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Endothelium-derived endothelin-1

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Abstract

One year after the revelation by Dr. Furchgott in 1980 that the endothelium was obligatory for acetylcholine to relax isolated arteries, it was clearly shown that the endothelium could also promote contraction. In 1988, Dr. Yanagisawa's group identified endothelin-1 (ET-1) as the first endothelium-derived contracting factor. The circulating levels of this short (21-amino acid) peptide were quickly determined in humans, and it was reported that, in most cardiovascular diseases, circulating levels of ET-1 were increased, and ET-1 was then tagged as "a bad guy." The discovery of two receptor subtypes in 1990, ET_A and ET_B, permitted optimization of the first dual ET-1 receptor antagonist in 1993 by Dr. Clozel's team, who entered clinical development with bosentan, which was offered to patients with pulmonary arterial hypertension in 2001. The revelation of Dr. Furchgott opened a Pandora's box with ET-1 as one of the actors. In this brief review, we will discuss the physiological and pathophysiological role of endothelium-derived ET-1 focusing on the regulation of the vascular tone, and as much as possible in humans. The coronary bed will be used as a running example in this review because it is the most susceptible to endothelial dysfunction, but references to the cerebral and renal circulation will also be made. Many of the cardiovascular complications associated with aging and cardiovascular risk factors are initially attributable, at least in part, to endothelial dysfunction, particularly dysregulation of the vascular function associated with an imbalance in the close interdependence of nitric oxide and ET-1.

Keywords

Vascular tone; Inflammation; Cardiovascular diseases

Introduction

Dr. Furchgott rediscovered an extraordinary organ, the endothelium, that protects the arterial wall through the release of nitric oxide (NO) [1, 2] among other factors. Before 1980, the endothelium was merely considered as an anticoagulant sheet of cellophane. The presence of an endothelium-derived constricting factor (EDCF) was perceived 1 year after the revelation of the endothelium-dependent relaxant properties of the endothelium [3, 4]. But, it was 8 years later that Yanagisawa and colleagues identified an EDCF, endothelin-1 (ET-1) [5, 6].

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Two receptors for ET-1 were identified 2 years later [7, 8]. Then, shortly after the discovery of ET_A and ET_B receptors, Martine Clozel's team presented in 1993 the first orally active endothelin-1 receptor antagonist, Ro 46-2005 [9], and the same team made a structurally modified analog, bosentan (Tracleer), available to patients with pulmonary arterial hypertension at the end of 2001. In less than 15 years, a new factor was identified, its receptors were cloned, and their pharmacology was characterized, a pathology associated with the abnormal function of the ET-1 system and an effective treatment offered to patients in need. It all started from the "so-obvious-today" rediscovery of the endothelial cells by Dr. Furchgott.

In this short review, we will focus on ET-1 and the endothelium and thus, review the role of ET-1 on the vasculature with as much as possible references to the human pathophysiology.

Physiological effects of ET-1 in arteries

Endothelin-1 is one of the most potent endogenous vasoconstrictor identified so far [6]. This peptide induces a long-lasting contraction of isolated porcine coronary arteries with a half maximum effective concentration (EC₅₀) of 0.4± 0.2 nM, which is at least one order of magnitude lower than values reported for other contracting peptides such as angiotensin II, with the exception of urotensin II [10]. In contrast to urotensin II, however, ET-1 induces contraction of almost all arteries and veins tested. There are two additional isoforms of endothelins, ET-2 and ET-3. Among these, ET-1 is the prominent isoform synthesized by the vasculature [6].

ET-1 is released continuously, mostly from endothelial cells (EC), by a constitutive and regulated pathway [11] and contributes to the maintenance of vascular tone [12–15]. NO strongly inhibits the release of ET-1 from the native endothelium [16, 17], which makes ET-1 and NO functionally closely interdependent, with a strong inhibitory effect of ET-1 on NO-mediated dilation, including in human coronary and cerebral arteries, and vice versa [14, 18–21]. The inhibitory effects of ET-1 are not limited to the dilations induced by NO since ET-1, through activation of the protein kinase C pathway, also reduces β-adrenergic receptor-dependent relaxation both in vitro and in vivo [22, 23]. This peptide is therefore a potent "anti-vasodilatory" factor released by the endothelium.

