Recent Advances in Endothelin Research on Cardiovascular and Endocrine Systems

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Introduction

Since the quite exciting discovery in 1988 of endothelin (ET) in the culture medium of aortic endothelial cells by Yanagisawa and coworkers [1], evidence has accumulated to support its important roles in the regulation of blood pressure and body fluid homeostasis and its pathophysiological significance in various cardiovascular diseases. The discovery of ET and the opponent substances including nitric oxide [2] and natriuretic peptides [3] during the last decade was epoch-making in the field of “Cardiovascular Endocrinology”.

Because of its impressive potency and the long duration of its pressor action [1], the roles of ET in the regulation of cardiovascular homeostasis have been extensively studied. However, the co-expression or close localization of ET and its receptors in various tissues other than the vascular vessels suggest non-vascular roles of ET [4–6]. In the present review, we firstly integrate the information relevant to its physiological role in blood pressure regulation and possible pathogenic role in hypertension. We secondarily focus on the possible roles of ET in the endocrine system, especially in the pituitary and adrenal. Substantial review articles on ET in the kidney [7, 8], ET and the heart [9], the growth regulating properties of ET [10], and ET in bronchial diseases [11, 12] have been published.

Structure of ET and ET Receptor

The molecular and biochemical aspects of ET family peptides and ET receptors have been reviewed by Masaki and Yanagisawa [13] and others [14–16]. We here describe the fundamentals.

1. ET Peptides

The ET molecule consists of 21 amino acid residues with two intramolecular disulfide bonds between cystein residues at 1 and 15 and 3 and 11, respectively. The ring structure and the hydrophobic amino acid residues at the C-terminus are indispensable to its full biological activities. There are three distinct isoforms of ET: ET-1, ET-2, and ET-3 [13]. The difference in the amino acid sequence is seen in the ring structure, while they share the same sequence in the linear C-terminal portion. Interspecies differences are minimal [13–16]. Interestingly, the amino acid sequence of a family of rare snake venoms, the sarafotoxins, is very similar to the sequence of ET [16], composing the ET-sarafotoxin family and suggesting the importance of the peptide in evolution.

PreproET-1, the precursor of the peptide, is synthesized through the gene transcription and translation. Expression of the preproET-1 mRNA in the endothelial cells is stimulated by various humoral and physical factors: thrombin, TGF-β, interleukin-1, TNF-α, angiotensin II (Ang II), vasopressin, and shear stress (for review see [13–15]). Production of ET-1 in the endothelial cells is mediated by an increase in [Ca^{2+}]i and activation of protein kinase C. On the other hand, increased cGMP caused by natriuretic peptides and nitric oxide inhibits the production of ET-1 [17–19], suggesting a close interrelationship between the vasoconstrictive and...
vasodilatory systems.

After deletion of the signal peptide, the pro-segment of the proET-1 is initially cleaved by an endopeptidase specific for a pair of dibasic amino acids to produce an inactive 38-amino acid peptide, big-ET-1, which is finally cleaved into the mature form, ET-1, by a specific ET-converting enzyme. The enzyme is a phosphoramidon-sensitive membrane-bound metalloprotease [20]. Both big ET-1 and ET-1 are secreted into the culture medium. The immunocytochemical demonstration of ET-1 in cytoplasmic vesicles suggests a constitutive pathway for the secretion of ET-1 [21].

Northern blot analysis demonstrated that mRNAs corresponding to the three distinct ETs are expressed in different proportions in non-vascular tissues as well as vascular tissues where ET-1 is predominantly produced. In addition, the existence of ET was also shown by immunoassay in a variety of tissues other than the vascular endothelium [22]. The ET-1 content is the highest in the posterior pituitary and the lung followed by the anterior pituitary, brain, and adrenal gland. The ET-3 content is highest in the posterior and anterior pituitaries. The ET-1 content is significantly higher than that of ET-3 in all the tissues except for the anterior pituitary (Fig. 1).

