PHARMACOLOGICAL POTENTIAL OF ENDOTHELIN RECEPTORS AGONISTS AND ANTAGONISTS

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Summary: Endothelins are potent predominantly vasoconstricting agents that act as local autocrine and paracrine mediators. Endothelin-1 is the most potent and sustained vasoconstrictor and pressor substance yet identified. Abnormalities of the endothelin system occur in a range of diseases associated with vasoconstriction, vasospasm, and vascular hypertrophy. ET receptor antagonists were until recently regarded as drugs of great promise in patients with congestive heart failure, pulmonary hypertension and others. The aim of this article is a survey of compounds that affect the endothelin receptors and clinical trials with these agents.

Introduction

The endothelin (ET) family of peptides are very potent endogenous vasoconstrictors and pressor agents, secreted by various cells and tissues in the human. The ET family consists of 3 structurally similar isopeptides: ET-l,-2, and -3. The genes that encode these peptides are cloned and are found to be on chromosomes 6, 1 and 20, respectively (14). Of the three isoforms, endothelin ET-1 is the predominant isoform produced by the vascular endothelium. ET-l is synthesized in the human vasculature and is the most potent vasoconstrictor substance known (32). ET-2 has similar vasoconstrictor potency to ET-l and appears to be synthesized predominantly in the kidney and intestines (13). ET-3 is the least potent vasoconstrictor. It is detectable in the central nervous system, lungs, kidneys, pancreas and spleen (12). All the three endogenous isoforms of ET in humans mediate their actions via two ET receptor subtypes: ET_A and ET_B. These ETs play an important role in human physiology and in the pathophysiology of vasospastic conditions, renal failure, chronic heart failure and asthma (10). Chemical compounds that interact with ET receptors, natural and/or synthetic agonists and antagonists, respectively, represent important group of substances with weighty pharmacological potential (23). Because ETs were thought to be important in cardiovascular homeostasis, many investigators focused on the physiological and pathophysiological significance of ET. Accordingly, ET receptor antagonists have been developed rapidly, mostly for the treatment of cardiovascular diseases (11). The current review will focus on the recent developments in the endothelin field, with special emphasis on the ET-1 antagonists and their clinical use.

Endothelins

ETs represent a family of 21–amino acid peptides whose structure consists of two rings formed by intra-chain disulfide bonds and a linear C-terminal tail. These peptides were originally described on the basis of their potent vasoconstrictor activity (27). The three peptides are encoded by three different genes. Biologically active endothelins are produced from pre-pro-polypeptides through two steps of proteolytic processing. The approximately 200-residue pre-pro-endothelins are first processed by a furin-like processing protease(s) into biologically inactive intermediates termed big endothelins (big ETs) (25). These are further proteolytically cleaved between Trp^{21} and Val/Ile^{22} to produce active endothelins. This proteolytic conversion is catalyzed by specific endothelin-converting enzymes (ECEs). Two isozymes of ECE, ECE-1 and ECE-2, have been molecularly identified. Both these enzymes are membrane proteins with highly conserved Zn^{2+} metalloprotease motifs.

The ECE-1 cleaves big endothelins at a neutral pH, while ECE-2 functions in an acidic pH range. This implies that these enzymes function in different subcellular locations: ECE-1 may act in early components of the secretory path-

way, presumably in the Golgi apparatus, as well as on the cell surface. In contrast, ECE-2 probably functions in highly acidified compartments of the secretory pathway, including a portion of the *trans*-Golgi apparatus. These isozymes also appear to have different functions during embryonic development (9). Targeted disruption of the ECE-1 gene in mice revealed that ECE-1 is the major enzyme involved in the activation of big ET-1 and big ET-3 at specific developmental stages. Importantly, it appears that loss of ECE-1 protein cannot be compensated for by ECE-2.

