

International Union of Pharmacology. XXIX. Update on Endothelin Receptor Nomenclature

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Abstract	219
I. Introduction	220
II. Cloned endothelin receptors	220
III. Mammalian splice variants of endothelin _A and endothelin _B receptors	221
IV. Physiological role of receptors	222
V. Endogenous and synthetic agonists	223
VI. Radiolabeled agonists	223
VII. Antagonists	223
VIII. Radiolabeled endothelin _A -selective antagonists	223
IX. Endothelin _B -selective antagonists	223
X. Endothelin _A /endothelin _B antagonists	223
XI. Conclusions	225
Acknowledgments	225
References	225

Abstract—In mammals, the endothelin (ET) family comprises three endogenous isoforms, ET-1, ET-2, and ET-3. ET-1 is the principal isoform in the human cardiovascular system and remains the most potent and long-lasting constrictor of human vessels discovered. In humans, endothelins mediate their actions via only two receptor types that have been cloned and classified as the ET_A and ET_B receptors in the first NC-IUPHAR (International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification) report on nomenclature in 1994. This report was compiled before the discovery of the majority of endothelin receptor antagonists (particularly nonpeptides) currently used in the characterization of receptors and now updated in the present review. Endothelin receptors continue to be classified according to their rank order of potency for the three endogenous isoforms of endothelin. A selective ET_A receptor agonist has not been discovered, but highly selective

antagonists include peptides (BQ123, cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]; FR139317, *N*-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp-3 (2-pyridyl)-D-Ala) and the generally more potent nonpeptides, such as PD156707, SB234551, L754142, A127722, and TBC11251. Sarafotoxin S6c, BQ3020 ([Ala^{11,15}]Ac-ET-1₍₆₋₂₁₎), and IRL1620 [Suc-(Glu⁹, Ala^{11,15})-ET-1₍₈₋₂₁₎] are widely used synthetic ET_B receptor agonists. A limited number of peptide (BQ788) and nonpeptide (A192621) ET_B antagonists have also been developed. They are generally less potent than ET_A antagonists and display lower selectivity (usually only 1 to 2 orders of magnitude) for the ET_B receptor. Radioligands highly selective for either ET_A (¹²⁵I-PD151242, ¹²⁵I-PD164333, and ³H-BQ123) or ET_B receptors (¹²⁵I-BQ3020 and ¹²⁵I-IRL1620) have further consolidated classification into only these two types, with no strong molecular or pharmacological evidence to support the existence of further receptors in mammals.

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I. Introduction

In mammals, the endothelin (ET¹) family comprises three endogenous isoforms, ET-1, ET-2, and ET-3 (Yanagisawa et al., 1988; Inoue et al., 1989). These peptides mediate their actions via two receptor types, classified as the ET_A and ET_B receptors in the first NC-IUPHAR (International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification) receptors report on nomenclature by Masaki et al. (1994). This report was compiled before the discovery of the majority of ET receptor antagonists (particularly nonpeptides) currently used in the characterization of receptors and now updated in the present review. The single proposed ET_B receptor antagonist included by Masaki et al. (1994) was subsequently shown to lack efficacy and was withdrawn by the original discoverers (Urade et al., 1994). This review reflects both peptide and nonpeptide ET_B antagonists that are the compounds of choice. A second major change is the inclusion of radioligands that are highly selective for either ET_A (¹²⁵I-PD151242, ¹²⁵I-PD164333, and ³H-BQ123 (cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]) or ET_B receptors [¹²⁵I-BQ3020 ([Ala^{11,15}]Ac-ET-1₍₆₋₂₁₎) and ¹²⁵I-IRL1620 (Suc-(Glu⁹, Ala^{11,15})-ET-1₍₈₋₂₁₎)] that have been crucial to consolidating the present classification. Masaki et al. (1994) referred to a gene encoding a third receptor (P32940) cloned from the amphibian *Xenopus laevis* dermal melanophores, which was reported to be ET-3 specific (ET-3 > ET-1), the so-called ET_C receptor. However, to date, molecular and ligand binding techniques have failed to identify a mammalian homolog. Since NC-IUPHAR is predominantly interested in human receptors, with an extension to mammalian receptors, this review follows the recommendation to exclude consideration of nonmammalian receptors in the classification.

ET-1 is the principal isoform in the human cardiovascular system and remains the most ubiquitous, potent, and unusually long-lasting constrictor of human vessels discovered. ET-1 is unusual among the mammalian bioactive peptides in being released from a dual secretory pathway (Russell et al., 1998). The peptide is continuously released from vascular endothelial cells by the constitutive pathway, producing intense constriction of the underlying smooth muscle and contributing to the maintenance of endogenous vascular tone (Haynes and Webb, 1994). The peptide is also released from endothelial cell-specific storage granules (Weibel-Palade bodies) in response to external physiological, or perhaps patho-

physiological, stimuli producing further vasoconstriction (Russell et al., 1998). Thus, ET-1 functions as a locally released, rather than circulating, hormone, and concentrations are comparatively low in plasma and other tissues. ET-2 has been less extensively studied than other ET peptides, but it is present in human cardiovascular tissues and was as potent a vasoconstrictor as ET-1 in human arteries and veins (Maguire and Davenport, 1995). Endothelial cells do not synthesize ET-3, but the mature peptide is detectable in plasma (Matsumoto et al., 1994) and other tissues, including heart and brain. ET-3 is unique in that it is the only endogenous isoform that distinguishes between the two endothelin receptors. It has the same affinity at the ET_B receptor as ET-1 but, at physiological concentrations, has little or no affinity for the ET_A.

The only endogenous peptides with a high degree of sequence similarity to the ETs are the sarafotoxins (S6a, S6b, S6c, and S6d). This family of 21-amino acid (aa) peptides was originally discovered in the venom of a snake, *Atractaspis engadensis* (Takasaki et al., 1988).