In addition to its genesis by EC, ET-1 is produced by vascular smooth muscle cells (VSMC), cardiomyocytes, leukocytes, macrophages, various neurons, and other cells [24]. ET-1 is not only a potent vasoconstrictor, but at elevated concentrations—pharmacological and pathological—it is also proinflammatory and promotes VSMC proliferation [25–30]. These latter properties of ET-1 are important in pulmonary arterial hypertension and probably other vascular diseases such as atherosclerosis and venous graft occlusion as we will see later. In the physiological environment, ET-1 certainly contributes to cardiovascular homeostasis through several pathways that impact on the regulation of basal vascular tone [12, 24] (Fig. 1).

The production of ET-1 is regulated at the gene level [31]: ET-1 messenger RNA (mRNA) is upregulated by inflammatory factors such as transforming growth factor beta, tumor necrosis factor alpha, interleukins, insulin, and angiotensin II, and downregulated by NO, PGI₂, hypoxia, and shear stress [12, 24]. It is synthesized as a large protein, the pre-proET-1 or Big ET-1 that is cleaved first to pro-ET-1 and second to ET-1 by ET-converting enzymes (ECE-1 and ECE-2), but additional peptidases can generate ET-1 as demonstrated by experiments in ECE1/2 knockout mice [32]. The biological effects of ET-1 are mediated through activation of the two known ET-1 receptors, ET_A and ET_B [7, 8]. Although both receptors are important for normal function, the debate in the literature is whether or not both receptors should be blocked to provide most clinical benefit [33, 34]. The ET_A has

been classified as the bad one, while the ET_B is considered as the good one; this distinction is based on the role of ET_B in the clearance of circulating ET-1 and on the observation that activation of endothelial ET_B induces dilatation by stimulating the release of endothelial prostacyclin and NO. Inhibition of ET_B increases circulating ET-1 levels and blood pressure in healthy subjects [35] demonstrating that ET_B stimulation is overall vasodilatory. This debate will not be closed unless we learn more on the pathophysiology of ET-1.

Vascular effects of ET-1

In this section, we will mostly, but not exclusively, report data collected in the coronary arteries since excellent reviews have been recently published on the pulmonary vascular bed [36, 37]. The coronary vascular bed is unique because in this vascular territory shear stress, the tangential force per unit area created by flow of blood over the luminal surface of the endothelium is the most unstable. The vascular endothelium-dependent dilation to shear stress permits fine tuning of nutrient delivery to accommodate metabolic demand [38, 39], and the stability of shear stress, rather than its level, contributes to the maintenance of a healthy endothelium [39]. In coronary arteries, blood flow varies throughout the cardiac cycle [40]: blood flows in the coronary arteries during diastole and reverses during systole as the contracting myocardium squeezes the subendocardial coronary arteries. Mechanical stress is nowhere else more pronounced in the circulation. In turn, the coronary circulation is the prime site for endothelial dysfunction. Because of these unique physiological hemodynamic features, coronary arteries display an unusual gene pattern when compared to the aorta: a fivefold lower endothelial NO synthase and a 2.5-fold higher ET-1 mRNA expressions [41]. Based on our current knowledge, this pattern would predispose to endothelial dysfunction and atherosclerosis and is directly in line with the functional interaction existing between NO and ET-1, in favor of ET-1.

Systemic exogenous administration of ET-1 induces a biphasic response, an initial and transient reduction in blood pressure followed by a sustained hypertensive phase. The depressor phase involves endothelial ET_B receptors whereas the hypertensive phase is mediated by ET_A receptors [42]. Similarly, isolated rat hearts perfused with exogenous ET-1 display a biphasic response: a drop in coronary perfusion pressure associated with coronary dilation was observed at low concentrations of ET-1, while a rise in coronary perfusion pressure associated with a coronary constriction was observed at high concentrations of ET-1 [12]. In addition, the effect of exogenous ET-1 on the coronary circulation may vary according to the mode of administration (bolus, infusions) and the experimental conditions (conscious versus anesthetized) [43]. It has been reported that, in coronary arteries, ET_A are far more dominant in VSMC than ET_B, and thus that ET-1 acts mainly as a vasoconstrictor [44].

