Endothelin: role in hypertension

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Endothelins (ET) are 21-aminoacid peptides produced ubiquitously, which were discovered originally as endothelial products. These peptides may play important roles in cardiovascular physiology and pathophysiology. As the pathophysiologic roles of endothelins in cardiovascular disease become increasingly apparent, the potential therapeutic use of endothelin antagonists or endothelin converting enzyme inhibitors is recognized. The main endothelin produced by the endothelium is ET-1. Endothelin-1 is overexpressed in the vascular wall of salt-dependent models of hypertension, such as DOCA-salt hypertensive rats, DOCA-salt-treated spontaneously hypertensive rats (SHR) and Dahl salt-sensitive rats, and in stroke-prone SHR, angiotensin II-infused rats and 1-kidney 1 clip Goldblatt hypertensive rats, but not in SHR, 2-K 1C hypertensive rats or L-NAME-treated rats. The vasoconstrictor effect of ET-1 may contribute to blood pressure elevation and its growth-promoting action to vascular hypertrophy in the hypertensive models which overexpress ET-1 in blood vessels. In rats without generalized activation of the endothelin system, expression of ET-1 is often enhanced in coronary arteries, which suggests a role for ET-1 in myocardial ischemia in hypertension. In rats overexpressing ET-1, $ET_{A/B}$ and ET_A-selective antagonists lowered blood pressure slightly, and significantly reduced vascular growth, particularly of small arteries, suggesting that ET-1 has a direct effect on growth. Protection from renal injury and from stroke has also been demonstrated in hypertensive rats treated with endothelin antagonists. In normotensive human subjects endothelin-dependent tone can be shown in the forearm. In a study of mild hypertensive patients, the $ET_{A/B}$ antagonist bosentan reduced blood pressure similarly to an ACE inhibitor. Moderate to severe hypertensive patients presented enhanced expression of ET-1 mRNA in the endothelium of subcutaneous resistance arteries. In blacks with familial hypertension increased plasma levels of endothelin have been found. Thus, ET-1 may play a role in some experimental hypertensive models and in human hypertension. In summary, endothelial ET-1 may be overexpressed in the more severe forms of hypertension, and in certain special populations which may respond particularly well to endothelin antagonism. Endothelin antagonists may prove to be effective disease-modifying agents if in future clinical trials they are shown clinically to blunt vascular growth and endothelial dysfunction, reduce stroke and exert the cardioprotective and renal protective effects already reported in experimental hypertension. These agents could contribute to reduce the long-term complications of hypertension, which remains to be demonstrated in humans.

Key terms: *arterial blood pressure, cardiac hypertrophy, endothelium, smooth muscle cells, vascular hypertrophy.*

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INTRODUCTION

Endothelins (ET) are 21-aminoacid peptides produced in many different tissues. They were discovered as a product of vascular endothelial cells (69), but have since been shown to be produced by vascular smooth muscle cells in culture (19), and by many cells in different organs. Although initially thought to be essentially a cardiovascular hormone because of its potent vasoconstrictor effects and its origin in the vasculature, it is now apparent that endothelins are important regulators of non cardiovascular functions, including those of airway smooth muscle, the digestive tract, endocrine glands, the renal and genitourinary system and the nervous system. They may participate as important mediators in numerous pathological processes, and play as well an important role in development. The role of the endothelin system in development has been emphasized by the results of gene disruption experiments (30) of the ET-1 and ET-3 genes, and of the genes of the endothelin ET_A and ET_B receptors (22). Inactivation in mice of the ET-1 or the ET_A receptor genes results in branchial arch abnormalities such as malformations of the mandibula, upper airway, and aortic arch, which resemble the Pierre Robin syndrome, with associated hypoxia and hypercapnia (30). Surprisingly for the inactivation of genes encoding components of a vasoconstrictor system, disruption of the ET-1 gene results in elevation rather than reduction of blood pressure. This paradoxical result could be explained by the disappearance of the relaxant effects of ET-1 (via endothelial ET_B receptors and release of nitric oxide and prostacyclin), which will be described below in greater detail, but is more probably the result of the hypoxia and hypercapnia, which through sympathetic activation elevates blood pressure. Indeed, correction of the hypoxia normalizes blood pressure. The inactivation of ET-3 or the ET_B receptor gene results in pigmentary abnormalities and aganglionic megacolon (22), underlining the role of ET-3 as a neuropeptide, and its participation through the ET_B receptor in the migration of neural crest cells (melanocytes and neurons of the myenteric plexus). In humans, mutations in the ET_B receptor gene have been discovered in some of the familial and in sporadic forms of Hirschsprung's disease (44). These results serve to show that ET-1 is the main ligand of the ET_A receptor (which binds it with greater affinity than for ET-3), whereas ET-3 is the main endogenous ligand of the ET_B receptor (this subtype binds all isopeptides with similar affinity). The discussion in this review will address the potential involvement of endothelin in hypertension, disorder in which the participation of endothelin has been subject of considerable controversy (39, 65). Only recently has new evidence (reviewed in 49, 51) provided a basis for understanding the possible contribution of ET-1 to the pathophysiology of elevated blood pressure.