II. Cloned Endothelin Receptors

Receptors can be identified by their amino acid structure and provide unambiguous evidence for expression of a gene encoding a particular type in specific cells or tissues. An increasing number of mammalian species have been studied, but only two ET receptors have been isolated and cloned (Table 1; Arai et al., 1990; Sakurai et al., 1990; Adachi et al., 1991; Lin et al., 1991; Nakamura et al., 1991; Saito et al., 1991; Baynash et al., 1994). The deduced amino acid sequences for the two human receptors display only 59% similarity and are shown in Table 2. The amino acid sequences of ET_A receptors also differ between humans and other species, for example by 9% between human and rat ET_A receptors and by 12% for the ET_B. These may contribute to differences in efficacy and potency of selective agonists and antagonists.

The structures of the mature receptors have been deduced from the nucleotide sequences of the cDNAs.

TABLE 1
Cloned mammalian endothelin receptors

Potency:	Mammalian	
	ET _A	ET _B
	ET-1 = ET-2 > ET-3	ET-1 = ET-2 = ET-3
Human	427	442
Bovine	427	441
Rat	426	442
Mouse		442
Porcine	427	443
Equine		443

Values are the number of amino acids in the predicted receptor protein. Percentages indicate the degree of sequence similarity between receptor types. References: Arai et al., 1990; Sakurai et al., 1990; Hosoda et al., 1991; Kozuka et al., 1991; Lin et al., 1991; Nakamura et al., 1991; Elshourbagy et al., 1992; Hosoda et al., 1994; Nishimura et al., 1995; Yang et al., 1998.

¹ Abbreviations: ET, endothelin; aa, amino acid(s); NC-IUPHAR, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification; 7TM, seven-transmembrane; FR139317, N-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp-3(2-pyridyl)-D-Ala; BQ123, cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]; IRL1620, Suc-(Glu⁹, Ala^{11,15})-ET-1₍₈₋₂₁₎; BQ3020, [Ala^{11,15}]Ac-ET-1₍₆₋₂₁₎; PD142893, Ac-(β-Phenyl) D-Phe-L-Leu-L-Asp-L-Ile-L-Trp; SB209670, (1RS,2SR,3RS)-3-(2-carboxymethoxy-4-methoxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid.

TABLE 2
Amino acid sequences of the human ET_A and ET_B receptors

ET_A	M-----ETLCLRAS
ET_B	MQPPSLCGRALVALVLACGLSRIWGEERGFPPDRA---TP- LLQTAEIMTPPTKTL
ET_A	WLALVGCVISDNPERYSTNLSNHVDDFTFRGTELSFLVTHQPTNLVLPNSGSMHNYC
ET_B	W-----PKGSNASLARSLAPAEVFKGDR---TAGSPRTISPPP-----C
I	
ET_A	PQQTKITSAFKYINTVISCTIFIVGMVGNATLLRRIYQNKCMRNGPNALIASLALGDLI
ET_B	QGPTEIKETFKYINTVVSCLVFLGIIGNSTLLRRIYKNCMRNGPNILIASLALGDLI
II	
ET_A	<u>YVVIDLPINVFKLLAGRWPFHDHDFGVFLCKLFPFLQKSSVGITVLNLCALSVDRYRAV</u>
ET_B	<u>HIVIDIPINVKLLAEDWPFGE-----MCKLVFPIQKASVGITVLSLICALSIDRYRAV</u>
III	
IV	
ET_A	ASWSRVQIGIPLVTAIEIVSIWILSFILAIPEAIGFVMVPPFYRGEQHKTCMLNATSK
ET_B	ASWSRIKIGIVPKWTAVEIVLIWVSVVLAVPEAIGFDIITMDYKGSYLRIICLLHPVQK
ET_A	--FMEFYQDVKDWWLFGFYFCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRRE
ET_B	TAFMQFYKTAKDWWLFSFYFCLPLAITAFFYTLMTCEML-RKKSQMIALNDHLKQRRE
V	
ET_A	<u>VAKTVFCLVVFALCWFLHLSRILKKTIVYNEMDKNRCELLSFLLLMDYIGINLATMNS</u>
ET_B	<u>VAKTVFCLVLFALCWFLHLSRILKLTLYNQNDPNRCELLSFLLVLDYIGINMASLNS</u>
ET_A	<u>CINPIALYFVSKKFKNCQFQSCCLCCCYQSKSLMSTVPMNGTSTIQWKNHDQNNHNTDRSS</u>
ET_B	<u>CINPIALYLVSKRFKNCFKSCCLCCWCQSFEEKQSLEEKQSCLKFKANDHGYDNFRSSNK</u>
ET_A	HKDSMN
ET_B	YSSS

Membrane-spanning domains (I-VII) are shown underlined.

The encoded proteins contain seven stretches of 20 to 27 hydrophobic aa residues in both receptors, consistent with both subtypes belonging to the seven-transmembrane (7TM) domain, G protein-coupled rhodopsin-type receptor superfamily. Both receptors have an N-terminal signal sequence, which is rare among heptahelical receptors, with a relatively long extracellular N-terminal portion preceding the first transmembrane domain. There are two separate ligand interaction subdomains on each endothelin receptor. The extracellular loops, particularly between TM 4 to 6, determine selectivity.

At present, there is no justification for further types beyond the current classification into ET_A and ET_B in mammalian tissue. Functional studies have suggested that PD142893 [Ac-(beta-phenyl) D-Phe-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp], a hexapeptide antagonist, can block the vasodilator actions of ET-1 at endothelial ET_B receptors but not constrictor responses mediated by ET_B smooth muscle receptors (Warner et al., 1993; Douglas et al., 1995). However, in the ET_B receptor gene knockout mouse, both the PD142893-sensitive vasodilator response and the PD142893-resistant contractile response to the ET_B agonist sarafotoxin S6c were completely absent. These results indicate that the pharmacologically heterogeneous responses to S6c are mediated by ET_B receptors derived from the same gene (Mizuguchi et al., 1997). In agreement, a very detailed binding study (including PD142893) was unable to distinguish between ET_B receptors expressed by human isolated endothelial cells compared with smooth muscle cells in culture (Flynn et al., 1998). Furthermore, in human tissue, both ET_A - and ET_B -selective radiolabeled ligands bound with a single affinity and Hill slopes close to unity (Molenaar et al., 1992; Davenport et al., 1994, 1998; Davenport,

1997). Similarly, competition studies using unlabeled ligands provided no evidence for further subtypes (Peter and Davenport, 1996; Russell and Davenport, 1996).

