Cloned AT_1 Receptors

The relatively low abundance of the AT_1 receptor in most Ang II target tissues, and the instability of the solubilized receptor molecule, impeded efforts to isolate and sequence the receptor protein. For this reason, expression cloning from bovine adrenal and rat smooth muscle cells was necessary to isolate the cDNAs encoding the receptor proteins of these species (Sasaki et al., 1991; Murphy et al., 1991). Both AT_1 receptors were found to be typical seven transmembrane domain proteins, composed of 359 amino acids and with a molecular mass of about 41 kDa. The extracellular regions, composed of the N terminus and the three extracellular loops, contain three *N*-glycosylation sites and four cysteine residues (Fig. 1). Each of the consensus sites is glycosylated in the native AT_1 receptor (Jayadev et al., 1999), which has a molecular mass of about 65 kDa. In addition to the two conserved cysteines that form a disulfide bond between the first and second extracellular loops of all GPCRs, the AT_1 receptor contains an additional pair of extracellular cysteine residues. These are located in the N-terminal region and the third extracellular loop, and form a second disulfide bond that maintains the conformation of the AT_1 receptor protein (Ohyama et al., 1995). The latter disulfide bond, which is not present in the AT_2 receptor, renders the AT_1 receptor susceptible to inactivation by dithiothreitol and other reducing agents. The cytoplasmic region of the receptor, which is composed of the three intracellular loops and the C-terminal cytoplasmic tail, contains consensus sites for phosphorylation by several serine/threonine kinases, including protein kinase C (PKC) and GPCR kinases. Several of the specific residues that are phosphorylated during AT_1 receptor activation have been identified, but there are no confirmed reports of agonist-induced tyrosine phosphorylation of the AT_1 receptor or other GPCRs.

Similar structural features are present in several other cloned mammalian and nonmammalian AT receptors. The rat and mouse AT_1 receptors exist as two distinct subtypes, termed AT_{1A} and AT_{1B}, that are 95% identical in their amino acid sequences. The two subtypes are also similar in terms of their ligand binding and activation properties but differ in their tissue distribution, chromosomal localization, genomic structure, and transcriptional regulation. None of the other cloned mammalian AT_1 receptors, including those from cow (Sasaki et al., 1991), human (Bergsma et al., 1992; Curnow et al., 1992), pig (Itazaki et al., 1993), rabbit (Burns et al., 1993), and dog (Burns et al., 1994) appear to have subtypes. The two AT_1 subtypes in the rodent genome may be the consequence of a gene duplication event that occurred during evolution after the branching of rodents from the mammalian phylogenetic tree (Aiyar et al., 1994b).

C. Genomic Organization of Rat AT_{1A} and AT_{1B} Receptor Genes

The rat AT_{1A} receptor gene is 84 kb in length and contains three introns and four exons, the third of which (~2 kb) includes the entire 1077-base pair (bp) coding sequence of the receptor protein as well as 5′ and 3′ untranslated sequences (Langford et al., 1992; Murasawa et al., 1993; Takeuchi et al., 1993). The first two small exons encode alternatively spliced 5′ untranslated sequences, and the fourth exon (1 kb) encodes an additional 3′ untranslated sequence. A 2.3-kb transcript is found in all AT_{1A}-expressing tissues and contains exons 2 and 3. An additional 3.3-kb transcript containing exons 2, 3, and 4 is present in vascular smooth muscle cells and several other tissues but is not found in the brain. The transcription start site of the AT_{1A} receptor gene is located about 70 kb upstream from the exon that encodes the receptor protein. The rat AT_{1B} receptor gene is about 15 kb in length and contains two introns and three exons, the first two of which encode 5′ untranslated sequences. The third exon contains the entire coding region of the receptor and the 3′ untranslated sequence. The AT_{1B} receptor has 92 and 95% homology with the AT_{1A} at the nucleotide level and amino acid levels, respectively (Guo and Inagami, 1994) and is expressed in relatively few tissues as a 2.4-kb transcript. The rat AT_{1A} and AT_{1B} receptor genes are located on chromosomes 17q12 and 2q24, respectively (Tissir et al., 1995).

D. Expression and Regulation of Rat AT_{1A} and AT_{1B} Receptor

AT_{1A} and AT_{1B} receptors exhibit similar ligand binding and signal transduction properties but differ in their tissue distribution and transcriptional regulation. In the rat, AT_{1A} and AT_{1B} receptor mRNAs are expressed in numerous tissues, including adrenal, kidney, heart, aorta, lung, liver, testis, pituitary gland, and brain. AT_{1A} transcripts are predominantly expressed in all tissues except the adrenal and pituitary glands, where the AT_{1B} message is the major subtype. AT_{1A} receptors are abundantly expressed in vascular smooth muscle cells, in which their properties and regulation have been extensively investigated. In the adult mouse, AT_{1A} receptors are expressed in the kidney, liver, adrenal gland, ovary, brain, testis, lung, heart, and adipose tissue. In contrast, AT_{1B} receptors are confined to the adrenal gland, brain, and testis (Burson et al., 1994).

Studies on the tissue-specific expression of AT_1 receptor by in situ hybridization revealed that liver, heart, and lung contain solely AT_{1A} receptors, whereas the anterior pituitary gland contains only AT_{1B} receptors (Gasc et al., 1994). In the adrenal gland, the zona glomerulosa contains both AT_{1A} and AT_{1B} transcripts, the zona fasciculata contains little of either subtype, and only AT_{1A} mRNA is present in the medulla. In the kidney, AT_{1A} mRNA is present in mesangial and juxtaglomerular cells, proximal tubules, vasa recta, and interstitial cells, whereas AT_{1B} mRNA is found only in mesangial and juxtaglomerular cells, and in the renal pelvis. In male rats, quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) showed that the relative abundance of AT_{1A} transcripts is 100% in liver, 85% in lung, 73% in kidney, 48% in adrenals, and 15% in the pituitary gland (Llorens-Cortes et al., 1994). In contrast to the adult animal, only AT_{1A} receptors are expressed in the pituitary gland during fetal and postnatal life.

The expression of the AT_{1A} receptor is stimulated by glucocorticoids, which act via one of three putative glucocorticoid responsive elements located in its promoter region (Guo et al., 1995). In the rat heart, where the AT_{1A} receptor is expressed in 10-fold excess over the AT_{1B} receptor, treatment with dexamethasone increased AT_{1A} and AT_{1B} mRNA levels by 100 and 300%, respectively (Della Bruna et al., 1995). Conversely, deoxycorticosterone acetate suppressed AT_{1A} mRNA levels by 70%, indicating that glucocorticoids and mineralocorticoids exert reciprocal actions on AT_{1A} receptor levels in the heart. In the heart and aorta, transcripts for both AT_1 subtypes were reduced by treatment with an AT_1 receptor antagonist. However, the AT_{1B} subtype was preferentially reduced, suggesting that the expression of AT_{1B} receptors in the adrenal is dependent on the activity of the renin-angiotensin system (Kitami et al., 1992).

Estrogens also influence the expression of AT_1 receptors, and exert divergent actions on subtype abundance in the pituitary gland and vascular smooth muscle. Estrogen treatment suppresses the expression of AT_{1B} but not AT_{1A} mRNA in the pituitary gland (Kakar et al., 1992). On the other hand, AT_{1A} receptor expression in vascular smooth muscle is elevated in ovariectomized rats and restored to normal by estrogen replacement (Nickenig et al., 1996). In cultured vascular smooth muscle cells, a high concentration of estradiol (1 μM) reduced AT_{1A} mRNA by about 30%. Whether estrogen deficiency leads to increased vascular AT_1 receptor expression in the human has yet to be determined.

Other forms of hormonal regulation of AT_1 receptor expression include the insulin-induced up-regulation of vascular AT_1 receptor expression, which has been attributed to a post-translational mechanism (Nickenig et al., 1998). In cultured vascular smooth muscle cells, insulin caused a doubling of AT_1 receptor density and a concomitant increase in the Ang II-induced intracellular Ca^{2+} response. This increase in receptor content, which was dependent on tyrosine phosphorylation and the intracellular Ca^{2+} response, was due to an increase in receptor mRNA stability rather than increased gene transcription. In rat astrocytes, growth hormone but not insulin-like growth factor 1 (IGF-1) also increased AT_{1A} receptor expression. This was associated with an increase in gene transcription and elevated mRNA levels. AT_{1B} receptors, which were much less abundant than the AT_{1A} subtype, were not affected by growth hormone treatment (Wyse and Sernia, 1997). On the other hand, nitric oxide (NO) caused a marked decrease in AT_{1A} gene expression in vascular smooth muscle cell (VSMC) that was independent of changes in cyclic GMP. This was accompanied by an inhibitory action of NO on the expression of a reporter gene containing 616 bp of the AT_1 receptor gene promoter, and reduced association with a DNA binding protein that interacts with this region (Ichiki et al., 1998).

E. The Human AT_1 Receptor

The human AT_1 receptor contains 359 amino acids, and its deduced amino acid sequence is 95% identical with those of the rat and bovine AT_1 receptors (Curnow et al., 1992; Bergsma et al., 1992; Furuta et al., 1992). The receptor is derived from a single large gene that contains five exons ranging in size from 59 to 2014 bp (Guo et al., 1994). The open reading frame of the AT_1 receptor is located on exon 5. The other four exons participate to varying degrees in alternative splicing to produce mature RNAs that encode two receptor isoforms that are translated with different efficiencies (Curnow et al., 1995). The inclusion of exon 2 occurs in up to 50% of AT_1 mRNAs and inhibits the translation of the downstream AT_1 receptor sequence. In about one-third of AT_1 transcripts, the splicing of exon 3 to exon 5 yields a receptor with a 32 amino acid N-terminal extension. The

ligand binding and signaling properties of this receptor are similar to those of the predominant shorter isoform of the AT_1 receptor.

The human AT_1 receptor gene is located on the q22 band of chromosome 3 (MEM number 106165) (Curnow et al., 1992; Davies et al., 1994). An additional human AT_1 receptor gene was suggested by the report of a human cDNA clone that differed from the known sequence in 10 of its 359 residues (Konishi et al., 1994), but subsequent studies have not confirmed the existence of a second gene (Curnow, 1996; Su et al., 1996). However, most human Ang II target tissues also express the slightly longer and functionally similar AT_1 receptor that results from alternative splicing of exons 3/5 as noted above. The longer isoform appears to be better expressed at the plasma membrane in cell transfection studies, but there is no evidence to suggest that it has a significant physiological role in AT_1 receptor function (Curnow, 1995).

Expression of the human AT_1 receptor is enhanced by epidermal growth factor in transfected COS-7 cells (Guo and Inagami, 1994b). Relatively little is known about the control of expression of the AT_1 receptor in most Ang II target tissues in the human. In the reproductive system, both Ang II and its AT_1 and AT_2 receptor types are present in the endometrium and exhibit cyclic changes during the menstrual cycle with a maximum in the early secretory phase (Ahmed et al., 1995). AT_1 receptors are expressed in the glands and the endometrial blood vessels and may participate in uterine vascular regulation and regeneration of the endometrium after menstruation. The human placenta expresses the AT_1 receptor and all other components of the renin-angiotensin system. The receptors are present throughout gestation in the syncytiotrophoblast and cytotrophoblast, and in the fetal vascular endothelial cells (Cooper et al., 1999). AT_1 receptor mRNA transcripts (2.4 kb) and receptor protein (83 kDa) increase progressively during pregnancy and reach their maximal level in the term placenta (Petit et al., 1996).

A local renin-angiotensin system is also present in human adipose tissue, with expression of angiotensinogen, ACE, and AT_1 receptor genes in omental and s.c. fat and cultured adipocytes (Engeli et al., 1999). The extent to which these components are related to the development of hypertension and obesity-related disorders has yet to be established. In the human kidney, AT_1 receptors are expressed in the renal vasculature, glomeruli, and the vasa recta bundles in the inner stripe of the outer medulla (Goldfarb et al., 1994). AT_1 receptors are diminished in the glomeruli of patients with chronic renal disease (Wagner et al., 1999). The AT_1 receptors expressed in cultured human mesangial cells mediate Ang II-induced hypertrophy and proliferative responses, implying that Ang II may be involved in the pathogenesis of glomerulosclerosis (Orth et al., 1995).

Similar effects of Ang II are mediated by AT_1 receptors in human pulmonary artery smooth muscle cells, in which Ang II stimulates DNA and protein synthesis. This response was associated with activation of mitogen-activated protein kinase (MAPK) and was prevented by losartan and by the MAPK inhibitor, PD-98059. These findings suggest that Ang II-induced activation of the AT_1 receptor initiates signaling pathways that participate in growth and remodeling of the human vascular system (Morrell et al., 1999).

In erythroid progenitor cells, which express both AT_1 and erythropoietin (EPO) receptors, Ang II enhances EPO-stimulated erythroid proliferation in vitro (Mrug et al., 1997). In vivo, the β_2-adrenergic receptor-induced production of EPO in normal subjects was inhibited by losartan treatment, implying that Ang II is a physiological regulator of EPO production in the human (Freudenthaler et al., 1999).

1. AT_1 Receptor Gene Polymorphisms and Cardiovascular Disease. The discovery of several polymorphisms in the human AT_1 receptor gene, one of which (A1166C) was more frequent in hypertensive subjects (Bonnardeaux et al., 1994), initiated a series of studies on the role of such mutations in the genesis of hypertension and other cardiovascular disorders. Subsequently, this polymorphism was reported to act synergistically with the angiotensin converting enzyme DD genotype on the risk of myocardial infarction (Tiret et al., 1994). However, the results of subsequent reports on this topic have not been consistent. In some studies, the A1166C polymorphism had no effect on ambulatory blood pressure, left ventricular mass, or carotid arterial wall thickness (Castellano et al., 1996; Schmidt et al., 1997). In other reports, the same AT_1 receptor gene polymorphism was associated with increased coronary arterial vasoconstriction in response to methylergonovine maleate (Amant et al., 1997), essential hypertension (Szombathy et al., 1998; Kainulamen et al., 1999), and increased left ventricular mass but not hypertension (Takami et al., 1998). An analysis of the role of this polymorphism in rats overexpressing the mutant human AT_1 receptor in the myocardium suggested that it is associated with increased responsiveness to Ang II. This may lead to cardiac hypertrophy under high-renin conditions or during pressure and volume overload (Van Geel et al., 1998).

F. The Amphibian AT_1 Receptor

In the *Xenopus laevis* oocyte, endogenous Ang II receptors were detected in the ovarian follicular cells that surround the oocyte. These receptors mediate Ang II-induced elevations of cytoplasmic Ca^{2+} in the oocyte via gap junctions between follicular cells and oocyte (Sandberg et al., 1990, 1992b) and are thus functionally identifiable as AT_1 receptors. However, the amphibian (xAT) receptor for Ang II did not recognize the nonpeptide antagonist, DuP753, that inhibits the binding and ac-

tions of Ang II at the mammalian AT_1 receptor (Sandberg et al., 1991). The xAT receptor cDNA was cloned from a *Xenopus* myocardial cDNA library to investigate the structural basis of this functional distinction in ligand binding. The xAT receptor is a 41-kDa protein containing 362 amino acids that has 60% amino acid identity and 65% nucleotide homology with the coding regions of known mammalian AT_1 receptors (Ji et al., 1993; Aiyar et al., 1994a). When expressed in *Xenopus* oocytes, xAT receptors mediate Ang II-induced Ca^{2+} mobilization and are pharmacologically distinct from mammalian AT_1 receptors. Receptor transcripts are present in *Xenopus* lung, liver, kidney, spleen, and heart, but not in adrenal, intestine, and smooth muscle. Mutational analyses of xAT and rat AT_1 receptors have largely elucidated the structural basis of their individual ligand binding properties, as described below.

G. The AT_1 Receptor Null Mouse

Gene targeting experiments have provided several important insights into the physiological role of the renin-angiotensin system in cardiovascular regulation, fluid and electrolyte balance, and development. Deletion of the genes encoding angiotensinogen (Tanimoto et al., 1994; Kim et al., 1995) and ACE (Krege et al., 1995; Esther et al., 1996) revealed that the lack of Ang II in Agt−/− or *Ace*−/− mice was associated with hypotension, reduced survival, and marked abnormalities in renal development. Most of the Agt null animals died before weaning and most of the ACE null mice died within 12 months. The Agt and *ACE* null animals that survived until adult life had severe renal lesions. In both cases, the kidneys showed focal areas of cortical inflammation, thickened arterial walls, and medullary hypoplasia with a consequent deficit in urinary concentration. An additional feature of interest in the *Ace*−/− animals was impaired fertility in the male animals, although histologically the sperm appeared to be normal and had normal motility (Krege et al., 1995; Esther et al., 1997). This was dependent on the loss of a testis-specific ACE isozyme that is expressed in round and elongating spermatids and was associated with defects in sperm transport in the oviduct and binding to the zona pellucida of the oocyte (Hagaman et al., 1998). In mice lacking both ACE isozymes, male fertility was selectively restored by sperm-specific expression of the testicular isoenzyme (Ramaraj et al., 1998).

The effects of disruption of the mouse gene encoding the AT_1 receptor types were in part predictable from the results of deleting the genes encoding angiotensinogen and ACE. Mice lacking a functional AT_{1A} receptor had a significant reduction of resting blood pressure (ca. 20 mm Hg) and lacked the pressor/depressor responses to infused Ang II that occur in normal animals. However, the Agtr1a(−/−) animals showed no marked impairment of development and survival and had no major abnormalities in the heart, vascular system, and kidney (Ito et al., 1995; Sugaya et al., 1995a,b; Chen et al., 1997). Closer examination of the Agtr1a(−/−) animals revealed a slight decrease in survival, marked hypertrophy of the renal juxtaglomerular cells, and a moderate degree of mesangial expansion (Oliviero et al., 1997). Also, the tubuloglomerular feedback loop that regulates sodium delivery to the distal tubule was not detectable in AT_{1A} knockout mice, indicating a specific role of AT_1 receptor in the operation of this homeostatic mechanism (Schnermann et al., 1997). The absence of the severe renal lesions observed in animals lacking angiotensinogen or ACE at first suggested that the AT_{1A} receptor is not a critical determinant of normal renal development and structure. However, the more severe effects of angiotensinogen and ACE deficiency, and the prominent inhibitory action of losartan treatment on renal structure and function in neonatal mice, indicated that Ang II action through the AT_1 receptor is essential for normal renal growth and development (Tufro-McReddie et al., 1995).

Subsequent studies revealed that Ang II infusions in Agtr1a(−/−) mice treated with enalapril to reduce endogenous Ang II production cause dose-related elevations in blood pressure that were prevented by AT_1 receptor antagonists. These pressor responses were much smaller than those observed in wild-type mice, but nevertheless demonstrated that AT_{1B} receptors participate in blood pressure regulation in the absence of the AT_{1A} receptor (Oliverio et al., 1997). This was confirmed by the finding that Ang II-induced calcium mobilization was similar in vascular smooth muscle cells from AT_{1A}-deficient and wild-type mice, and was blocked by losartan (Zhu et al., 1998b). Thus, the AT_{1B} receptor contributes substantially to Ang II action in the cardiovascular system in the absence of the AT_{1A} receptor, and presumably is subsidiary to the major AT_{1A} subtype in normal animals. This is supported by data obtained in mice with a double knockout of the AT_{1A} and AT_{1B} receptors, which have a more severe phenotype and lower blood pressure than mice lacking only the AT_{1A} receptor (Oliviero et al., 1998a; Tsuchida et al., 1998).

These conclusions were confirmed by the finding that mice lacking both AT_{1A} and AT_{1B} receptors showed impaired growth and marked abnormalities in renal structure and function. The renal abnormalities in the double knockout animals were similar to those seen in Agtr−/− and *Ace*−/− mice and were accompanied by comparable decreases in blood pressure and the complete absence of pressor responses to Ang II. In these animals, inhibition of converting enzyme by enalapril caused a paradoxical increase in blood pressure that could result from impairment of AT_2 receptor signaling and possibly an inhibitory effect on renal sodium excretion (Oliviero et al., 1998). These observations demonstrated that although the AT_{1B} receptor has a relatively minor role in normal animals, its contributions to growth, renal development, and cardiovascular regulation can compensate for much

of the loss of the major regulatory actions of the AT_{1A} receptors in Agtr1a−/− animals.

Studies on the role of the AT_1 receptors in sodium homeostasis revealed that Agtr1a−/− mice have a further fall in blood pressure during sodium restriction and, unlike wild-type mice, develop negative sodium balance. However, these animals showed normal increases in plasma aldosterone levels during sodium depletion, consistent with the abundance of AT_{1B} receptors in the adrenal zona glomerulosa. These findings suggest that the hypotension observed in Agrt1a−/− mice results from sodium deficiency and blood volume depletion and are consistent with the major role of AT_{1A} receptors in renal sodium resorption. The mechanisms by which Ang II regulates water homeostasis through its actions in the kidney and the brain were also clarified by observations in AT_{1A} receptor-deficient mice (Oliviero et al., 1998a,b). Agtr1a−/− mice have a significant defect in urinary sodium concentration and develop marked serum hypotonicity during water deprivation. This does not result from impairment of vasopressin action on water permeability in the distal tubule but from the inability to maintain a maximal sodium gradient in the kidney. This change is not increased by losartan treatment and appears to be solely dependent on AT_{1A} receptor function. In contrast, the central action of Ang II on drinking responses appears to be mediated by AT_{1B} receptors, since it is largely retained in Agtr1a−/− mice but is almost abolished in the mice lacking AT_{1B} receptor (Davisson et al., 1998).

In contrast to the hypertension and impaired vascular responses observed in AT_1-deficient mice, knockout of the AT_2 receptor leads to elevation of blood pressure and increased vascular sensitivity to Ang II (Hein et al., 1995a; Ichiki et al., 1995b). This has suggested that the AT_2 receptor may exert a protective action in blood pressure regulation by counteracting AT_1 receptor function. Such an action could be exerted in part by reduced expression of the AT_1 receptor, which is increased in the vascular smooth muscle of AT_2-deficient mice (Tanaka et al., 1999). However, the sustained hypersensitivity to Ang II in such animals is also attributable to loss of the counter-regulatory action of renal bradykinin and cyclic GMP formation (an index of NO production) (Siragy et al., 1999). The relative contributions of these two factors to AT_2-dependent vascular regulation, and the extent to which AT_2 receptor deficiency could contribute to sustained blood pressure elevation, as in human hypertension, have yet to be determined (see *Section III, D*).

H. Structural Basis of Ligand Binding to the AT_1 Receptor

The cloning of Ang II receptors from several species was followed by extensive studies on the structural features that are responsible for many of the specific functional properties of the AT_1 receptor. Mutational analyses of AT_1 receptors have identified many of the amino acid sequences and residues that are involved in the processes of ligand binding, agonist activation, G protein coupling, and internalization of agonist-receptor complexes.

1. Determinants of Ang II Bioactivity. The major features of the Ang II octapeptide (*Asp*^{1}-*Arg-Val-Tyr-Ile/Val-His-Pro-Phe*^{8}) that determine its biological activity were identified in early studies on the in vivo and in vitro actions of structurally modified Ang II peptides (Khosla et al., 1974). All of the biological responses that were analyzed in these early reports were mediated by what is now defined as the AT_1 receptor. These findings were extended by the development of radioligand-receptor binding assays that used radioiodinated Ang II or its peptide analogs for in vitro analysis of the hormone-receptor interaction. More recent studies on the properties of cloned and mutant AT_1 receptors have led to the development of ligand-receptor models that incorporate the major features currently believed to be important in agonist binding to the AT_1 receptor. They have also provided further insights into the nature of the peptide binding site and the structural features that determine receptor activation, G protein coupling, and agonist-induced desensitization and internalization of the receptor.

The biological activity of Ang II is highly dependent on the aromaticity of its *Phe*^{8} C-terminal residue. The aromatic side groups of *Tyr*^{4} and *His*^{6}, the guanidine group of *Arg*^{2}, and the charged carboxyl terminus, are also essential for receptor activation (Khosla et al., 1974). In contrast, the N-terminal residues are important for receptor binding and the duration of action of Ang II agonists but are not specifically required for biological activity. The Ang II (2–8) heptapeptide (Ang III) formed by deletion of the *Asp*^{1} residue is almost as potent as the native octapeptide. The Ang II (3–8) hexapeptide (Ang IV) and the Ang II (4–8) pentapeptide also retain full biological efficacy but are weak agonists due to their low-binding affinity for the AT_1 receptor. The des-*Phe*^{8} Ang II (1–7) heptapeptide also binds to the receptor with low affinity, but has no agonist activity (Capponi and Catt, 1979) in most Ang II target cells, and is accordingly a weak Ang II antagonist in vitro (Mahon et al., 1994).

NMR analyses of Ang II and its peptide analogs yielded models for the spatial arrangement of the four pharmacophore groups, Tyr^4, His^6, Phe^8, and the C-terminal carboxyl group, that determine the biologically active conformation of the peptide (Nikiforovich and Marshall, 1993; Matsoukas et al., 1994; Nikiforovich et al., 1994). These studies indicated that the three aromatic rings cluster together and suggested that a bend in the Tyr-Ile-His region of the molecule is a prominent feature of its agonistic conformation. The charged amino- and carboxyl-terminal regions of the Ang II molecule are believed to form an ion pair that maintains the hairpin shape of the Ang II molecule and also stabilizes

the binding of Ang II to the receptor. Structural comparisons of Ang II and its nonpeptide antagonist analogs by overlapping the imidazole, phenyl ring, and acidic moieties confirmed the model of Ang II as a twisted hairpin shape with about 9 Å between the His^6 imidazole and terminal carboxylate groups (Pierson and Freer, 1992). Also, significant interactions between the Asp^1 and Arg^2 side chains of Ang II were suggested by proton NMR studies (Zhou et al., 1991).

Subsequent studies confirmed the compact folded shape of the Ang II peptide backbone, and the relative positions of the four pharmacophore groups. This shape resulted from electrostatic interactions between the N- and C-terminal groups and was similar to that for antibody-bound Ang II as determined by crystallography (Joseph et al., 1995a,b). The conformation of Ang II in phospholipid micelles also confirmed the hairpin structure of the molecule and suggested the presence of a stable hydrogen bond between the Phe^8 NH and His^6 carbonyl group, with an inverse gamma turn centered on Pro^7 (Carpenter et al., 1998).

The Phe^8 residue of Ang II has long been recognized to be crucial for its biological activity. This residue is important for both the binding activity and the intrinsic activity of Ang II, and even minor charges in its structure have marked effects on biological activity. Its aromaticity and steric influence determine the biologically active conformation of the C terminus of Ang II, which requires an appropriate orientation of the position 8 carboxyl group relative to the aromatic group of the phenylalanine residue. Replacement of Phe^8 by nonaromatic residues endows antagonist properties that result from distortion of the orientation of the C-terminal carboxyl group (Aumelas et al., 1985). Consistent with its central role in receptor activation, replacement of Phe^8 by D-Phe^8 also caused antagonist activity, and replacement by *Phe(Br5)8* causes prolonged pressor responses attributed to slow dissociation of the more hydrophobic peptide from the Ang II receptor (Bosse et al., 1990).

2. Agonist Binding Site of the AT_1 Receptor. Amino acids in the AT_1 receptor that are essential for Ang II binding include the four cysteine residues that form the two external disulfide bonds and several other residues located in the exposed surface regions of the receptor (Fig. 2). In addition, polar or charged residues located within the hydrophobic transmembrane domains, including Lys^{102} at the top of TM helix III and Lys^{199} near the top of TM helix V, participate in agonist binding. Some of the extracellular residues contribute to ligand interaction and stabilization of Ang II binding and others to the conformational change that causes receptor activation. The additional disulfide bridge between the amino terminal region and the third extracellular loop of the AT_1 receptor appears to stabilize the receptor and may be necessary to maintain the proximity of the extracellular amino acids that are involved in peptide binding. Cleavage of this disulfide bond probably ac-

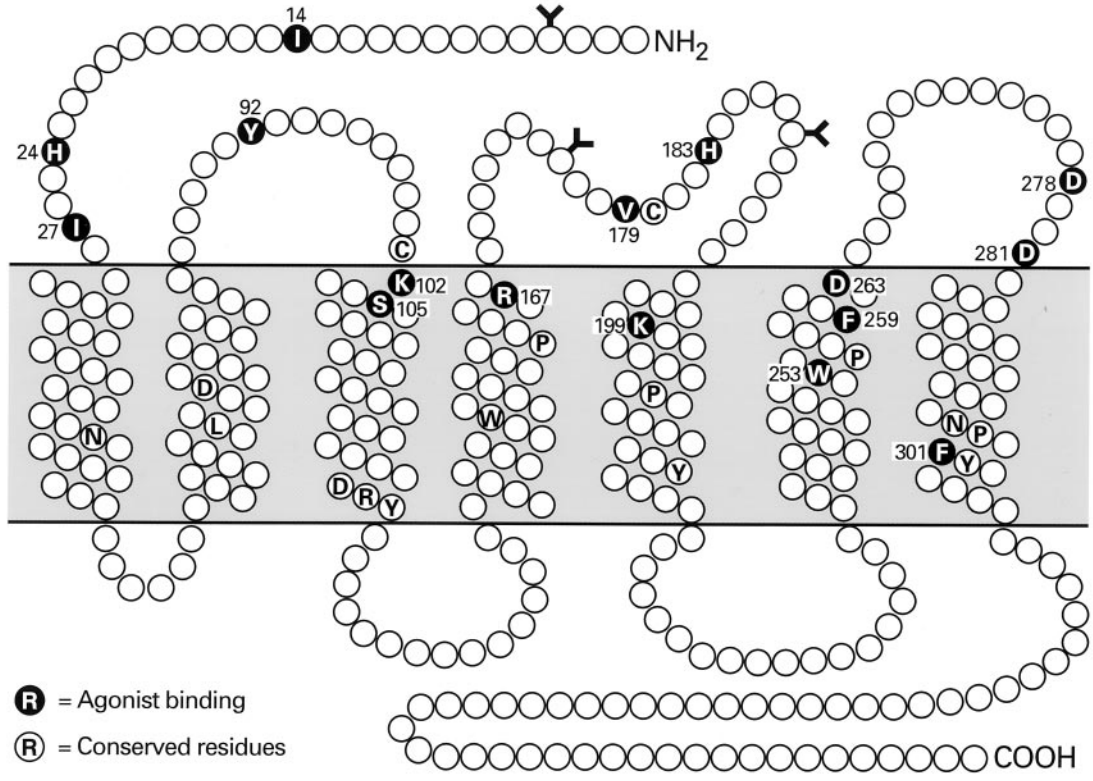


FIG. 2. Amino acids that contribute to the agonist binding site of the AT_1 receptor. Residues implicated in Ang II binding are shown as blue letters on pink background. The positions of the glycosylation sites, and of the conserved residues among GPCRs, are also shown.

counts for the impairment of AT_1 receptor binding by reducing agents (Ohyama et al., 1995).

Ang II binds primarily to the extracellular region of the AT_1 receptor (Hjorth et al., 1994) by interacting with residues in its N terminus and its first and third extracellular loops. However the transmembrane helices also participate in Ang II binding, since its C-terminal carboxyl group interacts with Lys^{199} in the upper part of helix 5 of the receptor (Underwood et al., 1994; Noda et al., 1995a; Yamano et al., 1995). This could involve the formation of a salt-linked triad between Lys^{199} of the receptor and the carboxyl groups of Asp^1 and Phe^8 of the Ang II peptide (Joseph et al., 1995a,b). The Trp^{253} residue has been proposed to stabilize the ionic bridge formed between Lys^{199} and the carboxyl-terminal group of the Phe^8 residue. In addition, Phe^{259} and Asp^{263} in transmembrane helix VI could provide the docking site for His^6 of the ligand (Yamano et al., 1995). Two other residues (Lys^{102} and Ser^{105}) in the outer region of transmembrane helix III of the receptor have also been implicated in Ang II binding (Groblewski et al., 1995; Noda et al., 1995a). This region may participate in the formation of the intramembrane binding pocket and possibly in stabilization of the receptor's conformation.

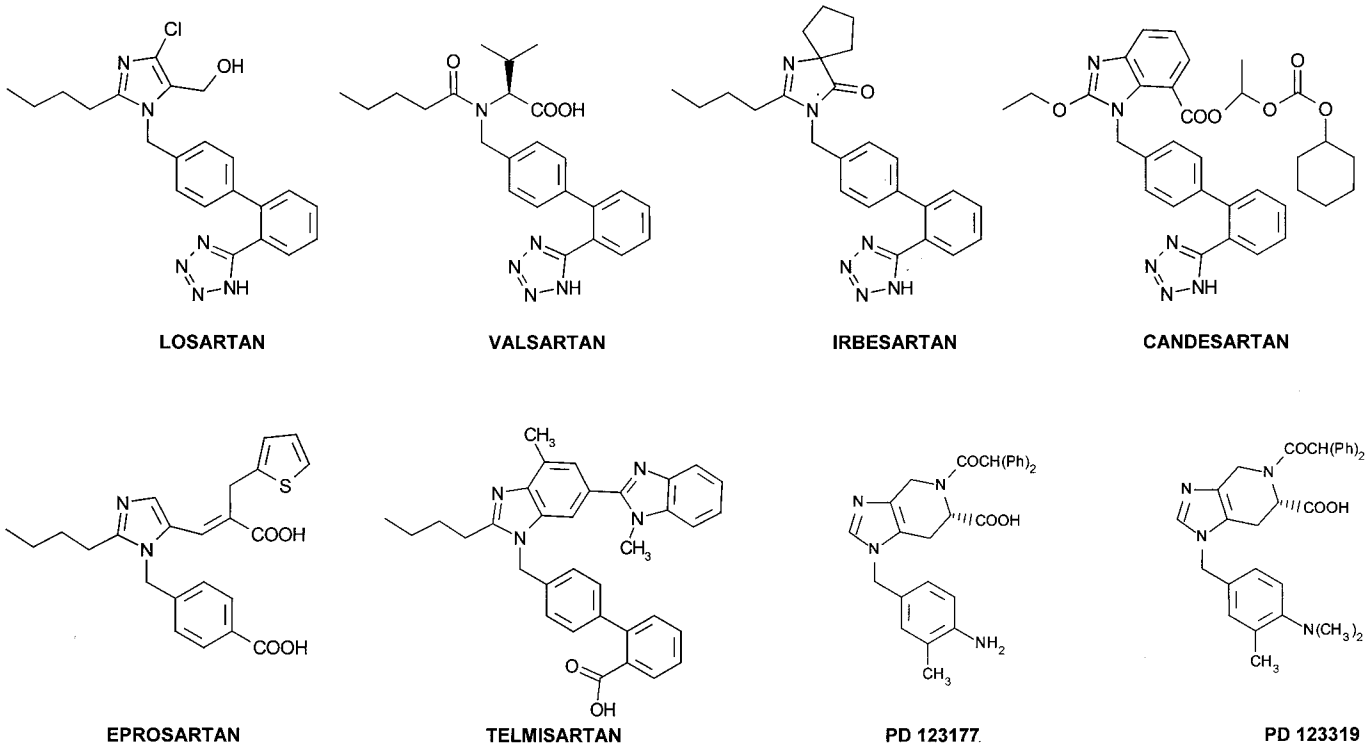
The Asp^{281} residue, located at the C-terminal end of the third extracellular loop of the AT_1 receptor, serves as a major docking point for Ang II through its charge interaction with Arg^2 of the ligand (Feng et al., 1995). The Asp^{278} residue could also be important in this regard, since its mutation causes an even greater loss of receptor binding affinity (Hjorth et al., 1994). The N-terminal Asp^1 residue of Ang II has been proposed to interact with His^{123} in the second extracellular loop of the AT_1 receptor (Yamano et al., 1995). These findings support the view that Ang II attaches primarily via its charged amino-terminal end to the extracellular binding region of the receptor. The major docking points for the amino- and carboxyl-terminal ends of Ang II, Asp^{281} and Lys^{199}, respectively, are located at the outer ends of helices 7 and 5, respectively. The intramembrane binding pocket lies between these proposed contact points, and is adjacent to the cleft that contains the binding sites of receptors for smaller ligands, as well as the nonpeptide binding site of the AT_1 receptor. This region contains docking sites for the apolar/aromatic mid-portion of the Ang II molecule and for the carboxyl-terminal phenyl group that elicits the conformational change(s) leading to receptor activation.

The apolar nature of the essential aromatic ring of the Phe^8 residue of Ang II binds to the AT_1 receptor suggests that the phenyl group could interact with residues located within the membrane-spanning helices. This is consistent with NMR-based predictions of the receptor-bound conformation of Ang II that position the phenyl group in an appropriate location for such interactions (Nikiforovich et al., 1993, 1994). The phenyl group could interact with aromatic residues in the helices, some of which have been suggested to form an aromatic floor for charge interactions between ligand and receptor (Trumpp-Kallmeyer et al., 1992; Findlay et al., 1993). Modeling studies on the AT_1 receptor have also suggested that conserved aromatic residues in helices IV and VI could form the base of the ligand binding site, as in other G protein-coupled receptors (Underwood et al., 1995). Mutations of two of these residues, Phe^{259} and Trp^{253}, reduces Ang II binding to the receptor (Yamano et al., 1995). Whether the aromatic group of Phe^8 of the ligand interacts with the binding pocket of the receptor via these amino acids, or with aromatic residues in other segments of the membrane domains, has yet to be determined. The Phe^8 residue could also form a polar/aromatic interaction with the hydroxyl group of Ser^{105} in the third transmembrane domain of the receptor (Joseph et al., 1995a,b).

The Tyr^4 residue of Ang II is an important determinant of its binding and biological activities (Bumpus et al., 1977; Capponi and Catt, 1979; Nikiforivich et al., 1993, 1994). In fact, reversing the Tyr^4 and Phe^8 residues of the Ang II molecule (Marshall et al., 1970) formed the first Ang II antagonist. The Tyr^4 residue has been proposed to interact with Arg^{167} at the top of the fourth transmembrane helix (Yamano et al., 1995), and could also disrupt the hydrogen bonding between Asn^{111} and Tyr^{292} in transmembrane domains 3 and 7 of the unoccupied receptor by competing with Tyr^{292}. This would permit the latter to interact with Asp^{74} in the second transmembrane domain during receptor activation. In this mechanism, the loss of a proton from the Tyr^4 phenolic hydroxyl group to the carboxyl group of Glu^{91} could be part of a relay system initiated by the interaction of His^6 with Thr^{88} and Glu^{91} of the receptor (Joseph et al., 1995a,b).

3. Antagonist Binding of the AT_1 Receptor. Since Ang II is a major regulator of blood pressure, aldosterone secretion, and fluid homeostasis, and is also an important etiological factor in hypertension and other cardiovascular disorders, blockade of Ang II formation or action by ACE inhibitors or receptor antagonists is of major therapeutic importance. Early attempt to develop therapeutic agents able to block the Ang II receptor impeded by the peptidic nature of antagonists such as saralasin, which lacked oral activity and showed agonistic properties (Pals et al., 1979). More recently, based on imidazole derivatives first described by Furukawa et al. (1982), it became possible to develop specific nonpeptide Ang II receptor antagonists that specifically and selectively block the angiotensin AT_1 receptor (Timmermans et al., 1993; Goodfriend et al., 1996). The first of this series to reach the clinic, losartan, was followed by a large number of orally active AT_1 antagonists (Table 2).These can be classified in two groups depending on the presence of a biphenyltetrazole moiety, as in the prototype drug, losartan, in their structure. Receptor binding of nonpeptide Ang II antagonists is saturable

TABLE 2
Structure of AT_1 and AT_2 receptor nonpeptidic antagonists



and usually reversible and is independent of the pathway responsible for the synthesis of Ang II. This could be relevant to comparisons with ACE inhibitors, given the possible role of alternative Ang II-generating enzymes such as chymase, in human tissues (Urata et al., 1996).

In pharmacological studies on the properties of angiotensin and its synthetic analogs, certain AT_1 receptor antagonists not only cause a rightward shift in the Ang II dose-response curve but also reduce the maximal response to agonist stimulation (Wienen et al., 1993; Morimoto and Ogihara, 1994; Criscione et al., 1995; Goa and Wagstaff, 1996; Gillis and Markham, 1997; McClellan and Balfour, 1998). The latter compounds (candesartan, EXP 3174, valsartan, irbesartan) are termed insurmountable antagonists, in contrast to surmountable antagonists such as losartan, eprosartan, and telmisartan, which do not impair the maximum response to Ang II. One explanation for this difference is that nonpeptide antagonists can act by interfering with receptor activation by occupying an intramembrane site that overlaps with the space occupied by the agonist (competitive or surmountable antagonists) or by inducing conformational changes that prevent agonist binding (noncompetitive or insurmountable antagonists). Another proposal is that surmountable antagonists such as losartan dissociate rapidly from the receptor, whereas insurmountable antagonists, exemplified by candesartan, bind tightly and dissociate so slowly as to cause functional loss of the occluded receptors. Recent studies on the properties of the human AT_1 receptor expressed in Chinese hamster ovary (CHO) cells have shown that the agonist-receptor complexes are divisible into a rapidly reversible, surmountable population, and a tightly binding, insurmountable population (Fierens et al., 1999).

Although losartan is a potent antagonist in its own right, about 10% of the dose is metabolized to EXP 3174, which has 10-fold higher affinity for the AT_1 receptor and is responsible for the 24 h decrease in blood pressure. Candesartan cilexetil is an inactive ester prodrug and is completely cleaved during absorption in the gastrointestinal tract. Twenty-five percent of candesartan is eliminated by metabolism to an inactive metabolite. Among the other antagonists, irbesartan, valsartan, and eprosartan do not require metabolism to be active. Irbesartan is mainly eliminated by the liver (75%) and less than 2% is excreted unchanged in the urine. Sixty percent of eprosartan is cleared unchanged via the bile. Valsartan is essentially eliminated by biliary excretion and 10% of the dosage appears intact in urine.

As expected, significant increases in renin activity, Ang I and Ang II are observed after blockade of the AT_1 receptor. It is conceivable that the increased circulating Ang II level could stimulate the AT_2 receptor, which appears to counterbalance the effect of the AT_1 receptor (see below). Blockade of the AT_1 receptor not only inhibits its smooth muscle contraction but also reduces the production of pressor agents including aldosterone, vasopressin, catecholamine, and endothelin. AT_1 receptor

antagonists have shown exceptionally good tolerability and their incidence of adverse effects is similar to that of placebos. The AT_1 receptor antagonists are approved for the treatment of hypertension, and in early clinical studies also appear to be of use in the treatment of congestive heart failure, postmyocardial infarction, and renal failure. Several large clinical trials are in progress such as Scope, Life and Value for hypertension, Regaal and Silver for left ventricular hypertrophy, ValHeft and Charm for congestive heart failure, Optimaal and Valiant for postmyocardial infarction, and Irma, IDNT, Renaal and ABCD 2V for diabetic nephropathy.

Identification of the losartan binding region of the mammalian AT_1 receptor was facilitated by the finding that the amphibian Ang II receptor, which resembles the mammalian AT_1 receptor in its signal transduction mechanisms, does not recognize nonpeptide antagonists such as losartan. This enabled the amino acid residues involved in the binding of losartan to the mammalian AT_1 receptor to be determined by analysis of the ligand binding properties of mutant rat AT_1 receptors in which nonconserved amino acids were replaced by the corresponding amphibian residues (Ji et al., 1994). Most of these mutant receptors showed only minor changes in binding affinity for Ang II and its peptide antagonist [Sar^1,Ile^8]Ang II, indicating that the overall conformation of the receptor was unaltered by such replacements. However, several residues located in the transmembrane domains (TMDs) of the receptor were found to be required for binding of the nonpeptide antagonist. These included Val^{108} in TMD III, Ala^{163} in TMD IV, Pro^{192} and Thr^{198} in TMD V, Ser^{252} in TMD VI, and Leu^{300} and Phe^{301} in TMD VII.

These findings demonstrated that the nonpeptide AT_1 antagonist binds to a site defined by amino acids located within the membrane-spanning regions of the receptor. Also, the nonpeptide binding site was largely distinct from the receptor domain that is involved in binding of Ang II and other peptide ligands. This conclusion is consistent with the presence of a primordial binding site for small ligands between the transmembrane helices of all GPCRs that can be used for the development of nonpeptide analogs for a wide variety of peptide hormones. Other amino acid residues in the rat AT_{1B} receptor that influence losartan binding include Ala^{73} in TMD II; Ser^{104}, Ala^{114}, and Ser^{115} in TMD III; Lys^{199} in TMD V; Phe^{248} in TMD VI; and Asn^{295} in TMD VII (Schambye et al., 1994; Ji et al., 1994; Noda et al., 1995). These and other observations have implicated TMD III in losartan binding to the mammalian AT_1 receptor. The locations of the multiple amino acids that contribute to losartan binding in the receptor are shown in Fig. 3.

A mutant amphibian receptor formed by exchanging these residues for the corresponding amino acids in the *Xenopus* AT receptor bound losartan with the same high affinity as the rat AT_1 receptor (IC_{50} values: rat AT 2.2 ± 0.2 nM; xAT > 50 :M: mutant xAT 2.0 ± 0.1 nM) (Ji et al., 1993, 1995). This gain-of-function mutation, in

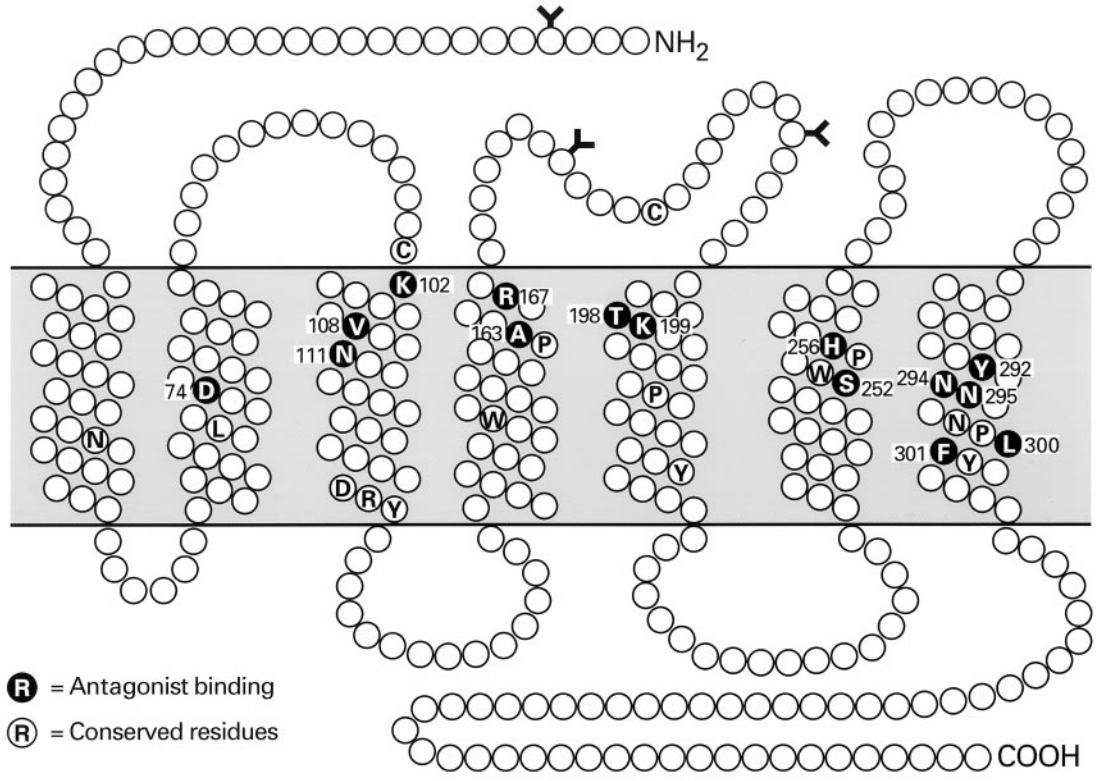


FIG. 3. Amino acids that contribute to the nonpeptide antagonist binding site of the AT_1 receptor. Residues implicated in losartan binding are indicated by green letters on orange background. The positions of the glycosylation sites, and of the conserved residues among GPCRs, are also shown.

which the residues known to participate in the formation of the ligand binding site in a mammalian hormone receptor were transferred to an unresponsive amphibian receptor, confirms the validity of the proposed losartan binding site. The identification of residues involved in receptor binding of nonpeptide Ang II analogs should aid in modeling studies on the structural basis of ligand recognition and activation of the Ang II receptor.