Endothelin receptors

ET_A and ET_B have been isolated and cloned from human tissues (2). Both subtypes belong to the seven-trans-membrane-domain-spanning, G-protein-coupled receptor superfamily (6). ET receptors are widely expressed in all tissues, consistent with the physiological role of ET-1, the most abundant isoform, as a ubiquitous endothelium-derived vasoactive p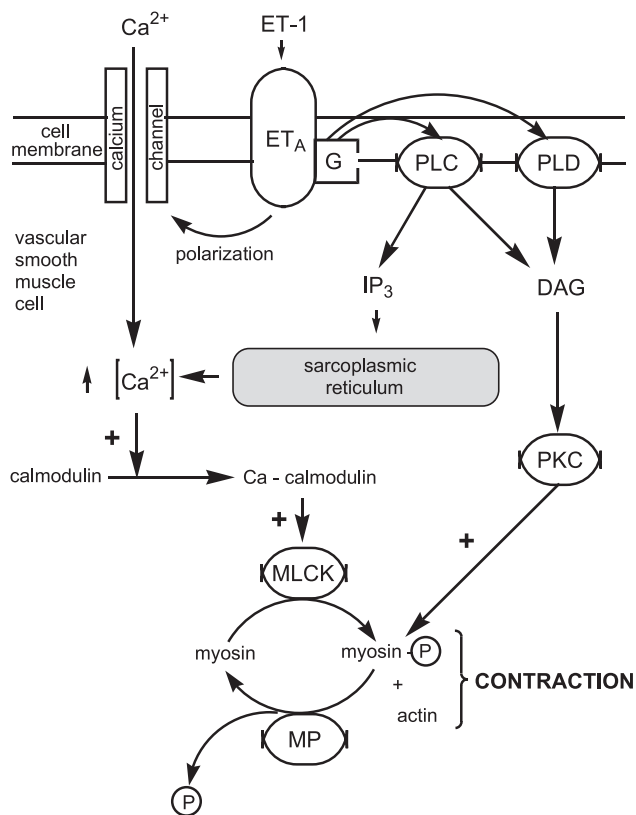eptide contributing to the maintenance of normal vascular tone. ET receptors are also localized to non-vascular structures, such as epithelial cells, and expressed in the central nervous system. ET-1 stimulates proliferation in a number of different cell types, including smooth muscle cells (mainly via the ET_A subtype) and astrocytes (via ET_B). In human blood vessels, ET_A receptors are present mainly on vascular smooth muscle cells, and are chiefly responsible for contraction whereas ET_B receptors mediate vasodilatation. The mechanisms of ET_A activation by endothelins and signal transfer mechanism of vascular smooth muscle contraction is schematically shown in Fig 1 [according to Potaczek and Sanak (26)]. ET_B receptors are also localized in the single layer of endothelial cells lining the vessel wall. Activation of endothelial ET_B receptors may lead to the release of endothelium-derived relaxing factors (nitric oxide and prostanoids).

ET-1 has dual vasoactive effects, mediating vasoconstriction via ET_A receptor activation of vascular smooth muscle cells and vasorelaxation via ET_{B1} receptor activation of endothelial cells. ET_B–receptor is also present in vascular smooth musle like ET_A–receptor. The receptor on endothelium has been called ET_{B1}, while the vascular smooth muscle subtype is termed ET_{B2}. Agonists acting on ET_{B1} cause, as already mentioned, vasodilation. In contrast, ET_{B2}–mediated responses are vasoconstrictor, like responses mediated by ET_A. Vasodilation is, at least partly, caused by stimulation of nitric oxide (NO) synthesis and PGI_2 synthesis,, however, the precise signal tranduction is unknown (21).

Non-vascular ET_B receptors in organs including the kidney may beneficially clear ET-1 from the circulation (6). All these receptors have become important targets for drugs developed to inhibit vasoconstrictor actions (19).

It is widely accepted that the vascular, cardiac, and renal adverse effects of ET-1 are mediated by ET_A, while activation of ET_B receptors leads to beneficial effects such as: attenuating the vascular and cardiac hypertrophic effects of ET-1 as well as the vasodilatory action of this peptide (1).

