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REVIEW

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Potential role of endothelin-1 and endothelin antagonists in cardiovascular diseases

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Abstract The endothelins comprise a family of three isopeptides ET-1, ET-2 and ET-3, whereby ET-1 appears to be the most relevant in humans. They act in a paracrine manner on ET_A and ET_B receptors. ET-1 plays an important role in the cardiovascular system. In addition, it modulates vasomotion and growth processes, and it participates in thrombogenesis and neutrophil adhesion. This review summarizes some of the current literature pertaining to the physiological and pathophysiological significance of ET-1, focusing the assets and drawbacks of elevated ET-1 levels. In this regard, modulation of the endothelin system by either receptor blockade or by inhibition of endothelin converting enzyme is expected to provide novel therapeutic drug strategies.

Key words Endothelin – cardiovascular system – receptor – pathophysiology – physiology

Introduction

Endothelin-1 (ET-1) is an ubiquitous endothelium-derived peptide with a long-lasting and profound vasoconstrictive activity. It is a potent antagonist of another endothelium derived compound: nitric oxide. Although an abundance of papers on the endothelin family have been published from 1988 to date (about 10500), ET-1 is still an intensively investigated peptide which plays an important role in the physiology and pathophysiology of several organs. This review displays some of the manifold facts of this fascinating compound with respect to its effects on the heart and the circulatory system (see Fig. 1). In addition, some potential therapeutic options are explored.

Synthesis and regulation

Some ten years after its first description (85), three structurally and pharmacologically distinct endothelin (ET) isopeptides are known to exist: ET-1, ET-2 and ET-3, each produced by a different gene. ET-1 appears to be the most relevant in humans (50). A pre-propeptide is cleaved to form the big ET-1 (39 amino acids), which is further cleaved by the endothelin-converting enzyme (ECE) to form the 21-amino-acid active peptide (68). ET-1 is the only vasoconstrictor known to be primarily produced by endothelial cells in humans, where it can be stored in secretory vesicles (70). Furthermore, ET-1 is present in cardiomyocytes of both normal and failing hearts (15). Most ET-1 is released abluminally; hence, while the

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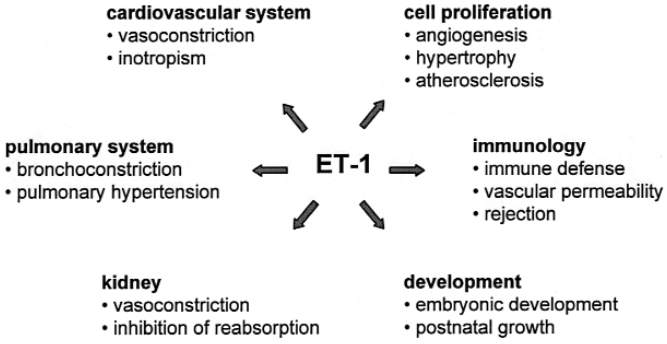

Fig. 1 Physiological and pathophysiological significance of ET-1.

circulating levels are in the pM range, the effective ET-1 concentration on the effector site is unknown.

Cloning of endothelin receptor cDNA has revealed two subtypes: ET_A receptors, found mainly on vascular smooth muscle cells and cardiomyocytes (23), and ET_B receptors, located predominantly on endothelial cells and vascular smooth muscle cells (58). In addition, an ET_C receptor has been cloned (31), although its existence and function remains controversial.

Based on their susceptibility to the ET_A antagonist BQ123, ET_A receptors can be further subclassified as ET_{A1} and ET_{A2} receptors (9). ET_B receptors can also be subclassified: ET_{B1} receptors mainly mediate vasorelaxation through the release of nitric oxide, while ET_{B2} receptors mediate vasoconstriction (9). Furthermore, ET_B receptors mediate the clearance of ET-1 and its subsequent intracellular degrading (16).