2. ET Receptors

There are at least two subtypes of ET receptors: ETA receptor with a higher affinity for ET-1 and ET-2 than for ET-3 and ETβ receptor with an equivalent affinity for all three ETs [23, 24]. The vascular smooth muscle cells predominantly express the ETA receptor, while there is also an ETβ receptor [25, 26]. The vascular endothelial cells predominantly express ETβ receptor. In addition, both receptors are expressed in various tissues including lung, heart, brain, kidney and adrenal gland.

The primary structures of the receptors determined from the sequences of the cDNA clones indicate that both receptors are single chain peptides containing seven stretches of hydrophobic amino acid residues. The homology of the amino acid sequence of ETA and ETβ is about 60%. The sequence of the transmembrane region is very similar for each receptor, while that of the N-terminal extracellular domain shows practically no homology. Both receptors have two N-linked glycositation sites in the N-terminal domain, and serine/threonine residues in the intracellular third loop and C-terminus. The third type of receptor more specific to ET-3 has been predicted but is still unidentified.

The distribution of the ET determined from peptide content and mRNA expression is very similar to that of the ET receptor determined by the binding study and the expression of mRNA [4-6]. These findings suggest that ET acts mainly in a paracrine/autocrine fashion rather than in a classical endocrine fashion.

Biological Actions

The three ETs show somewhat different biological activities in relation to the affinity to and the distribution of each receptor subtype; ET-1 and ET-2 are more potent than ET-3 in pressor response, while ET-3 is relatively more potent in vasodilation. In addition to the vasoconstrictive actions, ETs have diverse biological actions on the cardiovascular, neural, renal, endocrine, metabolic, gastrointestinal, and genitourinary systems (Table 1), but the physiological significance of these effects remains to be elucidated.

ET elicits the biological actions by activating phospholipase C to produce inositol triphosphate and diacylglycerol which in turn activates protein kinase C, and by increasing the influx of calcium ion via activation of the calcium channel. These two major mechanisms are closely interrelated to each other. The ET-stimulated intracellular signal
transduction mechanism is detailed in other review articles [13-15].

**ET in the Cardiovascular System**

The anatomically close site of production to the vascular smooth muscle cells and the potent vasoconstrictive property indicate the potential importance of ETs in the regulation of cardiovascular homeostasis and their pathophysiological significance in various cardiovascular diseases, especially in hypertension.

1. **Significance of ETs in Systemic Hypertension**

1) Essential Hypertension

The acute administration of ET-1 to rats produces a transient decrease in blood pressure followed by a strong and long-lasting increase in blood pressure [1]. The first depressor effect is attributed to the release of vasodilator substances including prostacyclin and endothelium-derived nitric oxide through the ETB receptor on the endothelial cells, while the latter pressor effect is through the ETA receptor on the vascular smooth muscle cells. The ETB receptor on the vascular smooth muscle cells was recently shown to contribute also to vasoconstriction [25, 26].

In addition to the direct action on the vascular smooth muscle cells, ET-1 has non-vascular actions which might affect blood pressure: central activation of the sympathetic nervous system [27], secretion of vasopressin [28], aldosterone [29] and...
catecholamine [30], positive inotropic and chronotropic actions [31], and a decrease in the glomerular filtration rate and renal blood flow (for review see [6]). ET-1 potentiates the actions of other vasoconstrictive substances such as norepinephrine and serotonin [32]. In addition, ET-1 promotes a proliferation of vascular smooth muscle cells and fibroblasts (for review see [9]), which may result in the morphological changes in the vascular wall. All these direct and indirect actions contribute to the acute and chronic increase in blood pressure.

On the other hand, ET-1 stimulates ANP secretion, while it suppresses renin secretion [33], Na⁺/K⁺ ATPase activity [34], and vasopressin action in the kidney [35]. These actions in addition to the vasodilatory action through the ETs receptor on the endothelial cells may contribute to the decrease in the blood pressure. Pressor effects of ET-1 could be partly counterbalanced by these depressor effects. Fig. 2 shows the biological actions of ET possibly involved in the blood pressure control.

