Interactions between endothelin-1 and the renin–angiotensin–aldosterone system

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Abstract

The renin–angiotensin–aldosterone (RAA) system and the endothelin (ET) system entail the most potent vasopressor mechanisms identified to date. Although they were studied in depth in relation to arterial hypertension and cardiovascular diseases, limited information on their interrelationships in causing hypertension and related target organ damage exists. The identification of consensus sequences for jun in the regulatory region of the preproendothelin-1 (ppET-1) gene raised the possibility of its transcriptional regulation by angiotensin II (Ang II). This was confirmed by the finding that stimulation with Ang II of cultured vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) induced expression of the ppET-1 gene and synthesis of ET-1. Endogenously produced ET-1 was found to contribute to the hypertrophic response of cardiomyocytes to Ang II and thereby to cardiac hypertrophy. Furthermore, ET-1 exerts multifaceted effects on the RAA system, such as dose-dependent inhibition of renin synthesis, and stimulation of aldosterone secretion. The finding of abundant specific ET-1 receptors in the adrenocortical zona glomerulosa (ZG) suggested a direct secretagogue effect of ET-1. In rats, ET receptors mediate such an effect, whilst in humans, both ET and ET receptor subtypes intervene in regulating the transcripion of the aldosterone synthase gene. In addition, ET-1 stimulates DNA synthesis and proliferation of ZG cells via ET receptors and, therefore, might play a role in cell turnover of the normal adrenal cortex and in the onset of adrenal tumours. Studies on the in vivo interactions between ETs and the RAA system have given conflicting results, insofar as some suggested a participation of ET-1 in the pressor and cellular effects of exogenously administered Ang II, whereas others did not in the transgenic TGR(Ren 2m)27 rats and in the two-kidney, one clip. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The renin–angiotensin–aldosterone system (RAAS) and the endothelin (ET) system are two of the most potent vasopressor mechanisms identified to date (for a review, see [1–3]). Therefore, it is not surprising that they have been investigated extensively in relation to arterial hypertension and related cardiovascular disease. However, despite this vast body of investigations, only limited information exists on their reciprocal relationships. Thus, in this paper, we shall review available knowledge on the interactions between these two important pressor systems, with emphasis on the role of endothelin-1 (ET-1) in the regulation of the RAAS and on in vivo studies supporting a co-operation of both systems in the regulation of cardiovascular and adrenocortical function.

2. Potential sites of interaction

Several potential sites of interaction between the RAAS and ET system, spanning from the molecular level to the in vivo hormonal and haemodynamic regulation, can be envisaged. It has been hypothesised that Ang II, the active peptide of the RAAS, affects the synthesis of ET-1, which, in turn, can influence the RAAS by acting at different steps...
Fig. 2. Schematic representation of the biosynthetic pathways of the endothelins showing a role for chymase, the main Ang II-forming enzyme in the human heart, in ET(1–31) formation.
might be relevant for the regulation of both function and cell turnover of the adrenal cortex under physiological and pathophysiological conditions.

3. Effect of the RAAS on endothelin-1

The identification of consensus sequences for regulatory elements in the regulatory region of both the ppET-1 gene (Fig. 3) and the endothelin converting enzyme 1 (ECE-1) gene suggested the possibility that both of these key-genes involved in ET-1 biosynthesis are regulated by the same factors. The hypothesis that Ang II can turn on the transcription of the ppET-1 gene is supported by the in vitro findings that human and porcine cultured vascular smooth muscle cells (VSMCs) [12–14] and endothelial cells (ECs) [15,16] can express the preproET-1 gene and synthesise immunoreactive (ir) ET-1 upon stimulation with Ang II. Evidence indicates that Ang II turns on the transcription of the ppET-1 gene by acting via AT-1 receptors linked to activation of transcription via activator protein-1/kinase C-mediated mechanisms [14,15,17,18].

However, the possibility of complex cross-talk between several signalling pathways that can be activated by a number of humoral factors, such as insulin, catecholamines, components of the clotting/fibrinolysis cascade, and cytokines, as depicted in Fig. 3, should be kept in mind when interpreting results of in vivo studies.

