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Chemical names

SDZ208911: *N*-[(8α)-2,6-dimethylergoline-8-yl]-2,2-diethylpropanamide

SDZ208912: *N*-[(8α)-2-chloro-6-methylergoline-8-yl]-2,2-diethylpropanamide

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The current endothelin receptor classification: time for reconsideration?

Willem A. Bax and Pramod R. Saxena

The possible involvement of endothelins in a variety of diseases has attracted the attention of many pharmacologists in search of a novel therapeutic approach. The rapid development of endothelin research has resulted in the molecular characterization and pharmacological recognition of ET_A and ET_B receptors, and in the development of compounds selective for these receptors. However, the characterization of receptors in various assays has shown that a number of effects are mediated by receptors that do not fit the present criteria for ET_A or ET_B receptors. In this article, **Willem Bax and Pramod Saxena** address endothelin receptors in general, and atypical receptors in particular.

Endothelin was discovered and recognized as a potent vasoconstrictor peptide only six years ago[1]. Three distinct endogenous endothelin isoforms endothelin 1 (ET-1), ET-2 and ET-3 are cleaved from the endothelin precursors big-ET-1, big-ET-2 and big-ET-3 by an endothelin converting enzyme. Increased concentrations of endothelins have been observed after myocardial infarction, in atherosclerosis, (pulmonary) hypertension, migraine and many other diseases[2]. Although recent advances towards the elucidation of the molecular structure of endothelin converting enzymes[3] will undoubtedly be followed by the development of inhibitors of endothelin converting enzymes, efforts have so far primarily been directed to the development of endothelin receptor antagonists for clinical purposes. Indeed ET_A and ET_B receptors were cloned[4,5], and selective ligands for these receptors have been recognized.

The current criteria for endothelin receptor classification

In general, receptor classification should be based on three criteria[6,7]: (1) gene nucleotide and amino acid sequence of the receptor protein, (2) receptor–effect coupling, and (3) interaction between receptors and agonists or antagonists. Endothelin receptors are currently classified by the consensus view of the subcommittee of the International Union of Pharmacology (IUPHAR), primarily

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according to the potency order of endothelin isopeptides, but also according to the potency of some antagonists. In so-called type I responses, ET-1 is more potent than ET-3, whereas in type II responses, both isopeptides have similar potency. In studies applying techniques to express cDNA for the currently known endothelin receptors, it was established that the type I and type II responses correspond to ET_A and ET_B receptors, respectively[8].

Gene nucleotide and amino acid sequence of the receptor protein

ET_A and ET_B receptors have approximately 63% amino acid homology. Southern blots of the human genomic DNA, using cDNA probes for the ET_A and ET_B receptor under low stringency, revealed only two signals, probably corresponding to human ET_A and ET_B receptor genes. Thus, it appeared that other endothelin receptors, if existent, would probably have a considerably different amino acid sequence[9]. However, it should be noted that although amino acid homology may be indicative of pharmacological similarity, this is not a general prerequisite. For example, 5-HT_{1B} and 5-$HT_{1D\beta}$ receptors have a clearly different pharmacological profile for a number of compounds, despite a 96% amino acid homology in the transmembrane region. By contrast, 5-$HT_{1D\beta}$ and 5-$HT_{1D\alpha}$ receptors are pharmacologically almost indistinguishable, but have a relatively moderate 77% amino acid homology in the transmembrane region[7].

Recently, the identification of cDNA for a receptor with relatively high affinity for ET-3 was reported in *Xenopus* dermal melanophores. This receptor had approximately 50% amino acid homology with ET_A and ET_B receptors[10]. However, it is not yet certain whether it actually represented the putative ET_C receptor, which is highly selective for ET-3 and has been reported in pharmacological studies in bovine endothelial cells[11], or whether it represented the *Xenopus* variant of, for example, ET_B receptors. Because of the scarcity of functional correlates and the lack of selective ET_C receptor ligands other than ET-3, ET_C receptors will not be discussed in further detail.

Second messenger mechanisms

Both ET_A and ET_B receptors have been described to be coupled to phosphatidylinositol (4,5)-bisphosphate (PtdInsP_2) hydrolysis via G protein-coupled phospholipase C, and to the generation of inositol phosphates (Ins*P*) and diacylglycerol, resulting in an increased concentration of intracellular Ca^{2+} (Ref. 8). In transfected Chinese hamster ovary cells, it was observed that ET_A receptors induced accumulation of cAMP, whereas ET_B receptors inhibited forskolin-stimulated cAMP production[12]. However, the stimulation of adenylate cyclase, mediated by ET_A receptors, was less efficient than the stimulation of Ins*P* formation, which raises questions about the physiological relevance of adenylate cyclase as a second messenger system in these cells[12].

