Biochemical Pharmacology, Vol. 38, No. 12, pp. 1877–1883, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

COMMENTARY

ENDOTHELIN, A NOVEL ENDOTHELIUM-DERIVED PEPTIDE

PHARMACOLOGICAL ACTIVITIES, REGULATION AND POSSIBLE ROLES IN CARDIOVASCULAR CONTROL

MASASHI YANAGISAWA* and TOMOH MASAKI
Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

The contractility of blood vessels is regulated by various neural and hormonal signals together with the local regulatory mechanisms intrinsic to the blood vessel wall. Since the discovery of prostacyclin by Moncada and Vane [1], much attention has been paid to the importance of the vasoactive substances produced within the vessel wall. The discovery by Furchgott and Zawadzki of the obligatory role of vascular endothelium in the vasodilatation induced by acetylcholine, and the demonstration that an extremely short-lived diffusible substance, named "endothelium-derived relaxing factor" (EDRF), was involved in this response [2], have stimulated intense interest in the direct role of the endothelium in modulating vascular responsiveness. Since then, the characterization of numerous chemical and physical stimuli which induce endothelium-dependent relaxation [3], followed by the identification of one EDRF as nitric oxide or a closely related nitroso compound [4], has made the investigation of the endothelial function one of the most exciting fields in current cardiovascular research.

Substantial data are now available which demonstrate that, in addition to mediating vasodilatation, endothelium can also facilitate contractile responses of the vascular smooth muscle [5]. Various chemical and mechanical factors, including noradrenaline, thrombin, neuropeptide Y, calcium ionophore A23187, arachidonic acid, hypoxia, stretch, and increased transmural pressure, have been found to cause vasoconstriction dependent on, and/or enhanced by, the intact endothelium. These observations have led to the idea that endothelial cells under various stimuli can also secrete vasoconstrictor substance(s), in addition to potent vasodilators such as EDRF and prostacyclin. Although substances from several different chemical categories including eicosanoids, peptides and free radicals [6] have been proposed as these constrictor factors, none was rigorously evaluated previously.

Endothelin (ET) is a potent vasoconstrictor peptide that was recently characterized from the supernatant fraction of cultured vascular endothelial cells [7] and is the only endothelium-derived vasoconstrictor substance convincingly identified to date. In this article we will review the identification and the current status of knowledge of this novel peptide, and briefly discuss the possible physiological role of ET.

Identification of endothelin

Several investigators have described previously a protease-sensitive vasoconstrictor activity within the supernatant fraction of cultured bovine aortic endothelial cells [8–10]. This substance induced a slow-onset and extremely long-lasting vasoconstriction, apparently by a direct action on the vascular smooth muscle cells. Stimulated by our experience that the confluent monolayer culture of certain vascular endothelial cells can be maintained for weeks even in protein-free medium (which facilitates the purification of the secreted substances of minute content), we set up a large-scale culture of porcine aortic endothelial cells and successfully purified the vasoconstrictor from about 10 liters of the serum-free conditioned medium [7]. The purification was done by three steps of anion-exchange and reverse-phase HPLC, the constrictor activity contained in the fractions from each step being monitored by bioassay with porcine coronary artery strips. The active substance, porcine ET, was an acidic 21-residue (M_r 2492) peptide with free amino- and carboxy-termini containing two sets of intrachain disulfide bridges (Fig. 1). Synthetic ET was prepared by a solid-phase and solution chemistry with selective crosslinking of the four Cys residues [11]. The primary structure, as well as the disulfide topology, was confirmed by comparing the biological activities and the chromatographic properties of the natural and synthetic ETs.