Considering the long duration of its effects and the strong binding to the receptors, ET is expected to be involved in the long-term regulation of the vascular tone. However, passive immunization of normotensive rats with antiserum to ET-1 does not affect the blood pressure, providing evidence against the physiological role of ET-1 in regulating vascular tone in a steady state condition [36]. Even an increase in blood pressure in rats with the ET-1 gene knocked out by gene targeting techniques is in agreement with this concept [37]. This is quite in contrast to the case of nitric oxide which plays an important role in the maintenance of basal vascular tone [38, 39].

The chronic infusion of ET-1 for one week resulted in a sustained increase in blood pressure [40, 41]. Interestingly, however, the administration of ET-1 over a period of 4 weeks in normal rats was associated with normalization of the once elevated blood pressure despite the sustained increase in plasma ET-1 [42]. This suggests that only the increase in plasma ET is not a factor eliciting chronic hypertension. This is supported by the fact that transgenic rats harboring the human ET-2 gene do not show any hypertension despite an increase in plasma ET-2 [43]. Counter-regulatory mechanism(s) including endothelium-dependent vasorelaxation may suppress the increase in blood pressure in intact rats.

The pathophysiological role of ET in systemic hypertension is still controversial [44, 45]. Reports on plasma levels of ET-1 are not unanimous: a moderate but a significant increase in some reports [46–48] and no change in others [49, 50]. We demonstrated that plasma ET-1 is increased in patients with essential hypertension of the stage III of the WHO classification. There was a close correlation between impaired renal function and the plasma levels [51]. Although Saito et al. [46] have shown that plasma levels are increased even in the stage III patients with normal renal function, increased plasma levels of ET-1 are generally associated with renal failure [47, 51, 52] and/or atherosclerosis [52, 53]. In addition, plasma levels of ET-1 are not high or even decreased in rat models of hypertension [54].

Most of the ET produced by the endothelial cells is secreted on the abluminal side rather than on the luminal side [55]. It is therefore not surprising that plasma ET levels do not accurately reflect the local production of ET. It is more precise to say that plasma ET levels partly reflect the local events. Therefore, caution must be taken especially when interpreting marginally changed plasma levels.

The next issue to be taken into account is the
responsiveness to ET, since the biological action of ET could also be determined by sensitivity on the receptor side. However, changes in the pressor response in vivo [56, 57] and in the vasoconstrictive response of isolated arteries in vitro [58-61] of SHR are inconclusive. Increased sensitivity to ET-1 despite the normal plasma levels reported in some studies in SHR [56, 59] could be partly involved in the maintenance of hypertension.

How can the role of endogenous ET be assessed in vivo? To better address the issue, we have utilized anti-ET antiserum to block the action of ET [36]. The infusion of ET-specific antibodies into SHR decreased the mean arterial pressure by approximately 10% and renal vascular resistance by approximately 35%. Both the glomerular filtration rate and renal plasma flow increased by approximately 50% over the control. By contrast to this, the infusion of normal rabbit serum in SHR or of ET-specific antibodies in WKY rats did not result in any significant changes in these hemodynamic parameters. These findings suggest that endogenous ET plays an important role in the modulation of systemic blood pressure and renal function in SHR. The fact that the plasma ET-1 level in SHR was even lower than that in WKY indicates difficulty in ascertaining the role of ET from the plasma levels. The profound effect of ET-1 on renal function may play a key role in the development of hypertension, since the kidneys play a central role in pressure-volume homeostasis [62].