III. Mammalian Splice Variants of Endothelin_A and Endothelin_B Receptors

Alternative splice variants of ET receptors have been reported but to date these variants either show little or no change in binding characteristics and their physiological or pathophysiological significance is unclear. The following is intended to be a guide only because the field has not developed sufficiently with unequivocal quantitative evidence for significant expression and function in native tissues rather than artificial cell lines, to make any firm recommendation for classification.

The existence of alternative splice variants of the ET_B receptor in human and porcine tissue has been reported. A variant human ET_B receptor that results in a 10-aa increase in the length of the second cytoplasmic domain has been described (Shyamala et al., 1994). Messenger RNA measured by reverse transcription-polymerase chain reaction in a limited number of human tissues was found only in low abundance in human brain (which expresses one of the highest densities of ET_B receptors) as well as the heart, lung, and placenta but was not detected in other species tested (bovine, porcine, and rat). The increase in amino acids did not result in any change in either ligand affinities or signal transduction (cAMP and inositol phosphate turnover), and the physiological importance of this variant receptor is unclear.

Elshourbagy et al. (1996) discovered a second splice variant from a human placental library. Analysis indicated that the deduced polypeptide was identical to the

native ET_B sequence except that the 42 aa of the intracellular carboxy terminus of the former was replaced with an alternative 36-aa sequence, bearing no significant homology with other known proteins. Northern blot analysis indicated an mRNA species of 2.7 kilobases, which was expressed in all of a limited number of human tissues tested (lung, placenta, kidney, and skeletal muscle) in addition to mRNA encoding the native ET_B receptor. However, mRNA encoding the variant was not particularly abundant. The relative ratio of each individual variant mRNA was less than 10% of the total ET_B mRNA, with the intriguing exception of skeletal muscle where it represented more than 40%. Two cell types were also examined, endothelial and smooth muscle cells, but only mRNA encoding the native receptor was detected. The cloned variant receptors expressed in COS cells displayed similar binding properties for ET peptides compared with expressed native receptors in the same cells, indicating unsurprisingly that the splice variant had little or no effect on ligand binding. However, functional studies showed that ET-stimulated inositol phosphate accumulation in expressed native receptors was abolished in cells transfected with the splice variant. These data suggest the difference in the amino acid sequences between the two receptors may alter functional coupling in the variant receptor.

Nambi et al. (2000) detected a novel cDNA from another species, porcine cerebellum, that was predicted to encode an ET_B receptor also with alternate splicing of the carboxy terminus, resulting in a deduced polypeptide of 429 aa, 14 residues shorter than the wild-type receptor. The relative abundance of mRNA encoding the splice variant compared with the wild-type receptor was not reported, but mRNA was detected in ET_B-rich tissues including porcine lung, kidney, and cerebellum. However, the splice variant did not alter the binding of radiolabeled ET-1 or functional coupling when expressed in COS cells. The lack of effect on inositol phosphate accumulation is in marked contrast to the human variant (see above) previously described by this group. Combined with the lack of sequence similarity between the human (38 aa) and porcine (29 aa) carboxy terminal splice variants, it is not clear whether the porcine variant is a homolog of the human or whether these are distinct splice variants.

Cheng et al. (1993) identified cDNA from rat brain, which they described as producing a receptor protein with four amino acid substitutions that displayed equal affinity for the three ET isoforms. However, Cheng et al. (1993) probably described the correct rat ET_B sequence, correcting a sequencing error in the previously deduced sequence of Sakurai et al. (1990), for the following reasons. The Cheng et al. (1993) sequence has 3 extra bases in a 9-base span, which corrects a pair of adjacent frame-shifts in the Sakurai et al. sequence, making the DNA sequence identical to the mouse sequence (Hosoda et al., 1994) in the same region and matching 3 of 4 amino

acids in the human sequence as opposed to 0 of 4 with the Sakurai et al. sequence. Cheng et al. (1993) also report a different sequence in the 5'-untranslated region, which could be an alternative first exon, reflecting transcription initiating from an alternative promoter. It is also possible, although less likely, that one of the 5'-untranslated region sequences is an artifact, such as a chimeric cDNA or sequencing assembly error.

The human ET_A receptor gene has been proposed to give rise to at least three alternatively spliced ET_A receptor transcripts corresponding to deletion of exon 3 (producing a protein with two membrane-spanning domains), exon 4 (producing a protein with three membrane-spanning domains), and exon 3 plus exon 4 (producing a protein lacking the third and fourth domain) (Miyamoto et al., 1996; Bourgeois et al., 1997). Although alternative transcripts were identified in human tissues including lung, aorta, and atrium, the truncated receptors when expressed in COS cell lines did not bind ET-1 (Miyamoto et al., 1996), and a physiological role remains unclear. Intriguingly, mRNA encoding the putative truncated receptor with the deletion of exon 3 plus 4 was more abundant than the wild type in human melanoma cell lines and melanoma tissue (Zhang et al., 1998).