PHYSIOLOGY OF THE CARDIOVASCULAR ENDOTHELIN SYSTEM

Endothelial cells in blood vessels produce endothelin, mainly ET-1, in response to pressure, low shear stress (high shear inhibits ET-1 production), angiotensin II, vasopressin, catecholamines. Endothelin-1 is mainly secreted abluminally and acts in a paracrine or autocrine fashion on cells (endothelial or smooth muscle) in its immediate vicinity. Immunoreactive endothelin in the circulation is the result of spillover from the vascular wall or is secreted by the pituitary. Endothelin-1 secreted toward underlying smooth muscle cells acts on ET_A and ET_B receptors to induce contraction, proliferation and cell hypertrophy (38). Endothelin-1 may also act on endothelial ET_B receptors, inducing release of nitric oxide and prostacyclin, which explains that it is also a vasorelaxant. It is unknown whether the vasoconstrictor or the vasorelaxant action of endothelins is their most important physiological function, although this probably varies according to the vascular bed examined. In some vascular beds such as the coronary circulation, the virtual absence of endothelial ET_B receptors

has the result that endothelins act mainly as coronary vasoconstrictor agents. In other vascular beds it is possible that ET-1 acts on smooth muscle cells as a paracrine constrictor and growth promotor only when it is overexpressed in endothelial cells under pathological conditions, and that normally it is functioning predominantly as an autocrine or paracrine vasorelaxant through its effect on endothelial ET_B receptors, and nitric oxide or prostacyclin release.

In the heart, ET-1 is produced by various cell types, including endothelial cells, smooth muscle cells, fibroblasts and cardiomyocytes (5, 16, 23, 24, 68). In these cells, upregulation of the ET-1 gene may occur in response to angiotensin II (16, 24), wall stretch (68), and ischemia (23). The ET_A subtype is the predominant receptor present in cultured neonatal (63) and adult cardiomyocytes (15). A mixed population of ET_A and ET_B receptors is found in cultured cardiac fibroblasts (15, 28). Endothelins have chronotropic and inotropic effects on cardiac muscle. Following receptor activation, endothelins influence not only contraction of cardiomyocytes but may also stimulate expression of fetal genes, protein synthesis and growth (2, 16, 56, 68).