Although there is little overlap between the intramembrane residues that influence Ang II and losartan binding, both ligands appear to interact with the region located between helices III, V, VI, and VII of the AT_1 receptor. This area also contains the binding sites of GPCRs for small ligands such as catecholamines and acetyl choline, as well as other nonpeptide antagonist analogs. In addition to sharing the binding pocket formed by the transmembrane helices of the receptor, Ang II and its nonpeptide analogs probably interact with common contact points at other locations in the receptor. In addition to the Lys^{102}, Ser^{105}, Arg^{167}, and Lys^{199} residues, this could apply to Asn^{295}, replacement of which by serine impairs the binding of Ang II as well as its nonpeptide agonist and peptide antagonist analogs (Hunyady et al., 1998). It is noteworthy that peptide agonist binding is also influenced by Phe^{301}, an aromatic residue located near the end of helix VII (Hunyady et al., 1996a, 1999). Mutation of this residue to alanine impairs Ang II and losartan binding, as well as that of the peptide antagonist, [Sar^1,Ile^8]Ang II. These findings indicate that multiple residues contribute to the integrity of the binding pocket and are required for optimal agonist and antagonist binding.

Although the extracellular loops of the receptor contribute significantly to the binding of Ang II and its peptide antagonists, the intramembrane binding pocket has a major functional role in the AT_1 and probably most other peptide hormone receptors. In receptors for large and/or bulky ligands, the intramembrane binding pocket per se may not permit efficient interaction without additional stabilization of the ligand through binding to residues in the extracellular surface of the receptor. Nevertheless, the binding pocket is essential for the initiation of receptor activation, and its interaction of agonists triggers the change in receptor conformation that leads to G protein coupling and second messenger generation.

The importance of the transmembrane domains in receptor activation also applies to glycoprotein hormone receptors, which bind extremely large ligands. These data suggest that a common mechanism for activation of GPCRs is based on critical interactions within the intramembrane binding pocket that evoke specific changes in receptor conformation. The extracellular domains of the receptor provide additional interactions with the ligand that are necessary for high-affinity binding. This is a prominent feature of receptors for larger agonists such as glycoprotein hormones, but also occurs in those for small peptide hormones such as angiotensin II, bradykinin, and vasopressin.

I. AT_1 Receptor Signaling Mechanisms

1. AT_1 Receptor Activation and Signal Transduction. The AT_1 receptor, like other GPCRs, has been proposed to undergo spontaneous isomerization between its inactive (R) and active (R*) states. The inactive R state is favored in the absence of agonist ligands and is in equilibrium with a small proportion of the active R* state in its unliganded form. In the presence of Ang II, the active R* form of the receptor is either selected or induced by agonist binding. The shift in equilibrium during agonist activation favors the R* state, in which the altered conformation of the receptor permits coupling to one or more G proteins that mediate intracellular signaling via phospholipase C and other pathways. Most receptors appear to be kept in their inactive state by structural restraints that are removed by agonist binding, leading to the formation of the activated R* state. Recent evidence suggests that the agonist-activated AT_1 receptor, and possibly other GPCRs, undergoes transitions into multiple conformational states that are associated with the individual stages of receptor activation and regulation (Thomas et al., 2000).

The proportion of receptors in the activated state varies among individual GPCRs and is manifested by varying degrees of basal signaling activity when receptors are overexpressed in transfected cells. Significant degrees of agonist-independent or constitutive activity have been observed in wild-type thyroid-stimulating hormone and muscarinic receptors (Burstein et al., 1997; Wang and Gershengorn, 1999) but are not evident in the AT_1 and most other GPCRs. Agonist-induced activation of the AT_1 receptor appears to be triggered by an interaction between the Tyr^4 residue of Ang II and the Asn^{111} residue located in the third transmembrane domain of the receptor (Noda et al., 1996). This, and the interaction of Phe^8 of the angiotensin molecule with His^{256} in the sixth transmembrane domain of the receptor, appear to drive the AT_1 receptor into its fully active R* state (Noda et al., 1995a).

The importance of Asn^{111} as a switch residue in agonist-induced AT_1 receptor activation is further indicated by the ability of its mutation to Gly^{111} or other small residues to induce constitutive activation of the receptor (Feng et al., 1998). This suggests that the ability of Asn^{111} to act as a conformational switch is related to its side chain size, rather than polarity or hydrogen binding. On the other hand, it is the aromaticity rather than the size of the Tyr^4 side chain that is important in receptor activation, which probably depends on amino-aromatic binding between Tyr^4 and Asn^{111} of the receptor (Miura et al., 1999a). Aromaticity is likewise important in the interaction between Phe^6 and His^{256}, which is critical for full receptor activation by agonist ligands.

Like other constitutively activated GPCRs, the $Asn^{111}Gly$ and $Asn^{111}Ala$ mutant receptors show increased affinity and efficacy for agonist ligands, and exhibit marked increases in the sensitivity of their biological responses to agonist stimulation. They also discriminate between inverse agonists, which stabilize the inactive conformation of the receptor, and neutral antagonists. For these reasons, constitutively active AT_1 receptors are highly sensitive to Ang II derivatives that are weak or partial agonists at the wild-type receptor (Miura et al., 1999b).

A proposed model of GPCR activation predicts that rigid body movement of the third, sixth, and seventh transmembrane domains induces conformational changes in the cytoplasmic loops that permit G protein interaction with the agonist-activated receptor (Gether and Kobilka, 1998). However, the manner in which the activating regions in the cytoplasmic domains of individual GPCRs interact with specific subsets of G proteins is not yet known. Although many amino acids in the transmembrane and cytoplasmic regions of a wide range of receptors have been implicated in G protein coupling, there are very few cases in which individual residues can be correlated with binding to specific types of G proteins (Hunyady et al., 1996b). The limited degree of selectivity of most such residues presumably reflects the precise conformational requirements for specific interaction of receptors with their cognate G proteins.

Although the AT_1 receptor has been reported to interact with several G proteins, its major physiological functions are expressed through G_q-mediated activation of phospholipase C followed by phosphoinositide hydrolysis and Ca^{2+} signaling. Although all GPCRs possess the basic seven transmembrane structure, only a few amino acids are highly conserved among the superfamily of G protein-coupled receptors (Fig. 1). One such conserved motif is the characteristic $NPX_{2-3}Y$ sequence that is located in the seventh transmembrane domain of most receptors, and in the rat AT_{1A} receptor is Asn^{298}-Pro^{299}-Leu^{300}-Phe^{301}-Tyr^{302}. A recent model of aminergic GPCRs suggests that the $NPX_{2-3}Y$ sequence is ideally placed to receive a signal from agonist-induced conformational changes in the ligand binding region (Donnelly et al., 1994). In the three-dimensional structure of the receptor, this sequence is in close proximity to the asparagine-aspartic acid pair located in transmembrane segments 1 and 2 and may form functionally important hydrogen bonding interactions with this region. Such models assume that the highly conserved proline residues, which disrupt the α-helical structure of the transmembrane domains, could serve as hinges during the conformational changes that occur in agonist-activated receptors. One such proline residue is located within the seventh transmembrane domain in the conserved $NPX_{2-3}Y$ sequence and has been found to be important for signal transduction by the m_3 muscarinic receptor (Wess et al., 1993). However, this residue does not appear to be essential for signaling by Ang II and gonadotropin-releasing hormone (GnRH) receptors.

Another interesting feature of the $NPX_{2-3}Y$ sequence is its similarity to the NPXY internalization sequence that is present in the cytoplasmic segment of receptors for low-density lipoprotein and several growth factors. The tyrosine residue in this sequence has been reported to be essential for sequestration of the β-adrenergic receptor (Barak et al., 1994). However, this does not apply to the gastrin-releasing peptide receptor (Slice et al., 1994) and the AT_1 receptor, both of which contain an additional aromatic amino acid (Phe^{321} and Phe^{301}, respectively) in their $NPX_{2-3}Y$ sequences. The presence of such residues might be important since phenylalanine can substitute for the tyrosine residue in the NPXY internalization sequence of nutrient receptors.

An analysis of the functional role of the Asn^{298}-Pro^{299}-Leu^{300}-Phe^{301}-Tyr^{302} sequence (Hunyady et al., 1995b) revealed that the ability of the receptor to interact with G proteins and to stimulate inositol phosphate responses was markedly impaired by alanine replacement of Asn^{298} and was reduced by replacement of Pro^{299} or Tyr^{302}. The $Phe^{301}Ala$ mutant receptor exhibited normal G protein coupling and inositol phosphate responses, and the binding of the peptide antagonist, [Sar^1,Ile^8]Ang II, was only slightly affected. However, its affinity for Ang II and the nonpeptide antagonist losartan was reduced by an order of magnitude, suggesting that Ang II and losartan share an intramembrane binding site, possibly through their aromatic moieties, in this region. None of the agonist-occupied mutant receptors, including $Tyr^{302}Ala$ and triple alanine replacement of Phe^{301}, Tyr^{302}, and Phe^{304} showed substantial changes in their internalization kinetics. These findings demonstrate that the NPLFY sequence of the AT_1 receptor is not an important determinant of agonist-induced internalization. However, the Phe^{301} residue contributes significantly to agonist binding, and Asn^{298} is required for normal receptor activation and signal transduction.

Another highly conserved amino acid in the superfamily of G protein-coupled receptors is a tyrosine residue located in the fifth transmembrane helix, adjacent to the amino-terminal end of the third cytoplasmic loop. The location of this amino acid, which is Tyr^{215} in the rat AT_1 receptor, suggests that it could be involved in receptor activation. The amino-terminal region of the third cytoplasmic loop adjacent to this residue has been shown to be important in the signal generation and internalization of several G protein-coupled receptors. Furthermore, modeling of G protein-coupled receptors based on the crystal structure of bovine rhodopsin has suggested that this residue is in molecular proximity to regions that are important in receptor activation, including the conserved acidic-arginine-aromatic (DRY) triplet of the second intracellular loop (Baldwin et al., 1997). Although AT_1 receptor internalization and signal transduction have different structural requirements, a

6-amino acid deletion of the amino-terminal end of the third cytoplasmic loop, which included Tyr^{215}, was found to prevent both receptor internalization and signaling responses. The role of the highly conserved Tyr^{215} residue in the activation of GPCRs was analyzed in a mutant AT_1 receptor created by replacing this amino acid with a phenylalanine residue (Hunyady et al., 1995b).

The resulting Tyr^{215}Phe mutant receptor was normally expressed in transfected COS-7 cells, and its binding affinity for the peptide antagonist [Sar^1, Ile^8]Ang II was similar to that of the wild-type receptor. However, its affinity for Ang II was significantly reduced and its ability to mediate inositol phosphate responses to Ang II stimulation was abolished. These changes were associated with loss of coupling of the mutant receptor to G proteins, as indicated by the lack of effect of GTPγS on agonist binding to the receptor. The agonist-induced internalization of the mutant receptor was also impaired. The concomitant decreases in receptor internalization and G protein-mediated signaling of the Tyr^{215}Phe mutant receptor indicate that this residue has a critical role in AT_1 receptor activation. It is possible that the Tyr^{215} residue takes part in propagation of the agonist-induced conformational change to the intracellular loops that participate in G protein coupling. In view of its wide conservation among members of the seven transmembrane domain receptor superfamily, this residue is likely to be of general importance in signal transduction from G protein-coupled receptors. The AT_1 receptor, like many other Ca^{2+}-mobilizing GPCRs, signals primarily through coupling to a $G_{q/11}$ protein that activates phospholipase C-β (PLC-β), leading to polyphosphoinositide hydrolysis, stimulation of $InsP_3$/calcium signaling, and activation of protein kinase C. However, in cultured rat VSMC it is predominantly PLC-γ rather than PLC-β that is activated during Ang II action. This response, which is dependent on tyrosine phosphorylation of the PLC-γ isozyme, is distinct from the usual process of activation of PLC-β_1 and β_2 by GPCRs through α and $\beta\gamma$ subunits derived from the heterotrimeric G proteins, $G_{q/11}$ and $G_{i/o}$, respectively. AT_1 receptors have also been reported to couple to the $G_{12/13}$ family of pertussis-insensitive G proteins, which appear to mediate Ang II-induced L-channel activation in rat portal vein myocytes (Macrez et al., 1997). In the rat, the AT_1 receptor is also coupled to pertussive-sensitive $G_{i/o}$ protein(s) that inhibit adenylyl cyclase in several Ang II target tissues, including the adrenal glomerulosa zone, liver, kidney, and pituitary gland. Ang II has also been found to stimulate modest increases in cyclic AMP production in some of its target tissues. However, this may result from activation of Ca^{2+}-sensitive adenylyl cyclases rather than coupling of the AT_1 receptor to G_s. Interestingly, although the cloned rat AT_{1A} and AT_{1B} receptors expressed in COS-7 and Y-1 cells show the expected coupling to phosphoinositide hydrolysis and other $G_{q/11}$-mediated responses, they do not exhibit coupling to G_i-mediated responses such as inhibition of adenylyl cyclase (Tian et al., 1996).

2. AT_1 Receptor and Tyrosine Phosphorylation. In addition to its rapid actions on phosphoinositide/calcium signaling, Ang II elicits many of the intracellular signaling responses that are typically associated with activation of growth factors. These include tyrosine phosphorylation, most immediately of PLC-γ and subsequently of several other downstream proteins and effector enzymes. These include $pp60^{c\text{-}src}$, pp120, $pp125^{FAK}$, JAK2, STATs, paxillin, TYK2, and MAPK (Bernstein and Marrero, 1996). Because GPCRs do not have intrinsic tyrosine kinase activity, the ability of the AT_1 receptor to induce tyrosine phosphorylation and activation of PLC-γ in rat VSMC, with consequent increases in $InsP_3$ production and Ca^{2+} signaling, must depend on other tyrosine kinases. Such ligand-induced activation of PLC-γ by tyrosine phosphorylation may occur during the actions of growth factors on their target cells, and accounts for the associated elevations in $[Ca^{2+}]_i$. This aspect of AT_1 receptor function has been controversial, because the mechanisms by which the receptor couples to PLC in VSMC are poorly defined. In most cell types, calcium-mobilizing GPCRs are coupled through their cognate G proteins to activation of the β isozymes of PLC. The β1 and β2 enzymes are activated by G_α and $G_{\beta\gamma}$ subunits, respectively, the former derived from $G_{q/11}$ and the latter largely from $G_{i/o}$ proteins. In contrast, AT_1 receptor-mediated activation of PLC-γ is dependent on tyrosine phosphorylation and consequently on signaling from receptor or nonreceptor tyrosine kinases.

The mechanism by which Ang II stimulates tyrosine phosphorylation and activation of PLC-γ involves binding of the enzyme to the C-terminal cytoplasmic domain of the AT_1 receptor. This interaction occurs between the C-terminal cytoplasmic domain of PLC-γ1 and a phosphorylated YIPP motif located within the intracellular tail of the receptor (Venema et al., 1998a). The same YIPP motif has been implicated in the binding of JAK2 to the AT_1 receptor, probably as a complex with the phosphotyrosine phosphatase, SHP-2 (Ali et al., 1997a). The way by which AT_1 receptor activation regulates ligand-dependent binding of two distinct intracellular signaling proteins to the same site in its cytoplasmic tail has yet to be determined. The relative roles of PLC-β and PLC-γ in Ang II-induced phosphoinositide hydrolysis in VSMC have been difficult to determine, and studies on the relative abundance of the PLC isoforms in VSMC have given variable results. Relatively little PLC-β_1 was detected in rat and rabbit VSMC in some reports (Homma et al., 1993; Marrero et al., 1994), but others have found that all three isozymes (α, β, and γ) are abundant in rat VSMC (Ushio-Fukai et al., 1998). In the latter study, AT_1 receptors were proposed to couple initially to PLC-β by heterotrimeric G proteins, and subsequently to PLC-γ. The G proteins responsible for the early activation of PLC-β, and the initial maximum

$InsP_3$ response at 15 to 20 s, included both $G_{q/11}$ and G_{12}. The subsequent activation of PLC-γ and its contribution to the delayed $InsP_3$ response are attributed to its tyrosine phosphorylation by a downstream kinase. This study also suggested that $\beta\gamma$ subunits derived from G_{12} are involved in the activation of PLC in VSMC, and mediate Ang II-induced activation of PLD in these cells.

3. AT_1 Receptor-Activated Growth Responses. In addition to its contractile and secretory actions in smooth muscle and adrenal cells, Ang II stimulates growth and/or proliferative responses in these and others of its target cells. This effect was first documented in rat adrenal glomerulosa cells, which respond to sodium deficiency with hypertrophy and hyperplasia, and undergo regression in animals on high sodium intake (Gross, 1968). These changes are dependent on the circulating Ang II level, which is a major determinant of trophic changes in the adrenal glomerulosa zone (Aguilera et al., 1978). Ang II also has trophic actions on renal mesangial cells and vascular smooth muscle cells, leading to cellular hypertrophy and sometimes to cell division (Huckle and Earp, 1994). Cardiac myocytes and fibroblasts also exhibit hypertrophic responses to Ang II. However, cardiac fibroblasts undergo both hypertrophy and proliferation during stimulation by Ang II, an action that contributes to the development of ventricular hypertrophy. Treatment with AT_1 antagonists has been found to abolish the growth-promoting actions of Ang II in vitro and in vivo. As discussed below, many of the growth-related actions mediated by the AT_1 receptor are inhibited by concomitant binding of Ang II to the AT_2 receptor.

The growth factor-like effects of Ang II include increases in tyrosine phosphorylation of numerous intracellular proteins, activation of MAPK and related pathways, and increased expression of several early response genes including c-*fos*, c-*jun*, and c-*myc* (Clark et al., 1992; Berk and Corson, 1997). These changes are associated with cell growth, increased thymidine incorporation, and cell proliferation (Tian et al., 1995). Such actions of Ang II are consistent with its well defined growth effects in adrenal glomerulosa and VSMC, and its role in the vascular and cardiac lesions associated with endothelial damage, atherosclerosis, hypertension, and cardiac failure. One of the earliest Ang II-induced events in VSMC is the rapid tyrosine phosphorylation and activation of PLC-γ, and the ensuing phosphoinositide/Ca^{2+} signaling responses that initiate many of the actions of Ang II in these cells (Marrero et al., 1994). This is accompanied by the rapid activation of Ras, and its dependent phosphorylation cascades that extend through the cytoplasm and into the nucleus, with consequent effects on cell growth and differentiation. These include the MAPK and related pathways, in which sequential phosphorylations that are initiated at the plasma membrane lead to subsequent actions in the nucleus and other regions of the cell.

The consequences of Ang II-induced activation of Ras in cardiac myocytes resemble those elicited by agonist activation of growth factor receptors, in which the MAPK cascade is initiated by the binding of adaptor proteins, such as Grb2 and Shc, to the autophosphorylated receptors (Sadoshima et al., 1995). These adaptors interact with guanine nucleotide exchange factors that enhance the activities of small GTP binding proteins, including Ras, Rac, and Rho. The small G proteins of the Ras superfamily are activated by recruitment of guanine nucleotide exchange factors such as Sos, which promotes the exchange of GTP for Ras-bound GDP to form the active Ras-GTP complex. Ras-GTP then recruits and activates serine/threonine kinases such Raf-1 or Mos, which in turn activate dual-specificity MAPK kinases, such as MEKs, by serine phosphorylation. MEKs in turn phosphorylate the MAPKs, $p44^{MAPK}$ and $p42^{MAPK}$ (also known as extracellular signal-regulated kinases, or ERK1 and ERK2) on threonine and tyrosine residues that are located within a TEY phosphorylation motif in their activation loop. The activated MAPKs are translocated into the nucleus, where they phosphorylate other kinases and transcription factors that regulate the coordinated expression of genetic programs that control a wide variety of cellular functions. These include several early response genes and others such as c-myc, Elk1, Ets, RSK, and Mnk. MAPKs also exert regulatory actions on other signaling pathways in the cytoplasm and at the cell membrane. The multiple actions of activated MAPKs are terminated by a group of dual-specificity phosphatases that selectively dephosphorylate the individual MAPKs and related enzymes (Neel and Tonks, 1997).

Ang II is but one of several calcium-mobilizing GPCRs that mimic the effects of growth factors and other receptor kinases on the activation of MAPKs and early response genes. The way by which this response is linked to the well defined phosphoinositide/calcium signaling system that is used by many such receptors has only recently been established, and some aspects of this process still remain to be determined. In addition to the need for Ca^{2+} signaling (Sadoshima et al., 1995) a role of PKC was suggested by reports of phorbol ester-induced activation of Raf and Ras in certain cell types. In addition, the phorbol ester insensitive PKC isoform, PKC-ζ, was found to mediate Ang II-induced activation of ERK1/2 in VSMC and to associate with Ras during Ang II stimulation (Liao et al., 1997). Such findings indicated that additional upstream factors must be of primary importance in MAPK activation by calcium-mobilizing GPCRs. In rat VSMC, Ang II also activates Ras (Schieffer et al., 1996), an early and cardinal intermediate in stimulation of the MAPK pathway by growth factors. The Ang II-induced activation of Ras is more rapid and less prominent than that elicited by epidermal growth factor (EGF) and is substantially reduced by treatment with pertussis toxin. However, the concomitant in-

creases in MAPK activity and c-fos expression are not impaired by pertussis toxin (Okuda et al., 1996), suggesting that these responses are not dependent on the G_i-mediated activation of Ras that occurs in Ang II-stimulated VSMC.

Ang II not only increases Ras-GTP levels but also stimulates the formation of Ras-Raf-1 complexes and tyrosine phosphorylation of GTPase-activating proteins (GAPs) such as p120 Ras-GAP and p190 Rho-GAP. These effects are prevented by the introduction of $pp60^{c\text{-}src}$ antibodies into the cultured cells, as is the tyrosine phosphorylation of PLC-γ1 and a substantial fraction of the Ang II-induced Ins(1,4,5)P_3 response (Marrero et al., 1995; Schieffer et al., 1996). These and other findings have shown that Ang II partially mimics the action of growth factors by using the Ras pathway to activate MAPK. Furthermore, this appears to involve the nonreceptor tyrosine kinase, Src ($pp60^{src}$), which probably acts through tyrosine phosphorylation and activation of a nucleotide exchange factor. Further evidence for the importance of Src in Ang II-stimulated activation of ERK has come from studies with tyrosine kinase inhibitors, and in Src-deficient and Src-overexpressing VSMC (Ishida et al., 1998).

4. Transactivation of Growth Factor Signaling by the AT_1 Receptor, In addition to Src, a calcium-dependent tyrosine kinase has been implicated in the Ang II-induced stimulation of the Ras/MAPK cascade in VSMC (Eguchi et al., 1996). This observation led to the recognition that transactivation of the EGF receptor contributes to the activation of MAPK by Ang II in these cells. This process involves the calcium-dependent phosphorylation of the EGF receptor to form docking sites for Src and the adaptor protein, Shc, which then recruits Grb2 and Sos to activate Ras (Eguchi et al., 1998).

These findings account for much of the similarity between the actions of Ang II and growth factors on the activation of PLC-γ, tyrosine kinases, and MAPKs, and increased expression of nuclear proto-oncogenes. They are also consistent with the observation that Ang and platelet-derived growth factor (PDGF) signaling cascades converge in VSMC, with activation of the PDGF-β receptor (Linseman et al., 1995). Ang II also stimulates the tyrosine phosphorylation and activation of the IGF-1 receptor, as well as insulin receptor substrate 1, in VSMC (Ali et al., 1997b). Recently, activation of the EGF receptor by Ang II has been implicated in the Ang II-induced synthesis of TGF-β and fibronectin in cardiac fibroblasts. This effect was mediated by downstream signaling from the EGF receptor, which was transactivated by Ang II via a Ca^{2+}-dependent tyrosine kinase. The Ang II-induced expression of fibronectin mRNA was regulated at the transcriptional level by binding of the fos/jun complex to an AP-1 site and also by stabilization of the message through actions of TGF-β (Moriguchi et al., 1999).

These observations are relevant to the proposed role of AT_1 receptor activation in the remodeling of cardiac interstitial tissue during ventricular hypertrophy. Ang II has been shown to stimulate the expression of collagen and fibronectin in cardiac fibroblasts, and to promote the release of newly formed collagen from the cells (Villareal et al., 1993; Crabos et al., 1994). At least one of the calcium-dependent tyrosine kinases that could mediate the transactivation of the EGF receptor by Ang II has been identified as Pyk2 (also termed CAKβ and RAFTK). However, it appears that Pyk2 does not fully account for this process and that other mechanisms are involved in AT_1 receptor-mediated transactivation of the EGF receptor (Murasawa et al., 1993). Another example of cross-talk from the AT_1 receptor is its ability to influence the activity of the LOX-1 receptor for oxidized low-density lipoprotein in human coronary artery endothelial cells (Li et al., 1999). Since oxidized low-density lipoprotein has been implicated in the pathogenesis of atherosclerosis, and attenuates nitric oxide-induced dilatation, and promotes leukocyte deposition in the vascular wall, the ability of Ang II to up-regulate LOX-1 expression via AT_1 receptors suggests another potential role for AT_1 antagonists in the management of cardiovascular disease.

5. Other AT_1 Receptor-Mediated Signaling Pathways. In addition to activating phosphoinositide/calcium signaling, tyrosine phosphorylation, and MAPK pathways, Ang II also acts through the AT_1 receptor to stimulate the Jak/STAT signaling pathway. This was first observed in neonatal cardiac fibroblasts and transfected CHO cells, in which the AT_{1A} receptor activates the STAT signaling pathway and stimulates sis-inducing factor-like DNA binding activity (Bhat et al., 1994). The stimulatory effect of Ang II on SIF/STAT activation was slower than that elicited by IL-6, and was preceded by an initial inhibitory phase that was associated with concomitant suppression of IL-6-induced STAT tyrosine phosphorylation and SIF responses (Bhat et al., 1995). The inhibitory action of Ang II on IL-6-induced Stat3 signaling was attributed to activation of the MAPK pathway and could be attenuated by the MAPK inhibitor, PD98059 (Bhat et al., 1996). Ang II was subsequently found to stimulate rapid serine phosphorylation of Stat3 via a MAPK1-dependent pathway (Bhat and Baker, 1997). In neonatal cardiac myocytes, the AT_1 receptor is coupled through Jak2 kinase to the activation of Stat1 and Stat3, and to the formation of SIF complexes (McWhinney et al., 1997).

Ang II also causes rapid and transient activation of the Jak/STAT pathway in rat aortic smooth muscle cells (Marrero et al., 1995). This is initiated by tyrosine phosphorylation and increased catalytic activity of Jak2, and is dependent on its physical association with the agonist-activated AT_1 receptor. This interaction occurs at a YIPP sequence that is located in the carboxy terminal tail of the receptor (Ali et al., 1997a,b), and is also

present in the PDGF receptor. This motif has also been implicated in the binding of SH2 domains in PLC-γ to the AT_1 receptor (Venema et al., 1998a). The binding of Jak2 to this motif has been attributed to the participation of SHP-2 phosphotyrosine phosphatase, which also contains an SH2 domain and functions as an adaptor protein in the association between JAK2 and the AT_1 receptor (Marrero et al., 1998).

In addition to ERK1 and ERK2, Ang II stimulates the activity of c-Jun N-terminal kinase (JNK) members of the MAPK family. In vascular smooth muscle cells, p21-activated kinase (PAK), which mediates JNK activation by IL-1 and tumor necrosis factor, was rapidly stimulated by Ang II before the activation of JNK. Ang II-induced activation of both PAK and JNK was dependent on calcium and PKC, and partially on a tyrosine kinase other than Src. These findings, and the ability of a dominant negative PAK to attenuate the JNK response to Ang II in transfected CHO and COS cells, indicated that PAK is a mediator of JNK activation by Ang II in VSMC (Schmitz and Berk, 1997). In this regard, Ang II again behaves like inflammatory cytokines such as IL-2 and tumor necrosis factor-α, by acting through PAK as an upstream mediator of JNK.

Ang II also promotes hypertrophy of VSMC through an oxidant stress-dependent mechanism. Reactive oxygen species such as hydrogen peroxide and O_2^- appear to be involved in the pathogenesis of hypertension and atherosclerosis, and are released from endothelial cells and other vascular and circulating cells (Rajagopalan et al., 1996). These molecules have been implicated in vascular smooth muscle proliferation and in the development of hypertension (Laursen et al., 1997; Abe and Berk, 1998). The reactive oxygen species that are generated by xanthine oxidase are potent stimuli of VSMC proliferation (Rao and Berk, 1992). Conversely, antioxidants reduce the cell response to growth factors, and inhibit the proliferation of VSMC (Boscoboinik et al., 1995). In cultured rat VSMC, Ang II causes rapid increases in intracellular H_2O_2 and phosphorylation of p42/44 MAPK and p38 MAPK. Treatment with H_2O_2 activates only p38 MAPK, and an NADH/NADPH oxidase inhibitor reduced only p38 MAPK phosphorylation in Ang II-treated cells. Also, blockade of Ang II-induced p38 MAPK phosphorylation by transfected catalase inhibited solely p38 MAPK phosphorylation, and reduced Ang II-induced cell hypertrophy. These findings indicate that both the p38 MAPK and the p42/44 MAPK pathways are required for the hypertrophic response of VSMC to Ang II (Ushio-Fukai et al., 1998).

In addition to their role in Ang II-induced activation of p38 MAPK, reactive oxygen species mediate the activation by Ang II of Akt/protein kinase B (Akt/PKB) in VSMC (Ushio-Fukai et al., 1999b). This serine-threonine kinase has been implicated in protein synthesis and also appears to be an important component of a survival pathway that protects cells from apoptosis. Akt/PKB is activated by several growth factors via Ras and phosphatidylinositol-3 kinase, and also by heat shock and other stresses. In rat VSMC, the activation of Akt/PKB by Ang II is dependent on tyrosine phosphorylation and the activity of its upstream effector molecule, phosphatidylinositol-3 kinase (Takahashi et al., 1999). These findings suggest that Akt/PKB has an important role in the intracellular actions of Ang II and in particular in its effect on cell survival (Pollman et al., 1996).

NO inhibits several of the physiological actions of Ang II, and prevents its activation of ERK, JNK, and p38 MAPK (Wang and Murphy, 1998). In rat cardiac fibroblasts, NO inhibits the activation of PYK2 and causes a concomitant decrease in ERK phosphorylation in Ang II-stimulated cells. Since PYK2 is essential for Ang II-induced activation of ERK in VSMC (Sabri et al., 1998), these findings suggest that it could be a locus at which NO regulates calcium-dependent signaling by the AT_1 and other G_q-coupled receptors (Lev et al., 1995).

J. Receptor Activation and Endocytosis

Agonist activation of many plasma-membrane receptors is followed by clustering of the complexes in clathrin-coated pits, and subsequent internalization in clathrin-coated vesicles to undergo degradation or recycling to the plasma membrane. Although nutrient receptors (e.g., for low-density lipoprotein and transferrin) frequently exhibit constitutive internalization, the hormone and growth factor receptors that evoke intracellular signals usually undergo ligand-induced endocytosis. Growth factor receptors with intrinsic tyrosine kinase activity and G protein-coupled receptors are internalized by a similar clathrin-coated vesicular endocytic process. There is increasing evidence that the intracellular signaling mechanisms that are activated by these receptors do not necessarily participate in the internalization process. Such a dissociation between Ang II-induced G protein activation and receptor internalization was observed in studies on mutant and wild-type AT_{1A} receptors expressed in COS-7 cells (Hunyady et al., 1994b). Mutations of the third cytoplasmic loop revealed that the N-terminal part of this region is important for both receptor endocytosis and intracellular signaling. Also, three point mutations of the conserved Asp^{74} residue in TMD II, which has been implicated in signal transduction by the AT_{1A} receptor and other GPCRs (Bihoreau et al., 1993), significantly impaired G protein coupling and phosphoinositide hydrolysis. However, the Asp^{74} (D74N, D74H, and D74Y) showed markedly different internalization kinetics. The D74Y receptor showed the greatest impairment of internalization but retained the highest degree of inositol phosphate stimulation. In contrast, the D74N mutant, which showed the most impaired G protein coupling and inositol phosphate responses, had normal internalization kinetics. The combined mutant receptor containing the D74N substitution and deletion of residues 221–226 from the third cytoplasmic loop

showed no G protein coupling or inositol phosphate response but was internalized about 60% as rapidly as the wild-type receptor. These data demonstrate that endocytosis of the AT_1 receptor is independent of agonist-activated signal transduction and indicate that receptor internalization and activation phospholipase C have different structural requirements.

The demonstration that coupling to heterotrimeric G proteins and intracellular signaling responses is not required for internalization of the AT_1 receptor indicated that the conformational changes that result from agonist-induced receptor activation exert largely independent effects on signal transduction and receptor endocytosis. However, since the structural requirements for signaling and internalization overlap, mutations that cause changes in G protein coupling are frequently accompanied by impairment of endocytosis. Thus, the N-terminal region of the third cytoplasmic loop is important for both receptor signaling and internalization. However, the ability of internalization-deficient mutant receptors with specific changes in the cytoplasmic tail or third loop to activate their respective G proteins and signaling responses indicates that G protein activation alone is not sufficient to induce receptor endocytosis. The sequences in these regions that are important for receptor internalization are frequently enriched in serine and threonine residues. For example, the rat AT_1 receptor contains 12 serine/threonine residues in the 23-amino acid segment located between residues 326 and 348 of the cytoplasmic tail (Fig. 4).

A mutational analysis of this region of receptor revealed that deletion of the carboxyl-terminal 22 amino acids of the receptor did not affect agonist-induced endocytosis (Hunyady et al., 1994a). However, internalization was markedly impaired by removal of one additional residue (Leu^{337}) and was reduced by 95% after removal of the adjacent Ser^{335} and Thr^{336}. In addition, triple alanine replacement of the Ser-Thr-Leu residues reduced internalization to almost the same extent as the corresponding tail deletion mutant. The Ser-Thr-Leu motif is highly conserved in mammalian AT_1 receptors but is not present in the noninternalizing AT_2 receptor. These findings demonstrate that a serine/threonine-rich region including Leu^{337} in the cytoplasmic tail of the AT_1 receptor is a major requirement for endocytosis of the hormone-receptor complex, and support the concept that similar motifs in other G protein-coupled receptors are determinants of their agonist-induced internalization. An additional region in the cytoplasmic tail of the AT_1 receptor, involving hydrophobic and aromatic residues on a putative α-helix adjacent to the cell membrane, has also been found to be involved in agonist-stimulated endocytosis (Thomas et al., 1995). It is possible that these two regions in the cytoplasmic tail function cooperatively to trigger endocytosis, and that agonist-induced receptor activation leads to phosphorylation of the serine/threonine-rich region and causes a conformational change that exposes the more proximal hydrophobic residues for interaction with the endocytic mechanism.

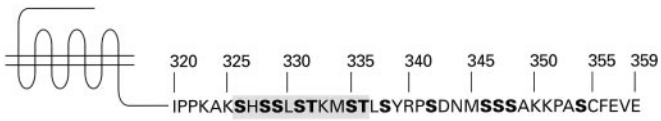


FIG. 4. Location of agonist-induced phosphorylation sites in the AT_1 receptor. The potential serine and threonine phosphorylation sites are shown in bold letters. The most highly phosphorylated region in the C-terminal cytoplasmic tail during Ang II action is indicated by the shaded box. Potential PKC sites are indicated by brackets, and the corresponding phosphorylated setrines are shown by asterisks. Recent evidence suggests that low agonist concentrations selectively induce PKC phosphorylation by other kinases is predominant at higher agonist concentrations (Quian et al., 1999).

K. AT_1 Receptor Function in Selected Tissues

1. The AT_1 Receptor and the Brain. Ang II regulates numerous physiological responses through its central actions in the brain, where it functions as a neurotransmitter or neuromodulator to influence blood pressure, drinking behavior, salt appetite, and several neuroendocrine processes. Some of these responses are induced by the actions of circulating Ang II at the circumventricular organs and other specialized regions, and others are influenced by locally formed Ang II generated within the brain itself. Although circulating hormones are effectively excluded from most parts of the brain by the blood-brain barrier, neurons in the circumventricular organs are accessible to many circulating ligands via the fenestrated endothelial cells of their dense capillary circulation. Due to the absence of the blood-brain barrier at these sites, neurons within the subfornical organ (SFO), organum vasculosum lamina terminales (OVLT), and area postrema are exposed to circulating hormones, ions, and other potential regulatory factors. These three structures, sometimes called the sensory circumventricular organs (Johnson and Gross, 1993), are rich in AT_1 receptors and have been implicated in numerous homeostatic processes. Ang II is but one of several peptides that bind to high-affinity receptors located on circumventricular neurons that project to hypothalamic nuclei and other brain regions. Other ligands with abundant circumventricular receptors include atrial natriuretic peptide, vasopressin, cholecystokinin, GLP-1, calcitonin, and relaxin (McKinley and Oldfield, 1998).

The pressor response to circulating Ang II is mediated by the area postrema and the SFO, and its stimulatory actions on water and salt ingestion, vasopressin secretion, and adrenocorticotropin (ACTH) release are mediated by the SFO. AT_1-expressing neurons in the SFO send projections to the supraoptic and paraventricular nuclei of the hypothalamus to influence vasopressin and ACTH release (Oldfield et al., 1994), and to the median optic nucleus to stimulate water drinking (Cunningham et al., 1991).

Like several other peptide hormones, Ang II exerts effects in the central nervous system that are complementary to its primary physiological actions in peripheral tissues. This is well exemplified by the presence of brain AT_1 receptors at sites that influence cardiovascular function, fluid and electrolyte homeostasis, and pituitary hormone secretion (Allen et al., 1998, 1999). The existence of an endogenous brain renin-angiotensin system that is distinct from the systemic system comprised of the kidneys, liver, and lungs has been demonstrated by numerous studies over the last 30 years. Early evidence for a central mechanism that contributes to the development of Ang II-induced hypertension (Bickerton and Buckley, 1961) was followed by the demonstration of renin and angiotensin II in the brain (Fisher-Ferraro et al., 1971). These and other studies led to the recognition of an autonomous brain renin-angiotensin system that has significant actions in cardiovascular regulation and several other brain functions (Ganten and Speck, 1978; Ganong, 1984; Phillips, 1987).

The ability of centrally administered Ang II to increase blood pressure, fluid intake, and vasopressin release presaged the existence of specific receptors in the brain regions controlling these functions. This was confirmed by the selective effects of Ang II on brain structures that influence blood pressure, sympathetic nerve activity, drinking behavior, salt appetite, and pituitary hormone secretion (Phillips, 1987; Ganong, 1993; Wright and Harding, 1994). Effects of angiotensin on learning and memory, as well as sensory function, have also been observed. The number and scope of these central effects of angiotensin indicate that the renin-angiotensin system has a major role in the regulation of neural components that control multiple physiological functions. Most of these actions are mediated by AT_1 receptors, which are expressed in brain regions known to be involved in the control of cardiovascular function and body fluid homeostasis.

At the cellular level, direct application of Ang II increases the firing rate of neurons in the supraoptic nucleus, subfornical organ, and paraventricular nucleus (Nicoll and Baker, 1971; Felix and Akert, 1974; Ambuhl et al., 1992a,b) as well as in the nucleus tractus solitarius, dorsal motor nucleus of the vagus, and thalamus (Wayner et al., 1973; Felix et al., 1988). In several of these studies, the angiotensin II (2–7) heptapeptide (Ang III) was found to be as or more effective than Ang II, raising the possibility that Ang III may be the centrally active form of Ang II (Harding et al., 1986). The application of topical autoradiography to receptor mapping in tissue sections revealed that angiotensin II receptors are present in many of the brain nuclei and other regions that are known to be involved in the central regulation of cardiovascular regulation and fluid homeostasis (Mendelsohn et al., 1984). The distributions of Ang II receptors in the brains of several mammalian species have been mapped by topical autoradiography with radioiodinated ligands, usually ^{125}I-[Sar^1,Ile^8]Ang II. Specific AT_1 and AT_2 receptors were identified by inhibition of radioligand binding by selective AT_1 (losartan) and AT_2 (PD123177) receptor antagonists. The distribution of AT_1 receptor messenger RNA in the rodent brain has also been analyzed by in situ hybridization using specific riboprobes to detect AT_{1A} and AT_{1B} receptor subtypes (Lenkei et al., 1998). Such studies have shown that all of the well defined physiological actions of Ang II in the brain are mediated by AT_1 receptors.

In all species studied, AT_1 receptors are most abundant in the hypothalamus and the circumventricular organs. In the hypothalamus, AT_1 receptors are localized to the parvocellular region of the paraventricular nucleus, which has been implicated in the control of anterior pituitary hormone secretion, ingestive behavior, and autonomic regulation of the cardiovascular system. In contrast, AT_1 receptor expression is low or absent in the magnocellular neurons of the paraventricular nucleus that control the release of vasopressin and oxytocin from the neurohypophysis. The circumventricular organs, which include the median eminence, arcuate nucleus, SFO, and OVLT, contain high densities of AT_1 receptors. At each of these sites, the local deficiency in the blood-brain barrier renders them accessible to Ang II and other regulatory ligands in the circulation. Together with the interposed median preoptic nucleus, which also contains abundant AT_1 receptors, the SFO and OVLT comprise the lamina terminalis of the forebrain. This structure has neural connections with the parvocellular neurons of the hypothalamus, as well as efferent projections to the supraoptic nucleus that subserve vasopressin secretion and other osmoregulatory responses including sodium excretion and thirst (McKinley et al., 1992).

In contrast to the relatively wide distribution of AT_{1A} receptor expression in the CNS, the AT_{1B} receptor is not detectable in most brain areas. However, AT_{1B} receptors are the predominant subtype expressed in the anterior pituitary gland, where they mediate the direct actions of Ang II on pituitary function. Both Ang II and Ang III can elicit a variety of physiological changes, including pressor and dipsogenic responses, and increased salt appetite, when injected into the cerebral ventricles (Wright and Harding, 1992). This is accompanied by increased firing of magnocellular neurons in the supraoptic nucleus (Okuya et al., 1987; Yang et al., 1992). Injection of Ang II or Ang III into the supraoptic and paraventricular nuclei also stimulates magnocellular neurons, leading to the release of vasopressin from the posterior pituitary gland (Phillips, 1987).

2. Ang II-Induced Neuronal Signaling Pathways. AT_1 receptor-mediated signaling pathways in neurons are generally similar to those observed in other Ang II target cells. In addition to phosphoinositide hydrolysis, with formation of $InsP_3$ and diacylglcerol, elevation of $[Ca^{2+}]_i$, and activation PKC, Ang II stimulates the ras/

raf/MAPK cascade and translocation of MAPK into the nucleus (Lu et al., 1996). It also increases the expression of c-*fos*, c-*jun*, and other early response genes. The MAPK pathway mediates Ang II-stimulated norepinephrine synthesis and reuptake mechanisms in cultured neurons (Yang et al., 1996). In addition, Ang II-induced increases in $[Ca^{2+}]_i$ activate calcium/calmodulin protein kinase II, which acts together with PKC to mediate the rapid actions of Ang II on neuronal membrane currents. These include a PKC-mediated increase in total neuronal Ca^{2+} current (I_{Ca}), as well as PKC- and calcium/calmodulin protein kinase II-mediated inhibition of delayed rectifier K^+ current (Kv) and transient K^+ current (I_A) (Zhu et al., 1997a, 1998a). In rat supraoptic neurons, AT_1 receptors mediate Ang II-induced activation of a nonselective Na^+/Ca^{2+} channel that promotes neuronal depolarization (Yang et al., 1992). Some of these effects of AT_1 receptor activation in neurons, including MAPK activation, are under the inhibitory control of AT_2 receptors via activation of serine/threonine type 2A (PP-2A) (Huang et al., 1996) and possibly tyrosine phosphatases such as MAPK phosphatase.

3. Role of Ang III in the Brain. Ang II is rapidly cleared from the circulation with a half-life of 13 s and is extensively metabolized in its target tissues to form Ang III, Ang IV, and Ang (4–8) (Harding et al., 1986; De Silva et al., 1988). Although the Ang II octapeptide is the major active species acting at AT_1 receptors in peripheral Ang II target tissues, the possibility that Ang III could be the predominant effector peptide in the brain has been recognized for several decades. This was first suggested by the observation that Ang III was twice as effective as Ang II in stimulating the firing rat of SFO neurons (Felix and Schlegel, 1978). This finding, and similar observations in paraventricular nucleus neurons, led to the suggestion that Ang III could be the centrally active form of Ang II (Harding et al., 1986). This proposal gained support from studies using nonselective aminopeptidase inhibitors such as bestatin, which increased the half-lives of Ang II and Ang III in the brain and potentiated the stimulatory action of Ang II and Ang III on paraventricular neurons (Harding and Felix, 1987).

The recent development of specific inhibitors of aminopeptidase A (APA) and aminopeptidase N (APN), which hydrolyze the N-terminal residues of Ang II and Ang III, respectively, has facilitated the investigation of the roles of Ang II and Ang III in the brain. The effects of i.c.v. administration of these compounds indicated that the central action of Ang II on vasopressin release in mice depends on its prior conversion to Ang III, confirming that Ang III acts as a major effector peptide of the renin-angiotensin system in the brain (Zini et al., 1996). Furthermore, the ability of centrally administered Ang II to stimulate the firing rate of magnocellular vasopressinergic neurons of the supraoptic nucleus (SON) of the rat was prevented by central administration of the selective APA inhibitor, EC33. Since the SON does not contain AT_1 receptors, this effect of EC33 presumably results from its inhibition of APA in the subfornical organ, from which Ang II-sensitive neurons project to the SON (Zini et al., 1998).

Recent studies on the effects of selective APA and APN inhibitors on blood pressure in the rat have indicated that Ang III is also the main effector peptide of the brain RAS in the regulation of blood pressure (Reaux et al., 1999). Central inhibition of APA by EC33 not only blocked the pressor action of Ang II but also caused a fall in basal blood pressure comparable to that caused by i.c.v. administration of losartan, indicating a requirement for Ang III in the tonic regulation of blood pressure. Furthermore, the inhibition of APN activity by PC18 caused an increase in blood pressure that was blocked by losartan. This suggests that inhibition of Ang III degradation by PC18 causes an increase in Ang III levels that elevates blood pressure through an action on AT_1 receptors. These observations indicate the potential applications of APA inhibitors as centrally acting compounds in the management of hypertension.

These observations raise the question of whether the central actions of Ang III are mediated by an as yet unidentified AT_1-type receptor with higher affinity for Ang III over Ang II. This seems unlikely in view of the probability that such a receptor with similarity to the AT_1 site would have been detected during isolation and cloning of the AT_1 and AT_2 subtypes. The alternative proposal, that blockade of the conversion of Ang II to Ang III by APA favors the activation of other Ang II-degrading pathways, appears to be a more likely explanation.