Less is known about transduction of receptors that do not resemble the ET_A or ET_B type. In the follicular membranes of *Xenopus laevis* oocytes, Kumar and co-workers[13] observed endothelin receptors that resemble human ET_A receptors in their affinity for ET-1, ET-3 and sarafotoxin

Table 1. The affinity (nM) of endothelin receptor ligands for endothelin ET_A and ET_B receptors

Ligand	ET_A		ET_B			Refs
	K_i	IC_{50}	K_i	IC_{50}	K_d	
ET-1	3.5, 0.58, 0.92[a]	0.16, 0.29	0.95, 0.12	1.6, 0.06, 0.44	0.003	4, 9, 57–61
ET-3	1000, 83, 900[a]	5.0, 150	2.0, 0.13	1.6, 0.06, 0.11	0.014	4, 9, 57–61
Sarafotoxin S6b	52[a]					4
Sarafotoxin S6c	2800	1300	0.29	0.3, 0.12	0.24	58–61
[Ala^{1,3,11,15}]ET-1		398		0.25	20	59, 61
BQ123	25, 17	13, 63	31 000, 11 100	>10 000, >100 000	285	57–61
FR139317	1[a]	6.3, 13	7300[a]	20 000, >100 000		59, 60, 62
BQ788		1300		1.2		28
Ro462005		200, 360		160, 530		59, 60
Bosentan	6.5		343			63
SB209670	0.4	2.0	15	32		59, 64
BMS182874	63	1600	55 000	>10 000		59, 65
97139	1		1000			66

Data obtained in cell lines transfected with human or bovine endothelin receptors. For BQ788 and 97139, only radioligand binding data obtained in membranes are available [ET_A: SK-N-MC human neuroblastoma cell line (BQ788), and A7r5 rat aortic smooth muscle cells (97139); ET_B: human Girardi heart cells]. In Ref. 59, pIC_{50} values were calculated to approximate IC_{50} values. ET-1, endothelin 1; ET-3, endothelin 3.

S6c, but exhibit an atypically low affinity for BQ123. Activation of these receptors, which were considered to be a subtype of ET_A receptors (ET_{AX}), resulted in mobilization of Ca^{2+}, which was blocked by treatment that uncouples gap junctions. By contrast, Ca^{2+} mobilization induced by expressed human ET_A receptors was not sensitive to such treatment. In a further study, [^{125}I]ET-1 binding to human brain endothelial cells revealed a high- and a low-affinity binding site[14]. The high-affinity binding site had the order of affinity ET-1 > ET-2 > sarafotoxin S6b > ET-3, which resembled the ET_A receptor and also matched the order of potency for Ins*P* accumulation in these cells. The order of affinity for the unidentified low-affinity binding site (sarafotoxin S6b > ET-2 > ET-1 = ET-3) did not match the order of potency for Ins*P* accumulation. Other second messenger systems were not examined in the latter study.

Pharmacological characterization of endothelin receptors

The present classification of endothelin receptors relies largely on data obtained in functional or radioligand binding experiments[8]. In addition to the frequently applied potency order of ET-1 and ET-3, a number of synthetic compounds has been identified with selectivity for ET_A or ET_B receptors (Table 1).

The pharmacological characterization of endothelin receptors is hampered by several pitfalls:

(1) Endothelin peptides can be internalized after binding to the receptor[15], and hence may not be available for ligand–receptor competition.

(2) The formation of ligand–receptor complexes with different dissociative behaviours depending on the ligand used may result in complicated receptor kinetics[16,17].

(3) Endothelin receptors may downregulate[18] or desensitize rapidly, possibly resulting in a differentially altered response to various endothelin peptides[19].

Responses mediated by endothelin receptors

Typical ET_A receptors

ET_A receptors have often been found to mediate contractile responses in isolated smooth muscle preparations. The involvement of ET_A receptors was typically established on the basis of the relative order of potency of ET-1 and ET-3, and on the inability of ET_B receptor-selective compounds (for example, sarafotoxin S6c or [Ala^{1,3,11,15}]ET-1) to act as agonists. Moreover, both BQ123 and FR139317 were generally used as ET_A receptor antagonists. Typical examples of preparations with ET_A receptors that mediate contractions include the rat[20] and guinea-pig[21] aorta (Table 2).

