

IV. International Union of Pharmacology Nomenclature of Adrenoceptors

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I. Introduction	121
II. α_1 -Adrenoceptor subtypes	123
A. Common α_1 -adrenoceptor characteristics	124
B. Pharmacologically defined α_1 -adrenoceptor subtypes	124
1. α_{1A} - and α_{1B} -adrenoceptors	124
2. α_{1H} -, α_{1L} -, and α_{1N} -adrenoceptors	124
C. Recombinant α_1 -adrenoceptor subtypes	124
1. $\alpha_{1a/d}$ -Adrenoceptors	124
2. α_{1b} -Adrenoceptors	125
3. α_{1c} -Adrenoceptors	125
D. Relationship between pharmacologically defined and recombinant α_1 -adrenoceptor subtypes	126
E. Additional α_1 -adrenoceptor subtypes?	126
III. α_2 -Adrenoceptor subtypes	126
A. Common α_2 -adrenoceptor characteristics	127
B. Pharmacologically defined α_2 -adrenoceptor subtypes	127
1. α_{2A}/α_{2B} -Adrenoceptors	127
2. α_{2C} - and α_{2D} -adrenoceptors	127
C. Recombinant α_2 -adrenoceptor subtypes	128
D. Relationship between pharmacologically defined and recombinant α_2 -adrenoceptor subtypes	129
E. Additional α_2 -adrenoceptor subtypes?	129
IV. β -Adrenoceptor subtypes	130
A. Common β -adrenoceptor characteristics	130
B. Pharmacologically defined β -adrenoceptor subtypes	130
C. Recombinant β -adrenoceptors subtypes	131
1. β_2 -Adrenoceptors	131
2. β_1 -Adrenoceptors	131
3. β_3 -Adrenoceptors	131
D. Relationship between pharmacologically defined and recombinant β -adrenoceptor subtypes	131
E. Are there additional β -adrenoceptor subtypes?	131
V. Signal transduction	132
A. α_1 -Adrenoceptor subtypes	133
B. α_2 -Adrenoceptor subtypes	133
C. β -Adrenoceptor subtypes	133
VI. Conclusions	133
VII. References	134

I. Introduction

Adrenoceptors mediate the central and peripheral actions of the primary sympathetic neurotransmitter, nor-

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adrenaline, and the primary adrenal medullary hormone (and central neurotransmitter), adrenaline. Adrenoceptors are found in nearly all peripheral tissues and on many neuronal populations within the central nervous system. Several types of neuronal varicosities have pre-junctional (or presynaptic) adrenoceptors serving as

auto- or heteroceptors that inhibit nerve-evoked release of a variety of neurotransmitters. Adrenoceptors mediate a variety of functions and have been of major interest for many years as targets for drug action. Both noradrenaline and adrenaline play important roles in the control of blood pressure, myocardial contractile rate and force, airway reactivity, and a variety of metabolic functions. Adrenoceptor activation in the locus coeruleus has direct and indirect effects on the activity of many other neuronal nuclei within the brain.

Adrenoceptors have divergent affinity for many synthetic drugs and, as new pharmacological tools have been identified, have been subdivided into an increasing number of distinct receptor subtypes. Drugs interacting with these subtypes have proven useful in a variety of diseases, involving all of the major organ systems. Some of these diseases include hypertension, angina pectoris, congestive heart failure, cardiac arrhythmia, asthma, depression, prostatic hypertrophy, and glaucoma.

For nearly a century it has been known that certain responses to adrenoceptor stimulation are insensitive to classical adrenoceptor antagonists such as the ergot alkaloids or β -haloalkylamines (Dale, 1906; Nickerson, 1949). In a manner analogous to the subclassification of the effects of acetylcholine as nicotinic or muscarinic, there were attempts to classify the effects of sympathomimetic amines as "excitatory" or "inhibitory." This qualitative distinction was not helpful in identifying adrenoceptor subtypes and even led to proposals of different neurotransmitters for excitatory and inhibitory responses. In contrast to this qualitative analysis, Ahlquist (1948) used a quantitative approach to propose the existence of two adrenoceptor subtypes, based on different rank orders of potency, within a series of structurally related natural and synthetic agonists, when responses to these agonists were evaluated in different tissues. Ahlquist designated these two subtypes of adrenoceptors as α and β . This mode of receptor subclassification was consistent with that based on antagonist sensitivity, with those responses designated by Ahlquist as mediated by β -adrenoceptors being insensitive to ergots or β -haloalkylamines. Final proof for this subclassification scheme came in 1957, with the description of the partial agonist dichloroisoprenaline, the first agent capable of antagonizing β - but not α -adrenoceptor-mediated responses (Powell and Slater, 1957; Moran and Perkins, 1958). Although there have been a few subsequent proposals for additional adrenoceptors mediating effects to sympathetic nerve stimulation (e.g., γ -adrenoceptors) it seems that many of these unexplained responses result from other neurotransmitters now known to be coreleased with noradrenaline.

In 1967, Lands and coworkers (1967), comparing rank orders of potency of agonists in a manner similar to that of Ahlquist, concluded that there were two subtypes of the β -adrenoceptor. The β_1 -adrenoceptor, the dominant

receptor in heart and adipose tissue, was equally sensitive to noradrenaline and adrenaline, whereas the β_2 -adrenoceptor, responsible for relaxation of vascular, uterine, and airway smooth muscle, was much less sensitive to noradrenaline vis-a-vis adrenaline. Highly selective antagonists for both β_1 - and β_2 -adrenoceptors have been developed, as well as many potent and selective β_2 -adrenoceptor agonists.

Soon after prejunctional α -adrenoceptors were identified in the early 1970s, it became apparent that pre- and postjunctional α -adrenoceptors had different pharmacological characteristics (Starke et al., 1974; Langer, 1974). Langer (1974) suggested the designation of α_2 and α_1 for pre- and postjunctional α -adrenoceptors, respectively. Studies of the interactions of agonists and antagonists with these α -adrenoceptors extended this subclassification scheme to a functional subdivision, as opposed to an anatomical subdivision, of α -adrenoceptors into the α_1 and α_2 subtypes (Berthelsen and Pettinger, 1977). Finally, with the identification of potent and highly selective α_1 - and α_2 -adrenoceptor agonists and antagonists, the subdivision of α_1 - and α_2 -adrenoceptors, as well as further subdivisions of each of these α -adrenoceptor subtypes (see sections II and III), has come to rely on a pharmacological subclassification rather than the anatomical or functional subdivisions used previously (for reviews, see Ruffolo et al., 1988, 1991).

With the development of additional pharmacological tools, as well as new techniques for studying drug-receptor interactions, such as radioligand-binding assays, it became apparent that the situation was significantly more complex, with further subdivision of both the α_1 -adrenoceptor (Morrow and Creese, 1986; Johnson and Minneman, 1987; Han et al., 1987) and α_2 -adrenoceptor (Bylund, 1985; Bylund et al., 1988; Michel et al., 1989b) being possible. Furthermore, it became apparent that not all of the β -adrenoceptor-mediated responses could be classified as either β_1 or β_2 , suggesting the existence of at least one additional β -adrenoceptor subtype (Arch et al., 1984; Bond and Clarke, 1988). Finally, tissues initially thought to represent examples of pure subtype populations were found to contain mixed adrenoceptor populations, with the subtype distribution often being highly species dependent (Hieble and Ruffolo, 1991).

Even more recently, the rapidly developing molecular biological techniques have had a major impact on adrenoceptor subclassification. Genes have been recombinant for many of the α - and β -adrenoceptor subtypes identified by functional or radioligand-binding studies in native tissues. Additional related subtypes have been identified by homologous hybridization of tissue mRNA with probes prepared from previously recombinant receptors. Transfection of genomic or cDNA clones into suitable mammalian cells has allowed the characterization of the pharmacology of pure populations of these recombinant receptor subtypes.

Although in some cases there is excellent correlation between the characteristics of recombinant and native receptors, there are several examples of recombinant receptors that cannot be assigned to one of the adrenoceptors identified in an isolated tissue or, conversely, there are adrenoceptors that have been identified by functional or radioligand-binding studies but have yet to be cloned. In some cases there is controversy between different laboratories regarding the subtype assignment of a particular adrenoceptor clone. Furthermore, subclassification schemes based on functional, radioligand-binding, and molecular biology studies are not completely consistent with one another. The existence of novel subtypes based on small differences in pharmacological properties is now being proposed with increasing frequency. One must be careful to assure that these differences are not a consequence of minor species differences in receptor characteristics or differences in accessibility of drug to the receptor (Kenakin, 1984).

Whereas the adrenoceptors have historically been divided into two major subtypes, the α - and β -adrenoceptors, it has recently become clear that it may be more useful to classify the adrenergic receptor into three major types: the α_1 -adrenoceptors, α_2 -adrenoceptors, and β -adrenoceptors. The rationale for this new classification scheme is based on three lines of evidence (Bylund, 1988). First, the difference in affinity of selective drugs is 3 to 4 orders of magnitude between major subtypes (e.g., α_1 , α_2 , and β), whereas the affinity ratios among subtypes of each of these major groups are generally only 10 to 100. Second, second-messenger responses are different for each of these three major types. Finally, the predicted amino acid sequences of the adrenoceptors are more consistent with three rather than two major types (Bylund, 1992).