This finding is in agreement with the findings obtained with phosphoramidon, a non-selective inhibitor of ET-converting enzyme (our unpublished data). The development of specific antagonists for ETA receptor [63, 64] enabled us to evaluate more specifically the role of ET-1 in hypertension. Despite the specificity of the blockade, the results do not completely coincide with each other. Bazil et al. [65] demonstrated that BQ-123 has a mild antihypertensive effect in SHR and DOCA/salt hypertensive rats but not in renin-dependent hypertensive rats. However, Nishikibe et al. [66] reported a significant reduction in blood pressure in SHR-SP, a model of malignant hypertension, especially after salt loading for 6 weeks, but not in SHR. Differences in the results may be partly attributed to the different pathogenesis in each hypertension model and dosage of the antagonists. ETA receptor-mediated action [25, 26] may also modify the effects of the ETA receptor antagonist.

It is tentatively concluded that ET-1 is partially involved in the development and maintenance of hypertension in some experimental models and human essential hypertension. The long term effects on the vascular structure are of potential importance in the development of hypertension. Studies with ET receptor antagonists for a longer period are obviously critical for the issue.

2) Secondary Hypertension

There are several clinical conditions in which ET is believed to play a crucial role in hypertension. One of the most interesting conditions is the ET-secreting malignant hemangioendothelioma [67]. Increased plasma ET, positive immunohistochemical staining for ET, the presence of immunoreactive ET, expression of ET mRNA, and normalization of blood pressure and plasma ET levels after removal of the tumor, all indicate an excess production of ET from the tumor as a cause of hypertension. Although this is definitely the discovery of a new type of secondary hypertension, the significance of the disease waits additional case reports.

Hypertension following erythropoietin therapy in patients under chronic hemodialysis may be another model of "ET-dependent hypertension" [68]. The plasma ET-1 levels were significantly higher in patients with hypertension than in those without hypertension. Changes in plasma ET-1 occurred in parallel to that of mean blood pressure. Discontinuance of the erythropoietin therapy resulted in a parallel decrease in plasma ET-1 and blood pressure. Since the endothelial cells possess a receptor for erythropoietin, erythropoietin may directly act to stimulate ET-1 production by the endothelial cells. This is supported by the fact that erythropoietin increases the secretion of ET from the cultured endothelial cells in vitro (our unpublished observation).

Cyclosporine therapy is often associated with hypertension [69]. Cyclosporine increases ET-1 release from cultured endothelial cells [70]. In addition, cyclosporine-induced renal vasoconstriction is mediated by ET-1 and is protected by prior administration of an ETA receptor antagonist [71]. Therefore, ET-1 may play an important part in cyclosporine-induced hypertension. Furthermore, hypertension associated with disseminated intravascular coagulation [72] and eclampsia [73] may be attributed, at least in part, to the increased
plasma ET-1.

However, whether an increase in plasma ET-1 is central to the pathogenesis of these secondary hypertension or merely a phenomenon secondary to vascular injury and/or atherosclerosis remains unclear. Recently, an orally active ET receptor antagonist has been developed [74]. This antagonist capable of blocking both ETA and ETB receptors decreases blood pressure in sodium-depleted monkeys and prevents renal vasospasm after renal ischemia and cerebral vasospasm after subarachnoid hemorrhage. Blockade of the ETB receptor as well as the ETA receptor appears to be of clinical significance.

2. Pulmonary Hypertension

The lung contains a large amount of ET peptides, expresses abundant ET mRNA, and takes up large quantities of the peptide during passage [75]. These findings indicate that the lung is one of the major sites of the production and action of ET. ET stimulates DNA synthesis and cell proliferation of pulmonary vascular smooth muscle cells [76]. In this regard, pulmonary hypertension is another pathological state in which the role of ET has been extensively studied. Hypoxic stress is associated with increases in the plasma ET-1 level, and in ET-1 content and the expression of ET-1 mRNA of the lung, suggesting that the lungs are a source of plasma ET-1 and may play a role in the development of hypoxia-induced pulmonary hypertension [77]. In addition, the rat model of idiopathic pulmonary hypertension named fawn-hooded rats shows increased ET-1 content and ET-1 mRNA expression in the lung prior to the development of pulmonary hypertension. Changes in lung ET-1 are therefore not the consequence but probably a cause of pulmonary hypertension [78].