Since Ang II is a well known promoter of cardiac hypertrophy, the data suggesting an effect of the peptide on ET-1 transcription raised the possibility that ET-1 might also intervene in hypertension-related left ventricular hypertrophy and remodelling. This contention is supported by the observation that endogenously produced ET-1 contributed to the hypertrophic response of cardiomyocytes to both Ang II and ET-3 [17]. Thus, ET-1 antagonism may offer an additional weapon for therapeutic interventions aimed at preventing cardiovascular damage. The demonstration that long-term treatment with ET antagonists not only greatly improved the survival of rats with chronic heart failure, but also prevented ventricular remodelling, i.e., the increase in left ventricular mass and cavity enlargement [19], as well as the increase in collagen density [20], strongly supports this view.

4. Effect of ET-1 on the RAAS

Besides its potent haemodynamic effects, the intravenous infusion of ET-1 in animals and humans was found to deeply affect the RAAS. Renin secretion from juxtaglomerular cells was generally decreased by ET-1 both in vitro and in vivo, as shown by seven out of eight available studies [21–28]. Collectively, available evidence is consistent with both a direct and an indirect inhibitory action of ET-1, through mechanisms that are depicted in Fig. 1. This inhibition may be relevant in a number of clinical

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**Fig. 3.** Schematic representation of the intracellular signalling mechanisms whereby Ang II can turn on the transcription of the preproET-1 gene via activation of the phospholipase C and protein kinase C pathways.
situations, for instance, in pregnancy-induced hypertension, a vasospastic and volume-reduced disorder where endothelial cell damage is suspected to play an important role. Compared to normal pregnancy, pregnancy-induced hypertension was found to be associated with a two-fold increase in plasma irET-1 levels, which may be responsible for the inappropriately low plasma renin activity in relation to the reduced plasma volume [28].

The observation that, despite this inhibitory effect on renin, ET-1 was found to markedly stimulate aldosterone secretion both in animals and in humans, suggested a direct effect of the peptide on the adrenal cortex (for review, see Ref. [10]). This contention was confirmed by findings of abundant specific ET\(_A\) and ET\(_\beta\) receptors in the human and animal adrenal ZG [29–31] as well as by in vitro studies [32–35], which clearly indicated that, in rats, the ET\(_\beta\) receptor subtype mediates the direct secretagogue effect of ET-1 on aldosterone [36]. At variance with findings in rats, available data in humans indicate that both the ET\(_A\) and ET\(_\beta\) receptors are involved [37] in the transcriptional regulation of the aldosterone synthase gene, both in adrenocortical carcinoma and in normal human adrenal cells [38]. Similar results were recently obtained by another group working independently [39]. Of interest, in vitro in dispersed normal human adrenocortical ZG cells, as well as in Conn’s adenoma cells, ET-1 was found to be equipotent to Ang II [38], the most important physiological secretagogue of aldosterone identified so far. Further experiments from our group provided unequivocal evidence that, in the rat adrenal ZG, by acting via ET\(_A\) receptors, ET-1 stimulates both DNA synthesis and cell proliferation [40]. Since these latter effects were dose-dependently blunted by the protein-kinase C inhibitor Ro31-8220 and by the tyrosine-kinase inhibitor tyrphostin-23, they are likely to involve activation of both of these kinase signalling pathways, but not the protein-kinase A, cyclooxygenase and lipoxygenase signalling pathways [40]. Based on these findings, we hypothesised that ET-1 may enhance the growth of the entire adrenal gland by acting on the ‘cambium’ layer of cells endowed with ET\(_A\) receptors, which are mainly located in the ZG. [41]. However, in recent in vivo experiments in rats, it was found that the administration of the ET\(_A\)-specific antagonist BQ-123 and the mixed ET\(_A\)/ET\(_\beta\) antagonist bosentan for four weeks did not affect adrenal gland weight, casting doubts on the relevance of ET-1 in the maintenance of the volume of the entire gland in this species (for review, see Ref. [42]).

Thus, collectively, these data indicate that ET-1 potently stimulates aldosterone secretion and suggest that it may affect adrenocortical growth through a direct action on specific receptors, albeit with differences among species. These effects of ET-1 are potentially relevant in several conditions where endothelial damage, hypertension and hyperaldosteronism coexist, such as congestive heart failure, malignant and severe hypertension, endotoxaemia and in transplant recipients.