It is the purpose of this review to present current knowledge regarding subtypes of α_1 -adrenoceptors, α_2 -adrenoceptors, and β -adrenoceptors and to present the rationale for the recommended nomenclature. Some α -adrenoceptor agonists and antagonists have affinity for novel sites apparently recognizing the imidazoline moiety present in these compounds (Hieble and Ruffolo, 1992). Because noradrenaline and adrenaline have essentially no affinity for these sites, they cannot be considered adrenoceptors and will not be considered here. Furthermore, with the exception of the turkey erythrocyte β -adrenoceptor, which has been used widely as a model system, the discussion will be limited to mammalian adrenoceptors.

II. α_1 -Adrenoceptor Subtypes

It is clear that α_1 -adrenoceptors are heterogeneous, although the number of discrete subtypes is still controversial. The first suggestion of multiple α_1 -adrenoceptor subtypes was based on differences in potency of the antagonists, prazosin and phenoxybenzamine, in func-

tional *in vitro* assays (Coates et al., 1982; Medgett and Langer, 1984; Flavahan and Vanhoutte, 1986). α_1 -Adrenoceptor subclassification based on sensitivity to prazosin blockade is consistent with the divergence of K_B for this antagonist between different tissues (Flavahan and Vanhoutte, 1986). Functional subclassification of α_1 -adrenoceptors based on prazosin affinity remains an active field of investigation (see section II.A.); nevertheless, radioligand-binding and molecular studies have identified several α_1 -adrenoceptor subtypes all having similar high affinity for prazosin but varying affinity for other α -adrenoceptor antagonists. The initial subdivision of the α_1 -adrenoceptor into the α_{1A} and α_{1B} classes was based on differential affinity of the competitive antagonist, WB 4101,† and the site-directed alkylating agent, chloroethylclonidine (Johnson and Minneman, 1987). Three α_1 -adrenoceptor cDNAs have been isolated and the receptor proteins expressed. Although the relationship of the recombinant subtypes to those in native tissues is still unclear, the data suggest the possible existence of four subtypes, which have been designated α_{1A} , α_{1B} , α_{1C} , and α_{1D} .

In the subclassification of α_1 -adrenoceptors, both upper case subscripts (e.g., α_{1A} , α_{1B}) and lower case subscripts (e.g., α_{1a} , α_{1b}) have been utilized. Although there has been some inference that the upper and lower case subscripts refer to results from subclassification based on radioligand-binding and functional assays, respectively, the usage has now become random, with upper case being currently more common. It is now suggested that, until the discrepancies between the receptor subclassification based on either functional or radioligand-binding assays in native tissues and that based on the

† Abbreviations: ARC-239, 2-[2,4-(2-methoxyphenyl)piperazin-1-yl]ethyl-4,4-dimethyl-1,3-(2H,4H)-isoquinolindione; BAM-1303, 8 β [(2-phenylimidazol-1-yl)methyl]-6-methylergoline; BE-2254 (HEAT), 2-[2-(4-hydroxyphenyl)ethyl-aminomethyl] tetralone; BRL 37344, 1-(3-chlorophenyl)-2-[2-(4-(carboxymethoxy)phenyl)-1-methyl-ethylamino]ethanol; BRL 44408, 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole; CGP 12177, (-)-4-(3-*t*-butylamino-2-hydroxypropoxy)-benzimidazol-2-one; CGP 20712A, 1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol; CL 316,243: (R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]1,3-benzodioxole-2,2-dicarboxylic acid; IBE-2254, 2-[2-(3-iodo, 4-hydroxyphenyl)ethyl-aminomethyl] tetralone; ICI 118,551, erythro(\pm)-1-[(7-methylindane-4-yl)-oxy]-3-isopropylamino-2-butanol; ICI 198,157, 1-[2(4-(2-carbomethoxyethoxy)phenoxy)ethylamino]-3-phenoxy-2-propanol; K_B , receptor dissociation constant; MK-912 (L-857,743), (2S,12bS) 1',3'-dimethylspiro (1,3,4,5',6',7,12b) octahydro-2H-benzo[b]furo[2,3-a]quinazoline)-2,4'-pyrimidin-2'one; RO 363, 1-(3,4-dihydroxyphenoxy)-3-[2-(3,4-dimethoxyphenyl)ethylamino]-2-propanol; RS-15385-197, (+)-(8a, 12a, 13a)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-methylsulfonyl-6H-isoquino[2,1-g][1,6] naphthyridine; SK&F 104078, 6-chloro-9-[(3-methyl-2-butanyl)oxy]-3-methyl-1H-2,3,4,5-tetrahydro-3-benzazepine; SK&F 104856, 2-vinyl-7-chloro-3,4,5,6-tetrahydro-4-methylthieno [4,3,2ef] [3]benzazepine; SZL-49, 1-(4-amino-6,7-dimethoxy-2-quinazoliny)-4-(2-bicyclo[2,2,2] octa-2,5-dienylcarbonyl)-piperazine; YM617 (tamsulosin), (-)-5-[2-[[2-(2-ethoxyphenoxy)ethyl]amino]propyl-2-methoxybenzenesulfonamide; WB-4101, 2(N[2,6-dimethoxyphenoxyethyl]) amino-methyl-1,4-benzodioxane.

cloning and expression of discrete receptor proteins have been resolved (see section II.D.), upper case subscripts be reserved for pharmacologically defined receptor subtypes and lower case be used for those recombinant receptors defined by molecular biology. This convention is also applicable to the α_2 -adrenoceptors and has precedent in the current nomenclature for native and recombinant muscarinic receptors. In view of the rapid progress in the cloning and expression of adrenoceptors from a variety of sources, and the identification of species-specific structural elements in these proteins controlling their pharmacology, it is likely that the pharmacological and molecular subclassification schemes will eventually converge.

A. Common α_1 -Adrenoceptor Characteristics

All of the α_1 -adrenoceptor subtypes are activated by the sympathetic neurotransmitters, noradrenaline and adrenaline. There is no evidence for selective affinity of either of these catecholamines for any of the α_1 -adrenoceptor subtypes identified to date. Although the functional K_B values span a broad range, all α_1 -adrenoceptor-mediated responses are sensitive to blockade by prazosin, and all show low affinity for selective α_2 -adrenoceptor antagonists such as yohimbine or rauwolscine. All known subtypes can be labeled with [3 H]prazosin or [125 I]IBE-2254 (I-HEAT), and activation of each subtype is associated with an increase in intracellular calcium.

B. Pharmacologically Defined α_1 -Adrenoceptor Subtypes

1. α_{1A} - and α_{1B} -Adrenoceptors. The α_{1A} - and α_{1B} -adrenoceptors were initially differentiated based on the affinities of phentolamine and WB 4101 (Morrow and Creese, 1986). An extensive series of α_1 -adrenoceptor assays, both biochemical and functional, were divided into those in which phentolamine showed a high (α_{1A}) or low (α_{1B}) affinity relative to prazosin. The proportion of high- and low-affinity sites for [3 H]WB 4101 was consistent with this subclassification scheme. Classification of α_1 -adrenoceptors into α_{1A} and α_{1B} subtypes has been supported by the identification of several antagonists showing at least 100-fold selectivity for the α_{1A} -adrenoceptor, such as 5-methylurapidil (Gross et al., 1989) and (+)-niguldipine (Boer et al., 1989; Han and Minneman, 1991), and by the discovery that chloroethylclonidine selectively alkylates the α_{1B} -subtype (Minneman et al., 1988). There is currently no competitive antagonist selective for the α_{1B} -adrenoceptor, although spiperone has been reported to have a 10-fold higher affinity for α_{1B} - than α_{1A} -adrenoceptors (Michel et al., 1989a). α_1 -Adrenoceptors are currently subclassified as α_{1A} or α_{1B} based on receptor dissociation constants for 5-methylurapidil or (+)-niguldipine and on sensitivity to irreversible inactivation by chloroethylclonidine.

Results of both functional and radioligand-binding studies suggest that the rat vas deferens contains a high density of α_{1A} -adrenoceptors relative to the α_{1B} . Other

tissues in which the α_{1A} -adrenoceptor predominates include the rat anococcygeus and rat submaxillary gland. The rat spleen and liver appear to represent tissues in which the α_1 -adrenoceptor response is mediated primarily by α_{1B} -adrenoceptors. Most tissues studied, including rat cerebral cortex, hippocampus, heart, and kidney, contain mixed populations of the two subtypes (Minneman et al., 1988). Although some studies have assigned receptors of blood vessels to the α_{1A} or α_{1B} subtypes (Han et al., 1990), several other reports have suggested that vascular α_1 -adrenoceptors have characteristics different from either of these subtypes (Muramatsu et al., 1990, 1991; Sulpizio and Hieble, 1991; Oriowo and Ruffolo, 1992).