IV. Physiological Role of Receptors

Endothelin receptors are widely expressed in all tissues, which is consistent with the physiological role of endothelins as ubiquitous endothelium-derived vasoactive peptides, contributing to the maintenance of vascular tone. In humans, ET_A receptors predominate on the smooth muscle of blood vessels, and the low density of ET_B receptors (<15%) also present on the smooth muscle contributes little to vasoconstriction in either normal or diseased tissue (Maguire and Davenport, 1995). ET_B receptors are the principal type in the kidney, localizing to nonvascular tissues. Evidence is emerging that the ET_B receptor functions as a "clearing receptor" to remove ET from the circulation. ET_B receptors localized to the single layer of endothelial cells that line all blood vessels, may play a role in the release of endothelium-derived relaxing factors, such as nitric oxide and prostanooids (Warner et al., 1989), where all three isoforms have a similar potency (de Nucci et al., 1988). Although ET_A receptors present on smooth muscle cells are mainly responsible for contraction throughout the human vasculature, the situation in animals is more complex since the relative contribution from activating constrictor ET_B receptors can vary, depending on the species and vascular bed. In some blood vessels, such as the rabbit saphenous vein, rabbit jugular vein, rat renal vascular bed, and porcine pulmonary vein, ET_B receptors mediate vasoconstriction. In other vessels, ET-1 is thought to mediate vasoconstriction by activating both receptors.

Receptors are also localized to nonvascular structures, such as epithelial cells, as well as occurring in the central nervous system on glia and neurones. Endothelin stimulates proliferation in a number of different cell types, including smooth muscle cells (mainly via the ET_A subtype) or astrocytes (ET_B). In most of these cells, ET is thought to be comitogenic, potentiating the actions of other growth factors such as platelet-derived growth factor.

V. Endogenous and Synthetic Agonists

ET receptors continue to be classified (Table 3; Davenport, 2000) according to their rank order of potency for the endogenous ET isoforms. A selective ET_A receptor agonist has not been discovered.

Sarafotoxin S6c is one of the most widely used ET_B-selective agonists, displaying over 200,000-fold selectivity in the rat (William et al., 1991), although the peptide is much less selective in human tissues, perhaps reflecting species differences in the receptors (Russell and Davenport, 1996). [Ala^{1,3,11,15}]ET-1 (Saeki et al., 1991), the linear analog of ET-1 in which the disulfide bridges have been removed by substitution of Ala for Cys residues, is ET_B-selective. The truncated linear synthetic analogs BQ3020 and IRL1620 are the most widely used selective synthetic agonists to characterize ET_B receptors. The compounds cause endothelium-dependent vasodilation in preparations such as porcine pulmonary artery, which is consistent with ET_B receptor-mediated release of relaxing factors from the endothelium.

VI. Radiolabeled Agonists

Most studies characterizing and localizing ET receptors use ¹²⁵I-ET-1, directly labeled via the Tyr¹³ (Table 3). This ligand binds with the same affinity to both ET_A and ET_B receptors and is stable under nonphysiological binding conditions with little or no degradation of labeled ET-1 being detected. ¹²⁵I-ET-2, ¹²⁵I-vasoactive intestinal contractor (the murine isoform of ET-2), and ¹²⁵I-sarafotoxin 6b have been labeled and used in saturation assays where they also bind to both receptors (Davenport and Morton, 1991; Maguire et al., 1996).

ET-3 can be labeled at Tyr⁶, Tyr¹³, and Tyr¹⁴. Tyr⁶ is generally used, as it is more difficult to separate ¹²⁵I-ET-3 labeled at the latter two Tyr residues, although all three ET-3 ligands have similar affinities. The selectivity of ET-3 for ET_B versus ET_A receptors is often only about two orders and it is difficult to precisely delineate the two receptors using this labeled peptide in saturation assays. ET_B receptors are usually characterized using ¹²⁵I-BQ3020 (Ihara et al., 1992b; Molenaar et al., 1992), which binds with subnanomolar affinity to the ET_B receptor, with at least 1500-fold selectivity for this receptor over the ET_A. Alternatively, the truncated analog ¹²⁵I-IRL1620 can also be used, particularly in animal tissues (Watakabe et al., 1992).

VII. Antagonists

Antagonists are currently classified as either ET_A-selective, ET_B-selective, or mixed antagonists that display similar affinity for both receptors. The most highly selective peptide antagonists (4 to 5 orders of selectivity) for the ET_A receptors are the cyclic pentapeptide BQ123 (Ihara et al., 1992a) and the modified linear peptide FR139317 (*N*-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp-3(2-pyridyl)-D-Ala; Aramori et al., 1993). Unlike peptide antagonists, many nonpeptide ET_A receptor-selective antagonists have oral bioavailability and some may cross the blood-brain barrier. The majority are more potent, with pA₂ values of up to 10 compared with 7 or 8 for BQ123 or FR139317, but are less selective, and plasma binding may also be significant in vivo.

VIII. Radiolabeled Endothelin_A Selective Antagonists

¹²⁵I-PD151242 is widely used to characterize and localize ET_A receptors. This linear tetrapeptide analog of FR139317, binds with subnanomolar affinity to the ET_A receptor and has about 10,000-fold selectivity for this receptor in human and animal tissues. A nonpeptide ET_A-selective ligand has also been developed, ¹²⁵I-PD164333 (Davenport et al., 1998) with comparable affinity as well as a tritiated ligand, ³H-BQ123 (Ihara et al., 1995). The above ligands are available commercially either as catalog items or custom syntheses.

IX. Endothelin_B Selective Antagonists

A more limited number of peptide (e.g., BQ788) and nonpeptide (e.g., A192621) ET_B antagonists have been developed, reflecting the lack of clinical need for this type of compound. They are less potent than ET_A antagonists and display lower selectivity (usually only 1 to 2 orders of magnitude) for the ET_B receptor (Table 3).

X. Endothelin_A/Endothelin_B Antagonists

The distinction between antagonists that are ET_A-selective and those that block both ET_A and ET_B receptors is not precise but generally the former display greater than 100-fold selectivity for the ET_A subtype, and the latter less than 100-fold. These compounds are seldom reported as having equal affinity for both receptors, and this should be taken into consideration in experimental designs. Nonpeptide compounds included bosentan (RO470203, Tracleer; Actelion, San Francisco, CA) (Clozel et al., 1994), SB209670 (Elliott et al., 1994), SB217242 (enrasentan; Ohlstein et al., 1996), and RO610612 (tezosentan; Clozel et al., 1999). Plasma binding may also be significant in vivo.