The mRNA encoding for preproendothelin was subsequently cloned by screening the cDNA libraries constructed for endothelial cells from porcine aorta and human umbilical vein [7, 12]. The presence in these endothelial cells of mRNA encoding the pre-pro-form of ET precursor indicates that this peptide is produced by *de novo* synthesis and is processed in a manner similar to that of many peptide hormones and neuropeptides. The porcine and human precursors show a strong similarity in both nucleotide

* Correspondence: Masashi Yanagisawa, M.D., Ph.D., Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

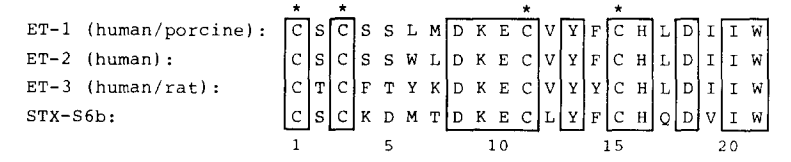

Fig. 1. Alignment of amino acid sequences of mammalian "ET family" peptides and an isoform of sarafotoxin S6 from venom of *Atractaspis engaddensis*. One-letter amino acid notation is used. Half-cystine residues are marked by asterisks; conserved amino acid residues are shown in boxes.

(79% identities) and amino acid (69% identities; 80% identities plus conservative substitutions) sequences. The sequences of the mature ET, in particular, are conserved perfectly. Although the structure of human ET has not yet been determined at the peptide level, the cDNA sequence shows that human mature ET is very likely identical to procine ET. The mature ET sequences were, as anticipated, directly preceded by paired basic residues Lys-Arg or Arg-Arg, which are known to be recognized by the processing endopeptidases. Interestingly, however, the carboxy-termini of both porcine and human mature ET lack the dibasic pairs. This indicates that the mature ET found in the EC-conditioned medium is generated through a very unusual proteolytic processing between the Trp^{21} and Val^{22} residues of the putative 38- (human) or 39- (porcine) residue "big endothelin," presumably involving an endopeptidase with a chymotrypsin-like specificity. The conversion of big ET to 21-residue ET is essential for the complete vasoconstrictor activity; in terms of molar potency, big ET is about 140 times less potent than ET as assayed on porcine coronary artery strips [13]. The localization and regulation of this putative "endothelin-converting enzyme" may have important implications for the control of the biosynthesis of ET, as well as on possible pharmacological intervention in the production of ET.

Analogous peptides

Sarafotoxin, a cognate peptide of endothelin in snake venom toxin. We [14] and others [15] have found a remarkable homology between ET and a class of 21-residue peptide toxins (sarafotoxins) recently purified and sequenced from the venom of the Israeli burrowing asp, *Atractaspis engaddensis* [16]. The strong sequence similarities, including the perfectly conserved four Cys residues and the C-terminal Trp residue (Fig. 1), convincingly suggest that ET and sarafotoxin have a common evolutionary origin. Sarafotoxins, like ET, have a potent coronary constrictor activity, to which the strong cardiac toxicity and lethality of the toxin are largely attributed [17]. In fact, intravenous injection of supra-pressor doses (4-6 nmol/kg) of ET is also highly lethal to rats, chiefly due to the severe coronary insufficiency induced by ET; the LD_{50} values for ET and sarafotoxins seem to be comparable. In this aspect, the extremely long-lasting vasoconstrictor activity of ET, which is quite difficult to wash out, may also exemplify the toxin-like property of this mammalian peptide. The existence of ET-related genes in a reptile may indicate the old evolutionary origin and the physiological importance of the ET/ET–receptor system. The evolutionary mechanism may have provided *A. engaddensis* with a weapon that targets this ligand–receptor system already in existence.