PATHOPHYSIOLOGY OF THE CARDIOVASCULAR ENDOTHELIN SYSTEM IN EXPERIMENTAL HYPERTENSION

The endothelin system is activated in salt-dependent models of hypertension, such as the DOCA-salt hypertensive rat and the DOCA-salt-treated spontaneously hypertensive rat (SHR). These models overexpress ET-1 in the vascular endothelium (9, 53), and ET-1 secretion is enhanced in cultured endothelial cells from DOCA-salt hypertensive rats (61). These models respond to endothelin antagonism with blood pressure lowering (34, 54). In Dahl salt-sensitive rats acute (12) or chronic administration of endothelin antagonists (7) resulted also in blood pressure lowering. In contrast, the endothelin system appeared not to be activated in SHR (35). Vascular hypertrophy may have an endothelin-dependent component in some hypertensive models, as demonstrated by greater regression of small artery hypertrophic remodeling than lowering of blood pressure after chronic administration of endothelin antagonists (7, 34, 52). Endothelin effects in the kidney of these hypertensive rats may contribute to water and sodium retention and renal vasoconstriction and hypertension in this model, and are mediated by ET_A receptors (18). Transgenic rats overexpressing human ET-2 have increased circulating ET-2 (21). Underlining the paracrine role of endothelins, blood pressure did not rise in spite of increased circulating endothelin. However, a recent study demonstrated that hepatic overexpression of human preproET-1 in rats resulted in some elevation of blood pressure, which was reduced by the ET_A antagonist FR139317 (42). In stroke-prone SHR (4, 57), in rats infused with angiotensin II (which is a known stimulant of ET-1 expression) (8, 40, 45) chronic administration of endothelin antagonists lowered blood pressure and reduced cardiac or small artery hypertrophic remodeling. In contrast, a genetic malignant model of hypertension did not show evidence of endothelin activation (67). In 2-kidney 1 clip Goldblatt hypertension (high renin and angiotensin II model resembling true renovascular hypertension in humans) the endothelin system is not activated (59), and blood pressure does not decrease under endothelin antagonism (33). In 1-kidney 1 clip Goldblatt hypertension (low renin model) there was some activation of vascular ET-1 gene expression (59), but endothelin antagonists failed to lower blood pressure (33), suggesting that an important degree of vascular overexpression of ET-1 is necessary for the system to play a demonstrable role. In many of these models responding to endothelin antagonism, plasma levels of endothelin are normal, even when significant increase in ET-1 production by endothelium is present, which underlines the abluminal character of endothelin secretion from vascular endothelium, and its paracrine effects. Thus the endothelin system seems to be activated

more often in low-renin, salt-sensitive, and severe forms of hypertension. Among other hypertensive models, cyclosporine-induced hypertension may exhibit an endothelin-dependent component, and bosentan, the ET_A/ET_B endothelin antagonist, lowers blood pressure in this model in rats and primates (1). Endothelin may also play a role in the fructose-fed hypertensive rat, a hypertensive model with hyperinsulinemia and insulin resistance in which chronic endothelin blockade with bosentan reduces blood pressure (66).

In the salt-sensitive, severe or exogenous angiotensin II-infused models of hypertension in which ET-1 expression is activated to some degree, there is often severe vascular hypertrophy, particularly of small arteries. This is in contrast to the hypertensive models in which ET-1 gene expression is not enhanced, such as SHR (53), 2-kidney 1 clip Goldblatt rats (59) and nitric oxide-deficient, L-NAME induced-hypertension (41, 60). When overexpression of ET-1 occurs, such as in the DOCA-salt hypertensive rat, endothelin antagonists reduce blood pressure and hypertrophic remodeling. As well, antihypertensive treatment with calcium channel antagonists may reduce tissue overexpression and regress vascular hypertrophy (36). In some models such as the DOCA-salt-treated SHR, a model of malignant hypertension, overexpression of endothelin in arteries and glomeruli (10) could play a role in fibrinoid necrosis and in renal failure occurring in malignant nephroangiosclerosis (37). In the absence of endothelin tissue overexpression, the endothelin system may play a role in perivascular fibrosis of the heart and in deterioration of renal function, as shown by the response to chronic endothelin antagonist treatment in SHR (26). In some experimental models of hypertension such as L-NAME-treated SHR, overexpression of endothelin may be regional (32, 58). In L-NAME-treated rats, preglomerular sudanophilic lesions have been attributed to nitric oxide synthesis inhibition resulting in enhanced endothelin secretion (3). Using *in situ* hybridization radioautography it has been possible to demonstrate that even in hypertensive models not presenting generalized overexpression of preproET-1 mRNA, such as the 2-kidney 1 clip Goldblatt hypertensive rat, glomerular overexpression occurred in the unclipped kidney but not in the clipped kidney (11), suggesting a role for local intravascular pressure in the triggering of increased production of ET-1. In a similar way, 1-kidney 1 clip hypertensive rats, which exhibit generalized vascular overexpression of the ET-1 gene, did not present enhanced expression of ET-1 in renal arteries or glomeruli of the remaining, clipped, kidney. L-NAME-treated SHR only overexpressed ET-1 in endothelium of aorta and in glomeruli, which suggests that renal overproduction of ET-1 may contribute to the progression of renal failure in these rats. L-NAME-treated SHR and 2-kidney 1 clip hypertensive rats, models without generalized vascular overexpression of ET-1, nevertheless had increased preproET-1 mRNA in the endothelium of coronary arteries (11), like models which do have generalized ET-1 overexpression (31). This finding may point to a role of ET-1 in myocardial ischemia in hypertension. This possibility is supported by the demonstration that only vasoconstrictor (smooth muscle) endothelin receptors are detected in coronary arteries, and no endothelial vasorelaxant receptors can be found (46). Thus, enhanced production without compensatory vasodilatation mediated by endothelial ET_B receptors could result in unopposed endothelin-dependent vasoconstriction of the coronary circulation.