2. α_{1H} -, α_{1L} -, and α_{1N} -Adrenoceptors. It was noted above that there is a wide affinity range for prazosin as an antagonist of α_1 -adrenoceptors. Flavahan and Vanhoutte (1986) differentiated α_1 -adrenoceptors into two groups, designated α_{1H} and α_{1L} , with high (α_{1H}) and low (α_{1L}) affinity for prazosin and yohimbine. This functional subclassification scheme has been recently extended by the addition of a third group, α_{1N} , which has a relatively low affinity for prazosin and a higher than expected affinity for yohimbine (Muramatsu et al., 1990). The use of phenylephrine as an agonist for determination of these affinity values would presumably eliminate contribution of a postjunctional α_2 -adrenoceptor. This subclassification scheme has thus far been mainly applied to vascular α_1 -adrenoceptors. It has been postulated (Muramatsu et al., 1991) that the α_{1A} - and α_{1B} -adrenoceptors are subtypes of the α_{1H} . Hence, blood vessels may contain additional α_1 -adrenoceptor subtypes (α_{1L} , α_{1N}) not found in many other tissues, which may explain why it is often difficult to reconcile vascular α_1 -adrenoceptor responses with the α_{1A}/α_{1B} classification scheme (Oriowo and Ruffolo, 1992). There does exist limited radioligand-binding data in support of this subclassification scheme, with some investigators reporting binding of [3 H]prazosin to two sites in membranes prepared from vascular smooth muscle, with K_D values consistent with those determined for α_{1H} - and α_{1L} -adrenoceptors in functional studies (Oshita et al., 1992).

The pharmacological tools used to characterize and subclassify α_1 -adrenoceptors are presented in table 1.

C. Recombinant α_1 -Adrenoceptor Subtypes

1. $\alpha_{1a/d}$ -Adrenoceptors. Screening of a rat brain library with a cDNA probe prepared from the hamster α_{1b} -adrenoceptor (see section II.C.2) revealed a cDNA clone for another α_1 -adrenoceptor subtype (Lomasney et al., 1991b). The amino acid sequence of the protein expressed by this clone is consistent with a seven transmembrane-spanning, G-protein-linked receptor. Northern analysis of the tissue distribution of mRNA transcribed by this clone suggested a similar distribution to that of the α_{1a} subtype, and the expressed receptor had a high affinity

TABLE 1
Pharmacological tools used to subclassify and characterize the α_1 -adrenoceptor subtypes

Compound	Mean K_i (nM)			
	α_{1A} *	α_{1B} †	α_{1C} ‡	α_{1D} §
WB 4101	0.08 ± 0.02 (5)	8.0 ± 2.8 (11)	0.65 ± 0.20 (4)	1.6 ± 0.3 (3)
5-Methylurapidil	0.70 ± 0.1 (5)	96 ± 26 (11)	7.6 ± 2.8 (4)	15 ± 0.4 (2)
Prazosin	0.13 ± 0.02 (4)	0.32 ± 0.09 (5)	0.54 ± 0.14 (4)	0.32 ± 0.01 (3)
Oxymetazoline	3.7 ± 0.83 (5)	280 ± 56 (12)	46 ± 20 (4)	2140 (1)
Spiperone	7.2 (1)	1.3 ± 0.72 (2)	22 (1)	No data available
Chloroethylclonidine (irreversible)¶	0	+++	+++	++

* Affinity for α_{1A} -adrenoceptors was determined in tissue homogenates. K_i values for WB-4101 and 5-methylurapidil represent either the high-affinity component of binding displacement in tissues containing both α_{1A} - and α_{1B} -adrenoceptors (e.g., rat cortex, rat vas deferens, human cortex) or the overall binding displacement in tissues containing the α_{1A} -adrenoceptor subtype only (rat submaxillary gland). Affinity represents mean ± SEM of several reported values. The number of experimental values used for the mean determination is noted in parentheses. Data from Michel et al., 1989a; Hanft and Gross, 1989; Gross et al., 1989; Hanft et al., 1989; Klijn et al., 1991.

† Affinity for α_{1B} -adrenoceptors was determined either in tissue homogenates or to membranes from cells expressing the rat, hamster, or human α_{1B} -adrenoceptor. K_i values for WB-4101 and 5-methylurapidil represent either the low-affinity component of binding displacement in tissues containing both α_{1A} - and α_{1B} -adrenoceptors (e.g., rat cortex, rat vas deferens, human cortex) or the overall binding displacement in tissues containing the α_{1B} -adrenoceptor subtype only (rat spleen, rat liver). Data presented as for the α_{1A} -adrenoceptor obtained from Cotecchia et al., 1988; Michel et al., 1989a; Hanft and Gross, 1989; Gross et al., 1989; Hanft et al., 1989; Lomasney et al., 1991a; Schwinn et al., 1990, 1991; Ramarao et al., 1992; Forray et al., 1993; Taddei et al., 1993.

‡ Affinity for α_{1C} -adrenoceptors was determined either in homogenates of rabbit liver or in membranes from cells expressing the recombinant bovine or human α_{1C} -adrenoceptor. Data presented as for the α_{1A} -adrenoceptor obtained from Schwinn et al., 1990, 1991; Taddei et al., 1993; Forray et al., 1993; Hirasawa et al., 1993.

§ Affinity for α_{1D} -adrenoceptors was determined in membranes from cells expressing the recombinant α_1 -adrenoceptor referred to as α_{1A} , from either a rat (Lomasney et al., 1991b) or human (Forray et al., 1993) source or the nearly identical recombinant rat receptor referred to as the α_{1D} -adrenoceptor (Perez et al., 1991). Data presented as for the α_{1A} -adrenoceptor.

¶ Sensitivity to irreversible receptor inactivation by chloroethylclonidine determined by the degree in reduction in B_{max} for α_1 -adrenoceptor binding following treatment of homogenates of either native tissues or cells expressing a particular recombinant α_1 -adrenoceptor. Ranking of sensitivity to chloroethylclonidine from Perez et al., 1991.

for WB 4101. This led to the conclusion that this clone represented the pharmacological α_{1A} -adrenoceptor (Lomasney et al., 1991b). However, Perez et al. (1991), studying an almost identical clone also isolated from rat brain, found low affinity for the more selective α_{1A} -adrenoceptor antagonists, 5-methylurapidil and (+)-niguldipine. They concluded that a clone for a novel α_1 -adrenoceptor subtype had been isolated and denoted it as α_{1D} . Because the clones isolated by Lomasney et al. (1991b) and Perez et al. (1991) encode for 560 amino acid proteins differing in sequence at only two sites (99.8% amino acid identity), it seems likely that they represent the same subtype. Because the expressed cDNA has pharmacological properties substantially different from those found for the α_{1A} -adrenoceptor in native tissues, this recombinant receptor should be referred to as the $\alpha_{1A/D}$ -adrenoceptor until these discrepancies can be explained and a native receptor having similar pharmacology identified. The human homolog of this α_1 -adrenoceptor subtype has been cloned recently (Forray et al., 1993).

2. α_{1B} -Adrenoceptors. The α_{1B} -adrenoceptor subtype was the first of the α_1 -adrenoceptor family to be cloned. This clone was isolated from a hamster vas deferens cell line (DD₁MF2) and encoded for a protein having a sequence consistent with a G-protein-coupled receptor (Cotecchia et al., 1988). Expression of this cDNA resulted in a protein with radioligand-binding properties consistent with an α_{1B} -adrenoceptor, with a high affinity

for prazosin and a low affinity for phentolamine, 5-methylurapidil, and yohimbine. Also consistent with the α_{1B} assignment was a high sensitivity to irreversible inactivation by chloroethylclonidine. Using a cDNA probe derived from the hamster α_{1B} -adrenoceptor, investigators (Voigt et al., 1990; Lomasney et al., 1991a) identified the rat α_{1B} -adrenoceptor and showed that it had a similar pharmacological profile and >98% amino acid identity with the hamster receptor. Northern analysis of rat tissues showed mRNA expression for this clone in a variety of tissues expected to express the α_{1B} -adrenoceptor, including liver, spleen, heart, and cerebral cortex. The human α_{1B} -adrenoceptor has been cloned recently and shown to have virtually identical pharmacology with the hamster α_{1B} -adrenoceptor (Ramarao et al., 1992).

3. α_{1C} -Adrenoceptors. Based on homology screening of a bovine cerebral cortex cDNA library with a probe derived from the hamster α_{1B} -adrenoceptor, an additional cDNA clone which apparently encoded for a novel α_1 -adrenoceptor subtype was identified (Schwinn et al., 1990). The protein expressed by this clone is distinct from the α_{1B} -adrenoceptor, with 65% amino acid identity in the membrane-spanning domains. The pharmacological profile is also distinct from either the α_{1B} - or $\alpha_{1A/D}$ -adrenoceptors, with a relatively high affinity for 5-methylurapidil (but less than that observed in most α_{1A} systems), a high affinity for WB 4101, and a high sensitivity to irreversible inactivation by chloroethylclonidine. In

view of its distinct pharmacological profile, this receptor was designated the α_{1c} -adrenoceptor. The cloning and expression of the human α_{1c} -adrenoceptor has been reported recently (Furray et al., 1993; Hirasawa et al., 1993).