In monocrotaline-induced pulmonary hypertension in rats, Miyauchi et al. [79] showed an increase in plasma ET-1 associated with an increase in the expression of ET-1 mRNA in the kidneys and hearts. On the contrary, the expression of ET-1 mRNA and ET-1 content in the lungs decreased gradually as pulmonary hypertension developed, suggesting that the lungs are not the source of plasma ET-1. In addition, continuous infusion of BQ-123 significantly inhibited the progression of both pulmonary hypertension and right ventricular hypertrophy. Although the results on ET-1 contents in the lungs differ from our preliminary results which showed a significant increase of lung ET-1 [80], endogenous ET-1 may contribute to the progression of hemodynamic changes in monocrotaline-induced pulmonary hypertension.

A similar close relationship between the plasma ET-1 levels and pulmonary vascular resistance was found in patients with pulmonary hypertension [81-85], suggesting a role of ET-1 in humans also. In addition, ET-1 is synthesized and released from respiratory epithelial cells, suggesting a potential pathological role in bronchial asthma (for review see [11, 12]). Blockade of the ETA receptor could therefore be a useful therapeutic tool in the treatment of various pulmonary diseases.

3. Disease States Associated with Hypotension

Although hypertensinogenic aspects have been emphasized in discussing the significance of ET, it may not always be a pathological factor, but a protective factor. The remarkable increase in plasma ET demonstrated in endotoxin shock [86], cardiogenic shock [87], and septic shock [88] may imply a physiological, defensive role rather than a pathological role of ET, although the possibility that the increased ET may act as a deteriorating factor by reducing the renal blood flow and renal function cannot be completely excluded.

We determined the plasma ET-1 levels during whole body hyperthermic therapy in patients with advanced lung cancer [89]. The hyperthermia induced a significant hyperdynamic state; approximately a 2-fold increase in the heart rate and cardiac output. By contrast, the mean arterial pressure and peripheral vascular resistance decreased significantly. These changes were reversed after cooling off the body. Plasma ET-1 showed a 5-fold increase as a function of time. The significant increase in plasma ET-1 associated with hypotension suggests a role of ET-1 in maintaining the peripheral circulation. Although not yet conclusive, the role of ET in the hypotensive state is obviously different from that in the hypertensive state.

ET in the Endocrine System

1. Adrenal Gland

Surgical and autopic specimens of human adre-
nal tissues contain a sizable amount of ET (Fig. 3). The highest content was seen in a patient who died of acute myocardial infarction, while the lowest value was seen in the adenoma tissue of a patient with Cushing's syndrome. The ET-1 content was significantly higher than the ET-3 content in all the tissues examined. The fact that the human adrenal contains ET and expresses ET mRNA [6] indicates local production of ET, but it is too early to conclude that there is any correlation between ET content and the disease state.

What is the function of ET in the adrenal gland? The immunohistochemical localization of ET-1 in the zona glomerulosa cells of the human adrenal cortex (Fig. 4) suggests a possible role of ET in the regulation of adrenocortical function, especially in the zona glomerulosa. This is supported by the fact that the addition of ET-1 stimulates aldosterone secretion in vitro from the adrenocortical tissue of various animal species [29, 90-94]. However, no data are available in man.

We examined the effects of ET-1 on the steroid secretion from human adrenocortical tissue [95]. ET-1 stimulated the secretion of aldosterone but not cortisol from normal adrenal tissues and adjacent tissue in primary aldosteronism. The effects were dose-dependent and Ca²⁺-dependent. There

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**Fig. 3.** ET content in autopsic and surgical specimens of human adrenal tissues. Nos. 4, 7 and 9 are the adenoma portion and Nos. 8 and 10 are the adjacent portion in primary aldosteronism. Nos. 4 and 8, and Nos. 9 and 10 are from the same patient, respectively.