Typical ET_B receptors

ET_B receptors were originally considered as 'vasodilator receptors' in contrast to the vasoconstrictor ET_A receptors. Warner and colleagues[22] showed that ET-3 and ET-1 were equipotent as vasodilators, whereas ET-1 had been shown

Table 2. Examples of studies in which the response was concluded to be mediated via an endothelin ET_A receptor

Species	Tissue and response	Characterization criteria		Ref.
		Agonist	Antagonist against ET-1	
Rat	Thoracic aorta contraction	ET-1 > ET-3; [Ala^{1,3,11,15}]ET-1: no effect	pA_2 BQ123: 6.93	20
Rabbit	Carotid artery contraction	ET-1 > ET-3;	pA_2 BQ123: 6.8	30
		[Ala^{1,3,11,15}]ET-1 and sarafotoxin S6c: no effect		30
		ET-1 > ET-3; sarafotoxin S6c: no effect	pK_b BQ123: 7.5	24
Guinea-pig	Pulmonary artery contraction	ET-1 > ET-3	pA_2 FR139317: 6.65	67
		Sarafotoxin S6c: no effect	pK_b BQ123: 6.7	21
	Aorta contraction	Sarafotoxin S6c: no effect	pK_b BQ123: 7.1	21
		ET-1 > ET-3;	pA_2 BQ123: 7.4	49
		[Ala^{1,3,11,15}]ET-1 and sarafotoxin S6c: no effect		49
	Iliac artery contraction	ET-1 > ET-3; sarafotoxin S6c: no effect	pK_b BQ123: 6.6–7.2	68
			pA_2 FR139317: 5.82	68
Goat	Cerebral artery contraction	ET-1 > ET-3	pK_b BQ123: 7.43	34
Human	Coronary artery contraction	ET-1 > ET-3	pA_2 BQ123: 6.4–7.47	52
	Omental artery contraction	ET-1 > ET-3	pA_2 BQ123: 7.09	26
	Pulmonary artery contraction	Sarafotoxin S6c: no effect	pK_b BQ123: 6.2–6.8	21

ET-1, endothelin 1; ET-3, endothelin 3.

Table 3. Examples of studies in which the response was concluded to be mediated via an endothelin ET_B receptor

Species	Tissue and response	Characterization criteria		Ref.
		Agonist	Antagonist[a]	
Rat	Aorta relaxation	IRL1620	BQ123: no effect (IRL1620)	69
Rabbit	Pulmonary artery contraction	ET-1 = ET-3;	BQ123: no effect (ET-1, ET-3, [Ala^{1,3,11,15}]ET-1)	30
		[Ala^{1,3,11,15}]ET-1 and sarafotoxin S6c		30
		ET-1 = ET-3; BQ3020	BQ123: no effect (BQ3020)	70
		ET-1 = sarafotoxin S6c	BQ123 and PD124893: no effect (ET-1)	43
			pA_2 BQ788: 8.4 (BQ3020)	29
	Jugular vein contraction	ET-1 = ET-3;	BQ123: no effect (ET-1, ET-3, [Ala^{1,3,11,15}]ET-1)	30
		[Ala^{1,3,11,15}]ET-1 and sarafotoxin S6c		30
	Saphenous vein contraction	ET-1 = ET-3; sarafotoxin S6c	BQ123: no effect (sarafotoxin S6c, ET-1)	24
Guinea-pig	Bronchus contraction	Sarafotoxin S6c	BQ123: no effect (sarafotoxin S6c, ET-1)	21
	Trachea contraction	IRL1620		71
		ET-1 = ET-3	FR139317: no effect (ET-1, ET-2, ET-3)	67
Pig	Pulmonary artery relaxation	[Ala^{1,3,11,15}]ET-1		72
		BQ3020		70
Canine	Coronary artery constriction	Sarafotoxin S6c	BQ123: no effect (sarafotoxin S6c)	44
Human	Bronchus contraction	Sarafotoxin S6c	BQ123: no effect (sarafotoxin S6c, ET-1)	21