Endothelin-1, together with angiotensin II and other factors, may participate not only in myocardial ischemia in hypertension but also in the development of cardiac hypertrophy. Cardiac endothelin expression increases in animal models of cardiac hypertrophy, and chronic administration of either selective ET_A or mixed antagonists may reduce the development of left ventricular hypertrophy. Catecholamines induce growth of cultured neonatal cardiac myocytes and increased expression of ET-1. Norepinephrine administered for 7 days

increased expression of ET-1 in the heart in rats, mainly in cardiomyocytes and in endothelial cells (25). Bosentan blocked some of the phenotypic manifestations of cardiac hypertrophy, suggesting that ET-1 may play a role in the development of hypertrophy of the heart. In the DOCA-salt hypertensive rat, preproET-1 mRNA overexpression occurs, as shown by *in situ* hybridization, mainly in endothelial cells of blood vessels and areas of endocardium (31). ET_A receptors in cardiomyocytes and fibroblasts are down-regulated and there is decreased responsiveness of intracellular Ca^{2+} to ET-1 (14), which is not found in other models of cardiac hypertrophy without activation of the endothelin system (SHR and aorto-caval shunt) (15, 64). Administration of bosentan (27, 34) or FR1393317 (17) reduced vascular hypertrophy (17, 34) and cardiac hypertrophy in one study (27) but not in another (34). Although SHR do not appear to present endothelin-dependent cardiovascular hypertrophy, treatment of these rats with bosentan reduced perivascular fibrosis of the heart, although cardiac hypertrophy was not significantly affected (26).

The endothelin system is activated in heart failure in rats (6, 62). After coronary artery ligation in rats, infusion of the ET_A antagonist BQ-123 for a short period of time decreased the rate and force of contraction of the heart, indicating an inotropic action of the activated endothelin system in this model (48). A 12-week infusion of BQ-123 in the same rat model reduced mortality by about 50%, and improved cardiac remodeling and function (47). Beneficial effects of bosentan on ventricular performance and vascular resistance have also been observed in a canine model of heart failure (55). Thus, activation of the endothelin system in the heart may have pathophysiological consequences, contributing initially to improve cardiac function, and later to cardiac hypertrophy and heart failure. Part of the latter is the result of endothelin-dependent vasoconstriction and increased afterload, rather than of direct effects of endothelins on the heart. Endothelin receptor antagonism may improve cardiac function in the later periods of cardiac failure, as already demonstrated in humans, as blockade of the activated endothelin system reduces vasoconstriction and afterload (29).

PATHOPHYSIOLOGY OF THE CARDIOVASCULAR ENDOTHELIN SYSTEM IN HUMAN HYPERTENSION

Endothelin plasma levels have been reported to be usually normal in human hypertension, although some severely hypertensive patients may present with elevated endothelin immunoreactivity in plasma (see references cited in ref 49). The acute intravenous administration of TAK-044, a balanced ET_A/ET_B endothelin receptor antagonist, increased forearm blood flow (20) and slightly lowered blood pressure in healthy subjects, which suggests that there may be an endothelin-dependent vascular tone. Normotensive offspring of hypertensive parents showed enhanced plasma endothelin responses to mental stress (43). Thus, a genetically-determined endothelial dysfunction may already be present in early stages. The expression of preproET-1 mRNA in small arteries obtained from gluteal subcutaneous biopsies, and demonstrated using *in situ* hybridization radioautography, was shown to be significantly enhanced in moderate to severe hypertensive patients, whereas in control subjects and untreated mild hypertensive patients expression was lower than in the former (fig 1) (50). Increased ET-1 production by endothelium of small arteries could thus play a role not only in elevated blood pressure but also in accentuating small artery hypertrophic remodeling in patients with moderate to severe hypertension. Certain populations of hypertensive patients, such as African Americans, in whom a severe and salt-sensitive form hypertension is often found, may present activation of the endothelin system (13). Severity of blood pressure elevation and salt-sensitivity appear to be common denominators for activation of the endothelin system in humans and experimental animals. Other forms of hypertension in which endothelins may be