**Fig. 4.** Immunohistochemical localization of ET-1 in human adrenal tissue. Note specific staining in the zona glomerulosa (ZG). ZF: Zona fasciculata.
was no such stimulatory effect in the adenoma tissue of primary aldosteronism. These findings suggest that ET-1 is also a stimulatory factor selective to aldosterone also in humans.

Whether the ET-1 in the adrenal tissue modifies the aldosterone secretion is the next question. We examined the effects of BQ-123 on aldosterone secretion from bovine zona glomerulosa cells. The ETA receptor antagonist showed a slight but significant suppression of basal aldosterone secretion, suggesting that adrenal ET is involved in the regulation of basal secretion of aldosterone [96]. Although ET was reported to potentiate the aldosterone production by ACTH [92] or Ang II [94] in cultured bovine zona glomerulosa cells, BQ-123 did not affect the ACTH- and Ang II-induced aldosterone secretion in our study. These findings suggest that the effects of ACTH and Ang II on aldosterone secretion are not mediated by adrenal ET.

Specific binding sites for ET-1 were initially demonstrated by autoradiography [4, 5, 97]. Although the receptor initially identified by the binding study has a higher affinity with ET-1 and ET-2 than ET-3 [90, 94, 98], thus corresponding to the ETA receptor, another receptor with similar affinity with all the ET family peptides, thus corresponding to the ETB receptor, was found [91]. Recent studies with Northern blot analysis on the expression of ET receptor mRNA showed that the ETB receptor is the predominant form [99, 100]. Stimulation of aldosterone secretion is therefore assumed to occur through the ETB receptor. However, the effectiveness of the ETA-specific antagonist in blocking aldosterone secretion also involves the involvement of the ETA receptor. The results of the in situ hybridization study demonstrating the presence of ETA mRNA at the corticomedullary junction and the diffuse localization of ETB mRNA in both the cortex and the medulla [101] indicate different roles of each receptor, although it is not yet possible to correlate the biological action and the localization.

To elucidate the role of adrenal ET in the response to the systemic changes in body fluid homeostasis, the effects of DOCA/salt or low salt treatments on adrenal ET content were studied. Contrary to our expectations, adrenal ET-1 and ET-3 contents showed a significant increase and decrease after one-week of DOCA/salt and low salt treatments, respectively. In contrast to the changes in the adrenal, no significant change was seen in the liver, indicating that the changes in the adrenal are organ specific (Fig. 5). The plasma renin-angiotensin-aldosterone system is suppressed in DOCA/salt treatment, while it is stimulated in low salt treatment. The reciprocal changes in the adrenal ET content and the plasma aldosterone levels allow of alternative explanations: 1) decreased secretion of ET and 2) accelerated production of ad-
renal ET to counterregulate the decreased aldosterone secretion.

To address this question, we determined the changes in the preproET-1 mRNA in the adrenal tissue with RT-PCR. The PCR products showed a significant increase after DOCA/salt treatment and a decrease after low salt treatment, respectively (Fig. 6). ET in the adrenal may be regulated to compensate for the changes in aldosterone production in response to systemic stimuli.

Fig. 7 illustrates the role of ET in the adrenal gland taking all these findings together. Aldosterone secretion is mainly regulated by systemic factors including ACTH, Ang II, and potassium as stimulatory factors and natriuretic peptides as inhibitory factors [102]. ET may serve as one of the local factors such as the adrenal renin-angiotensin system [103] and IGF-1 [104]. How these systemic and local factors interact and cooperate with each other remains to be elucidated. In addition, details of the physiological and pathophysiological significance of adrenal ET are not yet known, but a higher content of ET-1 in the adenoma portion than in the adjacent tissue in primary aldosteronism (Fig. 3) suggests a pathological role in the excess production of aldosterone. The reduced responsiveness to exogenous ET-1 [95] could be explained by prior exposure to an increased amount of endogenous ET leading to receptor occupancy, receptor down regulation, or tachyphylaxis. Adrenal ET may also be involved in disproportionately increased plasma aldosterone compared to plasma renin during the recovery period from malignant hypertension [105]. The remarkable increase in adrenal ET content in a patient with acute myocardial infarction suggests that adrenal ET is involved in the stress-mediated response, a speculation requiring further investigation.