[a]The agonist against which the antagonist was studied is indicated between brackets. ET-1, endothelin 1; ET-2, endothelin 2; ET-3, endothelin 3.

to be 20-fold more potent as a vasoconstrictor[23]. Thus, the involvement of ET_B receptors was first established on the basis of equipotency of ET-1 and ET-3. Later, it was shown that ET_B receptors were also involved in smooth muscle contraction in blood vessels such as the rabbit saphenous vein[24] and in the guinea-pig bronchus[21]. In these tissues, BQ123 failed to inhibit the contractile responses, and sarafotoxin S6c was observed to produce contraction. Other studies used [Ala^{1,3,11,15}]ET-1, IRL1620 and BQ3020 as agonists (Table 3). Until recently, only IRL1038 was available as a selective ET_B receptor antagonist[25,26]. Unfortunately, the affinity for ET_B receptors was reported to be highly variable between batches, and data obtained with this compound should be considered with caution[27]. However, the recent development of the potent and selective ET_B receptor antagonist BQ788 provided a novel tool to study the involvement of ET_B receptors[28] (Table 3).

Mixed ET_A and ET_B receptor populations

In the guinea-pig trachea, BQ123 was a weak antagonist of ET-1-induced contraction, sarafotoxin S6c was a partial agonist and the contractile effect of sarafotoxin S6c was resistant to antagonism by BQ123 (Ref. 21). Thus, it was concluded that both ET_A and ET_B receptors mediated contractile responses in the guinea-pig trachea[21]. Vasoconstriction in the isolated perfused rat kidney was also mediated by both ET_A and ET_B receptors[29]. In this preparation, BQ123 and FR139317 caused only partial attenuation of ET-1-induced contractions, whereas the nonselective ET_A and ET_B receptor antagonist PD145065 completely abolished the responses to ET-1 (Ref. 29).

Even in blood vessels such as the rabbit saphenous vein[24] and pulmonary artery[30] that had previously been considered to contract via ET_B receptors exclusively, a coexisting ET_A receptor mediating vasoconstriction has been demonstrated by showing that BQ123 attenuated part of the concentration–response curve to ET-1 in both vessels, despite an observed equipotency of ET-1 and ET-3 (Refs 31,32). The presence of ET_A receptors in the rabbit pulmonary artery has been confirmed by radioligand membrane-binding studies[32] (Table 4).

Atypical endothelin responses observed using ET_A receptor-selective compounds

A number of preparations exhibited an agonist order of potency of ET-1 > ET-3, which would imply the involvement of ET_A receptors. However, BQ123 was found to inhibit ET-3-induced contractions substantially more potently than ET-1-induced contractions, suggesting the presence of different receptors[20] (Table 5). Pierre and Clarke[33] suggested that the BQ123-sensitive contractions to ET-3 in the rat isolated renal artery were mediated via ET_A receptors, whereas the relatively BQ123-insensitive

Table 4. Examples of studies in which the response was concluded to be mediated via a mixed population of endothelin ET_A and ET_B receptors

Species	Tissue and response	Characterization criteria		Ref.
		Agonist	Antagonist[a]	
Rat	Kidney perfusion	ET-1 = ET-3 = sarafotoxin S6b = sarafotoxin S6c	BQ123: little effect (ET-1, sarafotoxin S6b)	73
		Sarafotoxin S6c	BQ123 and FR139317: partial inhibition (ET-1)	29
			PD145065: complete inhibition (ET-1)	29
Rabbit	Pulmonary artery contraction	Sarafotoxin S6c > ET-1	BQ123: antagonist (high concentrations ET-1)	32
		Sarafotoxin S6c: no effect[b]	pA_2 BQ123: 6.6 (ET-1)[b]	32
	Saphenous vein contraction	Sarafotoxin S6c > ET-1 = ET-3	10 μM BQ123: antagonist (high concentrations ET-1)	32
Guinea-pig	Trachea contraction	Sarafotoxin S6c	BQ123: no effect (sarafotoxin S6c)	21
			pK_b BQ123: 6.2 (ET-1)	21
Human	Internal mammary artery contraction	Sarafotoxin S6c: partial agonist	BQ123 and FR139317: antagonists (ET-1)	51

[a]The agonist against which the antagonist was studied is indicated between brackets. [b]Response determined after 30 min pretreatment with sarafotoxin S6c. ET-1, endothelin 1; ET-3, endothelin 3.