An increasing number of agents have been found to contribute to the regulation of the entire ET system. For example, the induction of ET-1 mRNA and the rate of peptide release is stimulated by thrombin, transforming growth factor, angiotensin II, vasopressin, interleukin-1, hypoxia and intravascular shear stress (38, 68). ET receptor density can also vary, e.g., incubation with ET-1 downregulated ET receptors (47). In contrast, ischemia and heart failure upregulated ET receptors (30, 64). Even the expression of ECE mRNA changes under certain circumstances, e.g., it is increased in atrial tissue of patients after myocardial infarction (5).

Physiological significance

The biological activities of ET-1 are manifold, involving such diverse areas as embryonic development, normal postnatal growth and cardiovascular homeostasis (24, 41). In particular, ET-1 plays an important role in the cardiovascular system. For example, it modulates vasomotion and growth processes, participates in thrombogenesis and neutrophil adhesion, and it plays an autocrine-paracrine role in the endothelial vasoactive system (18). Moreover, ET-1 exerts inotropic effects, influences vascular permeability (11), and appears to modify nociception (65).

Vascular system

Vasomotion is affected by ET_A and ET_B receptors located on endothelial and smooth muscle cells (44). ET-1 can activate ET_{B1} receptors on the endothelium that release factors, such as nitric oxide and prostacyclin, which then lead to an initial relaxation mediated by increased cGMP and cAMP levels in smooth muscle cells with subsequent inhibition of L-type Ca^{2+} channels (1). In addition, ET-1 acts in a paracrine manner on ET_A and ET_{B2} receptors located on vascular smooth muscle cells, eliciting a longlasting vasoconstriction mediated by increased intracellular calcium (46) (see Fig. 2). Under physiological conditions, endogenous generation of ET-1 appears to contribute to the maintenance of basal vascular tone and blood pressure through activation of ET_A receptors on vascular smooth muscle (74).

Heart

The influence of ET-1 on cardiac function and its mode of action is still controversial. The cellular basis for the actions of ET-1 is highly complex; however, alteration in Ca^{2+} home-

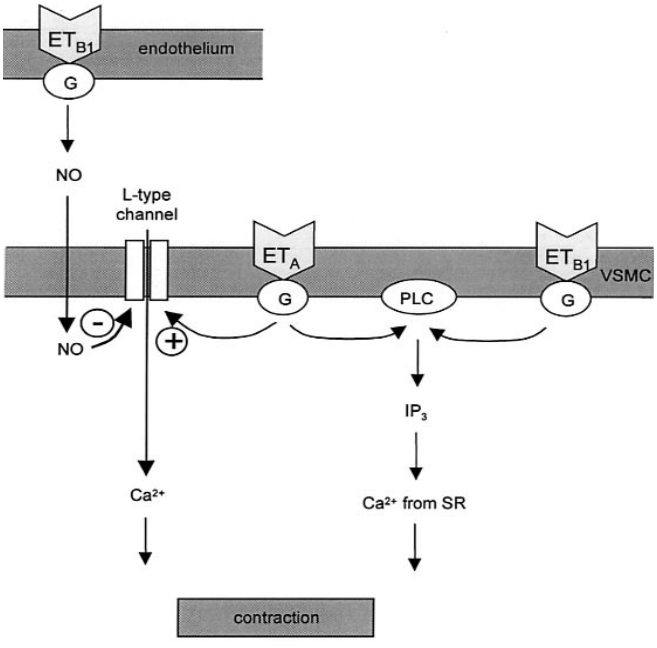

Fig. 2 Schematic of pathways activated by endothelin-receptor subtypes in the vascular system. *G* G-protein, *PLC* phospholipase C, IP_3 inositol 1,4,5-triphosphate, *SR* sarcoplasmatic reticulum