Endothelin receptors agonists

Natural ET agonists are all endogenous ET isoforms and ET receptors to be classified according to their rank order of potency for the individual ETs (5). Other natural ET agonists are sarafotoxins, toxic peptides from the venom of mole viper *Atractaspis engaddensis* (burrowing asp), which may pose a serious threat to humans. At present, four sarafotoxins (STX) are known: S6a, S6b, S6c, and S6d (3,30). A new member of the endothelin/sarafotoxin family of vasoconstrictor peptides, bibrotoxin (BTX), was isolated from the venom of the burrowing asp *Atractaspis bibroni* (4). The primary structure of all known endothelins, sarafotoxins and bibrotoxin are summarized in Tab. 1. They are 21-amino acid peptides with high degree of similarity whose structure consists of two rings formed by intra-chain disulphide bonds.

Fig. 1: Mechanism of contraction of vascular smooth muscle caused by endothelin-1 (According to Potaczek and Sanak, 2002). Abbreviations used: **ETA** – endothelin receptor of type A, **G** – G-protein coupled receptor, **PLC** – phospholipase C, **PLD** – phospholipase D, **IP₃** – inositol trisphosphate, **DAG** – diacylglycerol, **PKC** – protein kinase C, **MLCK** – myosinlight-chain dinase, **MP** – myosin phosphatase.

Tab. 1: The sequence of amino acid residues in endothelin family peptides.

Nowadays three endothelins (ET), four sarafotoxins (SFTX) and bibrotoxins are known. All these peptides have 21 amino acids and two disulphide bridges between Cys^1 – Cys^{15} and between Cys^3 – Cys^{11}.

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            S ------------------------------------------------ S
            |                                                  |
            |       S ------------------------- S              |
            |       |                           |              |
            |       |                           |              |
ET-1        Cys-Ser-Cys-Ser-Ser-Leu-Met-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Try
ET-2        Cys-Ser-Cys-Ser-Ser-Tyr-Leu-Asp-Lys-Glu-Cys-Val-Tyr-Phe-Cys-His-Leu-Asp-Ile-Ile-Try
ET-3        Cys-Thr-Cys-Phe-Thr-Tyr-Lys-Asp-Lys-Glu-Cys-Val-Tyr-Tyr-Cys-His-Leu-Asp-Ile-Ile-Try
SFTX S6a    Cys-Ser-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Asn-Phe-Cys-His-Gln-Asp-Val-Ile-Try
SFTX S6b    Cys-Ser-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Tyr-Phe-Cys-His-Gln-Asp-Val-Ile-Try
SFTX S6c    Cys-Ser-Cys-Asn-Asp-Met-Thr-Asp-Glu-Glu-Cys-Leu-Asn-Phe-Cys-His-Gln-Asp-Val-Ile-Try
SFTX S6d    Cys-Thr-Cys-Lys-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Tyr-Phe-Cys-His-Gln-Asp-Ile-Ile-Try
Bibrotoxin  Cys-Ser-Cys-Ala-Asp-Met-Thr-Asp-Lys-Glu-Cys-Leu-Tyr-Phe-Cys-His-Gln-Asp-Val-Ile-Try
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Tab. 2: Classification of ET receptors agonists and antagonists.