ET-1-induced contractile responses were mediated via non-ET_A receptors. Although this is a plausible explanation with regard to the antagonist, it is yet unclear why ET-3 recognizes an ET_A receptor that is not recognized by ET-1. Similarly, in the goat cerebral artery, contractile responses induced by sarafotoxin S6b were antagonized more potently by BQ123 than those induced by ET-1 (Ref. 34) (Table 5). It has been argued that the reversibility of receptor binding of ET-1 is different from that of ET-3 or sarafotoxin S6b, and that this could account for the differences in antagonist potencies against these agonists[35]. However, this does not explain the biphasic antagonism of sarafotoxin S6b-induced contractions of the human saphenous vein by BQ123 (Ref. 36), or the biphasic displacement of binding with 30 pM [^{125}I]sarafotoxin S6b by BQ123 in the media of human coronary arteries[37]. Furthermore, it should be noted that in other investigations, the antagonist potency did not differ between these particular agonists[38–40], or was even higher against ET-1 than against the other agonist[41,42]. Thus, although the possibility of interference by complex endothelin receptor kinetics[15–19] should not entirely be disregarded, it appears that ET-1 on the one hand, and sarafotoxin S6b and ET-3 on the other hand, may exert their effects via different receptors that do not fit the current classification of ET_A and ET_B receptors.

Atypical endothelin responses observed using ET_B receptor-selective compounds

Warner and colleagues[43] observed an ET_B receptor that mediated constriction of the rabbit pulmonary artery and rat stomach strip that was relatively insensitive to the non-selective endothelin receptor antagonist PD142893. By contrast, PD142893 potently antagonized the ET_B receptor-mediated vasodilator effect in the perfused mesentery, which indicated receptor heterogeneity among ET_B receptors[43]. In addition, a study in swine pulmonary blood vessels revealed differences between ET_B receptors mediating contraction and endothelium-dependent relaxation[35]. Only receptors mediating endothelium-dependent relaxation were sensitive to antagonism by the ET_B receptor antagonist IRL1038 (Ref. 25). However, as mentioned above, it should be noted that questions have arisen over the use of IRL1038 as an ET_B receptor antagonist[27], and these experiments need verification using alternative antagonists with affinity for ET_B receptors. Radioligand binding studies in canine coronary artery membranes also indicated the possibility of ET_B receptor subtypes[44]. These binding sites had either high or low affinity for both ET-1 and ET-3. In addition, the high-affinity ET_B site showed high affinity for sarafotoxin S6c, but not for BQ123, whereas the low-affinity ET_B site had moderate affinity for both sarafotoxin S6c and BQ123. Coronary vasoconstriction induced by sarafotoxin S6c was insensitive to BQ123, indicating involvement of the high-affinity ET_B site. No functional correlate for the low-affinity ET_B site is known at present[44]. In the rat left atrium, equipotent contractile responses to ET-1, ET-2, ET-3 and sarafotoxin S6b were observed, indicating the involvement of ET_B receptors. However, the ineffectiveness of the ET_B receptor agonists [Ala^{1,3,11,15}]ET-1 and sarafotoxin S6c would suggest the involvement of receptors other than conventional ET_B receptors[45]. Similarly, *Xenopus laevis* liver membranes revealed a binding site with identical affinity for ET-1 and ET-3. As expected for ET_B receptors, BQ123 was ineffective in displacing

Table 5. Examples of studies in which the response was concluded to be mediated by a single or mixed receptor population consisting of receptors characterized as partly atypical or as subtypes of endothelin ET_A and/or ET_B receptors