4. The AT_1 Receptor and the Pituitary Gland. The presence of Ang II receptors in the anterior pituitary gland was suggested by early reports of actions of the octapeptide on pituitary hormone secretion (Steele et al., 1981). This was later confirmed by binding studies with ^{125}I-Ang II, which demonstrated specific, high-affinity binding sites in the anterior pituitary glands of rat, rabbit, and dogs. No receptors were found in the posterior pituitary gland or in GH3 pituitary tumor cells (Hauger et al., 1982; Mukherjee et al., 1982). The sites were nanomolar affinity, and bound the heptapeptide des-Asp^1-Ang II with about one-tenth the affinity of the octapeptide. Ang II and the heptapeptide showed a similar potency ratio in their ability to stimulate ACTH release from pituitary cells (Capponi et al., 1982). Fractionation of rat pituitary cells revealed that Ang II receptors were associated with lactotrophs and stimulated prolactin as well as ACTH release. These findings suggested that Ang II contributes to the physiological control of prolactin secretion (Aguilera et al., 1982).

The stimulatory action of Ang II on prolactin production by pituitary lactotrophs is mediated by the AT_1 receptor, which is coupled to phosphoinositide hydroly-

sis and PKC activation, and also affects cyclic AMP production (Audinot et al., 1991). In addition to these stimulatory actions, Ang II also exerts AT_1-mediated inhibitory actions on adenylyl cyclase activity in pituitary cell membranes. In intact cells, blockade of AT_1 receptors by losartan prevented AT_1-induced prolactin release, as well as the phospholipase C and PKC-dependent increase in cyclic AMP production (Moreau et al., 1994). During development the responsiveness of rat pituitary lactotrophs to Ang II increases with age, and by 28 days was more prominent in female than in male rats (Diaz-Torga et al., 1994). The ability of the hypothalamic GnRH to stimulate prolactin release in perifused pituitary cells, but not gonadotropin release, was abolished by losartan, consistent with a paracrine role of Ang II in GnRH-stimulated prolactin release (Becu-Villalobos et al., 1994). Interestingly, the ability of Ang II to stimulate prolactin release from cultured pituitary cells from animals fed on an essential fatty acid-free diet was markedly decreased, although the stimulatory action of thyrotropin-releasing hormone, and the inhibitory action of dopamine were not affected (Alessio et al., 1994).

Ang II also increases the proliferation of cultured mammatrophs, and this effect is suppressed by tamoxifen and restored by estradiol-17β. Treatment with the peptide Ang II antagonist, saralasin, inhibited not only the Ang II-stimulated proliferative response but also that of GnRH, again indicating that GnRH-stimulated release of Ang II, together with estradiol, regulates lactotroph proliferation in the rat pituitary gland (Shinkai and Ooka, 1995). The stimulatory action of Ang II on prolactin release was associated with a rapid increase in intracellular calcium concentration that was abolished by losartan. However, in the same report no increase in [^{3}H]thymidine incorporation was observed (Diaz-Torga et al., 1998). An analysis of the distribution of the AT_1 receptor subtypes in the rat anterior pituitary gland, which expresses predominantly (80%) AT_{1B} receptors, revealed that the majority of the AT_{1B} receptor-expressing cells are lactotrophs, and the remainder are corticotrophs. In adult male rats, AT_{1B} receptors are located in about 50% of the pituitary lactotrophs and about 25% of the corticotrophs. These findings suggest that Ang II synthesized in gonadotrophs can directly stimulate prolactin and ACTH release from lactotrophs or corticotrophs through activation of AT_{1B} receptors (Lenkei et al., 1999). This AT_{1B}-mediated paracrine mechanism in the pituitary gland is subsidiary to its endocrine regulation by AT_{1A}-mediated changes in the hypothalamic production of releasing hormones and neurotransmitters including dopamine and catecholamines (Denef, 1986; Ganong, 1993).

5. The AT_1 Receptor and the Heart. The role of the renin-angiotensin system in cardiovascular control and body water homeostasis is well documented (reviewed in Saavedra, 1992; Wright and Harding, 1994, 1997; Höhle et al., 1995; Mosimann et al., 1996; Unger et al., 1996). Ang II acts primarily at the AT_1 receptor type in a variety of tissues including vascular smooth muscle, kidney, and adrenal gland to influence vasoconstriction and sodium reabsorption. Furthermore, it is known that Ang II acts locally within cardiac tissues to influence protein synthesis and cellular growth (Baker and Aceto, 1990; Lindpainter and Ganten, 1991; Baker et al., 1992; Dostal and Baker, 1992; Dostal et al., 1992, 1996). There is accumulating evidence that the AT_1 receptor may be involved in the development of cardiac hypertrophy via the regulation of extracellular matrix accumulation (Weber and Brilla, 1991; Weber et al., 1993, 1995a,b; Brilla et al., 1993, 1995b). Specifically, cardiac fibroblast stimulation appears to facilitate the accumulation of collagen in the extracellular matrix of the heart (Brilla et al., 1995a). Furthermore, Ang II appears to block MMP-1 activity, an enzyme directly involved in the degradation of fibrillar collagen. Thus, interference with MMP-1 results in a proliferation of extracellular matrix. Ang II-induced hypertrophy in cardiac myocytes and vascular smooth muscle cells follows the pattern of an initial increase in protein synthesis and cell size without an elevation in numbers of cells (Geisterfer et al., 1988; Berk et al., 1989). After prolonged exposure to Ang II, mitogenic changes were also observed (Weber et al., 1994). The ability of ACE inhibitors and AT_1 receptor antagonists to prevent the onset of cardiac hypertrophy in animal models (Zhu et al., 1997b), and to promote its regression in hypertensive patients (Thürmann et al., 1998), has also demonstrated the role of AT_1 receptors in ventricular hypertrophy. These agents also prevent the onset of ventricular dilatation in rats after myocardial infarction by suppressing the expression of gene expression (ANF, β-myosin heavy chain, collagen) associated with cardiac remodeling (Yoshiyama et al., 1999). To determine whether this effect results from inhibition of the direct action of angiotensin on the heart, or reduction of pressure overload, Paradis et al. (2000) created transgenic mice over-expressing the AT_1 receptor in cardiac myocytes. These animals spontaneously developed cardiac hypertrophy and remodeling, with increased expression of cardiac ANF and interstitial collagen deposition. They showed no change in heart rate or blood pressure, but died of heart failure at an early age. These findings indicate that the AT_1 receptor mediates the direct actions of Ang II in the development of ventricular hypertrophy and heart failure. However, it should be noted that some of the beneficial effects of ACE inhibitors and AT_1 antagonists on cardiac function could result from activation of B_2 kinin and AT_2 receptor-dependent pathways that modulate myocyte contractility and growth (Wollert and Drexler, 1999). The role of the bradykinin B_2 receptor remains, however, disputed (Madeddu et al., 2000).

III. The Type 2 (AT_2) Angiotensin Receptor

Whereas differential stability to dithiothreitol (Chang et al., 1982; Gunther, 1984; Chiu 1989b; Speth et al., 1991) suggested the presence of more than a single form of angiotensin II binding or receptor site (Chiu et al., 1989a; Whitebread et al., 1989; Chang and Lotti, 1990), cloning and expression of the receptor types AT_1 (Murphy et al., 1991; Sasaki et al., 1991) and AT_2 (Kambayashi et al., 1993b; Mukoyama et al., 1993) provided clear evidence for the presence of the second isoform, AT_2. The identification and characterization of the biochemical and physiological functions of the AT_2 receptor is still a matter of intense research.

A. Cloning, Purification, and Properties of the AT_2 Receptor

The AT_2 receptor is clearly distinct from the AT_1 receptor in tissue-specific expression, signaling mechanisms, and diversity in molecular weight. In the early phase of AT_2 receptor research it was suspected that the AT_2 receptor may not even be a G protein-coupled seven transmembrane domain receptor (Bottari et al., 1991). Clearly, its structure had to be determined by cloning and purification. Whether it was a single entity or had related isoforms was not apparent in the beginning. Undoubtedly, more fundamental studies were needed, which should be based on the cloning of the AT_2 cDNA and purification of the AT_2 receptor.

There are cell lines expressing the AT_2 but not the AT_1 receptor, such as PC12W, R3T3, and some lines of N1E-115. Fetal rats express a large amount of the AT_2 receptor protein and its mRNA. However, the cloning of the AT_2 receptor turned out to be difficult. Attempts to screen cDNA libraries with AT_1 cDNA as a probe did not work, indicating a lack of homology between the AT_1 and AT_2 receptor gene. Thus, the expression cloning method had to be used. For some unknown reason, cDNA detected by autoradiographic identification of transfected COS-7 cells presumably expressing AT_2 mRNA could not be recovered from these cells. After several technical refinements, a 4.5-kb rat AT_2 cDNA was cloned from rat PC12W cells by Kambayashi et al. (1993b), and from rat fetuses by Nakajima et al. (1993). It contained seven hydrophobic sequences compatible with the structural theme of the seven transmembrane domain receptors super family (or GPCR) (see Fig. 5) but showed only a 32% amino acid sequence identity with the rat AT_1 receptor.

Since targeted gene deletion can be performed only in the mouse, Ichiki et al. (1994) and Nakajima et al. (1993) cloned mouse AT_2 cDNA from a fetal mouse cDNA library by a plaque hybridization method using rat AT_2

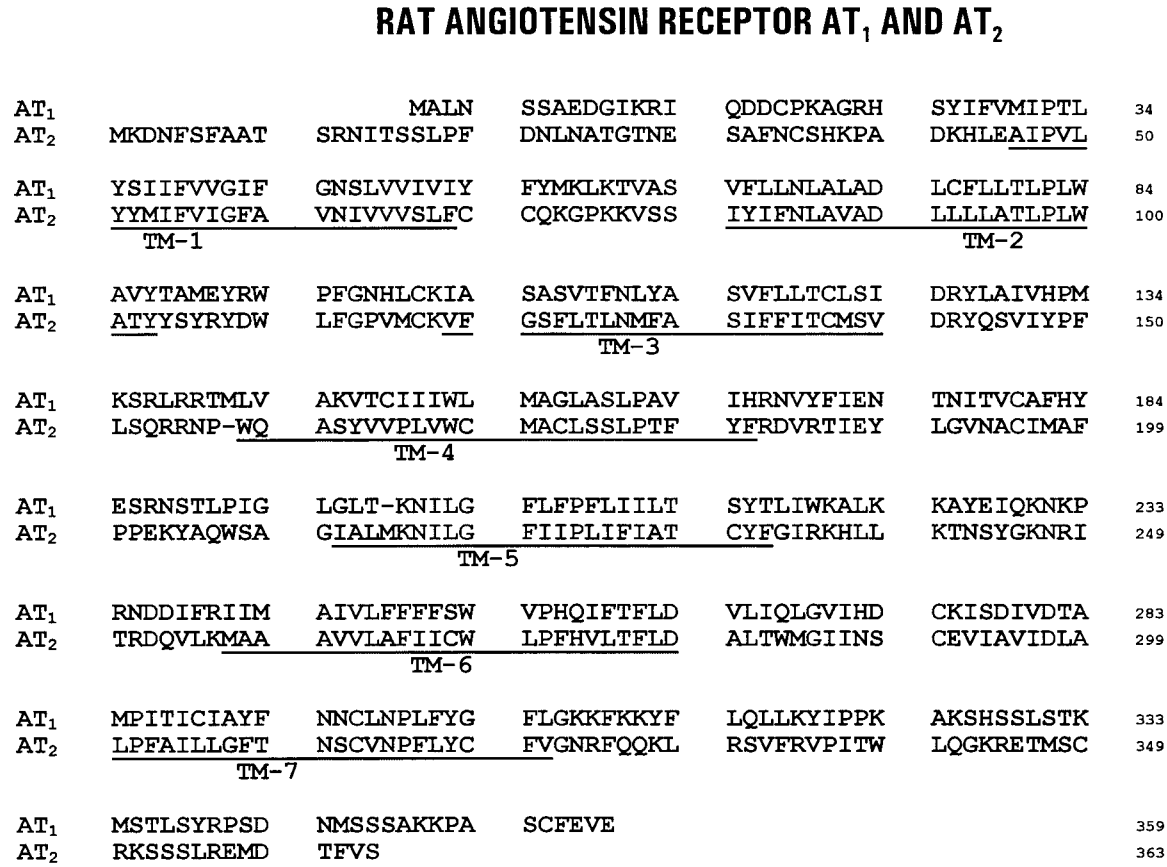


FIG. 5. Amino acid sequences of rat AT_{1A} and AT_2 deduced from respective cDNA. Sequences in shaded boxes are identical. Of the overall 32% sequence identity, the transmembrane regions show a somewhat higher degree of homology.

cDNA as a probe. A mouse AT_2 genomic 4.4-kb DNA fragment was cloned by Ichiki et al. (1994) and Koike et al. (1994). Since these studies show that the entire coding sequence is contained in one exon, the human AT_2 coding sequence (Tsuzuki et al., 1994) or fragments containing more extensive sequences could be cloned (Martin et al., 1994; Koike et al., 1994). The genomic DNA of all three species consist of three exons with an uninterrupted coding region being confined to the third exon. The genes encoding the AT_2 receptor are localized in human chromosome Xq22-q2, rat chromosome Xq3, and mouse chromosome X (Koike et al., 1994; Hein et al., 1995b; Tissir et al., 1995). The localization in the long arm of the X chromosome throughout species may have important implications in targeted gene deletion as well as in the genetics of the AT_2 receptor gene.

The open reading frame of AT_2 cDNA encodes 363 amino acid residues in all three species with 99% sequence identity between rat and mouse and 72% identity between rat and human. The divergence between rodent and human AT_2 occurs mainly in the N-terminal region. AT_1 and AT_2 receptors have only 33 to 24% amino acid sequence identity (Kambayashi et al., 1993b; Nakajima et al., 1993). The homology was mainly localized in the transmembrane hydrophobic domains, which are believed to form seven transmembrane helical columns. Residues located in these helical column domains and considered to be essential for Ang II binding to the AT_1 receptor are also preserved in the AT_2 receptor. These include Lys^{118} (Lys^{102} of AT_1) at the top of TM3, Arg^{183} (Arg^{167} of AT_1) at the top of TM4 and Lys^{216} (Lys^{199} of AT_1) and more.

Almost complete divergence between AT_1 and AT_2 receptors is seen in the third intracellular loop and more extensive differences in the carboxyl terminal tail (C-tail). The amino acid sequence of the rat AT_2 receptor indicates that it has five potential *N*-glycosylation sites with the consensus amino acid sequences -Asn-X-Ser/Thr. It is possible that carbohydrate chains would give rise to increased and diverse molecular weights of the AT_2 receptor. Southern hybridization analysis of restriction enzyme fragments of rat genomic DNA using rat AT_2 cDNA as a probe did not produce evidence for a subtype of the AT_2 receptor. Thus, the molecular weight divergence for the AT_2 receptor ranging from 60 to 140 kDa may be due to a difference in the extent of glycosylation, as shown by Servant et al. (1994).

An earlier discriminator of the AT_1 and AT_2 receptors, before the isoform-specific antagonist became available, was the reducing agent dithiothreitol (DTT). The DTT sensitive Ang II receptor turned out to be the AT_1 receptor, whereas DTT increased the ligand binding capability of the AT_2 receptor and the AT_2 receptor remained stable for hours (Chang, Lotti, Keegan, 1982; Whitebread et al., 1989; Chang and Lotti, 1990; Speth et al., 1991). This property was exploited for the direct purification of the AT_2 receptor protein (see below). Another discriminator is Sar-*p*-benzoylphenylalanine^8-angiotensin II, which can be used for selective labeling of AT_2 by a photoaffinity technique. Although it binds to the bovine adrenal AT_1 receptor with a K_d value of 6.5 nM and human myometrium with a K_d value of 0.39 nM, its covalent binding upon illumination takes place only with the AT_2 receptor (Bosse et al., 1990). The selective labeling of the AT_2 receptor is in contrast to another photoaffinity labeling peptide, Sar^1-D-azido Phe^8-Ang II, which is specific for the AT_1 receptor (Guillemette et al., 1986). The ^{125}I-radiolabeled peptide was used to visualize AT_2 receptors in various cells to determine their molecular sizes by SDS gel electrophoresis. Cells known to contain the AT_2 receptor produced radiolabeled AT_2 receptor bands of 68 kDa (human uterine myometrium), 91 kDa (R3T3 cells), and 113 kDa (PC12 cells). However, endoglycosydase treatment reduced the myometrial 68-kDa receptor to 40- and 31-kDa proteins, the 91-kDa R3T3 receptor was reduced to 46- and 31-kDa and the 113-kDa PC12 AT_2 receptor to 55 and 31 kDa. These results may involve artifacts of proteolytic digestion. More decisive evidence for glycosylation diversity came from the inhibition of *N*-glycosylation by treatment of rat PC12 cells with tunicamycin (Servant et al., 1996). The 140-kDa AT_2 receptor in untreated PC12 cells was reduced to 63- and 47-kDa glycosylated and 32-kDa unglycosylated receptors. An interesting byproduct of these studies is the observation of nonglycosylated receptor expression on the cell surface.

The structural diversity of the AT_2 receptor is still a complex and unresolved issue. Attempts have been made to purify the AT_2 receptor protein (Ciuffo et al., 1993a) by combining affinity chromatography on CGP42112-Sepharose and size separation by gel filtration and SDS gel electrophoresis from AT_2 solubilized by CHAPS from neonatal rat kidney which had a 71-kDa molecular mass. The murine neuroblastoma cell line N1E-115 cell line contains both AT_1 and AT_2 receptors. The latter increases upon cellular differentiation. Ang II receptors were solubilized from membranes by the detergent CHAPS (1%). The labile AT_1 receptor could be removed by denaturation and the AT_2 receptor was affinity purified on an Ang II-Sepharose affinity column by Siemens et al. (1991). They found that the AT_2 receptor from N1E 115 can be separated to two different forms, peak I and peak III, by a Sepharose column chromatography. Peak III seemed to be an AT_2 receptor whose ligand binding activity is enhanced by the reducing agent DTT, but the stable GTP analog, GTPγS, did not suppress the binding. Ligand binding to a receptor under peak I was clearly reduced by GTPγS, but treatment with DTT reduced the ligand binding function (Siemens et al., 1994b). The peak I components consisted of two proteins 110 and 60 kDa. It has been proposed that the 110 kDa is a dimer of 60 kDa that is stabilized by the agonist (Yee et al., 1994). Interestingly, stabilization of the AT_2 receptor on the cell membrane of R3T3 cells by

an as yet undefined endogenous ligand, which is not identical with Ang II, has recently been described by Csikos et al. (1998).

B. Regulation of the AT_2 Receptor

The AT_2 receptor gene is expressed ubiquitously at a very high level in the fetus and it declines precipitously after birth in many but not all tissues. In the skin, for instance, it decreases to undetectable levels, but in certain tissues such as the adrenal and heart, the decline stops at certain levels, and AT_2 receptor expression persists for the remainder of life. In uterine myometrium, the AT_2 receptor is expressed under nonpregnant conditions and declines during pregnancy, but returns to nonpregnant levels after parturition (de Gasparo et al., 1994). These few examples demonstrate clearly that the AT_2 receptor expression is regulated. Theoretically, such regulatory changes may be controlled by transcription and/or by changing the stability of mRNA.

Post-transcriptional (translational) regulation also seems to take place. After confluency, the AT_2 binding sites increase in R3T3 cells without an increase in mRNA (Dudley et al., 1991), whereas mRNA is low in the growth phase and increases in the preconfluent state. The AT_2 receptor in these cells may signal to activate protein phosphotyrosine phosphatases (PTP), which inactivates mitotic or hypertrophic growth. Teleologically, its activity should then be expressed more or less constantly in normal nongrowing cells. That this receptor lacks an internalization and desensitization mechanism is compatible with its role to maintain differentiated cells in a quiescent state (Unger, 1999).

Attempts to investigate the transcriptional regulation have begun. AT_2 genomic DNA have been cloned from mouse (Nakajima et al., 1993; Ichiki et al., 1994) and humans (Koike et al., 1994). The behavior of the AT_2 receptor gene expression in rat vascular smooth muscle cells from the spontaneously hypertensive (SHR) and Wistar Kyoto or Sprague-Dawley rats are markedly different. This finding is in line with the observation that AT_2 receptor binding in endothelial cells from SHR is markedly higher than in those from Wistar Kyoto rats (Unger, 1999). The 5′-flanking region of rat genomic DNA was also cloned to determine its functional elements (Ichiki and Inagami, 1995a).

The expression of the AT_2 receptor was shown to be dependent on growth factors or growth states. In mouse R3T3 cells, AT_2 mRNA is expressed when growth is arrested in the confluent state and is decreased in the vigorous growth phase (Dudley et al., 1991; Dudley and Summerfeldt, 1993). Prolonged serum depletion in the presence of insulin or IGF-1 stimulates the expression of AT_2 receptor in vascular smooth muscle cells, whereas serum growth factors, like PDGF, lysophospatidic acid, and phorbol ester rapidly abolished AT_2 receptor expression (Kambayashi et al., 1993a, 1996). Similar observations with respect to IGF-1 as well as interleukin-1β (IL-1β) were made in R3T3 fibroblast cells (Ichiki et al., 1995; Kambayashi et al., 1996). In contrast, growth factors (phorbol ester, lysophosphatidic acid, and basic fibroblast growth factor) markedly suppressed mouse AT_2 mRNA expression in the fibroblast cells but did not completely eliminate it as they did in vascular smooth muscle cells (Ichiki et al., 1995a,b, 1996).

The transcriptional activity of the 5′-flanking region of the mouse AT_2 receptor gene was investigated further by using constructs consisting of truncated 5′-flanking sequences (up to 1.2 kb) fused to the luciferase gene in R3T3 cells. An AP-1 site may transmit the suppressive effects of noninsulin growth factors. Thus, PKC-activating growth factors and phorbol esters rapidly downregulate the AT_2 receptor expression (Kambayashi et al., 1993a; Kizima et al., 1996). The C/EBP site may mediate the positive transcription effect of IL-1β as it stimulates the expression of C/EBPβ and γ (Ichiki and Inagami, 1995a,b, 1996).

AT_2 receptor expression by insulin or IGF-1 is interesting in that the process has a very slow onset, taking several days. A putative insulin response sequence (IRS) is located at -126 to -117 of the 5′-flanking region of the rat AT_2 receptor gene. This sequence TAATGTTTTG is 80% homologous to the IRS in the phosphoenolpyruvate carboxykinase gene TGGTGTTTTG (O'Brien and Granner, 1991). An interesting aspect of the effect of insulin is that the aorta of obese (fa/fa) Zucker rats, which have about 10 times higher plasma insulin concentrations than Sprague-Dawley rats, expresses AT_2 mRNA at a level clearly detectable by Northern blotting, whereas it was not detectable by the Northern blotting in the aorta of Sprague-Dawley rats (Kambayashi et al., 1996).

In these studies, an enhancer activity of the AP-1, C/EBP, NF/IL-6, IRS was not examined rigorously. Horiuchi et al. (1995) demonstrated by elegant methods that interferon regulatory factor IRF-2 attenuated AT_2 receptor expression, both in the confluent and growing cells, whereas IRF-1 enhanced AT_2 expression in confluent cells only. Several IRF binding motifs were found in the promoter in positions between -453 and -225. Whether these elements alone account for up-regulation is not yet known. Tissue-specific expression and widespread low level expression of the AT_2 receptor rather point to complex mechanisms of AT_2 receptor regulation.

C. AT_2 Receptor Diversity

Although the AT_2 receptor was cloned and purified, there remains a nagging question as to whether the AT_2 receptor is a single entity, because there is evidence for divergence in the molecular and functional properties (Siemens et al., 1994a). As discussed above, the large disparity of molecular size was attributed to a difference in the size of carbohydrate structure (Servant et al., 1994) but it could be also due to dimerization or oligomerization (Yee et al., 1994). Although DTT enhances

ligand binding of the AT_2 receptor in most tissues investigated, in some this is not the case (Tsutsumi and Saavedra, 1992b). Most intriguing is the observation that agonist binding of typical AT_2 receptors, including those that had been cloned and expressed, is not suppressed by GTPγS, or pertussis toxin. Some AT_2 receptors in rat locus ceruleus and thalamic or geniculate nuclei are markedly affected by these reagents, which affect the heterotrimeric G proteins, G_i and G_o. On the other hand, GTPγS has no effect on the AT_2 receptor in R3T3 cells or the inferior olive (Table 3). The lack of effect has also been seen in other cells expressing the AT_2 receptor (Tsutsumi and Saavedra, 1991). Moreover, AT_2 mRNA was not detected by in situ hybridization using a sequence of the cloned AT_2 receptor as an antisense oligonucleotide probe (Jöhren et al., 1996). The question as to whether diversity in the functional properties of the AT_2 receptor in different types of cells and tissues is due to another molecular species, or to additional factors associated with it, may be addressed by investigating various modes of the signal transduction mechanism of the AT_2 receptor associated with the molecular diversity.

D. Targeted AT_2 Receptor Gene Overexpression and Deletion

The use of AT_2 receptor antagonists has provided evidence for a variety of functions of the AT_2 receptor in several different types of cells and tissues including cardiovascular, renal, adrenal, central nervous, as well as dermal mesenchymal systems. Gene manipulation to increase, decrease, or abolish the expression of a gene in question are effective approaches to assess the pathophysiological roles of the gene or its product. Transgenic transfection of AT_2 receptor expression vectors into COS-7 cells (Kambayashi et al., 1993b; Mukoyama et al., 1993), and vascular smooth muscle in vivo (Nakajima et al., 1995) in "gain of function" approach have proven to be efficient methods. Whereas cardiac targeted overexpression of the AT_2 receptor in mice did not cause obvious morphological or functional changes, Ang II infusion paradoxically decreased blood pressure and produced a negative chronotropic effect (Masaki et al., 1998). Moreover, Tsutsumi et al. (1999) reported stimulation of bradykinin activity and nitric oxide production in AT_2 transgenic mice following inhibition of the Na^+/H^+ exchanger.

The targeted gene deletion (AT_2 knockout mouse or AT_2 null mouse) method produced a very interesting strain of mice (Hein et al., 1995a; Ichiki et al., 1995a). In contrast to the hypertension and impaired vascular responses observed in AT_1-deficient mice, knockout of the AT_2 receptor leads to elevation of blood pressure and increased vascular sensitivity to Ang II (Hein et al., 1995a; Ichiki et al., 1995). This has suggested that the AT_2 receptor may exert a protective action in blood pressure regulation by counteracting AT_1 receptor function. Such an action could be exerted in part by reduced expression of the AT_1 receptor, which is increased in the vascular smooth muscle of AT_2-deficient mice (Tanaka et al., 1999). However, the sustained hypersensitivity to Ang II in such animals is also attributable to loss of the counter-regulatory action of renal bradykinin and cyclic GMP formation (an index of NO production) (Siragy et al., 1999). The relative contributions of these two factors to AT_2-dependent vascular regulation, and the extent to which AT_2 receptor deficiency could contribute to sustained blood pressure elevation, as in human hypertension, have yet to be determined.

The AT_2 receptor null mouse Agtr2(−/−) also showed behavioral changes and proclivity to have renal diseases. However, despite its massive expression in the mesenchymal tissues of skin, kidney, as well as in the brain and heart in fetal stages of development, the AT_2 receptor null mouse does not show discernible gross anatomical defects or deformity to date. The early fetal form of the kidney mesonephros is programmed to disappear during the fetal development. Its tubular tissue is densely surrounded by mesenchymal cells expressing AT_2 mRNA (Kakuchi et al., 1995). However, its disappearance also takes place in the fetus of the AT_2 receptor null mouse. The medulla of the metanephros is also filled with AT_2 receptor-expressing mesenchymal cells, which undergo apoptosis as tubulogenesis proceeds (Kakuchi et al., 1995). Again, kidney formation in the AT_2 receptor null mice takes place normally and newborn mice grew to adult mice with a normal pair of kidneys (Ichiki et al., 1995b). These studies with AT_2 receptor gene deleted mice indicate that the AT_2 receptor is not essential for apoptosis in nephrogenesis.

Functionally, the AT_2 receptor seems to exert a hypotensive effect—although this function is still discussed controversially—and to blunt the sensitivity of AT_1 receptors to Ang II. Its role in the regulation of cardiac,

TABLE 3
Binding characteristics of the AT_2 receptor[a]

Treatment	Atypical AT_2		AT_{2A}	
	Ventral Thalamic Nuclei	Medial Geniculate Nucleus	Locus Ceruleus	Inferior Olive
GTPγS effect	K_d: 100% increase	Slight increase	120% increase	No increase
Pertussis toxin treatment	Agonist binding reduced to 45%	Agonist binding reduced to 63%	Agonist binding reduced to 45%	

[a] Taken from Tsutsumi and Saavedra (1992a).

renal, and adrenal function is still under investigation. As for the renal function, the role of the AT_2 receptor as a regulator (or modulator) of pressure natriuresis is still controversial (Keiser et al., 1992; Lo et al., 1995; Siragy and Carey, 1996). Whereas studies with AT_2 receptor agonists or antagonists in intact rats suggested an antidiuretic and antinatriuretic effect of the AT_2 receptor, results in AT_2 receptor knockout mice showed the opposite effects (Siragy et al., 1999).

Although AT_2 receptor null mice did not produce gross morphological abnormalities, vascular differentiation driving the expression of the constituents of the contractile apparatus was altered. The expression of h-caldesmon and calponin are delayed in the aorta, suggesting that the AT_2 receptor enhances the differentiation of vascular smooth muscle cells and is involved in vasculogenesis (Yamada et al., 1999). In addition, more detailed studies with a larger population of AT_2 receptor null mice are producing exciting evidence that a loss of the AT_2 receptor in the AT_2 null mice results in the development of congenital urinary tract anomalies with a 23% penetrance.

1. Behavioral Changes in AT_2 Receptor Null Mice. In the brain, the AT_2 receptor is expressed in distinct areas (Obermüller et al., 1991), prominently in the locus ceruleus (Rowe et al., 1990a,b; Saavedra, 1992). The locus ceruleus contains a high concentration of noradrenergic nerve endings that release norepinephrine upon treatment with Ang II (Sumners and Phillips, 1983) and sends out long noradrenergic projections to the cerebral cortex, hypothalamus, and hippocampus. The AT_2 receptor is also expressed in the central amygdaloid nucleus (Song et al., 1992), and/or medial amygdaloid nucleus (Saavedra, 1992). Loss of the AT_2 receptor in these areas could entail behavioral effects.

AT_2 receptor null mice showed a markedly reduced exploratory behavior when placed in a new environment with ambulation reduced by 40% (Hein et al., 1995a; Ichiki et al., 1995). After water deprivation, AT_2 null mice show a greater stimulation of dipsogenesis (Hein et al., 1995a).

E. Signaling Mechanisms of the AT_2 Receptor

Studies on hormone receptor signaling mechanisms are aimed at explaining cellular and physiologic responses by intracellular biochemical processes. As Nahmias and Strosberg (1995) point out, the search for major physiologic functions of the AT_2 receptor is progressing and several interesting clues are at hand. The AT_2 receptor seems to open a delayed rectifier potassium channel at least in hypothalamic neuronal tissues (Kang et al., 1994, 1995), to close a T-type Ca^{2+} channel (Buisson et al., 1992, 1995), to suppress tissue and cellular growth (Nakajima et al., 1995; Meffert et al., 1996; Munzenmaier and Green, 1996; Stoll et al., 1995; Tsuzuki et al., 1996a,b), to induce (neuronal) cell differentiation (Laflamme et al., 1996; Meffert et al., 1996; Gallinat et al., 1997; Stroth et al., 1998; Côté et al., 1999; Gendron et al., 1999) and to support apoptosis (Yamada et al., 1996; Chamoux et al., 1999; Gallinat et al., 1999). In addition, the AT_2 receptor may exert hypotensive effects (Scheuer and Perrone, 1993; Ichiki et al., 1995) and appears to influence behavior (Hein et al., 1995a; Ichiki et al., 1995).

The consensus among investigators working with cells expressing the AT_2 receptor exclusively without the AT_1 receptor, such as R3T3 (Dudley et al., 1990, 1991), PC12W (Bottari et al., 1991, 1992; Leung et al., 1992; Webb et al., 1992), ovarian granulosa cells (Pucell et al., 1991), and COS-7 cells expressing cloned AT_2 cDNA (Kambayashi et al., 1993b; Mukoyama et al., 1993) is that the AT_2 receptor does not modulate cytosolic Ca^{2+} or cyclic AMP, which are sensitive indicators of the heterotrimeric G_q protein-coupled phospholipase Cβ activation and G_s or G_i-coupled activation or inhibition of adenylyl cyclase, respectively. Furthermore, agonist binding did not induce receptor internalization (Dudley et al., 1991; Csikos et al., 1998), nor did treatment of plasma membrane with stable GTP analogs (e.g., GTPγS or GDP-NH-P) result in a decrease of ligand binding. Heterotrimeric G proteins show a binding of a stable analog of GTP upon stimulation by agonist to their respective receptor. Stimulation of the AT_2 receptor in membranes from human myometrium, bovine cerebellar cortex, and rat adrenal glomerulosa did not result in an increase in the binding of [^{35}S]GTPγS (Bottari et al., 1991). These findings, obtained before the molecular structure of the AT_2 receptor had been disclosed, led the authors to propose that the AT_2 receptor was not coupled to a heterotrimeric G protein since the above are general criteria for the family of G protein-coupled receptors. Thus, even though the AT_2 receptor, as we know today, has basic structural features commonly shared by GPCR (seven transmembrane domain receptors), it did not reveal any functional features ascribable to this class of receptors.

An elaborate study on the binding of the AT_2 labeled with ^{125}I-Sar^1-Ang II indicated that the AT_2 receptor binds to $G_{i\alpha2}$ or $G_{i\alpha3}$ (Zhang and Pratt, 1996). However, the mode of interaction may be somewhat different from those seen with other G_i-coupling receptors.

There is evidence that the AT_2 receptor inhibits the G_q-coupled phospholipase C activation by the AT_1 receptor. Wounded rat skin expresses both AT_1 and AT_2 receptors (Kimura et al., 1992; Viswanathan and Saavedra, 1992). When rat skin slices are incubated with Ang II (10^{-7}–10^{-4} M), the production of inositol-monophosphate is stimulated and is abolished by losartan. The AT_1 receptor-mediated inositol monophosphate production is markedly potentiated by the AT_2 antagonist PD123319 at 10^{-3} M (Gyurko et al., 1992). Although the results were obtained at high concentrations of Ang II and AT_2 antagonist, these observations indicate that

AT_2 receptor could negatively modulate the AT_1 receptor-mediated phospholipase C activation.

The effect of Ang II on cGMP in AT_2 receptor-expressing cells has been controversial (Dudley et al., 1991; Sumners et al., 1991; Sumners and Myers, 1991; Bottari et al., 1992; Leung et al., 1992; Webb et al., 1992; Brechler et al., 1993; Kambayashi et al., 1993b; Reagan et al., 1993b; Israel et al., 1995). The reason for this discrepancy is not yet clear. More recent in vivo studies on the effect of the AT_2 receptor in the kidney indicate that the stimulation of the AT_2 receptor increases renal medullary and cortical interstitial cGMP by a bradykinin-dependent mechanism (Siragy and Carey, 1996, 1997a,b; Siragy et al., 2000). Similar observations were made by Golke et al. (1998) in the aorta of stroke prone SHR infused with Ang II, and some therapeutic effects of AT_2 receptor blockade are clearly mediated by the AT_2 receptor and kinin stimulation (Liu et al., 1997). Mice overexpressing the AT_2 receptor have confirmed these findings (Tsutsumi et al., 1999). Thus, the signaling mechanisms of the AT_2 receptor have been less clearly defined as yet compared with those of the AT_1 receptor. In addition to the above-mentioned signaling pathways including G_i protein coupling and cGMP (NO) generation, activation of protein tyrosine phosphatase, serine/threonine phosphatase 2A (PP2A), protein kinase phosphatase (MKP-1) acting on both phosphotyrosine and phosphothreonine as well as SHP-1 tyrosine phosphatase have been reported (for details see Blume et al., 1999; Horiuchi et al., 1999; Millat et al., 1999).

1. Dephosphorylation and Inactivation of the Mitogen-Activated Protein Kinases ERK1 and ERK2. MAPK plays a key role in cellular proliferation, and its activation can be detected by highly sensitive methods. VSMC transfected with an AT_2-expressing vector responded to Ang II by a very slight increase in MAPK activity, whereas untransfected VSMC clearly increases MAPK. The AT_2 antagonist markedly increased the MAPK activity (both ERK1 and ERK2 isoenzymes) close to the level attained in untransfected VSMC when stimulated with -10^{-7} M Ang II. These results indicate that stimulation of the AT_2 receptor by Ang II suppresses the AT_1 receptor-mediated activation of MAPK (Nakajima et al., 1995). Although adult aortic media do not seem to expresses AT_2 at measurable levels, the carotid artery may express it to a limited degree (Viswanathan et al., 1991). AT_2 receptor expressed, even in a limited quantity, at the edge of neointima, (or, for that matter, in a healing wound) may suppress MAPK in the growing edge. The serine/threonine phosphatase, PP2A, which is activated by Ang II stimulation of the AT_2 receptor in the neonatal hypothalamic neurons, was recently shown to inactivate MAPK following the stimulation of the AT_1 receptor. In the presence of the AT_2 receptor antagonist, PD123319, MAPK activity was markedly enhanced. Thus, the AT_2 receptor is able to block MAPK activation either by the dephosphorylation of serine/threonine phosphate by PP2A or tyrosine phosphate by PTPs, most likely by MAPK phosphatase, MKP-1 (Huang et al., 1996).

The influence of the AT_2 receptor on MAPK activity may also depend on the biological program engaged by the receptor under defined experimental conditions. Thus in PC12W cells expressing AT_2, but not AT_1, receptors, treatment with Ang II under conditions allowing for cell differentiation induced a short-lasting increase in ERK1 and ERK2 activity as was seen with NGF, a classical differentiation factor. Costimulation with NGF and Ang II led to initial MAPK stimulation but the subsequent NGF-induced plateau of ERK1 and ERK2 stimulation was suppressed by the AT_2 receptor (Stroht et al., 2000). Altered ERK activity was also observed in the heart of AT_2 transgenic mice suggesting that the ERK inactivated by the AT_2 receptor has a physiological role in vivo (Masaki et al., 1998).

Further evidence for a G_i-coupled activation of PTP was obtained in vascular smooth muscle cells in culture, even if the AT_2 receptor was not expressed in these cells. Instead of using Ang II to stimulate G_i, it was activated by a 22 amino acid residue peptide with the sequence of the third intracellular loop of the AT_2 receptor protein, which was transferred into the cells expressing the AT receptor by LipofectAMINE liposome. Cells which received the AT_2-related peptide showed small but significant attenuation of serum-stimulated MAPK and thymidine incorporation. The suppression of thymidine incorporation was reversed by pretreatment of cells with pertussis toxin, which indicates the role of a G_i or G_o protein. The inhibition of MAPK activation by serum was also reversed by sodium orthovanadate, a tyrosine phosphatase inhibitor but not by okadaic acid, a serine/threonine phosphatase inhibitor, which indicates that the third intracellular loop of AT_2 receptor activates a PTP via a G_i or G_o protein (Hayashida et al.,1996). In the neuroblastoma cell line, N1E-115, the AT_2 receptor has been found to activate the catalytic activity of SH-PTP1, a soluble PTP implicated in termination of signaling by cytokine and growth factor receptors, resulting in ERK inactivation (Bedecs et al., 1997).

Recently, ceramide, which is linked to phosphatase activation, was proposed to be the second messenger in AT_2 receptor-mediated apoptosis, (Gallinat et al., 1999; Lehtonen et al., 1999), and pertussis toxin and orthovanadate blocked its production. A negative crosstalk appears to exist between AT_2 and AT_1 receptors not only on a functional level as has been observed by various authors (see Unger, 1999) but also on the level of intracellular signaling. For instance, stimulation by Ang II, interferon, EGF, or PDGF of rat adult vascular smooth muscle cells transfected with AT_2 receptor cDNA inhibits AT_1 receptor-mediated ERK activation and tyrosine phosphorylation of STAT without influence on Janus kinase (Horiuchi et al., 1999). However, these results also indicate that the G_i-coupled activation of protein tyrosine phosphatase(s) or protein serine/threo-

nine phosphatase is not targeted narrowly to specific channel proteins and that many other proteins can be their substrates.

2. Activation of Phospholipase A_2 and Prostacyclin Generation. In contrast to cells in which the AT_2 receptor is the predominant or exclusive Ang II receptor, cardiac ventricular myocytes are interesting as they express both AT_1 and AT_2 receptors (Busche et al., 2000). In the hypertrophic ventricles of SHR and two-kidney one-clip hypertensive rats, AT_1 receptor expression is increased along with AT_2 receptor density (Suzuki et al., 1993). Thus, the heart offers unique materials for studies on interactive regulation of AT_1 and AT_2 receptors. The AT_2 receptor mediates sustained arachidonic acid release in isolated pure cardiac myocytes, and this effect is completely blocked by the AT_2-specific antagonist PD123317. Losartan suppresses it by about 50%. On the other hand, the AT_1 receptor supports largely the release of inositol phosphates. The fact that 1 mM DTT does not completely abolish arachidonic acid release, but completely eliminates inositol phosphate, supports the role of the AT_2 receptor in the activation of phospholipase A_2 but not phospholipase C (Lokuta et al., 1994).

Kohout and Rogers (1995) found evidence indicating that the AT_2 receptor-mediated release of arachidonic acid may contribute to the activation of Na^+/HCO^- symporter system (NBC), which increases the pH value of myocytes by 0.08 pH unit. This stimulation of the symporter system by Ang II can be blocked by the AT_2 blocker PD123319, but not by the AT_1 blocker losartan. Furthermore, superfusion of myocytes with exogenous arachidonic acid (5 μM) mimicked the Ang II-mediated alkalinization. Thus, the arachidonate release and symporter activation is a unique function of the AT_2 receptor in the heart. These observations were extended by Sandmann et al. (1998) in an ex vivo study in rats after myocardial infarction. Whereas pretreatment with an ACE inhibitor prevented the postinfarct up-regulation of both ion transporter systems, the Na^+/H^+-exchanger (NHE-1) and NBC, in cardiac tissue, the AT_1 receptor antagonist, valsartan, selectively blocked the increase in NHE-1 and the AT_2 antagonist, PD123319, selectively blocked the increase of the NBC.

AT_2 receptor-dependent production of prostacyclin (PGI_2) was reported in differentiated adipocytes Ob1771 in culture. The PGI_2 formation is seen only in differentiated Ob1771 cells, and PGI_2 thus formed induces differentiation of undifferentiated Ob1771 cells in coculture by a paracrine mechanism. This action is blocked by PD123177, but not by losartan, indicating that the prostacyclin formation is mediated by the AT_2 receptor (Darimont et al., 1994). The mechanism involved in this signaling pathway remains to be clarified.

In summary, diverse AT_2 receptor signaling pathways were unveiled, which include activation of serine/threonine phosphatase PP2A and subsequent opening of the delayed rectifier K^+-channel, activation of cytosolic PTPs, which may lead to closing of the T-type Ca^{2+} channel, inactivation, but in some cases also transient activation, of MAPK, the inhibitory effect presumably through PP2A, MKP-1, or other PTP, and the activation of phospholipase A_2 (Nouet and Nahmias, 2000). With respect to the specific pathways or substrates involved in AT_2 receptor-mediated intracellular signaling, there are still numerous unresolved problems such as GTPγS sensitivity, PTP activation, or cGMP production.

F. Tissue Distribution of the AT_2 Receptor

To identify the physiologic functions of the AT_2 receptor, investigations were initiated on tissue-specific expression, changes in AT_2 expression in relation to ontogenic stages and tissue development, and identification of cell types in vivo and in cell lines carrying the AT_2 receptor. In these studies, the use of isoform selective antagonists for the AT_1 and AT_2 receptors enabled investigators to detect biphasic binding of Ang II, which indicated the coexistence of AT_1 and AT_2 receptors. Later, cloning of AT_2 cDNA allowed for specific identification of AT_2 mRNA expressed in various tissues (Kakuchi et al., 1995; Shanmugam et al., 1995; Jöhren et al., 1996). Distribution of the AT_2 receptor appears to be tissue- and species-specific. Both AT_1 and AT_2 receptors are expressed in the adrenal gland at varying ratios in different regions. In the rat adrenal medulla, the ratio of AT_1 to AT_2 receptors was approximately 20:80 (Chang and Lotti, 1990), whereas in rat, rabbit, monkey and human adrenal cortex, the AT_2 receptor comprised ~10 to 40% of the total angiotensin binding sites (Chiu et al., 1989a; Whitebread et al., 1989; Chang and Lotti, 1990). Other tissues that expressed the AT_2 receptor at a high proportion, as compared to the AT_1 receptor, were the nonpregnant human uterus (Whitebread et al., 1989; Criscione et al., 1990; Bottari et al., 1991; de Gasparo and Levens, 1994); sheep uterine myometrium (Cox et al., 1993); bovine cerebellar cortex (Bottari et al., 1991); and rat ovarian follicular granulosa cells (Pucell et al., 1991). Species dependence exists, as only 40% and 60% of Ang II binding sites are AT_2 in rat and rabbit uterus, respectively (Dudley et al., 1990; Bottari et al., 1991). The proportion of the AT_2 receptor in the kidney cortex is less than 10% of the AT_1 receptor in the rat and rabbit, and ~55% in monkey (Chang and Lotti, 1991). On the other hand, the heart in the rat, rabbit, and monkey contains the AT_2 receptor in small but finite and measurable amounts, which comprises about 30% of the total Ang II binding sites (Chang and Lotti, 1991). The rat and primate pancreas were found to contain AT_1 and AT_2 receptors (Chappell et al., 1992, 1994). Expression of the AT_2 receptor was particularly high in pancreatic acinar cells (Chappell et al., 1995). Table 4 summarizes adult and fetal tissues that express AT_2 receptors.

Some fetal tissues express the AT_2 receptor at high levels. As summarized in Table 4, in many of these tissues the AT_2 receptor emerges on embryonic days 11

to 13 (E11–E13) and reaches a maximal level on E19. The AT_2 receptor then rapidly declines in newborn animals to lower levels or to undetectable levels. As examined by autoradiographic techniques and/or in situ hybridization, expression of the AT_2 receptor was particularly dense in differentiated mesenchymes, such as mesenchymal tissues in the tongue, subdermal, and s.c. regions of the skin and the diaphragm. In these tissues, the preponderant fetal Ang II binding sites are AT_2 (>97%) (Grady et al., 1991; Feuillan et al., 1993; Grady and Kalinyak, 1993; Shanmugam et al., 1995). The skin and tongue are the tissues in which fetal expression of AT_2 receptor is particularly intense, however, rapid postpartum disappearance is seen. In many other tissues where fetal AT_2 receptor expression level is detectable, a precipitous lowering of the AT_2 receptor is observed. The very dense expression of Ang II receptors in rat fetal skin cells noted by Millan et al. (1989) seems to be AT_2 receptor (Feuillan et al.,1993). These cells were isolated and cultured. Earlier passage cells express more AT_2 than AT_1 receptor. Switching of AT_2 to AT_1 expression, or AT_2-expressing cells to AT_1-expressing cells seems to take place (Johnson and Aguilera, 1991). In some tissues, AT_2 receptors reach undetectable levels (submucosal cells of the stomach and intestine or trachea). On the other hand, in certain tissues the process of rapid postpartum decrease is arrested, and the AT_2 receptor remains at detectable levels in the adrenal medulla, zona glomerulosa, (Shanmugam et al., 1995), pancreas (Chappell et al., 1994), uterus (Cox et al., 1993), and heart (see below).

