-CHAPTER 6-

Endothelin Plays a Role in the Pathogenesis of Hypertension: Fact or Fiction?

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Abstract

Endothelin-1 was discovered 10 years ago. Because it is one of the most potent vasoconstrictors *in vivo*, a pathophysiological role for the peptide as a mediator of hypertension has been postulated. Several clinical studies, however, have been unable to identify elevated endothelin levels in the plasma of hypertensive patients, suggesting that it does not play a prominent role in this disease. More recently, evidence has been presented that endothelines act predominantly at the autocrine/paracrine level and that measurements of plasma levels can give only an indirect view of the activity of the system. In addition, transgenic technology has uncovered new actions of the peptide system in recent years, which point to a key function of the system in prenatal development. Moreover, investigation of conditions associated with hypertensive end-organ damage, such as chronic renal failure, has led to a re-evaluation of the role of the endothelin system in hypertension. This article discusses this recent evidence and defines the exact role of the endothelin system in hypertension and hypertensive end-organ damage.

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Introduction

During the past decade, The role of the endothelial cells in the regulation of vascular tone has become clear. Moncada et al.[1] described in 1976 the existence of prostacyclin within the blood vessel wall, and in 1980, Furchgott and Zawadzki[2] reported that acetylcholine (Ach)-mediated vasodilatation depended on the presence of the endothelium. In other words, several investigators showed that the endothelium serves as a regulator of vascular smooth muscle tone by elaborating a potent endothelial-dependent vasodilator.

A vasoconstrictor role for the endothelium has also been suggested. In 1985, Rubanyi and Vanhoutte[3] reported that hypoxia caused the release of a diffusible vasoconstrictor substance(s) from endothelial cells, independently of prostaglandin synthesis. In 1988, Yanagisawa et al.[4] discovered a 21-amino acid peptide, subsequently named endothelin (ET), in the supernatant of cultured porcine aortic endothelial cells. In 1989, Miller et al.[5] reported that systemic administration of synthetic ET resulted in pronounced systemic, coronary and renal vasoconstriction in association with activation of the renin-angiotensin-aldosteron system. These investigators postulated an important pathophysiological role for ET in diseases such as congestive heart failure, hypertension, atherosclerosis and cerebrovascular diseases. This review focuses on the role of the ET system in hypertension and hypertensive end-organ damage.

The endothelin system

The ET family consists of three distinct 21-amino acid peptides (ET-1, -2 and –3), all with very similar peptide structure[4]. The genes encoding the different ETs have been cloned and their regulation has been investigated[6-8]. ET-1, the most important ET, is synthesized in both the endothelium[4,6] and vascular smooth muscle cells (VSMCs)[9]. Stimuli for ET-1 release are hypoxia, thrombin, angiotensin II, vasopressin, norepinephrine, bradykinin[4], transforming growth factor-ß1[10,11] and low shear stress[12]. High shear stress potently inhibits ET-1 secretion[13].

Besides the different ETs, two

types of endothelin receptors are found in the vascularture[14]: type A (ET_A) and type B (ET_B). The ET_A receptor is present on VSMCs mediates predominantly contraction by endothelin-1[15]. The ET_B receptor is present both on the endothelium, where it release nitric oxide and prostacyclin, thereby mediating relaxation, and also on VSMCs where it mediates vasoconstriction[17,18]. The ET_A receptor shows high selectivity for ET-1; the ET_B receptor is equally sensitive for all three ETs[16]. ET-1 infusion into the circulation produces a transient vasodilatation and a short hypotensive response, followed by a long-lasting, ET_A receptor-mediated vasoconstriction and pressure increase.

Besides the abilities of ET to change vascular tone, they also induce hypertrophy in smooth muscle cells and function as mitogens as well[19,20].

