

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

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**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<sub>A</sub> and ET<sub>B</sub> 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<sub>A</sub> 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<sup>11,15</sup>]Ac-ET-1<sub>(6-21)</sub>), and IRL1620 [Suc-(Glu<sup>9</sup>, Ala<sup>11,15</sup>)-ET-1<sub>(8-21)</sub>] are widely used synthetic ET<sub>B</sub> receptor agonists. A limited number of peptide (BQ788) and nonpeptide (A192621) ET<sub>B</sub> antagonists have also been developed. They are generally less potent than ET<sub>A</sub> antagonists and display lower selectivity (usually only 1 to 2 orders of magnitude) for the ET<sub>B</sub> receptor. Radioligands highly selective for either ET<sub>A</sub> (<sup>125</sup>I-PD151242, <sup>125</sup>I-PD164333, and <sup>3</sup>H-BQ123) or ET<sub>B</sub> receptors (<sup>125</sup>I-BQ3020 and <sup>125</sup>I-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 (ET<sup>1</sup>) 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<sub>A</sub> and ET<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> antagonists that are the compounds of choice. A second major change is the inclusion of radioligands that are highly selective for either ET<sub>A</sub> (<sup>125</sup>I-PD151242, <sup>125</sup>I-PD164333, and <sup>3</sup>H-BQ123 (cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]) or ET<sub>B</sub> receptors [<sup>125</sup>I-BQ3020 ([Ala<sup>11,15</sup>)Ac-ET-1<sub>(6-21)</sub>] and <sup>125</sup>I-IRL1620 (Suc-(Glu<sup>9</sup>, Ala<sup>11,15</sup>)-ET-1<sub>(8-21)</sub>)] 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<sub>C</sub> 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<sub>B</sub> receptor as ET-1 but, at physiological concentrations, has little or no affinity for the ET<sub>A</sub>.

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 egadensis* (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<sub>A</sub> receptors also differ between humans and other species, for example by 9% between human and rat ET<sub>A</sub> receptors and by 12% for the ET<sub>B</sub>. 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 <sub>A</sub>    | ET <sub>B</sub>    |
|          | 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.

<sup>1</sup> 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-(Glu<sup>9</sup>, Ala<sup>11,15</sup>)-ET-1<sub>(8-21)</sub>; BQ3020, [Ala<sup>11,15</sup>)Ac-ET-1<sub>(6-21)</sub>; 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         |
| $ET_B$ | QGPTEIKETFKYINTVVSCLVFLGIIGNSTLLRRIYQNKCMRNGPNILIASLALGDLI          |
|        | II  |
| $ET_A$ | <u>YVVIDLPINVFKLLAGRWPFHDHDFGVFLCKLFPFLQKSSVGITVLNLCALSVDRYRAV</u>  |
| $ET_B$ | <u>HIVIDIPINVKLLAEDWPFGE-----MCKLVFPIQKASVGITVLSLICALSIDRYRAV</u>   |
|        | IV  |
| $ET_A$ | ASWSRVQIGIPLVTAIEIVSIWILSFILAIPEAIGFVMVPPFEYRGEQHKTCMLNATSK         |
| $ET_B$ | ASWSRIKIGIVPKWTAVEIVLIWVSVVLAVPEAIGFDIITMDYKGSYLRIICLLHPVQK         |
| $ET_A$ | --FMEFYQDVKDWLWLFYFCMPLVCTAIFYTLMTCEMLNRRNGSLRIALSEHLKQRRE          |
| $ET_B$ | TAFMQFYKTAKDWLWLFYFCPLAITAFFYTLMTCEML-RKKSQMIALNDHLKQRRE            |
|        | VII   |
| $ET_A$ | <u>VAKTVFCLVVFALCWFLHLSRILKKTVYNEMDKNRCELLSFLLLMDYIGINLATMNS</u>    |
| $ET_B$ | <u>VAKTVFCLVLFALCWFLHLSRILKLTLYNQNDPNRCELLSFLLVLDYIGINMASLNS</u>    |
| $ET_A$ | <u>CINPIALYFVSKKFKNCFQSCCLCCCYQSKSLMSTVPMNGTSTIQWKNHDQNNHNTDRSS</u> |
| $ET_B$ | <u>CINPIALYLVSKRFKCNFKSCCLCCWCQSFEEKQSLEEKQSCLKFKANDHGYDNFRSSNK</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<sub>A</sub> and Endothelin<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub> 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<sub>B</sub>-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<sub>B</sub> 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<sub>A</sub> receptor gene has been proposed to give rise to at least three alternatively spliced ET<sub>A</sub> 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<sub>A</sub> receptors predominate on the smooth muscle of blood vessels, and the low density of ET<sub>B</sub> receptors (<15%) also present on the smooth muscle contributes little to vasoconstriction in either normal or diseased tissue (Maguire and Davenport, 1995). ET<sub>B</sub> receptors are the principal type in the kidney, localizing to nonvascular tissues. Evidence is emerging that the ET<sub>B</sub> receptor functions as a "clearing receptor" to remove ET from the circulation. ET<sub>B</sub> 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<sub>A</sub> 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<sub>B</sub> 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<sub>B</sub> 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<sub>A</sub> subtype) or astrocytes (ET<sub>B</sub>). 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<sub>A</sub> receptor agonist has not been discovered.