In the fetal kidney, AT_2 receptor expression is seen mostly in the mesenchymal cells of differentiating cortex and medulla, which are surrounding glomeruli in the cortex and tubular tissues in the medulla (Kakuchi et al., 1995). The mesenchymal cells will undergo apoptosis and will be replaced by tubular tissues. The role of the AT_2 receptor in fetal development is not yet clearly established. Although the apoptosis of the AT_2 receptor-expressing cells in fetal tissue development is an attractive concept, hemizygotic male AT_2 receptor gene deleted mice (the AT_2 receptor gene is localized to the X-chromosome) and homozygotic female AT_2 receptor gene null mice undergo normal fetal development, and newborns and adults do not show any abnormality in the gross morphology of the skin, tongue, kidney, or adrenal. A delay in vasculogenesis was observed, however (Yamada et al., 1998).

Although in many tissues the AT_2 receptor undergoes a unidirectional decrease in its expression, this receptor also undergoes reversible changes. The myometria of the human, sheep, and rat express measurable AT_2 receptors at a high level (Criscione et al., 1990; Bottari et al., 1991; Cox et al., 1993; de Gasparo et al., 1994). In sheep uterus, for instance, the ratio of AT_1:AT_2 receptor is 15:85. In the pregnant ewe, the AT_2 receptor is reduced by more than 90% and AT_1 is reduced by 60% with the AT_1 to AT_2 ratio being reversed to 80:20. However, upon parturition, the AT_2 receptor rapidly returns to the high prepregnancy levels (Cox et al., 1993). Similar observation was made in human myometrium during pregnancy (de Gasparo et al., 1994). The AT_2 receptor also emerges during wound healing of the skin (Kimura et al., 1992; Viswanathan and Saavedra, 1992). This increase occurs particularly in the superficial dermis. A low level of AT_2 receptor expression was also seen in the neointimal tissue of rat carotid artery following balloon catheterization (Janiak et al., 1992; Viswanathan et al., 1994b). These changes in the uterus during pregnancy or in wound healing in the skin and brain indicate that the AT_2 receptor has a definitive but not yet identified regulatory function in these tissues.

1. Brain. Although circumventricular organs of the brain respond to circulating Ang II, an endogenous brain

TABLE 4
Tissue distribution of the AT_2 receptor and ontogenic change

Tissues	Fetus	Newborn	3 wk	8 wk	Reference
Adrenal					
Cortex	+	+	+	+	Shanmugam et al., 1995
Medulla	−	+	+	+	Feuillan et al., 1993
Ovary					
Follicular granulosa	+	+		+	Pucell et al., 1991
Kidney					
Cortex	+++	+			Shanmugam et al., 1995
Medulla (outer strip)	+	++			Shanmugam et al., 1995; Kakuchi et al., 1995
Heart	±				Shanmugam et al., 1995
Uterus					
Myometrium				+	
Blood vessels					
Heart	±				Shanmugam et al., 1995
Aorta	+			+	Feuillan et al., 1997; Shanmugam et al., 1995
Pancreas	+				Chappell et al., 1992
Trachea	+				Shanmugam et al., 1995
Stomach	+				Shanmugam et al., 1995
Mesenchyme	+				
Skin	+++	±			Grady et al., 1991; Shanmugam et al., 1995; Feuillan et al., 1993
Tongue	+++	±			Grady et al., 1991; Feuillan et al., 1993
Skeletal muscle	+				Feuillan et al., 1993

renin-angiotensin system can generate Ang II in tissues protected from circulating Ang II by the blood-brain barrier. Ang II generated by this system reacts with angiotensin receptors within the brain. The brain angiotensin receptors have been studied and reviewed extensively by Gehlert et al. (1991a,b), Rowe et al. (1992), Saavedra (1992), Song et al. (1992), and Höhle et al. (1995). An expression of the AT_2 receptor in fetal (E18) brain tissues was reported in several areas: inferior olive, paratrigeminal nucleus, and hypoglossal nucleus. Saavedra (1992) determined AT_1 and AT_2 receptor expression in various regions of the brain, and its age dependence in 2- and 8-week-old rat brains. Although AT_1 receptor expression did not show marked age dependence, the AT_2 receptor showed a significant decrease from 2 to 8 weeks of age (see Table 5).

Obermüller et al. (1991) investigated the distribution of Ang II receptor types in adult rat brain nuclei using competitive radioligand binding. Whereas midbrain and brain stem contained AT_1 and AT_2 receptors in comparable concentrations, AT_1 receptors were by far predominant in several hypothalamic nuclei, although a limited amount of AT_2 receptors was detectable in most of them. Although minor differences may exist among these studies, they share the following observations: AT_2 receptor expression is consistently high in the cerebellar nuclei, inferior olive, and locus ceruleus in the brain stem, which is rich in noradrenergic neurons. In contrast to the AT_1 receptor, which participates in various central cardiovascular functions and is expressed in the hypothalamus and in brain stem nuclei (nucleus of the solitary tract, dorsal motor nucleus of vagus at a low level), the AT_2 receptor is much less present in distinct hypothalamic and brain stem nuclei associated with the regulation of cardiovascular functions. The presence of the AT_2 receptor in the dorsal motor nucleus of vagus is not completely settled.

Although the distribution of AT_2 receptors in the brain is well known, their effects are still not clear. As outlined above, brain AT_2 receptors may play a role in cognitive functions and certain types of behavior such as exploration or drinking (Hein et al., 1995a). On the other hand, they may also antagonize the central effects of angiotensin peptides in osmoregulation mediated via AT_1 receptors (Höhle et al., 1995, 1996). A possibly important role of AT_2 receptors in neuroregeneration and neuroprotection will be dealt with below.

2. Heart. Earlier studies suggested that myocytes of rabbit and rat hearts contain AT_1 and AT_2 receptors in comparable quantities as shown in Table 6 (Rogg et al., 1990; Scott et al., 1992; Sechi et al., 1992b). However, questions remained as to the cell-specific localization of these receptors in situ. A recent study using single cell RT-PCR has demonstrated that in the adult rat about 50% of cardiomyocytes contain the AT_1 receptor, whereas the AT_2 receptor is much more scarce with only about 10% carrying this receptor type (Busche et al., 2000).

Autoradiographic studies of the rat heart show about a 2-fold increase in AT_1 and AT_2 receptors after birth, and their receptor numbers are about equal from E16 through 10 to 16 weeks of age (Sechi et al., 1992b). They are expressed in the myocardium of all four chambers, as well as in the vascular smooth muscles of the aorta and pulmonary arteries. However, very little ligand binding was seen in the coronary artery. The conduction system (the atrioventricular and sino-aortic nodes) was reported to contain both AT_1 and AT_2 receptors by Sechi et al. (1992b), whereas Saavedra et al. (1993), Brink et al. (1996), and Wharton et al. (1998), showed only AT_1 receptors in these tissues.

Primary culture of cardiomyocytes from neonatal rat left ventricles showed about a 50% decrease in the AT_2

TABLE 5
Distribution of the AT_2 receptor in the brain—effect of age

	2-wk-old	8-wk-old
	fmol/mg protein (mean ± S.E.M.)	
Regions containing only the AT_2 receptor		
Persistent AT_2 receptor with age		
Lateral septal nucleus	58 ± 6	18 ± 3
Ventral thalamic nuclei	101 ± 8	24 ± 3
Mediodorsal thalamic nucleus	165 ± 11	38 ± 13
Locus ceruleus	289 ± 19	98 ± 13
Principal sensory trigeminal nucleus	75 ± 6	15 ± 4
Parasolitary nucleus	220 ± 15	57 ± 14
Inferior olive	1328 ± 61	181 ± 32
Medial amygdaloid nucleus	159 ± 8	94 ± 9
Medial geniculate nucleus	338 ± 24	71 ± 6
Transient expression of the AT_2 receptor		
Anterior pretectal nucleus	53 ± 8	N.D.
Nucleus of the optic tract	101 ± 13	N.D.
Ventral tegmental area	101 ± 11	N.D.
Posteodorsal tegmental nucleus	110 ± 21	N.D.
Hypoglossal nucleus	141 ± 11	N.D.
Central medial and paracentral thalamic nucleus	202 ± 14	N.D.
Laterodorsal thalamic nucleus	110 ± 10	N.D.
Oculomotor nucleus	98 ± 13	N.D.
Regions containing both AT_1 and AT_2 receptors		
Persistent AT_2 receptor with age		
Superior collicus	145 ± 5	65 ± 8
Cingulate cortex	19 ± 4	8 ± 4
Transient expression of the AT_2 receptor		
Cerebellar cortex	59 ± 6	N.D.

N.D., not determined.

TABLE 6
Coexistence of AT_1 and AT_2 in cardiac tissues

	% of AT_1 + AT_2		References
	AT_1	AT_2	
Rabbit ventricle	60	40	Rogg et al., 1990
Rat (10-wk-old)	50	50	Sechi et al., 1992b
Myocytes	65	35	Matsubara et al., 1994
Fibroblasts	>95	<5	Villareal et al., 1993
Fibroblasts (adult)	100		
Bovine	67	33	Nozaki et al., 1994
Human	33	69	Regitz-Zagrosek, 1995

receptor from those of E19 fetuses, whereas the AT_1 receptor did not decrease (Matsubara et al., 1994). In contrast, the AT_2 receptor in cultured fibroblasts collected from 1-day-old rat heart was less than 10% of the AT_2 receptor in fibroblasts cultured from E19 fetal heart, whereas there was no change in AT_1 receptor number. In cardiac fibroblasts cultured from 7-day-old rats, AT_2 receptor density was negligible and the majority of the Ang II receptor population was reported to be AT_1, whereas in cultured cardiomyocytes AT_2 receptor numbers are maintained at a finite and measurable level (about 50% of AT_1). Bovine and human ventricular and atrial myocardium also contain both AT_2 and AT_1 receptors (Rogg et al., 1991; Nozawa et al., 1994; Regitz-Zagrosek et al., 1995). Although the AT_2 receptor is usually expressed at low density in adult, it is up-regulated to different extents in pathological circumstances such as cardiac hypertrophy, myocardial infarction, cardiomyopathy, and congestive heart failure (Matsubara, 1998; Unger, 1999). Interestingly, both in nonfailing and explanted end-stage human heart the AT_2 receptor population measured in a binding assay (65% of total Ang II receptor) is greater than AT_1, and there may be a correlation between the density of the AT_2 receptors and the severity of heart failure (Rogg et al., 1996). Wharton et al. (1998) also observed a significant increased density of high-affinity binding sites in endocardial, interstitial, perivascular, and infarcted regions of the ventricle of patients with end-stage ischemic heart disease or dilated cardiomyopathy, greater than in adjacent noninfarcted myocardium. The border zone between noninfarcted and infarcted myocardium was rich in microvessels with perivascular AT_2 receptors. Ohkubo et al. (1997) reported that in the heart of cardiomyopathic hamster (Bio 146), both AT_1 and AT_2 receptors were increased during heart failure (153% and 72%, respectively). In human, the expression of the AT_2 receptor was markedly (3-fold) increased in patients with dilated cardiomyopathy at both protein and mRNA levels compared with patients with acute or well organized old myocardial infarction (Tsutsumi et al., 1998). In contrast, the AT_1 receptor expression was significantly down-regulated. The AT_2 receptor sites were highly localized in the interstitial region in the fibrotic areas where fibroblasts are present. Collagen and fibronectin formation from fibroblasts were suppressed by the AT_1 antagonist TCV116, and increased by the inhibition of AT_2 receptor with PD123319 in the cardiomyopathic hamster. These results are intriguing as they suggest the possibility that the AT_1 and AT_2 receptors modulate extracellular matrix formation in an opposite way as has already been suggested from experiments using cardiac microvascular enothelial cells (Fischer et al., 1996).

Although much remains to be learned about the various functions of cardiac AT_2 receptors, their distribution and time- and event-driven regulation suggest roles in cardiac development, repair and remodeling with actions opposing those mediated by cardiac AT_1 receptors.

3. Kidney. The kidney is a major target organ of Ang II. Ang II exerts its regulatory function on both hemodynamic and tubular functions, and most of its actions seem to be explained by AT_1 receptor functions. The AT_1 receptors are indeed abundantly expressed in small cortical arteries, glomeruli, proximal tubules, and interstitium. The AT_2 receptor is, however, also expressed in the kidney. Ontogenic studies on rat and humans by autoradiography, in the presence of losartan (Sechi et al., 1992a; Ciuffo et al., 1993b), on rat (Shanmugan et al., 1995), and on mouse (Kakuchi et al., 1995) showed marked development-dependent changes in AT_2 receptor expression in the kidney.

In the mouse (Kakuchi et al., 1995), on embryonic days 12 to 16 (E12–E16) AT_2 mRNA was densely expressed in the mesenchymal cells of the mesonephros surrounding the mesonephric tubules. In this developmental period, the mesonephros begins regression, and a possible function of the AT_2 receptor in the apoptotic loss of mesonephros can be envisioned. On E14, the AT_2 receptor emerges in the interstitial mesenchymes of the kidney, but not in the glomeruli or the S-body, whereas the AT_1 receptor is richly expressed in the preglomeruli and S-bodies. On E16, AT_2 mRNA is seen in the renal capsule and inner medulla where it is prominent along the papillary duct and between the collecting ducts. AT_2 receptor on the medullary ray extend into the cortex, whereas the detection signals are reduced to undetectable levels after birth (Kakuchi et al., 1995; Shanmugam et al., 1995).

About 10 to 15% of the renal Ang II binding sites were blocked by CGP42112 (de Gasparo et al., 1990; Zhuo et al., 1992, 1993, 1998). The outermost layer of the cortex outside of the glomeruli and inner layer of medulla seem to show signaling for the AT_2 receptor, the proximal convoluted tubule is a candidate site for AT_2 receptor localization, which is functional in the renal tubular system, as its existence has been reported by Dulin et al. (1994). The same group also reported the presence of the AT_2 receptor in cultured rat mesangial cells by binding studies (Ernsberger et al., 1992). However, quantitative autoradiographic studies of rat renal glomeruli by Ciuffo et al. (1993b) did not detect the AT_2 receptor in rat renal glomeruli. Reviewing many of these studies, it appears that the competitive ligand binding autoradiography or in situ hybridization does not provide sufficient sensitivity for detecting a low-level expression of AT_2, comprising only 10 to 20% of total binding. Along this line, earlier studies by a similar technique by Gibson et al. (1991) did not detect AT_2 receptor in the rat renal cortex. By contrast, in rhesus monkey kidneys, AT_2 receptors (PD12198 sensitive sites) were seen in the juxtaglomerular apparatus and in arterial smooth muscle cells.

Ozono et al. (1997) examined the expression of the AT_2 receptor in the fetal, newborn, and adult kidney by im-

munohistochemistry using antibodies raised against the peptide epitope with the amino acid sequence of the N terminus of the rat AT_2 receptor. Positive immunohistochemical staining of the AT_2 receptor was observed in the mesenchymal cells and ureteral bands of the 14-day-old fetal kidney and in the glomeruli, tubules, and vessels in the 19-day-old fetal and newborn kidney. Glomeruli expressing the AT_2 receptor were localized mainly in the outer layers of the renal cortex. In the young and adult rat, AT_2 receptors were present in glomeruli at a substantially diminished level. Lowering dietary sodium intake increased glomerular and interstitial AT_2 receptors. In human kidney, the AT_2 receptor is clearly present in large preglomerular vessels of the renal cortex (Grone et al., 1992) and in the tubular interstitium (Chansel et al., 1992; Goldfarb et al., 1994). Possible renal functions of the AT_2 receptor have recently been reviewed (Carey et al., 2000).

4. Vasculature. Although it is well known that the AT_1 receptor, present in vascular smooth muscle, mediates the contractile and hypertrophic effects of Ang II, the presence of the AT_2 receptor in the vasculature in vivo was seen only by autoradiography. Its exact site of localization and functional roles are yet to be clarified. In coronary endothelial cells derived from SHR, AT_2 receptor mRNA as well as AT_2 binding were present with a ratio AT_1/AT_2 of about 80%/20% (Stoll et al., 1995). In ontogenic studies on AT_2 receptor expression in fetal (E18), 2-week-, and 8-week-old rat aorta autoradiographic determination of specific binding of ^{125}I-Sar^1-Ang II in the presence of losartan and PD123177 was performed (Viswanathan et al., 1991). Interestingly, the AT_2 receptor was the major or almost exclusive Ang II receptor in the fetal aorta (E18), accounting for 85% of the total binding. Even at 2 weeks of age the AT_2 was still the dominant form but it became a minor Ang II receptor at 8 weeks of age (25%). However, the total Ang II receptor sites decrease after birth and throughout the process of maturation from a very high level (300 fmol/mg of protein) in the fetus down to less than 10 fmol/mg of protein in 8 weeks. Autoradiography showed the AT_2 receptor in the medial layer even at 8 weeks of age. Similar results were obtained by competitive binding of the vascular membrane fraction (Chang and Lotti, 1991). This small but finite population of AT_2 receptors in the media of the vasculature may explain a rapid pressor and subsequent depressor effect of Ang III infused into rats pretreated with losartan. The AT_2 blocker almost completely eliminated the depressor phase (Scheuer and Perrone, 1993). The presence of AT_2 receptors even at a low level may be compatible with the increased sensitivity to the pressor action of Ang II at a low concentration (10^{-12}-10^{-10}M) under AT_2 blockade. Thus, it has been speculated that the AT_2 blockade unmasks the sensitivity of the vascular AT_1 receptor to a low concentration of Ang II (Hong et al., 1994).

Formation of neointima following balloon catheterization of the rat carotid artery can be suppressed by angiotensin converting enzyme inhibitors or AT_1 antagonists (see above). However, there was a report that an AT_2 agonist was efficient in preventing neointima formation, but not an AT_1 antagonist (Janiak et al., 1992). Autoradiographic studies of Ang II receptor expression indicated a marked increase in AT_1 and a decrease in AT_2 receptors in the balloon catheter-treated aorta, and equally elevated AT_1 but no expression of AT_2 receptors in the normal and injured carotid artery (Viswanathan et al., 1994b). However, the neointimal area of the vessels transfected with and expressing the AT_2 receptor transgene was decreased by 70% compared to untransfected or control-vector transfected vessels. This inhibitory effect on the development of the neointima was blocked by an AT_2 receptor antagonist, PD123319 (Nakajima et al., 1995). The in vivo localization of the AT_2 receptor in the aorta of rats may provide a basis for a possible interpretation of the interesting report that chronic blockade of AT_2 rather than AT_1 receptor antagonizes Ang II-induced aortic hypertrophy and fibrosis (Levy et al., 1996). In a model of chronic Ang II infusion, Cao et al. (1999) reported on an increased mesenteric weight and wall-lumen ratio of the vessels as well as proliferation of smooth muscle cells. These vascular changes were attenuated by both AT_1 and AT_2 receptor antagonists. Moreover, AT_1 receptor blockade was associated with down-regulation of both receptors, whereas AT_1 receptor blockade was associated with reduced Ang II binding to only the AT_2 receptor. Although these observations suggest that vasotrophic effects of Ang II could, at least partially and under certain experimental conditions, be mediated by the AT_2 receptor type, this topic is still controversially discussed. The marked AT_2-mediated increase of aortic cGMP under Ang II infusion in SHR as observed by Gohlke et al. (1998) is just one example speaking against AT_2 as a vasotrophic factor.

Despite the fact that the media of rat thoracic aorta express the AT_2 receptor, smooth muscle cells derived from the aorta and cultured in vitro do not (Kambayashi et al., 1996; Ichiki et al., 1996). This is possibly due to down-regulation of the AT_2 receptor gene by growth factors in serum-containing culture medium since AT_2 receptor expression can be regained upon prolonged serum depletion in the presence of insulin (Kambayashi et al., 1996).

Coronary endothelial cells cultured from adult SHR contain AT_1 and AT_2 receptors in a ratio of about 80/20% as determined by the demonstration of AT_2 mRNA, AT_2 binding and functional studies (Stoll et al., 1995). The same authors showed that in these cells, serum- or basic fibroblast growth factor-induced proliferation is dose-dependently inhibited by Ang II (Metsärinne et al., 1992; Stoll et al., 1995) and that this antiproliferative effect is sensitive to AT_2 receptor blockade. Furthermore, that under nonstimulated conditions, the mito-

genic activity of Ang II is suppressed by the AT_1 antagonist losartan but is markedly enhanced by the AT_2 antagonist PD123177 (Stoll et al., 1995). This is presumably because the mitogenic action of AT_1 receptor is partially suppressed by the AT_2 receptor. The antiproliferative effect of the AT_2 receptor has subsequently been confirmed in several laboratories and also extended to nonvascular cell systems (Meffert et al., 1996; Munzenmaier et al., 1999; Goto et al., 1997; Van Kesteren et al., 1997; Maric et al., 1998).

5. Pancreas, Lung, Thymus, and Other Tissues. Canine and primate pancreas were found to contain the AT_2 receptor at a relatively high level (Chappell et al., 1992, 1994). This receptor was characterized by high binding affinity ($K_d \sim 0.48$ nM) for Ang II, a 2-fold enhancement of binding by DTT and inhibition of radioactive Ang II binding by AT_2-specific antagonists. Of the total Ang II binding sites approximately 70% seems to be AT_2. Autoradiographic studies showed a predominance of AT_2 over AT_1 receptors throughout the pancreas including islet cells, acinar and duct cells, as well as vasculature cells (Chappell et al., 1991, 1992). Subsequently, the rat pancreatic acinar cell line AR42J was shown to contain a high level of AT_2 receptors (250 fmol/mg of protein) with concomitant AT_1 receptors at ~10 to 15% of the level of the AT_2 receptor (Chappell et al., 1995). The pancreas also contains a large amount of angiotensinogen and its mRNA studied (Chappell et al., 1991).

The distribution of the AT_1 and AT_2 receptor in human lung was studied using immunohistochemistry with specific polyclonal antibodies and with in situ hybridization. The AT_1 receptor mRNA and protein were localized on vascular smooth muscle cells, macrophages, and, in particular, in the stroma underlying the airway epithelium. In contrast, the AT_2 receptor mRNA and protein was observed in the epithelium, with strong staining on the bronchial epithelial cell brush border and also on many of the underlying mucous glands. The AT_2 receptor was also present on some endothelial cells (Bullock et al., 1999). The thymus was shown to contain sites able to bind Sar^1-Ang II at a high affinity. This binding is inhibited by an AT_2 antagonist (Correa et al., 1994).

There are tissues or cells that are devoid of AT_2 receptor when using either ^{125}I-Ang II in the presence of AT_1 selective antagonists or the AT_2-specific binding agent ^{125}I-CGP 42112. These tissues are the liver (Whitebread et al., 1989; Dudley et al., 1990) and the pituitary (Leung et al., 1991).

6. Cells in Primary Culture and Cell Lines Expressing the AT_2 Receptor. To clarify the signaling mechanism and pathophysiological significance of the AT_2 receptor, numerous types of cells derived from tissues known to contain the AT_2 receptor were prepared or identified. They provided useful materials for studies on AT_2 receptor signaling, pathophysiologic roles, and regulation for cloning and purification.

Among primary cultured cells were neuronal cells dispersed from rat neonatal hypothalamus (Sumners and Myers, 1991), neonatal rat cardiomyocytes (Lokuta et al., 1994; Suzuki et al., 1993), adult rat aortic endothelial cells (Stoll et al., 1995), rat fetal skin cells (Tsutsumi et al., 1991), and rat fetal fibroblasts (Johnson and Aguilera, 1991).

Rat adrenomedullary pheochromocytoma PC12 cells express both AT_1 and AT_2 receptors reflecting their adrenal medullar origin where both AT_1 and AT_2 receptors are coexpressed. A subline, PC12W, which expresses a large amount of AT_2 but not AT_1 receptors (Speth and Kim, 1990), were extensively used for studies of AT_2 signaling mechanisms and for AT_2 cloning. A subline of the Swiss mouse 3T3 fibroblast cell line, R3T3, also expresses AT_2 without AT_1 receptor (Dudley et al., 1991; Csikos et al., 1998). Although in both of these cell lines expression of the AT_2 receptor is suppressed in the cellular growth phase, AT_2 mRNA increases as they approach the subconfluent or confluent phase. When R3T3 cells are shifted to a quiescent condition by serum deprivation, the AT_2 receptor protein production increases without proportional increase in mRNA (Dudley and Summerfeldt, 1993).

An undifferentiated mouse neuroblastoma cell line, NG108-15, was shown to express the AT_2 but not the AT_1 receptor (Buisson et al., 1992) and was used in studies of the signaling mechanism: AT_2 receptor activation by Ang II resulted in phosphotyrosine phosphatase activation and closing of calcium T-channel (Buisson et al., 1995).

Another mouse neuroblastoma cell, N1E-115, expresses both AT_1 and AT_2 receptors (Reagan et al., 1990). Since AT_1 receptors can be rapidly destroyed by dithiothreitol, AT_2 receptors could be preserved in fractions solubilized by the detergent CHAPS and were used as a source for purification of the AT_2 receptor. The transformed rat pancreatic acinar cell line, AR42J, contains both AT_1 and AT_2 receptors and shows a marked rise in Ca^{2+} characteristic of the AT_1 receptor response (Chappell et al., 1995). Thus, this cell line may not be very convenient for the study of the role of AT_2 receptor. However, AR42J cells have an exocrine function that may or may not be regulated by AT_2. Ovarian granulosa cells isolated to a high degree of purity and maintained in primary culture were also very useful to study AT_2 receptor-dependent differentiation and apoptosis (Pucell et al., 1991; Tanaka et al., 1995). The cloned mouse preadipocyte cell line, Ob1771, expresses the AT_2 receptor upon differentiation in a serum-free medium and responds to Ang II by producing prostacyclin, which promotes the differentiation of the preadipocytes by a paracrine mechanism (Darimont et al., 1994). Rat VSMC in culture do not express the AT_2 receptor under regular culture conditions in the presence of fetal calf

serum (Stoll et al., 1995; Ichiki et al., 1996; Kambayashi et al., 1996), but, as outlined above, the AT_2 receptor can emerge in rat thoracic VSMC when cultured in serum-depleted medium supplemented with insulin or other insulin-like growth factors such as IGF (Ichiki et al., 1996; Kambayashi et al., 1996). Thus rat VSMC grown and maintained under ordinary culture conditions do not seem to provide convenient materials for the study of the role of the AT_2 receptor. However, VSMC can be transfected with an artificial AT_2 receptor gene consisting of an AT_2 receptor coding region with a myosin heavy chain promoter (Nakajima et al., 1995).

G. Pathophysiological Aspects of AT_2 Receptor Activation

The widespread distribution of AT_2 receptors in various brain nuclei, heart, vascular tissues, adrenal, kidney, skin, and during wound healing suggests a physiological role for the AT_2 receptor. The identification of several AT_2 receptor signaling pathways as reviewed in preceding sections implies diverse pathophysiologic consequences. Studies to identify the role of the AT_2 receptor in pathophysiology have been conducted using diverse approaches, which include the use of cultured cells, in vivo vascular tissues injured by balloon catheter, transgenic expression of AT_2 receptor, targeted AT_2 receptor gene deletion (gene knockout), and chronic administration of AT_2 antagonists and agonists (Horiuchi et al.,1998; Matsubara, 1998; Unger, 1999; Carey et al., 2000).

1. The AT_2 Receptor Can Induce Apoptosis. The effect of the AT_2 receptor does not stop at the inhibition of cellular proliferation but is associated with cellular programs such as differentiation and regeneration (see below) and, under appropriate conditions, also with apoptosis.

In PC12W cells, expressing only AT_2 but not AT_1 receptors, nuclear condensation, fragmentation, and marginalization were observed in serum-free medium containing NGF (1 ng/ml) after treatment with 10^{-7} M Ang II for 2 days (Yamada et al., 1996; Gallinat et al., 1999). Characteristic internucleosomal DNA fragmentation was also observed. In R3T3 cells, serum depletion seemed to be the major contributor to apoptotic DNA fragmentation although activation of AT_2 receptor has an additional effect.

Skin fibroblasts collected from mice embryos with genetic deletion of the AT_2 receptor did not show any DNA fragmentation characteristic of apoptosis after stimulation with Ang II, whereas fibroblasts from wild mice did (Li et al., 1998b). Ovarian granulosa cells undergo apoptosis during follicular atresia. These cells contain the AT_2 but not the AT_1 receptor (Pucell et al., 1991). Tanaka et al. (1995) showed that these cells in culture underwent apoptotic DNA fragmentation when depleted of follicle-stimulating hormone. Although no direct effect of added Ang II on the apoptosis was observed, the AT_2 receptor level in these cells was increased by the removal of follicle-stimulating hormone and the addition of Ang II, indirectly suggesting a possible role of the AT_2 receptor in apoptosis and even perhaps in ovulation.

"Programmed cell death" is an important concept in developmental morphogenesis. The fetal kidney expresses the AT_1 receptor in the preglomerular S-bodies, whereas the AT_2 receptor is expressed in fetal renal mesenchymal cells, which are replaced by tubular tissues in the later stages of renal development. Mesonephros also express the AT_2 receptor in the mesenchyme surrounding its tubular system. Mesonephros are known to disappear by apoptosis (Kakuchi et al., 1995). However, the nephrogenesis occurs even in AT_2 receptor null mice. Thus, triggering of the AT_2 receptor-mediated activation of MKP-1 and apoptosis may be contingent on additional growth suppressive measures such as serum depletion.

The mechanism behind AT_2-mediated apoptosis appear complex as both AT_1 and AT_2 receptors are involved in the process (de Gasparo and Siragy, 1999). The calcium flow between endoplasmic reticulum and mitochondria and the ratio *Bcl*2/Bax, which is modulated by both AT_1 and AT_2 receptors play a key role in homeostasis between cell growth and cell death (Kajstura et al., 1997; Berridge et al., 1998; Fortuno et al., 1998; Tea et al., 1998). Horiuchi et al. (1997b) reported that AT_2 receptor stimulation in PC12W cells dephosphorylates *Bcl*2 by activation of MKP-1. In PC12W cells, AT_2 stimulation markedly lowered MAPK. This effect was suppressed by pretreatment with vanadate and pertussis toxin, indicating that a G protein-driven PTP decreased MAPK activity. The PTP was shown to be MKP-1, since the elimination of MKP-1 by an antisense oligonucleotide transfection abolished DNA fragmentation (Yamada et al., 1996). Activation of caspase may be involved in AT_2 receptor-induced apoptosis in umbilical venous endothelial cells (Dimmeler et al., 1997). A stimulation of ceramide, which replaces the JNK-stress-activated protein kinase pathway leads to caspase stimulation (Hayashida et al., 1996).

Gallinat et al. (1999) observed that in PC12W cells, kept under conditions allowing for apoptosis, the AT_2 receptor-induced apoptosis was associated with an Ang II concentration-dependent ceramide production. Because sphingomyelin concentrations were unaltered by Ang II these findings suggested a de novo synthesis of ceramide by AT_2 receptor stimulation. This report was subsequently confirmed by Lehtonen et al. (1999).

Stretch also activates apoptosis following increased expression of the transcriptional factor p53 and up-regulation of the local renin-angiotensin system, whereas *Bcl*2 expression is decreased. p53 indeed binds to the promoter of the angiotensinogen and the AT_1 receptor genes and stimulates production of Ang II and expression of the AT_1 receptor (Pierzchalski et al., 1997; Leri et al., 1998).

Together, these reports clearly indicate that the AT_2 receptor is able to induce apoptosis, although the signaling pathways await further investigation. It should be noted, however, that the proapoptotic features of the AT_2 receptor can only be unveiled under specific experimental conditions, for instance serum deprivation/NGF dependence, which generally prepare the grounds for apoptosis.

2. Effects on Vascular Tone. The presence of AT_2 receptors in the vasculature has been discussed above. Whereas the vascular antiproliferative and neointima-reducing effects of AT_2 receptor stimulation are now well established (Horiuchi et al., 1998; Unger, 1999), the participation of the AT_2 receptor in the regulation of vascular tone is still controversial. When a bolus injection of Ang III was given i.v. into anesthetized rats, both pressor and depressor effects were observed. Under AT_1 receptor blockade, Ang III exerted a dose-dependent depressor effect, which was abolished when both AT_1 and AT_2 receptors were blocked (Scheuer and Perrone, 1993). In rabbit abdominal aorta, the AT_2 blocker PD123177 unmasked losartan-sensitive pressor effects of Ang II at a low-concentration range of 10^{-12} to 10^{-10} M where the peptide alone was not pressoric. These in vivo results suggested that a pressor action of the AT_1 receptor was masked by the AT_2 receptor (Hong et al., 1994). AT_2 receptor gene null mice prepared by Ichiki et al. (1995) show an elevated systolic and diastolic pressure, indicating that the AT_2 receptor has a depressor effect. In a renal wrap Grollman hypertension model, AT_1 receptor blockade normalized blood pressure, whereas AT_2 receptor antagonism significantly increased blood pressure but decreased renal interstitial concentration of bradykinin and nitric oxide (Siragy and Carey, 1999). These results suggest that the AT_2 receptor mediates counterregulatory vasodilation. Similarly, AT_2 receptor activation with CGP42112 facilitates in SHR the depressor response caused by an AT_1 receptor antagonist (Barber et al., 1999). On the other hand, in adult conscious SHR a concomitant infusion of Ang II and the AT_2 antagonist, PD123319, did not result in blood pressure elevation, whereas the AT_1 antagonist, losartan, consistently lowered blood pressure in the presence or absence of Ang II (Gohlke et al., 1998).

Thus, although the results of several studies suggest that the AT_2 receptor exerts depressor effects upon stimulation by Ang II or III, there is also evidence against the idea of AT_2 as a vasodilator. It appears that, under normal conditions, the pressor effect of the AT_1 receptor exerts a dominant effect, and overall effect of Ang II is directed to blood pressure elevation. A defect of the AT_2 receptor, either genetic or otherwise, could lead to an elevation in blood pressure or increased sensitivity to Ang II or III. On the other hand, blockade of AT_1 receptor in juxtaglomerular cells by selective AT_1 receptor antagonists increases circulating Ang II, which will then interact with the unopposed AT_2 receptor. Stimulation of AT_2 receptors could thus contribute to a further decrease of blood pressure. However, the exact mechanism of AT_2 receptor-mediated depressor effect is still not clear.

3. Vascular Hypertrophy and Fibrosis and the AT_2 Receptor. Arterial hypertrophy, remodeling, and fibrotic changes induced by chronic administration of Ang II have been believed to be due to diverse effects mediated by the AT_1 receptor. However, when normotensive rats received a chronic (3 weeks) s.c. infusion of Ang II (120 ng/kg/min), Ang II + losartan (10 mg/kg/day) or Ang + PD123319, it was the AT_1 receptor blocked (with losartan) rat that showed a marked increase in collagen and elastin in the thoracic aorta, despite normal blood pressure maintained by losartan. By contrast, collagen and elastin in the Ang II-PD123319-treated rats were at low control levels despite markedly elevated blood pressure. Media thickness was also increased in the AT_2-blocked rats rather than AT_1-blocked animals (Levy et al., 1996; Cao et al., 1999). A persuasive explanation for these intriguing observations, which were not confirmed by Li et al. (1998a) and are in disagreement with a host of reports on the antigrowth effects of the AT_2 receptor in many tissues including the vasculature (Horiuchi et al., 1999; Unger, 1999) is not yet available.

4. Renal Tubular Function. The presence of an AT_2-like (termed AT_{1B}) receptor in renal tubules particularly in the proximal tubules have been reported by Douglas and associates (Douglas, 1987; Ernsberger et al., 1992; Dulin et al., 1994). However, AT_2 mRNA is not readily detected in adult rat kidney. On the other hand, the AT_1 receptor is clearly expressed in the glomeruli and in the inner stripe of the outer medulla. Glomerular AT_1 receptors may control the hemodynamic function of the kidney.

The AT_2 receptor seems to have some detectable effect on renal tubular function. The AT_2 selective antagonist, PD123319, infused i.v. at 300 μg/kg/min into anesthetized dogs increased free water clearance 4-fold and sodium excretion 3-fold, whereas the AT_1 selective losartan had only insignificant effects on the tubular functions (Keiser et al., 1992). In these experiments, renal blood flow was decreased only by 10%. On the other hand, salt replete dogs infused with PD123177 into the renal artery did not show appreciable changes in free water clearance and natriuresis (Clark et al., 1993). When the hemodynamic function and tubular function are dissociated by maintaining the renal blood flow constant using inflatable aortic cuffs above and below the renal artery of an unilaterally nephrectomized rat (Roman et al., 1984), infusion of the AT_2 blocker, PD123319, rapidly induces an increase in diuresis and natriuresis. As the extent of diuresis was perfusion pressure-dependent, it is considered to represent "pressure natriuresis". CGP42112, an AT_2 agonist, suppresses the diuretic response. The glomerular filtration rate remained remarkably constant (Lo et al., 1995). These results can be

interpreted to mean that AT_2 receptor stimulation by Ang II induces Na^+ retention when kidney perfusion pressure is increased. In contrast, in conscious rats with a chronic microdialysis cannula in the renal medullary interstitium, AT_1 receptor blockade showed a marked diuretic effect, whereas AT_2 receptor blockade by PD123319 did not exert any actions on water clearance or natriuresis. When animals were placed on a low-salt diet, cGMP and PGE_2 in the microdialysate from the kidney interstitial fluid were generally increased 2- to 3-fold over a 5-day period. Upon infusion of losartan, PGE_2 was reduced to the basal level of rats on regular diet, whereas cGMP was not affected. PD123319 markedly increased (~4-fold) PGE_2 and reduced cGMP to basal levels. Losartan plus PD123319 reduced PGE_2 to basal levels overriding the effect of PD123319, whereas cGMP was essentially regulated by PD123319. These results indicate that the AT_2 blocker, PD123319, rapidly induces an increase in diuresis and production of cGMP, which is regulated primarily by the AT_2 receptor, whereas the AT_1 receptor has no effect. On the other hand, PGE_2 production was abolished by losartan and was markedly increased by PD123319 (Siragy and Carey, 1996, 1997a,b). Similar microdialysis has indicated that tissue bradykinin, nitric oxide, and PGF2α formation were released upon stimulation of the AT_2 receptor (Siragy et al., 2000).

In AT_2 receptor knockout mice, a subpressor dose of Ang II inhibits natriuresis and diuresis suggesting that the AT_2 receptor stimulation physiologically increase pressure natriuresis and that this effect is sustained over a prolonged period (Siragy et al., 1999). An explanation for the discrepancy with the data of Lo et al. (1995) is not obvious as yet.

5. Neuronal Cell Differentiation and Nerve Regeneration. Cultured Schwann cells express both AT_1 and AT_2 receptors, and Ang II decreases the expression of the neurite-promoting protease nexin-1. Blockade of the AT_1 receptor or stimulation of the AT_2 receptor leads to a severalfold increase of nexin-1 favoring nerve regeneration (Bleuel et al., 1995). Similarly, treatment of nondifferentiated NG108-15 or PC12W cells with Ang II or nerve growth factor induces growth arrest and morphological differentiation of neuronal cells including neurite outgrowth and up-regulation of polymerized tubulin as well as microtubule-associated protein MAP2c expression and differential regulation of neurofilament M (Laflamme et al., 1996; Meffert et al., 1996; Gallinat et al., 1997; Stroth et al., 1998). These effects were mimicked by the AT_2 receptor agonist, CGP42112A, and abolished by coincubation with the antagonist, PD123319. The underlying signaling mechanisms may include inhibition of $p21^{ras}$ as well as activation of MAPK (Gendron et al., 1999; Stroth et al., 2000). Repression of c-fos and c-jun expression does not seem to be involved in AT_2 receptor-mediated growth arrest and cell differentiation in PC12W cells (Steckelings et al., 1998). Moreover, there is a marked up-regulation of AT_2 receptor mRNA in regenerating neurons in response to nerve crush coinciding, as an AT_2 expression wave from proximal to distal, with the regeneration of nerve fibers (Gallinat et al., 1998). Both in vitro and in vivo, Ang II promotes axonal elongation of postnatal retinal explants and dorsal root ganglia neurons and, in adult rats, axonal regeneration of retinal ganglion cells after optic nerve crush. This effect is completely abolished by an AT_2 receptor antagonist but not affected by an AT_1 receptor antagonist (Lucius et al., 1998). Thus, the AT_2 receptor plays a role in neuronal cell differentiation and nerve regeneration via regulation of the cytoskeleton, whereas the AT_1 receptor may inhibit this process (Unger, 1999).

H. Summary

AT_2 receptor expression is widespread. However, in several tissues it disappears after birth or when cells are transferred in culture in the presence of serum and growth factor. On the other hand, a sometimes dramatic up-regulation of the AT_2 receptor can occur after tissue injury. Research on the AT_2 receptor has revealed actions differing greatly from those of the AT_1 receptor such as antiproliferative effects in several tissues, cellular differentiation, nerve regeneration, and apoptosis. It appears that the AT_2 receptor often plays the role of a modulator of biological programs in tissue development or repair.

The signaling mechanisms of the AT_2 receptor are diverse, and only a few of them have as yet been characterized reasonably well. In some cases they are coupled to G_i proteins. One pathway in neurons (and perhaps other tissues) involves activation of protein serine/threonine phosphatase PP2A, which leads to the activation of the delayed rectifier K^+-channel (outward K^+ current in neuronal cells). This will result in hyperpolarization of plasma membranes that suppress cellular activities stimulated by depolarization. A second signaling pathway involves the activation of protein phosphotyrosine phosphatases (PTPases), which are of pivotal importance to prevent or rapidly shut off undesired and uncontrolled growth of normal tissues.

The AT_2 receptor lacks the mechanism of rapid desensitization, internalization, and low-affinity shift by GTP. Molecular mechanisms underlying such a unique signaling mechanism have not been clarified and warrant further intensive investigation.

Arachidonic acid release from cardiac myocytes leading to the activation of Na^+/HCO_3 symporter may be a unique regulatory mechanism of cardiac myocytes. Release of nitric oxide with subsequent formation of cGMP appears to be another important intracellular effect of AT_2 stimulation occurring in vascular and renal tissues. In many adult tissues, the modulatory or suppressive actions of the AT_2 receptor, often counteracting the AT_1 receptor-mediated stimulatory actions, require measure

of reduction in activities, which is technically more difficult than measurement of increased activity from a very low level. This may have been the reason for which the AT_2 receptor has sometimes escaped the attention of investigators. Chronic application of AT_2 antagonists and AT_2 receptor gene deletion are beginning to reveal the role of AT_2 receptor in the maintenance of, or restoring normality not only in the cardiovascular system but also in other tissues e.g., the central nervous system. A possible role of AT_2 receptor in apoptosis of mesenchymal cells in conjunction with other factors is beginning to be clarified as an important basis of organogenesis, failure of which may lead to some childhood diseases.

IV. The AT_4 Receptor

In 1990, during the process of purifying and sequencing the AT_1 receptor type, it was noticed that heat-denatured purified receptor from the bovine adrenal gland lost binding with ^{125}I-Sar^1,Ile^{8}Ang II, whereas ^{125}I-Ang III binding persisted. At first it was suspected that an angiotensin receptor specific to Ang III was isolated. However, with sufficient peptidase inhibitors added to prevent the conversion of Ang III to shorter fragments, this binding activity was lost. This was puzzling given that the two known receptor types at that time, AT_1 and AT_2, each accepted Ang II and Ang III as ligands, albeit with different affinities. A fragment of Ang III was suspected to be acting at this new site because Sar^1,Ang II, Sar^1,Ile^{8}Ang II (Sarile), Sar^1,Ala^{8}Ang II (Saralasin), DuP753 (losartan), PD123177, CGP42112A, Ang II(1–7) and Ang III did not serve as ligands (Harding et al., 1992; Swanson et al., 1992). In fact, ^{125}I-Ang II(3–8)[Ang IV] did bind at this site reversibly, saturably, and with high affinity (K_d = 1 nM) (Harding et al., 1992; Swanson et al., 1992; Sardinia et al., 1993). Having identified a ligand that bound at this site it was possible to determine its brain distribution using in vitro autoradiographic techniques. The brain distribution of this receptor site was unlike that of either the AT_1 or AT_2 receptors. The greatest concentrations of what has now been termed the AT_4 site (IUPHAR Nomenclature Committee, de Gasparo et al., 1995) were in structures classically associated with cognitive processes and sensory and motor functions, not in structures associated with the functions of body water balance, cardiovascular regulation, and control of reproductive hormones and behaviors where the AT_1 receptor is predominant. The following sections summarize what is presently known about this new Ang IV/AT_4 system with emphasis on signaling mechanisms, tissue distributions, the development of analogs that act as agonists and antagonists, and the physiologies and behaviors associated with this AT_4 site.

A. Signaling Mechanisms

Given that small peptides are able to activate the AT_4 receptor and that the vast majority of small peptide receptors are G protein-linked, it would seem logical to predict that the AT_4 receptor might be a serpentine, G protein-linked receptor as well. This, however, does not appear to be the case since the AT_4 receptor exhibits a molecular weight between 160 and 190 kDa as determined by reduced SDS-polyacrylamide gel electrophoresis. Of particular interest is the observation that the adrenal AT_4 receptor may be multimeric. This suggestion comes as a result of nonreducing gels that indicate a second specifically labeled band at 225 kDa. A similar molecular weight has been observed for other bovine tissues including heart, thymus, kidney, bladder, aorta, and hippocampus (Zhang et al., 1999). Bernier et al. (1995) have reported a similar molecular weight for the binding subunit of the AT_4 receptor in bovine aortic endothelial cells. Together these data all but preclude the linkage of AT_4 receptors to G proteins. The lack of linkage to G proteins is further supported by the observation that GTPγS fails to alter ^{125}I-Ang IV binding in rabbit heart (Hanesworth et al., 1993), guinea pig brain (Miller-Wing et al., 1993), and rat vascular smooth muscle (Hall et al., 1993). However, a single report by Dulin et al. (1995) indicates that GTPγS can inhibit binding in opossum kidney cells. As experienced with the AT_2 receptor, a definite conclusion has to wait the cloning of the AT_4 receptor.

The initial events that characterize AT_4 receptor intracellular signaling mechanisms are presently unknown. Nevertheless, downstream targets appear to include immediate early genes. Intracerebroventricular infusion of Ang IV in rats induces c-Fos expression in brain regions associated with cognition (Roberts et al., 1995). Angiotensin IV agonists can also stimulate c-Fos, c-Jun, and egr-1 in isolated, nonstimulated rabbit hearts (Slinker, personal communication). Studies carried out in the laboratory of Vaughan (Kerins et al., 1995) indicate that AT_4 activation increases the expression of plasminogen activator inhibitor, PAI-1, and is blocked by coapplication of an AT_4 receptor antagonist. However, a possible involvement of the AT_1 receptor has been postulated both in vitro and in vivo (Brown et al., 1999; Chabielska et al., 1999; Goodfield et al., 1999; Sironi et al., 1999).

A final area of investigation indirectly associated with AT_4 signaling is the identification of endogenous ligands for the AT_4 receptor. The first putative ligand identified for the AT_4 receptor was the hexapeptide, Ang IV. Although Ang IV is known to be present in the circulation (Semple et al., 1976) and is generated from Ang II or Ang III (Abhold and Harding, 1988), recent studies (Møeller et al., 1997) indicate that other peptides like LVV-hemorphin-7 are also capable of binding and activating AT_4 receptors. This is not surprising given that the structural requirements for AT_4 ligands are fairly minimal. Presumably, other putative ligands will be found in the future.

B. Tissue Distribution of the AT_4 Receptor

1. Brain. To date, brain distributions of the AT_4 binding site using in vitro autoradiography have been completed in rat (Roberts et al., 1995), guinea pig (Miller-Wing et al., 1993), *macaca fascicularis* (Møeller et al., 1996a), rhesus monkey (Wright et al., 1995), and human (hippocampus only; Harding, unpublished observations), and there is cross-species consistency. The predominant brain distribution of AT_4 receptor is presented in Table 6 by comparison with the AT_1 and AT_2 receptors. The highest densities of the AT_4 site are located in regions involved in cognitive processing, and motor and sensory functions. Specifically, the AT_4 receptor site is prominent in structures associated with the cholinergic system (Møeller et al., 1996a). This system is composed of two major pathways: one from the basal forebrain with cell bodies located in the nucleus basalis magnocellularis, which project primarily to the neocortex, whereas the other originates in the medial septum-diagonal band of Broca complex and projects primarily to the hippocampus (Wenk et al., 1980; Mesulam et al., 1983; Dutar et al., 1995). There are also additional projections to the amygdala and thalamus presumed to be involved in the integration of subcortical contributions to this system (Goldman and Cote, 1991), and the piriform cortex.