Selectivity/Company	Company code and chemical structure
ET_A selective	
Peptide agonists:	[D-Lys^9]cyclo(11–15)ET-1(9–21)
	[Ala^{11,15}, Trp(For)^{21}]ET-1(9–21)
Peptide antagonists:	BQ123 = BE18257A = cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]
	BQ153
	BQ485 = hexahydro-1H-azepinyl)carbonyl- D-Leu-D-Trp-OH
	BQ610 = homopiperidenyl-CO-Leu-D-Trp-CHO-D-Trp-OH
	FR139317 = N-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp^3 (2-pyridyl)-D-Ala
	PD151242 = Tyr^3-(N-[(hexahydro-1H-azepin-1-yl)carbonyl]-L-Leu]-1Me)-D-Trp
Non-peptide antagonists:	A127722
Abbott (Knoll)	A216546
	ABT627 (A147627) = active enantiomer of the racemate A127722
	LU127043 = 2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid
	LU135252 = (+)-S-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid
Bristol-Myers Squibb	BMS182874 = 5-(dimethylamino)-N-(3,4-dimethyl-5-isoxazolyd)-1-naphthalene sulfonamide
	BMS184696
	BMS184697
ImmunoPharmaceuticals Inc	IPI413
Pfizer (Parke-Davis)	PD156707 (=CI1020; 8–8.7) = sodium 2-benzo(1,3)dioxol-5-yl-4-(4-methoxy-phenyl)-4-oxo-3-(3,4,5-trimethoxybenzyl)-but-2-enoate
	PD155080 (8–8.5), PD155719 = sodium 2-benzo{1,3}dioxol-5-yl-3-benzyl-4-(4-methoxy-phenyl-)-4-oxobut-2-enoate
	PD159433
	PD163070
	PD164893
Peptide International	PED3512PI
Hoffman-LaRoche	RO611790

Merck (Banyu)	L744453
	L754142
GlaxoSmithKline	SB234551
Novartis	34-Sulfatobastadin
Shionogi	S-0139
Takeda	TTA101
	TTA386
Texas Biotech Corp.	TBC11251
Fujisawa	WS7338A
	WS009A
	WS009B
AstraZeneca	ZD1611, ZD2574

ET_B selective
Peptide agonists:

[Ala^{1,3,11,15}]ET-1
BQ788 = (N-*cis*-2,6-di-methylpiperidinocarbonyl)-L-γ-MeLeu-D-Trp-COOCH$_3$-Nle
BQ3020 = N-Acetyl-[Ala^{11,15}]-ET-1 (8–21)
IRL1620 = N-Succinyl-[Glu^9,Ala^{11,15}]-ET-1 (8–21)
Sarafotoxin S6c
IRL2500
RES7011
BQ788 = *N-cis*-2,6-di-methylpiperidinocarbonyl L-γ-MeLeu-D-Trp(COOCH$_{3)}$Nle

Non-peptide antagonists	
Hoffman-LaRoche	RO468443
Abbott (Knoll)	A192621

$ET_{A/B}$ (mixed)
Antagonists:

Abbott (Knoll)	Aselacins A, B and C
	A182086
Actelion (Hoffman-LaRoche)	RO470203 = 4–(1,1–dimethylethyl)-N-[6–(2–hydroxy-ethoxy)-5–(2–methoxyphenoxy) [2,2'-bipyrimidin]-4–yl]-benzenesulfonamide (BOSENTAN)
GlaxoSmithKline	SB209670
	SB217742
Hoffman LaRoche	RO462005
ImmunoPharmaceutics Inc.	Sulfamethoxazole
Merck (Banyu)	Cochinmicin I, II and III
	L749329
	L754142 = active enantiomer of L749329
	L751281 = carbon isostere of L749329
	J104132
Novartis (Ciba-Geigy)	CGS27830
Pfizer (Parke-Davis)	PD142893= Acetyl-D-10,11–dihydro-5H-dibenzo[a,d] cycloheptene-glycine-3,3–D-diphenyl-Ala-Leu-Asp-Ile-Ile-Trp
	PD143296
	PD145065 (N-Acetyl-α-[10,11–dihydro-5H-dibenzo[a,d]cycloheptadien-5–yl]-D-Gly-Leu-Asp-Ile-Ile-Trp
	PD151242 = Tyr^{3-}(N-[(hexahydro-1H-azepin-1–yl)carbonyl]-L-Leu]-1Me)-D-Trp
	PD156252
	PD159020
	PD160672
	PD160874
Takeda	TAK044

The venom from the snake *A. engaddensis* has a very high lethal potency, with an i.v. LD_{50} of 0.06–0.075 mg.kg^{-1} body weight in mice. The onset of action of the venom is rapid (30–45 min) and death results from seemingly neurotoxic effects. However, even at high concentrations, the venom does not block contractions of skeletal muscles that are directly or indirectly stimulated. The changes observed in the ECG are similar to those recorded in human victims (18) and are the result of an A-V block that is caused by an apparent direct action of the venom on the heart (34). Concerning ETs already a low dose of ET-1 has a lethal effect in experimental animals by inducing disturbances in the cardiovascular system (16).