5. In vivo interactions of ET-1 and the RAAS

To investigate whether the aforementioned in vitro interactions between ET-1 and RAAS have an in vivo counterpart, a number of studies have been carried out. Among the original observations stimulating these investigations was the report that subthreshold concentrations of ET-1, i.e., lower than the concentration range (30–300 pM) needed to elicit a contractile response, were able to potentiate the vasoconstrictor response to well known vasoconstrictors, such as serotonin and histamine [43]. In male HanRen2/Edin rats derived from crossing the homozygote transgenic TGR(mREN-2)27 with Edinburgh Sprague-Dawley rats, which show a 73.5% incidence of the malignant phase of hypertension, an increased ET-1 mRNA content was found in the kidney in the animals that developed malignant hypertension [44]. Nonetheless, no effect of the mixed ET\(_A\)/ET\(_\beta\) endothelin antagonist bosentan [45] was seen on blood pressure. At variance with these findings, bosentan pre-treatment was capable of blunting the increase in blood pressure, the fall in cardiac output, and the decrease in arterial conductance evoked by an infusion of Ang II, both in WKY and in SHR rats [46]. This blunting was more pronounced in SHR rats, even though it was seen in both strains, but it was evident at the lower (2–10 ng/kg/min) but not at the higher (>30 ng/kg/min) doses of Ang II, thereby suggesting the possibility of an ET-1 component only in the initial pressor effect of Ang II. In partial agreement with this contention, a five-day-infusion of Ang II (0.7 mg/kg/day) to Sprague-Dawley rats was found to markedly enhance immunostaining for ET-1 in VSMCs; furthermore, administration of the ET\(_A\)-selective antagonist PD 155080 abolished the rise in blood pressure, as did the Ang II AT-1 receptor antagonist losartan [47]. In 1997, a number of studies carried out in Dr. Lüscher’s laboratory suggested that ET-1 could play an important role in the structural changes induced by Ang II infusion. Two weeks of Ang II infusion (200 ng/kg/min) increased media thickness, the media–lumen ratio and the cross-sectional area of the mesenteric and cerebral arterioles, and nearly doubled the content of ET-1 in the mesenteric tissue, through mechanisms most likely involving the ET\(_A\) receptor, inasmuch as the specific antagonist LU135252 was able to prevent both the development of medial hypertrophy and increased peptide content in both types of vessels [48]. It was also shown that a two-week-infusion of a similar dose of Ang II led to enhanced vascular endothelin converting enzyme activity and renal ET-1 content in WKY rats [49] and that LU135252 lowered the Ang II-induced pressure increase and improved endothelium-dependent relaxation [50]. The
hypothesis that ET-1 might play a role not only in the haemodynamic effects of Ang II but also in the mechanisms of related cardiovascular damage, is further supported by three sets of observations. First, bosentan entirely prevented the development of hypertension, the reduction in renal blood flow and the marked increase in albuminuria and heart weight induced by a ten-day infusion of Ang II (200 ng/kg/min) [51]. Second, in a canine model of Page (kidney wrapping) hypertension, bosentan was found to exert a hypotensive effect in addition to that of the AT-1 receptor antagonist losartan [52]. This additive hypotensive effect was found also in a study on rats with renin-dependent hypertension [53].

An animal model that lends itself to the investigation of the interactions between the RAA and ET systems in vivo is the TGR(mREN-2)27 rat. In this model, the introduction into the rat genome of the mouse Ren-2 gene, encoding for renin, causes severe hypertension, which is associated with overexpression of the transgene in several tissues, particularly in the adrenal cortex and the vessel wall, with ensuing enhanced endogenous production of Ang II in tissues and low plasma renin activity [54]. A marked sexual dimorphism of blood pressure changes exists in this model. In fact, while males develop severe hypertension, which is fulminant in homozygotes and somehow less severe in heterozygotes, the female TGR(mREN-2)27 rats have a milder form of hypertension, which in heterozygotes peaks at approximately ten weeks of age and then spontaneously returns to normal values by 35 weeks of age. Thus, these heterozygote female rats can be maintained without any antihypertensive treatment. Of interest, we recently reported that, in these female animals, the aortic responsiveness to ET-1 followed changes that closely paralleled those of their systolic blood pressure, thereby suggesting a role for ET-1 in this form of renin-dependent hypertension [55], in agreement with previous reports [56]. However, in contrast with this contention and in keeping with the aforementioned results in the HanRen2/Edin rats cross [44], bosentan did not have either a blood-pressure-lowering effect or any effectiveness in preventing left ventricular and vascular hypertrophy in male heterozygote TGR(mREN-2)27 rats. These findings were in sharp contrast with the efficacy of the angiotensin II AT-1 receptor antagonist irbesartan in lowering blood pressure and preventing left ventricular and arteriolar hypertrophy in the same model. Thus, it would appear that in this form of severe hypertension, due to overexpression of a renin gene, ET-1 does not play any major role. This conclusion is only partially supported by findings in two-kidney one-clip hypertension rats. In this animal model of renovascular hypertension, the orally active endothelin antagonist Ro-00203, whilst having no significant effect on renin secretion and renin gene expression, attenuated the rise in blood pressure elicited by clipping one renal artery, although it did not abolish it [57].