The contribution of endogenously generated ET-1 to the coronary vessel tone in humans was assessed in patients undergoing routine diagnostic cardiac catheterization for suspected coronary artery disease (CAD), by intracoronary infusion of BQ123 (100 nmol/min, for 60 min), a selective antagonist of ET_A receptors [45]. Inhibition of ET_A receptors caused a coronary dilation in proximal and distal segments, an increase in coronary blood flow, and a decrease in coronary resistance. This result was confirmed by Ganz's group showing that BQ123 (40 nmol/min, for 60 min) increased coronary artery diameter by 7% compared to 21% with nitroglycerine [46]. In this study, ET-1 was estimated to contribute to 39% of total vasomotor tone in healthy subjects. Similarly, MacCarthy et al. reported that intracoronary infusion of BQ123 (40 nmol/min, for 15 min) in patients with angiographically normal coronary arteries induced a reversible dilation of proximal, mid, and distal vessels, an increase in coronary blood flow, but no changes in systemic hemodynamic [47]. The vasodilatory effect of BQ123 appeared greater in distal vessels, likely explicable by the

distribution of ET_A in human coronary arteries. BQ123 highlights the importance in ET_A-mediated contraction in coronary arteries, but also unmasks the role of endothelial ET_B-dependent dilation mediated by NO and prostacyclin release [13, 15].

ET-1 not only exerts direct constrictor effects, but is also able, at low concentrations, to potentiate contractile responses to other vasoconstrictor substances such as norepinephrine and serotonin; on the other hand, the presence of small concentrations of vasoconstrictor substances can amplify the response to ET-1 [15, 48]. In isolated human cerebral arteries, endothelium-derived ET-1 augmented significantly serotonin-induced contraction, and BQ-123 prevented the rise in tone induced by inhibition of NO production [49]. Thus, even subthreshold concentrations of ET-1 may regulate vascular tone and reactivity. This is likely the physiological function of ET-1.

The role of ET_B beside clearance of ET-1 and its endothelium-dependent dilatory activity is poorly understood. This is complicated by the possible change in expression of ET-1 receptors during the development of pathologies as evidenced in pulmonary hypertension [50] and renal diseases [51, 52] or by chronically doubling the circulating levels of ET-1 [53]. For example, doubling the circulating levels of ET-1 in dogs induces, likely under the control of ET_B receptors, a reduction in cardiac output and heart rate combined with an increased systemic and renal vascular resistance and an antinatriuretic effect [54]. In addition, it has been reported that ET_B receptors may play a role in the potentiation of the contraction induced by neurohormones such as angiotensin II and norepinephrine by low doses of ET-1 [55].

In summary, the endogenous ET-1-dependent control of tone in normal arteries depends on the balance between ET_A- and ET_B-mediated effects and on factors such as receptor distribution and endothelial integrity.

Physiological control of vascular remodeling

It has been reported that ET-1 modulates the expression of extracellular matrix (ECM) and matrix metalloproteinases, the main enzymes that degrade ECM molecules [56]. This confers to ET-1 a role in vascular remodeling. Vessel remodeling, however, should be considered as an adaptive response and is usually associated with endothelial dysfunction and vascular disease. ET-1, via ET_A, mediates intimal hyperplasia in human saphenous vein graft [26, 27]. Similarly, when compared to control healthy internal mammary artery segments, segments isolated from patients with CAD displayed increased in situ immunostaining to ET-1 and ET_A/ET_B in VSMC, increased total and type 1 collagen, and a higher rate of VSMC proliferation, suggesting that ET-1 plays a role in arterial remodeling associated with CAD [57]. Recently, it has been reported that, in vascular endothelial cell ET-1 knockout mice, neointima formation induced by arterial (carotid) ligation was reduced, SMC proliferation decreased, and expression of endothelial adhesion molecules was inhibited [25]. This is a direct evidence for the role of endothelial ET-1 in mediating neointima formation following vascular injury. Hence, beyond its known vasoconstrictor effects, ET-1 is an important mediator of vascular remodeling. The links between ET-1-induced signaling events and the vascular remodeling are largely unknown, since the role of ET-1 in remodeling has been evidenced under stress conditions only.