Species	Tissue and response	Observations	Refs
		ET_A receptor-selective compounds	
Rat	Aorta contraction	BQ123 more potent versus ET-3 than versus ET-1; [Ala^{1,3,11,15}]ET-1: no effect	20
	Vas deferens increased twitch	BQ123 and PD142893 more potent versus ET-3 and sarafotoxin S6b than versus ET-1; sarafotoxin S6c: no effect	56, 74
Goat	Cerebral artery contraction	BQ123 more potent versus sarafotoxin S6b than versus ET-1	34
Human	Small omental vein contraction	BQ123 more potent versus ET-3 than versus ET-1	26
		BQ123 more potent versus high than versus low concentrations of ET-3	26
		IRL1038 no effect against ET-1[a]; sarafotoxin S6c: no effect	26
	Coronary artery contraction	BQ123 more potent versus ET-3 than versus ET-1	52
		BQ123 and FR139317 more potent versus sarafotoxin S6b than versus ET-1; [Ala^{1,3,11,15}]ET-1: no effect	53
	Saphenous vein contraction	BQ123 more potent versus sarafotoxin S6b than versus ET-1	36
		BQ123 more potent versus high than versus low concentrations of sarafotoxin S6b	36
	Umbilical artery contraction	BQ123 more potent versus sarafotoxin S6b than versus ET-1	54
		ET_B receptor-selective compounds	
Rat	Stomach strip contraction	Contraction to sarafotoxin S6c (more potent than ET-1) weakly antagonized by PD142893	43
	Perfused mesentery dilatation	Dilatation to sarafotoxin S6c (equipotent ET-1) strongly antagonized by PD142893	43
	Atrium contraction	ET-1, ET-3, sarafotoxin S6b equipotent; sarafotoxin S6c and [Ala^{1,3,11,15}]ET-1 no effect	45
Pig	Pulmonary vein contraction[a]	Isopeptide nonselective receptor, resistant to IRL1038	25
	Pulmonary artery relaxation[a]	Isopeptide nonselective receptor, sensitive to IRL1038	25
	Coronary artery contraction	Sarafotoxin S6c-sensitive receptor recognizes ET-3, but not ET-1 or sarafotoxin S6b	48
		Sarafotoxin S6c and [Ala^{1,3,11,15}]ET-1 sensitive receptor [pK_b BQ123: ≈5 (ET-1)], and another receptor resistant to BQ123	49

[a] The receptor affinity and selectivity for ET_B receptors of IRL1038 have been described to be highly variable. Therefore, these data must be interpreted with caution[27].

[^{125}I]ET-1 labeling of this site, but sarafotoxin S6c was also ineffective, suggesting the presence of a subtype of ET_B receptors[46].

Other atypical endothelin responses

The nature of endothelin receptors that mediate contraction of the porcine coronary artery is still controversial, but part of the receptor population does not appear to correspond to either ET_A or ET_B receptors. Ihara and co-workers[47] observed ET-1-induced contractile responses that were sensitive to antagonism by BQ123 ($pA_2 = 7.4$) and thus considered to be mediated by ET_A receptors. The small BQ123 nonsensitive part of the concentration–response curve to ET-1 was assigned to be mediated by ET_B receptors[47]. Further studies agreed on the ET_A receptor component on the basis of the agonist order of potency, but also observed a receptor that recognized sarafotoxin S6c and ET-3, but not ET-1 and sarafotoxin S6b (Ref. 48). Later, it was shown that the contractile effects of both sarafotoxin S6c and [Ala^{1,3,11,15}]ET-1 were likely to be mediated via the same ET_B receptor, whereas a non-ET_A, non-ET_B type of receptor contributed to the contractile response induced by ET-1 (Ref. 49) (Table 5).

Endothelin receptors in human blood vessels

Endothelin receptors mediating contractions in human blood vessels were recently reviewed by Davenport and Maguire[50]. Although the contractile responses may be mediated via typical ET_A receptors[50] (perhaps in addition to ET_B receptors[51]), there are several reports focusing on non-ET_A, non-ET_B receptors in human blood vessels.

In parallel with the rat aorta[20] and the goat cerebral artery[34] (Table 5), BQ123 has been observed to be more potent against ET-3- and sarafotoxin S6b-induced contractions than against ET-1-induced contractile responses in the human isolated saphenous vein[36], coronary artery[52,53], umbilical artery[54] and in small omental veins[26]. Recent data obtained in the human isolated coronary artery

suggest that the same discrepancy between ET-1- and sarafotoxin S6b-induced contractile responses is also observed with other ET_A receptor antagonists, such as FR139317 (Ref. 53).

It is unclear whether the ET_B receptor plays a significant role in vasoconstriction of human blood vessels. Indeed, the endogenous ligand ET-3 is a less potent vasoconstrictor agonist than ET-1. Moreover, both ET_B receptor agonists BQ3020 and [Ala^{1,3,11,15}]ET-1 hardly contracted the human isolated coronary artery[50]. However, sarafotoxin S6c induced contractile responses in some (but not all) coronary artery[50], internal mammary artery[51] or saphenous vein[55] segments. Although this may be due to a relatively low ET_B receptor density[50], these observations could also be related to the isopeptide nonselective ET_B receptors, with low affinity for the ET_B receptor agonists [Ala^{1,3,11,15}]ET-1 or sarafotoxin S6c (Refs 45,46).

