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COMMENTARY

ENDOTHELIN RECEPTOR ANTAGONISTS: ACTIONS AND RATIONALE FOR THEIR DEVELOPMENT

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The endothelins were first isolated in 1988 [1], and it has become apparent that they are produced and active in almost all tissues. *In vitro* and *in vivo*, the endothelins are potent vasoconstrictors and pressor agents, although there may also be accompanying vasodilatation, particularly at low concentrations. Among numerous other effects, the endothelins stimulate the release of autocoids and hormones, contract non-vascular smooth muscle, both potentiate and reduce neurotransmitter release, decrease glomerular filtration, and act as cardiac inotropes and chronotropes. They also strongly affect numerous isolated cells and can be shown, for example, to increase smooth muscle proliferation and neutrophil superoxide generation. The purpose of this review, however, will not be to discuss the range of endothelin activities, which has already been ably achieved [see Refs. 2–8]. Rather, it will be to draw attention to current research leading to the production of endothelin receptor antagonists.

It is fair to say that a large portion of endothelin research has been aimed at elucidating the regulatory effects of these peptides in the cardiovascular system. This research target is suggested by the observations that endothelin is an extremely potent vasoconstrictor that causes very prolonged responses, together with findings that the circulating levels of endothelins are increased in numerous cardiovascular disease states. Thus, one readily arrives at the commonly held notion that the generation of selective endothelin antagonists or inhibitors of endothelin production could be of benefit in a range of diseases from hypertension to renal failure and stroke. Endothelin receptor antagonists are, therefore, currently being tested in animal models of these pathologies, and also most probably in some limited human trials. However, before discussing the type of compounds that might be the most useful in these studies, it is necessary, first, to briefly review our current knowledge about the regulatory systems controlling endothelin production and the receptors that mediate its effects.

Endothelin family of peptides

The endothelins (ET-1, ET-2 and ET-3)§ constitute a family of three peptides that are very closely related structurally [1, 9]. They have a common structure of 21 amino acids with four cysteine residues at positions 1, 3, 11 and 15. These cysteine residues link to form two intrachain disulfide bridges between residues 1 and 15, and 3 and 11. ET-2 differs from ET-1 by three amino acids and ET-3 by six residues. These differences are all contained within the region 2 to 14, and the endothelins therefore share a common tail region, residues 16 to 21. Probably all mammalian species produce endothelins, for the genes for ET-1, ET-2 and ET-3 are present in human, porcine, rat and murine tissues [9, 10]. Although less work has been carried out on non-mammalian species, these may also produce closely related peptides. In particular, the endothelins are very similar structurally to the sarafotoxins, which are found in the venom of *Atractaspis engaddensis* and which are potent agonists of endothelin receptors [11, 12]. Interestingly, experiments with these compounds indicate that marked stimulation of endothelin receptors can be lethal!

Synthesis of the endothelins

The biosynthesis of ET-1, as suggested in the initial report [1], is similar to that of many biologically active peptides. After synthesis of the prepro-endothelin (203 a.a.), removal of the signal sequence generates pro-endothelin. Pro-endothelin is processed to release the intermediate referred to as big ET-1, which in the human is 38 a.a. in length. The mature and active form of ET-1 is then formed by the action of a putative endopeptidase referred to as ECE. Less is known about the formation of the other endothelins, although they are generally considered to be produced by similar processing. Interestingly, however, sarafotoxins do not appear

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§ Abbreviations: ET-1, ET-2 and ET-3, endothelin-1, -2 and -3; ECE, endothelin-converting enzyme; and ACE, angiotensin-converting enzyme.

to be cleaved from a "big sarafotoxin" precursor but rather to be synthesized more directly by the processing of a large protein precursor containing multiple sarafotoxin sequences [13].