Several mechanisms for ET involvement in the pathophysiology of hypertension are discussed here. Hypertension could be mediated by high ET levels in the circulation or the vascular wall or by alterations in response to ET at the receptor level. A decrease in the response to the ET_B receptor, such as in the dysfunctional endothelium, might attenuate the vasodilator response. Structural alterations of the vessel wall, such as an increase in vessel wall thickness (vascular hypertrophy due to ETs mitogenic effects), could play a role. Alternatively, ETs may elevated blood pressure by causing renal sodium and water retention[21], resulting in volume expansion. Finally, ET effects on the central and peripheral nervous can also cause vasoconstriction through the release of other substances[22].

Endothelin in the etiology of hypertension

Prerequisites for proposing a role for ET in the pathophysiology of hypertension are augmentation of the peptide levels or potentiation of its vasoconstrictor response and lowering of arterial blood pressure in hypertensive diseases by reasonably selective ET antagonists. A causal relationship between ET and hypertension has been shown by the development of a pressure response after infusing ET in animal[23-26] and human[27] experiments. ET infusion

transiently lowers blood pressure, followed by a prolonged rise in blood pressure. Niranjan et al.[28] showed that viral transfer of human preproET-1 cDNA, into the rat liver, increases plasma endothelin levels sixfold and significantly increases blood pressure.

Similarly, the prerequisites for an important role for ET in animal models of hypertension would be increased ET levels in the vasculature or serum. However, most hypertensive animal models investigated have normal or only slightly increased plasma endothelin levels[29]. Only induction of malignant hypertension, such as in the spontaneously hypertensive rats (SHR) treated with deoxycorticosterone acetate (DOCA) and salt[30] or in the two kidney-one clip (2K-1C) hypertensive rat treated with caffeine, significant elevations of plasma ET levels are found[31]. Under normal conditions, only small amounts of ET-1 circulates. This may be due to rapid removal of ET-1 from the circulation or the preferential release towards the medial smooth muscle via a paracrine mechanism. Approximately 80% of the total amount of ET-1 synthesized by endothelial cells is found on the abluminal side[32], implying that circulating levels of ET-1 do not reflect true local concentrations in blood vessel walls and that ET-1 is primarily a locally acting, rather than a circulating, hormone[33]. Among animal models of hypertension, only the DOCA-salt rat and the DOCA-salt SHR have increased ET-1 mRNA levels in the vessel wall. Surprisingly, SHR have similar or lower ET-1 mRNA than normotensive controls[34]. In 2K-1C hypertensive rats, however, blood vessel wall ET-1 mRNA is slightly elevated[35], and in Dahl rats, the ET-3 locus cosegregates with blood pressure[36].

Elevated vessel ET mRNA may mediate important structural effects, in the different hypertensive models, such as vascular hypertrophy, due to its growth promoting properties[19,37]. Interestingly, DOCA-salt hypertensive rat arteries show severe vascular hypertrophy with prominent medial thickening[38] and overexpression of the ET-1 gene[34]. In contrast, little vascular hypertrophy and no ET-1 gene expression are seen in SHR[39].

Additional information has been provided by experiments on

the effects of ET inhibitors on blood pressure in hypertensive models. In the DOCA-salt hypertensive rat, ET antagonists lower blood pressure and almost normalize vascular hypertrophy[34]. Also in the DOCA-salt treated SHR with malignant hypertension[40] and in the stroke prone SHR[41], endothelin antagonists were able to lower blood pressure. In Dahl-salt hypertensive rats, ET blockade decreases blood pressure, but did not normalize it[42]. In contrast, in the 2K-1C or 1K-1C hypertensive rat[35] and in SHR[43] ET antagonist do not lower blood pressure or affect vascular hypertrophy. This discrepancy may reflect strain-specific mechanisms in the pathogenesis of hypertension. Whether an ET antagonist influences blood pressure possibly depends on the relative importance of the ET system in the specific hypertensive models (Table 1).

In conclusion, it seems that in the DOCA-salt hypertensive rat and in rats with malignant hypertension, ET plays a more important role than in other models; that is not all animal hypertensive models are the same. The different hypertensive diseases have different etiologies in which ET plays different roles, but in more severe forms of hypertension, such as malignant and in the stroke-prone SHR, ET plays a clear central role.