TABLE 3
Classification of endothelin receptors

Receptor	ET _A
Receptor Code	2.1:ET:1:ETA:
Previous names	None
Structural information	7TM h 427 aa, P25101, chr, 4; (Adachi et al., 1991) r 426 aa, P26684; (Lin et al., 1991)
Functional assays	Vasoconstriction in rat aorta
Agonists	Selective: none
Agonist potencies	ET-1 = ET-2 > S6b >> ET-3 (human coronary artery)
Antagonist potencies	BQ123 (pA ₂ 6.9–7.4; Ihara et al., 1992a) PD155080 (8–8.5; Maguire et al., 1995) FR139317 (7.3–7.9; Aramori et al., 1993) PD156707 (8–8.7; = CI1020; Doherty et al., 1995) SB234551 (9; Ohlstein et al., 1998) L754142 (7.7–8.7; Williams et al., 1995) BMS182874 (6.2; Stein et al., 1994) A127722 (9–10.5; ABT627; Opgenorth et al., 1996) TBC11251 (8.0; Wu et al., 1997) LU127043 (7.3; Raschack et al., 1995) LU135252; (Münter et al., 1996)
Radioligand assays	human, rat and porcine heart; A10 smooth muscle cells
Radioligands	¹²⁵ I-ET-1 ($K_d = 0.01\text{--}5$ nM) Davenport, 1997 ¹²⁵ I-PD151242 (0.5 nM) Davenport et al., 1994 ¹²⁵ I-PD164333 (0.2 nM) Davenport et al., 1998 ³ H-BQ123 (3.2 nM) Ihara et al., 1995
Transduction mechanisms	G protein-coupled: increase in phosphatidyl inositol turnover with elevation of [Ca ²⁺] _i ; activation of Ca ²⁺ influx
Receptor distribution	Mainly vascular smooth muscle and therefore in all tissues receiving a blood supply, including heart, lung, and brain
Tissue functions	Vasoconstriction; positive inotrope, cell proliferation (e.g., smooth muscle, mesangial cells)
Phenotypes	Craniofacial and cardiovascular malformations in ET _A knockout mice (Clouthier et al., 1998)
Receptor	ET _B
Receptor Code	2.1:ET:2:ETB:
Previous names	None
Structural information	7TM h 442 aa, P24530, chr. 13; (Nakamuta et al., 1991) r 441 aa, P21451; (Sakurai et al., 1990) m 442 aa, P48302; (Baynash et al., 1994)
Functional assays	Initial depressor response in vivo, NO release, PI generation; vasoconstriction in some vascular beds depending on species (e.g., rabbit pulmonary artery)
Agonists	selective: [Ala ^{1,3,11,15}]ET-1; (Saeki et al., 1991) BQ3020; (Ihara et al., 1992b) IRL 1620; (Takai et al., 1992) S6c; (William et al., 1991)
Agonist potencies	ET-1 = ET-2 = ET-3 = S6b (rat glomeruli)
Antagonist potencies	IRL2500 (pA ₂ 7.8; Balwierczak et al., 1995) RES7011 (6.0; Tanaka et al., 1994) BQ788 (6.9; Ishikawa et al., 1994) Ro468443 (pA ₂ 8.1; Clozel and Breu, 1996) A192621 (8.1; von Geldern et al., 1999)
Radioligand assays	Brain, lung, placenta, and kidney
Radioligands	¹²⁵ I-ET-1 ($K_d = 0.01\text{--}5$ nM) Davenport, 1997 ¹²⁵ I-BQ3020 (0.1 nM) Ihara et al., 1992b ¹²⁵ I-[Ala ^{1,3,11,15}]ET-1 (0.2 nM) Molenaar et al., 1992 ¹²⁵ I-IRL 1620 (0.02 nM) Watakabe et al., 1992
Transduction mechanisms	G protein-coupled: increase in phosphatidyl inositol turnover with elevation of [Ca ²⁺] _i ; activation of Ca ²⁺ influx
Receptor distribution	Vascular, endothelial cells; high densities present in the brain, lung, heart, and intestine
Tissue functions	Vasodilatation, bronchoconstriction, vasoconstriction, cell proliferation (e.g., astrocytes)
Phenotypes	Polymorphism (N104I; Tanaka et al., 1998) and mutations (S390R and C109R, Tanaka et al., 1998; W276C, Puffenburger et al., 1994) in human ET _B receptor gene in Hirschsprung's disease. ET _B knockout mice have aganglionic megacolon (Hosoda et al., 1994; resembling Hirschsprung's disease), associated with coat color spotting, and are deficient in sensing inflammatory pain (Griswold et al., 1999)

XI. Conclusions

In humans, ET peptides mediate their actions via only two receptor types, classified as ET_A and ET_B. There is no strong evidence to support the existence of further receptors in mammals. Further research is required to establish whether any of the potential splice variants in the ET_B receptor have a physiological or pathophysiological role.