Equally impressive binding has been reported in structures associated with motor function including the ventral horn of the spinal cord (i.e., spinal motor nuclei) (Møeller et al., 1995, 1996a; Wright and Harding, 1995) inferior olivary nucleus, motor trigeminal nucleus, vestibular and reticular nuclei of the hindbrain, red nucleus, oculomotor nucleus, substantia nigra, and ventral tegmentum of the midbrain. In the forebrain, considerable binding is present in the globus pallidus, caudate-putamen, and nucleus accumbens (Miller-Wing et al., 1993; Møeller et al., 1995, 1996a). There are also high densities of AT_4 receptors in the granular cell layer of the cerebellum and deep cerebellar nuclei (Miller-Wing et al., 1993), as well as Betz cells of the primary motor neocortex (Møeller et al., 1996a). AT_4 receptors have also been identified in brain autonomic nuclei such as the dorsal motor nucleus of the vagus, nucleus ambiguus, rostral ventral lateral medulla, and paraventricular nucleus of the hypothalamus (Møeller et al., 1995, 1996a).

Finally, less dense distributions of AT_4 sites have been identified in sensory associated structures including spinal trigeminal nuclei, the colliculi, gracile and cuneate nuclei, and lateral geniculate nuclei, thalamic nuclei (anterior, lateral, and ventral), lateral olfactory tract, and primary sensory neocortex (Miller-Wing et al., 1993; Møeller et al., 1995, 1996a).

A comparison of the adult brain structures most densely distributed with AT_1, AT_2, and AT_4 receptors reveal some overlap (Table 7). Most notably in the dorsal motor nucleus of the vagus, inferior olivary nucleus, cerebellum, superior colliculus, lateral geniculate nucleus, and paraventricular nucleus (Höhle et al., 1995; Wright and Harding, 1995, 1997; Møeller et al., 1996a). However, there are structures rather uniquely distributed with AT_4 receptors. These include: reticular formation (motor areas), motor trigeminal and vestibular nuclei, cuneate and gracile nuclei, ventral tegmental area, periaqueductal gray, caudate-putamen, medial habenula, nucleus basalis of Meynert, hippocampus, piriform cortex, Betz cells in neocortex, and granular layer of the cerebellum.

2. Peripheral Tissue. Table 8 provides the binding constants for AT_4 receptors located in several peripheral tissues. Bovine adrenal cortex revealed a mean (± S.D.) K_d value of 0.7 (± 0.14) nM and a B_{max} value of 3.82 (± 1.12) pmol/mg of protein for ^{125}I-Ang IV (Harding et al., 1994). The binding of ^{125}I-Ang IV at this AT_4 site could not be inhibited with Ang II, Sar^1,Ile^{8}Ang II, Ang II(1–7), DuP753, CGP42112A, PD123177. The metabolically resistant form of Ang III, [D-Arg^1],Ang III had a significantly lower apparent binding affinity for this site.

Monkey kidney displayed a K_d value of 1.5 (± 0.31) nM and a B_{max} value of approximately 1.0 (± 0.21) pmol/mg of protein (Harding et al., 1994). Autoradiographic analyses of rat kidney indicated heavy concentrations of AT_4 receptors in the outer stripe of the medulla and a few receptors in glomeruli and the core of the medulla (Harding et al., 1994; Coleman et al., 1998). The reverse pattern was observed for ^{125}I-Sar^1,Ile^{8}Ang II binding to the AT_1 and AT_2 receptor sites.

Rat, guinea pig, and rabbit hearts revealed heavy concentrations of AT_4 receptors with K_d values of 3.3(± 1.1), 1.33(± 0.02), and 1.75(± 0.5) nM, respectively. Corresponding B_{max} values were: 0.32(± 0.03), 0.14(± 0.02), and 0.73(± 0.16) pmol/mg of protein (Wright et al., 1995). Autoradiographic analyses indicated heavy [^{125}I]Ang IV binding throughout the heart muscle. Guinea pig and bovine vascular smooth muscle evidenced considerable binding with K_d values of 0.40(± 0.09) and 1.85(± 0.45) nM, respectively. The B_{max} values were: 1.04(± 0.24) and 0.96(± 0.1) pmol/mg of protein, respectively. Binding constants for other tissues of interest, such as guinea pig colon and spleen, human bladder and prostate, are also included in Table 8.

C. Development of Agonists and Antagonists

1. Binding Requirements of AT_4 Receptor. The characteristics of the Ang IV molecule that result in high-affinity binding at the AT_4 type are much different than those described above for the AT_1 receptor. Specifically, they include the following. 1) Removal of the N-terminal valine significantly reduces binding affinity as compared with Ang IV ($K_d \approx 2.6 \cdot 10^{-9}$ M) (reviewed in Wright et al., 1995). 2) Glycine substitution in positions 1, 2, or 3 of the Ang IV molecule or the use of D-isomers at these positions, greatly reduces affinity (Sardinia et al., 1993). 3) On the other hand, positions 4, 5, and 6 can accom-

TABLE 7
Predominant brain distributions of the three angiotensin receptor types identified in the mammalian brain[a]

	AT_1	AT_2	AT_4
Structures:	Amygdala		Amygdala
	Anterior pituitary gland		Anterior pituitary gland
	Area postrema	Amygdala	Caudate-putamen
	Dorsal motor nucleus of the vagus	Caudate putamen	Cerebellum
	Habenula	Globus pallidus	Cerebral neocortex
	Inferior olivary nucleus	Hypoglossal nucleus	Dorsal motor nucleus of the vagus
	Interpeduncular nucleus	Inferior colliculus	Globus pallidus
	Lateral geniculate	Inferior olivary nucleus	Habenula
	Lateral olfactory tract	Lateral habenula	Hippocampus
	Locus ceruleus	Locus ceruleus	Inferior colliculus
	Medial preoptic area	Medial geniculate pons	Inferior olivary nucleus
	Median eminence	Reticular formation	Lateral geniculate
	Nucleus of the solitary tract	Septum	Lateral olfactory tract
	Olfactory bulbs organum	Thalamus	Locus ceruleus
	Vasculosum of the lateral terminalis	Ventral tegmental area	Nucleus basalis of magnocellularis
	Paraventricular nucleus		Nucleus accumbens
	Septum		Nucleus cuneatus
	Subforical organ		Nucleus gracilus
	Superior colliculus		Paraventricular nucleus of hypothalamus
	Supraoptic nucleus		Periaqueductal gray piriform cortex
	Ventromedial hypothalamic nucleus		Septum
	Red nucleus		Spinal cord-ventral horn
			Substantia nigra
			Superior colliculus
			Thalamus
			Ventral tegmental area

[a] Adapted from Wright and Harding (1997).

TABLE 8
Binding constants for AT_4 receptors in various species and tissues

Species	Tissue	K_d	B_{max}
		nM	*fmol/mg protein*
Rabbit	Heart	1.75 ± a5[a]	731 ± 163
Rat	Heart	3.3 ± 1.1	320 ± 66
Bovine	Adrenals	0.74 ± 0.14	3820 ± 1120
Guinea pig	Heart	1.33 ± 0.02	144 ± 19
Guinea pig	Hippocampus	1.29 ± 0.18	499 ± 62
Bovine	Vascular smooth muscle	1.85 ± 0.45	960 ± 100
Bovine	Vascular smooth muscle	0.70 ± 1.0	476 ± 57
Guinea pig	Vascular smooth muscle	0.40 ± 0.09	1040 ± 239
Monkey	Kidney	1.5 ± 0.31	1000 ± 212
Guinea pig	Colon	0.84 ± 0.08	659 ± 75
Human	Prostate	0.60 ± 0.15	1399 ± 152

[a] Mean ± S.D., n = 2–4. Study performed with ^{125}I-Ang IV.

modate several amino acid constituents without significantly impacting affinity. A detailed study of position 1 of Ang IV revealed the following specific characteristics. 1) High affinity binding necessitates the presence of a primary amine in position 1. Methylation, cyclization, or removal of the N-terminal amine yields a dramatic loss of affinity. 2) Hydrophobic residues in position 1 produce the greatest binding affinity. Thus, norleucine^1-Ang IV with a 4-carbon straight chain aliphatic group in position 1 results in a very high affinity ($K_d \approx$ 3,zmd.10^{-12} M). The addition of the amino group in position 1 significantly lowered affinity as compared with analogous straight chain analogs. 3) The amino acids substituted in position 1 must be in the L-configuration. For example, replacing L-norleucine with D-norleucine in position 1 significantly reduces affinity ($K_d \approx 6 \cdot 10^{-7}$ M). Reasonably high affinity can be achieved with a CH_2-NH isostere substituted for the 1–2 bond. These isosteres permit increased rotation around the peptide bond (Sardinia et al., 1994). They also introduce a formal positive charge with a bond length similar to the normal peptide bond accompanied by increased metabolic stability. Position 2 of the Ang IV molecule requires an aromatic amino acid in the L-configuration to achieve high-affinity binding. Position 3 requires a hydrophobic amino acid also in the L-configuration. Thus, the requirements for significant occupancy and activation of the AT_4 receptor type include a cluster group of Val-Tyr-Ile-R_1-R_2-R_3. Removal of phenylalanine from the C terminus of Ang IV yields a binding affinity, which is nearly equivalent with that of native angiotensin IV, but is physiologically inactive as measured by its influence on blood flow. With further successive removal of amino acids, binding affinity declines such that the K_d value for Ang IV(1–4) $\approx 5.7 \cdot 10^{-8}$ M, and for Ang IV(1–3) $\approx 4.8 \cdot 10^{-7}$ M (reviewed in Wright et al., 1995). In sum, the minimal ligand requirements for an Ang IV-like peptide to bind with reasonably high affinity at the AT_4 receptor type appears to be Val-Tyr-Ile-R_1-R_2, although an agonist appears to require the addition of phenylalanine at the C terminus yielding a required structure of Val-Tyr-Ile-R_1-R_2-Phe.

2. Antagonists of the AT_4 Receptor. Antagonists of the AT_4 receptor type have been reasonably difficult to design and synthesize. Harding's laboratory has had success with the substitution of Val for Ile in the third position of Ang IV accompanied by reduced peptide bonds between Val-Tyr and Tyr-Val (i.e., Val-Tyr-Val-His-Pro-Phe). This compound called divalinal-Ang IV (Krebs et al., 1996) binds at the AT_4 receptor type with high affinity to a similar number of binding sites as ^{125}I-Ang IV. It does not bind at the AT_1 and AT_2 receptor

types and has been shown to prevent i.c.v. Ang IV-induced c-Fos expression in rats (Roberts et al., 1995), Ang IV-induced elevation in cerebral (Kramár et al., 1997) and kidney cortical blood flow (Coleman et al., 1998), and Ang IV-induced inhibition of proximal tubule Na^+ transport (Handa et al., 1998).

D. Physiology Associated with the AT_4 Receptor

1. Regulation of Blood Flow. Haberl et al. (1991) have reported dilation of rabbit pial arterioles with topical application of Ang IV using the closed cranial window technique (Haberl, 1994). Ang IV-induced dilation was dependent upon pretreatment with exogenous L-arginine. Application of L-arginine alone resulted in only minimal vasodilation (+4% at 10^{-5} M), whereas coapplication of L-arginine and Ang IV produced a 20+% vasodilation. Pretreatment with methylene blue blocks vasodilation suggesting that it is dependent upon an endothelial-dependent factor, probably nitric oxide (Riedel et al., 1995). A degradation product of Ang II could therefore induce this endothelium-dependent dilation (Haberl et al., 1991).

Laser Doppler flowmetry was used to measure Ang IV-induced increase in blood flow within the cerebral cortex (Kramár et al., 1997) and kidney cortex (Swanson et al., 1992; Harding et al., 1994; Coleman et al., 1998). Angiotensin IV was intra-arterially administered via the internal carotid or renal arteries, respectively. Angiotensin II induced decreases in blood flow in these preparations. Pretreatment with DuP753 blocked subsequent Ang II-induced reductions in cerebral or renal cortical blood flow, whereas both PD123177 and divalinal-Ang IV failed to inhibit these responses. In contrast, pretreatment with the antagonist divalinal-Ang IV blocked the Ang IV-induced elevation in cerebral or renal cortical blood flow, whereas pretreatment with DuP753 or PD123177 failed to influence this response. In a related study, Kramár et al. (1998) have noted that pretreatment with the NO synthase inhibitor, N^{ω}-nitro-L-arginine (L-NAME) blocked the vasodilatory effect of Ang IV suggesting that this Ang IV-induced elevation in cerebral blood flow is dependent upon the synthesis and release of NO from vascular endothelial cells. Similar results have been obtained by Coleman et al. (1998) concerning kidney cortical blood flow.

Finally, Näveri et al. (1994a,b) have noted that i.v. administration of Ang IV reversed the decreases in cerebral blood flow that accompanies experimentally induced subarachnoid hemorrhage. Pretreatment with the nonspecific AT_1 and AT_2 receptor antagonist, Sar^1-Ile^8 Ang II, failed to influence this Ang IV-induced effect suggesting that the elevation in blood flow was mediated by the AT_4 receptor type. Taken together these results argue in favor of important roles for angiotensins II, III, and IV in the control of blood flow. In contrast with the above findings, Gardiner et al. (1993) have noted Ang IV-induced reductions in renal and mesenteric blood flows and vascular conductances in conscious rats using implanted pulsed Doppler flow probes. Pretreatment with DuP753 blocked these responses suggesting mediation by the AT_1 receptor type. The authors concluded that Ang IV acts as a weak agonist at the AT_1 receptor type. In agreement with these findings, Fitzgerald et al. (1999) infused Ang IV via the left renal artery and noted dose-dependent reductions in renal artery blood flow via transit-time flow probes placed around the artery. Once again these responses could be blocked by pretreatment with DuP753. Infusion of LVV-hemorphin-7 failed to alter blood flow. Presently it is difficult to resolve these conflicting results. Those reports noting Ang IV-mediated vasoconstriction determined that it was mediated by the AT_1 receptor type. Those reports noting Ang IV-induced vasodilation attributed it to the AT_4 receptor type. These Ang IV responses have been further separated into direct and indirect effects. Specifically, pretreatment with L-NAME prevented subsequent Ang IV-induced elevations in cerebral (Kramár et al., 1998) and renal cortical blood flow (Coleman et al., 1998). Nossaman et al. (1995) have shown that L-NAME and the cyclooxygenase inhibitor, meclofenamate, shift the Ang IV-induced vasoconstrictor dose-response curve in the pulmonary circulation. These researchers concluded that Ang IV could be promoting the release of vasodilators such as prostaglandins and nitric oxide. Consistent with these results, Yoshida et al. (1996) found the vasoconstriction induced by Ang IV to be initially facilitated by L-NAME, and by L-NAME and indomethacin (another cyclooxygenase inhibitor). In contrast, Li et al. (1997) have reported that indomethacin failed to impact the Ang IV dose-response curve in isolated human saphenous veins. Recently, Patel et al. (1998) and Hill-Kapturczak et al. (1999) determined that blockade of AT_1 and AT_2 receptor types failed to influence Ang II-induced NO release, or the release of NO synthase (NOS). Ang IV stimulated significantly greater NO release and greater endothelial-type constitutive NOS activity than the equivalent dose of Ang II. Divalinal-Ang IV blocked these Ang II and Ang IV-mediated NO effects and NOS activation. The authors concluded that the Ang IV/AT_4 system is primarily responsible for the Ang II facilitation of NO release and endothelium-dependent vasorelaxation. This endothelial cell NOS activation appears to be regulated by intracellular Ca^{2+} release and increased expression of calreticulin (Patel et al., 1999). Resolution of these issues awaits further investigation.

2. Cardiac Hypertrophy. Autoradiographic analyses utilizing ^{125}I-Ang IV reveals considerable binding in guinea pig and rabbit heart membranes that is distinct from AT_1 or AT_2 receptor types (Hanesworth et al., 1993). Angiotensin IV binding has also been shown using cultured vascular smooth muscle cells (Hall et al., 1993), coronary microvascular endothelial cells (Jarvis et al., 1992; Hall et al., 1995), and cultured rabbit cardiac fibroblasts (Wang et al., 1995b). Baker et al. (1990,

1992) have reported that Ang IV blocks increases in RNA and protein synthesis initiated by Ang II in chick cardiocytes. Rats, predisposed to left ventricular hypertrophy via abdominal aortic coarctation, revealed significant accumulations of collagen that is facilitated with the AT_4 receptor antagonist, divalinal-Ang IV, and significantly reduced when treated with the AT_4 receptor agonist norleucine[1]-Ang IV. These results point to a potentially important role for the Ang IV/AT_4 system in collagen accumulation in hypertrophied hearts.

3. Renal Tubular Reabsorption. Autoradiograms utilizing ^{125}I-Ang IV demonstrate heavy distributions of AT_4 sites in the outer stripe of the outer medulla with diffuse labeling of the cortex (Coleman et al., 1998). This binding of Ang IV was not influenced by the specific AT_1 receptor antagonist losartan, or the AT_2 receptor antagonist PD123177. However, the AT_4 receptor antagonist, divalinal-Ang IV, or Ang IV, completely displaced this binding. Emulsion autoradiography determined that these AT_4 receptors are localized on cell bodies and apical membranes of convoluted and straight proximal tubules (Handa et al., 1998). Activation of these receptors by Ang IV produced a concentration-dependent decline in sodium transport as measured by rate of tissue oxygen consumption (Handa et al., 1998). Thus, these results provide strong evidence that Ang IV acts on tubular epithelium to inhibit sodium reabsorption. This natriuretic effect of Ang IV has been confirmed using in vivo infusion via the renal artery in anesthetized rats. Urine sodium concentration was found to significantly increase, whereas urine volume and glomerular filtration rate were not affected. This Ang IV-induced natriuresis was unaffected by pretreatment with DuP753 but was blocked with divalinal-Ang IV. These results support an important role for Ang IV in the control of sodium transport by the kidney (Ardaillou and Chansel, 1996).

4. Electrophysiological Analysis. Albrecht and colleagues (1997a) have investigated the ability of microiontophoretic administration of Ang IV to influence neurons in the hippocampus and in the dorsal lateral geniculate nucleus (Albrecht et al., 1997b). Of 43 hippocampal neurons tested, Ang IV produced a greater than 40% increase in firing frequency in 16 of these cells (37%) with a decrease in three neurons. Of the 72 hippocampal neurons tested with Ang II, 21 evidenced a greater than 40% elevation in firing rate (29%), with a decrease in eight cells. With both Ang II and Ang IV the excitation effects were seen primarily in neurons that evidenced slower spontaneous discharge rates, whereas decreases in firing rate were usually seen in cells with higher spontaneous discharge rates. These changes in firing rates induced by Ang II could be blocked with the specific AT_1 receptor antagonist DuP753 (eight of ten neurons examined), however the specific AT_4 receptor antagonist divalinal-Ang IV was ineffective. On the other hand divalinal-Ang IV was effective at blocking the Ang IV-induced increases in discharge rate in six of nine neurons tested, while DuP753 was ineffective. These results suggest that Ang II and Ang IV are acting at different receptors, i.e., AT_1 and AT_4, respectively. Along these lines, the authors saw no evidence of cross-desensitization between Ang II and Ang IV. The authors suggest that the presumed colocalization of AT_1 and AT_4 receptor types on the same hippocampal neurons supports the notion that both Ang II and Ang IV are capable of influencing hippocampal neurons.

Similar findings were obtained with angiotensin-sensitive geniculate neurons (Albrecht et al.,1997b). These investigators further reported that Ang II application produced a potent inhibition of *N*-methyl-D-aspartate- and kainate-induced facilitation of firing rates in some neurons, whereas increased discharge frequencies were observed in others. Ang IV was also found to block such glutamate receptor excitation in three neurons, whereas this excitation was facilitated in two neurons. Finally, these investigators found the iontophoretic application of Ang II onto these geniculate neurons suppressed light-evoked excitation (nine neurons) in some neurons, although other units also revealed facilitation (eight neurons). A similar evaluation of Ang IV indicated a facilitation of this light-evoked excitation in ten neurons and a suppression in seven neurons. These angiotensin effects could be blocked by the appropriate AT_1 or AT_4 receptor antagonists. Furthermore, the suppression effect of Ang II and Ang IV on light-evoked activity could itself be blocked by γ-aminobutyric acid receptor antagonists. Albrecht et al. (1976) suggested that Ang II and Ang IV appear to act as neuromodulators in the dorsal lateral geniculate nucleus. Determination of the precise role of each ligand will require further investigation.

5. Role of Ang IV in Learning and Memory. Intracerebroventricular injection of Ang IV leads to c-Fos expression in the hippocampus and piriform cortex, whereas similar injection of Ang II failed to induce c-Fos-like immunoreactivity in these structures but did activate c-Fos expression in circumventricular organs (Roberts et al., 1995) as well as paraventricular nucleus, SON, and the medial preoptic nucleus (Zhu and Herbert, 1996, 1997). Pretreatment with losartan prevented this Ang II-induced c-Fos-like immunoreactivity, whereas pretreatment with divalinal-Ang IV blocked the Ang IV-induced c-Fos expression (Roberts et al., 1995). There were no crossover effects demonstrated by these antagonists. Along these lines, Braszko and colleagues (1988, 1991) established that i.c.v. injected Ang II and Ang IV (1 nmol) were equivalent at facilitating exploratory behavior in rats tested in an open field, and furthermore, improved recall of passive avoidance conditioning and acquisition of active avoidance conditioning. Intracerebroventricular treatment with divalinal-Ang IV (10 nmol), disrupted recall of this response (Wright et al., 1995). Metabolically resistant analogs of Ang IV injected i.c.v. can be used to facilitate acquisition of successful

search patterns in a circular water maze task in scopolamine-treated rats (Pederson et al., 1998). Taken together, these results suggest an important role for the Ang IV/AT_4 system in learning and memory processes. Recently Møeller et al. (1997) have isolated the globin fragment LVV-hemorphin-7, a decapeptide (Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Phe) that binds at the brain AT_4 receptor type, which could be the endogenous ligand that acts at this receptor.

Finally, Delorenzi and colleagues (1997) have reported Ang IV facilitation of long-term memory of an escape response in the crab *Chasmagnathus*. Angiotensin II was also shown to facilitate long-term memory in this species, although not as robustly; however, this Ang II effect could not be blocked by losartan or PD123177. These results suggest that the Ang IV/AT_4 system may have been involved in invertebrate memory processes long before the emergence of mammals.

E. Summary

Recently a new angiotensin receptor type, AT_4, has been discovered and characterized that preferentially binds Ang II(3–8), a fragment of angiotensin II, now referred to as Ang IV. This receptor site is prominent among brain structures concerned with cognitive processing, motor and sensory functions. Specifically, high densities of AT_4 sites have been localized in neocortex, piriform cortex, hippocampus, amygdala, and nucleus basalis of Meynert. Major motor structures with high levels of AT_4 receptors include basal ganglia, red nucleus, substantia nigra, ventral tegmentum, vestibular and reticular nuclei of the hindbrain, motor trigeminal nucleus, Betz cells of primary motor neocortex, cerebellum, and ventral horn of the spinal cord. Significant sensory structures include thalamus, the colliculi, gracile and cuneate nuclei, lateral geniculate, lateral olfactory tract, and primary sensory neocortex. Peripheral tissues that reveal heavy distributions of AT_4 sites are kidney, bladder, heart, spleen, prostate, adrenals, and colon.

The primary functions thus far associated with this Ang IV/AT_4 system include memory acquisition and recall, the regulation of blood flow, inhibition of renal tubular sodium reabsorption, and cardiac hypertrophy. There are preliminary indications that this system may also be involved in neurite outgrowth (Møeller et al., 1996b), angiogenesis, and stimulation of endothelial cell expression of PAI-1 (Kerins et al., 1995). The identification of additional functions awaits further elucidation of this new receptor system. Cloning of the AT_4 receptor will certainly substantiate its potential role in pathophysiology of the renin-angiotensin system.

V. General Conclusions

Most of the known effects of Ang II are mediated through the AT_1 receptor, e.g., vasoconstriction, aldosterone and vasopressin release, salt and water retention, and sympathetic activation without neglecting the important autocrine and paracrine effects of Ang II on cell proliferation and migration and on extracellular matrix formation. The function of the AT_2 receptor has become unraveled over the last few years owing to various sophisticated approaches including gene transfection and deletion. Accumulated published data suggests that the AT_2 receptor counterbalances the effect of the AT_1 receptor in vitro as well as in vivo. There is an inactivation of MAPK, antiproliferation, promotion of apoptosis, differentiation and regeneration, opening of delayed-rectifier K^+ channels and closing of T-type Ca^{2+} channels. The re-expression of the AT_2 receptor in various diseases suggests a role of this receptor in pathophysiology.

The AT_4 receptor appears to be involved in memory acquisition and recall. Like the AT_2 receptor, it may also oppose the effect of the AT_1 receptor as it regulates renal blood flow, inhibits tubular sodium reabsorption and affects cardiac hypertrophy.

Cloning of the described angiotensin receptors and the ability to express these clones in mammalian cells will allow exhaustive structure/function studies. Further pharmacological and molecular studies will allow for a better and more complete understanding of the role of the renin-angiotensin system in pathology.

REFERENCES

Abe J-I and Berk BC (1998) Reactive oxygen species as mediators of signal transduction in cardiovascular disease. *Trends Cardiovasc Med* **8:**59–64.

Abhold RH and Harding JW (1988) Metabolism of angiotensins II and III by membrane-bound peptidases from rat brain. *J Pharmacol Exp Ther* **245:**171–177.

Adams B, Obertone TS, Wang X and Murphy TJ (1999) Relationship between internalization and mRNA decay in down-regulation of recombinant type 1 angiotensin II receptor (AT_1) expression in smooth muscle cells. *Mol Pharmacol* **55:** 1028–1036.

Aguilera G, Capponi A, Baukal A, Fujita K, Hauger R and Catt KJ (1979) Metabolism and biological actions of angiotensin II and des-Asp1-angiotensin II in isolated adrenal glomerulosa cells. *Endocrinology* **104:**1279–1285.

Aguilera G and Catt KJ (1978) Regulation of aldosterone secretion by the renin-angiotensin system during sodium restriction in rats. *Proc Natl Acad Sci USA* **75:**4057–4061.

Aguilera G, Hauger RL and Catt KJ (1978) Control of a aldosterone secretion during sodium restriction: adrenal receptor regulation and increased adrenal sensitivity to angiotensin II. *Proc Natl Acad Sci USA* **75:**975–979.

Aguilera G, Hyde CL and Catt KJ (1982) Angiotensin II receptors and prolactin release in pituitary lactotrophs. *Endocrinology* **111:**1045–1050.

Aguilera G, Young WS, Kiss A and Bathia A (1995) Direct regulation of hypothalamic corticotropin-releasing-hormone neurons by angiotensin II. *Neuroendocrinology* **61:**437–444.

Ahmed A, Li XF, Shams M, Gregory J, Rollason T, Barnes NM and Newton JR (1995) Localization of the angiotensin II and its receptor subtype expression in human endometrium and identification of a novel high-affinity angiotensin II binding site. *J Clin Invest* **96:**848–857.

Aiyar N, Baker E, Pullen M, Nuthulaganti P, Bergsma DJ, Kumar C and Nambi P (1994a) Characterization of a functional angiotensin II receptor in Xenopus laevis heart. *Pharmacology* **48:**242–249.

Aiyar N, Baker E, Wu HL, Nambi P, Edwards RM, Trill JJ, Ellis C and Bergsma DJ (1994b) Human AT_1 receptor is a single copy gene: Characterization in a stable cell line. *Mol Cell Biochem* **131:**75–86.

Albrecht D, Broser M and Kruger H (1997a) Excitatory action of angiotensins II and IV on hippocampal neuronal activity in urethane anesthetized rats. *Regul Pept* **70:**105–109.

Albrecht D, Broser MK, Kruger H and Bader M (1997b) Effects of angiotensin II and IV on geniculate activity in nontransgenic and transgenic rats. *Eur J Pharmacol* **332:**53–63.

Alessio ML, Leger CL, Rasolonjanahary R, Wandscheer DE, Clauser H, Enjalbert A and Kardon C (1994) Selective effect of a diet-induced decrease in the arachidonic acid membrane-phospholipid content on in vitro phospholipase C and adenylate cyclase-mediated pituitary response to angiotensin II. *Neuroendocrinology* **60:** 400–409.

Ali MS, Sayeski PP, Dirksen LB, Hayzer DJ, Marrero MB and Bernstein KE (1997a) Dependence on the motif YIPP for the physical association of Jak2 kinase with the intracellular carboxyl tail of the angiotensin II AT_1 receptor. *J Biol Chem* **272:** 23382–23388.

Ali MS, Schieffer B, Delafontaine P, Bernstein KE, Ling BN and Marrero MB (1997b) Angiotensin II stimulates tyrosine phosphorylation and activation of insulin receptor substrate 1 and protein-tyrosine phosphatase 1D in vascular smooth muscle cells. *J Biol Chem* **272:**12373–12379.

Allen AM, MacGregor DP, McKinley MJ and Mendelsohn FAO (1999a) Angiotensin II receptors in the human brain. *Regul Pept* **79:**1–7.

Allen AM, Moeller I, Jenkins TA, Zhuo J, Aldred GP, Chai S-Y and Mendelsohn FAO (1998) Angiotensin receptors in the nervous system. *Brain Res Bull* **47:**17–28.

Allen AM, Oldfield BJ, Giles ME, Paxinos GG, McKinley MJ and Mendelsohn FAO (1999b) Localization of angiotensin receptors in the nervous system. *Handbook of Chemical Neuroanatomy*, in press.

Amant C, Hamon M, Bauters C, Richard F, Helbecque N, McFadden EP, Escudero X, Lablanche JM, Amouyel P and Bertrand ME (1997) The angiotensin II type 1 receptor gene polymorphism is associated with coronary artery vasoconstriction. *J Am Coll Cardiol* **29:**486–490.

Ambroz C and Catt KJ (1992) Angiotensin II receptor-mediated calcium influx in bovine adrenal glomerulosa cells. *Endocrinology* **131:**408–414.

Ambroz C, Clark AJL and Catt KJ (1991) The mas oncogene enhances angiotensin-induced [Ca^{2+}] responses in cells with pre-existing angiotensin II receptors. *Biochim Biophys Acta* **1133:**107–111.

Ambuhl P, Felix D, Imboden H, Khosla H and Ferrario CM (1992a) Effects of angiotensin II and its selective antagonist on inferior olivary neurons. *Regul Pept* **41:**19–26.

Ambuhl P, Felix D, Imboden H, Khosla M and Ferrario CM (1992b) Effects of angiotensin analogues and angiotensin receptor antagonists on paraventricular neurons. *Regul Pept* **38:**111–120.

Anderson KM, Murahashi T, Dostal DE and Peach MJ (1993) Morphological and biochemical analysis of angiotensin II internalization in cultured rat aortic smooth muscle cells. *Am J Physiol* **264:**C179–C188.

Ardaillou R and Chansel D (1996) Angiotensin IV, a new component of the renin-angiotensin system, which acts on kidney cells. *Bull Acad Natl Med* **180:**475–486.

Armstrong DL, Garcia EA, Ma T, Quinones B and Wayner MJ (1996) Angiotensin II blockade of long-term potentiation at the perforant path-granule cell synapse in vitro. *Peptides* **17:**689–693.

Audinot V, Rasolonjanahary R, Bertrand P, Priam M, Kordon C and Enjalbert A (1991) Involvement of protein kinase-C in the effect of angiotensin-II on adenosine 3′,5′-monophosphate production in lactotroph cells. *Endocrinology* **129:**2231–2239.

Aumelas A, Sakarellos C, Lintner K, Fermandjian S, Khosla MC, Smeby RR and Bumpus FM (1985) Studies on angiotensin II and analogs: Impact of substitution in position 8 on conformation and activity. *Proc Natl Acad Sci USA* **82:**1881–1885.

Baker KM and Aceto JF (1990) Angiotensin II stimulation of protein synthesis and cell growth in chick heart cells. *Am J Physiol* **259:**H610–H618.

Baker KM, Booz GW and Dostal DE (1992) Cardiac actions of angiotensin II: Role of an intracardiac renin-angiotensin system. *Annu Rev Physiol* **54:**227–241.

Baldwin JM, Schertler GFX and Unger VM (1997) An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. *J Mol Biol* **272:**144–164.

Barak LS, Tiberi M, Freedman NJ, Kwatara MM, Lefkowitz RJ and Caron MG (1994) A highly conserved residue in G protein-coupled receptors is required for agonist-mediated beta 2 adrenergic receptor sequestration. *J Biol Chem* **269:**2790–2795.

Barber MN, Sampey DB and Widdop RE (1999) AT_2 receptor stimulation enhances antihypertensive effects of AT_1 receptor antagonist in hypertensive rats. *Hypertension* **34:**1117–1122.

Baukal AJ, Hunyady L, Catt KJ and Balla T (1994) Evidence for participation of calcineurin in potentiation of agonist-stimulated cyclic AMP formation by the calcium-mobilizing hormone, angiotensin II. *J Biol Chem* **269:**24546–24549.

Becu-Villalobos D, Lacau-Mengido IM, Thyssen SM, Diaz-Torga GS and Libertun C (1994) Effects of LHRH and ANG II on prolactin stimulation are mediated by hypophysial AT_1 receptor subtype. *Am J Physiol* **266:**E274–E278.

Bedecs K, Elbaz N, Sutren M, Masson M, Susini C, Strosberg AD and Nahmias C (1997) Angiotensin II type 2 receptors mediate inhibition of mitogen-activated protein kinase cascade and functional activation of SHP-1 tyrosine phosphatase. *Biochem J* **325:**449–454.

Begeot M, Langlois D, Vilgrain I and Saez JM (1987) Angiotensin II (A-II) steroidogenic refractoriness in Y-1 cells in the presence of A-II receptors negatively coupled to adenylate cyclase. *Endocr Res* **13:**301–316.

Berecek KH, Olpe HR, Jones RSG and Hofbauer KG (1984) Microinjection of vasopressin into the locus coeruleus of conscious rats. *Am J Physiol* **247:**H675–H681.

Bergsma DJ, Ellis C, Kumar C, Nuthulaganti P, Kerstein H, Elshourbagy N, Griffin E, Stadel JM and Aiyar N (1992) Cloning and characterization of a human angiotensin II type 1 receptor. *Biochem Biophys Res Commun* **183:**989–995.

Berk BC and Corson MA (1997) Angiotensin II signal transduction in vascular smooth muscle: Role of tyrosine kinases. *Circ Res* **80:**607–616.

Berk BC, Vekshtein V, Gordon HM and Tsuda T (1989) Antiotensin II-stimulated protein synthesis in cultured vascular smooth muscle cells. *Hypertension* **13:**305–314.

Bernier SG, Servant G, Boudreau M, Fournier A and Guillemette G (1995) Characterization of binding site for antiotensin IV on bovine aortic endothelial cells. *Eur J Pharmacol* **291:**191–200.

Bernstein KE and Marrero MB (1996) The importance of tyrosine phosphorylation in angiotensin II signaling. *Trends Cardiovasc Med* **6:**179–187.

Berridge MJ, Bootman MD and Lipp P (1998) Calcium – a life and death signal. *Nature (Lond)* **395:**645–648.

Bhat GJ, Abraham ST and Baker KM (1996) Angiotensin II interferes with interleukin 6-induced Stat3 signaling by a pathway involving mitogen-activated protein kinase kinase 1. *J Biol Chem* **271:**22447–22452.

Bhat GJ and Baker KM (1997) Angiotensin II stimulates rapid serine phosphorylation of transcription factor Stat3. *Mol Cell Biochem* **170:**171–176.

Bhat GJ, Thekkumkara TJ, Thomas WG, Conrad KM and Baker KM (1994) Angiotensin II stimulates sis-including factor-like DNA binding activity. Evidence that the AT_{1A} receptor activates transcription factor-Stat91 and/or a related protein. *J Biol Chem* **269:**31443–31449.

Bhat GJ, Thekkumkara TJ, Thomas WG, Conrad KM and Baker KM (1995) Activation of the STAT pathway by angiotensin II in T3CHO/AT_{1A} cells. Cross-talk between angiotensin II and interleukin-6 nuclear signaling. *J Biol Chem* **270:**19059–19065.

Bickerton RK and Buckley JP (1961) Evidence for a central mechanism of agonist-induced hypertension. *Proc Soc Exp Biol Med* **106:**834–837.

Bihoreau C, Monnot C, Davies E, Teursch B, Bernstein KE, Corvol P and Clauser E (1993) Mutation of Asp74 of the rat angiotensin II receptor confers changes in antagonist affinities and abolishes G-protein coupling. *Proc Natl Acad Sci USA* **90:**5133–5137.

Birabeau MA, Capponi AM and Vallotton MB (1984) Solubilized adrenal angiotensin II receptors: Studies on the site of action of sodium and calcium ions, and on the role of disulfide bridges. *Mol Cell Endocrinol* **37:**181–189.

Bleuel A, de Gasparo M, Whitebread S, Puttner I and Monard D (1995) Regulation of protease nexin-1 expression in cultured Schwann cells is mediated by angiotensin II receptors. *J Neurosci* **15:**750–761.

Blume A, Herdegen T and Unger T (1999) Angiotensin peptides and inducible transcription factors. *J Mol Med* **77:**339–357.

Bonnardeaux A, Davies E, Jeunemaitre X, Fery I, Charru A, Clauser E, Tiret L, Cambien F, Corvol P and Soubrier F (1994) Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension* **24:**63–69.

Booz GW, Conrad KM, Hess AL, Singer HA and Baker KM (1992) Angiotensin-II-binding sites on hepatocyte nuclei. *Endocrinology* **130:**3641–3649.

Boscoboinik D, Ozer NK, Moser U and Azzi A (1995) Tocopherols and 6-hydroxychroman-2-carbonitrile derivatives inhibit vascular smooth muscle cell proliferation by a nonantioxidant mechanism. *Arch Biochem Biophys* **318:**241–246.

Bosse R, Gerold M, Fischli W, Holck M and Escher E (1990) An angiotensin with prolonged action and blood pressure-lowering properties. *J Cardiovasc Pharmacol* **16 (Suppl 4):**50–55.

Bottari SP, King IN, Reichlin S, Dahlstroem I, Lydon N and de Gasparo M (1992) The angiotensin AT_2 receptor stimulates protein tyrosine phosphatase activity and mediates inhibition of particulate guanylate cyclase. *Biochem Biophys Res Commun* **183:**206–211.

Bottari SP, Taylor V, King IN, Bogdal Y, Whitebread S and de Gasparo M (1991) Angiotensin II AT_2 receptors do not interact with guanine nucleotide binding proteins. *Eur J Pharmacol* **207:**157–163.

Boulay G, Chretien L, Richard DE and Guillemette G (1994) Short-term desensitization of the angiotensin II receptor of bovine adrenal glomerulosa cells corresponds to a shift from a high to a low affinity state. *Endocrinology* **135:**2130–2136.

Bouscarel B, Blackmore PF and Exton JH (1988) Characterization of the angiotensin II receptor in primary cultures of rat hepatocytes. Evidence that a single population is coupled to two different responses. *J Biol Chem* **263:**14913–14919.

Braszko JJ, Kupryszewski G, Witczuk B and Wisniewski K (1988) Angiotensin II (3–8)-hexapeptide affects motor activity, performance of passive avoidance and a conditioned avoidance response in rats. *Neuroscience* **27:**777–783.

Braszko JJ, Wlasienko J, Koziolkiewicz W, Janecka A and Wisniewski K (1991) The 3–7 fragment of angiotensin II is probably responsible for its psychoactive properties. *Brain Res* **542:**49–54.

Braun-Menendez E, Fasciolo JC, Leloir LF and Muñoz JM (1940) The substance causing renal hypertension. *J Physiol* **98:**283–298.

Brechler V, Jones PW, Levens NR, de Gasparo M and Bottari SP (1993) Agonistic and antagonistic properties of angiotensin analogs at the AT_2 receptor in PC12W cells. *Regul Pept* **44:**207–213.

Brilla CG, Reams GP, Maisch B and Weber KT (1993) Renin-angiotensin system and myocardial fibrosis in hypertension: Regulation of the myocardial collagen matrix. *Eur Heart J* **14 (Suppl J):**57–61.

Brilla CG, Rupp H, Funck R and Maisch B (1995a) The renin-angiotensin-aldosterone system and myocardial collagen matrix remodeling in congestive heart failure. *Eur Heart J* **16:**107–109.

Brilla CG, Zhou G, Rupp H, Maisch B and Weber KT (1995b) Role of angiotensin II and prostaglandin E_2 in regulating cardiac fibroblast collagen turnover. *Am J Cardiol* **76:**80–130.

Brink M, Erne P, de Gasparo M, Rogg H, Schmid A, Stulz P and Bullock G (1996) Localization of the angiotensin II receptor subtypes in the human atrium. *J Mol Cell Cardiol* **28:**1789–1799.

Brown NJ, Agirbasli M and Vaughan DE (1999) Comparative effect of angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor antagonism on plasma fibrinolytic balance in humans. *Hypertension* **34:**285–290.

Buisson B, Bottari SP, de Gasparo M, Gallet-Payet N and Payet MD (1992) The angiotensin AT_2 receptor modulates T-type calcium current in non-differentiated NG108 cells. *FEBS Lett* **309:**161–164.

Buisson B, Laflamme L, Bottari SP, de Gasparo M, Gallo-Payet N and Payet MD (1995) A G-protein is involved in the angiotensin AT_2 receptor inhibition of the T-type calcium current in non-differential NG108-15 cells. *J Biol Chem* **270:**1670–1674.

Bullock GR, Steyaert I, Carey R, Siragy H, Bilbe G, Praet M and de Gasparo M (1999) Distribution of angiotensin II type 1 and type 2 receptors in the human lung. *Eur Respir J* **Suppl 30:**229.

Bumpus FM (1997) Mechanisms and sites of action of newer angiotensin agonists and antagonists in terms of activity and receptor. *Fed Proc* **36:**2128–2132.

Bumpus FM, Catt KJ, Chiu AT, de Gasparo M, Goodfriend T, Husain A, Peach MJ, Taylor DG and Timmermans PbmwM (1991) Nomenclature for angiotensin receptors. *Hypertension* **17:**720–721.

Bumpus FM, Schwartz H and Page IH (1957) Synthesis and pharmacology of the octapeptide angiotonin. *Science (Wash DC)* **125:**886–887.

Burns KD, Inagami T and Harris RC (1993) Cloning of a rabbit kidney cortex AT_1 angiotensin II receptor that is present in proximal tubule epithelium. *Am J Physiol* **264:**F645–F654.