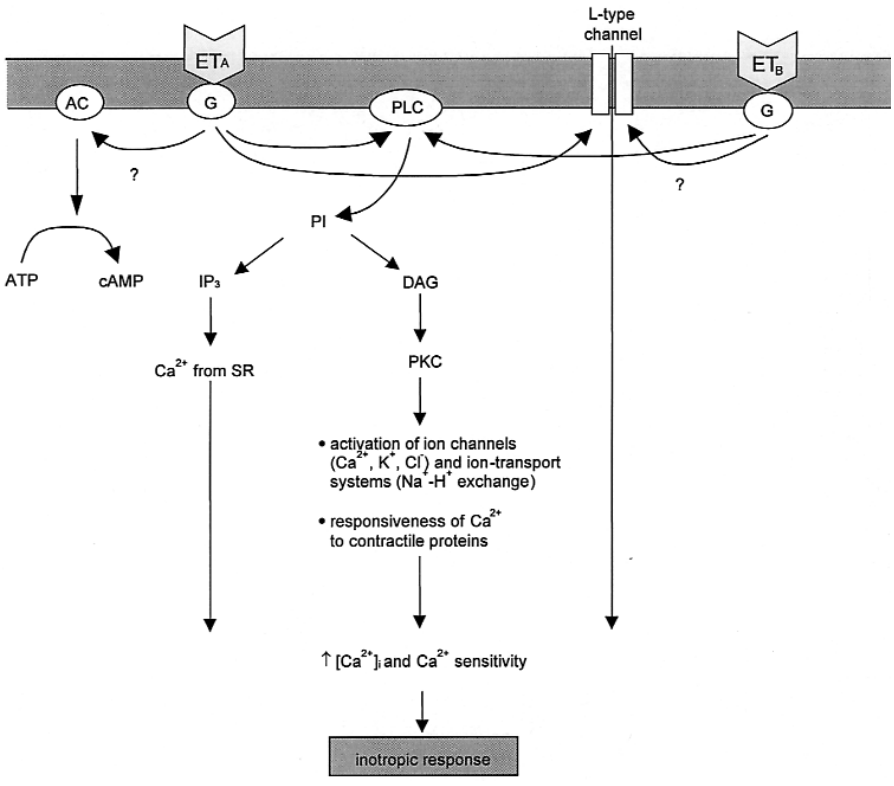

Fig. 3 Schematic of pathways activated by endothelin-receptor subtypes in cardiomyocytes. *AC* adenylyl cyclase, *G* G-protein, *PLC* phospholipase C, *PI* phosphatidylinositol, IP_3 inositol 1,4,5-triphosphate, *DAG* diacylglycerol, *PKC* proteinkinase C

ostasis appears to be central to the cardiac actions of this peptide (see Fig. 3).

Both ET receptor subtypes are linked via Gα-proteins to two effector systems: phospholipase C and adenylyl cyclase (48). In cardiomyocytes, ET-1 appears to stimulate predominately the phospholipase C pathway via the ET_A receptor (81). Stimulation of the ET receptor results in a phosphatidylinositol (PI) hydrolysis which leads to production of inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+} from internal stores. This increase in the intracellular free Ca^{2+} concentration together with elevated DAG levels activates proteinkinase C (PKC), a key enzyme in regulation of cellular function, which includes the operation of various types of ion channels (Ca^{2+}, K^+, Cl^-) and ion-transport systems that include the Na^+-H^+ exchange (79). The latter results in a rise in pH_i and sensitization of cardiac myofilaments to intracellular Ca^{2+} (39). Both mechanisms are expected to induce a positive inotropic effect.

Furthermore, ET receptors regulate adenylyl cyclase activity (AC). Regarding the manner of this regulation, conflicting results exist depending on the cell type examined. ET-1 is reported to inhibit the accumulation of cyclic AMP (cAMP) via the ET_A receptor in guinea pig ventricular myocytes (29, 62). However, in other cell systems, ET-1 can stimulate cAMP generation. ET-1 stimulates cAMP formation via the ET_A receptor and inhibits it via the ET_B receptor in vascular smooth cells (8). Interestingly, ET-1 increases cAMP levels in neonatal rat ventricular cardiomyocytes that solely express the ET_A receptor subtype alone (66).