It is important to note that big ET-1 is at least 100-fold less active than ET-1 at constricting isolated vascular preparations and displacing ET-1-receptor binding [14]. The conversion of big ET-1 to ET-1 is, therefore, a crucial step in the formation of the biologically active peptide, and, consequently, inhibition of ECE has been viewed as a route by which the biological effects of endogenous endothelin could be suppressed. This rationale is clearly supported by the widespread understanding that inhibition of ACE is a very good limiter of the effects of endogenous angiotensin II. Interestingly, and also in direct analogy to ACE, ECE activity is widespread in the vasculature for, although big ET-1 is much less active *in vitro* than ET-1, it is almost equiactive as a pressor agent when administered intravenously to the rat [15], guinea pig [16] or rabbit [17].

Numerous candidates have been proposed as the ECE, although the one of greatest interest is a phosphoramidon-inhibitable activity first described in endothelial cells [18–20]. This may well be the most relevant form of ECE for phosphoramidon decreases the release of ET-1 from cultured endothelial cells [21, 22], the conversion of exogenous big ET-1 to ET-1 by endothelial cells in culture [23], and the physiological responses to big ET-1 *in vitro* [24, 25] and *in vivo* [26, 27]. Although there have been preliminary reports of partial purifications of ECE, it is only very recently that an enzyme with the characteristics of that present in endothelial cells has been isolated. This protein has been purified from rat lung and found to be a metalloprotease with a molecular weight of 130 kDa. It converts big ET-1 with a K_m of 0.2 μM and a maximal velocity of 3.1 nmol$\cdot$min$^{-1}\cdot$mg protein^{-1}, and may well be the "physiological" ECE [28]. Other "non-physiological" ECEs have also been characterized, including cathepsins [29], mast cell chymase [30] and elastase from neutrophils [31]. However, there is no support for the idea that they are involved in the production of ET-1 by endothelial cells in culture or in the conversion of exogenous big ET-1 in the normal circulation [32, 33].

Endothelin receptors

Before the synthesis of selective endothelin agonists and antagonists, experimenters characterizing endothelin receptors had to content themselves with comparing the activities of the naturally occurring peptides. Early studies with the endothelins indicated that ET-3 was less potent than either ET-1 or ET-2 as a pressor agent [9]. Conversely, the three endothelins were equipotent at producing the initial hypotension that followed intravenous injection [9] and stimulating the release of nitric oxide from the endothelium of isolated vascular preparations [34, 35]. This clearly suggested the presence of different endothelin receptors: an isopeptide selective receptor (ET-1 > ET-3) that mediated the pressor effects of the endothelins and a non-selective receptor (ET-1 = ET-3) present on the endothelium. At the same time, experiments employing as an agonist the common C-terminal portion of the endothelins, $ET_{(16-21)}$, found clear differences between the receptors mediating constrictions of the guinea-pig bronchus or rabbit pulmonary artery, on which $ET_{(16-21)}$ was a full agonist, and those on the rat thoracic aorta or human renal artery, where $ET_{(16-21)}$ was without effect [36]. Similarly, receptor binding assays indicated the presence of heterogenous populations of endothelin receptors in a variety of tissues [see Ref. 7]. Such has been the pace of endothelin research that these functional indications were closely followed by the cloning and expression of two endothelin receptors: the ET_A receptor that is selective for ET-1, ET-2 or SX6b over ET-3 or SX6c [37], and the ET_B receptor that does not discriminate between the endothelin/sarafotoxin peptides [38]. These receptors have very similar predicted molecular weights of approximately 47 kDa and contain seven transmembrane domains of 20–27 hydrophobic amino acid residues, typical of the rhodopsin-type superfamily of G-protein-coupled receptors. As may be expected, the genes encoding these receptors have been suggested to be expressed in a wide variety of tissues and species [see Ref. 8].