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REFERENCES

- Adachi M, Yang YY, Furuichi Y, and Miyamoto C (1991) Cloning and characterization of cDNA encoding human A-type endothelin receptor. *Biochem Biophys Res Commun* **180**:1265–1272.
- Arai H, Hori S, Aramori I, Ohkubo H, and Nakanishi S (1990) Cloning and expression of a cDNA encoding an endothelin receptor. *Nature (Lond)* **348**:730–732.
- Aramori I, Nirei H, Shoubo M, Sogabe K, Nakamura K, Kojo H, Notsu Y, Ono T, and Nakanishi S (1993) Subtype selectivity of a novel endothelin antagonist, FR139317, for the two endothelin receptors in transfected Chinese hamster ovary cells. *Mol Pharmacol* **43**:127–131.
- Balwierczak JL, Brusseau CW, DeGrande D, Jeng AY, Savage P, and Shetty SS (1995) Characterization of a potent and selective endothelin-B receptor antagonist, IRL 2500. *J Cardiovasc Pharmacol* **26** (Suppl 3):S393–S396.
- Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, and Yanagisawa M (1994) Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* **79**:1277–1285.
- Bourgeois C, Robert B, Rebouret R, Mondon F, Mignot T-M, Duc-Goiran P, and Ferre F (1997) Endothelin-1 and ETA receptor expression in vascular smooth muscle cells from human placenta: a new ETA receptor messenger ribonucleic acid is generated by alternative splicing of exon 3. *J Clin Endocrinol Metab* **82**:3116–3123.
- Cheng HF, Su YM, Yeh JR, and Chang KJ (1993) Alternative transcript of the nonselective-type endothelin receptor from rat brain. *Mol Pharmacol* **44**:533–538.
- Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, and Yanagisawa M (1998) Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. *Development* **125**:813–824.
- Clozel M and Breu V (1996) The role of ET_B receptors in normotensive and hypertensive rats as revealed by the non-peptide selective ET_B receptor antagonist Ro 46–8443. *FEBS Lett* **383**:42–45.
- Clozel M, Breu V, Gray GA, Kalina B, Löffler BM, Burri K, Cassal JM, Hirth G, Muller M, Neidhart W, and Ramuz H (1994) Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J Pharmacol Exp Ther* **270**:228–235.
- Clozel M, Ramuz H, Clozel J-P, Breu V, Hess P, Löffler B-M, Coassolo P, and Roux S (1999) Pharmacology of tezosentan, new endothelin receptor antagonist designed for parenteral use. *J Pharmacol Exp Ther* **290**:840–846.
- Davenport AP (1997) Distribution of endothelin receptors, in *Endothelin in Biology and Medicine* (Miller R, Pelton JT, and Huggins J eds) pp 45–68, CRC Press, NY.
- Davenport AP (2000) Endothelin receptors, in *The IUPHAR Compendium of Receptor Characterization and Classification*, 2nd ed, pp 182–188, IUPHAR Media, London, UK.
- Davenport AP, Kuc RE, Ashby MJ, Patt WC, and Doherty AM (1998) Characterization of [¹²⁵I]-PD164333, an ET_A selective non-peptide radiolabelled antagonist, in normal and diseased human tissues. *Br J Pharmacol* **123**:223–230.
- Davenport AP, Kuc RE, Fitzgerald F, Maguire JJ, Berryman K, and Doherty AM (1994) [¹²⁵I]-PD15242, a selective radioligand for human ET_A receptors. *Br J Pharmacol* **111**:4–6.
- Davenport AP and Morton AJ (1991) Binding sites for ¹²⁵I ET-1, ET-2, ET-3 and vasoactive intestinal contractor are present in adult rat brain and neurone-enriched primary cultures of embryonic brain cells. *Brain Res* **554**:278–285.
- de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD, and Vane JR (1988) Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelin-derived relaxing factor. *Proc Natl Acad Sci USA* **85**:9797–9800.
- Doherty AM, Patt WC, Edmunds JJ, Berryman KA, Reisdorph BR, Plummer MS, Shahripour A, Lee C, Cheng XM, Walker DM, et al. (1995) Discovery of a novel series of orally active non-peptide endothelin-A (ET_A) receptor-selective antagonists. *J Med Chem* **38**:1259–1263.
- Douglas SA, Beck GR Jr, Elliott JD, and Ohlstein EH (1995) Pharmacologic evidence for the presence of three functional endothelin receptor subtypes in rabbit saphenous vein. *J Cardiovasc Pharmacol* **26** (Suppl 3):S163–S168.
- Elliott JD, Lago MA, Cousins RD, Gao A, Leber JD, Erhard KF, Nambi P, Elshourbagy NA, Kumar C, Lee JA, et al. (1994) 1,3-Diarylindan-2-carboxylic acids, potent and selective non-peptide endothelin receptor antagonists. *J Med Chem* **37**:1553–1557.
- Elshourbagy NA, Adamou JE, Gagnon AW, Wu HL, Pullen M, and Nambi P (1996) Molecular characterization of a novel human endothelin receptor splice variant. *J Biol Chem* **271**:25300–25307.
- Elshourbagy NA, Lee JA, Korman DR, Nuthalaganti P, Sylvester DR, Dilella AG, Sutiphong JA, and Kumar CS (1992) Molecular cloning and characterization of the major endothelin receptor subtype in porcine cerebellum. *Mol Pharmacol* **41**:465–473.
- Flynn MA, Haleen SJ, Welch KM, Cheng XM, and Reynolds EE (1998) Endothelin B receptors on human endothelial and smooth-muscle cells show equivalent binding pharmacology. *J Cardiovasc Pharmacol* **32**:106–116.
- Griswold DE, Douglas SA, Martin LD, Davis TG, Davis L, Ao Z, Luttmann MA, Pullen M, Nambi P, Hay DWP, and Ohlstein EH (1999) Endothelin B receptor modulates inflammatory pain and cutaneous inflammation. *Mol Pharmacol* **56**:807–812.