ET-1 stimulates angiogenesis

Another mean by which ET-1 may be beneficial to tissue perfusion is through its ability to stimulate angiogenesis. Angiogenesis is the final common pathway in ischemia-induced neovascularization as well as formation of collateral vessels in cardiovascular diseases. Both in vitro experiments using isolated endothelial cells and in vivo models showed that ET-1

induces angiogenesis via ET_B [58, 59]. Tissue hypoxia is a physiological stimulus for angiogenesis, and ET-1 production is also enhanced by hypoxia. In cardiomyocytes, ET-1 triggers connective tissue growth factor, a fibrotic mediator that regulates cell proliferation, migration, and ECM accumulation and thus plays a role in angiogenesis and tissue repair [60]. The angiogenic property of ET-1 could potentially be involved in the neovascularization described in atherosclerotic human coronary arteries.

In summary, the physiological roles of ET-1 involve maintenance of normal blood vessel tone, cellular proliferation, tissue development and repair, and angiogenesis.

Pathophysiological vascular effects of ET-1

The rise in circulating ET-1 levels

Under normal physiological conditions, the plasmatic concentration of ET-1 is around 1 pM, but it has been reported that the local concentration of ET-1 within the vascular wall is 100-fold higher than that of plasma levels [24]. The changes in plasmatic concentration of ET-1 may therefore not directly reflect changes in ET-1 release/clearance/breakdown associated with pathological conditions. Nonetheless, it has been shown that doubling circulating ET-1 levels acutely in dogs decreased heart rate and cardiac output without affecting blood pressure, and therefore increasing systemic vascular resistance [54]. This experimental increase in ET-1 was also associated with a rise in renal vascular resistance in association with a decreased glomerular filtration rate, without affecting coronary flow and pulmonary wedge pressure. On the other hand, doubling circulating levels of ET-1 chronically (7 days) in rats increased systolic blood pressure [53]. Furthermore, α_2 -adrenergic receptor-dependent contraction of isolated mesenteric arteries was increased, blunted by dual ET_{A/B} inhibition but not by selective ET_A inhibition with BQ123. Therefore, chronic elevation of ET-1 leads to changes in vascular reactivity possibly by affecting ET_B-dependent responses.

Circulating levels of ET-1 have been reported to be elevated in patients with CAD and atherosclerosis, pulmonary hypertension, diabetes [29, 30, 34, 61–64], in patients with hypertension associated with renal failure [24], and in patients with heart failure [13, 65–68]. Many of the cardiovascular complications associated with aging and cardiovascular risk factors are attributable, at least in part, to endothelial dysfunction, particularly dysregulation of the vascular tone induced by an imbalance between NO and ET-1. Therefore, the known reduction of NO bioavailability in association with risk factors for cardiovascular diseases and disease states could also explain the rise in ET-1. Subsequently, ET-1 may feed forward by stimulating the production of damaging reactive oxygen species (ROS) [69] and hasten the decline in endothelial function [39]; on the one hand, prevention of NO production increases ET-1 release [16], while on the other hand, blockade of ET-1 receptors improves NO-dependent, flow-mediated dilation in patients with CAD [61]. The chronic rise in ET-1 may then lead to a molecular remodeling of the endothelin system (receptors, enzyme, associated proteins, etc.) and affect the cardiovascular system. By opposition, chronic blockade of ET_A (4 weeks) in rats with congestive heart failure partially restored guanylate cyclase sensitivity and the loss of endothelial cell/smooth muscle cell communication [70]. Therefore, changes in ET-1 levels or blockade of its receptors have functional consequences involving proteins and pathways outside of the endothelin system itself.