Early experiments had suggested that the isopeptide-selective (ET_A) receptor would be present predominantly on smooth muscle, and in particular on vascular smooth muscle, where it would mediate the contractile effects of the endothelins, while the ET_B receptor would be present, for instance, on endothelial cells and mediate vasodilatation. However, it is now clear that there are no exacting rules as to the distribution of endothelin receptors, for contractions of smooth muscle may be mediated by either ET_A or ET_B receptors [39–47]. Furthermore, there are marked species variations in the receptor subtypes mediating the effects of the endothelins in a large number of tissues. However, it still appears to be generally true that only ET_B receptors mediate vasodilatation, irrespective of species or tissue.

Additional endothelin receptors

Although only two endothelin receptors have been cloned and expressed, there is much functional evidence for receptor subtypes in addition to the ET_A or ET_B subtype. For instance, a receptor selective for ET-3 over ET-1, tentatively named an ET_C receptor [48], appears to be expressed on some bovine endothelial cells. Activation of these receptors by ET-3, but not ET-1, results in the selective [49] and maintained [50] release of nitric oxide. This receptor subtype may be expressed in other species and tissues, since high-affinity receptors selective for ET-3 have been identified by receptor binding assays using membranes prepared from rat brain and atria [51, 52]. Furthermore, ET-3 selective receptors have been shown to be present in anterior pituitary cells [53]. There is also a growing body of data suggesting that the ET_B receptor present on the endothelium, which mediates the release of nitric oxide in response to the endothelins, is functionally different from the ET_B receptor mediating the vasoconstrictor effects of the endothelins [45, 47, 54]. Thus, these have tentatively been classified as ET_{B1} (present on the endothelium) and ET_{B2} (present on vascular and

Table 1. Disease models in which endothelin antibodies are effective

Model	Species	Reference
Renal ischaemia/reperfusion	Rat	66,67
Cyclosporine nephrotoxicity	Rat	69
Gastric ulceration induced by ethanol	Rat	74,75
Gastric ulceration induced by indomethacin	Rat	76
Myocardial ischaemia/reperfusion	Rat	77
	Rabbit	78
Hypertension	Rat	79

non-vascular smooth muscle) [47]. Additionally, other functional studies have also suggested that non-ET_A/ET_B receptors mediate responses to the endothelins in preparations from the rat, guinea pig and rabbit [see Ref. 8]. Full classification of these receptor subtypes awaits the purification or cloning of these additional receptors.

Endothelin in disease: effects of endothelin antibodies

The circulating level of endothelin in healthy subjects has been reported to be about 1.5 pmol/L, which is at least one order of magnitude less than that of circulating human atrial natriuretic peptide and several times less than that of angiotensin II [see Ref. 55]. Elevated levels of endothelin-immunoreactivity have been reported in a wide number of disease states, including acute myocardial infarction, hypertension, atherosclerosis, congestive heart failure, Raynaud's phenomenon, and surgery [see Refs. 55 and 56]. However, it is still not clear whether these increases, which are almost without exception in the order of 2 to 3-fold, are simply a symptom of the underlying disease or are causally related. It may be that these small changes indicate that endothelin has very little involvement in these disease processes. However, it should also be remembered that the half-life of endothelin within the circulation is extremely brief [57, 58], and it appears to be rapidly cleared by the lung in both humans [59] and other animals [60, 61]. In addition, it also appears that endothelial cells release endothelin predominantly in an abluminal direction [62], i.e. towards the underlying smooth muscle. Once bound to its receptors, endothelin dissociates very slowly [63–65] and is therefore lost to the circulation. Once these factors are taken into account, it is very difficult to judge the degree of increase in local endothelin production that would result in a 2 to 3-fold elevation in circulating endothelin. Therefore, it is probable that the relatively small changes in circulating levels of endothelin reflect very poorly the large local increases in endothelin formation.