Northern analysis showed no α_{1c} -mRNA in any rat tissue examined, with hybridization detected only in rabbit liver and human hippocampus (Schwinn et al., 1991). Despite the apparent lack of mRNA transcription, Southern hybridization suggests the existence of the α_{1c} gene in the rat, and a rat homolog of the bovine α_{1c} -adrenoceptor has been cloned recently (Laz et al., 1993).

D. Relationship between Pharmacologically Defined and Recombinant α_1 -Adrenoceptor Subtypes

The close correspondence between the pharmacological properties of the expressed α_{1b} -adrenoceptor cDNA from rat or hamster, or the gene fusion product constructed from the human clone, with those recognized for the α_{1B} -adrenoceptor indicates that the protein encoded by this clone represents the α_{1B} -adrenoceptor that has been identified by functional and radioligand-binding studies in native tissues.

On the other hand, there is currently some confusion regarding the assignment of the recombinant $\alpha_{1a/d}$ - and α_{1c} -adrenoceptors to one of the pharmacologically defined α_1 -adrenoceptor subtypes. It is clear that the $\alpha_{1a/d}$ -adrenoceptor clone does not correspond with the pharmacologically defined α_{1A} -adrenoceptor. It is possible that this clone represents a novel subtype (α_{1D}) and that the α_{1A} -adrenoceptor remains to be cloned; alternatively, recent data suggest that the α_{1c} clone, at least its rat homolog, may correspond to the pharmacologically defined α_{1A} -adrenoceptor (Laz et al., 1993). The recombinant rat α_{1c} -receptor, when compared to the other two recombinant rat α_1 -adrenoceptors, has significantly lower sensitivity to irreversible inactivation by chloroethylclonidine and relative antagonist affinities correlating well with that of the native α_{1A} -adrenoceptor (Laz et al., 1993).

Although a direct comparison between species homologs of the recombinant α_{1c} -adrenoceptor has not been performed, there may be species differences in the sensitivity of this receptor subtype to chloroethylclonidine.

Recently, mRNA for the α_{1c} -adrenoceptor has been identified in human prostate (Price et al., 1993), and the contractile response induced by α_1 -adrenoceptor activation in this tissue appears to be mediated by an adrenoceptor having pharmacological characteristics corresponding to those of the expressed recombinant α_{1c} -adrenoceptor (Marshall et al., 1992; Furray et al., 1993). Interestingly, the functional pharmacological profile of the α_1 -adrenoceptor in the rat aorta (high affinity for WB 4101, 5-methylurapidil affinity intermediate between α_{1A} and α_{1B} , sensitivity to irreversible inactivation by chloroethylclonidine) is similar to that observed for

the expressed bovine α_{1c} -adrenoceptor clone (Muramatsu et al., 1991; Oriowo and Ruffolo, 1992).

A correlation, as yet incomplete, between recombinant and pharmacologically defined α_1 -adrenoceptors is shown in table 2.

E. Additional α_1 -Adrenoceptor Subtypes?

A new α_1 -adrenoceptor antagonist radioligand, [3 H]YM617, identifies two α_1 -adrenoceptor sites in rat brain, one of which can be inhibited by several α_1 -adrenoceptor antagonists, including 5-methylurapidil, WB-4101, and phentolamine, but is highly insensitive to prazosin (Yazawa et al., 1992). The affinity of prazosin for this novel site is several orders of magnitude lower than its functional K_B at α_{1L} -receptors. There is also some evidence for differential alkylation of α_1 -adrenoceptors by the prazosin analog, SZL 49 (Piascik et al., 1990), although whether the alkylation pattern produced by this compound is inconsistent with the α_{1A}/α_{1B} subclassification scheme, or indeed whether this agent shows true subtype selectivity, has yet to be established. The classical irreversible α -adrenoceptor antagonist, phenoxybenzamine, can apparently produce a functional subclassification of α_1 -adrenoceptor-mediated responses (Coates et al., 1982) and has recently been reported to block selectively one component of [3 H]prazosin binding to rat brain (Tsuchihashi et al., 1991).

III. α_2 -Adrenoceptor Subtypes

As described for the α_1 -adrenoceptor, the α_2 -adrenoceptor has been subdivided based on functional and radioligand-binding studies, and several distinct α_2 -adrenoceptor proteins have been cloned and expressed. Although the relationships between the receptor subtypes identified by these three modes of subclassification have not been completely established, it is clear that there are multiple α_2 -adrenoceptor subtypes, with distinct drug specificities.

The subclassification of α_2 -adrenoceptors was initially

TABLE 2
Correspondence between the characteristics of recombinant and pharmacologically defined α_1 -adrenoceptors

Clone	Species	Pharmacology
$\alpha_{1a/d}$	Human	*
	Rat	*
α_{1b}	Human	α_{1B}
	Rat	α_{1B}
	Hamster	α_{1B}
α_{1c}	Human	No data available
	Rat	α_{1A}
	Bovine	*

* The pharmacology of these clones cannot be definitively assigned to one of the functional α_1 -adrenoceptor subtypes based on currently available data. These expressed clones all show relatively high affinity for 5-methylurapidil and WB-4101 and are sensitive to irreversible inactivation by chloroethylclonidine.

based on the ability of prazosin to inhibit the binding of [³H]yohimbine or [³H]rauwolscine to tissue homogenates from a variety of isolated tissues or cell culture lines (Bylund, 1985; Nahorski et al., 1985; Petrash and Bylund, 1986). Prazosin and another α_1 -adrenoceptor antagonist, ARC 239, have high affinity for one group of α_2 -adrenoceptors, designated α_{2B} , and low affinity for another, designated α_{2A} . The partial α -adrenoceptor agonist, oxymetazoline, and the recently discovered antagonist, BRL 44408, selectively inhibit binding to the α_{2A} -adrenoceptor (Bylund et al., 1988; Young et al., 1989). Although there is some functional evidence to support this subclassification scheme (Bylund and Ray-Prenger, 1989), it has been difficult to find functional responses clearly showing an α_{2B} -adrenoceptor pharmacological profile. By correlation of antagonist affinity for inhibition of [³H]rauwolscine binding in different cell and tissue preparations, two additional α_2 -adrenoceptor subtypes have been proposed, designated α_{2C} and α_{2D} .

Independent of the above α_2 -adrenoceptor subclassification, several novel antagonists, SK&F 104078 and SK&F 104856, have been found to produce functional blockade of certain α_2 -adrenoceptor-mediated responses, such as vascular contraction, while having no effect on others, such as inhibition of adrenergic neurotransmission at atrial neuroeffector junctions (Ruffolo et al., 1987; Hieble et al., 1991).

Three α_2 -adrenoceptor cDNA clones have been isolated from human libraries, and three highly homologous clones, which appear to be species homologs of the three human clones, have been isolated from the rat and the mouse. Although some controversy remains regarding the subtype assignment of one of the rat clones, the recombinant α_2 -adrenoceptors appear to correspond to the α_2 -adrenoceptor subtypes identified by radioligand-binding studies (Bylund et al., 1992).

A. Common α_2 -Adrenoceptor Characteristics

All known α_2 -adrenoceptor subtypes can be activated by noradrenaline and adrenaline, and there is no evidence that these physiological catecholamines show significant selectivity between any of the known α_2 -adrenoceptor subtypes. All subtypes can be blocked by yohimbine and rauwolscine ($K_B < 40$ nM) and labeled with ³H analogs of these antagonists, although the affinity can vary substantially between subtypes. All α_2 -adrenoceptors appear to be linked to inhibition of adenylate cyclase as one, but not the only, mechanism of signal transduction.

B. Pharmacologically Defined α_2 -Adrenoceptor Subtypes

1. α_{2A}/α_{2B} -Adrenoceptors. Radioligand-binding studies first established the presence of two distinct subtypes of the α_2 -adrenoceptor, based on differential affinity of prazosin, which had previously been considered as a selective antagonist of the α_1 -adrenoceptor. Studies comparing human platelet and neonatal rat lung showed

each tissue to have a homogeneous α_2 -adrenoceptor population, but the ability of prazosin to inhibit [³H]rauwolscine or [³H]yohimbine binding differed markedly between the two tissues. The human platelet receptor was designated as α_{2A} (low prazosin affinity) and that in the neonatal rat lung as α_{2B} (high prazosin affinity). The partial agonist, oxymetazoline, showed the opposite selectivity, with preferential affinity for the α_{2A} -adrenoceptor. Subsequent experiments showed that this subclassification scheme was not a result of species differences, with both subtypes being detected in both rat (Kawahara and Bylund, 1985) and human cortex (Petrash and Bylund, 1986), and several tissue culture lines have been identified that have pure populations of either α_{2A} -adrenoceptors (HT 29) or α_{2B} -adrenoceptors (NG 108). Although this subclassification is based primarily on data from radioligand-binding studies, the ability of prazosin to produce functional blockade of α_2 -adrenoceptor-mediated inhibition of adenylate cyclase in cells containing α_{2A} - or α_{2B} -adrenoceptors correlates with its binding affinity (Bylund and Ray-Prenger, 1989). Other antagonists have been shown to have selective action at α_{2B} -adrenoceptors (ARC 239, spiroxatrine, imiloxan), and an antagonist selective for the α_{2A} subtype, BRL 44408, has been identified (Young et al., 1989). The physiological catecholamines, adrenaline and noradrenaline, do not discriminate between α_{2A} - and α_{2B} -adrenoceptors, and, with the exception of oxymetazoline, selective agonists have not been identified.