Subsequent studies have yielded conflicting results concerning the ET-1 action on L-type Ca^{2+} current (I_{CaL}). ET-1 has been reported to increase basal L-type Ca^{2+} current via the ET_A receptor in cardiac cells. The stimulation of the Ca^{2+} current appears to be mediated by a sensitive G-protein (4, 42). Otherwise, ET receptor stimulation causes in guinea pig ventricular myocytes an increase in I_{CaL} via PKC activation (83). ET-1 consistently decreases the isoprenaline-enhanced I_{CaL} and PKA-dependent Cl^- current in a pertussis toxin sensitive manner (29, 62). In contrast, ET-1 exerts a dual effect on the I_{CaL} in rabbit ventricular myocytes (34). At 1 nM, ET-1 increases the I_{CaL} whereas at a higher concentration, ET-1 decreases the I_{CaL}. The same authors find in a later study that ET_A receptor mediates the decrease and ET_B receptor the increase in I_{CaL} (35).

ET-1 activates via its receptor subtypes at least two distinct intracellular pathways in cardiomyocytes. The ET_A receptor appears to predominate in mediating the positive inotropic response of ET-1 (45). However, the ET_B receptor was also shown to mediate a positive inotropic effect (3).

Nevertheless, ET-1 induces extremely controversial responses depending on the experimental setting: ET-1 exerts in more reductionistic experimental models like cardiomy-

ocytes and papillary muscles a clear-cut positive inotropic effect (10). Negative inotropic effects predominate in isolated hearts and on in situ hearts (12, 36) although positive inotropic effects have been observed (75). At least two different explanations exist for these controversial findings. The negative inotropic reactions of ET-1 in the more holistic models are most likely owing to the ET-induced reduction in oxygen supply that masks the positive inotropic effect. It should be mentioned that ET-1 levels in the experimental setting are relatively high compared with physiological conditions, where the lower levels exclusively exert positive inotropic effects. On the other hand, ET-1 decreases cAMP levels in ventricular myocytes (29, 62), an effect more classically associated with a negative inotropic response. However, it is imaginable that ET-1 protects in this way the heart from the consequences of a sympathetic stimulation, e.g., by preventing potentially arrhythmogenic shortening of the action potential.

Pathophysiological significance

Upon observing an overactivity of the ET system, the central question arises: what makes an elevated plasma ET-1 level a critical indicator for numerous diseases? Some answers to this question are given below.

CAD/restenosis

The vessel wall is a major target of ET-1. In addition to its impressive, long-lasting modulation of vascular tone, ET-1 induces proliferation of vascular smooth muscle and the expression of adhesion molecules (21). Furthermore, ET-1 enhances microvascular permeability and albumin extravasation. These effects seem to be mediated through the release of secondary mediators like thromboxane A_2 and platelet-activating factor via stimulation of the ET_A receptor (11). Thus, ET-1 appears to play an important role in vascular diseases like atherosclerosis and in restenosis after percunateous transluminal angioplasty (PTCA) (27). In atherosclerosis, ET-1 can lead to vasospasm and to the progression of atherosclerosis in coronary plaque tissue (86, 87), and it can promote tissue inflammation in ischemic myocardium. The inflammatory response, once established, may further impair oxygenation through edema and coagulation disturbances leading to myocardial reperfusion injury.

Several processes are involved in the development of pathological restenosis after PTCA, like adhesion of monocytes, invasion of macrophages, and changes of the phenotype of the medial smooth muscle cells which lead finally to neointimal hyperplasie (2). ET-1 seems to be involved in several steps of this development, with the ET-1 receptor density being upregulated in coronary arteries after PTCA (37).