Evidence from animal models also supports the idea that endothelin may have a role in certain disease processes. For instance, the deleterious effects of renal ischaemia and reperfusion in the rat are decreased by the local infusion of endothelin antibodies [66, 67] (Table 1). Similarly, the harmful effects of cyclosporine in the renal circulation, which are associated with damage of the endothelium and/or increased endothelin gene expression [see Ref. 68], are attenuated by endothelin antibodies [69, 70]. The idea that endothelin may be involved in various renal pathologies is supported by the observation that chronic renal failure in the rat is associated with an increased urinary excretion of endothelin [71, 72]. Other experiments have indicated that ET-1 is a potent pro-ulcerogenic agent in the rat stomach [73]. This result may be considered somewhat predictable, bearing in mind that ET-1 induces strong vasoconstrictions that would potentiate the effects of any locally harmful agent by reducing the blood flow. However, locally produced endothelin may also be an important mediator of gastric ulceration, for endothelin antibodies decrease the ulcerogenic effects of alcohol [74, 75] or indomethacin [76] applied to the inner surface of the stomach. Other experiments have also revealed that infusion of endothelin antibodies reduces coronary infarct size in both the rat and rabbit [77, 78] and have also suggested that this treatment may lower blood pressure [79]. It is fair to say, however, that not all investigators find endothelin antibodies to be anti-hypertensive [80].

Endothelin antagonists

There are, therefore, clear indications that an increase in endothelin production may be a promoting factor in a number of disease states. For this reason, the discovery of endothelin receptor antagonists that could be therapeutically beneficial agents in these pathologies has been the clear target of a large number of research groups (Table 2). Various compounds have been reported as ET-receptor antagonists, such as [Dpr^1, Asp^{15}]-ET-1, which is a full-length ET-1 derivative [81], and [D-Arg^1, D-Phe^5, D-$Trp^{7,9}$, Leu^{11}]-substance P, which is a non-endothelin-related peptide antagonist [82]. However, many of these agents have not been studied extensively and are not necessarily very selective. For instance, [D-Arg^1, D-Phe^5, D-$Trp^{7,9}$, Leu^{11}]-substance P also inhibits the effects of bombesin, arginine-vasopressin and bradykinin. This review will not attempt to survey all these putative antagonists, but will rather concentrate on compounds that have been studied more widely.

ET_A receptor selective antagonists

Many of the currently available endothelin antagonists have been discovered as a result of natural product and/or compound library screening.

Table 2. Currently disclosed endothelin receptor antagonists

Compound	Selectivity	Reference
[D-Arg^1, D-Phe^5,D-Trp^{7,9},Leu^{11}]-SP	ET_A?	82
[Dpr^1,Asp^{15}]-ET-1	ET_A?	81
BQ-123	ET_A	84
BQ-153	ET_A	84
BQ-485	ET_A	126
FR 139317	ET_A	90
50-235	ET_A	94
TTA-386	ET_A	93
IRL 1038	ET_B	95
PD 142893	ET_A/ET_B	96
PD 145065	ET_A/ET_B	97
Cochinmicins	ET_A/ET_B	102
Ro 46-2005	ET_A/ET_B	101

Good examples of these are the members of a series of compounds, e.g. BQ-123 and BQ-153, synthesised by the Banyu Pharmaceutical Co. in Japan, which were developed from antagonists discovered in the broth from the mycelium of *Streptomyces misakiensis* [83–85]. These modified compounds, which like the progenitor molecules are cyclic pentapeptides, have a high affinity for ET_A receptors in porcine and rat vascular smooth muscle [83–87], whereas they are ineffective at displacing binding from ET_B receptors, e.g. in cerebellar membranes. Interestingly, starting from the same cyclic pentapeptide natural product, two linear tripeptidic, selective ET_A receptor antagonists, FR 139317 [88–91] and BQ-610, were also developed [92].

A number of other ET_A selective antagonists apart from BQ-123, BQ-153 and FR 139317 have also been reported. These include TTA-101 (Bu^t OCO-Leu-Trp-Ala) and TTA-386 [93] and myriceron caffeoyl ester (50-235), a non-peptide isolated from the bayberry *Myrica cerifera* [94]. However, in contrast to BQ-123 and FR 139317, which are the compounds used most often, very few studies have been published using these latter antagonists and, as such, it is difficult to discuss their selectivity or possible utility.