2. α_{2C} - and α_{2D} -Adrenoceptors. By the correlation of the affinities for a series of α -adrenoceptor antagonists as inhibitors of [³H]rauwolscine binding in different tissues, two additional α_2 -adrenoceptor subtypes have been identified. The α_{2C} -adrenoceptor is similar to the α_{2B} -adrenoceptor with respect to a relatively high affinity for prazosin, ARC 239, and spiroxatrine, but it has a higher affinity for rauwolscine. There is some confusion in the literature regarding assignment of a receptor to the α_{2B} or α_{2C} groups. A receptor must be subclassified using more than one or even two selective drugs to identify subtle but yet important differences. Several other antagonists are selective for α_{2C} - versus α_{2B} -adrenoceptors (BAM 1303, WB 4101). The α_{2C} -adrenoceptor was first found in a tissue culture line derived from the opossum kidney (Murphy and Bylund, 1988) but has now been shown to be present in opossum kidney tissue (Blaxall et al., 1991), and a human retinoblastoma cell line, Y79, has been found to have similar pharmacological characteristics (Gleason and Hieble, 1992). A fourth subtype, designated α_{2D} , has been found in bovine pineal, rat submaxillary gland, and a cell line derived from a rat pancreatic islet cell tumor (RINm5F) (Simonneaux et al., 1991; Michel et al., 1989b; Remaury and Paris, 1992). This subtype has a lower affinity for [³H]rauwolscine than the other subtypes and, like the α_{2A} -adrenoceptor, a low affinity for prazosin, spiroxatrine and ARC 239.

Several other tissues possess an α_2 -adrenoceptor having low affinity for yohimbine or rauwolscine, including adipocytes from several species and rabbit jejunal enterocytes. Although the pharmacological profile of the α_2 -adrenoceptor in these tissues has not been studied extensively, these receptors may represent additional examples of the α_{2D} -adrenoceptor. Other than yohimbine and rauwolscine, only BAM 1303 has moderate selectivity between α_{2D} - and α_{2A} -adrenoceptors, but the two subtypes can be distinguished when the potency ratios for several antagonist pairs are compared (Simonneaux et al., 1991). Studies in the rat brain using two new highly potent and selective α_2 -adrenoceptor radioligands, [3 H]RS-15385-197 (MacKinnon et al., 1992) and [3 H]MK-912 (Uhlen et al., 1992) show the presence of a site having low affinity for yohimbine and rauwolscine, consistent with an α_{2D} -adrenoceptor. This site is designated either α_{2D} (MacKinnon et al., 1992) or α_{2A} (Uhlen et al., 1992), demonstrating that confusion remains in the assignment between these two subtypes, although it now seems clear that the α_{2D} -adrenoceptor is the rat homolog of the human α_{2A} -adrenoceptor.

The pharmacological tools used to characterize and subclassify α_2 -adrenoceptors are summarized in table 3.

C. Recombinant α_2 -Adrenoceptor Subtypes

An α_2 -adrenoceptor gene was first isolated from human platelet (Kobilka et al., 1987b). This clone was designated α_2C10 , based on its location on human chromo-

some C10. Southern analysis with a fragment of the α_2C10 cDNA revealed the presence of related genes on chromosomes 2 and 4. These genes have subsequently been cloned, expressed, and designated α_2C2 and α_2C4 (Regan et al., 1988, Lomasney et al., 1990). The pharmacological characteristics of these three receptor proteins are consistent with α_2 -adrenoceptors. A porcine analog of α_2C10 , showing >93% amino acid identity with the human receptor, and similar pharmacological characteristics, has also been isolated (Guyer et al., 1990).

Three α_2 -adrenoceptors have also been cloned from the rat. One, designated RNG, clearly appears to be an analog of α_2C2 , based on similar pharmacological profiles and the unique lack of consensus sequences for N-linked glycosylation on the amino terminus (although showing only 82% amino acid identity). Another receptor, designated either pA₂d (Voigt et al., 1991) or RG10 (Lanier et al., 1991), appears to be a species homolog of the α_2C4 -adrenoceptor, with 88% identity of primary structure. The third rat clone, designated cA₂-47 (Chalberg et al., 1990) or RG20 (Lanier et al., 1991) shares 89% amino acid identity with the α_2C10 and key similarities in pharmacological profile, such as a low affinity for prazosin. However, some investigators suggest that, rather than being a rat homolog of the human α_2C10 , the RG20 clone represents a distinct α_2 -adrenoceptor subtype. This is based primarily on the low affinity of the expressed RG20 clone for yohimbine and rauwolscine.

Three α_2 -adrenoceptors have been cloned from the

TABLE 3
Pharmacological tools used to subclassify and characterize the α_2 -adrenoceptor subtypes

Compound	Mean K_i (nM)			
	α_{2A} *	α_{2D} †	α_{2B} ‡	α_{2C} §
Rauwolscine	3.7 ± 1.6 (5)	33 ± 10 (7)	1.2 ± 0.5 (5)	0.18 ± 0.03 (4)
Prazosin	1034 ± 403 (5)	1127 ± 337 (8)	30 ± 7 (5)	61 ± 17 (6)
ARC-239	256 ± 86 (3)	285 ± 135 (2)	4.6 ± 2.0 (3)	51 ± 28 (4)
BAM-1303	4.8 ± 1 (3)	70 ± 16 (2)	21 ± 12 (3)	0.73 ± 0.37 (4)
BRL 44408	3.6 ± 1.9 (2)	16 (1)	174 ± 30 (2)	187 (1)
Oxymetazoline	5.6 ± 1.9 (5)	34 ± 14 (7)	350 ± 91 (5)	72 ± 23 (7)
Imiloxan	1750 ± 1250 (2)	79 (1)	50 ± 6 (2)	No data available

* Affinity for α_{2A} -adrenoceptors was determined as the ability to inhibit radioligand binding to the receptor, either in homogenates of tissues containing a relatively pure receptor subtype population (e.g., human platelet or HT29 cell line) or to membrane from cells expressing the recombinant human α_{2A} -adrenoceptor. Affinity represents mean ± SEM of several reported values. The number of experimental values used for the mean determination is noted in parentheses. Data from Michel et al., 1989b; Gleason and Hieble, 1991; Lomasney et al., 1991b; Link et al., 1992; Bylund et al., 1992; MacKinnon et al., 1992.

† Affinity for α_{2D} -adrenoceptors was determined in homogenates of tissues containing a relatively pure receptor subtype population (e.g., bovine pineal gland, rat sublingual gland, or RINm5F cell line) or from cells expressing the rat RG20 or mouse M α_2 -10H recombinant receptors. The α_{2D} -adrenoceptor can be considered a species homolog of the human α_{2A} -adrenoceptor, with recombinant receptors from rat and mouse (α_{2D} pharmacology) showing a high degree of amino acid identity with those from human and porcine sources (α_{2A} pharmacology). Data presented as for the α_{2A} -adrenoceptor, obtained from Lanier et al., 1991; Harrison et al., 1991; Gleason and Hieble, 1992; Bylund et al., 1992; MacKinnon et al., 1992; Remaury and Paris, 1992; Link et al., 1992.

‡ Affinity for α_{2B} -adrenoceptors was determined either in homogenates of tissues containing a relatively pure receptor subtype population (e.g., neonatal rat lung, rat kidney, or NG-108 cell line) or to membranes from cells expressing the human α_{2B} -adrenoceptor. Data presented as for the α_{2A} -adrenoceptor, obtained from Michel et al., 1989b; Weinshank et al., 1990; Gleason and Hieble, 1991; Bylund et al., 1992; MacKinnon et al., 1992.

§ Affinity for α_{2C} -adrenoceptors was determined either in homogenates of tissues containing a relatively pure receptor subtype population (e.g., opossum kidney or OK cell line) or in membrane from cells expressing the recombinant mouse, opossum, or human α_{2C} -adrenoceptor. Data presented as for the α_{2A} -adrenoceptor, obtained from Regan et al., 1988; Gleason and Hieble, 1992; Bylund et al., 1992; Link et al., 1992; Blaxall et al., 1994.

mouse, having a high degree of amino acid identity with the human α_2C2 , α_2C4 , and α_2C10 . The mouse homologs of α_2C2 (Chruscinski et al., 1992) and α_2C4 (Link et al., 1992) have similar pharmacology to the human clones. As observed in the rat, the mouse homolog of the human α_2C10 has a low affinity for yohimbine and rauwolscine (Link et al., 1992).