Myocardial infarction/heart failure

Although the underlying mechanisms are still unclear, ET-1 is involved in a variety of both acute and chronic cardiovascular diseases. For example, ECE-1 and plasma ET-1 levels are elevated in patients who suffered from myocardial infarction (5), and ET-1 levels at 72 h post myocardial infarction accurately predict long-term survival. Similar to patients with acute infarction, ET-1 levels in CHF patients are elevated and correlate with the long-term outcome. As a consequence, the big ET-1 plasma levels were superior in predicting the 1-year mortality over such well-established predictors as plasma atrial natriuretic peptide, norepinephrine, NYHA class, age, and echocardiographic left ventricular parameters (51). Likewise, plasma ET-1 levels correlate closely with functional classes of heart failure and thus become strong and independent predictors of mortality in both acute and chronic heart failure. The predictive power of plasma ET-1 is insofar unexpected as circulating ET-1 is degraded by about 80 % after a single passage through the lung. Thus, an elevated ET-1 level at 72 h post infarctum speaks for a long-lasting ET-1 release.

In an experimental model of chronic heart failure in rats, the expression of preproendothelin-1 (ppET-1) mRNA was markedly increased in ischemic myocardium, and ET-1-like immunoreactivity was localized in cardiomyocytes, vascular endothelial cells, macrophages, and proliferating fibroblasts (61). Several mechanisms may explain the increased ppET-1 mRNA expression in the ischemic myocardium: 1) hypoxia induces ppET-1 mRNA expression in endothelial cells and in cardiomyocytes (25, 28); 2) hemodynamic overload results in mechanical stress which increases ppET-1 mRNA levels (84); and 3) neurohumoral mediators are activated after myocardial infarction, e.g., the renin-angiotensin system and the sympathetic catecholamine system, either of which can trigger ET-1 release (19).

Given the complex ET-1 actions, it is not easy to decide whether increased ET-1 levels are beneficial or deleterious. Some actions suggest that ET-1 is involved in the healing process after myocardial infarction, since it exhibits mitogenic properties (48, 76), releases proinflammatory cytokines (69), and stimulates proliferation of cardiac fibroblasts and vascular smooth muscle cells (14, 57). In particular, the ET_A receptor seems to be responsible for the scar formation because the early use of ET_A antagonists (LU127043, LU135252, EMD94246) shows detrimental effects on cardiac function (26, 56). Unlike other peptides, which act rapidly upon demand and then vanish, the ET-1 system is probably not intended to act as an emergency agent, because maximum upregulation of ppET-1 mRNA requires up to 7 days after acute myocardial infarction (61).

Besides these beneficial effects, ET-1 seems to be an important tool in managing the functional consequences of myocardial infarction. In support of this notion, ET-1 levels

correlate with increased contractility in nonischemic myocardial areas in a canine model of coronary occlusion (33). In parallel, intravenous infusion of an ET_A receptor antagonist (BQ123) significantly reduced both heart rate and contractile state in CHF rats (73). In another study, an ET receptor antagonist (bosentan) reduced arterial pressure and total peripheral resistance, indicating that ET-1 participates in the maintenance of cardiovascular function in chronic myocardial infarction (59).

In contrast, other animal experiments provide evidence for detrimental ET actions. The release of ET-1 seems to correlate with the infarct size (33). Similarly, administration of an ET receptor antagonist (PD145065) reduced infarct size and ventricular arrhythmias in anesthetized rabbits (80). In pacing-induced CHF in pigs, ET_A receptor activation appears to contribute to the development and progression of left ventricular dysfunction (71). Moreover, in rats with myocardial infarction, a combined ET_A/ET_B receptor antagonist (bosentan) partially prevented cardiac remodeling and improved hemodynamics (13, 54, 78). Long-term administration of ET_A receptor antagonists (BQ123, LU135,252) also improved survival of rats with chronic heart failure (53, 72). It is worth mentioning that the effects of ET receptor antagonists as such on dysfunctional human hearts remain to be elucidated.