ET_B receptor selective antagonists

There is very little information available on the activities of selective ET_B antagonists. Indeed, IRL 1038, ([Cys^{11}-Cys^{15}]-ET-1 (11-21)), which has been shown to have a much higher affinity for ET_B receptors (K_i = 6–11 nM) than for ET_A receptors (K_i = 400–700 nM) [95] and to antagonize functional responses mediated by ET_B but not ET_A receptors [95], is currently the only well characterized ET_B antagonist for which data have been published.

$ET_{A/B}$ receptor non-selective antagonists

$ET_{A/B}$ receptor non-selective antagonists may well be the most effective in a range of disease states as these will be active irrespective of variations in the endothelin receptor population. In contrast to the random screening that has led to the effective ET_A receptor antagonists discussed above, others have developed non-selective endothelin antagonists by a rational approach starting with $ET_{(16-21)}$, which is known to interact with ET receptors [96–99]. Modification of the C-terminal hexapeptide portion of ET-1 by substituting His^{16} with (B-phenyl)-D-Phe produced PD 142893, the first disclosed ET_A and ET_B functional receptor antagonist in this series of compounds. Incorporation of D-Bhg in position 16, produced another compound, PD 145065, that in comparison to PD 142893, had a further increased binding affinity to both ET_A and ET_B receptors. Other ET-1-derivatives, such as [Thr^{18}, γ-methyl Leu^{19}]ET-1, have also been shown to bind with high affinities to both ET_A and ET_B receptors, although, once again, there is little additional information as to the usefulness of this latter compound as a functional antagonist of endothelin responses [100].

Perhaps most interestingly, compound library screening has also led to the discovery of the orally active non-peptide endothelin receptor antagonist, Ro 46-2005 [101]. This sulfonamide derivative was derived by structural modification of a compound first synthesized as part of an antidiabetic project. Ro 46-2005 inhibits binding to both ET_A and ET_B receptors, although it would be fair to say that it does so with considerably less affinity than the non-selective peptidic antagonist PD 145065. On the other hand, Ro 46-2005 has the advantage of being orally active (30% bioavailability in the rat), which the peptidic PD 145065 probably is not, and of having a longer plasma half-life.

Natural product screening of the products of *Microbispora sp.* ATCC55140 has also revealed the cochinmicins I, II and III, cyclic depsipeptides, which have been described as non-selective $ET_{A/B}$ antagonists [102, 103]. However, these are much less potent antagonists than the compounds in the PD series.

Effects of endothelin receptor antagonists: distribution of endothelin receptors

Of all the antagonists discussed above, the most widely studied are the ET_A receptor selective antagonists of the "BQ" series, in particular BQ-123, and FR 139317, and the $ET_{A/B}$ receptor non-selective antagonists of the "PD" series, e.g. PD 142893 and PD 145065.

BQ-123 antagonizes constrictions of the isolated porcine coronary artery induced by ET-1, inhibits binding to endothelin receptors on vascular smooth muscle cells, and blunts, but does not ablate, the pressor effects of ET-1 in anaesthetized rats [84]. Further studies have indicated that the inability of BQ-123 to block entirely the pressor effects of ET-1 is explained by the presence of non-ET_A constrictor receptors within the rat circulation, most notably within the mesenteric and renal beds [104–107]. Interestingly, treatment with PD 145065 blocks the renal constrictor effects of ET-1 in the anaesthetised rat, illustrating the importance of ET_B vasoconstrictor receptors. At the same time, PD 142893 and PD 145065 also block the depressor effects of intravenously administered ET-1 in this same species [96, 97, 107], whereas BQ-123 and FR 139317 do not affect or tend to potentiate this portion of the ET-1 response [90, 108]. Thus, experiments using the endothelin receptor antagonists confirm the suggestion that within the rat circulation both ET_A and ET_B receptors mediate the vasoconstrictor or pressor effects of the endothelins and that ET_B receptors, most probably present on the endothelium [34, 35, 45, 47], mediate the transient depressor response to the endothelins.