Burns L, Clark KL, Bradley J, Robertson MJ and Clarke AJ (1994) Molecular cloning of the canine angiotensin II receptor. An AT_1-like receptor with reduced affinity for DuP753. *FEBS Lett* **343:**146–150.
Burson JM, Aguilera G, Gross KW and Sigmund CD (1994) Differential expression of angiotensin receptor 1_A and 1_B in mouse. *Am J Physiol* **276:**E260–E267.
Burstein ES, Spalding TA and Brann MR (1997) Pharmacology of muscarinic receptor subtypes constitutively activated by G proteins. *Mol Pharmacol* **51:**312–319.
Busche S, Gallinat S, Bohle RM, Reinecke A, Seebeck J, Franke F, Fink L, Zhu M, Sumners C and Unger T (2000) Expression of angiotensin AT1- and AT2-receptors in adult rat cardiomyocytes after myocardial infarction: a single-cell RT-PCR study. *Am J Pathol,* in press.
Bussé R, Servant LM, Zhou LM, Bouley G, Guillemette G and Escher E (1993) Sar^1-p-benzoyl-phenfalanine^8-angiotensin, a new photo affinity probe for selective labeling of the type 2 angiotensin receptor. *Regul Pept* **44:**215–223.
Campanile CP, Crane JK, Peach MJ and Garrison JC (1982) The hepatic angiotensin II receptor. I. Characterization of the membrane-binding site and correlation with physiological response in hepatocytes. *J Biol Chem* **257:**4951–4958.
Cao Z, Dean R, Wu L, Casley D and Cooper ME (1999) Role of angiotensin receptor subtypes in mesenteric vascular proliferation and hypertrophy. *Hypertension* **34:** 404–414.
Capponi AM and Catt KJ (1979) Angiotensin II receptors in adrenal cortex and uterus. *J Biol Chem* **254:**5120–5127.
Capponi AM, Favrod-Coune CA, Gaillard RC and Muller AF (1982) Binding and activation properties of angiotensin II in dispersed rat anterior pituitary cells. *Endocrinology* **110:**1043–1045.
Carey RM, Wang Z-Q and Siragy HM (2000) Role of the angiotensin type 2 receptor in the regulation of blood pressure and renal function. *Hypertension* **35:**155–163.
Carpenter KA, Wilkes BC and Schiller PW (1998) The octapeptide angiotensin II adopts a well-defined structure in a phospholipid environment. *Eur J Biochem* **251:**448–453.
Carsia RV, McIlroy PJ, Kowalski KI and Tilly JL (1993) Isolation of turkey adrenocortical cell angiotensin II (AII) receptor partial cDNA: evidence for a single-copy gene expressed predominantly in the adrenal gland. *Biochem Biophys Res Commun* **31:**1073–1080.
Carson MC, Leach-Harper CM, Baukal AJ, Aguilera G and Catt KJ (1987) Physicochemical characterization of photoaffinity-labeled angiotensin II receptors. *Mol Endocrinol* **1:**147–153.
Castellano M, Muiesan ML, Beschi M, Rizzoni D, Cinelli A, Salvetti M, Pasini G, Porteri E, Bettoni G, Zulli R and Agabiti-Rosei E (1996) The angiotensin II type 1 receptor A/C 1166 polymorphism. Relationships with blood pressure and cardiovascular structure. *Hypertension* **28:**1076–1080.
Catt KJ, Mendelsohn FA, Millan MA and Aguilera G (1984) The role of angiotensin II receptors in vascular regulation. *J Cardiovasc Pharmacol* **6 (Suppl 4):**575–586.
Chabielska E, Pawlak R, Wollny T, Rolkowski R and Buczko W (1999) Antithrombotic activity of losartan in two kidney, one clip hypertensive rats. A study on the mechanism of action. *J Physiol Pharmacol* **50:**99–109.
Chaki S, Guo DF, Yamano Y, Ohyama K, Tani M, Mizukoshi M, Shirai H and Inagami T (1994) Role of carboxyl tail of the rat angiotensin II type 1A receptor in agonist-induced internalization of the receptor. *Kidney Int* **46:**1492–1495.
Chaki S and Inagami T (1992) Identification and characterization of a new binding site for angiotensin II in mouse neuroblastoma Neuro-2A cells. *Biochem Biophys Res Commun* **182:**388–394.
Chamoux E, Breault L, Lehoux J-G and Gallo-Payet N (1999) Involvement of the angiotensin II type 2 receptor in apoptosis during human fetal adrenal gland development. *J Clin Endocrinol Soc* **84:**4722–4730.
Chang RS, Lotti VJ and Keegan ME (1982) Inactivation of angiotensin II receptors in bovine adrenal cortex by dithiothreitol: Further evidence for the essential nature of disulfide bonds. *Biochem Pharmacol* **31:**1903–1906.
Chang RSL and Lotti VJ (1990) Two distinct angiotensin II receptor binding sites in adrenal revealed by new selective nonpeptide ligands. *Mol Pharmacol* **29:**347–351.
Chang RSL and Lotti VJ (1991) Angiotensin receptor subtypes in rat, rabbit and monkey tissues: Relative distribution and species dependency. *Life Sci* **49:**1485–1490.
Chansel D, Czekalski S, Pham P and Ardaillou R (1992) Characterization of angiotensin II receptor subtypes in human glomeruli and mesangial cells. *Am J Physiol* **262:**F432–F441.
Chappell MC, Millsted A, Diz DI, Brosnihan KB and Ferrario CM (1991) Evidence for an intrinsic angiotensin system in the canine pancreas. *J Hypertens* **9:**751–759.
Chappell MC, Bosch SM, Hansen RC, Ferrario CM and Diz DI (1994) Angiotensin II receptor subtype expression in the primate pancreas. *Am J Hypertens* **4:**92A.
Chappell MC, Diz DI and Jacobsen DW (1992) Pharmacological characterization of angiotensin II binding sites in the pancreas. *Peptides* **13:**313–318.
Chappell MD, Jacobsen DW and Tallant EA (1995) Characterization of angiotensin II receptor subtypes in pancreatic acinar AR42J cells. *Peptides* **16:**741–747.
Chen X, Li W, Yoshida H, Tsuchida S, Nishimura H, Takemoto F, Okubo S, Fogo A, Matsusaka T and Ichikawa I (1997) Targeting deletion of angiotensin type 1B receptor gene in the mouse. *Am J Physiol* **272:**F299–F304.
Chiu AT, Herblin WF, Ardecky RJ, McCall DE, Carini DJ, Duncia JV, Pease LJ, Wexler RR, Wong P, Johnson AL and Timmermans PBMWM (1989a) Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Commun* **165:**196–203.
Chiu AT, McCall DE, Nguyen TT, Carini DJ, Duncia JV, Wexler RR, Yoo SE, Johnson AL and Timmermans PBMWM (1989b) Discrimination of angiotensin II receptor subtypes by dithiothreitol. *Eur J Pharmacol* **170:**117–118.
Ciuffo GM, Heemskerk FMJ and Saavedra JM (1993a) Purification and characterization of angiotensin II AT_2 receptor from neonatal rat kidney. *Proc Natl Acad Sci USA* **90:**11009–11013.
Ciuffo GM, Viswanathan M, Seltzer AM, Tsutsumi K and Saavedra JM (1993b) Glomerular angiotensin II receptor subtypes during development of rat kidney. *Am J Physiol* **265:**F264–F271.
Clark AJL, Balla T, Jones MR and Catt KJ (1992) Stimulation of early gene expression angiotensin II in glomerulosa cells: Roles of calcium and protein kinase C. *Mol Endocrinol* **6:**1889–1898.
Clark K, Robertson MJ and Drew GM (1993) Role of angiotensin AT_1 and AT_2 receptors in mediating the renal effect of angiotensin II in the anesthetized dog. *Br J Pharmacol* **109:**148–156.
Clauser E, Curnow KM, Davies E, Conchon S, Teutsch B, Vianello B and Corvol P (1996) Angiotensin II receptors: protein and gene structures, expression and potential pathological involvements. *Eur J Endocrinol* **134:**403–411.
Coffman TM (1997) A genetic approach for studying the physiology of the type 1A (AT_{1A}) angiotensin receptor. *Semin Nephrol* **17:**404–411.
Coffman TM (1998) Gene targeting in physiological investigations: Studies on the renin-angiotensin system. *Am J Physiol* **274:**F999–F1005.
Coleman JKM, Krebs LT, Hamilton TA, Ong B, Lawrence KA, Sardinia MF, Harding JW and Wright JW (1998) Autoradiographic identification of kidney angiotensin IV binding sites and angiotensin IV-induced renal cortical blood flow changes in rats. *Peptides* **19:**269–277.
Colley PA, Sheu FS and Routtenberg A (1990) Inhibition of proteinkinase C blocks two components of LTP persistence, leaving initial potentiation intact. *J Neurosci* **10:**3353–3360.
Conchon S, Barrault M-B, Miserey S, Corvol P and Clauser E (1997) The C-terminal third intracellular loop of the rat AT_{1A} angiotensin receptor plays a key role in G protein coupling specificity and transduction of the mitogenic signal. *J Biol Chem* **272:**25566–25572.
Conchon S, Miserey S, Parnot C, Monnot C, Corvol P and Clauser E (1999) Several interesting phenotypes of the AT_1 receptor produced by site-directed mutagenesis. *J Am Soc Nephrol* **10 (Suppl 11):**8–14.
Conchon S, Peltier N, Corvol P and Clauser E (1998) A noninternalized nondesensitized truncated AT_{1A} receptor transduces an amplified ANG II signal. *Am J Physiol* **274:**E336–E345.
Cooper AC, Robinson G, Vinson GP, Cheung WT and Broughton Pipkin F (1999) The localization and expression of the renin-angiotensin system in the human placenta throughout pregnancy. *Placenta* **20:**467–474.
Correa FMA, de Oliveria AM, Viswanathan M and Saavedra JM (1994) Autoradiographic localization and characterization of angiotensin II receptor subtypes in rat thymus. *Peptides* **15:**821–824.
Coté F, Do TH, Laflamme L, Gallo J-M and Gallo-Payet N (1999) Activation of the AT_2 receptor of angiotensin II induces neurite outgrowth and cell migration in microexplant cultures of the cerebellum. *J Biol Chem* **274:**31686–31692.
Cox BE, Ipson MA, Shaul PW, Kamm KE and Rosenfeld CR (1993) Myometrial angiotensin II receptor subtypes change during ovine pregnancy. *J Clin Invest* **92:**2240–2248.
Crabos M, Roth M, Hahn AW and Erne P (1994) Characterization of angiotensin II receptors in cultured adult rat cardiac fibroblasts. Coupling to signaling systems and gene expression. *J Clin Invest* **93:**2372–2378.
Crane JK, Campanile CP and Garrison JC (1982) The hepatic angiotensin II receptor. II. Effect of guanine nucleotides and interaction with cyclic AMP production. *J Biol Chem* **257:**4959–4965.
Crawford KW, Frey EA and Cote TE (1992) Angiotensin II receptor recognized by DuP753 regulates two distinct guanine nucleotide-binding protein signaling pathways. *Mol Pharmacol* **41:**154–162.
Criscione L, Bradley WA, Buehlmayer P, Whitebread S, Glazer R, Lloyd P, Mueller P and de Gasparo M (1995) Valsartan: Preclinical and clinical profile of an antihypertensive angiotensin II antagonist. *Cardiovasc Drug Rev* **13:**230–250.
Criscione L, Thomann H and Whitebread S (1990) Binding characteristics and vascular effects of various angiotensin II antagonists. *J Cardiovasc Pharmacol* **16 (Suppl 4):**56–59.
Csikós T, Balmforth A, Grojec M, Gohlke P, Culman J and Unger T (1998) Angiotensin AT_2 degradation is prevented by ligand occupation. *Biochem Biophys Res Commun* **243:**142–147.
Cunningham JT, Sullivan MJ, Edwards GL, Farinpour R, Betz TG and Johnson AK (1999) Dissociation of experimentally induced drinking behavior by ibotenate injection into the median preoptic nucleus. *Brain Res* **554:**153–158.
Curnow KM, Pascoe L and White PC (1992) Genetic analysis of the human type-1 angiotensin II receptor. *Mol Endocrinol* **6:**1113–1118.
Curnow KM (1996) Human type-1 angiotensin II (AT_1) receptor gene structure and function. *Clin Exp Pharmacol Physiol* (**Suppl 3):**67–73.
Curnow KM, Pascoe L, Davies E, White PC, Corvol P and Clauser E (1995) Alternatively spliced human type 1 angiotensin II receptor mRNAs are translated at different efficiencies and encode two receptor isoforms. *Mol Endocrinol* **9:**1250–1262.
Damron DS, Nadim HS, Hong SJ, Darvish A and Murray PA (1998) Intracellular translocation of PKC isoforms in canine pulmonary artery smooth muscle cells by Ang II. *Am J Physiol* **274:**L278–L288.
Darimont C, Vassaux G, Alhand G and Negrel R (1994) Differentiation of preadipose cells: Paracrine role of prostacyclin upon stimulation of adipose cells by angiotensin II. *Endocrinology* **135:**2030–2036.
Davies E, Bonnardeaux A, Lathrop GM, Corvol P, Clauser E and Soubrier F (1994) Angiotensin II (type 1) receptor locus: CA repeat polymorphism and genetic mapping. *Hum Mol Genet* **3:**83–84.
Daviett L, Lehtonen JYA, Tamura K, Griese DP, Horiuchi M and Dzau VJ (1999) Cloning and characterization of ATRAP, a novel protein that interacts with the angiotensin II type 1 receptor. *J Biol Chem* **274:**17058–17062.
Davisson RL, Yang G, Beltz TG, Cassell MD, Johnson AK and Sigmund CD (1998) The brain renin-angiotensin system contributes to the hypertension in mice containing both the human renin and human angiotensinogen transgenes. *Circ Res* **83:**1047–1058.
de Gasparo M, Husain A, Alexander W, Cat KJ, Chiu AT, Drew M, Goodfriend T, Harding JW, Inagami T and Timmermans PBMWM (1995) Proposed update of angiotensin receptor nomenclature. *Hypertension* **25:**924–939.
de Gasparo M and Siragy HM (1999) The AT_2 receptor: Fact, fancy and fantasy. *Regul Pept* **81:**11–24.

de Gasparo M, Whitebread S, Kalenga MK, de Hertogh R, Crevoisier P and Thomas K (1994) Down regulation of the angiotensin II receptor subtype AT_2 in human myometrium during pregancy. *Regul Pept* **53:**39–45.
de Gasparo M, Whitebread S, Mele M, Motani AS, Whitecombe PJ, Ramjoue HP and Kamber B (1990) Biochemical characterization of two angiotensin II receptor subtypes in the rat. *J Cardiovasc Pharmacol* **16 (Suppl 4):**31–35.
De Lean A, Ong H, Gutkowska J, Schiller PW and McNicol N (1984) Evidence for agonist-induced interaction of angiotensin receptor with a guanine nucleotide-binding protein in bovine adrenal zona glomerulosa. *Mol Pharmacol* **26:**498–508.
Denef C (1986) Paracrine interactions in the anterior pituitary. *Clin Endocrinol Metab* **15:**1–32.
Della Bruna R, Ries S, Himmelstoss C and Kurtz A (1995) Expression of cardiac angiotensin II AT_1 receptor genes in rat hearts is regulated by steroids but not by angiotensin II. *J Hypertens* **13:**763–769.
Delorenzi A, Locatelli F, Romano A, Nahmod V and Maldonado H (1997) Angiotensin II (3–8) induces long-term memory improvement in the crab *Chasmagnathus*. *Neurosci Lett* **226:**143–146.
de Oliveria AM, Viswanathan M, Heemskerk FMJ, Correa FMA and Saavedra JM (1994) Specific non-angiotensin, [^{125}I]-CGP42112 binding sites in rat spleen macrophages. *Biochem Biophys Res Commun* **200:**1049–1058.
DeSilva PE, Husain A, Smeby RR and Kairallah PA (1988) Measurements of immunoreactive angiotensin peptides in rat tissues: Some pitfalls in angiotensin II analysis. *Anal Biochem* **174:**80–87.
Devynck MA and Meyer P (1978) Angiotensin receptors. *Biochem Pharmacol* **27:**1–5.
Diaz-Torga G, Gonzalez Iglesias A, Achaval-Zaia R, Libertun C and Becu-Villalobos D (1998) Angiotensin II-induced Ca^{2+} mobilization and prolactin release in normal and hyperplastic pituitary cells. *Am J Physiol* **274:**E534–E540.
Diaz-Torga GS, Becu-Villalobos D and Libertun C (1994) Ontogeny of angiotensin-II-induced prolactin release in vivo and in vitro in female and male rats. *Neuroendocrinology* **59:**57–62.
Dimmeler S, Rippmann V, Weiland U, Haendeler J and Zeiher AM (1997) Angiotensin II induces apoptosis of human endothelial cells: Protective effect of nitric oxide. *Circ Res* **81:**970–976.
Dohlman HG, Caron MG, DeBlasi A, Frielle T and Lefkowitz RJ (1990) Role of extracellular disulfide-binded cysteines in the ligand binding function of the beta 2-adrenergic receptor. *Biochemistry* **29:**2335–2342.
Donnelly D, Findlay JB and Blundell TL (1994) The evolution and structure of aminergic G protein coupled receptors. *Receptors Channels* **2:**61–78.
Dostal DE and Baker KM (1992a) Angiotensin II stimulation of left ventricular hypertrophy in adult rat heart. *Am J Hypertens* **5:**276–280.
Dostal DE, Booz GW and Baker KM (1996) Angiotensin II signalling pathways in cardiac fibroblasts: Conventional versus novel mechanisms in mediating cardiac growth and function. *Mol Cell Biochem* **157:**15–21.
Dostal DE, Rothblum KN, Chernin MI, Cooper GR and Baker KM (1992b) Intracardiac detection of angiotensinogen and renin: A localized renin-angiotensin system in neonatal rat heart. *Am J Physiol* **263:**C838–C850.
Douglas J and Catt KJ (1976) Regulation of angiotensin II receptors in the rat adrenal cortex by dietary electrolytes. *J Clin Invest* **58:**834–843.
Douglas JG (1987) Angiotensin receptor subtypes of the kidney cortex. *Am J Physiol* **253:**F1–F7.
Dudley DT, Panek RI and Major TC (1990) Subclasses of angiotensin II binding sites and their functional significance. *Mol Pharmacol* **38:**370–377.
Dudley DT, Hubbell SE and Summerfelt RM (1991) Characterization of angiotensin II (AT_2) binding sites in R3T3 cells. *Mol Pharmacol* **40:**360–367.
Dudley DT and Summerfeldt RM (1993) Regulated expression of angiotensin II (AT_2) binding sites in R3T3 cells. *Regul Pept* **44:**199–206.
Dulin NO, Ernsberger P, Suciu DJ and Douglas JC (1994) Rabbit renal epithelial angiotensin II receptors. *Am J Physiol* **267:**F776–F782.
Dulin N, Madhun ZT, Chang CH, Berti-Mattera L, Dickens D and Douglas JG (1995) Angiotensin IV receptors and signaling in opossum kidney cells. *Am J Physiol* **269:**F644–F652.
Dutar P, Bassant MH, Senut MC and Lamour Y (1995) The septohippocampal pathway: Structure and function of a central cholinergic system. *Physiol Rev* **75:**393–427.
Dzau VJ, Baxter JA, Cantin M, de Bold A, Ganten D, Gross K, Husain A, Inagami T, Menard J, Poole S, Robertson JI, Tang J and Yamamoto K (1987) Report of the Joint Nomenclature and Standardization Committee of the International Society of Hypertension, American Heart Association and the World Health Organization. *Hypertension* **5:**507–511.
Dzau VJ and Gibbons GH (1987) Autocrine and paracrine mechanisms of vascular myocytes in systemic hypertension. *Amer J Cardiol* **60:**991–1031.
Eguchi S, Matsumoto T, Motley ED, Utsunomiya H and Inagami T (1996) Identification of an essential signaling cascade for mitogen-activated protein kinase activation by angiotensin II in cultured rat vascular smooth muscle cells. *J Biol Chem* **271:**14169–14175.
Eguchi S, Numaguchi K, Iwasaki H, Matsumoto T, Yamakawa T, Utsunomiya H, Motley ED, Kawakatsu H, Owada KM, Hirata Y, Marumo F and Inagami T (1998) Calcium-dependent epidermal growth factor receptor transactivation mediates the angiotensin II-induced mitogen-activated protein kinase activation in vascular smooth muscle cells. *J Biol Chem* **273:**8890–8896.
Elliott DF and Peart WS (1956) Amino acid sequence in a hypertensin. *Nature (Lond)* **177:**527–528.
Engeli S, Gorzelniak K, Kreutz R, Runkel N, Distler A and Sharma AM (1999) Co-expression of renin-angiotensin system genes in human adipose tissue. *J Hypertens* **17:**555–560.
Ernsberger P, Zhuo J, Damon T and Douglas JG (1992) Angiotensin II receptor subtypes in cultured rat mesangial cells. *Am J Physiol* **263:**F411–F416.
Esther CR, Howard TE, Marino EM, Goddard JM, Capecchi MR and Bernstein KE (1996) Mice lacking angiotensin-converting enzyme have loww blood pressure, renal pathology, and reduced male fertility. *Lab Invest* **74:**953–965.
Esther CR, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi MR and Bernstein KE (1997) The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest* **15:**2375–2385.
Felix D and Akert K (1974) The effect of angiotensin II on neurons of the cat subfornical organ. *Brain Res* **76:**350–353.
Felix D and Harding JW (1986) Manipulation of aminopeptidase activities: differential effects of iontophoretically applied angiotensins in rat brain. *J Hypertens* **4:**S398–S401.
Felix D, Harding JW and Imboden H (1988) The hypothalamic-angiotensin system: location and functional considerations. *Clin Exp Hypertens [A]* **10 (Suppl 1):**45–62.
Felix D and Schlegel W (1978) Angiotensin receptive neurons in the subfornical organ-structure-activity relations. *Brain Res* **149:**107–116.
Feng Y-H, Miura S-I, Husain A and Karnik SS (1998) Mechanism of constitutive activation of the AT_1 receptor: Influence of the size of the agonist switch binding residue Asn111. *Biochemistry* **57:**15791–13798.
Feng Y-H, Noda K, Saad Y, Liu XP, Husain A and Karnik SS (1995) The docking of Arg2 of angiotensin II with Asp281 of AT_1 receptor is essential for full agonism. *J Biol Chem* **270:**12846–12850.
Ferguson AV and Bains JS (1997) Actions of angiotensin in the subfornical organ and area postrema: Implications for long term control of autonomic output. *Clin Exp Pharmacol* Physiol **24:**96–101.
Ferrario CM, Brosnihan KB, Diz DI, Jaiswal N, Khosla MC, Milsted A and Tallant EA (1991) Angiotensin-(1–7): A new hormone of the angiotensin system. *Hypertension* **18 (Suppl 3):**123–133.
Ferrario CM and Iyer SN (1998) Angiotensin-(1–7): A bioactive fragment of the renin-angiotensin system. *Regul Pept* **78:**13–18.
Feuillan PP, Millan MA and Aguilera G (1993) Angiotensin II binding sites in the rat fetus characterization of receptor subtypes and interaction with guanyl nucleotides. *Regul Pept* **44:**159–169.
Fierens FL, Vanderheyden PM, De Backer JP and Vauquelin G (1999) Insurmountable angiotensin AT_1 receptor antagonists: The role of tight antagonist binding. *Eur J Pharmacol* **372:**199–206.
Findlay JB, Donnelly D, Bhogal N, Hurrell C and Attwood TK (1993) Structure of G-protein-linked receptor. *Biochem Soc Trans* **21:**869–873.
Fischer J, Stoll M and Unger T (1996) Thrombospondin mRNA expression is increased in endothelial cells following stimulation of AT_2-receptors. *J Vasc Res* **33 (Suppl 1):**26.
Fisher-Ferraro C, Nahmod VE, Goldstein DJ and Finkielman S (1971) Angiotensin and renin in the dog brain. *J Exp Med* **133:**353–361.
Fitzgerald SM, Evans RG, Bergstrom G and Anderson WP (1999) Renal hemodynamic response to intrarenal infusion of ligands for the putative angiotensin IV receptor in anesthetized rats. *J Cardiovasc Pharmacol* **34:**206–211.
Florio T, Pan MG, Newman B, Hershberger RE, Civelli O and Stork PJC (1992) Dopaminergic inhibition of DNA synthesis in pituitary tumor cells is associated with phosphotyrosine phosphatase activity. *J Biol Chem* **267:**24169–24172.
Fortuno MA, Ravassa S, Etayo JC and Diez J (1998) Overexpression of Bax protein and enhanced apoptosis in the left ventricle of spontaneously hypertensive rats –effects of AT_1 blockade with losartan. *Hypertension* **32:**280–286.
Freis ED (1995) Historical development of antihypertensive treatment, in *Hypertension: Pathophysiology, Diagnosis, and Management* (Laragh JH and Brenner BM eds) pp 2741–2751, Raven Press Ltd, New York.
Freudenthaler SM, Schenck T, Lucht I and Gleiter CH (1999) Fenoterol stimulates human erythropoietin production via activation of the renin angiotensin system. *Br J Clin Pharmacol* **48:**631–634.
Friberg P, Sundelin B, Bohman S-O, Bobik A, Nilson H, Wickman A, Gustafsson H, Petersen J and Adams M (1994) Renin-angiotensin system in neonatal rats: Induction of a renal abnormality in response to ACE inhibition or angiotensin II antagonism. *Kidney Int* **45:**485–492.
Fu ML, Schulze W, Wallukat G, Elies R, Eftekhari P, Hjalmarson A and Hoebeke J (1998) Immunohistochemical localization of angiotensin II receptors (AT_1) in the heart with anti-peptide antibodies showing a positive chronotropic effect. *Recept Channels* **6:**99–111.
Furukawa Y, Kishimoto S and Nishikawa T (1982) Hypotensive imidazole derivatives. US Patent 4,340,598 issued to Takeda Chenical Industries, Ltd, Osaka, Japan.
Furuta H, Guo DF and Inagami T (1992) Molecular cloning and sequencing of the gene encoding human angiotensin II type 1 receptor. *Biochem Biophys Res Commun* **28:**8–13.
Gáborik Z, Mihalik B, Jayadev S, Jagadeesh G, Catt KJ and Hunyady L (1998) Requirement of membrane-proximal amino acids in the carboxyl-terminal tail for expression of the rat AT_{1A} angiotensin receptor. *FEBS Letters* **428:**147–151.
Gallinat S, Busche S, Schütze S, Krönke M and Unger T (1999) AT_2 receptor stimulation induces generation of ceramides in PC12W cells. *FEBS Lett* **443:**75–79.
Gallinat S, Csikós T, Meffert S, Herdegen T, Stoll M and Unger T (1997) The angiotensin AT_2 receptor down-regulates neurofilament M in PC12W cells. *Neurosci Lett* **227:**29–32.
Gallinat S, Yu M, Dorst A, Unger T and Herdegen T (1998) Sciatic nerve transection evokes lasting up-regulation of angiotensin AT_2 and AT_1 receptor mRNA in adult rat dorsal root ganglia and sciatic nerves. *Brain Res* **57:**111–122.
Ganong WF (1984) The brain renin-angiotensin system. *Annu Rev Physiol* **46:**17–31.
Ganong WF (1993) Blood, pituitary, and brain renin-angiotensin systems and regulation of secretion of anterior pituitary gland. *Front Neuroendocrinol* **14:**233–249.
Ganten D, Hutchinson S and Schelling P (1975) The intrinsic brain iso-renin angiotensin system: Its possible role in central mechanisms of blood pressure. *Clin Mol Med* **48:**2655–2685.
Ganten D and Speck G (1978) The brain renin-angiotensin system: A model for the synthesis of peptides in the brain. *Biochem Pharmacol* **27:**2379–2389.
Gardiner SM, Kemp PA, March JE and Bennett T (1993) Regional haemodynamic effects of angiotensin II (3–8) in conscious rats. *Br J Pharmacol* **110:**159–162.
Gasc JM, Shanmugam S, Sibony M and Corvol P (1994) Tissue-specific expression of

type 1 angiotensin II receptor subtypes. An in situ hybridization study. *Hypertension* **24:**531–537.
Gehlert DR, Gackenheimer SL and Schober DA (1991a) Angiotensin II receptor subtypes in rat brain: Dithiotherital inhibits ligand binding to AII-1 and enhances binding to AII-2. *Brain Res* **546:**161–165.
Gehlert DR, Gackenheimer SL and Schober DA (1991b) Autoradiographic localization of subtypes of angiotensin II antagonist binding in the rat brain. *Neuroscience* **44:**501–514.
Gehlert DR, Speth RC and Wamsley JK (1986) Quantitative autoradiography of angiotensin II receptors in SHR brain. *Peptides* **7:**1021–1027.
Geisterfer AA, Peach MJ and Owens GK (1988) Angiotensin II induces hypertrophy, not hyperplasia, of cultured rat aortic smooth muscle cells. *Circ Res* **62:**749–856.
Gendron L, Laflamme L, Rivard N, Asselin C, Payet MD and Gallo-Payet N (1999) Signals from the AT_2 (angiotensin type 2) receptor of angiotensin II inhibit $p21^{ras}$ and activate MAPK (mitogen-activated protein kinase) to induce morphological neuronal differentiation in NG108-15 cells. *Mol Endocrinol* **13:**1615–1626.
Gether U and Kobilka BK (1998) G protein-coupled receptors. II. Mechanism of agonist activation. *J Biol Chem* **273:**17979–17982.
Gibson RE, Thorpe HH, Cartwrite ME, Frank, JD, Shoru TW, Bunting PB and Siegl PKS (1991) Angiotensin II receptor subtypes in renal cortex of rats and rhesus monkeys. *Am J Physiol* **261:**F512–F518.
Gillis JC and Markham A (1997) Irbesartan, a review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in the management of hypertension. *Drugs* **54:**885–902.
Glossmann H, Baukal A and Catt KJ (1974a) Angiotensin II receptors in bovine adrenal cortex. Modification of angiotensin II binding by guanyl nucleotides. *J Biol Chem* **249:**664–666.
Glossmann H, Baukal A and Catt KJ (1974b) Cation dependence of high-affinity angiotensin II binding to adrenal cortex receptors. *Science (Wash DC)* **185:**281–283.
Glossmann H, Baukal A and Catt KJ (1974c) Properties of angiotensin II receptors in the bovine and rat adrenal cortex. *J Biol Chem* **249:**825–831.
Goa KL and Wagstaff AJ (1996) Losartan potassium. A review of its pharmacology, clinical efficacy and tolerability in the management of hypertension. *Drugs* **51:**820–845.
Gohlke P, Pees C and Unger T (1998) AT_2 receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism. *Hypertension* **81:**349–355.
Goldblatt H, Lynch J, Hanzal RF and Summerville WW (1934) Studies on experimental hypertension: I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. *J Exp Med* **59:**347–349.
Goldfarb DA, Diz DI, Tubbs RR, Ferrario CM and Novock AC (1994) Angiotensin-II receptor subtypes in the human renal cortex and renal cell carcinoma. *J Urol* **151:**208–213.
Goldman J and Cote L (1991) Aging of the brain: Dementia of the Alzheimer's type, in *Principles of Neural Science*, (Kandel ER, Schwartz JH and Jessell TM eds) pp 974–983, Elsevier Science Publishing, New York.
Goodfield NE, Newby DE, Ludlam CA and Flapan AD (1999) Effects of acute angiotensin II type 1 receptor antagonism and angiotensin converting enzyme inhibition of plasma fibrinolytic parameters in patients with heart failure. *Circulation* **99:**2983–2985.
Goodfriend TL, Elliott ME and Catt KJ (1996) Angiotensin receptors and their antagonists. *New Engl J Med* **344:**1649–1654.
Goodfriend TL and Lin SY (1970) Receptors for angiotensin I and II. *Circ Res* **27:**163–174.
Goto M, Mukoyama M, Suga S, Matsumoto T, Nakagawa M, Ishibashi R, Kasahara M, Sugawara I, Tanaka I and Nakao K (1997) Growth-dependent induction of angiotensin type 2 receptor in rat mesangial cells. *Hypertension* **30:**358–362.
Govantes C and Marin J (1996) Effect of angiotensin converting enzyme inhibitors on quality of life in hypertensive patients. Pharmacodynamic basis. *Fundam Clin Pharmacol* **10:**400–405.
Grady EF and Kalinyak JE (1993) Expression of AT_2 receptors in rat fetal subdermal cells. *Regul Pept* **44:**171–180.
Grady EF, Sechi LA, Griffin CA, Schambelan M and Kalinyak JE (1991) Expression of AT_2 receptors in the developing rat fetus. *J Clin Invest* **88:**921–933.
Griendling KK, Ushio-Fukai M, Lassegue B and Alexander RW (1997) Angiotensin II signaling in vascular smooth muscle. New concepts. *Hypertension* **29:**366–373.
Griffin SA, Brown WC, MacPherson F, McGrath JC, Wilson VG, Korsgaard N, Mulvany MJ and Lever AF (1991) Angiotensin II causes vascular hypertrophy in part by a non-pressor mechanism. *Hypertension* **17:**626–635.
Groblewski T, Maigret B, Nouet S, Larguier R, Lombard C, Bonnafous JC and Marie J (1995) Amino acids of the third transmembrane domain of the AT_{1A} angiotensin II receptor are involved in the differential recognition of peptide and nonpeptide ligands. *Biochem Biophys Res Commun* **209:**153–160.
Gröne HJ, Simon M and Fuchs E (1992) Autoradiographic characterization of angiotensin receptor subtypes in fetal and adult human kidney. *Am J Physiol* **262:**F326–F331.
Gross F (1968) The regulation of aldosterone secretion by the renin-angiotensin system under various conditions. *Acta Endocrinol (Copenh)* **(Suppl 124):**141.
Guillemette G, Guillon G, Marie J, Balestre MN, Escher E and Jard S (1986) High yield photoaffinity labeling of angiotensin II receptors. *Mol Pharmacol* **30:**544–551.
Gunther S (1984) Characterization of angiotensin II receptor subtypes in rat liver. *J Biol Chem* **259:**7622–7629.
Guo DF and Inagami T (1994a) The genomic organization of the rat angiotensin II receptor AT_{1B}. *Biochim Biophys Acta* **1218:**91–94.
Guo DF, Furuta H, Mizukoshi M and Inagami T (1994) The genomic organization of human angiotensin II type 1 receptor. *Biochem Biophys Res Commun* **200:**313–319.
Guo DF and Inagami T (1994b) Epidermal growth factor-enhanced human angiotensin II type 1 receptor. *Hypertension* **23:**1032–1035.
Guo DF, Uno S and Inagami T (1995) Steroid hormones upregulate rat angiotensin II type 1A receptor gene: Role of glucocorticoid responsive elements in rat angiotensin II type 1A promoter. *J Steroid Biochem Mol Biol* **53:**69–73.
Gyurko R, Kimura B, Kurian P, Crews FT and Phillips MI (1992) Angiotensin II receptor subtypes play opposite roles in regulating phosphatidylinositol hydrolysis in rat skin slices. *Biochem Biophys Res Commun* **188:**285–292.
Haberl RL (1994) Role of angiotensin receptor subtypes in the response of rabbit brain arterioles to angiotensin. *Stroke* **25:**1476–1480.
Haberl RL, Anneser F, Villringer A and Einhäupl KM (1990) Angiotensin II induces endothelium-dependent vasodilation of rat cerebral arterioles. *Am J Physiol* **258:**H1840–H1846.
Haberl RL, Decker PJ and Einhaupl KM (1991) Angiotensin degradation products mediate endothelium-dependent dilation of rabbit brain arterioles. *Circ Res* **68:**1621–1627.
Hagaman JR, Moyer JS, Bachman ES, Sibony M, Magyar PL, Welch JE, Smithies O, Krege JH and O'Brien DA (1998) Angiotensin-converting enzyme and male fertility. *Proc Natl Acad Sci USA* **95:**2552–2557.
Hall KL, Hanesworth JM, Ball AE, Felgenhauer GP, Hosick HL and Harding JW (1993) Identification and characterization of a novel angiotensin binding site in cultured vascular smooth muscle cells that is specific for the hexapeptide (3–8) fragment of angiotensin II, angiotensin IV. *Regul Pept* **44:**225–232.
Hall KL, Ventkateswaran S, Hanesworth JM, Schelling ME and Harding JW (1995) Characterization of a functional antiotensin IV receptor on coronary microvascular endothelial cells. *Regul Pept* **58:**107–115.
Hamawaki M, Coffman TM, Lashus A, Koide M, Zile MR, Oliverio MI, DeReyte G, Cooper G and Carabello BA (1998) Pressure-overload hypertrophy is unabated in mice devoid of AT_{1A} receptors. *Am J Physiol* **274:**H868–H873.
Han HM, Shimuta SI, Kanashiro CA, Oliveira L, Han SW and Paiva AC (1998) Residues Val254, His256, and Phe259 of the angiotensin II AT_1 receptor are not involved in ligand binding but participate in signal transduction. *Mol Endocrinol* **12:**810–814.
Handa RK, Krebs LT, Harding JW and Handa SE (1998) The angiotensin IV-AT_4 receptor system in the rat kidney. *Am J Physiol* **274:**F290–F299.
Hanesworth JM, Sardinia MF, Krebs LT, Hall KL and Harding JW (1993) Elucidation of a specific binding site for angiotensin II (3–8), angiotensin IV, in mammalian heart membranes. *J Pharmacol Exp Ther* **266:**1036–1042.
Harding JW, Cook VI, Miller-Wing AV, Hanesworth JM, Sardinia MF, Hall KL, Stobb JW, Swanson GN, Coleman JK, Wright JW and Harding EC (1992) Identification of an AII (3–8) [AIV] binding site in guinea pig hippocampus. *Brain Res* **583:**340–343.
Harding JW and Felix D (1987) The effects of the aminopeptidase inhibitors amastatin and bestatin on angiotensin-evoked neuronal activity in brain. *Brain Research* **424:**299–304.
Harding JW, Imboden H and Felix D (1986) Is angiotensin III the centrally active form of angiotensin? *Experientia* **42:**706–709.
Harding JW, Wright JW, Swanson GN, Hanesworth JM and Krebs LT (1994) AT_4 receptors: Specificity and distribution. *Kidney Int* **46:**1510–1512.
Hauger RL, Aguilera G, Baukal AJ and Catt KJ (1982) Characterization of angiotensin II receptors in the anterior pituitary gland. *Mol Cell Endocrinol* **25:**203–212.
Hayashida W, Horiuchi M and Dzau VJ (1996) Intracellular third loop domain of angiotensin II type-2 receptor: Role in mediating signal transduction and cellular function. *J Biol Chem* **271:**21985–21992.
Healy DP, Ye MQ and Troyanovskaya M (1995) Localization of angiotensin II type 1 receptor subtype mRNA in rat kidney. *Am J Physiol* **268:**F220–F226.
Heemskerk FMJ and Saavedra JM (1995) Quantitative autoradiography of angiotensin II AT_2 receptors with $[^{125}I]$CGP 42112. *Brain Res* **671:**29–38.
Hein L (1998) Genetic deletion and overexpression of angiotensin II receptors. *J Mol Med* **76:**756–763.
Hein L, Barsk GS, Pratt RE, Dzau VJ and Kobilka BK (1995a) Behavioral and cardiovascular effects of disruption the angiotensin II type-2 receptor gene in mice. *Nature (Lond)* **377:**744–747.
Hein L, Dzau VJ and Barsh GS (1995b) Linkage mapping of the angiotensin AT_2 receptor gene (Agtr2) to the mouse X chromosome. *Genomics* **30:**369–371.
Hill-Kapturczak N, Kapturczak MH, Block ER, Patel JM, Malinski T, Madsen KM and Tisher CC (1999) Angiotensin II-stimulated nitric oxide release from porcine pulmonary endothelium is mediated by angiotensin IV. *J Am Soc Nephrol* **10:**481–491.
Hirai K, Song K, Kanehara H, Shiota N, Ueda H, Kim S, Miyazaki H, Katsuoka Y and Miyazaki M (1998) Pituitary-dependent expression of the testicular angiotensin II receptor and its subtypes in rats. *Int J Androl* **21:**177–185.
Hiruma Y, Inoue A, Hirose S and Hagiwara H (1997) Angiotensin II stimulates the proliferation of osteoblast-rich populations of cells from rat calvariae. *Biochem Biophys Res* Commun **230:**176–178.
Hjorth SA, Schambye HT, Greenlee WJ and Schwartz TW (1994) Identification of peptide binding residues in the extracellular domains of the AT_1 receptor. *J Biol Chem* **269:**30953–30959.
Höhle S, Blume A, Lebrun C, Culman J and Unger T (1995) Angiotensin receptors in the brain. *Pharmacol Toxicol* **77:**306–315.
Höhle S, Culman J, Boser M, Qadri F and Unger T (1996) Effect of angiotensin AT_2 and muscarinic receptor blockade on osmotically induced vasopressin release. *Eur J Pharmacol* **300:**119–123.
Holubarsch C, Schmidt-Schwedn SL, Knorr A, Duis J, Pieske B, Ruf T, Fasol R, Hasenfus G and Just H (1994) Functional significance of angiotensin receptors in human myocardium: Sufficient difference between atrial and ventricular myocardium. *Eur Heart J* **15 (Suppl D):**88–91.
Holzmeister J, Graf K, Warnecke C and Fleck Eand Regitz-Zagrosek V (1997) Protein kinase C-dependent regulation of the human AT_1 promoter in vascular smooth muscle cells. *Am J Physiol* **273:**H655–H664.
Homma Y, Sakamoto H, Tsunoda M, Aoki M, Takenawa T and Ooyama T (1993) Evidence for involvement of phospholipase C-gamma 2 in signal transduction of

platelet-derived growth factor in vascular smooth-muscle cells. *Biochem J* **290:** 649–653.
Hong KW, Rhim BY, Shin YW and Yoo SE (1994) Characterization of PD12198 and CGP421120 induced unmasking of low concentration effects of angiotensin II in rabbit abdominal aorta. *J Pharmacol Exp Ther* **271:**1591–1596.
Horiuchi M, Aksishita M and Dzau VJ (1999) Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension* **33:**613–621.
Horiuchi M, Hayashida W, Akishiba M, Tamura K, Daviet J, Lehtonen JY and Dzau VJ (1998) Stimulation of different subtypes of angiotensin II receptors, AT_1 and AT_2 receptors, regulates STAT activation by negative cross-talk. *Circ Res* **84:**876–882.
Horiuchi M, Hayashida W, Kambe T, Yamada T and Dzau VJ (1997a) Angiotensin type 2 receptor dephosphorylates Bcl2 by activating mitogen-activated protein kinase phosphatase-1 and induces apoptosis. *J Biol Chem* **272:**19022–19026.
Horiuchi M, Koike G, Yamada T, Mukoyama M, Nakajima M and Dzau VJ (1995) The growth dependent expression of angiotensin II type 2 receptor is regulated by transcription factor interferon regulatory factor-1 and -2. *J Biol Chem* **270:**20225–20230.
Horiuchi M, Yamada T, Hayashida W and Dzau VJ (1997b) Interferon regulatatory factor –1 up-regulates angiotensin II type 2 receptor and induces apoptosis. *J Biol Chem* **272:**11952–11958.
Huang XC, Richards EM and Sumners C (1996) Mitogen activated protein kinases in rat brain neuronal cultures are activated by angiotensin II type 1 receptors and inhibited by angiotensin II type 2 receptors. *J Biol Chem* **271:**15635–15641.
Huckle WR and Earp HS (1994) Regulation of cell proliferation and growth by angiotensin II. *Prog Growth Factor Res* **5:**177–194.
Humphrey PPA and Barnard EA (1998) International Union of Pharmacology. XIX. The IUPHAR receptor code: A proposal for an alphanumeric classification system. *Pharmacol Rev* **50:**271–277.
Humphrey PPA, Spedding M and Vanhoutte P (1994) Receptor classification and nomenclature: The revolution and the resolution. *Trends Pharmaco Sci* **15:**203–204.
Hunyady L (1999) Molecular mechanisms of angiotensin II receptor internalization. *J Am Soc Nephrol* **10:**S47–S56.
Hunyady L, Balla T and Catt KJ (1996a) The ligand binding site of the AT_1 receptor. *Trends Pharmacol Sci* **17:**135–140.
Hunyady L, Baukal AJ, Balla T and Catt KJ (1994a) Independence of type I angiotensin II receptor endocytosis from G protein coupling and signal transduction. *J Biol Chem* **269:**24798–24804.
Hunyady L, Bor M, Balla T and Catt KJ (1994b) Identification of a cytoplasmic ser-thr-leu motif that determines-agonist-induced internalization of the AT_1 angiotensin receptor. *J Biol Chem* **269:**31378–31382.
Hunyady L, Bor M, Balla T and Catt KJ (1995a) A conserved NPLFY sequence contributes to agonist binding and signal transduction but is not an internalization signal for the AT_1 angiotensin receptor. *J Biol Chem* **270:**16602–16616.
Hunyady L, Bor M, Balla T and Catt KJ (1995b) Critical role of a conserved intramembrane tyrosine residue in angiotensin II receptor activation. *J Biol Chem* **270:**9702–9705.
Hunyady L, Ji H, Jagadeesh G, Zhang M, Gaborik Z, Mihalik B and Catt KJ (1998) Dependence of AT_1 angiotensin receptor function on adjacent asparagine residues in the seventh transmembrane helix. *Mol Pharmacol* **54:**427–434.
Hunyady L, Zhang M, Jagadeesh G, Bor M, Balla T and Catt KJ (1996b) Dependence of agonist activation on a conserved apolar residue in the third intracellular loop of the AT_1 angiotensin receptor. *Proc Natl Acad Sci USA* **93:**10040–10045.
Huwiler A, van Rossum G, Wartmann M and Pfeilschifter J (1998) Angiotensin II stimulation of the stress-activated protein kinases in renal messangial cells is mediated by the angiotensin AT_1 receptor subtype. *Eur J Pharmacol* **343:**297–302.
Ichiki T, Herold CL, Kambayashi Y, Bardhan S and Inagami T (1994) Cloning of the cDNA and the genomic DNA of the mouse angiotensin II type 2 receptor. *Biochim Biophys Acta* **1218:**91–94.
Ichiki T and Inagami T (1995a) Expression genomic organization and transcription of the mouse angiotensin II type 2 receptor gene. *Circ Res* **76:**693–700.
Ichiki T and Inagami T (1995b) Transcriptional regulation of the mouse angiotensin II type 2 receptor gene. *Hypertension* **25:**720–725.
Ichiki T, Kambayashi Y and Inagami T (1996) Differential inducibility of angiotensin II AT_2-receptor between SHR and WKY vascular smooth muscle cells. *Kidney Int* **49 (Suppl 55):**14–17.
Ichiki T, Kambayashi Y and Inagami T (1995a) Multiple growth factors modulate mRNA expression of angiotensin II type-2 receptor in R3T3 cells. *Circ Res* **77:** 1070–1076.
Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BLM and Inagami T (1995b) Effects on blood pressure and reduced exploratory behavior in mice lacking angiotensin II type 2 receptor. *Nature (Lond)* **377:**748–750.
Ichiki T, Usui M, Kato M, Funakoshi Y, Ito K, Egashira K and Takeshita A (1998) Downregulation of angiotensin II type 1 receptor gene transcription by nitric oxide. *Hypertension* **31:**342–348.
Inagami T, Guo DF and Kitami Y (1994) Molecular biology of angiotensin II receptors: Overview. *J Hypertens* **12 (Suppl 10):**83–94.
Inoue Y, Nakamura N and Inagami T (1997) A review of mutagenesis studies of angiotensin II type 1 receptor, the three-dimensional receptor model in search of the agonist and antagonist binding site and the hypothesis of a receptor activation mechanism. *J Hypertension* **15:**703–714.
Ishida M, Ishida T, Thomas SM and Berk BC (1998) Activation of extracellular signal-regulated kinases (ERK1/2) by angiotensin II is dependent on c-Src in vascular smooth muscle cells. *Circ Res* **82:**7–12.
Ishizaka N, Griendling KK, Lassegue B and Alexander RW (1998) Angiotensin II type 1 receptor: Relationship with caveolae and caveolin after initial agonist stimulation. *Hypertension* **32:**459–466.
Israel A, Strömberg C, Tsutsumi K, Garrido MDR, Torres M and Saavedra JM (1995) Angiotensin II receptor subtypes and phosphoinositide hydrolysis in rat adrenal medulla. *Brain Res* **38:**441–446,.
Itazaki K**,** Shigeri Y and Fujimoto M (1993) Molecular cloning and characterization of the angiotensin receptor subtype in porcine aortic smooth muscle. *Eur J Pharmacol* **245:**147–156.
Ito M, Oliverio MI, Mannon PJ, Best CF, Maeda N, Smithies O and Coffman TM (1995) Regulation of blood pressure by the type 1_A angiotensin II receptor gene. *Proc Natl Acad Sci USA* **92:**3521–3525.
Janiak P, Pillan A, Prost JT and Vilaine JP (1992) Role of angiotensin subtype 2 receptor in neointima formation after vascular injury. *Hypertension* **20:**737–745.
Jarvis MF, Gessner GW and Lyn CQ (1992) The angiotensin hexapeptide 3–8 fragment potently inhibits [^{125}1] angiotensin II binding to non-AT_1 or -AT_2 recognition sites in bovine adrenal cortex. *Eur J Pharmacol* **219:**319–322.
Jayadev S, Smith RD, Jagadeesh G, Baukal AJ, Hunyady L and Catt KJ (1999) N-linked glycosylation is required for optimal AT_{1a} angiotensin receptor expression in COS-7 cells. *Endocrinology* **140:**2010–2017.
Jezova D, Ochedalski T, Kiss A and Aguilera G (1998) Brain angiotensin II modulates sympathoadrenal and hypothalamic pituitary adrenocortical activation during stress. *J Neuroendocrinol* **10:**67–72.
Ji H, Leung M, Zhang YL, Catt KJ and Sandberg K (1994) Differential structural requirements for specific binding of nonpeptide and peptide antagonists to the AT_1 angiotensin receptor. *J Biol Chem* **269:**16533–16536.
Ji H, Sandberg K, Zhang Y and Catt KJ (1993) Molecular cloning, sequencing and functional expression of an amphibian angiotensin II receptor. *Biochem Biophys Res Commun* **194:**756–762.
Ji H, Zheng W, Zhang Y, Catt KJ and Sandberg K (1995) Genetic transfer of a nonpeptide binding site to a previously unresponsive angiotensin receptor. *Proc Natl Acad Sci USA* **92:**9240–9244.
Johnson AK and Gross PM (1993) Sensory circumventricular organs and brain homeostatic pathways. *FASEB J* **7:**678–686.
Johnson MC and Aguilera G (1991) Angiotensin-II receptor subtypes and coupling to signaling systems in cultured fetal fibroblasts. *Endocrinology* **129:**1266–1274.
Jöhren O, Imboden H, Hauser W, Maye I, Sanvitto GL and Saavedra JM (1997a) Localization of angiotensin-converting enzyme, angiotensin II, angiotensin II receptor subtypes, and vasopressin in the mouse hypothalamus. *Brain Res* **757:**218–227.
Jöhren O, Inagami T and Saavedra JM (1995) AT_{1a}, AT_b and AT_2 angiotensin II receptor subtype gene expression in rat brain. *Mol Neurosci* **6:**2549–2552.
Jöhren O, Inagami T and Saavedra JM (1996) Localization of AT_2 angiotensin II receptor gene expression in rat brain by *in situ* hybridization histochemistry. *Mol Brain Res* **37:**192–200.
Jöhren O, Sanvitto GL, Egidy G and Saavedra JM (1997b) Angiotensin II AT_{1A} receptor mRNA expression is induced by estrogen-progesterone in dopaminergic neurons of the female rat arcuate nucleus. *J Neurosci* **17:**8283–8292.
Joseph MP, Maigret B, Bonnafous JC, Marie J and Scheraga HA (1995a) A computer modeling postulated mechanism for angiotensin II receptor activation. *J Protein Chem* **14:**381–398.
Joseph MP, Maigret B and Scheraga HA (1995b) Proposals for the angiotensin II receptor-bound conformation by comparative computer modeling of AII and cyclic analogs. *Int J Pept Protein Res* **46:**515–526.
Kai H, Alexander RW, Ushio-Fukai M, Lyons PR, Akers M and Griendling KK (1998) G-Protein binding domains of the angiotensin II AT_{1A} receptors mapped with synthetic peptides selected from the receptor sequence. *Biochem J* **332:**781–787.
Kainulainen K, Perola M, Terwilliger J, Kaprio J, Koskenvuo M, Syvanen AC, Vartianen E, Peltonen L and Kontula K (1999) Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. *Hypertension* **33:** 844–849.
Kajstura J, Cigola E, Malhotra A, Li P, Cheng W, Meggs LG and Anversa P (1997) Angiotensin II induces apoptosis of adult ventricular myocytes in vitro. *J Mol Cell Cardiol* **29:**859–870.
Kakar SS, Sellers JC, Devor DC, Musgrove LC and Neill JD (1992) Angiotensin II type-1 receptor subtype cDNAs: Differential tissue expression and hormonal regulation. *Biochem Biophys Res Commun* **31:**1090–1096.
Kakuchi J, Ichiki T, Kiyama S, Hogan BLM, Fogo A, Inagami T and Ichikawa I (1995) Developmental expression of renal angiotensin receptor gene in the mouse. *Kidney Int* **47:**140–147.
Kambayashi Y, Bardhan S and Inagami T (1993a) Peptide growth factors markedly decrease the ligand binding of angiotensin II type 2 receptor in rat cultured vascular smooth muscle cells. *Biochem Biophys Res Commun* **194:**478–482.
Kambayashi Y, Bardhan S, Takahashi K, Tsuzuki S, Inui H, Hamakubo T and Inagami T (1993b) Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. *J Biol Chem* **268:**24543–24546.
Kambayashi Y, Nagata K, Ichiki T and Inagami T (1996) Insulin and insulin-like growth factor-induced expression of angiotensin type 2 receptor in vascular smooth muscle cells. *Eur J Biochem* **239:**558–565.
Kang J, Posner P and Sumners C (1994) Angiotensin II type 2 receptor stimulation of neuronal K^+ currents involves an inhibitory GTP binding protein. *Am J Physiol* **267:**C1389–C1397.
Kang J, Richards EM, Posner P and Sumners C (1995) Modulation of the delayed rectifier K^+ current in neurons by an angiotensin II type 2 receptor fragment. *Am J Physiol* **268:**C278–C282.
Kang J, Sumners C and Posner P (1992) Modulation of net outward ionic current in cultured neurons by angiotensin II: Involvement of AT_1 and AT_2 receptors. *Brain Res* **580:**317–324.
Kang J, Sumners C and Posner P (1993) Angiotensin II type 2 receptor-modulated changes in potassium current in cultured neurons. *Am J Physiol* **265:**C607–C616.
Karnik SS, Husain A and Graham RM (1996) Molecular determinants of peptide and non-peptide binding to the AT_1 receptor. *Clin Exp Pharmacol Physiol* **(Suppl 3):**58–66.
Keiser JA, Bjork FA, Hodges JC and Taylor DG Jr (1992) Renal hemodynamic and