Coronary arterial vasospasm

The evidence that ET-1 plasma levels are elevated in the coronary circulation of patients during angina, that ET-1 induces a long-lasting contraction in coronary arteries, and that subthreshold concentrations of ET-1 potentiate the coronary constrictor effects of other vasoconstrictors make ET-1 an ideal candidate for the initiation and the maintenance of coronary arterial spasm [15]. This hypothesis has been validated recently in a case report

[71]. In this report, a 46-year-old patient with severe and treatment-resistant coronary vasospasm was treated successfully with the endothelin receptor antagonist bosentan. In acute coronary syndrome, it was reported that the concentration of ET-1 in the thrombus exceeded 280 times that of angiotensin II, norepinephrine, and serotonin [72]. Importantly, thrombus homogenates exerted vasoconstrictions of isolated porcine coronary artery rings that were inhibited by the dual ET-1 receptor antagonist tezosentan. The recent demonstration that following coronary artery endothelial dysfunction ET-1 is essential for a ROS-dependent coronary vasospasm [69], a mechanism that could also be involved in coronary spasm postcardiac transplantation [73], provides a strong rationale for testing ET_{A/B} antagonists in spastic angina.

Endothelial dysfunction and atherosclerosis

In 1995, it was shown that treatment of cholesterol-fed hamsters with the selective ET_A receptor antagonist BMS-182874 decreased the area of the fatty streak by reducing the number and size of macrophage foam cells [74], strongly suggesting a proinflammatory role of ET_A activation. Lüscher's group demonstrated that ET_A inhibition improved endothelial dysfunction and reduced atherosclerosis development in ApoE knockout mice [75]. In pigs fed with a high-cholesterol diet, both selective ET_A and dual ET_{A/B} chronic treatment selectively increased coronary blood flow induced by intracoronary infusion of acetylcholine [76], while following myocardial infarction in rats, treatment with both the selective ET_A antagonist LU 135252 and the dual ET_{A/B} antagonist bosentan improved acetylcholine-induced relaxation [77]. Several studies have reported the effects of intracoronary infusion of the ET_A antagonist BQ123 on coronary diameter and coronary flow in patients with CAD. As in healthy patients, Dr. Webb's group showed that BQ123 (100 nmol/min, for 60 min) increased diameter and coronary flow [45]. In contrast, Ganz's group reported that BQ123 (40 nmol/min, for 60 min) increased more coronary artery diameter in patients with CAD than without [46]. In this latter study, compared with the dilation to nitroglycerin, ET-1 contributed to 39% of coronary vasomotor tone in healthy subjects, 74% of tone in CAD arteries, and 106% of tone at stenoses. The contribution of NO, that is the endothelial dilatory function, was, however, not determined: one would assume that the more severe the diseases state the less NO would be produced (if any), and the more ET-1 would contribute to tone. This hypothesis was tested in 44 patients with CAD in a study published that same year in 2001: Halcox et al. provided the first evidence that ET-1, via ET_A, contributed to reduce the endothelial dilatory function [63]. Intracoronary infusion of BQ123 (200 nmol/min, for 1 h) in patients with atherosclerosis resulted in coronary artery dilation and an improvement of acetylcholine-induced endothelium-dependent dilation. The patients with the greatest endothelial dysfunction benefited the most from the intracoronary infusion of the ET_A antagonist [63]. In pig coronary arteries subjected to ischemia reperfusion, another model of endothelial dysfunction, the vasoreactivity to exogenous ET-1 was increased and associated with a reduction in endothelial ET_B-mediated dilation and an increase in vascular smooth muscle ET_B-dependent contraction [78]. Bohm et al. reported that both selective ET_A (BQ123) and the combination of selective ET_A (BQ123) and ET_B (BQ788) antagonists improved endothelial-dependent dilation in coronary arteries from patients with CAD [61]. Again, the dilation was higher in severely stenotic segments, demonstrating that the importance of ET-1 in the control of vascular tone is increased in atherosclerosis. In agreement with these data, using isolated human coronary arteries from idiopathic and atherosclerotic cardiomyopathic hearts, we demonstrated that ET-1-dependent constriction became more important when endothelial function was altered [21]. Taken together, these data strongly suggest that ET-1 is an important contributor to endothelial dysfunction in the coronary arterial bed. The role of the ET_B needs also to be considered because its expression increases in experimental hypercholesterolemia, promoting contraction of isolated pig coronary arterial rings at low