An opossum homolog of the human α_2C4 receptor has recently been cloned from the OK cell line (Blaxall et al., 1994). This recombinant receptor, although having pharmacology characteristic of the α_{2C} -adrenoceptor, has a lower degree of amino acid identity (64%) with the human α_2C4 -receptor than does the corresponding rat homolog (RG10).

D. Relationship between Pharmacologically Defined and Recombinant α_2 -Adrenoceptor Subtypes

Several clear correlations between the α_2 -adrenoceptor subtypes identified in native tissues and cell lines have been established with the recombinant receptor proteins expressed from cDNA clones. These correlations are supported by hybridization of the α_2 -adrenoceptor clones with tissues known to contain a particular α_2 -adrenoceptor subtype. The α_2C10 clone was isolated from human platelets. The expressed receptor has radioligand-binding characteristics in good agreement with those of the platelet α_{2A} -adrenoceptor. Although the α_2C4 clone was initially thought to correspond to the α_{2B} -adrenoceptor, based on a high affinity for prazosin, comparison of affinities for an extensive series of antagonists shows this clone to correspond more closely to the α_{2C} -adrenoceptor (Bylund et al., 1992). Northern analysis shows a strong signal when the α_2C4 gene is hybridized with mRNA prepared from OK cells, the source from which the α_{2C} subtype was identified (Lorenz et al., 1990). The rat RG10 clone also appears to have the pharmacological characteristics of the α_{2C} -adrenoceptor (Zeng and Lynch, 1991; Uhlen et al., 1992). The α_2C2 clone and the corresponding RNG correspond well to the α_{2B} -adrenoceptor, based on protein structure and pharmacological profile. Furthermore, this clone is detected in neonatal rat lung and adult rat kidney, tissues known to possess the α_{2B} subtype (Zeng et al., 1990; Lomasney et al., 1990).

The assignment of the rat RG20 clone remains unclear. As noted in II.C, this clone has 89% amino acid identity with the human α_2C10 ; however, the pharmacological profile of the RG20, unlike that of α_2C10 , does not clearly correspond to the α_{2A} -adrenoceptor. A consistent characteristic of the RG20 clone is a relatively low affinity (>10 nM) for yohimbine and rauwolscine. This has led some investigators to assign this clone to the α_{2D} subtype, where these antagonists have lower affinity than at the other three α_2 -adrenoceptor subtypes. Other groups consider the low rauwolscine affinity as a species variation and assign the RG20, like the α_2C10 , to the α_{2A} subtype. One group (Lanier et al., 1991) has observed a relatively

low affinity ($K_i = 531$ nM) of the RG20 clone for SK&F 104078, a novel antagonist that can discriminate between some α_2 -adrenoceptors in functional studies. This was used to suggest that this clone represented the prejunctional, neuronal α_2 -adrenoceptor, which has also been postulated to have α_{2D} characteristics (Bylund and Iversen, 1990). However, other investigators (Harrison et al., 1991) have not observed any substantial difference in SK&F 104078 affinity between rat α_2 -adrenoceptor clones.

It has been suggested that a single receptor subtype may have slightly different pharmacological characteristics in each species studied, thus causing a proliferation of subtypes. Although the RG20 (rat) and α_2C10 (human) clones have distinct pharmacological profiles, the corresponding clones from the mouse (Link et al., 1992) and cow (D. B. Bylund, unpublished data) appear to resemble closely the rat RG20. The low affinity of the RG20 clone, and its mouse homolog, for yohimbine and rauwolscine has been demonstrated to result from a serine at position 201, as opposed to a cysteine in the human α_2C10 and its porcine homolog (Link et al., 1992). The bovine analog of this α_2 -adrenoceptor subtype also contains a serine at this position. Hence, evidence is accumulating in support of the premise that the α_{2A} - and α_{2D} -adrenoceptor are species variants.

The postulated relationships between recombinant and pharmacologically defined α_2 -adrenoceptor subtypes are shown in table 4.

E. Additional α_2 -Adrenoceptor Subtypes?

One antagonist, guanoxabenz, has been reported to differentiate the α_{2B} -adrenoceptor into two subtypes (Uhlen and Wikberg, 1991), and it has recently been suggested that this antagonist can likewise subdivide the α_{2A} -adrenoceptor but not the α_{2C} -adrenoceptor (Uhlen et al., 1992).

In addition to α_2 -adrenoceptor subclassification based primarily on correlations between antagonist potency for inhibition of radioligand binding, a functional α_2 -adrenoceptor subclassification has been postulated, based on

TABLE 4
Correspondence between the characteristics of recombinant and pharmacologically defined α_2 -adrenoceptors

Clone	Species	Name	Pharmacology
α_{2a}	Human	α_2 -C10	α_{2A}
	Pig		α_{2A}
	Rat	RG20	α_{2D}
	Mouse	M α_2 -10H	α_{2D}
α_{2b}	Human	α_2 -C2	α_{2B}
	Rat	RNG	α_{2B}
	Mouse	M α_2 -2H	α_{2B}
α_{2c}	Human	α_2 -C4	α_{2C}
	Rat	RG10 (pA2d, RB α_{2B})	α_{2C}
	Mouse	M α_2 -4H	α_{2C}
	Opossum		α_{2C}

novel α -adrenoceptor antagonists. SK&F 104078 and SK&F 104856 have such a profile, being capable of blocking some α_2 -adrenoceptor-mediated responses, such as constriction of peripheral blood vessels, while having no effect on the neuroinhibitory effect of α_2 -adrenoceptor agonists in atria from several species or in guinea pig ileum (Ruffolo et al., 1987; Hieble et al., 1991). In several *in vivo* models, neither SK&F 104078 nor SK&F 104856 produces evidence of prejunctional α_2 -adrenoceptor blockade. It was initially suggested that these compounds could differentiate between pre- or postjunctional α_2 -adrenoceptors. However, in the field-stimulated rat vas deferens, SK&F 104078 can produce relatively potent blockade of the neuroinhibitory action of some, but not all, α_2 -adrenoceptor agonists (Oriowo et al., 1991; Akers et al., 1991). It is possibly more appropriate to assume that these antagonists can discriminate between α_2 -adrenoceptors on a functional, rather than an anatomical, basis. SK&F 104078 has equal affinity for α_{2A} - and α_{2B} -adrenoceptors, as well as a relatively high affinity for the expressed human (Lomasney et al., 1991a) and rat (Harrison et al., 1991) α_2 -adrenoceptor clones. It has been suggested that the prejunctional α_2 -adrenoceptor has α_{2D} characteristics, because SK&F 104078 has relatively low affinity against [³H]rauwolscine binding in the bovine pineal gland (Bylund and Iversen, 1990). However, SK&F 104078 has high affinity in other test systems assigned to the α_{2D} subtype, and the other functionally selective α_2 -adrenoceptor antagonist, SK&F 104856, has relatively high affinity for the α_{2D} -adrenoceptor in the bovine pineal or rat submaxillary gland (Simonneaux et al., 1991). Hence, no relationship can yet be established between the functional α_2 -adrenoceptor subclassification produced by SK&F 104078 and SK&F 104856 and the subclassification established by molecular and radioligand-binding assays.

IV. β -Adrenoceptor Subtypes

The existence of two subtypes of the β -adrenoceptor is generally accepted (Lands et al., 1967). The β_1 - and β_2 -adrenoceptor subclassification is supported by the development of subtype-selective agonists and antagonists and the therapeutic application of several of these pharmacological classes (i.e., selective β_1 -adrenoceptor antagonists and selective β_2 -adrenoceptor agonists). The avian β -adrenoceptor, as exemplified by that of the turkey erythrocyte, has many similarities, but some significant differences, compared to mammalian β_1 -adrenoceptors (Neve et al., 1986). Evidence has accumulated throughout the years for the existence of a β -adrenoceptor that is insensitive to the commonly used antagonists. This receptor has often been referred to as the "atypical β -adrenoceptor," but with the identification of selective agonists, and the expression of a recombinant receptor having similar characteristics, it now is appropriate to refer to this receptor as the β_3 -adrenoceptor.

All of the β -adrenoceptors identified in pharmacological studies have been recombinant and expressed. β_1 - and β_2 -adrenoceptor cDNA has been obtained from a variety of tissue sources, including turkey, hamster, mouse, rat, and human. The human β_3 -adrenoceptor has recently been recombinant. The pharmacological characteristics of the recombinant receptors appear to correspond well with those of the three receptor subtypes identified in native tissues, although there are some differences in the case of the β_3 -adrenoceptor. The molecular pharmacology of the β -adrenoceptors has been studied extensively, serving as one of the prototypes for the use of techniques such as site-directed mutagenesis to study the mode of interaction between the receptor and either agonists/antagonists or second-messenger regulatory proteins.