Mammalian "endothelin family" predicted by three separate endothelin-related genes. Three distinct ET-related genes were recently cloned by screening a human genomic DNA library under a low hybridization stringency [18]. Genomic Southern blot analysis demonstrated three corresponding genes not only in human but also in rat and porcine genomes. The amino acid sequences of the 21-residue peptides predicted by the three human genes were similar to but distinct from each other at several amino acid residues (Fig. 1). These peptides are called endothelin-1 (ET-1 or the original porcine/human ET), endothelin-2 (ET-2, with two amino acid substitutions from ET-1) and endothelin-3 (ET-3, with six amino acid substitutions). In this article, however, the term "ET" hereafter means ET-1 unless otherwise stated. A rat gene encoding a peptide identical to human ET-3 has also been cloned [19]. Synthetic human ET-2 and ET-3, like ET-1, caused a strong vasoconstriction *in vitro* and a transient depressor response followed by a sustained pressor response *in vivo*. The *in vitro* constrictor activity and the *in vivo* pressor activity was: ET-2 > ET-1 > ET-3. Interestingly, however, the initial transient depressor response *in vivo* was most profound in ET-3 [18]. These findings suggest the existence in many mammalian species of three distinct peptides from the "endothelin family", which might play differential physiological roles possibly by interacting preferentially with different subtypes of ET receptors. However, tissue localization of ET-2 and ET-3 is currently unknown; the vascular endothelium appears to express ET-1 only.

Structure–activity relationships. The amino acid sequences of the ETs and a sarafotoxin are compared in Fig. 1. Although these peptides are very similar to each other overall, the amino acid residues at positions 4–7 are quite variable. In contrast, (i) the four Cys residues, (ii) the C-terminal hydrophobic moiety, and (iii) the cluster of charged residues Asp^8-Lys^9-Glu^{10} are almost perfectly conserved, suggesting the importance of these regions for biological activity. Experiments with synthetic derivatives of ET demonstrated that these moieties are, in fact, essential for the expression of complete biological activity [20]: the removal of the C-terminal Trp residue or the substitution of the Trp residue with D-Trp reduced the vasoconstrictor activity by more than 100-fold; the destruction of the loop structure either

by the hydrolysis at Lys^9 with lysyl endopeptidase or by the reduction and alkylation of the Cys residues also attenuated the activity by two orders of magnitude. The topology of the disulfide bonds is also important for the activity; the two non-natural disulfide isomers of ET ([Cys^1-Cys^{11}/Cys^3-Cys^{15}]ET and [Cys^1-Cys^3/Cys^{11}-Cys^{15}]ET) were at least 100 times less active as compared with the native ET, which has the disulfide bonds Cys^1-Cys^{15} and Cys^{13}-Cys^{11} [11].

Pharmacological activities

Although ET was identified originally as a potent constrictor of vascular smooth muscle, as mentioned above, this peptide has been demonstrated to possess a spectrum of activities much wider than initially inferred.

Contraction of vascular smooth muscle. ET (10^{-11}–10^{-8} M) induces a dose-dependent contraction of isolated arterial and venous strips from various mammalian species including rat, guinea pig, rabbit, pig, dog, cat and human, almost irrespectively of the anatomical location of the blood vessels. In general, veins appear to be more sensitive to ET than arteries [21]. The vasoconstrictor activity is resistant to the antagonists of α-adrenergic, H_1-histaminergic, serotonergic and muscarinic receptors, and the inhibitors of cyclooxygenase and lipoxygenase, suggesting that ET acts directly on the smooth muscle cells. On porcine coronary artery strips, the dose–response relation of the constrictor effect of ET showed that the maximum tensions developed are comparable to those of KCl-induced contraction, and the EC_{50} value was 4–5 $\times$ 10^{-10} M, which is at least one order of magnitude lower than any other known vasoconstrictors effective on this artery. The EC_{50} values are also within the range of 2 $\times$ 10^{-10}–5 $\times$ 10^{-9} M on many other arteries/veins, indicating that ET is one of the most potent vasoconstrictors known to date.

Interestingly, ET constricts renal arteries from 12-week-old spontaneously hypertensive rats (SHR) at concentrations significantly lower than it constricts the arteries from age-matched Wistar Kyoto rats (WKY) [22]. The EC_{50} values were 6.6 $\times$ 10^{-10} M and 1.7 $\times$ 10^{-9} M in SHR and WKY respectively. The increased sensitivity to ET of the renal arteries from SHR could contribute to the maintenance of the hypertensive state in SHR.