How can the discrepant findings be interpreted? One could speculate that a low ET-1 level could ameliorate cardiac dysfunction in a number of ways. It is a fact, however, that unphysiologically high ET-1 levels are clearly not desirable: 1) ET-1 induces a hypertrophic gene program (myocardial ANP, BNP, β-MHC, and skeletal α-actin mRNAs) during chronic heart failure (60), thus, contributing to excessive hypertrophy. 2) ET-1 contributes to the progression of chronic heart failure via long-term stimulation of myocardial contractility. 3) ET-1 drastically increases the peripheral vascular tone leading to increased preload and afterload and a subsequent increase in myocardial wall stress (60). 4) ET-1 drastically increases cardiac vascular tone and further reduces oxygen supply to the ischemic myocardium.

With this in mind, ET-1 seems to be a "witches brew", in as much as it exerts a variety of detrimental effects during ischemic heart failure. On the other hand, ET-1 is involved in the healing program. From a teleological point of view, this discrepancy is unsatisfying, but maybe we have to accept the possibility that ET-1 is a double edged sword.

Hypertension

In the early stage of ET-1 research, the impressive vasoconstriction followed by the administration of ET-1 led to the concept that ET-1 is important in the maintenance of physiological vascular tone and in hypertension. The results of many studies quenched the initial enthusiasm. For example, the blockade of ET_A receptors had no effect on forearm blood flow in healthy men (6). In other studies investigating ET receptor antagonists, hypotensive activity was observed in DOCA-salt hypertensive rats, in rats under nitric oxide blockade, and in renal hypertensive dogs, but not in spontaneously hypertensive rats (52). On the other hand, a combined ET_A/ET_B antagonist (bosentan) reduced blood pressure in patients with essential hypertension (40).

These results suggest that the role of ET-1 in the maintenance of basal vascular tone and blood pressure is still unclear. Nonetheless, the deleterious prolonged vascular effects of endogenous ET-1 could play a major role in pathophysiological situations in which formation of nitric oxide is impaired. It also appears likely that ET-1 participates in the adverse cardiac and vascular remodeling of hypertension, as well as in hypertensive end-organ damage (22).

Potential therapeutic strategies

Relatively few clinical studies have examined the effect of ET receptor antagonists in cardiovascular diseases in humans (Table 1), although the experimental findings strongly suggest that modulating the endothelin system should become a fruitful field for pharmacological interventions. To date, antagonists of ET_A and ET_B receptors and inhibitors of the endothelin converting enzyme are available as pharmacological tools. On the other hand, transgenic animal models are available to investigate additional avenues for therapeutic interventions, including ET-1 and ET_A and ET_B knockout mice, and rats in which either ET-1 and ET-2 or ET receptors can be overexpressed (63).

Cardiovascular diseases

The predominantly beneficial effect of blocking ET-receptors in a variety of cardiovascular diseases seems to result from the reduction of vascular resistance without increasing heart rate or neurohumoral activation, as demonstrated by the following examples (Table 1).

Bosentan increased coronary artery diameter in patients with coronary artery disease, particularly in vessels with no or mild angiographic changes. Because coronary flow velocity was unaffected (82), coronary blood flow and thus oxygen supply must have increased. In CHF patients already receiving treatment with an ACE inhibitor, infusion of a combined ECE and neutral endopeptidase inhibitor (phosphoramidon) and an ET_A receptor antagonist (BQ123) increased forearm blood flow (43). In another study, patients with symptomatic heart failure (NYHA III) received oral bosentan in addition to a conventional triple therapy (diuretics, digoxin, ACE inhibitors). After two weeks, cardiac output had increased and systemic