Endothelins and sarafotoxins are not selective agonists of ET_A and ET_B receptors. Until now, non-selective ET_A receptor agonists have been described in the literature. Recently, [D-Lys^9]cyclo(11–15) ET-1(9–21) was described as a specific full agonist of ET_A receptors, being under two orders less potent than ET-1 (EC_{50}:2.3 x 10^{-7} vs 6.8 x 10^{-9} M). Interestingly, even a linear formylated analogue, [Ala^{11,15}, Trp(For)^{21}]ET-1(9–21), showed a selective ET_A activity (EC_{50}:3.0 x 10^{-6} M). These peptides are the first compounds showing a significant bioactivity in an ET_A pharmacological preparation while being inactive in an ET_B paradigm (20).

As regard ET_B receptor, sarafotoxin S6c and the truncated, linear synthetic analogues BQ3020 ([Ala^{11,15}]acetyl-ET-1-(6–21)) (15) and IRL1620 (Suc-[Glu^9,Ala^{11,15}]ET-1-(8–21)]) (33) are the most widely used selective agonists for characterizing ET_B receptors. Radiolabelled analogues [^{125}I]BQ3020 and [^{125}I]IRL1620 are also commercially available. Some other peptide and/or non-peptide selective agonists of ET_B receptor are given in Tab. 2.

Endothelin receptors antagonists

Antagonists are classified as ET_A-selective, ET_B-selective, or non-selective mixed antagonists that display similar affinities for both receptor subtypes. However, only ET_A-selective or ET_A/ET_B antagonists are currently being evaluated in clinical trials. Nevertheless, ET_B-selective antagonists are also known.

The most highly selective peptide antagonists (4–5 orders of selectivity) for the ET_A receptors are the cyclic pentapeptide BQ123 (cyclo-[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]) (24) and the modified linear peptide FR139317 (N-[(hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp^3(2-pyridyl)-D-Ala)) (29). A peptide structurally similar to FR139317 has been radiolabelled, [^{125}I]PD151242, which displays subnanomolar affinity for the ET_A subtype (7). BQ123 is also available as a tritiated analogue, and a non-peptide antagonist, [^{125}I]PD164333, displays high affinity and selectivity for this subtype.

Generally more potent non-peptide antagonists for the ET_A receptors, such as PD156707, SB234551, L754142,

A127722, and TBC11251 are known and some of them are characterized in Tab. 2.

A limited number of peptide (BQ788) and non-peptide (A192621) ET_B antagonists have also been developed. Some of them are summarized in Tab. 2. 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 (^{125}I-PD151242, ^{125}I-PD164333, and ^{3}H-BQ123) or ET_B receptors (^{125}I-BQ3020 and ^{125}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.

Endothelin receptors antagonists in clinical development

While peptide antagonists are useful as research tools, their therapeutic use is limited by the fact that they can only be administered i.v. and have a short duration of action. Being peptides, they are degraded in the gastrointestinal tract by proteolytic enzymes.

The limitations of peptide antagonists have been partly overcome by non-peptide antagonists (5). ET receptors antagonists in clinical trial are summarized in Tab. 3. Manipulation of the activity of ET-1, especially using ET receptor antagonist, has allowed the further elucidation of the role of this neurohormonal system and development of novel therapeutic strategies in the treatment of heart failure (28). To date, published clinical studies of these agents have involved relatively small numbers of patients with severe heart failure, followed for a relatively short time period, and have mainly examined surrogate endpoints. Large-scale trials that address to significant clinical outcomes are ongoing and their results forthcoming. A key question that remains is whether selective ET_A or dual ET_A/ET_B receptor blockade will be more effective (1).