In other species it may also be safe to assume that ET_A and ET_B receptors mediate vasoconstriction and ET_B receptors vasodilatation, although it is worth noting that the relative importance of different endothelin receptors in various tissues is not common between different species. For instance, as outlined above, the renal constrictor effects of the endothelins in the rat are mediated by a mixed population of ET_A and ET_B receptors. However, in the pig these responses are more sensitive to ET_A receptor blockade [109], and in the rabbit BQ-123 blocks entirely ET-1-induced renal vasoconstriction [110]. This means, unfortunately, that there is no particular rule that one can follow to predict the efficacy of these compounds as antagonists of the effects of the endothelins in human tissues. One answer is to use receptor binding, immunohistochemical or autoradiographical techniques to study the endothelin receptors present in smaller sections of human tissue, which are often more easily obtained than intact preparations. However, there are clear difficulties in drawing conclusions about function from these types of experiments. For instance, although receptor binding assays indicate that the populations of ET_A and ET_B receptors in the rat kidney are divided 50:50, which is in accord with the vascular responses, the ratio of ET_A to ET_B receptors in the dog kidney is 20:80. However, in this latter species ET_A receptors are the predominant subtype that mediate renal vasoconstriction [111, 112]. One obvious explanation for this difference is that many of these endothelin receptors in the canine kidney mediate responses other than vasoconstriction. Unfortunately, immunological techniques do not necessarily assist us in understanding what functions these may be. Similarly, we find that the populations of endothelin receptors on isolated blood vessels vary between species, although in this case functional studies with human tissue are somewhat easier to perform. As an example of this heterogeneity, contractions of the pulmonary artery from the rabbit are mediated by ET_B receptors and are not sensitive to BQ-123 [45, 47, 113, 114], whereas in porcine, guinea pig and human vessels ET_A receptors predominate [40, 54, 115]. Similarly, although current knowledge makes it a safe assumption that ET_B receptors mediate the release of nitric oxide from the endothelium of all species [45, 47, 54], the release of prostanoids from various vascular beds may or may not be sensitive to ET_A receptor blockade [110, 116]. This species heterogeneity in the distribution of endothelin receptors is not confined to the cardiovascular system, as non-vascular responses to the endothelins also vary in their sensitivity to the various antagonists. For instance, BQ-123 or FR 139317 do not affect contractions induced by ET-1 in trachea or upper bronchi from the guinea pig [117] or bronchi from the human [118], whereas these antagonists may reduce the contractions induced by ET-1 in the rat trachea [119].

Table 3. Disease models in which endothelin receptor antagonists are effective

Model	Species	Reference
Hypertension	Rat	120,121
	Monkey	101
Renal ischaemia/reperfusion	Rat	101,122
	Dog	123
Cyclosporine nephrotoxicity	Rat	124
Subarachnoid haemorrhage	Dog	125,126
	Rat	101
Cardiac ischaemia/reperfusion	Dog	127

Endothelin receptor antagonists in disease models

Although the endothelin antagonists have been tested in many systems against exogenous endothelins [see Ref. 8], there is less information about their effectiveness in disease models (Table 3). The few available reports contain data from models very similar to those in which endothelin antibodies have been found to be beneficial. For instance, as mentioned above, endothelin antibodies have been reported to be beneficial in models of myocardial ischaemia and reperfusion, and application of a monoclonal antibody against ET-1 significantly reduces the infarct size in a rabbit model of coronary artery occlusion (30 min) and reperfusion (24 hr) from 29 to 17% [78]. However, in a similar study, FR 139317, the selective ET_A receptor antagonist, was found to be ineffective after 2 hr in protecting against the extension of infarct in the same species [128]. This does not appear to be explained by the presence of other receptors in this vascular bed, as FR 139317 very effectively antagonizes the vasoconstrictor effects of ET-1 in the isolated perfused heart of the rabbit [128]. Possibly this indicates that the extension of infarct in the rabbit is not dependent on vasoconstrictor receptors or that the differences in the duration of these protocols are important. Interestingly, experiments using the dog as an experimental species do indicate that BQ-123 is capable of decreasing infarct size following 90 min of ischaemia and 5 hr of reperfusion [127]. Once again this illustrates inter-species variation in the sensitivity to modulations of the endothelin system.