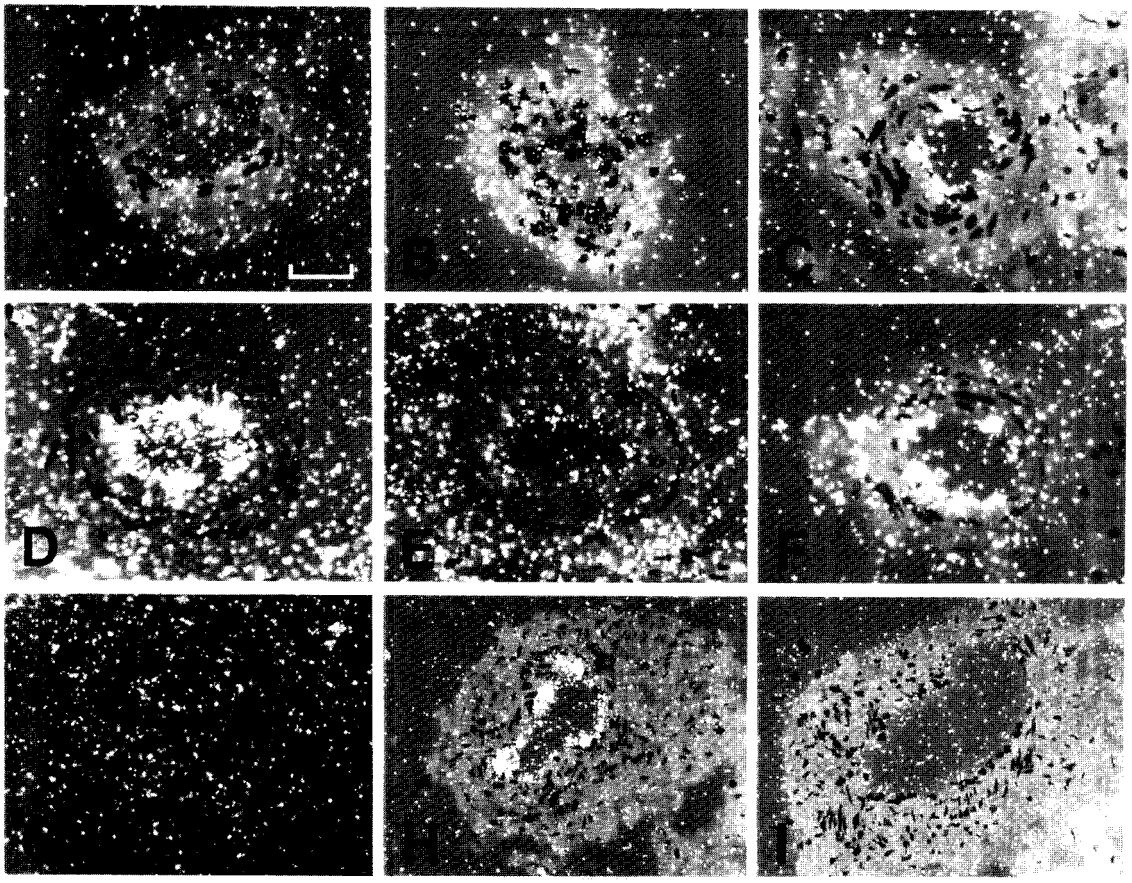

Fig 1. Representative microphotographs of *in situ* hybridization of gluteal subcutaneous fat biopsies from normotensive subjects, and mild and severe hypertensive patients, using human endothelin-1 cRNA probes. Original magnification of microphotographs shown was x100, bar in A corresponds to 30 μm for all panels, except for panels H and I, for which original magnification was x50 and bar in A represents 60 μm. Panels A and B show a small artery from gluteal subcutaneous fat from a normotensive subject (A) and a mild hypertensive patient (B). Small arteries of these subjects exhibit scant labeling when using the antisense endothelin-1 cRNA probe, similar to labeling found with the sense endothelin-1 cRNA probe (not shown). Panels C, D, F, H show small arteries from subcutaneous fat in biopsy material from severe hypertensive patients, hybridized with the endothelin-1 antisense probe, demonstrating increased density of labeling of the endothelium by specific grains, not found with the sense endothelin-1 cRNA probe on adjacent sections of the same vessels (panels E, G and I). (From ref 50, with permission of the publisher).

involved in humans include rare cases of hemangioendothelioma producing endothelin, chronic renal failure, erythropoietin and cyclosporine-induced hypertension, pheochromocytoma and pregnancy-induced hypertension (reviewed in ref 49).