- Haynes WG and Webb DJ (1994) Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet* **344**:852–854.
- Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, and Yanagisawa M (1994) Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* **79**:1267–1276.
- Hosoda K, Nakao K, Arai H, Suga S, Ogawa Y, Mukoyama M, Shirakami G, Saito Y, Nakanishi S, and Imura H (1991) Cloning and expression of human endothelin-1 receptor cDNA. *FEBS Lett* **287**:23–26.
- Ihara M, Noguchi K, Saeiki T, Fukuroda T, Tsuchida S, Kimura S, Fukami T, Ishikawa K, Nishibe M and Yano M (1992a) Biological profiles of highly potent novel endothelin antagonists selective for the ET(A) receptor. *Life Sci* **50**:247–255.
- Ihara M, Saeiki T, Fukuroda T, Kimura S, Ozaki S, Patel AC, and Yano MA (1992b) A novel radioligand [¹²⁵I]BQ-3020 selective for endothelin (ET_B) receptors. *Life Sci* **51**:47–52.
- Ihara M, Yamanaka R, Ohwaki K, Ozaki S, Fukami T, Ishikawa K, Towers P, and Yano M (1995) [³H]BQ-123, a highly specific and reversible radioligand for the endothelin ET_A receptor subtype. *Eur J Pharmacol* **274**:1–6.
- Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyachi T, Goto K, and Masaki T (1989) The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA* **86**:2863–2867.
- Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeiki T, Fukuroda T, Fukami T, Ozaki S, Nagase T, et al. (1994) Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Natl Acad Sci USA* **91**:4892–4896.
- Kozuka M, Ito T, Hirose S, Lodhi KM, and Hagiwara H (1991) Purification and characterization of bovine lung endothelin receptor. *J Biol Chem* **266**:16892–16896.
- Lin HY, Kaji EH, Winkel GK, Ives HE, and Lodish HF (1991) Cloning and functional expression of a vascular smooth muscle endothelin 1 receptor. *Proc Natl Acad Sci USA* **88**:3185–3189.
- Maguire JJ and Davenport AP (1995) ET_A receptors mediate the constrictor responses to endothelin peptides in human blood vessels *in vitro*. *Br J Pharmacol* **115**:191–197.
- Maguire JJ, Kuc RE, Doherty AM, and Davenport AP (1995) Potency of PD155080, an orally active ET_B receptor antagonist, determined for human endothelin receptors. *J Cardiovasc Pharmacol* **26** (Suppl 3):S362–S364.
- Maguire JJ, Kuc RE, Rous BA, and Davenport AP (1996) Failure of BQ123, a more potent antagonist of sarafotoxin S6b than of endothelin-1, to distinguish between these agonists in binding experiments. *Br J Pharmacol* **118**:335–342.
- Masaki T, Vane JR, and Vanhoutte PM (1994) International Union of Pharmacology nomenclature of endothelin receptors. *Pharmacol Rev* **46**:137–142.
- Matsumoto H, Suzuki N, Kitada C, and Fujino M (1994) Endothelin family peptides in human plasma and urine: their molecular forms and concentrations. *Peptides* **15**:505–510.
- Miyamoto Y, Yoshimasa T, Arai H, Takaya K, Ogawa Y, Itoh H, and Nakao K (1996) Alternative RNA splicing of the human endothelin-A receptor generates multiple transcripts. *Biochem J* **313**:795–801.
- Mizuguchi T, Nishiyama M, Moroi K, Tanaka H, Saito T, Masuda Y, Masaki T, de Wit D, Yanagisawa M, and Kimura S (1997) Analysis of two pharmacologically predicted endothelin B receptor subtypes by using the endothelin B receptor gene knockout mouse. *Br J Pharmacol* **120**:1427–1430.
- Molenaar P, Kuc RE, and Davenport AP (1992) Characterization of two new ET_B selective radioligands, [¹²⁵I]-BQ3020 and [¹²⁵I]-[Ala^{1,3,11,15}]ET-1 in human heart. *Br J Pharmacol* **107**:637–639.
- Münter K, Hergenröder S, Unger L, and Kirchengast M (1996) Oral treatment with an ET_A receptor antagonist inhibits neointima formation induced endothelial injury. *Pharm Pharmacol Lett* **6**:90–92.
- Nakamura M, Takayanagi R, Sakai Y, Sakamoto S, Hagiwara H, Mizuno T, Saito Y, Hirose S, Yamamoto M, and Nawata H (1991) Cloning and sequence analysis of a cDNA encoding human non-selective type of endothelin receptor. *Biochem Biophys Res Commun* **177**:34–39.
- Nambi P, Wu H-L, Ye D, Gagnon A, and Elshourbagy N (2000) Characterization of a novel porcine endothelin(B) receptor splice variant. *J Pharmacol Exp Ther* **292**:247–253.
- Nishimura J, Aoki H, Chen X, Shikasho T, Kobayashi S, and Kanaide H (1995) Evidence for the presence of endothelin ETA receptors in endothelial cells in situ on the aortic side of porcine aortic valve. *Br J Pharmacol* **115**:1369–1376.
- Ohlstein EH, Nambi P, Hay DWP, Gellai M, Brooks DP, Luengo J, Xiang J-N, and Elliott JD (1998) Nonpeptide endothelin receptor antagonists. XI. Pharmacological characterization of SB 234551, a high-affinity and selective nonpeptide ET_A receptor antagonist. *J Pharmacol Exp Ther* **286**:650–656.
- Ohlstein EH, Nambi P, Lago A, Hay DW, Beck G, Fong KL, Eddy EP, Smith P, Ellens H, and Elliott JD (1996) Nonpeptide endothelin receptor antagonists. VI. Pharmacological characterization of SB 217242, a potent and highly bioavailable endothelin receptor antagonist. *J Pharmacol Exp Ther* **276**:609–615.
- Ogenorth TJ, Adler AL, Calzadilla SV, Chiou WJ, Dayton BD, Dixon DB, Gehrke LJ, Hernandez L, Magnuson SR, Marsh KC, et al. (1996) Pharmacological Characterization of A-127722, an orally active and highly potent ET_A-selective receptor antagonist. *J Pharmacol Exp Ther* **276**:473–481.