The organ in which the protective effects of endothelin antibodies have been studied most extensively is the kidney, where they decrease the deleterious effects of both ischaemia reperfusion and cyclosporine (Table 1). As might be expected, some of the endothelin antagonists have already been tested in these models, and it has been reported that a high dose ($0.5\,mg\cdot kg^{-1}\cdot min^{-1}$) of BQ-123 is protective in ischaemic acute renal failure in the rat [122]. However, it would be fair to say that the protective effects seen were not impressive, which could indicate that the endothelins have very little involvement in damage progression in this protocol. More precisely, it is indicative of a lack of involvement of ET_A receptors in these effects. This may not be surprising for, as outlined above, the vasoconstrictor responses of the rat kidney are predominantly mediated by ET_B receptors. Furthermore, in the rat kidney as a whole the ratio of ET_A to ET_B receptors is 50:50. With this knowledge in mind, it may not appear too surprising that BQ-123 had only a slight protective effect. Interestingly, and as we might predict, the non-selective antagonist Ro 46-2005 partially restores the initial (20–30 min) fall in renal blood flow that follows ischaemia and reperfusion [101]. On the other hand, in *in vivo* and *in vitro* models of acute cyclosporine nephrotoxicity, where endothelin antibodies are also effective, BQ-123 is found to be protective [70, 124]. This may be because in this model there is a role for endothelin at sites such as the mesangial cell, where BQ-123 protects against the increase in myosin light chain phosphorylation induced by cyclosporine *in vitro* [93]. However, it would probably be safest to state that any conclusions about the role of endothelin in these models await the use of the $ET_{A/B}$ receptor non-selective antagonists in more exacting studies.

As might be expected, endothelin antagonists have been tested for their effects in animal models of hypertension, and some investigators have reported hypotensive effects. For instance, in one comparative study, BQ-123 has been shown to lower blood pressure in spontaneously hypertensive stroke prone rats, but not in spontaneously hypertensive or normotensive controls [120]. Another more complete study has suggested that blockade of ET_A receptors does not lower blood pressure in high-renin models of hypertension but may produce a moderate anti-hypertensive effect in low/normal-renin models, such as deoxycorticosterone-salt rats or spontaneously hypertensive animals [129]. This is in contrast to the reports that BQ-123 will reduce blood pressure and peripheral resistance in spontaneously hypertensive rats [121] and that the $ET_{A/B}$ receptor non-selective antagonist Ro 46-2005 will lower blood pressure in sodium-depleted squirrel monkeys [101], which is more likely to provoke a high-renin state. However, it may be best to be cautious in reviewing the data obtained with Ro 46-2005 for further studies may be required to substantiate that Ro 46-2005 is lowering blood pressure through antagonism of endothelin and not through an unrelated mechanism. Thus, as with the above models, it appears that there is as yet no consensus as to the role of endothelin in hypertension. However, it is not to be doubted that this will be answered in the very near future, and even if endothelin is not found to have a central role in hypertension, it may be linked to the progression of the disease, particularly when there is endothelial dysfunction or renal disease.

One pathological model in which there does appear to be agreement as to the efficacy of endothelin antagonists, even in different species, is subarachnoid haemorrhage. In both the dog and rat, FR 139317 [125], BQ-485 (an ET_A-receptor selective antagonist [126]) and Ro 46-2005 [101] attenuate the reduction in basilar artery diameter following local injection of autologous blood. In contrast to the previous models, in which antibodies were the indicators, the initial evidence that endothelin might be involved in the pathological *sequelae* of subarachnoid haemorrhage was the finding that phosphoramidon, which inhibits ECE (see above), is beneficial in a canine model of this disorder [26].