Endothelin in human hypertension

In humans with mild to moderate hypertension, plasma ET-1 levels are normal or only slightly increased[44,45]. End-organ damage, however, appears to be a confounding factor because hypertensive patients with renal disease have higher plasma ET levels than those without renal involvement[46]. Similarly, ET levels are higher in salt sensitive essential hypertension[47] and in obese hypertensives[48] than in normal controls. Racial differences for ET levels in human hypertension have also been suggested, by showing significantly higher plasma concentrations of immunoreactive ET-1 levels in black than in white hypertensives[49]. Furthermore, the severity of hypertension correlates with plasma ET levels[46,50], and in malignant hypertension, plasma

Table 1. Endothelin expression in hypertensive rat models

Strain Model	Vascular ET-1 mRNA	plasma ET	ET inhibition
DOCA-Salt	high[34]	normal[29]	↓ blood pressure[34]
2K-1C/1K-1C	slightly elevated[35]	normal	no effect[35]
2K-1C + caffeine	not known	elevated[31]	not known
SHR	normal or low[34]	normal[29]	no effect[43]
SHRsp	not known	not known	↓ blood pressure[41]
SHR + DOCA/Salt	high[34]	elevated[30]	↓ blood pressure[40]
Dahl	not known	not known	slightly ↓ blood pressure[42]

2K-1C = two kidney one clip, 1K-1C = one kidney one clip.

ET-1 levels are extremely high[51,52]. In addition, Schiffrin et al.[53] have demonstrated increased vascular ET-1 expression in patients with severe hypertension. Another form of hypertension in which ETs play a major role is that seen in a small group of patients with hemangioendothelioma. These patients have elevated blood pressure and increased plasma ET-1 levels. Surgery reduces blood pressure and plasma ET-1 levels, and after tumor relapse, blood pressure and ET-1 levels increase again[54]. ET-1 gene expression has been detected also in human pheochromocytoma[55], and also plasma ET-1 levels are significantly higher in such patients than in patients with essential hypertension or normal subjects[56]. Another hypertensive disease reportedly accompanied by elevated ET-1 levels is preeclampsia or eclampsia[57,58], with even higher levels in patients with severe pregnancy-induced hypertension[58] or the hypertension, elevated liver enzymes, and low platelet count (HELLP) syndrome[59]. In contrast, plasma ET-1 and big ET-1 levels are not elevated in pregnant women with chronic hypertension[60]. Not only are different hypertensive diseases associated with elevated plasma ET levels, but several substances, such as cyclosporine and erythropoeitin, can also increase endothelin plasma levels. In patients receiving cyclosporine after heart,

liver, or kidney transplantation, plasma ET-1 concentration are elevated[61,62]. Because cyclosporines induce hypertension, ET may play an important role in the etiology of this hypertension. Administration of human recombinant erythropoeitin to patients with chronic renal failure on hemodyalisis may be associated with increased plasma levels of ET-1, which, in turn, could contribute to the hypertension seen in these patients[63] (Table 2).

Although there are no studies concerning ET antagonists and blood pressure in humans, experiments on ET_A receptor antagonists in the human forearm circulation[64] and skin microcirculation[65] suggests that ETs contribute to basal vascular tone. The role for ET in human hypertension thus appears similar as in animal models: a more important pathophysiological role in the more severe forms or in hypertension with end-organ damage.

Endothelin and hypertensive end-organ damage

Based on the evidence presented earlier here, ET could play an important role in end-organ damage in hypertensive diseases. Chronic hypertension is commonly characterized by three types of end-organ damage namely, renal failure, heart failure and vascular disease.

Renal failure

Endothelin is importance in the kidney. ET infusion reduces renal blood flow, glomerular filtration rate (GFR) and sodium excretion[24,66]. Intravenous infusion of anti-endothelin-1/3 antibodies into rat kidney increases renal blood flow, suggesting ET involvement in the physiological control of renovascular tone[67]. Infusion of anti-ET antibodies exert a renal protective effect in ischemia[68] and in cyclosporine nephrotoxicity[69]. In a further study, ET-1 infusion in humans participates in volume homeostasis, where pathophysiolocigal concentrations contributed to renal vasoconstriction and sodium retention. These could be prevented by a calcium channel antagonist that blocks the elevation of intracellular [Ca^{2+}] or prevents the direct opening of ion channels[70,71]. Similarly, infusion of an ET antagonist into the renal interstitium in SHR improves

Table 2. Endothelin plasma levels in human hypertensive diseases.