Sarafotoxin S6c is one of the most widely used ET<sub>B</sub>-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<sup>1,3,11,15</sup>]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<sub>B</sub>-selective. The truncated linear synthetic analogs BQ3020 and IRL1620 are the most widely used selective synthetic agonists to characterize ET<sub>B</sub> receptors. The compounds cause endothelium-dependent vasodilation in preparations such as porcine pulmonary artery, which is consistent with ET<sub>B</sub> receptor-mediated release of relaxing factors from the endothelium.

### VI. Radiolabeled Agonists

Most studies characterizing and localizing ET receptors use <sup>125</sup>I-ET-1, directly labeled via the Tyr<sup>13</sup> (Table 3). This ligand binds with the same affinity to both ET<sub>A</sub> and ET<sub>B</sub> receptors and is stable under nonphysiological binding conditions with little or no degradation of labeled ET-1 being detected. <sup>125</sup>I-ET-2, <sup>125</sup>I-vasoactive intestinal contractor (the murine isoform of ET-2), and <sup>125</sup>I-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 Tyr<sup>6</sup>, Tyr<sup>13</sup>, and Tyr<sup>14</sup>. Tyr<sup>6</sup> is generally used, as it is more difficult to separate <sup>125</sup>I-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<sub>B</sub> versus ET<sub>A</sub> 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<sub>B</sub> receptors are usually characterized using <sup>125</sup>I-BQ3020 (Ihara et al., 1992b; Molenaar et al., 1992), which binds with subnanomolar affinity to the ET<sub>B</sub> receptor, with at least 1500-fold selectivity for this receptor over the ET<sub>A</sub>. Alternatively, the truncated analog <sup>125</sup>I-IRL1620 can also be used, particularly in animal tissues (Watakabe et al., 1992).

### VII. Antagonists

Antagonists are currently classified as either ET<sub>A</sub>-selective, ET<sub>B</sub>-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<sub>A</sub> 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<sub>A</sub> receptor-selective antagonists have oral bioavailability and some may cross the blood-brain barrier. The majority are more potent, with pA<sub>2</sub> 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<sub>A</sub> Selective Antagonists