CONCLUSION

The definitive place of endothelins in the pathophysiology of hypertension is still unclear. Figure 2 summarizes our current view of the potential implication of ET-1 in blood pressure elevation and vascular hypertrophy in moderate to severe hypertension, and probably in particular in salt-sensitive forms. As the physiological and pathophysiological implications of endothelin in the cardiovascular system have become clearer, it has been recognized that involvement of endothelin is not primary, but secondary to altered cardiovascular pathophysiology. With worsening endothelial damage, expression of endothelin is accentuated. Stretch and pressure may activate the endothelin system in the heart. By contributing to thicken the walls of blood vessels and reducing wall stress, and through inotropic effects on the heart, endothelin activation may be initially

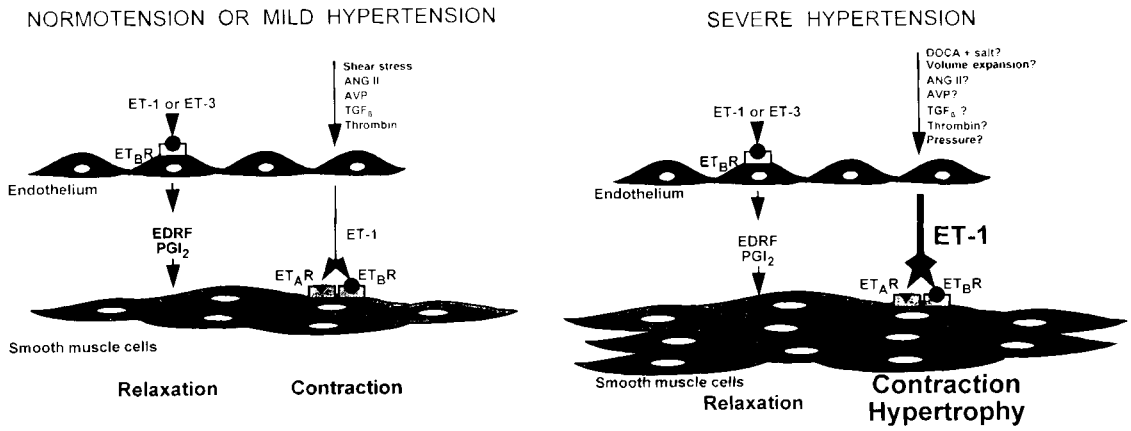

Fig 2. Endothelin-1 may play either a vasorelaxant role or a vasoconstrictor role in different vascular beds in normotension and in mild hypertension (left hand side of figure). In moderate to severe hypertension, enhanced expression of endothelin-1 produces a predominant vasoconstrictor effect associated with enhanced growth, resulting in a contribution to elevated blood pressure. Growth of the vascular wall is accentuated, and contributes both to further elevate blood pressure and to complications of hypertension.

beneficial. However, as the process progresses, endothelin may start to exert pathophysiologically significant deleterious effects on the cardiovascular system. Endothelin antagonists may prove useful at this point. Moderate to severe hypertension, particularly in certain subsets of patients such as salt-sensitive hypertensive patients or African-Americans, prevention of progression of nephroangiosclerosis and renal failure in hypertension, protection from ischemic heart disease and stroke, are some conditions in which there may be a role for endothelin antagonism in human cardiovascular therapeutics. To date, limited preliminary clinical studies have been performed, mostly in heart failure, and one in hypertension. As these clinical trials develop with the increasing number of anti-endothelin agents, either balanced ET_A/ET_B antagonists, selective ET_A antagonists or endothelin converting enzyme inhibitors, we will not only learn more about the therapeutic utility of these agents, but also about the pathophysiological implication of the endothelin system in human disease, and its role in the short and long-term regulation of cardiovascular function.

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