Disease	Plasma ET-1	Correlation with HT
Ess. HT	normal/slightly elevated[44,45]	low
Ess. HT salt sensitivity/renal involvement	elevated[47,46]	high
Ess. HT obese /black patients	elevated[48,49]	intermediate
Malignant HT	high[51,52]	high
Pheochromocytoma	elevated[55,56]	difficult to interpret
Hemangioendothelioma	high[54]	high
Preeclampsia/eclampsia	elevated[57,58]	high
HELLP syndrome	high[59]	high
Cyclosporine HT	elevated[61,62]	high
Erythropoeitine HT	normal or elevated[63]	difficult to interpret

Ess. HT = essential hypertension. Summary of effect as discussed in the text. References are sited in the text.

renal hemodynamics and excretory function[72]. As mentioned earlier here, hypertensive diseases with renal involvement have elevated ET levels, so it may be clear that the renal ET system may play an important role in the dynamic regulation of renal flow not only in normal physiology but also in chronic hypertension.

Heart failure

Endothelin infusion increases coronary vascular resistance and decreases cardiac output and heart rate[24,73]. Schott et al.[74] showed after transfection with the preproET-1 gene, contraction of porcine arteries *in vivo* is augmented due to increased sensitivity to angiotensin I, implying a role for ET-1 in the pathogenesis of cardiovascular disease. Increased ET levels are reported in heart failure, an important constituent of hypertensive end-organ damage, suggesting a possible role for ET in this disease[75]. In this context, mortality decreases and cardiac function improves after treatment of rats with chronic heart failure with the ET_A inhibitor BQ-123. These benefits are accompanied by significant amelioration of left

ventricular dysfunction and prevention of ventricular remodeling, in which there is usually an increase in the ventricular mass and ventricular cavity enlargement[76]. ET-1 increases after cardiac infarction in rats, and pretreatment with an anti-ET-γ-globulin in this model can reduces infarct size by up to 40%[77]. In addition, infusion of ET_A receptor antagonists reduces infarct size in animals when infused before the ischemic event[78]. Myocardial infarction increases plasma ET not only in animals, but also in patients[79], and ET levels are lower in patients with early coronary artery reperfusion than in patients without early reperfusion. Plasma ET levels also may predict hemodynamic complications in patients with myocardial infarction[80], as plasma ET levels apparently correlate with highest creatinine phosphokinase (CPK), CPK-MB-isoenzyme levels, and low ejection fraction[81]. Accordingly, serum ET levels may be a prognostic indicator of survival after acute myocardial infarction.

Vascular diseases

In all hypertensive rat models, impaired endothelium-dependent relaxation occurs in large conduit arteries like the aorta[82]. Two independent groups have reported impaired endothelium-dependent vasodilation to Ach with preserved or only slightly altered responsiveness to sodium nitroprusside in the human forearm circulation of patients with essential hypertension[83,84]. In some hypertensive diseases, increased ET-1 levels accompany elevated von Willebrand factor, indicating general endothelial damage[47,85] ET-1 levels are markedly raised in diseases with diffuse endothelial dysfunction, such as diseases with hypertension, artheroscleroses, renal insufficiency, congested heart failure[86,87] and eclampsia or the HELLP syndrome[58,59]. Although high ET levels are often viewed as a marker of endothelial damage, ET may itself cause endothelial dysfunction. We recently found that high ET-2 levels cause endothelial dysfunction in the ET-2 transgenic rat. This rat has a 2.5-fold increase in serum ET-2, a level comparable to that found in pathophysiological conditions. In these animals endothelium-dependent Ach-mediated relaxation is attenuated[88]. These data show that ET is not only a marker