excretory responses to PD123319 and losartan, nonpeptide AT_1 and AT_2 subtype-specific angiotensin II ligands. *J Pharmacol Exp Ther* **263:**1154–1160.
Kerins DM, Hao Q and Vaughn DE (1995) Angiotensin induction of PAI-1 expression in endothelial cells is mediated by the hexapeptide angiotensin IV. *J Clin Invest* **96:**2515–2520.
Khosla MC, Smeby RR and Bumpus FM (1974) Structure activity relationship in angiotensin II analogs, in *Handbook of Experimental Pharmacology*, (Page IH and Bumpus RM eds) XXXVII, pp 126–156, Springer-Verlag, New York.
Kim H, Krege J, Kluckman K, Hagaman J, Hodgin J, Best C, Jennette J, Coffman T, Maeda N and Smithies O (1995) Genetic control of blood pressure and the angiotensinogen locus. *Proc Natl Acad Sci USA* **92:**2735–2739.
Kimura B, Sumners C and Phillips MI (1992) Changes in skin angiotensin II receptors in rats during wound healing. *Biochem Biophys Res Commun* **187:**1083–1090.
Kiron MA and Soffer RL (1989) Purification and properties of a soluble angiotensin II-binding protein from rabbit liver. *J Biol Chem* **264:**4138–4142.
Kitami Y, Okura T, Marumoto K, Wakamiya R and Hiwada K (1992) Differential gene expression and regulation of type-1 angiotensin II receptor subtypes in the rat. *Biochem Biophys Res Commun* **188:**446–452.
Kizima K, Matsubara H, Murasawa S, Ohkubo N, Mori Y and Inada M (1996) Regulation of angiotensin II type 2 receptor gene by the protein kinase C-calcium pathway. *Hypertension* **27:**529–534.
Klett C, Nobiling R, Gierschik P and Hackenthal E (1993) Angiotensin II stimulates the synthesis of angiotensinogen in hepatocytes by inhibiting adenylyl cyclase activity and stabilizing angiotensinogen mRNA. *J Biol Chem* **268:**25095–25107.
Kohout TA and Rogers TB (1995) Angiotensin II activates the Na^+/NCO_3 symport through a phosphoinositide-independent mechanism in cardiac cells. *J Biol Chem* **269:**20432–20438.
Koike G, Horiuchi M, Yamada T, Szpirere C, Jacob HJ and Dzau VJ (1994) Human type 2 angiotensin receptor gene cloned, mapped to the X chromosome, and its mRNA is expressed in human lung. *Biochem Biophys Res Commun* **203:**1842–1850.
Konishi H, Kuroda S, Inada Y and Fujisawa Y (1994) Novel subtype of human angiotensin II type 1 receptor: cDNA cloning and expression. *Biochem Biophys Res Comm* **199:**467–474.
Konvicka K, Guarnieri F, Ballesteros JA and Weinstein H (1998) A proposed structure for transmembrane segment 7 of G protein-coupled receptors incorporating an Asn-Pro/Asp-Pro motif. *Biophys J* **75:**601–611.
Kramár EA, Harding JW and Wright JW (1997) Angiotensin II- and IV-induced changes in cerebral blood flow: Roles of AT_1, AT_2, and AT_4 receptor subtypes. *Regul Pept* **68:**131–138.
Kramár EA, Krishnan R, Harding JW and Wright JW (1998) Role of nitric oxide in angiotensin IV-induced increases in cerebral blood flow. *Regul Pept* **74:**185–192.
Krebs LT, Kramár EA, Hanesworth JM, Sardinia MF, Ball AE, Wright JW and Harding JW (1996) Characterization of the binding properties and physiological action of divalinal-angiotensin IV, a putative AT_4 receptor antagonist. *Regul Pept* **67:**123–130.
Krege J, John S, Langenbach L, Hodgin J, Hagaman J, Bachman E, Jennette J, O'Brien D and Smithies O (1995) Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature (Lond)* **375:**146–149.
Krege J, Kim H, Moyer J, Jenette J, Peng L, Hiller S and Smithies O (1997) Angiotensin converting enzyme gene mutations, blood pressures, and cardiovascular homeostasis. *Hypertension* **29:**150–157.
Kudoh S, Komuro I, Hiroi Y, Zou Y, Harada K, Sugaya T, Takekoshi N, Murakami K, Kaowaki T and Yazaki Y (1998) Mechanical stretch induces hypertrophic responses in cardiac myocytes of angiotensin II type 1a receptor knockout mice. *J Biol Chem* **273:**24037–24043.
Kulakowska A, Karwowska W, Wisniewski K and Braszko JJ (1996) Losartan influences behavioural effects of angiotensin II in rats. *Pharmacol Res* **34:**109–115.
Laflamme L, de Gasparo M, Gallo JM, Payet MD and Gallopayet N (1996) Angiotensin II induction of neurite outgrowth by AT_2 receptors in NG108-15 cells - effect counteracted by the AT_1 receptors. *J Biol Chem* **271:**22729–22735.
Langford K, Frenzel K, Martin BM and Bernstein KE (1992) The genomic organization of the rat AT_1 angiotensin receptor. *Biochem Biophys Res Commun* **183:**1025–1032.
Laporte SA, Boucard AA, Servant G, Guillemette G, Leduc R and Escher E (1999) Determination of peptide contact points in the human angiotensin II type I receptor (AT_1) with photosensitive analogs of angiotensin II. *Mol Endocrinol* **13:**578–586.
Laursen DA, Benjamin CW and Jones DA (1997) Role of superoxide in angiotensin-induced but not catecholamine-induced hypertension. *Circulation* **95:**588–593.
Lazard D, Briend-Sutren MM, Villageois P, Mattei MG, Strosberg AD and Nahmias C (1994) Molecular characterization and chromosome localization of a human angiotensin II AT_2 receptor gene highly expressed in fetal tissues. *Receptors Channels* **2:**71–80.
Lehtonen JY, Horiuchi M, Daviet L, Akishita M and Dzau VJ (1999) Activation of the de novo biosynthesis of sphyngolipids mediates angiotensin II type 2 receptor-induced apoptosis. *J Biol Chem* **274:**16901–16906.
Lenkei Z, Corvol P and Llorens-Cortes C (1995) The angiotensin receptor subtype AT_{1A} predominates in rat forebrain areas involved in blood pressure, body fluid homeostasis and neuroendocrine control. *Brain Res Mol Brain Res* **30:**53–60.
Lenkei Z, Nuyt AM, Grouselle D, Corvol P and Llorens-Cortes C (1999) Identification of endocrine cell populations expressing the AT_{1B} subtype of angiotensin II receptors in the anterior pituitary. *Endocrinology* **140:**472–477.
Lenkei Z, Palkovits M, Corvol P and Llorens-Cortes C (1988) Distribution of angiotensin type-1 receptor messenger RNA in the adult rat brain. *Neuroscience* **82:**827–841.
Lenkei Z, Palkovits M, Corvol P and Llorens-Cortes C (1997) Expression of angiotensin type-1 (AT_1) and type-2 (AT_2) receptor mRNAs in the adult rat brain: A functional neuroanatomical review. *Front Neuroendocrinol* **18:**383–439.
Leri A, Claudio PP, Li Q, Wang XW, Reiss K, Wang SG, Malhotra A, Kajstura J and Anversa P (1998) Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. *J Clin Invest* **101:**1326–1342.
Leung KH, Roscoe WA, Smith RD, Timmermans PBMWM and Chiu AT (1992) Characterization of biochemical responses of angiotensin II (AT_2) binding sites in the rat pheochromocytoma PC12W cells. *Eur J Pharmacol* **227:**63–70.
Leung KH, Smith RD, Timmermans BMWM and Chiu AT (1991) Regional distribution of the two types of angiotensin II receptor in rat brain using selective non-peptide antagonists. *Neurosci Lett* **123:**95–98.
Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, Plowman GD, Rudy B and Schlessinger J (1995) Protein tyrosine kinase PYK2 involved in $Ca^{(2+)}$-induced regulation of ion channel and MAP kinase functions. *Nature (Lond)* **376:**737–745.
Levens NR, Peach MJ, Carey RM, Poat JA and Munday KA (1980) Stimulation of intestinal sodium and water transport in vivo ny angiotensin II and analogs. *Endocrinology* **107:**1946–1953.
Levy BI, Benessiano J, Henrion D, Caputo L, Heymes C, Durier M, Poitevin P and Samuel JL (1996) Chronic blockage of AT_2-subtype receptors prevents the effect of angiotensin II on the rat ventricular structure. *J Clin Invest* **98:**418–425.
Li DY, Zhang YC, Philips MI, Sawamura T and Mehta JL (1999) Upregulation of endothelial receptor for oxidized low-density lipoprotein (LOX-1) in cultured human coronary artery endothelial cells by angiotensin II type 1 receptor activation. *Circ Res* **84:**1043–1049.
Li JS, Touyz RM and Schiffrin EL (1998a) Effect of AT_1 and AT_2 angiotensin receptor antagonists in angiotensin II-infused rats. *Hypertension* **31:**487–492.
Li Q, Feenstra M, Pfaffendorf M, Eijsman L and van Zwieten PA (1997) Comparative vasoconstrictor effects of angiotensin II, III, IV in human isolated saphenous vein. *J Cardiovasc Pharmacol* **29:**451–456.
Li WG, Ye YH, Fu B, Wang JZ, Yu LF, Ichiki T, Inagami T, Ichikawa I and Chen XM (1998b) Genetic deletion of AST2 receptor antagonizes angiotensin II-induced apoptosis in fibroblasts of the mouse embryo. *Biochem Biophys Res Commun* **250:**72–76.
Liang H, Venema VJ, Wang X, Ju H, Venema RC and Marrero MB (1999) Regulation of angiotensin II-induced phosphorylation of STAT3 in vascular smoth muscle cells. *J Biol Chem* **274:**19846–19851.
Liao DF, Duff JL, Daum G, Pelech SL and Berk BC (1996) Angiotensin II stimulates MAP kinase kinase kinase activity in vascular smooth muscle cells –Role of Raf. *Circ Res* **79:**1007–1014.
Liao DF, Monia B, Dean N and Berk BC (1997) Protein kinase C-zeta mediates angiotensin II activation of ERK1/2 in vascular smooth muscle cells. *J Biol Chem* **272:**6146–6150.
Lin SY and Goodfriend TL (1970) Angiotensin receptors. *Am J Physiol* **218:**1319–1328.
Lindpainter K and Ganten D (1991) The cardiac renin-angiotensin system; an appraisal of present experimental and clinical evidence. *Circ Res* **68:**905–921.
Linseman DA, Benjamin CW and Jones DA (1995) Convergence of angiotensin II and platelet-derived growth factor receptor signaling cascades in vascular smooth muscle cells. *J Biol Chem* **270:**12563–12568.
Liu YH, Yang XP, Sharov VG, Nass O, Sabbah HN, Peterson E and Carretero OA (1997) Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure—Role of kinins and angiotensin II type 2 receptors. *J Clin Invest* **99:**1926–1935.
Liyou N, Davis D, James K, Simons L, Friedlander Y, Simons J, McCallum J and Johnson A (1999) The A1166C mutation in the angiotensin II type I receptor and hypertension in the elderly. *Clin Exp Pharmacol Physiol* **26:**525–526.
Llorens-Cortes C, Greenberg B, Huang H and Corvol P (1994) Tissular expression and regulation of type 1 angiotensin II receptor subtypes by quantitative reverse transcriptase-polymerase chain reaction analysis. *Hypertension* **24:**538–548.
Lo M, Liu KL, Lanteime P and Sassard J (1995) Subtype 2 of angiotensin II receptors controls pressure natriuresis in rats. *J Clin Invest* **95:**1394–1397.
Lokuta AJ, Cooper C, Gaa ST, Wang HE and Rogers TB (1994) Angiotensin II stimulates the release of phospholipid derived second messengers through multiple subtypes in heart cells. *J Biol Chem* **269:**4832–4838.
Lu D, Yang H and Raizada MK (1996) Angiotensin II regulation of neuromodulation: Downstream signaling mechanism from activation of mitogen-activated protein kinase. *J Cell Biol* **135:**1609–1617.
Lucius R, Gallinat S, Rosenstiel P, Herdegen T, Sievers J and Unger T (1998) The angiotensin II type 2 (AT2) receptor promotes axonal regeneration in the optic nerve of adult rats. *J Exp Med* **188:**661–670.
Macari D, Whitebread S, Cumin F, de Gasparo M and Levens N (1994) Renal actions of the angiotensin AT_2 receptor ligands CGP 42112 and PD123319 after blockade of the renin-angiotensin system. *Eur J Pharmacol* **259:**27–36.
Macrez N, Morel JL, Kalkbrenner F, Viard P, Schultz G and Mironneau J (1997) A betagamma dimer derived from G_{13} transduces the angiotensin AT_1 receptor signal to stimulation of Ca^{2+} channels in rat portal vein myocytes. *J Biol Chem* **272:**23180–23185.
Macrez-Lepretre N, Kalkbrenner F, Morel JL, Schultz G and Mironneau J (1997) G protein heterotrimer Galphal13beta1 gamma3 couples the angiotensin AT_{1A} receptor to increases in cytoplasmic Ca^{2+} in rat portal vein myocytes. *J Biol Chem* **272:**10095–100102.
Madeddu P, Emanueli C, Maestri R, Salis MB, Minasi A, Capogrossi MC and Olivetti G (2000) Angiotensin II type 1: Receptor blockade prevents cardiac remodeling in Bradykinin B2 receptor knockout mice. *Hypertension* **35:**391–396.
Mahon JM, Carr RD, Nicol AK and Henderson IW (1994) Angiotensin (1–7) is an antagonist at the type 1 angiotensin II receptor. *J Hypertens* **12:**1377–1381.
Maloney JA, Tsygankova O, Szot A, Yang L, Li Q and Williamson JR (1998) Differential translocation of protein kinase C isozymes by phorbol esters, EGF, and Ang II in rat liver WB cells. *Am J Physiol* **274:**C974–C982.
Maric C, Aldred GP, Harris PJ and Alcorn D (1998) Angiotensin II inhibits growth

of cultured embryonic renomedullary interstitial cells through the AT_2 receptor. *Kidney Int* **53:**92–99.
Marie J and Jard S (1983) Angiotensin II inhibits adenylate cyclase from adrenal cortex glomerulosa zone. *FEBS Lett* **159:**97–101.
Marie J, Maigret B, Joseph MP, Larguier R, Nouet S, Lombard C and Bonnafous JC (1994) Tyr292 in the seventh transmembrane domain of the AT_{1A} angiotensin II receptor is essential for its coupling to phospholipase C. *J Biol Chem* **269:**20815–20818.
Marrero MB, Paxton WG, Duff JL, Berk BC and Bernstein KE (1994) Angiotensin II stimulates tyrosine phosphorylation of phospholipase C-γ1 in vascular smooth muscle cells. *J Biol Chem* **269:**10935–10939.
Marrero MB, Schieffer B, Li B, Sun J, Harp JB and Ling BN (1997) Role of Janus kinase/signal transducer and activator of transcription and mitogen-activated protein kinase cascades in angiotensin II- and platelet-derived growth factor-induced vascular smooth muscle cell proliferation. *J Biol Chem* **272:**24684–24690.
Marrero MB, Schieffer B and Paxton WG (1995) Direct stimulation of JAK/STAT pathway by the angiotensin II AT_1 receptor. *Nature (Lond)* **375:**247–250.
Marrero MB, Venema VJ, Ju H, Eaton DC and Venema RC (1998) Regulation of angiotensin II-induced JAK2 tyrosine phosphorylation: Roles of SHP-1 and SHP-1. *Am J Physiol* **275:**C1216–C1223.
Marshall GR, Vine W and Needlemann P (1970) A specific competitive inhibitor of angiotensin II. *Proc Natl Acad Sci USA* **67:**1624–1630.
Martin J and Govantes C (1995) Angiotensin converting enzyme inhibitors and the role of nitric oxide and excitatory amino acids in improvement of cognition and memory. *J Auton Pharmacol* **15:**129–149.
Martin MM, Su B and Elton TS (1994) Molecular cloning of the human angiotensin II type 2-receptor. *Biochem Biophys Res Commun* **205:**645–651.
Masaki H, Kurihara T, Yamaki A, Inimata N, Nazawa Y, Mori Y, Murasawa S, Kizima K, Maruyama K, Horiuchi M, Dzau VJ, Takahashi H, Iwasaka T, Inada M and Marsubara H (1998) Cardiac-specific overexpression of angiotensin II AT_2 receptor causes attenuated response to AT1 receptor-mediated pressor and chronotropic effects. *J Clin Invest* **101:**527–535.
Matsoukas JM, Bigam G, Zhou N and Moore GJ (1990) ^{1}H-NMR studies of [Sar^1] angiotensin II conformation by nuclear Overhauser effect spectroscopy in the rotating frame (Roesy) clustering of the aromatic rings in dimethylsulfoxide. *Peptides* **11:**359–366.
Matsoukas JM, Hanrelis J, Keramida M, Marromoustados T, Makrixannis A, Yamdagni R, Wu Q and Moore GJ (1994) Role of the NH_2-terminal domain of angiotensin II (AngII) and [Sar^1]angiotensin II on conformation and activity. NMR evidence for aromatic virus clustering and peptide backbone folding compared with [des-1,2,3] angiotensin II. *J Biol Chem* **269:**5303–5312.
Matsubara H (1998) Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases. *Circ Res* **83:**1182–1191.
Matsubara H, Kanasaki M, Murasawa S, Tsukaguchi Y, Nio Y and Inada M (1994) Differential gene expression and regulation of angiotensin II receptor subtypes in rat cardiac fibroblasts and cardiomyocytes in culture. *J Clin Invest* **93:**1592–1601.
Matsusaka T, Nishimura H, Utsunomiya H, Kakuchi J, Niimura F, Inagami T, Fogo A and Ichikawa I (1996) Chimeric mice carrying 'regional targeted' deletion of the angiotensin type 1A receptor gene. Evidence against the role for local angiotensin in the in vivo feedback regulation of renin synthesis in juxtaglomerular cells. *J Clin Invest* **98:**1867–1877.
Matsusaka T and Ichikawa I (1997) Biological functions of angiotensin and its receptors. *Annu Rev Physiol* **59:**395–412.
Maturana AD, Burnay MM, Capponi AM, Vallotton MB and Rossier MF (1999a) Angiotensin II type 1 receptor activation modulates L- and T-type calcium channel activity through distinct mechanisms in bovine adrenal glomerulosa cells. *J Recept Signal Transduct Res* **19:**509–520.
Maturana AD, Casal AJ, Demaurex N, Vallotton MB, Capponi AM and Rossier MF (1999b) Angiotensin II negatively modulates L-type calcium channels through a pertussis toxin-sensitive G protein in adrenal glomerulosa cells. *J Biol Chem* **274:**19943–19948.
Mauzy CA, Hwang O, Egloff AM, Wu LH and Chung FZ (1992) Cloning, expression, and characterization of a gene encoding the human angiotensin II type 1A receptor. *Biochem Biophys Res Commun* **186:**277–284.
McClellan KJ and Balfour JA (1998) Eprosartan. *Drugs* **55:**713–718.
McKinley MJ, Bicknell RJ, Hards D, McAllen RM, Vivas L, Weisinger RS and Oldfield BJ (1992) Efferent neural pathways of the lamina terminalis subserving osmoregulation. *Prog Brain Res* **91:**395–402.
McKinley MJ and Oldfield BJ (1998) The brain as an endocrine target for peptide hormones. *Trends Endocrinol Metab* **9:**349–353.
McWhinney CD, Hunt RA, Conrad KM, Dostal DE and Baker KM (1997) The type I angiotensin II receptor couples to Stat1 and Stat3 activation through Jak2 kinase in neonatal rat cardiac myocytes. *J Mol Cell Cardiol* **29:**2513–2524.
Meffert S, Stoll M, Steckelings UM, Bottari SP and Unger T (1996) The angiotensin AT_2 receptor inhibits proliferation and promotes differentiation in PC12W cells. *Mol Cell Endocrinol* **122:**59–67.
Mendelsohn FA, Quirion R, Saavedra JM, Aguilera G and Catt KJ (1984) Autoradiographic localization of angiotensin II receptors in rat brain. *Proc Natl Acad Sci USA* **81:**1575–1579.
Mesulam MM, Mufson EJ, Wainer BH and Levey AI (1983) Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience* **10:**1185–1201.
Metsärinne KP, Stoll M, Gohlke P, Paul M and Unger T (1992) Angiotensin II is antiproliferative for coronary endothelial cells in vitro. *Pharm Pharmacol Lett* **2:**150–152.
Millan MA, Carvallo P, Izumi S, Zemel S, Catt KJ and Aguilera G (1989) Novel sites of expression of functional angiotensin II receptors in the late gestation fetus. *Science (Wash DC)* **244:**1340–1342.
Millan M, Jacobowitz DM, Aguilera G and Catt KJ (1991) Differential distribution of AT_1 and AT_2 angiotensin II receptor subtypes in the rat brain during development. *Proc Natl Acad Sci USA* **88:**11440–11444.
Millan MA, Jacobowitz DM, Catt KJ and Aguilera G (1990) Distribution of angiotensin II receptors in the brain of subhuman primates. *Peptides* **11:**243–253.
Millatt LJ, Abdel-Rahman EM and Siragy HM (1999) Angiotensin II and nitric oxide: A question of balance. *Regul Pept* **81:**1–10.
Miller-Wing AV, Hanesworth JM, Sardinia MF, Wright JW, Speth RC, Grove KL and Harding JW (1993) Central angiotensin IV receptors: Distribution and specificity in guinea pig brain. *J Pharmacol Exp Ther* **266:**1718–1726.
Miura S, Feng YH, Husain A and Karnik SS (1999a) Role of aromaticity of agonist switches of angiotensin II in the activation of the AT_1 receptor. *J Biol Chem* **274:**7103–7110.
Miura SI and Karnik SS (1999b) Angiotensin II type 1 and type 2 receptors bind angiotensin II through different types of epitope recognition. *J Hypertens* **17:**397–401.
Mlinar B, Biagi BA and Enyeart JJ (1995) Losartan-sensitive AII receptors linked to depolarization-dependent cortisol secretion through a novel signaling pathway. *J Biol Chem* **270:**20942–20951.
Moeller I, Allen AM, Chai SY, Zhuo J and Mendelsohn FAO (1998) Bioactive angiotensin peptides. *J Hum Hypertens* **12:**289–293.
Moeller I, Chai S, Oldfield BJ, McKinley MJ, Casley D and Mendelsohn FAO (1995) Localization of angiotensin IV binding sites to motor and sensory neurons in the sheep spinal cord and hindbrain. *Brain Res* **701:**301–306.
Moeller I, Lew RA, Mendelsohn FA, Smith AI, Brennan ME, Tetaz TJ and Chai SY (1997) The globin fragment LVV-hemorphin-7 is an endogenous ligand for the AT_4 receptor in the brain. *J Neurochem* **68:**2530–2537.
Moeller I, Paxinos G, Mendelsohn FA, Aldred GP, Casley D and Chai SY (1996a) Distribution of AT_4 receptors in the *Macaca fascicularis* brain. *Brain Res* **712:**307–324.
Moeller I, Small DH, Reed G, Harding JW, Mendelsohn FA and Chai SY (1996b) Angiotensin IV inhibits neurite outgrowth in cultured embryonic chicken sympathetic neurones. *Brain Res* **726:**61–66.
Monck JR, Williamson RE, Rogulia WI, Fluharty SJ and Williamson JR (1990) Angiotensin II effects on the cytosolic free Ca^{2+} concentration in N1E-115 neuroblastoma cells: Kinetic properties of the Ca^{2+} transient measured in single Fura-2-Loaded cells. *J Neurochem* **54:**278–287.
Monnot C, Bihoreau C, Conchon S, Curnow KM, Corvol P and Clauser E (1996) Polar residues in the transmembrane domains of the type 1 angiotensin II receptor are required for binding and coupling. Reconstitution of the binding site by co-expression of two deficient mutants. *J Biol Chem* **271:**1507–1513.
Monnot C, Weber V, Stinnakre J, Bihoreau C, Teutsch B, Corvol P and Clauser E (1991) Cloning and functional characterization of a novel mas-related gene, modulating intracellular angiotensin II actions. *Mol Endocrinol* **5:**1477–1487.
Moreau C, Rasolonjanahary R, Audinot V, Kordon C and Enjalbert A (1994) Angiotensin II effects on second messengers involved in prolactin secretion are mediated by AT_1 receptor in anterior pituitary cels. *Mol Cell Neurosci* **5:**597–603.
Moriguchi Y, Matsubara H, Mori Y, Murasawa S, Masaki H, Maruyama K, Tsutsumi Y, Shibasaki Y, Tanaka Y, Nakajima T, Oda K and Iwasaka T (1999) Angiotensin II-induced transactivation of epidermal growth factor receptor regulates fibronectin and transforming growth factor-β synthesis via transcriptional and posttranscriptional mechanisms. *Circ Res* **84:**1073–1084.
Morimoto S and Ogihara T (1994) TCV-116 A new angiotensin II type-1 receptor antagonist. *Cardiovasc Drug Rev* **12:**153–164.
Moriuchi R, Shibata S, Himeno A, Johren O, Hoe KL and Saavedra JM (1998) Molecular cloning and pharmacological characterization of an atypical gerbil angiotensin II type-1 receptor and its mRNA expression in brain and peripheral tissues. *Brain Res Mol Brain Res* **60:**234–246.
Morrell NW, Upton PD, Kotecha S, Huntley A, Yacoub MH, Polak JM and Wharton J (1999) Angiotensin II activates MAPK and stimulates growth of human pulmonary artery smooth muscle via AT_1 receptors. *Am J Physiol* **277:**L440–L448.
Morris M, Li P, Callahan MF, Oliverio MI, Coffman TM, Bosch SM and Diz DI (1999) Neuroendocrine effects of dehydration in mice lacking the angiotensin AT_{1A} receptor. *Hypertension* **33:**482–486.
Mosimann R, Imboden H and Felix D (1996) The neuronal role of angiotensin II in thirst, sodium appetite, cognition and memory. *Biol Rev Camb Philos Soc* **71:**545–559.
Mrug M, Stopka T, Julian BA, Prchal JF and Prchal JT (1997) Angiotensin II stimulates proliferation of normal early erythroid progenitors. *J Clin Invest* **100:**2310–2314.
Mukherjee A, Kulkarni P, McCann SM and Negro-Vilar A (1982) Evidence for the presence and characterizaton of angiotensin II receptors in rat anterior pituitary membranes. *Endocrinology* **110:**665–667.
Mukoyama M, Kakajima M, Horiuchi M, Sasamura H, Pratt RE and Dzau VJ (1993) Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven transmembrane receptors. *J Biol Chem* **268:**24539–24542.
Munzenmaier DH and Green AS (1999) Opposing actions of angiotensin II on microvascular growth and arterial blood pressure. *Hypertension* **27:**760–765.
Murasawa S, Matsubara H, Urakami M and Inada M (1993) Regulatory elements that mediate expression of the gene for the angiotensin II type 1a receptor for the rat. *J Biol Chem* **268:**26996–27003.
Murphy TJ, Alexander RW, Griendling KK, Runge MSand Bernstein KE (1991) Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature (Lond)* **16:**233–236.
Murphy TJ, Nakamura Y, Takeuchi K and Alexander RW (1993) A cloned angiotensin receptor isoform from the turkey adrenal gland is pharmacologically distinct from mammalian angiotensin receptors. *Mol Pharmacol* **44:**1–7.
Nahmias C, Cazaubon SM, Brion-Sutren M, Lazard D, Villageois P and Strosberg AD (1995) Angiotensin II AT_2 receptors are functionally coupled to protein tyrosine dephosphorylation in N1E-115 neuroblastoma cells. *Biochem J* **306:**87–92.
Nahmias C and Strosberg AD (1995) The angiotensin AT_2 receptor: Searching for signal-transduction pathway and physiological function. *Trends Pharmacol Sci* **16:**223–225.
Najmoutin G, Abdulaev NG and Ridge KD (1998) Light-induced exposure of the

cytoplasmic end of transmembrane helix seven in rhodopsin. *Biochemistry* **95:** 12854–12859.
Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horichi M, Pratt R and Dzau VJ (1995) The angiotensin II (AT_2) receptor antagonizes the growth effects of the AT_1 receptor: Gain-of-function study using gene transfer. *Proc Natl Acad Sci USA* **92:**10663–10667.
Nakajima M, Mukoyama M, Pratt RE, Horiuchi M and Dzau VJ (1993) Cloning of cDNA and analysis of the gene for mouse angiotensin II type 2 receptor. *Biochem Biophys Res Commun* **197:**393–399.
Natarajan R, Lanting L, Xu L and Nadler J (1994) Role of specific isoforms of protein kinase C in angiotensin II and lipoxygenase action in rat adrenal glomerulosa cells. *Mol Cell Endocrinol* **101:**59–66.
Näveri L, Strömberg C and Saavedra JM (1994a) Angiotensin II AT_2 receptor stimulation extends the upper limit of cerebral blood flow autoregulation: Agonist effects of CGP 42112 and PD123119. *J Cereb Blood Flow Metab* **14:**38–44.
Näveri L, Strömberg C and Saavedra JM (1994b) Angiotensin II AT_2 receptor stimulation increases cerebrovascular resistance during hemorrhagic hypotension in rats. *Regul Pept* **52:**21–29.
Neel BG and Tonks NK (1997) Protein tyrosine phosphatases in signal transduction. *Curr Opin Cell Biol* **9:**193–204.
Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosenkranz S, Stablein A, Beckers F, Smits JF, Daemen MJ, Vetter H and Bohm M (1996) Estrogen modulates AT_1 receptor gene expression in vitro and in vivo. *Circulation* **97:**2197–2201.
Nickenig G, Laufs U, Schnabel P, Knorr, Paul M and Bohm MP (1997) Downregulation of aortic and cardiac AT_1 receptor gene expression in transgenic (mRen-2) 27 rats. *Br J Pharmacol* **121:**134–140.
Nickenig G, Roling J, Strehlow K, Schnabel P and Bohm M (1998) Insulin induces upregulation of vascular AT_1 receptor gene expression by posttranscriptional mechanisms. *Circulation* **98:**2453–2460.
Nicoll RA and Baker JL (1971) Excitation of supraoptic neuroscretory cells by angiotensin II. *Nature New Biol* **233:**172–174.
Niimura F, Labosky P, Kakuchi J, Okubo S, Yoshida T, Oikawa T, Ichiki A, Naftilan A, Fogo A, Inagami T, Hogan B and Ichikawa I (1995) Gene targeting in mice reveals a requirement for angiotensin in the development and maintenance of kidney morphology and growth factor regulation. *J Clin Invest* **66:**2947–2954.
Nikiforovich GV and Marshall GR (1993) Three-dimensional recognition requirements for angiotensin agonists: A novel solution for an old problem. *Biochem Biophys Res Commun* **195:**222–228.
Nikiforovich GV, Kuo JL, Plucinska K, Zhang WJ and Marshall GR (1994) Conformational analysis of two cyclic analogs of angiotensin: Implications for the biologically active conformation. *Biochemistry* **33:**3591–3598.
Nio Y, Matsubara H, Murasawa S, Kanasaki M and Inada M (1995) Regulation of gene transcription of angiotensin II receptor subtypes in myocardial infarction. *J Clin Invest* **95:**46–54.
Nishimura H, Yerkes E, Hohenfellner K, Miyazaki Y, Ma J, Hunley TE, Yoshida H, Ichiki T, Threadgill D, Phillips JA 3rd, Hogan BM, Fogo A, Brock JW 3rd, Inagami T and Ichikawa I (1999) Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. *Mol Cell* **3:**1–10.
Noda K, Feng YH, Liu XP, Saad Y, Husain A and Karnik SS (1996) The active state of the AT_1 angiotensin receptor is generated by angiotensin II induction. *Biochemistry* **35:**16435–16442.
Noda K, Saad Y and Karnik SS (1995a) Interaction of Phe^8 of angiotensin II with Lys^{199} and His^{256} of AT_1 receptor in agonist activation. *J Biol Chem* **270:**28511–28514.
Noda K, Saad Y, Kinoshita A, Boyle TP, Graham RM, Husain A and Karnik SK (1995b) Tetrazole and carboxylate groups of angiotensin receptor antagonists bind to the same subsite by different mechanisms. *J Biol Chem* **270:**2284–2289.
Nossaman BD, Feng CJ, Kaye AD and Kadowitz PJ (1995) Analysis of responses to Ang IV: Effects of PD-123319 and Dup-753 in the pulmonary circulation of the rat. *Am J Physiol* **268:**L302–L308.
Nouet S and Nahmias C (2000) Signal transduction from the angiotensin AT_2 receptor. *Trends Endocrinol Metab* **11:**1–6.
Nozawa Y, Haruno A, Oda N, Yamasaki Y, Matsuura N, Yamada S, Inabe K, Kimura R, Suzuki H and Hoshino T (1994) Angiotensin II receptor subtypes in bovine and human ventricular myocardium. *J Pharmacol Exp Ther* **270:**566–571.
Obermüller N, Unger T, Culman J, Gohlke P, de Gasparo M and Bottari SP (1991) Distribution of angiotensin II receptor subtypes in rat brain nuclei. *Neuro Sci Lett* **132:**11–15.
O'Brien RM and Granner DK (1991) Regulation of gene expression by insulin. *Biochem J* **278:**609–619.
Ohkubo N, Matsubara H, Nozawa Y, Mori Y, Murasawa S, Kijima K, Maruyama K, Masaki H, Tsutumi Y, Shibazaki Y, Iwasaka T and Inada M (1997) Angiotensin type 2 receptors are reexpressed by cardiac fibroblasts from failing myopathic hamster hearts and inhibit cell growth and fibrillar collagen metabolism. *Circulation* **96:**3954–3962.
Ohnishi J, Ishido M, Shibata T, Inagami T, Murakami K and Miyazaki H (1992) The rat angiotensin II AT_{1a} receptor couples with three different signal transduction pathways. *Biochem Biophys Res Commun* **186:**1094–1101.
Ohyama K, Yamano Y, Sano T, Nakagomi Y, Hamakubo T, Morishima I and Inagami T (1995) Disulfide bridges in extracellular domains of angiotensin II receptor type IA. *Regul Pept* **57:**141–147.
Okuda M, Kawahara Y and Yokoyama M (1996) Angiotensin II type 1 receptor-mediated activation of Ras in cultured rat vascular smooth muscle cells. *Am J Physiol* **271:**H595–H601.
Okuya S, Inenago K, Kaneko T and Yamashita H (1987) Angiotensin II sensitive neurons in the supraoptic nucleus, subfornical organ and anteroventral third ventricle of rats *in vitro. Brain Res* **402:**58–67.
Oldfield BJ, Badoer E, Hards DK and McKinley MD (1994) Fos production in retrogradely labeled neurons of the lamina terminalis following intravenous infusion of either hypertonic saline or angiotensin II. *Neuroscience* **60:**255–262.
Oliverio MI, Best CF, Kim HS, Arendshorst WJ, Smithies O and Coffman TM (1997) Angiotensin II responses in AT_{1A} receptor-deficient mice: A role for AT_{1B} receptors in blood pressure regulation. *Am J Physiol* **272:**F515–F520.
Oliverio MI, Kim H-S, Ito M, Le T, Audoly L, Best CF, Hiller S, Kluckman K, Maeda N, Smithies O and Coffman TM (1998a) Reduced growth, abnormal kidney structure, and type 2 (AT_2) angiotensin receptor-mediated blood pressure regulation in mice lacking both AT_{1A} and AT_{1B} receptors for angiotensin II. *Proc Natl Acad Sci USA* **95:**15496–15501.
Oliverio MI, Madsen K, Best CF, Ito M, Maeda N, Smithies O and Coffman TM (1998b) Renal growth and development in mice lacking AT_{1A} receptors for angiotensin II. *Am J Physiol* **274:**F43–F50.
Orth SR, Weinreich T, Bonisch S, Weih M and Ritz E (1995) Angiotensin II induces hypertrophy and hyperplasia in adult human mesangial cells. *Exp Nephrol* **3:**23–33.
Ozono R, Wang ZQ, Moore AF, Inagami T, Siragy HM and Carey RM (1997) Expression of the Subtype 2 Angiotensin (AT_2) Receptor Protein in Rat Kidney. *Hypertension* **30:**1238–1246.
Page IH (1990) Hypertension research. A memoir 1920–1960. *Hypertension* **16:**199–200.
Page IH and Helmer OM (1940) A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin activator. *J Exp Med* **71:**29–42.
Pals DT, Denning GS Jr and Keenan RE (1979) Historical development of saralasin. *Kidney Int* **15:**S7–S10.
Pan MG, Florio T and Stork PJ (1992) G-protein activation of a hormone-stimulated phosphatase in human tumor cells. *Science (Wash DC)* **256:**1215–1217.
Papadimitriou A and Worcel M (1974) Dose-response curves for angiotensin II and synthetic analogues in three types of smooth muscle: Existence of different forms of receptor sites for angiotensin II. *Br J Pharmacol* **50:**292–297.
Paradis P, Dali-Youcef N, Paradis FW, Thibault G and Nemer M (2000) Overexpression of angiotensin II type 1 receptor in cardiomyocytes induces cardiac hypertrophy and remodelling. *Proc Natl Acad Sci USA* **97:**931–936.
Parnot C, Bardin S, Misery-Lenkei S, Guedin D, Corvol P and Clauser E (2000) Systematic identification of mutations that constitutively activate the angiotensin II type 1A receptor by screening a randomly mutated cDNA library with an original pharmacological bioassay. *Proc Natl Acad Sci USA* **97:**7615–7620.
Patel JM, Li YD, Zhang J, Gelband CH, Raizada MK and Block ER (1999) Increased expression of calreticulin is linked to Ang IV-mediated activation of lung endothelial NOS. *Am J Physiol* **277:**L794–L801.
Patel JM, Martens JR, Li YD, Gelband CH, Raizada MK and Block ER (1998) Angiotensin IV receptor-mediated activation of lung endothelial NOS is associated with vasorelaxation. *Am J Physiol* **275:**L1061–L1068.
Payet MD, Bilodeau L, Drolet P, Ibarrondo J, Guillon G and Gallo-Payet N (1995) Modulation of a Ca^{2+}-activated K^+ channel by angiotensin II in rat adrenal glomerulosa cells: Involvement of a G protein. *Mol Endocrinol* **9:**935–947.
Peach MJ (1977) Renin-angiotensin system: Biochemistry and mechanisms of action. *Physiol Rev* **57:**313–370.
Peach MJ and Levens RN (1980) Molecular approaches to the study of angiotensin receptors. *Adv Exp Med Biol* **130:**171–191.
Pederson ES, Harding JW and Wright JW (1998) Attenuation of scopolamine-induced spatial learning impairments by an Angiotensin IV analog. *Regul Pept* **74:**97–103.
Petit A, Geoffroy P and Belisle S (1996) Expression of angiotensin II type-I receptor and phospholipase C-linked G alpha q/11 protein in the human placenta. *J Soc Gynecol Investig* **3:**316–321.
Phillips MI (1987) Functions of angiotensin II in the central nervous system. *Annu Rev Physiol* **49:**413–435.
Phillips MI, Wang H, Kimura B, Speth RC and Ghazi N (1997) Brain angiotensin and the female reproductive cycle. *Adv Exp Med Biol* **377:**357–370.
Phillips MI and Sumners C (1998) Angiotensin II in central nervous system physiology. *Regul Pept* **78:**1–11.
Pierson ME and Freer RJ (1992) Analysis of the active conformation of angiotensin II: A comparison of AII and non-peptide AII antagonists. *Pept Res* **5:**102–125.
Pierzchalski P, Reiss K, Cheng W, Cirielli C, Kajstura J, Nitahara JA, Rizk M, Capogrossi MC and Anversa P (1997) p53 induces myocyte apoptosis via the activation of the renin-angiotensin system. *Exp Cell Res* **234:**57–65.
Pobiner BF, Hewlett EL and Garrison JC (1985) Role of Ni in coupling angiotensin receptors to inhibition of adenylate cyclase in hepatocytes. *J Biol Chem* **260:** 16200–16209.
Pobiner BF, Northup JK, Bauer PH, Fraser ED and Garrison JC (1991) Inhibitory GTP-binding regulatory protein G_{i3} can couple angiotensin II receptors to inhibition of adenylyl cyclase in hepatocytes. *Mol Pharmacol* **40:**156–167.
Poitras M, Sidibe A, Richard DE, Chretien L and Guillemette G (1998) Effect of uncoupling agents on AT_1 receptor affinity for antagonist analogs of angiotensin II. *Recept Channels* **6:**65–72.
Pollman MJ, Yamada T, Horiuchi M and Gibbons GH (1996) Vasoactive substances regulate vascular smooth muscle cell apoptosis. Countervailing influences of nitric oxide and angiotensin II. *Circ Res* **79:**748–756.
Pucell AG, Hodge JC, Sen I, Bumpus FM and Husain A (1991) Biochemical properties of the ovarian granulosa cell type 2 angiotensin II receptor. *Endocrinology* **128:**1291–1296.
Quian H, Pipolo L and Thomas WG (1999) Identification of protein kinase phosphorylation sites in the angiotensin II (AT_{1A}) receptor. *Biochem J* **343:**637–644.
Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK and Harrison DG (1996) Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* **97:**1916–1923.
Ramaraj P, Kessler SP, Colmenares C and Sen GC (1998) Selective restoration of male fertility in mice lacking angiotensin converting enzymes by sperm-specific expression of the testicular isozyme. *J Clin Invest* **102:**371–378.
Rangel LB, Caruso-Neves C, Lara LS, Brasil FL and Lopes AG (1999) Angiotensin II