A. Common β -Adrenoceptor Characteristics

All three β -adrenoceptor subtypes can be activated by noradrenaline and adrenaline. However, in contrast to the α -adrenoceptors, the endogenous catecholamines do have differential affinity for the β -adrenoceptor subtypes. A primary distinction between β_1 - and β_2 -adrenoceptors is the relative potencies of adrenaline and noradrenaline, with the two catecholamines being equipotent at the β_1 -adrenoceptor and adrenaline having up to 100-fold selectivity for the β_2 -adrenoceptor. Conversely, noradrenaline is more potent than adrenaline as a β_3 -adrenoceptor agonist. The synthetic catecholamine, isoprenaline, is a potent agonist at all β -adrenoceptor subtypes, with no consistent intrasubtype selectivity. Propranolol and its many analogs are potent antagonists at β_1 -adrenoceptors and β_2 -adrenoceptors; however, β_3 -adrenoceptor-mediated responses are much less sensitive to these antagonists. Both β_1 - and β_2 -adrenoceptors can be labeled with [³H]dihydroalprenolol or [¹²⁵I]iodopindolol and its analogs. Although its affinity is approximately 10-fold lower than for the β_1 - or β_2 -adrenoceptors, [¹²⁵I]iodocyanopindolol can be used to label the β_3 -adrenoceptor (Emorine et al., 1992). All three β -adrenoceptor subtypes activate adenylyl cyclase as a primary mechanism for signal transduction.

B. Pharmacologically Defined β -Adrenoceptor Subtypes

β_1 - and β_2 -adrenoceptors were originally distinguished by Lands and coworkers based on rank potency orders for a series of endogenous and synthetic agonists. Subsequently, selective antagonists have been identified for both β_1 -adrenoceptors (e.g., metoprolol, practolol, atenolol, betaxolol, CGP 20712A (Dooley et al., 1986)) and β_2 -adrenoceptors (e.g., butoxamine, ICI 118,551) (O'Donnell and Wanstall, 1980). Synthetic agonists having high selectivity for the β_2 -adrenoceptor are also available (e.g., terbutaline, salbutamol, salmeterol, zinterol), but the selective β_1 -adrenoceptor agonists thus far identified (e.g., denopamine, Ro 363, and xamoterol) have limited utility as pharmacological tools because of low selectivity

and/or efficacy. β_1 -Adrenoceptors mediate increases in cardiac rate and force of contraction, stimulation of renin secretion, relaxation of coronary arteries, and relaxation of gastrointestinal smooth muscle. β_2 -Adrenoceptors mediate smooth muscle relaxation at many sites, including the airways, most blood vessels, and uterus. The pre-junctional β -adrenoceptor modulating noradrenaline release from sympathetic nerve terminals also appears to have β_2 -adrenoceptor characteristics.

Several agonists producing selective activation of the β_3 -adrenoceptor have been identified, including BRL 37344, ICI 198,157, and CL 316,243. Interestingly, the β_1/β_2 -adrenoceptor antagonist, CGP 12177, is a partial agonist at the β_3 -adrenoceptor (Langin et al., 1991; Emorine et al., 1992). The β_3 -adrenoceptor is insensitive to blockade by most β -adrenoceptor antagonists, although β_3 -adrenoceptor-mediated stimulation of adenylyl cyclase in cells expressing the recombinant receptor can be antagonized by the β_2 -adrenoceptor antagonist, ICI 118,551. No selective β_3 -adrenoceptor antagonist has been identified to date. The primary actions mediated by the β_3 -adrenoceptor are lipolysis in white adipose tissue and thermogenesis in brown adipose tissue, although there is evidence that this receptor also contributes to the stimulation of insulin secretion from pancreatic islet cells, inhibition of glycogen synthesis in skeletal muscle, and inhibition of contractile activity in gastrointestinal smooth muscle. It has been suggested that activation of the β_3 -adrenoceptor contributes to the intrinsic sympathomimetic action of some β -adrenoceptor antagonists (Kaumann, 1989).

The pharmacological tools used to characterize and subclassify β -adrenoceptors are presented in table 5.

C. Recombinant β -Adrenoceptors Subtypes

1. β_2 -Adrenoceptors. Screening of a hamster genomic library with oligonucleotides complementary to peptide fragments of the purified hamster lung β_2 -adrenoceptor yielded a clone that, when expressed in a variety of systems, had functional and radioligand-binding characteristics consistent with those of the β_2 -adrenoceptor (Dixon et al., 1986). Probes derived from this cDNA have been used, and β_2 -adrenoceptors from mouse, rat, and human sources have been recombinant. There are only minor species differences between these clones, with 87 to 93% overall amino acid identity.

2. β_1 -Adrenoceptors. It proved difficult to clone the β_1 -adrenoceptor, because the human β_2 -adrenoceptor cDNA did not cross-hybridize with the β_1 -adrenoceptor, even when the full-length coding sequence was used. A related receptor was isolated using the β_2 -adrenoceptor as a probe (Kobilka et al., 1987a), which proved to be the 5-HT_{1A} receptor (Fargin et al., 1988). Using the coding region of the 5-HT_{1A} receptor DNA to probe a human placental cDNA library, Frielle et al. (1987) finally identified the β_1 -adrenoceptor clone. The overall amino acid

identity of human β_1 - and β_2 -adrenoceptors is only 54%, although the amino acid identity between these receptors increases to 71% in the hydrophobic regions postulated to represent the membrane-spanning domains.

Again, using purified receptor protein as a source of oligonucleotide probes, the avian β -adrenoceptor of turkey erythrocyte was recombinant (Yarden et al., 1986). This clone closely resembles the mammalian β_1 -adrenoceptor (69% overall amino acid identity with the human receptor and 84% amino acid identity in the transmembrane-spanning region).

3. β_3 -Adrenoceptors. Screening of a human genomic library with probes prepared from the avian β -adrenoceptor and the human β_2 -adrenoceptor cDNA resulted in the identification of a novel clone having the predicted sequence for a G-protein-linked receptor (Emorine et al., 1989). The identity of primary structure was only 40 to 50% vis-a-vis the β_1 - or β_2 -adrenoceptors (64 to 69% in the putative transmembrane regions). Chinese hamster ovary cells expressing this DNA showed adenylyl cyclase activation in response to selective β_3 -adrenoceptor agonists and low affinity for propranolol, and several other classical β -adrenoceptor antagonists against isoprenaline induced adenylyl cyclase stimulation. Also, studies of radioligand binding to these cells were consistent with the expression of a β_3 -adrenoceptor. Southern analysis showed expression of mRNA hybridizing with this clone in rat tissues where "atypical" β -adrenoceptor-mediated responses have been shown, such as adipose tissue, liver, and skeletal muscle.

D. Relationship between Pharmacologically Defined and Recombinant β -Adrenoceptor Subtypes

For both the β_1 - and β_2 -adrenoceptors, there seems to be an excellent correlation between the properties of expressed receptor clones and the corresponding receptor as found in native tissues. This correlation is found both with respect to the ability to inhibit radioligand binding and the ability of agonists to stimulate adenylyl cyclase activity.

The properties of the β_3 -adrenoceptor, expressed in mammalian cells, also appears to correspond to those pharmacologically defined for the β_3 -adrenoceptor. Although the clone was derived from a human genomic library, some of the properties of the expressed receptor, such as the potency and intrinsic activity of the selective β_3 -adrenoceptor agonist, BRL 37344, appear to correspond more closely to those of the rat adipocyte than those of the human adipocyte (Zaagsma and Nahorski, 1990). This may reflect species differences in the β_3 -adrenoceptor density.