- Peter MG and Davenport AP (1996) Characterization of endothelin receptor selective agonist BQ3020 and antagonists BQ123, FR139317, BQ788, 50235, Ro462005 and bosentan in the heart. *Br J Pharmacol* **117**:455–462.
- Puffenberger EG, Hosoda K, Washington SS, Nakao K, deWit D, Yanagisawa M, and Chakravart A (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* **79**:1257–1266.
- Raschack M, Unger L, Riechers H, and Klinge D (1995) Receptor selectivity of endothelin antagonists and prevention of vasoconstriction and endothelin-induced sudden death. *J Cardiovasc Pharmacol* **26** (Suppl 3): S397–S399.
- Russell FD and Davenport AP (1996) Characterization of the binding of endothelin ET_B selective ligands in human and rat heart. *Br J Pharmacol* **119**:631–636.
- Russell FD, Skepper JN, and Davenport AP (1998) Human endothelial cell storage granules: a novel intracellular site for isoforms of the endothelin converting enzyme. *Circ Res* **83**:314–321.
- Saeki T, Ihara M, Fukuroda T, Yamagiwa M, and Yano M (1991) [Ala^{1,3,11,15}]endothelin-1 analogs with ET_B agonistic activity. *Biochem Biophys Res Commun* **179**:286–292.
- Saito Y, Mizuno T, Itakura M, Suzuki Y, Ito T, Hagiwara H, and Hirose S (1991) Primary structure of bovine endothelin ET_B receptor and identification of signal peptidase and metal proteinase cleavage sites. *J Biol Chem* **266**:23433–23437.
- Sakurai T, Yanagisawa M, Takawa Y, Miyazaki H, Kimura S, Goto K, and Masaki T (1990) Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature (Lond)* **348**:732–735.
- Shyamala V, Moulthrop TH, Stratton-Thomas J, and Tekamp-Olson P (1994) Two distinct human endothelin B receptors generated by alternative splicing from a single gene. *Cell Mol Biol Res* **40**:285–296.
- Stein PD, Hunt JT, Floyd DM, Moreland S, Dickinson KEJ, Mitchell C, Liu ECK, Webb ML, Murugesan N, Dickey J, et al. (1994) The discovery of sulfonamide endothelin antagonists and the development of the orally active ET_A antagonist 5-(dimethylamino)-N-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulfonamide. *J Med Chem* **37**:329–331.
- Takai M, Umemura I, Yamasaki K, Watakabe T, Fujitani Y, Oda K, Urade Y, Inui T, Yamamura T, and Okada T (1992) A potent and specific agonist, Suc-[Glu⁹, Ala^{11,15}]-endothelin-1_(8–21), IRL 1620, for the ET_B receptor. *Biochem Biophys Res Commun* **184**:953–959.
- Takasaki C, Tamiya N, Bdolah A, Wollberg Z, and Kochva E (1988) Sarafotoxins S6, several isotoxins from *Atractaspis engaddensis* (burrowing asp) venom that affect the heart. *Toxicol* **26**:543–548.
- Tanaka H, Moroi K, Iwai J, Takahashi H, Ohnuma N, Hori S, Takimoto M, Nishiyama M, Masaki T, Yanagisawa M, et al. (1998) Novel mutations of the endothelin B receptor gene in patients with Hirschsprung's disease and their characterization. *J Biol Chem* **273**:11378–11383.
- Tanaka T, Tsukuda E, Nozawa M, Nonaka H, Ohno T, Kase H, Yamada K, and Matsuda Y (1994) RES-701-1, a novel, potent, endothelin type B receptor-selective antagonist of microbial origin. *Mol Pharmacol* **45**:724–730.
- Urade Y, Fujitani Y, Oda K, Watakabe T, Umemura I, Takai M, Okada T, Sakata K, and Karaki H (1994) An endothelin B receptor-selective antagonist: IRL 1038, [Cys¹¹-Cys¹⁵]-endothelin-1_(11–21) *FEBS Lett* **342**:103. [Retraction of: Urade Y, Fujitani Y, Oda K, Watakabe T, Umemura I, Takai M, Okada T, Sakata K, and Karaki H. (1992) *FEBS Lett* **311**:12–16].
- von Geldern TW, Tasker AS, Sorensen BK, Winn M, Szczepankiewicz BG, Dixon B, Chiou WJ, Wang LM, Wessale JL, Adler A, et al. (1999) Pyrrolidine-3-carboxylic acids as endothelin antagonists. 4. Side chain conformational restriction leads to ET_B selectivity. *J Med Chem* **42**:3668–3678.
- Warner TD, Alcock GH, Mickley EJ, Corder R, and Vane JR (1993) Comparative studies with the endothelin receptor antagonists BQ-123 and PD142893 indicate at least three endothelin receptors. *J Cardiovasc Pharmacol* **22** (Suppl 8): S117–S120.
- Warner TD, de Nucci G, and Vane JR (1989) Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. *Eur J Pharmacol* **159**:325–326.
- Watakabe T, Urade Y, Takai M, Umemura I, and Okada T (1992) A reversible radioligand specific for the ET_B receptor, [¹²⁵I]Ty¹³-Suc-[Glu⁹, Ala^{11,15}]-endothelin-1_(8–21), [¹²⁵I]IRL1620. *Biochem Biophys Res Commun* **185**:867–873.
- William DL Jr, Jones KL, Pettibone DJ, Lis EV, and Clineschmidt BV (1991) Sarafotoxin S6c, an agonist which distinguishes between endothelin receptor subtypes. *Biochem Biophys Res Commun* **175**:556–561.
- Williams DL Jr, Murphy KL, Nolan NA, O'Brien JA, Pettibone DJ, Kivlighn SD, Krause SM, Lis EV Jr, Zingaro GJ, Gabel RA, et al. (1995) Pharmacology of L-754,142, a highly potent, orally active, nonpeptidyl endothelin antagonist. *J Pharmacol Exp Ther* **275**:1518–1526.
- Wu C, Chan MF, Stavros F, Raju B, Okun I, Mong S, Keller KM, Brock T, Kogan TP, and Dixon RA (1997) Discovery of TBC11251, a potent, long acting, orally active endothelin receptor-A selective antagonist. *J Med Chem* **40**:1690–1697.
- Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, and Masaki T (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature (Lond)* **332**:411–415.
- Yang GC, Croaker D, Zhang AL, Manglick P, Cartmill T, and Cass D (1998) A dinucleotide mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome (LWFS), a horse variant of Hirschsprung disease. *Hum Mol Genet* **7**:1047–1052.
- Zhang YF, Jeffery S, Burchill SA, Berry PA, Kaski JC, and Carter ND (1998) Truncated human endothelin receptor A produced by alternative splicing and its expression in melanoma. *Br J Cancer* **78**:1141–1146.