activates the ouabain-insensitive Na^+-ATPase from renal proximal tubules through a G-protein. *Biochim Biophys Acta* **1416:**309–319.
Rao GN and Berk BC (1992) Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. *Circ Res* **70:**593–599.
Re RN, Vizard DL, Brown J, LeGros L and Bryan SE (1984) Angiotensin II receptors in chromatin. *J Hypertens* **(Suppl 2):**S271–S273.
Reagan LP, Flanagan LM, Yee DK, Ma LY, Sakai RR and Fluherty SJ (1994) Immunohistochemical mapping of angiotensin type 2 (AT_2) receptors in rat brain. *Brain Res* **662:**45–59.
Reagan LP, Thevenian M, Yang XD, Siemens IR, Yee DK, Reisine T and Fluharty SJ (1993a) Development of polyclonal antibodies against angiotensin type 2 receptors. *Proc Natl Acad Sci USA* **90:**7956–7960.
Reagan LP, Ye X, Maretzski CH and Fluharty SJ (1993b) Down-regulation of angiotensin II receptor subtypes and desensitization of cyclic GMP production in neuroblastoma N1E-115 cells. *J Neurochem* **60:**24–31.
Reagan LP, Ye XH, Mir R, DePalo LR and Fluherty SJ (1990) Up-regulation of angiotensin II receptors by *in vitro* differentiation of murine N1E-115 neuroblastoma cells. *Mol Pharmacol* **38:**878–886.
Reaux A, Fournie-Zaluski MC, David C, Zini S, Roques BP, Corvol P and Llorens-Cortes C (1999) Aminopeptidase A inhibitors as potential central antihypertensive agents. *Proc Natl Acad Sci USA* **96:**13415–13420.
Regitz-Zagrosek V, Friedel N, Heymann A, Bauer P, Neuss M, Rolfs A, Steffen C, Hildebandt A, Hetzer R and Fleck E (1995) Regulation chamber localization and subtype distribution of angiotensin receptors in human hearts. *Circulation* **91:**1461–1471.
Regitz-Zagrosek V, Neuss M, Holzmeister J, Warnecke C and Fleck E (1996) Molecular biology of angiotensin receptors and their role in human cardiovascular disease. *J Mol Med* **74:**233–251.
Richard DE, Chretien L, Caron M and Guillemette G (1997a) Stimulation of the angiotensin II type I receptor on bovine adrenal glomerulosa cells activates a temperature-sensitive internalization-recycling pathway. *Mol Cell Endocrinol* **129:**209–218.
Richard DE, Laporte SA, Bernier SG, Leduc R and Guillemette G (1997b) Desensitization of AT_1 receptor-mediated cellular responses requires long term receptor down-regulation in bovine adrenal glomerulosa cells. *Endocrinology* **138:**3828–3835.
Riedel MW, Anneser F and Haberl RL (1995) Different mechanisms of L-arginine induced dilation of brain arterioles in normotensive and hypertensive rats. *Brain Res* **671:**21–26.
Rittel W, Iselin B, Kappeler H, Riniker B and Schwyzer R (1957) Synthesis of a highly effective angiotensin II amide (L-asparagynyl-L-arginyl-L-valyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine [in German]. *Helv Chim Acta* **40:**614–624.
Roberts KA, Krebs LT, Kramár EA, Shaffer MJ, Harding JW and Wright JW (1995) Autoradiographic identification of brain angiotensin IV binding sites and differential c-Fos expression following intracerebroventricular injection of angiotensin II and IV in rats. *Brain Res* **682:**13–21.
Robinson GS, Crooks GB, Shinkman PG and Gallagher M (1989) Behavioral effects of MK-801 mimic deficits associated with hippocampal damage. *Psychobiology* **17:**156–164.
Rogg H, de Gasparo M, Graedel E, Stulz P, Burkart F, Eberhard M and Erne P (1996) Angiotensin II-receptor subtypes in human atria and evidence for alterations in patients with cardiac dysfunction. *Eur Heart J* **17:**1112–1120.
Rogg H, de Gasparo M, Graedel E, Stulz P and Erne P (1991) Identifikation von Angiotensin II receptor subtype am human Vorhofgewebe. *Schweiz Med Wochenschr* **121:**23.
Rogg H, Schmid A and de Gasparo M (1990) Identification and characterization of angiotensin II receptor subtypes in rabbit ventricular myocardium. *Biochem Biophys Res Commun* **173:**416–422.
Rolfs A, Weber-Rolfs I, Regitz-Zagrosek V, Kallisch H, Riedel K and Fleck E (1994) Genetic polymorphisms of the angiotensin II type 1 (AT_1) receptor gene. *Eur Heart J* **15 (Suppl D):**108–112.
Roman RJ and Cowley AW Jr (1985) Characterization of a new model for the study of pressure natriuresis in the rat. *Am J Physiol* **248:**F190–F198.
Rondeau JJ, McNicoll N, Escher E, Meloche S, Ong H and DeLean A (1990) Hydrodynamic properties of the angiotensin II receptor from bovine adrenal zona glomerulosa. *Biochem J* **268:**443–448.
Röper D, Krüger P, Grötzinger J, Wollmer A and Strassburger W (1995) Models of G-protein coupled receptors revised for family-wide compliance with experimental data. A new sequence accommodation suggested for helix G. *Recept Channels* **3:**97–106.
Rowe BP, Grovwe DL, Saylor DL and Speth RC (1990a) Angiotensin II receptor subtypes in the rat brain. *Eur J Pharmacol* **186:**339–342.
Rowe BP, Kalivas PW and Speth RC (1990b) Autoradiographic localization of angiotensin II receptor 2 binding sites on noradrenergic neurons of the locus coeruleus of the rat. *J Neurochem* **55:**535–540.
Rowe BP, Saylor DL and Speth RC (1992) Analysis of angiotensin II receptor subtypes in individual rat brain nuclei. *Neuroendocrinology* **55:**563–573.
Ruan X, Wagner C, Chatziantoniou C, Kurtz A and Arendshorst W (1997) Regulation of angiotensin II receptor AT_1 subtypes in renal afferent arterioles during chronic changes in sodium diet. *J Clin Invest* **99:**1072–1081.
Saavedra JM (1992) Brain and pituitary angiotensin. *Endocr Rev* **13:**324–380.
Saavedra JM, Viswanathan M and Shigematsu K (1993) Localization of angiotensin AT_1 receptors in the rat heart conduction system. *Eur J Pharmacol* **235:**301–303.
Sabri A, Govindarajan G, Griffin TM, Byron KL, Samarel AM and Lucchesi PA (1998) Calcium- and protein kinase C-dependent activation of the tyrosine kinase PYK2 by angiotensin II in vascular smooth muscle. *Circ Res* **83:**841–851.
Sadoshima J, Qiu Z, Morgan JP and Izumo S (1995) Angiotensin II and other hypertrophic stimuli mediated by G protein-coupled receptors activate tyrosine kinase, mitogen-activated protein kinase, and 90-kD S6 kinase in cardiac myocytes. The critical role of Ca^{2+}-dependent signaling. *Circ Res* **76:**1–15.
Saltman S, Baukal A, Waters S, Bumpus FM and Catt KJ (1975) Competitive binding activity of angiotensin II analogues in an adrenal cortex radioligand-receptor assay. *Endocrinology* **97:**275–282.
Sandberg K (1994) Structural analysis and regulation of angiotensin II receptors. *Trends Endocrinol Metab* **5:**28–35.
Sandberg K, Bor M, Ji H, Markwick A, Millan MA and Catt KJ (1990) Angiotensin II-induced calcium mobilization in oocytes by signal transfer through gap junctions. *Science (Wash DC)* **249:**298–301.
Sandberg K, Ji H and Catt KJ (1994) Regulation of angiotensin II receptors in rat brain during dietary sodium changes. *Hypertension* **23:**I-137-I-141.
Sandberg K, Ji H, Clark AJ, Shapira H and Catt KJ (1992a) Cloning and expression of a novel rat angiotensin II receptor. *J Biol Chem* **267:**9455–9458.
Sandberg K, Ji H, Iida T and Catt KJ (1992b) Intercellular communication between follicular AII receptors and Xenopus laevis oocytes: Mediation by an inositol 1,4,5-trisphosphate-dependent mechanism. *Cell Biol* **4:**157–167.
Sandberg K, Ji H, Millan MA and Catt KJ (1991) Amphibian myocardial angiotensin II receptors are distinct from mammalian AT_1 and AT_2 receptor subtypes. *FEBS Lett* **284:**281–284.
Sandmann St, Yu MH, Kaschina E and Unger T (1999) Differential roles of AT_1 and AT_2 receptor subtypes in the expression of Na^+-HCO_3^--symporter and Na^+-H^+-exchanger in the rat heart after myocardial infarction. *Dtsch Med Wochschr* **124 (Suppl 3):**S97 (P71).
Sano T, Ohyama K, Yamano Y, Nakagomi Y, Nakazawa S, Kikyo M, Shirai H, Blank JS, Exton JH and Inagami T (1997) A domain for G protein coupling in carboxyl-terminal tail of rat angiotensin II receptor type 1_A. *J Biol Chem* **272:**23631–23636.
Sardinia MF, Hanesworth JM, Krebs LT and Harding JW (1993) AT_4 receptor binding characteristics: D-amino acid- and glycine-substituted peptides. *Peptides* **14:**949–954.
Sardinia MF, Hanesworth JM, Krishnan R and Harding JW (1994) AT_4 receptor structure-binding relationship: N-terminal modified angiotensin IV analogs. *Peptides* **15:**1399–1406.
Sasaki K, Yamano Y, Bardhan S, Iwai N, Murray JJ, Hasegawa M, Matsuda Y and Inagami T (1991) Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. *Nature (Lond)* **351:**230–233.
Sayeski PP, Ali MS, Semeniuk DJ, Doan TN and Bernstein KE (1998) Angiotensin II signal transduction pathways. *Regul Pept* **78:**19–29.
Schambye HT, Hjorth SA, Bergsma DJ, Sathe G and Schwartz TW (1994) Differentiation between binding sites for angiotensin II and nonpeptide antagonists on the angiotensin II type 1 receptor. *Proc Natl Acad Sci USA* **91:**7046–7050.
Scheuer DA and Perrone MH (1993) Angiotensin type 2 receptors mediate depressor phase of biphasic pressure response to angiotensin. *Am J Physiol* **264:**R917–R923.
Schiavone MT, Khosla MC and Ferrario CM (1990) Angiotensin-(1–7): Evidence for novel actions in the brain. *J Cardiovasc Pharmacol* **16 (Suppl 4):**19–24.
Schieffer B, Paxton WG, Chai Q, Marrero MB and Bernstein KE (1996) Angiotensin II controls p21ras activity via pp60c-src. *J Biol Chem* **271:**10329–10333.
Schmidt S, Beige J, Walla-Friedel M, Michel MC, Sharma AM and Ritz E (1997) A polymorphism in the gene for the angiotensin II type 1 receptor is not associated with hypertension. *J Hypertens* **15:**1385–1388.
Schmitz U and Berk BC (1997) Angiotensin II signal transduction. Stimulation of multiple mitogen-activated protein kinase pathways. *Trends Endocrinol Metab* **8:**261–266.
Schnermann JB, Traynor T, Yang T, Hhuang YG, Oliverio MI, Coffman T and Briggs JP (1997) Absence of tubuloglomerular feedback responses in AT_{1A} receptor-deficient mice. *Am J Physiol* **273:**F315–F320.
Schorb W, Booz GW, Dostal DE, Conrad KM, Chang KC and Baker KM (1993) Angiotensin II is mitogenic in neonatal rat cardiac fibroblasts. *Circ Res* **72:**1245–1254.
Schunkert H (1997) Polymorphism of the angiotensin-converting enzyme gene and cardiovascular disease. *J Mol Med* **75:**867–875.
Scott AL, Chang RS, Lotti VJ and Siegel PK (1992) Cardiac angiotensin receptors: Effects of selective angiotensin II receptor antagonists Dup 753 and PD123177 in rabbit heart. *J Pharmacol Exp Ther* **261:**931–935.
Sechi LA, Griffin CA, Giacchetti G, Valentin JP, Llorens-Cortes C, Corvol P and Schambelan M (1996) Tissue-specific regulation of type 1 angiotensin II receptor mRNA levels in the rat. *Hypertension* **28:**403–408.
Sechi LA, Grady EF, Griffin CA, Kalinyak JE and Schambelan M (1992a) Distribution of angiotensin II receptor subtypes in rat and human kidney. *Am J Physiol* **262:**F236–F240.
Sechi LA, Griffin CA, Grady EF, Kalinyak JE and Schambelan M (1992b) Characterization of angiotensin II receptor subtypes in rat heart. *Circ Res* **71:**1482–1489.
Semple PF, Boyd AS, Dawes PM and Morton JJ (1976) Angiotensin II and its heptapeptide (2–8) hexapeptide (3–8) and pentapeptide (4–8). Metabolites in arterial and venous blood of man. *Circ Res* **39:**671–678.
Sen I, Bull HG and Soffer RL (1984) Isolation of an angiotensin II-binding protein from liver. *Proc Natl Acad Sci USA* **81:**1679–1683.
Servant G, Dudley DT, Escher E and Guillemette G (1994) The marked disparity between the sizes of angiotensin type 2 receptors from different tissues is related to different degree of N-glycosylation. *Mol Pharmacol* **45:**1112–1118.
Servant G, Dudley DT, Escher E and Guillemette G (1996) Analysis of the role of N-glycosylation in cell surface expression and binding properties of angiotensin II type-2 of rat pheochromocytoma cells. *Biochem J* **313:**297–304.
Shanmugam S, Lenkel ZG, Gase JMR, Corvol PL and Llorent-Cortes CM (1995) Ontogeny of angiotensin II type 2 (AT_2) receptor mRNA in the rat. *Kidney Int* **47:**1095–1100.
Shanmugam S, Monnot C, Corvol P and Gasc JM (1994) Distribution of type 1 angiotensin II receptor subtype messenger RNAs in the rat fetus. *Hypertension* **23:**137–141.
Shapiro MS, Wollmuth LP and Hille B (1994) Angiotensin II inhibits calcium and M current channels in rat sympathetic neurons via G proteins. *Neuron* **12:**1319–1329.

Sharp M, Fettes D, Brooker G, Clark A, Peters J, Fleming S and Mullins J (1996) Targeted inactivation of the Ren-2 gene in mice. *Hypertension* **28:**1126–1131.
Shibata T, Suzuki C, Ohnishi J, Murakami K and Miyazaki H (1996) Identification of regions in the human angiotensin II receptor type 1 responsible for G_i and G_q coupling by mutagenesis study. *Biochem Biophys Res Commun* **218:**383–389.
Shinkai T and Ooka H (1995) Effect of angiotensin II on the proliferation of mammotrophs from the adult rat anterior pituitary in culture. *Peptides* **16:**25–29.
Shirai H, Takahashi K, Katada T and Inagami T (1995) Mapping of G protein coupling sites of the angiotensin II type 1 receptor. *Hypertension* **25(4 Pt 2):**726–730.
Siemens IR, Adler HJ, Addya Mah SJ and Fluharty SJ (1991) Biochemical analysis of solubilized angiotensin II receptors from murine neuroblastoma N1E-115 cells by covalent cross-linking and affinity purification. *Mol Pharmacol* **40:**717–726.
Siemens IR, Reagan LP, Yee DK and Fluharty SJ (1994a) Biochemical characterization of two distinct angiotensin AT_2 receptor population in murine neuroblastoma N1E-115 cells. *J Neurochem* **62:**2106–2115.
Siemens IR, Yee DK, Reagan LP and Fluharty SJ (1994b) Affinity purification of angiotensin type 2 receptors from N1E-115 cells: Evidence for agonist-induced formation of multimeric complexes. *J Neurochem* **62:**257–264.
Sims C, Ashby K and Douglas JG (1992) Angiotensin II-induced changes in guanine nucleotide binding and regulatory proteins. *Hypertension* **19:**146–152.
Siragy HM and Carey RM (1996) The subtype-2 (AT_2) angiotensin receptor regulates renal cyclic guanosine 3′, 5′-mono-phosphate and AT_1 receptor mediated prostaglandin E_2 production in conscious rats. *J Clin Invest* **97:**1978–1982.
Siragy HM and Carey RM (1997a) The subtype 2 (AT_2) angiotensin receptor mediates renal production of nitric oxide in conscious rats. *J Clin Invest* **100:**264–269.
Siragy HM and Carey RM (1997b) The subtype 2 angiotensin receptor regulates renal prostaglandin F-2-alpha formation in conscious rats. *Am J Physiol* **42:**R1103–R1107.
Siragy HM and Carey RM (1999) Protective role of the angiotensin AT_2 receptor in a renal wrap hypertension model. *Hypertension* **33:**1237–1242.
Siragy HM, de Gasparo M and Carey RM (2000) Angiotensin type 2 receptor mediates valsartan-induced hypotension in conscious rats. *Hypertension* **35:**1074–1077.
Siragy HM, Inagami T, Ichiki T and Carey RM (1999) Sustained hypersensitivity to angiotensin II and its mechanism in mice lacking the subytype-2 (AT2) angiotensin receptor. *Proc Natl Acad Sci USA* **25:**6506–6510.
Sironi L, Mussoni L, Calvio AM, Arnaboldi L, Corsini A and Tremoli E (1999) Effect of valsartan, a type-1 angiotensin II receptor (AT_1) antagonist, on PAI-1 accumulation in smooth muscle cells. *Thromb Haemost* Suppl A:2485.
Skeggs LT, Lentz KE, Kahn Jr, Shumway NP and Woods KR (1956) The amino acid sequence of hypertensin II. *J Exp Med* **104:**193–197.
Slice LW, Wong HC, Sternini C, Grady EF, Bunnett NW and Walsh JH (1994) The conserved NPX_nY motif present in the gastrin-releasing peptide receptor is not a general sequestration sequence. *J Biol Chem* **269:**21755–21762.
Smith RD (1999) Marked ion dependence of ^{125}I-Angiotensin I binding to atypical sites on *Mycoplasma hyorhinis*. *Peptides* **20:**165–169.
Smith RD, Baukal AJ, Dent P and Catt KJ (1999) Raf-1 kinase activation by angiotensin II in adrenal glomerulosa cells: Roles of G_i, phosphatidylinositol 3-kinase, and Ca^{2+} influx. *Endocrinology* **140:**1385–1391.
Smith RD, Hunyady L, Olivares-Reyes A, Mihalik B, Jayadev S and Catt KJ (1998) Agonist-induced phosphorylation of the angiotensin AT_{1a} receptor is localized to a serine/threonine-rich region of its cytoplasmic tail. *Mol Pharmacol* **54:**935–941.
Snyder SH and Bredt DS (1991) Nitric oxide as a neuronal messenger. *Trends Pharmacol Sci* **12:**125–128.
Song K, Allen AM, Paxinos G and Mendelsohn Faa (1992) Mapping of angiotensin II receptor subtype heterogeneity in rat brain. *J Comp Neurol* **316:**467–484.
Speth RC and Kim KH (1990) Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II p-aminophenylalanine^8 angiotensin II. *Biochem Biophys Res Commun* **169:**997–1006.
Speth RC, Rowe BP, Grove KL, Carter MR and Saylor D (1991) Sulfhydryl reducing agents distinguish two subtypes of angiotensin II receptors in the rat brain. *Brain Res* **548:**1–8.
Stanhope KJ, Choules M, Yudko E and Dourish CT (1994) Re-evaluation of the effects of putative cognitive disrupters in the reinforced-alternation T-maze task in the rat. *Br J Pharmacol* **112:**15P.
Steckelings UM, Bottari SP, Stoll M, Wagner J and Unger T (1998) Repression of c-fos and c-jun gene expression is not part of AT_2 receptor coupled signal transduction. *J Mol Med* **76:**202–207.
Steele MK, Negro-Vilar A and McCann SM (1981) Effect of angiotensin II on in vivo and in vitro release of anterior pituitary hormones in the female rat. *Endocrinology* **109:**893–899.
Stoll M, Steckelings UM, Paul M, Bottari SP, Metzger R and Unger T (1995) The angiotensin AT_2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. *J Clin Invest* **95:**651–657.
Streuli M, Krueger NX, Thai T, Tang M and Saito H (1990) Distinct functional roles of the two intracellular phosphatase like domains of the receptor-linked protein tyrosine phosphatase LCA and LAR. *EMBO J* **9:**2399–2407.
Stroth U, Meffert S, Gallinat S and Unger T (1998) Angiotensin II and NGF differentially influence microtubule proteins in PC12W cells –Role of the AT_2 receptor. *Mol Brain Res* **53:**187–195.
Stroth U, Blume A, Mielke K and Unger T (2000) Angiotensin AT_2 receptor stimulates Erk1 and Erk2 in quiscent but inhibits ERK in NGF-stimulated PC12W cells. *Mol Brain Res*, in press.
Su B, Martin MM, Beason KB, Miller PJ and Elton TS (1994) The genomic organization and functional analysis of the promoter for the human angiotensin II type 1 receptor. *Biochem Biophys Res Commun* **204:**1039–1046.
Sugaya T, Nishimatsu S, Tanimoto K, Takimoto E, Yamagishi T, Imamura K, Goto S, Imaizumi K, Hisada Y, Otsuka A, Uchida H, Sugiura M, Fukuta K, Fukamizu A and Murakami K (1995) Angiotensin II type 1a receptor-deficient mice with hypotension and hyperreninemia. *J Biol Chem* **270:**18719–18722.
Sugiura N, Hagiwara H and Hirose S (1992) Molecular cloning of porcine soluble angiotensin-binding protein. *J Biol Chem* **267:**18067–18072.
Sumners C and Myers LM (1991) Angiotensin II decreases cGMP levels in neuronal cultures from rat brain. *Am J Physiol* **260:**C79–C87.
Sumners C and Phillips MI (1983) Central injection of angiotensin II alters catecholamine activity in rat brain. *Am J Physiol* **244:**R257–R263.
Sumners C, Tang W, Zelezna B and Raizada MK (1991) Angiotensin II receptor subtypes and coupled with distinct signal-transduction mechanisms in neurons and astrocytes from rat brain. *Proc Natl Acad Sci USA* **88:**7567–7571.
Suzuki J, Matsubara H, Urakami M and Inada M (1993) Rat angiotensin II (Type 1A) receptor mRNA regulation and subtype expression in myocardial growth and hypertrophy. *Circ Res* **73:**439–447.
Swanson GN, Hanesworth JM, Sardinia MF, Coleman JK, Wright JW, Hall KL, Miller-Wing AV, Stobb JW, Cook VI, Harding EC and Harding JW (1992) Discovery of a distinct binding site for angiotensin II (3–8), a putative angiotensin IV receptor. *Regul Pept* **40:**409–419.
Szombathy T, Szalai C, Katalin B, Palicz T, Romics L and Csaszar A (1998) Association of angiotensin II type 1 receptor polymorphism with resistant essential hypertension. *Clin Chim Acta* **269:**91–100.
Takahashi T, Taniguchi T, Konishi H, Kikkawa U, Ishikawa Y and Yokoyama M (1999) Activation of Akt/protein kinase B after stimulation with angiotensin II in vascular smooth muscle cells. *Am J Physiol* **276:**H1927–H1934.
Takami S, Katsuya T, Rakugi H, Sato N, Nakata Y, Kamitani A, Miki T, Higaki J and Ogihara T (1998) Angiotensin II type 1 receptor gene polymorphism is associated with increase of left ventricular mass but not with hypertension. *Am J Hypertens* **11:**316–321.
Takayanagi R, Ohnaka K, Sakai Y, Ikuyama S and Nawata H (1994) Molecular cloning and characterization of the promoter for human type-1 angiotensin II receptor gene. *Biochem Biophys Res Commun* **200:**1264–1270.
Takeuchi K, Alexander RW, Nakamura Y, Tsujino T and Murphy TJ (1993) Molecular structure and transcriptional function of the rat vascular AT_{1a} angiotensin receptor gene. *Circ Res* **73:**612–621.
Tamaki K, Saku Y and Ogata J (1992) Effects of angiotensin and atrial natriuretic peptide on the cerebral circulation. *J Cereb Blood Flow Metab* **12:**318–325.
Tanaka M, Ohnishi J, Ozawa Y, Sugimoto M, Usuki S, Naruse M, Murakami K and Miyazake H (1995) Characterization of angiotensin II receptor type 2 during differentiation and apoptosis of rat ovarian cultured granulosa cells. *Biochem Biophys Res Commun* **207:**593–598.
Tanaka M, Tsuchida S, Imai T, Fujii N, Miyazaki H, Ichiki T, Naruse M and Inagami T (1999) Vascular response to angiotensin II is exaggerated through an upregulation of AT1 receptor in AT2 knockout mice. *Biochem Biophys Res Commun* **258:**194–198.
Tang H, Guo DF, Porter JP, Wanaka Y and Inagami T (1998) Role of cytoplasmic tail of the type 1A angiotensin II receptor in agonist- and phorbol ester-induced desensitization. *Circ Res* **82:**523–531.
Tang SS, Rogg H, Schumacher RM and Dzau VJ (1992) Characterization of nuclear angiotensin-II-binding sites in rat liver and comparison with plasma membrane receptors. *Endocrinology* **131:**374–380.
Tanimoto K, Sugiyama F, Goto Y, Ishida J, Takimoto E, Yagami K, Fukamizu A and Murakami K (1994) Angiotensinogen-deficient mice with hypotension. *J Biol Chem* **269:**31334–31337.
Tazawa S, Nakane T and Chiba S (1999) Angiotensin II type 1 receptor blockade prevents up-regulation of angiotensin II type 1A receptors in rat injured artery. *J Pharmacol Exp Ther* **288:**898–904.
Tea BS, Der Sarkissian S, Hamet P and Debois D (1998) Apoptotic and antiproliferative role of angiotensin II receptor subtype 2 in the heart of spontaneous hypertensive rats in vivo. *Hypertension* **232:**63.
Thekkumkara TJ, Thomas WG, Motel TJ and Baker KM (1998) Functional role for the angiotensin II receptor (AT_{1A}) 3N-untranslated region in determining cellular responses to agonist: Evidence for recognition by RNA binding proteins. *Biochem J* **15:**255–264.
Thomas WG (1999) Regulation of angiotensin II type 1 (AT_1) receptor function. *Regul Pept* **79:**9–23.
Thomas WG, Baker KM, Booz GW and Thekkumkara TJ (1996) Evidence against a role for protein kinase C in the regulation of the angiotensin II (AT_{1A}) receptor. *Eur J Pharmacol* **295:**119–122.
Thomas WG, Baker KM, Motel TJ and Thekkumkara TJ (1995) Angiotensin II receptor endocytosis involves two distinct regions of the cytoplasmic tail. A role for residues on the hydrophobic face of a putative amphipathic helix. *J Biol Chem* **270:**22153–22159.
Thomas WG, Motel TJ, Kule CE, Karoor V and Baker KM (1998) Phosphorylation of the angiotensin II (AT_{1A}) receptor carboxyl terminus: A role in receptor endocytosis. *Mol Endocrinol* **12:**1513–1524.
Thomas WG, Qian H, Chang CS and Karnik S (2000) Agonist-induced phosphorylation of the angiotensin II (AT(1A)) receptor requires generation of a conformation that is distinct from the inositol phosphate-signaling state. *J Biol Chem* **275:**2893–2900.
Thompson JB, Wade SM, Harrison JK, Salafranca MN and Neubig RR (1998) Cotransfection of second and third intracellular loop fragments inhibit angiotensin AT_{1a} receptor activation of phospholipase C in HEK-293 cells. *J Pharmacol Exp Ther* **285:**216–222.
Thürmann PA, Kenedi P, Schmidt A, Harder S and Rietbrock N (1998) Influence of the angiotensin antagonist valsartan on left ventricular hypertrophy in patients with essential hypertension. *Circulation* **98:**2037–2042.
Tian Y, Balla T, Baukal AJ and Catt KJ (1995) Growth responses to angiotensin II in bovine adrenal glomerulosa cells. *Am J Physiol* **268:**E135–E144.
Tian Y, Baukal AJ, Sandberg K, Bernstein KE, Balla T and Catt KJ (1996) Properties of AT_{1a} and AT_{1b} angiotensin receptors expressed in adrenocortical Y-1 cells. *Am J Physiol* **270:**E831–E839.
Tigerstedt R and Bergman PG (1898) Niere und Kreislauf. *Scand Arch Physiol* **8:**223–271.

Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Carini DJ, Lee RJ, Wexler RR, Saye JAM and Smith RD (1993) Angiotensin II receptor and angiotensin II receptor antagonists. *Pharmacol Rev* **45:**205–251.

Tiret L, Bonnardeaux A, Poirier O, Ricard S, Marques-Vidal P, Evans A, Arveiler D, Luc G, Kee F, Ducimetiere P, Soubrier F and Cambien F (1994) Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. *Lancet* **344:**910–913.

Tissir F, Riviere M, DF, Tsuzuki S, Inagami T, Levan G, Szpirer J and Szpirer C (1995) Localization of the genes encoding the three rat angiotensin II receptors, Agtr1a, Agtr1b, Agtr2, and the human AGTR2 receptor respectively to rat chromosomes 17q12, 2q24 and Xq34, and the human Xq22. *Cytogenet Cell Genet* **71:**77–80.

Trumpp-Kallmeyer S, Hoflack J, Bruinvels A and Hibert M (1992) Modeling of G-protein-coupled receptors: Application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. *J Med Chem* **35:**3448–3462.

Tsuchida S, Matsusaka T, Chen X, Okubo S, Niimura F, Nishimura H, Fogo A, Utsunomiya H, Inagami T and Ichikawa I (1998) Murine double nullizygotes of the angiotensin type 1A and 1B receptor genes duplicate severe abnormal phenotypes of angiotensinogen nullizygotes. *J Clin Invest* **101:**755–760.

Tsutsumi K and Saavedra JM (1992) Heterogeneity of angiotensin II AT_2 receptors in the rat brain. *Mol Pharmacol* **41:**290–297.

Tsutsumi K, Strömberg C, Viswanathan M and Saavedra JM (1991) Angiotensin-II receptor subtypes in fetal tissues of rat-autoradiography, guanine nucleotide sensitivity and association with phosphoinositide hydrolysis. *Endocrinology* **129:** 1075–1082.

Tsutsumi K, Zorad S and Saavedra JM (1992b) The AT_2 subtype of the angiotensin II receptors has differential sensitivity to dithiothreitol in specific brain nuclei of young rats. *Eur J Pharmacol Mol Pharmacol* **226:**169–173.

Tsutsumi Y, Matsubara H, Masaki H, Kurihara H, Murasawa S, Takai S, Miyazaki M, Nozawa Y, Ozono R, Nakagawa K, Miwa T, Kawada N, Mori Y, Shibasaki Y, Tanaka Y, Fujiyama S, Koyama Y, Fujiyama A, Takahashi H and Iwasaka T (1999) Angiotensin II type 2 receptor overexpression activates the vascular kinin system and causes vasodilation. *J Clin Invest* **104:**925–935.

Tsutsumi Y, Matsubara H, Ohkubo N, Mori Y, Nozawa Y, Murasawa S, Kijima K, Maruyama K, Masaki H, Moriguchi Y, Shibasaki Y, Kamihata H, Inada M and Iwasaka T (1998) Angiotensin II type 2 receptor is upregulated in human heart with interstitial fibrosis, and cardiac fibroblasts are the major cell type for its expression. *Circ Res* **83:**1035–1046.

Tsuzuki S, Eguchi S and Inagami T (1996a) Inhibition of cell proliferation and activation of protein tyrosine phosphatase mediated by angiotensin II type 2 (AT_2) receptor in R3T3 cells. *Biochem Biophys Res Commun* **228:**825–830.

Tsuzuki S, Ichiki T, Nakakubo H, Kitami Y, Guo DF, Shirai H and Inagami T (1994) Molecular cloning and expression of the gene encoding human angiotensin II type 2 receptor. *Biochem Biophys Res Commun* **200:**1449–1454.

Tsuzuki S, Matoba T, Eguchi S and Inagami T (1996b) Angiotensin II type 2 receptor inhibits cell proliferation and activates tyrosine phosphatase. *Hypertension* **28:** 916–918.

Tufro-McReddie A, Harrison AL, Everett A and Gomez R (1993) Ontogeny of type 1 angiotensin II receptor gene expression in the rat. *J Clin Invest* **91:**530–537.

Tufro-McReddie AL, Romano J, Harris L, Ferder L and Gomez R (1995) Angiotensin II regulates nephrogenesis and renal vascular development. *Am J Physiol* **269:** F110–F115.

Underwood DJ, Strader CD, Rivero R, Patchett AA and Prendergast K (1994) Structural model of antagonist and agonist binding to the angiotensin II, AT_1 subtype, G protein coupled receptor. *Chem Biol* **1:**211–221.

Unger T (1999) The angiotensin type 2 receptor: Variations on an enigmatic theme. *J Hypertens* **17:**1775–1786.

Unger T, Chung O, Csikos T, Culman J, Gallinat S, Gohlke P, Höhle S, Meffert S, Stoll M, Stroth U and Zhu Y (1996) Angiotensin receptors. *J Hypertens* **14 (Suppl):**95–103.

Urata H, Healy B, Stewart RW, Bumpus FM and Hussain A (1989) Angiotensin II receptors in normal and failing human hearts. *J Clin Endocrinol Metab* **69:**54–66.

Urata H, Nishimura H and Ganter D (1996) Chymase-dependent angiotensin II forming system in humans. *Am J Hypertens* **9:**277–284.

Ushio-Fukai M, Alexander RW, Akers M and Griendling KK (1998) p38 mitogen-activated protein kinase is a critical component of the redox-sensitive signaling pathways activated by angiotensin II. *J Biol Chem* **273:**15022–15029.

Ushio-Fukai M, Alexander RW, Akers M, Lyons PR, Lassegue B and Griendling KK (1999a) Angiotensin receptor coupling to phospholipase D is mediated by the betagamma subunits of heterotrimeric G proteins in vascular smooth muscle cells. *Mol Pharmacol* **55:**142–149.

Ushio-Fukai M, Alexander RW, Akers M, Yin Q, Fujio Y, Walsh K and Griendling KK (1999b) Reactive oxygen species mediate the activation of Akt/Protein kinase B by angiotensin II in vascular smooth muscle cells. *J Biol Chem* **274:**22699–22704.

Van Bilsen M (1997) Signal transduction revisited: Recent developments in angiotensin II signaling in the cardiovascular system. *Cardiovasc Res* **36:**310–322.

Van Geel PP, Pinto YM, Buikema H and van Gilst WH (1998) Is the A1166C polymorphism of the angiotensin II type 1 receptor involved in cardiovascular disease? *Eur Heart J* **19:**G13–G17.

Van Kesteren CAM, Vanheugten HAA, Lamers JMJ, Saxena PR, Schalekamp MADH and Danser AHJ (1997) Angiotensin II mediated growth and antigrowth effects in cultured neonatal rat cardiac myocytes and fibroblasts. *J Mol Cell Cardiol* **29:**2147–2157.

Venema RC, Ju H, Venema VJ, Schieffer B, Harp JB, Ling BN, Eaton DC and Marrero MB (1998a) Angiotensin II-induced association of phospholipase Cγ1 with the G-protein-coupled AT_1 receptor. *J Biol Chem* **273:**7703–7708.

Venema RC, Venema VJ, Eaton DC and Marrero MB (1998b) Angiotensin II-induced tyrosine phosphorylation of signal transducers and activators of transcription 1 is regulated by Janus-activated kinase 2 and Fyn kinases and mitogen-activated protein kinase phosphatase 1. *J Biol Chem* **273:**30795–30800.

Vianello B, Clauser E, Corvol P and Monnot C (1998) Functional interactions of L-162,313 with angiotensin II receptor subtypes and mutants. *Eur J Pharmacol* **347:**113–118.

Villareal FJ, Kim NN, Ungab GD, Printz MP and Dillman WH (1993) Identification of functional angiotensin II receptors on rat cardiac fibroblast. *Circulation* **88:** 2849–2861.

Viswanathan M, de Oliveria AM, Correa FMA and Saavedra JM (1994a) Expression of novel-non-angiotensin II [^{125}I-]CGP 42112 binding site in healing wounds of the rat brain. *Brain Res* **658:**265–270.

Viswanathan M and Saavedra JM (1992) Expression of angiotensin II AT_2 receptors in the rat skin during experimental wound healing. *Peptides* **13:**783–786.

Viswanathan M, Seltzer A and Saavedra JM (1994b) Heterogeneous expression of angiotensin II AT_2 receptor in neointima of rat carotid artery and aorta after balloon catheter injury. *Peptides* **15:**1205–1212.

Viswanathan M, Tsutsumi K, Correa FMA and Saavedra JM (1991) Changes in expression of angiotensin receptor subtypes in the rat aorta during development. *Biochem Biophys Res Commun* **179:**1361–1367.

Voisin L, Larose L and Meloche S (1999) Angiotensin II stimulates serine phosphorylation of the adapter protein Nck: Physical association with the serine/threonine kinases Pak1 and casein kinase I. *Biochem J* **341:**217–223.

Wagner J, Gehlen F, Ciechanowicz A and Ritz E (1999) Angiotensin II receptor type 1 gene expression in human glomerulonephritis and diabetes mellitus. *J Am Soc Nephrol* **10:**545–551.

Wallakat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A, Baur E, Nissen E, Vitter K, Neichel D, Dudenhausen JW, Haller H and Luft FC (1999) Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT_1 receptor. *J Clin Invest* **103:**945–952.

Wang C, Jayadev S and Escobedo JA (1995a) Identification of a domain in the angiotensin II type 1 receptor determining G_q coupling by the use of receptor chimeras. *J Biol Chem* **270:**16677–16682.

Wang D, Yu X and Brecher P (1999) Nitric oxide inhibits angiotensin II-induced activation of the calcium-sensitive tyrosine kinase proline-rich tyrosine kinase 2 without affecting epidermal growth factor receptor transactivation. *J Biol Chem* **274:**24342–24348.

Wang L, Eberhard M and Erne P (1995b) Stimulation of DNA and RNA synthesis in cultured rabbit cardiac fibroblasts by antgiotensin IV. *Clin Sci (Colch)* **88:**557–562.

Wang W and Gershengorn MC (1999) Rat TRH receptor type 2 exhibits higher basal signaling activity than TRH receptor type 1. *Endocrinology* **140:**4916–4919.

Wang X and Murphy TJ (1998) Inhibition of cyclic AMP-dependent kinase by expression of a protein kinase inhibitor/enhanced green fluorescent fusion protein attenuates angiotensin II-induced type 1 AT_1 receptor mRNA down-regulation in vascular smooth muscle cells. *Mol Pharmacol* **54:**514–524.

Wang X, Nickenig G and Murphy TJ (1997) The vascular smooth muscle type I angiotensin II receptor mRNA is destabilized by cyclic AMP-elevating agents. *Mol Pharmacol* **52:**781–787.

Wayner MJ, Armstrong DL, Polan-Curtain JL and Denny JB (1993) Ethanol and diazepam inhibition of hippocampal LTP is mediated by angiotensin II and AT_1 receptors. *Peptides* **14:**441–444.

Wayner MJ, Ono T and Nolley D (1973) Effects of angiotensin II on central neurons. *Pharmacol Biochem Behav* **1:**679–691.

Webb ML, Liu ECK, Cohen RB, Hedberg A, Bogosian EA, Monshizadegan H, Molloy C, Serafino R, Moreland S, Murphy TJ and Dickinson Kej (1992) Molecular characterization of angiotensin II type II receptors in rat pheochromocytoma cells. *Peptides* **13:**499–508.

Weber H, Taylor DS and Molloy CJ (1994) Angiotensin II induces delayed mitogenesis and cellular proliferation in rat aortic smooth muscle cells. Correlation with expression of specific growth factors and reversal by suramin. *J Clin Invest* **93:**788–798.

Weber KT and Brilla CG (1991) Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation* **83:**1849–1865.

Weber KT, Brilla CG, Campbell SE, Guarda E, Zhou G and Srivam K (1993) Myocardial fibrosis: Role of angiotensin II and aldosterone. *Basic Res Cardiol* **88 (Suppl 1):**107–124.

Weber KT, Sun Y and Campbell SE (1995a) Structural remodelling of the heart by fibrous tissue: Role of circulating hormones and locall produced peptides. *Eur Heart J* **16 (Suppl N):**12–18.

Weber KT, Sun Y, Katwa LC and Cleutjens JPM (1995b) Connective tissue - a metabolic entity [Review]. *J Mol Cell Cardiol* **27:**107–120.

Weber KT, Sun Y, Tyagi SC and Cleutjens JPM (1994) Collagen network of the myocardium: Function, structural remodeling and regulatory mechanisms. *J Mol Cell Cardiol* **26:**279–292.

Weerackody RP, Chatterjee PK, Mistry SK, McLaren J, Hawksworth GM and McLay JS (1997) Selective antagonism of the AT_1 receptor inhibits the effect of angiotensin II on DNA and protein synthesis of rat proximal tubular cells. *Exp Nephrol* **5:**253–262.

Wenk H, Bigl V and Meyer U (1980) Cholinergic projects from magnocellular nuclei of the basal forebrain to cortical areas in rats. *Brain Res Rev* **2:**295–316.

Wess J (1993) Molecular basis of muscarinic acetylcholine receptor function. *Trends Pharmacol Sci* **14:**308–313.

Wharton J, Morgan M, Rutherford RAD, Catravas JD, Chester A, Whitehead BF, De Leval MR, Yacoub MH and Polak JM (1998) Differential distribution of angiotensin AT_2 receptors in the normal and failing human heart. *J Pharmacol Exp Ther* **284:**323–336.

Whitebread S, Mele M, Kamber B and de Gasparo M (1989) Preliminary biochemical characterization of two angiotensin II receptor subtypes. *Biochem Biophys Res Commun* **163:**284–291.

Whitebread SE, Taylor V, Bottari SP, Kamber B and de Gasparo M (1992) Radioiodinated CGP 42 112A: A novel high affinity and highly selective ligand for the characterization of angiotensin AT_2 receptors. *Biochem Biophys Res Comm* **181:** 1365–1371.

Wienen W, Hauel N, vanMeel JC, Narr B, Ries U and Entzeroth M (1993) Pharma-

cological characterization of the novel nonpeptide angiotensin II receptor antagonist BIBR 277. *Br J Pharmacol* **110:**245–252.
Wollert KC and Drexler H (1999) The renin-angiotensin system and experimental heart failure. *Cardiovasc Res* **43:**838–849.
Woodcock EA and Johnston CI (1982) Inhibition of adenylate cyclase by angiotensin II in rat renal cortex. *Endocrinology* **111:**1687–1691.
Wright JW and Harding JW (1992) Regulatory role of brain angiotensins in the control of physiological and behavioral responses. *Brain Res Rev* **17:**227–262.
Wright JW and Harding JW (1994) Brain angiotensin receptor subtypes in the control of physiological and behavioral responses. *Neurosci Biobehav Rev* **18:**21–53.
Wright JW and Harding JW (1995) Brain angiotensin receptor subtypes AT_1, AT_2, and AT_4 and their functions. *Regul Pept* **59:**269–295.
Wright JW and Harding JW (1997) Important roles for angiotensin III and IV in the brain renin-angiotensin system. *Brain Res Rev* **25:**96–124.
Wright JW, Krebs LT, Stobb JW and Harding JW (1995) The angiotensin IV system: Functional implications. *Front Neuroendocrinol* **16:**23–52.
Wright JW, Mizutani S, Murray CE, Amir HZ and Harding JW (1990) Aminopeptidase-induced elevations and reductions in blood pressure in the spontaneously hypertensive rat. *J. Hypertens* **8:**969–974.
Wright JW, Sullivan MJ, Bredl CR, Hanesworth JM, Cushing LL and Harding JW (1987) Delayed cerebroventricular metabolism of [^{125}I] angiotensins in the spontaneously hypertensive rat. *J Neurochem* **49:**651–654.
Wright JW, Sullivan MJ and Harding JW (1985) Dysfunction of central angiotensinergic aminopeptidase activity in spontaneously hypertensive rats. *Neurosci Lett* **61:**351–356.
Wyse B and Sernia C (1997) Growth hormone regulates AT-1a angiotensin receptors in astrocytes. *Endocrinology* **138:**4176–4180.
Xi X-P, Graf K, Goetze S, Fleck E, Hsueh WA and Law RE (1999) Central role of the MAPK pathway in Ang II-mediated DNA synthesis and migration in rat vascular smooth muscle cells. *Arterioscler Throm Vasc Biol* **19:**73–82.
Xiong H and Marshall KC (1994) Angiotensin II depresses glutamate depolarizations and excitatory postsynaptic potentials in locus coeruleus through angiotensin II subtype 2 receptors. *Neuroscience* **62:**163–175.
Yamada H, Akishita M, Lto M, Tamura K, David L, Lehtonen JYA, Dzau VJ and Horiuchi M (1999) AT_2 receptor and vascular smooth muscle cell differentiation in vascular development. *Hypertension* **33:**1414–1419.
Yamada T, Horiuchi M and Dzau VJ (1996) Angiotensin II type 2 receptor mediates programmed cell death. *Proc Acad Sci USA* **93:**156–160.
Yamano Y, Ohyama K, Kikyo M, Sano T, Nakagomi Y, Inoue Y, Nakamura N, Morishima I, Guo D-F, Hamakubo T and Inagami T (1995) Mutagenesis and the molecular modeling of the rat angiotensin II receptor (AT_1). *J Biol Chem* **270:**14024–14030.
Yang CR, Phillips MI and Renaud LP (1992) Angiotensin II receptor activation depolarizes rat supraoptic neurons in vitro. *Am J Physiol Reg Integ Comp Physiol* **321:**R1333–R1338.
Yang H, Lu D, Yu K and Raizada MK (1996) Regulation of the neuromodulatory actions of angiotensin II in brain neurons by the RAS-dependent mitogen-activated protein kinase pathway. *J Neurosci* **16:**4047–4058.
Yee DK, Reagan LP, Moga CN, Siemens IR and Fluharty SJ (1994) Angiotensin II stabilizes a multimeric type 2 (AT_2) receptor complex in murine neuroblastoma N1E-115 cells. *Regul Pept* **54:**355–366.
Yoshida M, Kikukawa M, Hisa H and Satoh S (1996) Modulation of nitric oxide and prostaglandin of the renal vascular response to angiotensin II (3–8). *Br J Pharmacol* **117:**885–890.
Yoshiyama M, Takeuchi K, Omura T, Kim S, Yamagishi H, Toda I, Teragaki M, Akioka K, Iwao H and Yoshikawa J (1999) Effects of candesartan and cilazapril on rats with myocardial infarction assessed by echocardiography. *Hypertension* **33:**961–968.
Yu M, Sandmann S and Unger T (1998) Angiotensin AT_2 receptor mediates expression of Na^+-HCO_3 symporter in rat myocardium after experimental myocardial infarction. *Hypertension* **32:**807.
Yu SS, Lefkowitz RJ and Hausdorff WP (1993) Beta-adrenergic receptor sequestration. A potential mechanism of receptor resensitization. *J Biol Chem* **268:**337–341.
Zarahn ED, Ye X, Ades AM, Reagan LP and Fluharty SJ (1992) Angiotensin-induced cyclic GMP production is mediated by multiple receptor subtypes and nitric oxide in N1E-115 Neuroblastoma cells. *J Neurochem* **58:**1960–1963.
Zhang J and Pratt RE (1996) The AT_2 receptor selectively associates with $Gi\alpha_2$ and $Gi\alpha_3$ in the rat fetus. *J Biol Chem* **271:**15026–15033.
Zhang JH, Hanesworth JM, Sardinia M, Alt JA, Wright JW and Harding JW (1999) Structural analysis of angiotensin IV receptor (AT_4) from selected bovine tissues. *J Pharmacol Exp Ther* **289:**1075–1083.
Zhou H, Moore GJ and Vogel HJ (1991) Proton NMR studies of angiotensin II and its analogs in aqueous solution. *J Protein Chem* **10:**333–343.
Zhu B and Herbert J (1996) Central antagonism of atrial natriuretic peptides on behavioral and hormonal responses to angiotensin II: Mapping with c-Fos. *Brain Res* **734:**55–60.
Zhu B and Herbert J (1997) Angiotensin II interacts with nitric oxide-cyclic GmP pathway in the central control of drinking behaviour: Mapping with c-fos and NADPH-diaphorase. *Neuroscience* **79:**543–553.
Zhu M, Gelband CH, Posner P and Sumners C (1998a) Angiotensin II type 1 receptor-mediated inhibition of voltage-dependent K^+ current in cultured neurons: Role of calcium/calmodulin-dependent protein kinase II. *FASEB J* **12:**A54.
Zhu M, Neubig RR, Wade SM, Posner P, Gelband CH and Sumners C (1997a) Modulation of K^+ and Ca^{2+} currents in cultured neurons by an angiotensin II type 1a receptor peptide. *Am J Physiol* **273:**C1040–C1048.
Zhu YC, Zhu YZ, Gohlke P, Strauss HN and Unger T (1997b) Effects of angiotensin-converting enzyme inhibition and angiotensin II receptor antagonism on cardiac parameters in left ventricular hypertrophy (Review). *Am J Cardiol* **80:**110A–117A.
Zhu Z, Zhang SH, Wagner C, Kurtz A, Maeda N, Coffman T and Arendshorst WJ (1998b) Angiotensin AT_{1B} receptor mediates calcium signaling in vascular smooth muscle cells of AT_{1A} receptor-deficient mice. *Hypertension* **31:**1171–1177.
Zhuo J, Alcorn D, Harris PJ and Mendelsohn Fao (1993) Localization and properties of angiotensin II receptors in rat kidney. *Kidney Int* **44 (Suppl 42):**40–46.
Zhuo J, Maric C, Harris PJ, Alcorn D and Mendelsohn Fao (1997) Localization and functional properties of angiotensin II AT_1 receptors in the kidney: Focus on renomedullary interstitial cells. *Hypertens Res* **20:**233–250.
Zhuo J, Moeller I, Jenkins T, Chai SY, Allen AM, Ohishi M and Mendelsohn FAO (1998) Mapping tissue angiotensin-converting enzyme and angiotensin AT_1, AT_2 and AT_4 receptors. *J Hypertens* **16:**2027–2037.
Zhuo J, Song K, Harris PJ and Mendelsohn Fao (1992) In vitro autoradiography reveals predominantly AT1 angiotensin II receptor subtypes in rat kidney. *Renal Physiol Biochem* **15:**231–239.
Zini S, Fournie-Zaluski M-C, Chauvel E, Roques BP, Corvol P and Lorens-Cortes C (1996) Identification of metabolic pathways of brain angiotensin II and III using specific aminopeptidase inhibitors: Predominant role of angiotensin III in the control of vasopressin release. *Proc Natl Acad Sci USA* **93:**11968–11973.
Zini S, Demassey Y, Fournie-Zaluski M-C, Bischoff L, Corvol P, Llorens-Cortes C and Sanderson P (1998) Inhibition of vasopressinergic neurons by central injection of a specific aminopeptidase A inhibitor. *NeuroReport* **9:**825–828.