2. Hypothalamo-Pituitary Axis

1) Anterior Pituitary Gland

ET exists in a high concentration in the pituitary (Fig. 1). It is noteworthy that the anterior pituitary gland is one of the exceptional tissues where the ET-3 content is higher than that of ET-1 [22]. The expression of ET mRNA in the tissue [106] indicates its local synthesis. The rank order of the binding affinity of the isopeptides [107], the potency of the effects on the hormone secretion [108-
111], and in situ hybridization of ET mRNA [101], all indicate that ETα is the predominant ET receptor in the pituitary, whereas the presence of the ETβ receptor is suggested in some studies [106, 112]. Whatever the subtype of the ET receptor, local production of ET and the receptors in the same tissue suggest the role of ET as a paracrine/autocrine factor in the pituitary.

The functional interrelation between ET and the anterior pituitary was first described by Stojilkovic et al. [113]. ET was shown to increase the intracellular Ca\(^{2+}\) concentration and to stimulate the secretion of both LH and FSH in rats. Acute administration of rather higher doses of ET was subsequently demonstrated to stimulate the secretion of LH, FSH, GH, TSH, and PRL, while lower doses of ET for a longer period inhibited the secretion of PRL [110, 111, 114]. The biphasic effects of ET on PRL secretion, especially the inhibitory action of the later phase, coincide with other studies [108, 109, 112, 115]. The effect is influenced by specific ETα receptor antagonists [109, 111], but not by dopamine receptor antagonists [112, 116]. The PRL-inhibitory factor (PIF) found in the intermediate lobe was ET-like [117]. Therefore, the pituitary ET may be a PIF other than dopamine. Although the effects on the secretion of gonadotropin were demonstrated also in a gonadotroph-rich culture study [118], the effects on the pituitary hormones other than the PRL [108, 110, 111, 114, 116, 119] need further characterization.

The exact localization of ET in the pituitary is expected to provide information on the physiological role of ET. Samson et al. [117] showed intense immunohistochemical staining in the intermediate lobe and weak staining disseminated in the anterior pituitary of rats, suggesting paracrine control of anterior pituitary functions by ET from the intermediate lobe. By contrast, we found immunohistochemical staining of ET-3 in the cytoplasm of large ovoid cells of the human anterior pituitary. These cells were identified as gonadotrophs by means of a double staining technique and staining in the adjacent sections [120].

Despite of a possible interspecies difference, it is still fascinating to hypothesize the functional significance of the pituitary ET as illustrated in Fig. 8. Pituitary functions could be regulated by local factors in a paracrine/autocrine fashion. Of various types of pituitary cells, the folliculostellate cells [121] are quite intriguing as a possible center of local modulation. In addition, the gonadotrophs produce renin, Ang II [122], activin, ANP [123], and ET-3 [120] as well as gonadotropins. Since Ang II, activin, and ET-3 affect the secretion of gonadotropins, the gonadotrophs could be another candidate cells modulating locally the anterior pituitary functions. Close anatomical localization of gonadotrophs and lactotrophs provides further evidence to support the functional significance of ET-3-mediated paracrine regulation of PRL secretion.

2) Posterior Pituitary Gland

ET content is highest in the posterior pituitary. The interrelation between ET and posterior pituitary function was first described by Yoshizawa et al. [124]. ET was found immunohistochemically in the paraventricular and supraoptic nuclei of the hypothalamus and the axon terminals in the posterior pituitary. ET mRNA was also visualized in the paraventricular nucleus. After water deprivation for 4 days, ET in the posterior pituitary disappeared, suggesting a functional linkage between ET and vasopressin secretion. Since ET-1 stimulates the release of vasopressin from the perfused rat hypothalamus [125] and intracerebroventricular administration of ET-1 increases the plasma vaso-

![Fig. 8. ET-3 in gonadotrophs as a possible center of paracrine/autocrine regulation of anterior pituitary functions.](image-url)
pressin levels in conscious rabbits [126], ET-1 may act at the hypothalamic level on the perikarya of the magnocellular neurons. ET was also shown to potentiate the depolarization-induced vasopressin release from nerve endings in the posterior pituitary [127], suggesting that ET modulates vasopressin release at the pituitary level also. The ET receptor involved in this action is likely to be the ETA type [127], the same subtype as in the anterior pituitary.