Summary and concluding remarks: requirements for endothelin antagonists

From this brief survey of current knowledge, it is apparent that there are effects of the endothelins that can be antagonized selectively by available compounds, and that an increased production or activity of endothelin, e.g. secondary to an increase in endothelin receptor expression [130, 131], may underlie certain disease states. However, there are clearly problems in predicting the efficacy in humans of compounds discovered in experiments using animal models, particularly in defining the endothelin receptor populations that may need to be targeted. Before defining the criteria for endothelin receptor antagonist production, there are, therefore, a number of questions to be addressed. First, in which disease might the antagonists be beneficial; second, which receptor subtype should be targeted; and third, and maybe most important, is blockade of receptors the best approach to limiting the effects of the endothelins.

The disease targets against which endothelin antagonists may be effective are broadly outlined above. These would include hypertension, stroke, myocardial infarction, renal ischaemia, gastric ulceration and possibly airway disease. Data from experiments utilizing antibodies, and from initial studies with the available antagonists, suggest that inhibition of the endothelin system may be of some benefit. Unfortunately, the only data available are on the possible beneficial effects of these antagonists following acute application. This may be because the majority of compounds have to be present in relatively high concentrations and are not orally active, making their application over long periods technically difficult. Such a consideration may be important, for the lack of effect of endothelin antagonists over relatively short periods may be a function of the binding of ET-1 to its receptors. This is because in intact cells ET-1 becomes dissociated from its receptors only following receptor internalization, meaning that it takes 1 hr to recycle 40% of the receptors. Thus, the slow reversal by antagonists of the effects of ET-1 mediated by either ET_A or ET_B receptors [65, 132, 133] is a function of their ability to prevent new binding of ET-1 following re-externalization of the receptors [65] and is not explainable as a reversal of established endothelin binding. Clearly, this implies that application of endothelin receptor antagonists for short periods will not reverse the effects of locally released endothelin.

An alternative approach to antagonizing the effects of ET-1, second to its production, is to limit its synthesis, most obviously by inhibiting ECE. This has attractions as a therapeutic route for it would be effective irrespective of any alterations in the population of endothelin receptors that may occur with pathological changes [131]. Experiments to produce inhibitors of ECE have been complicated by a lack of purified enzyme, but fortunately this problem appears to have been surmounted with the report that ECE has finally been purified [28]. With this background it is interesting to speculate on the effects of blockade of ECE. If this is the enzyme responsible for the "physiological" formation of ET-1 from big ET-1, would its blockade lead to an increase in circulating levels of big ET-1, as would appear to be the case in cultured endothelial cells [22]. If so, this would present more substrate to other enzymes that can convert big ET-1 to ET-1 such as elastase, released from neutrophils [31], or chymase released from mast cells [30], both of which are more active converters of big ET-1 than the "physiological" ECE. These systems could become important in pathological states when these enzymes are released from cells. It is even interesting to speculate whether the final step in the conversion of big ET-1 to ET-1 does indeed take place outside the cell responsible for the synthesis of big ET-1, as the circulating levels of big ET-1 are 2 to 3-fold higher than the circulating levels of ET-1 [see Ref. 55]. Thus, it may be that conversion of big ET-1 outside the endothelial cell, either by other endothelial cells or alternative cell types such as vascular smooth muscle [23, 134], is of more importance in the body than intracellular conversion followed by release. Inhibitors of ECE could, therefore, regulate the activities of endothelin in both health and disease.

In conclusion, we may say that the initial results with compounds such as BQ-123, FR 139317 and Ro 46-2005 in animal disease models give encouragement to the belief that endothelin antagonists may be of future therapeutic benefit in humans. However, we must wait, in particular, for more data on their effectiveness following administration over longer periods before we can be confident in this judgment.

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