concentrations (0.1 nM) of ET-1 and sarafotoxin-6c, a selective ET_B agonist [79]. Hence, the functional contribution of ET-1 appears to rise with the severity of the disease. Raised plasma levels of ET-1 have been reported in patients with atherosclerosis, and upregulation of ET-1 and its receptors has been described in atherosclerotic arteries and plaques [64, 67, 80, 81]. Big ET-1 and ET-1 immunoreactivity has been found in regions of atherosclerosis [79, 82]. The constrictor, proinflammatory, chemoattractant, and mitogenic properties of ET-1 also clearly support a role for ET-1 in the pathogenesis of atherosclerosis [29, 83].

Additional studies brought another perspective on the potential clinical development of ET-1 receptor antagonists based on the interactions between ET-1 and angiotensin II. ET-1 receptor inhibition combined with angiotensin-converting enzyme (ACE) inhibitors has been shown to improve renal hemodynamics and sodium excretion in patients with chronic kidney diseases [84, 85]. Most interestingly, dual ET_{A/B} antagonism improved endothelium-dependent dilation in the forearm in atherosclerotic patients treated with an ACE inhibitor [86]. Similarly, ET_A antagonism and ACE inhibition were efficient at preventing diabetic renal lesions induced by diabetes in rats [51], and a recent study in treatment resistant hypertension [87] shows efficacy on blood pressure and proteinuria in patients already treated with renin–angiotensin system blockers. Since endothelin antagonists do not produce hyperkalemia, and there is tremendous unmet need in the field of chronic proteinuric nephropathy, this seems to be a very attractive area for further clinical development. This dual therapeutic approach may also be efficient in several other cardiovascular diseases including CAD, peripheral vascular diseases, and diabetes.

Conclusion

Although there are now a number of cardiovascular indications for endothelin receptor antagonists, and a number under consideration, the physiology of ET-1 needs more understanding. ET-1 is essential for normal physiological function, and its well-known interaction with NO should be sufficient to stimulate more research. More data may stimulate clinical development of ET receptors antagonists in atherosclerosis and diabetes among others [33, 34, 88]. The legacy of Dr. Furchgott is not an endothelium with a Janus face but a cellular layer that finely regulates tone and maintains wall integrity with high levels of control. Dysfunctions develop with age or prematurely with exposure to cardiovascular risk factors, and the loss of NO probably unbalances the system favoring ET-1-dependent pathogenesis.

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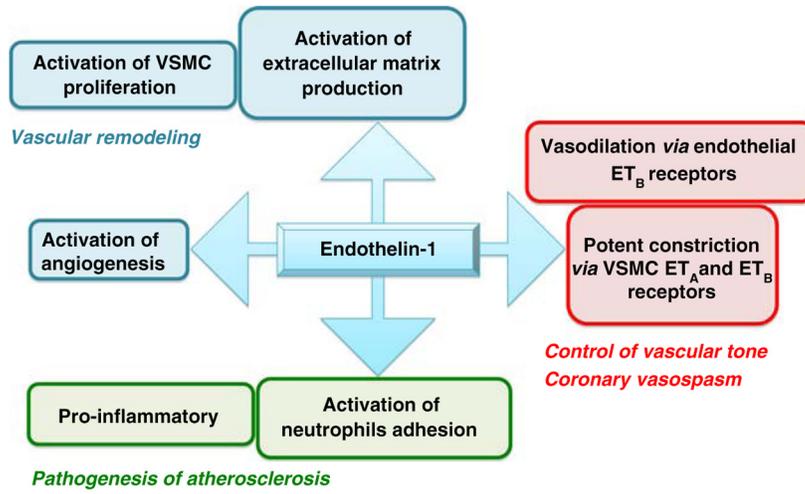


Fig. 1.
Multiple vascular properties of ET-1