E. Are There Additional β -Adrenoceptor Subtypes?

The properties of the avian β -adrenoceptor are sufficiently different from those of the mammalian β_1 -adrenoceptor to suggest that these two β -adrenoceptors should be considered different receptor subtypes. The

TABLE 5
Pharmacological tools used to subclassify and characterize the β -adrenoceptor subtypes

Compound	Index*	β_1	β_2	β_3	Reference
Agonists					
Nonspecific					
Isoprenaline	K_i	14	21	ND†	Naito et al., 1985
	EC_{50}	1.3	2.2	9.3	Arch et al., 1984
	K_{act}	ND	ND	3.9	Emorine et al., 1992
β_1 Selective					
Xamoterol‡	K_i	135	4533	ND	Brodde et al., 1990
Denopamine‡	K_i	545	2205	ND	Naito et al., 1985
β_2 Selective					
Salbutamol	EC_{50}	810	26	2300	Arch et al., 1984
Salmeterol	EC_{50}	>30,000	4	ND	Ball et al., 1987
Terbutaline	EC_{50}	18,000	120	ND	Waldeck et al., 1986
β_3 Selective					
BRL 37344	EC_{50}	680	35	1.7	Arch et al., 1984
	K_{act}	ND	ND	5.9	Emorine et al., 1992
CGP 12177‡	K_{act}	ND	ND	300	Emorine et al., 1992
Antagonists					
Nonspecific					
Propranolol	K_B	1	0.7	95	Harms et al., 1977
	K_B	2	5250		Wilson et al., 1984
CGP 12177	K_D	0.3	0.9	ND	Nanoff et al., 1987
β_1 Selective					
Betaxolol	K_i	3	610	ND	Mauriege et al., 1988
Atenolol	K_i	125	1250	ND	Daemmgen et al., 1985
Metoprolol	K_i	3	4200	ND	Mauriege et al., 1988
Bisoprolol	K_i	0.2	1020	ND	Mauriege et al., 1988
	K_B	ND	ND	630	Langin et al., 1991
CGP 20712	K_i	0.6	1500	ND	Mauriege et al., 1988
β_2 Selective					
ICI 118551	K_i	710	4.6	ND	Mauriege et al., 1988
	K_B	ND	ND	1600	Wilson et al., 1984

* Parameter used to express affinity for the β -adrenoceptor. All dissociation equilibrium constants expressed in nM. Dissociation equilibrium constant (K_i) for the inhibition of radioligand binding to the β -adrenoceptor. Inhibition of the binding of a nonselective radioligand in membrane homogenates from a tissue containing both β_1 - and β_2 -adrenoceptor subtypes is biphasic and can be used to calculate the K_i value at each subtype. Alternatively, binding can be studied in tissues containing a relatively pure population of β_1 (e.g., human atrium, rat ventricle) or β_2 (e.g., human or rat lung) adrenoceptors. EC_{50} for induction of a functional response in a tissue containing a relatively pure population of β_1 (e.g., rat atrium), β_2 (e.g., guinea pig trachea), or β_3 (e.g., rat white or brown adipocytes) adrenoceptors. Affinity constant (K_{act}) for stimulation of adenylyl cyclase in Chinese hamster ovary cells expressing the β_3 -adrenoceptor. Receptor dissociation constant (K_B) for blockade of a functional response to a β -adrenoceptor agonist in a tissue having a relatively pure population of a particular β -adrenoceptor subtype. K_D for the interaction of [³H]CGP 12177 with rat cardiac membranes in the presence of subtype-saturating concentrations of a selective β_1 - or β_2 -adrenoceptor antagonist.

† ND, no data available.

‡ Partial agonist activity.

avian receptor has been classified as a β_1 -adrenoceptor, based on approximately equal potencies of noradrenaline and adrenaline. However, correlation between the ability of the subtype-selective β -adrenoceptor antagonists to inhibit [¹²⁵I]iodohydroxypindolol binding to turkey erythrocyte membranes and their potency at either β_1 - or β_2 -adrenoceptors is low (Minneman et al., 1980).

It is unlikely that all of the "atypical" β -adrenoceptor responses observed have characteristics consistent with the β_3 -adrenoceptor, and hence, the possibility of additional subtypes cannot be excluded.

V. Signal Transduction

As noted in the Introduction, it has been suggested that the adrenoceptors be divided into three families, the

α_1 -adrenoceptors, the α_2 -adrenoceptors, and the β -adrenoceptors (Bylund, 1988). This subdivision is suggested, in part, by the observation that each of the three receptor groups is associated with a specific second-messenger system. α_1 -Adrenoceptors increase intracellular calcium concentrations, whereas α_2 -adrenoceptors and β -adrenoceptors inhibit and stimulate adenylyl cyclase, respectively. Although the α_2 -adrenoceptors are consistently associated with inhibition of adenylyl cyclase, this does not necessarily represent the mode of signal transduction between receptor occupation and functional response in all tissues. There do not appear to be differences in signal transduction mechanisms between individual subtypes in each of the three major families.

A. α_1 -Adrenoceptor Subtypes

Activation of all known α_1 -adrenoceptor subtypes results in an increase of the intracellular calcium concentration. This is often a result of calcium release from intracellular stores by a mechanism involving G-protein-mediated activation of phospholipase C. This enzyme hydrolyzes phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate, which acts, in turn, to release intracellular calcium stores, and diacylglycerol, which activates protein kinase C (Minneman, 1988). However, many α_1 -adrenoceptor-mediated responses, particularly those in vascular smooth muscle, depend on the influx of extracellular calcium through voltage-gated channels (Tsujiimoto et al., 1989). There is often a correlation between the relative contribution of intracellular versus extracellular calcium and the efficacy of an α_1 -adrenoceptor agonist. It has been postulated that the α_1 -adrenoceptor in vascular smooth muscle is linked to two G-proteins, one mediating protein kinase C activation and another linked to a membrane calcium channel. In this model, occupation of the α_1 -adrenoceptor by a full agonist results in activation of both G-proteins, whereas partial agonists can only activate the G-protein linked to the calcium channel (Ruffolo and Nichols, 1988).

Although it has been postulated that these two signal transduction mechanisms are subtype specific, with the α_{1A} -adrenoceptor acting via calcium influx and the α_{1B} -adrenoceptor via intracellular calcium release (Minneman, 1988; Wilson and Minneman, 1990), it is now clear that there is at least partial overlap between the signal transduction mechanisms of these two α_1 -adrenoceptor subtypes (Klijn et al., 1991; Minneman and Atkinson, 1991; Esbenshade and Minneman, 1992).

α_1 -Adrenoceptors have also been linked to other signal transduction mechanisms, including effects on cyclic nucleotides, activation of phospholipase A₂ and phospholipase D (Minneman, 1988). Whether these effects are secondary to effects on inositol phosphate levels and/or intracellular calcium, or whether they are selectively linked to any particular subtype, is not yet clear.

B. α_2 -Adrenoceptor Subtypes

Although it is clear that activation of α_2 -adrenoceptors results in an inhibition of adenylyl cyclase activity, mediated through an inhibitory G-protein (Limbird, 1988), this may not always represent the signal transduction mechanism responsible for the effect associated with receptor activation. For example, α_2 -adrenoceptor-mediated platelet aggregation and inhibition of platelet adenylyl cyclase are not always associated (Clare et al., 1984), and α_2 -adrenoceptor-mediated inhibition of neurotransmitter release is generally insensitive to inactivation of an inhibitory G-protein by pertussis toxin (Nichols et al., 1988). Furthermore, it is unlikely that the adenylyl cyclase of vascular smooth muscle could be sufficiently activated under basal conditions to account

for α_2 -adrenoceptor-mediated pressor effects as a result of inhibition of cyclase activity (Nichols, 1991). On the other hand, the action of α_2 -adrenoceptor agonists on the renal collecting duct to produce functional antagonism of the action of vasopressin does appear to result from the inhibition of vasopressin stimulated adenylyl cyclase.

In vascular smooth muscle, the postjunctional α_2 -adrenoceptor may be linked, in a manner similar to the α_1 -adrenoceptor, to a calcium channel, allowing translocation of extracellular calcium to mediate the vasoconstrictor response to receptor activation (Nichols, 1991). α_2 -Adrenoceptors have also been shown to activate other signal transduction mechanisms, including activation of potassium channels, phospholipase A₂, and Na⁺/H⁺ exchange.

There is no evidence for subtype selectivity in any of the signal transduction mechanisms associated with α_2 -adrenoceptor activation. Subtype-selective antagonists have been used to demonstrate that both α_{2A} - and α_{2B} -adrenoceptors can mediate inhibition of adenylyl cyclase (Bylund and Ray-Prenger, 1989), and inhibition of adenylyl cyclase activity in OK cells is presumably mediated by the α_{2C} -adrenoceptor (Murphy and Bylund, 1988). The subtype selectivity of the other signal transduction mechanisms associated with the α_2 -adrenoceptor has not been evaluated.

C. β -Adrenoceptor Subtypes

All three β -adrenoceptor subtypes appear to be linked to adenylyl cyclase activation through a stimulatory G-protein, with no evidence for subtype-related differences in receptor-cyclase interaction (Tate et al., 1991). The β -adrenoceptor has been the prototype for studies on the linkage between receptor, G-protein, and enzyme catalytic units, and much is known regarding the molecular interactions among these three proteins (Stadel, 1991). There is evidence to suggest that in certain tissues, such as cardiac muscle, there could be a direct coupling between a stimulatory G-protein and a voltage-sensitive calcium channel (Yatani et al., 1988).

VI. Conclusions

It is clear that there are multiple, closely related, adrenoceptor subtypes, although their exact number and the appropriate mode of grouping into major families is still controversial. The development of additional subtype-selective agonists and antagonists for use as pharmacological tools will help resolve some of the remaining discrepancies, as will additional correlation of the properties of recombinant and pharmacologically defined receptors.

Why there should be so many closely related subtypes is a great mystery. Multiple subtypes often coexist in a particular tissue or even on an individual cell and can mediate opposing (i.e., α_2 - versus β -adrenoceptors), redundant (β_1 - and β_2 -adrenoceptors), or synergistic (α_1 -

and β -adrenoceptors) responses. Elucidation of the functions and interactions of these many subtypes remains a major challenge.

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