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

### IX. Endothelin<sub>B</sub> Selective Antagonists

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

### X. Endothelin<sub>A</sub>/Endothelin<sub>B</sub> Antagonists

The distinction between antagonists that are ET<sub>A</sub>-selective and those that block both ET<sub>A</sub> and ET<sub>B</sub> receptors is not precise but generally the former display greater than 100-fold selectivity for the ET<sub>A</sub> 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 <sub>A</sub>   |
| Receptor Code           | 2.1:ET:1:ETA:   |
| Previous names          | None  |
| Structural information  | 7TM<br>h 427 aa, P25101, chr. 4; (Adachi et al., 1991)<br>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 <sub>2</sub> 6.9–7.4; Ihara et al., 1992a)<br>PD155080 (8–8.5; Maguire et al., 1995)<br>FR139317 (7.3–7.9; Aramori et al., 1993)<br>PD156707 (8–8.7; = CI1020; Doherty et al., 1995)<br>SB234551 (9; Ohlstein et al., 1998)<br>L754142 (7.7–8.7; Williams et al., 1995)<br>BMS182874 (6.2; Stein et al., 1994)<br>A127722 (9–10.5; ABT627; Opgenorth et al., 1996)<br>TBC11251 (8.0; Wu et al., 1997)<br>LU127043 (7.3; Raschack et al., 1995)<br>LU135252; (Münter et al., 1996) |
| Radioligand assays      | human, rat and porcine heart; A10 smooth muscle cells   |
| Radioligands            | <sup>125</sup> I-ET-1 ( <i>K<sub>d</sub></i> = 0.01–5 nM) Davenport, 1997<br><sup>125</sup> I-PD151242 (0.5 nM) Davenport et al., 1994<br><sup>125</sup> I-PD164333 (0.2 nM) Davenport et al., 1998<br><sup>3</sup> H-BQ123 (3.2 nM) Ihara et al., 1995   |
| Transduction mechanisms | G protein-coupled: increase in phosphatidyl inositol turnover with elevation of [Ca <sup>2+</sup> ] <sub>i</sub> ; activation of Ca <sup>2+</sup> 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 <sub>A</sub> knockout mice (Clouthier et al., 1998)   |
| Receptor                | ET <sub>B</sub>   |
| Receptor Code           | 2.1:ET:2:ETB:   |
| Previous names          | None  |
| Structural information  | 7TM<br>h 442 aa, P24530, chr. 13; (Nakamuta et al., 1991)<br>r 441 aa, P21451; (Sakurai et al., 1990)<br>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:<br>[Ala <sup>1,3,11,15</sup> ]ET-1; (Saeki et al., 1991)<br>BQ3020; (Ihara et al., 1992b)<br>IRL 1620; (Takai et al., 1992)<br>S6c; (William et al., 1991)   |
| Agonist potencies       | ET-1 = ET-2 = ET-3 = S6b (rat glomeruli)  |
| Antagonist potencies    | IRL2500 (pA <sub>2</sub> 7.8; Balwierczak et al., 1995)<br>RES7011 (6.0; Tanaka et al., 1994)<br>BQ788 (6.9; Ishikawa et al., 1994)<br>Ro468443 (pA <sub>2</sub> 8.1; Clozel and Breu, 1996)<br>A192621 (8.1; von Geldern et al., 1999)   |
| Radioligand assays      | Brain, lung, placenta, and kidney   |
| Radioligands            | <sup>125</sup> I-ET-1 ( <i>K<sub>d</sub></i> = 0.01–5 nM) Davenport, 1997<br><sup>125</sup> I-BQ3020 (0.1 nM) Ihara et al., 1992b<br><sup>125</sup> I-[Ala <sup>1,3,11,15</sup> ]ET-1 (0.2 nM) Molenaar et al., 1992<br><sup>125</sup> I-IRL 1620 (0.02 nM) Watakabe et al., 1992   |
| Transduction mechanisms | G protein-coupled: increase in phosphatidyl inositol turnover with elevation of [Ca <sup>2+</sup> ] <sub>i</sub> ; activation of Ca <sup>2+</sup> 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 <sub>B</sub> receptor gene in Hirschsprung's disease. ET <sub>B</sub> 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)   |

chr., chromosome; NO, nitric oxide; h, human; m, mouse; r, rat.

## XI. Conclusions

In humans, ET peptides mediate their actions via only two receptor types, classified as ET<sub>A</sub> and ET<sub>B</sub>. 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<sub>B</sub> receptor have a physiological or pathophysiological role.

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