Concluding remarks

The above-mentioned studies indicate that the current ET_A and ET_B endothelin receptor classification will have to be extended. A number of responses fit the present criteria for the ET_A receptor, but evidence indicating that ET_A receptor antagonists are sometimes more potent against ET-3 (Refs 20,26,52,56) or sarafotoxin S6b (Refs 34,36,53,54,56) than against ET-1 suggests further heterogeneity of endothelin receptors. These receptors may be classified as subtypes of the ET_A receptor, since ET_A receptor antagonists are moderately or highly potent in these assays and ET_B receptor agonists are usually inactive (Table 5). However, since it has only been possible to detect this heterogeneity in assays in which both receptors mediate the same effect, a detailed pharmacological analysis of the individual receptors has not yet been established, and a conclusive classification of these receptors is therefore best postponed.

ET_B receptors also appear to be heterogeneous since the relaxant but not the contractile responses were antagonized by PD142893. However, only the affinity for the antagonist should be used as a criterium for pharmacological receptor classification, and not whether the receptor mediates contraction or relaxation[6,7]. In view of the effect of ET_B receptor agonists and the ineffectiveness of BQ123, it would appear that these receptors may be designated ET_{B1} (PD142893-sensitive) and ET_{B2} (PD142893-insensitive) receptor subtypes. Whether additional ET_B receptor subtypes exist, for example, in canine coronary artery[44], or whether other atypical observations are related to the proposed ET_{B1} or ET_{B2} receptor subtypes, remains to be resolved (Table 5).

The currently available data are not yet sufficient to provide a conclusively extended endothelin receptor nomenclature. Indeed, additional data on second messenger mechanisms and possibly on the sequence of the corresponding DNA are eagerly awaited, and the use of more agonists and antagonists in functional studies is vital[6,7]. For now, it should be noted that, in particular, the current basic criterium of the potency difference of ET-1 and ET-3 should be applied with the utmost restraint. In some smooth muscle preparations, ET-1 is clearly more potent than ET-3, suggesting the involvement of ET_A receptors. However, considering the observed antagonist potency differences[20,26,52], the two peptides may in fact induce contractions via different receptors (Table 5). In other tissues, ET-1 and ET-3 are equipotent, suggesting the involvement of ET_B receptors. However, ET_B receptor agonists may in some cases be inactive[45], and detailed analysis using ET_A receptor antagonists has been used to demonstrate the presence of an additional ET_A receptor[31,32]. Given the relatively incomplete understanding of endothelin receptors and the limited number of selective receptor ligands, the use of a wide spectrum of both agonists and antagonists is required for endothelin receptor characterization. The nonpeptide receptor antagonists that are now becoming available will certainly help the process towards a conclusive, more detailed endothelin receptor classification.

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Chemical names

BQ123: *cyc*(D-Trp-D-Asp-Pro-D-Val-Leu)

BQ3020: *N*-acetyl-[Ala^{11,15}]endothelin-1(6–21)

FR139317: 2(R)-[2(R)-(2(S)-{[1-(hexahydro-1*H*-azepinyl)]carbonyl}amino-4-methylpentanoyl)-amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]-amino-3-(2-pyridyl)propionic acid

IRL1620: Suc-[Glu^{9},Ala^{11,15}]endothelin-1(8–21)

IRL1038: [Cys^{11,15}]endothelin-1(11–21)

PD145065: acetyl-(5*H*-dibenzyl[*a*,*d*]cycloheptane-10,11-dihydro-glycine)-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp

PD142893: acetyl-(3,3-D-diphenylalanine-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp

BQ788: *N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxy-carbonyltryptophanyl-D-norleucin

Ro462005: 4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(3-methoxy-phenoxy)-4-pyrimidinyl]-benzene-sulphonamide

SB209670: (+)-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxyphenyl)-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)-indane-2-carboxylic acid

BMS182874: 5-(dimethylamino)-*N*-(3,4-dimethyl-5-isoxazolyl)-1-naphthalenesulphonamide

97139: 27-*O*-3-[2-(3-carboxy-acryloyl-amino)-5-hydroxyphenyl]acryloyloxy-myricerone sodium salt

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