Table 1 Actions of ET antagonists in patients with various diseases

disease	ET-antagonist	type	Measurement / changes	author
essential hypertension	BQ123 i.a. BQ788 i.a.	A B	BQ123: + 33 % FBF (controls: no significant modification) BQ123 + BQ788: +63% FBF (controls: no significant modification)	(6)
essential hypertension	bosentan	A/B	↓ diastolic pressure, ⟷ HR, no activation of sympathetic nervous system or RAS	(40)
stable coronary artery disease	bosentan	A/B	bosentan: ↓ systolic blood pressure + ↑ HR + ↓ coronary diameter	(82)
CHF treated with ACE inhibitors	phosphoramidon (combined ECE and neutral endopeptidase inhibitor) BQ123	 A	phosphoramidon: + 52 % FBF BQ123: + 31 % FBF	(43)
NYHA III (conventional triple therapy)	bosentan	A/B	↓ MAP, ↓ pulm. artery mean, ↓ capillary wedge pressure, ↓ right atrial pressure, ↑ CO, ⟷ HR, ↓ systemic and vascular resistance ↑ plasma ET-1 level, ⟷ other hormones	(77)
syndrome X	BQ123 intrabrachial	A	+ 20 % FBF	(55)
chronic renal failure	BQ123 phosphoramidon (a combined ECE and neutral endopeptidase inhibitor)	A	BQ123: + 11 % FBF (controls + 44 %) phosphoramidon: + 68 % FBF (controls + 181 %) ET-1 level: – 35 % (controls: – 36 %)	(20)
migraine	bosentan	A/B	no effect on improvement of headache compared with placebo	(49)

FBF forearm blood flow; *HR* heart rate; *RAS* renin-angiotensin system; *MAP* mean arterial pressure; *CO* cardiac output; *CHF* chronic heart failure

and pulmonary vascular resistance had decreased without counter-regulatory neurohumoral activation or reflex increase in heart rate (77).

Hypertension

An ET_A receptor antagonist (BQ123) increased forearm blood flow in hypertensive patients, and combining this with the ET_B receptor antagonist (BQ788) resulted in an even greater vasodilator response in the same patients (6). Likewise, bosentan significantly lowered blood pressure in patients with essential hypertension (40). Both studies suggest that ET-1 contributes to the elevated blood pressure in hypertensive patients and that vasoconstriction is mediated by both ET_A and ET_B receptors.

Limitations

Despite the first promising results, many more experimental and clinical studies need to be completed for several reasons in order to make intervention via the ET system a safe therapy: 1) Immediate application of results of animal experiments to humans could disregard considerable differences in the distribution and density of receptor subtypes. 2) The receptor density might vary greatly between healthy subjects and patients. For example, in chronic renal failure, an ET_A receptor antagonist revealed that the contribution of endogenous ET-1 to resting vascular tone appeared to be reduced (20). Likewise, in patients with syndrome X, ET-1 caused a less pronounced reduction of forearm blood flow than in healthy subjects, suggesting an ET_A receptor downregulation (55). 3) ET-1 plasma concentrations in healthy subjects are in the pM range, while in the experimental setting, concentrations in the nM range are frequently investigated (11, 17, 32). Hence, these results might be misleading if one remembers the notion dosis facit venenum. 4) The strategy on what to antagonize is unclear. For example, blocking only ET_A receptors could well be superior to simultaneously blocking both ET_A and ET_B receptors, assuming that ET_B receptors are responsible for ET-1 clearance (16). 5) With respect to bosentan, the long-term impact of modulating the endothelin system on other organs like the

liver (67) strongly deserves further elucidation. 6) As long as the authentic effects of modulating the endothelin system are not entirely settled, any therapy in combination with other compounds seems precarious. For example, even if a combined ET-receptor/ACE inhibition might prove beneficial 7) it should be initiated at a later date.

Conclusion

The physiological significance of the ET system is not yet fully established and further effects are still coming to light. On the other hand, interactions with other compounds have been largely ignored. This can be said in particular of nitric oxide, a compound with almost mirror-like activities. The manifold and controversial effects of the ET system make research on it fascinating yet challenging at the same time. The pathophysiological significance of the endothelin system seems even more uncertain. Thus, despite their anticipated beneficial effects, any application of agents modulating the endothelin system will require further research before their routine clinical usage can be envisioned.

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