The same refers to utilization of ET antagonists in other pathological conditions, such as pulmonary arterial hypertension (PAH), subarrachnoid hemorrhage or hepatorenal syndrome. Bosentan (Tracleer) (Fig. 2) was the first ET antagonist approved by the Food and Drug Administration for the treatment of pulmonary arterial hypertension (8) and recently tezosentan (Veletri) has been designated for the same indication (17) and is under investigation for acute heart failure. The development of bosentan, a novel, well-tolerated, orally active endothelin antagonist, has significantly changed the therapeutic approach to PAH. Recent clinical trials have demonstrated that treatment with bosentan produces favourable effects on cardiopulmonary haemodynamics (22). Also tezosentan, dual ET_A/ET_B receptor antagonist that has demonstrated efficacy in improving cardiac index and reducing pulmonary capillary wedge pressure in patients with acute, decompensated heart failure, was introduced into clinical practice recently (31).

Tab. 3: Classification of ET receptor antagonists in clinical development.

ET receptors antagonists in II and III phase of clinical trials. CHF, congestive heart failure; PH, primary pulmonary hypertension; PC, prostate cancer; PORH, portal hypertension, SAH, subarachnoid hemorrhage; HRS, hepatorenal syndrome.

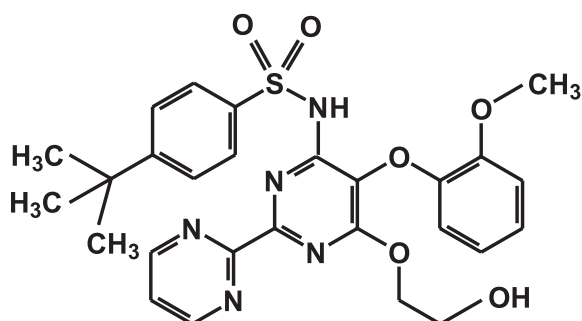| Commercial name	Generic name	Company code	Selectivity	Clinical trial	Target
Tracleer	Bosentan	RO470203	ET_A/ET_B	REACH-1	CHF
				EARLY	
				ENABLE-1 to -2	CHF
				BUILD-1 to -2	CHF
				RAPIDS-1 to -2	PH
				BREATHE-1 to -4	
		TAK044	ET_A/ET_B		PORH
		SB209670	ET_A/ET_B		
		PD156707 (=CI1020)	ET_A	CHF	
	Darusentan	LU135252	ET_A	EARTH	CHF
	Enrasentan	SB217242	ET_A/ET_B	ENCOR	
	Sitaxsentan	TBC11251	ET_A	PC	
	Atrasentan	ABT627	ET_A	PH	
	Ambrisentan	LU 208075 (=BSF-208075)	ET_A	ARIES I & II,	CHF
Veletri	Tezosentan	RO610612	ET_A/ET_B	RITZ-1 to -4	SAH
				VERITAS-1 to -2	HRS
	Clazosentan		ET_A		SAH

Fig. 2: Chemical structure of bosentan (Tracleer), the first endothelin receptor antagonist approved by the Food and Drug Administration for the treatment of pulmonary arterial hypertension.

Conclusions

The ET system plays an important role in the pathophysiology of a variety of cardiovascular diseases including congestive heart failure, essential and pulmonary hypertension, renal failure, and cerebrovascular disease. The biological effects of ET-1 on its target organs are mediated by two receptor types: ET_A and ET_B. It is widely accepted that the vascular, cardiac, and renal adverse effects of ET-1 are mediated by ET_A, while activation of ET_B receptors leads to beneficial effects such as: attenuating the vascular and cardiac hypertrophic effects of ET-1 as well as they mediate the vasodilatory action of this peptide (activation of ET_{B1}-

receptor subtype). In the last decade, a plenty of peptide and non-peptide ET-1 antagonists have been developed. Several clinical studies have revealed that ET-1 antagonists are clinically beneficial therapeutic agents for the treatment of several cardiovascular diseases, leading to the approval of bosentan (ET_A/ET_B antagonist) for the treatment of pulmonary hypertension.

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