for endothelial dysfunction, but that it may also impair endothelial function. High ET levels occur also in artherosclerosis[86], and *in vivo* ET has served as a chemotactic agent for monocytes and also stimulate the migration and proliferation of VSMCs, thereby sustaining the artherosclerotic process[7,89]. As described earlier here, ischemia, a complication of artherosclerotic vascular disease, further stimulates both the release of ET and externalization of ET binding sites[90,91]. This indirect evidence (which obviously awaits the results of trials with a specific antagonist) strongly implies participation of ET in complications of the hypertensive process. In all forms of hypertensive end-organ damage, ET is elevated, and in heart failure, there could even be an important role for ET antagonists. Not only does ET play a central role in the physiology of the different organs involved in end-organ damage, but also in severe pathology caused by hypertension. In summary, we propose that when hypertension progresses and organ damage develops, endothelin plays a central role in this process, irrespective of the initial hypertension etiology.

Discussion

Obviously, a causal relationship between ET and hypertension is difficult to prove. Not all hypertensive conditions have elevated ET levels, but it is highly conceivable that hypertension is a heterozygous disease entity. ET is clearly more relevant in some hypertensive disorders. ET levels are elevated in all severe forms of hypertensive diseases, especially those with end-organ damage. ET is elevated in rats and humans with malignant hypertension, in humans with essential hypertension with renal involvement, and in patients with the HELLP syndrome. It remains to be established whether the increase in circulating ET-1 is involved in the development of the disease state or is a secondary phenomenon due to activation of the endothelin system at the cellular level. One explanation proposes a vicious cycle in which end organ damage and ET levels mutually reinforce each other. ET has substantial effects in the renal circulation, reducing GFR and renal blood

flow, which, in turn, may increase renin activity[24]. Furthermore, already only minor elevations in ET concentrations potentiate effects of other vasoconstrictors, such as norepinephrine, angiotensin II, and serotonin. Stimulation of perfused mesenteric arteries by angiotensin II is associated with endothelium-dependent potentiation of the response to norepinephrine. As this response is prevented both by phosphoramidone, an endothelin converting enzyme inhibitor, and an anti-ET antibody, this strongly suggests that angiotensin II can stimulate local vascular ET production, in turn, augments contractile responses to norepinephrine. If this were to occur in vivo, such responses may participate in the pressure effects of very low concentrations of angiotensin II[92]. ET may, moreover, contribute to the hemodynamic effects of angiotensin II in both SHR and normotensive control rats and may contribute to the exaggerated pressure responsiveness of the SHR to angiotensin II[93]. This potentiation of vasoconstrictor may lead in some patients to hypertension without elevation of plasma ET levels. Therefore angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists can prevent the pressure response produced by ET[94]. ET levels could then rise, due to stimulation by angiotensin II, or to endothelial damage by hypertension itself or due to damaging effects of ETs. The elevated ET concentration then elicits further vasoconstriction and worsening of hypertension, acting together with an enhanced renin-angiotensin system in a vicious circle worsening hypertension. A further possible mechanism for ET in the etiology or attenuation of hypertension lies in the induction of vascular hypertrophy by ET, which can worsen hypertension. This hypothesis also explains why ET levels are not elevated in all hypertensive diseases. Another reason for low ET levels in some hypertensive diseases is that endothelial ET-1 secretion is mostly abluminal so that circulating ET-1 concentrations may not reflect vascular production. Only when malignant hypertension is induced in SHR by DOCA-salt treatment is the endothelin-1 gene overexpressed in the blood vessels[30]. It is possible, but as yet unproven, that the proportion of

hypertensive patients showing enhanced expression of endothelin-1 in the blood vessel walls will increase if severe forms of hypertension are investigated, because it is essentially in the more severe or malignant forms of hypertension that are accompanied by increases in plasma ET[51,52]. In conclusion, in severe hypertension with end-organ damage, a causal role for ET is very likely. Its pathophysiological role in milder forms of hypertension and in the absence of hypertension is more questionable.

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