Although a considerable number of neurosecretory granules in both types of axons were immunolabeled with antiserum to ET-1, the vasopressin-containing granules were more heavily labeled with anti-ET-1 antiserum than oxytocin-containing granules [128]. Double immunogold labeling clearly demonstrated intragranular colocalization of ET-1 and vasopressin or oxytocin. In addition, the posterior pituitary levels of ET-1, density of the neurosecretory granules, and the number of immunogold particles for ET-1 per neurosecretory granule, all showed a significant decrease after water deprivation (Table 2). Plasma vasopressin increased significantly after water deprivation, while plasma ET-1 was unchanged. All these findings support the notion that the ET-1 in the neurohypophyseal system is involved in the regulation of body fluid homeostasis by locally modulating the secretion of vasopressin at the level of the hypothalamus and/or the posterior pituitary.

Relevant to this issue, interaction between ET and vasopressin at the kidney level is noteworthy. ET increases urinary flow despite a decrease in renal blood flow and glomerular filtration rate [129, 130]. ET-1 inhibits vasopressin-evoked osmotic water permeability and cAMP accumulation in the cortical and medullary collecting ducts [35]. ET may therefore antagonize vasopressin action to increase urine volume despite the decrease in renal blood flow.

The interactions between ET and vasopressin in the hypothalamo-neurohypophyseal system and in the kidney seem to be independent of each other, since it is generally believed that ET acts locally rather than in an endocrine fashion. Both ET mRNA and ET receptor mRNA are expressed in these tissues. The fact that the plasma ET levels are unchanged after water deprivation also supports this view, but intravenous administration of ET provokes an increase in plasma vasopressin in dogs [131]. In addition, systemic ET acts at the subfornical organ which lacks the blood-brain barrier to have an excitatory action on vasopressin and oxytocin-secreting neurons in rats [132]. The possible involvement of circulating ET in the regulation of vasopressin secretion and/or vasopressin action can therefore not be completely excluded. The volume regulation by vasopressin may be under the dual control of the ET system-one in the brain and the other in the kidney.

Summary

Recent advances in ET research on the cardiovascular and endocrine systems were reviewed. Considering the potent vasculotropic actions, strategically advantageous localization, and recent findings with the specific receptor antagonist, ET is
potentially involved in the regulation of hemodynamic homeostasis and in the pathogenesis of essential and secondary hypertension. The mitogenic action on the vascular smooth muscle cells suggests its more chronic effect on the vascular structure. In addition to the hypertensinogenic aspects, the role of ET in maintaining blood pressure in a hypotensive condition should not be overlooked. The development of specific antagonists which block the action of locally operating ET in vivo will be a powerful tool in elucidating the pathophysiological significance of ET and will provide a new therapeutic approach for hypertension. The roles of ET in the endocrine system are also fascinating. Accumulating evidence supports the notion that ET modulates the secretion of pituitary and adrenal hormones. The mode of action is likely to be paracrine/autocrine rather than endocrine, although a possible role of circulating ET cannot be ruled out. The pathophysiological role of ET in the endocrine tissues remains to be clarified. The diversity of the action of ET on the blood pressure and endocrine functions provides further evidence of the complexity of the homeostatic mechanisms, leaving us an intriguing subject for future study.

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Note added in proof. After we submitted our manuscript in April 1994, an article by Shimada et al. describing cloning of endothelin-converting enzyme appeared in the J Biol Chem (269: 18275-18278).

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