The reasons why ET-1 may take part in hypertension induced by exogenously administered Ang II, but not in the models with an enhanced endogenous production of Ang II, are unclear at present. They might depend upon the different tissue levels attained by Ang II and/or the different gradients between the bloodstream, endothelium and VSMCs of the blood vessels’ tunica media, in these two situations. In fact, it is well documented that, in heterozygote TGR(mREN-2)27 rats, a high tissue expression of Ang II occurs together with low plasma Ang II, whilst in the models with exogenous Ang II administra-
tion, the peptide concentrations are likely to be high in plasma and lower in tissues.

In contrast with the controversial data on the involvement of ET-1 in renin-dependent forms of hypertension, rather concordant data on the role of the peptide in mineralocorticoid-dependent forms of hypertension exist (for a review, see Ref. [58]). Increased levels of ir-ET-1 were found both in plasma and in the arterial wall of rats with deoxycorticosterone acetate (DOCA)-salt-induced hypertension [59–61]. Elegant in situ hybridisation studies by Day et al. [62] showed enhanced expression of the ppET-1 gene in the vessel wall in the same model. Treatment with bosentan was quite effective in preventing vascular hypertrophy in this model. This finding lent further support to the contention of a direct involvement of ET-1 in target organ damage in this low-renin form of hypertension [63]. The contention that ET-1 plays an important role in salt-dependent forms of hypertension is further supported by the findings of Barton et al. [64], who reported that the ET_α-specific antagonist LU135252 was able to partially prevent the development of hypertension and the structural and functional alterations caused by chronic salt administration in Dahl salt-sensitive rats. A role for ET-1 in cardiovascular damage of salt-sensitive hypertension is suggested also by data obtained in humans [65].

Fig. 4 summarises available data on the participation of endothelin in experimental models of hypertension. It must be pointed out, however, that regardless of the initial ET-1 dependency of hypertension, the pathogenic role of the ET system is deemed to get more and more important as endothelial and target organ damage supervene.

6. Therapeutic implications

The identification of interactions at multiple levels between ET-1 and the RAAS in the pathogenesis of hypertension and related cardiovascular damage has suggested the potential usefulness of a therapeutic strategy targeted at both systems. The potential synergistic/antagonistic effects of a combined inhibition of these two systems on mean arterial blood pressure (MBP) was investigated by Löffler et al. [66] in spontaneously hypertensive rats (SHR) [66]. The endothelin system was inhibited using the ET_α-selective receptor antagonist Ro 61-1790 and/or the mixed NEP24.11/ECI inhibitor phosphoramidon, whilst angiotensin I converting enzyme (ACE) was inhibited using cilazapril. The inhibition of the ET system with maximally effective doses of either phosphoramidon or Ro 61-1790 similarly decreased MBP by about −30 mmHg potency; the two agents were equipotent to a maximal ACE inhibition. Most interestingly, both combined phosphoramidon/cilazapril and phosphoramidon/Ro 61-1790 infusion enhanced the maximal decrease in MBP by 100% to −60 mmHg and, thus, almost normalised blood pressure. Thus, the combined ET/RAAS inhibition may provide an interesting synergistic potential in terms of blood pressure reduction. Of further interest, this synergism was found also in patients with class III New York Heart Association (NYHA) congestive heart failure, who were haemodynamically stable on ACE inhibitors and diuretics, in whom the mixed ET_α/ET_β receptor antagonist bosentan elicited a clear-cut decrease in systemic and pulmonary vascular resistance along with an increase in the cardiac index [67].

7. Conclusions and perspectives

The identification of ET-1 by Yanagisawa et al. [68] one decade ago has added a novel important player to the ‘arena’ of potential mechanisms causing arterial hypertension and related cardiovascular disease. Over the years, it has been increasingly appreciated that any newly discovered agent cannot be investigated singularly, but rather should be assessed in the context of all the other known mechanisms. There are several interactions between the RAAS and the endothelin system, which may have relevant effects on blood pressure and on hypertension-related complications. Experimental research has started to unravel these interactions, and pilot clinical trials gave promising results, but the question of whether or not they are also relevant in humans in the clinical setting and have therapeutic implications needs to be investigated further.

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