The constrictor activity of ET on porcine coronary artery is slow in onset, long-lasting, and extremely difficult to wash out (usually lasting for hours despite repeated washing of the strips with the solution), although it is rapidly and completely reversed by the addition of agents such as isoproterenol, forskolin or glyceryl trinitrate, which raise the cellular cyclic nucleotide levels. The activity is dependent on extracellular Ca^{2+}; and the constrictive response is abolished in a Ca^{2+}-free ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA)-containing solution. The ET-induced contraction is inhibited very effectively by the preadministration of low doses (10^{-9}–10^{-8} M) of the dihydropyridine Ca^{2+}-channel blocker nicardipine. These observations suggest that the Ca^{2+}-influx through the voltage-dependent Ca^{2+} channels of the smooth muscle cells is important for the vasoconstrictor activity of ET.

In isolated perfused rat heart [19] and mesentery [21], the infusion of ET causes a sustained rise of perfusion pressure, suggesting that ET is also active on the resistance arterioles.

Pressor/depressor effects in vivo. Intravenous bolus injection of ET (0.1 to 3 nmol/kg) causes a transient, dose-related depressor response (lasting 0.5 to 2 min) followed by a sustained, dose-dependent rise in arterial pressure both in anesthetized rats pretreated with autonomic blockades [7, 23], and in intact, conscious rats [19]. The mechanism of the initial transient depressor response remains to be established, although ET has been found to induce the release of prostacyclin and/or EDRF from perfused tissues [21]. Moreover, in ganglion-blocked rats, the initial fall in arterial pressure was associated with increases in carotid and hindquarter vascular conductances but marked decreases in conductance in the renal and mesenteric vascular beds [23]. These effects were followed by a gradual but marked increase in arterial pressure accompanied by intense vasoconstriction in all four vascular beds. These observations indicate that the effects of ET on vascular beds *in vivo* vary in a complex manner depending on both time and the anatomical region of the vascular bed. The prominent feature of the pressor phase of the *in vivo* response to ET is its time course; more than 2–3 hr are typically required for return of arterial pressure to base-line levels after a 2 nmol/kg intravenous blous of ET to rats. Thus, the extremely long-lasting time course, both *in vivo* and *in vitro*, may be one of the most salient characteristics of the vascular effects of ET.

Contraction of non-vascular smooth muscles. ET also constricts various isolated non-vascular smooth muscle preparations, including intestinal and tracheal smooth muscles, in a dose-dependent manner [21]. ET (10^{-11}–10^{-8} M) induces a slow-developing and long-lasting contraction of guinea pig tracheal and human bronchial strips [24]. The EC_{50} value in these studies was in the range of 5 $\times$ 10^{-10} M, being more than 2 orders of magnitude lower than that of a well-known bronchoconstrictor, leukotriene D_4. The maximum response to ET was comparable to histamine and nearly twice that of leukotriene D_4. The bronchoconstrictor activity was also inhibited by low doses (10^{-8} M) of nicardipine.

Positive inotropic and chronotropic effects on myocardium. ET exerts a positive inotropic effect on isolated, electrically driven guinea pig left atria in a dose-dependent manner [25]. Although the maximum response (as increase in contractile tension) was only 30–40% of that to isoproterenol, the EC_{50} value was as low as about 10^{-9} M. The effect was not influenced by blockers of β-adrenergic, α-adrenergic, histaminergic or serotonergic receptors, or by indomethacin, suggesting that ET acted directly on the myocardium. However, the response was inhibited by nicardipine and was accompanied by an increase in duration of the action potentials (approximately 10%). In the atrial preparations in which the fast Na^+ channels were inactivated by high-K^+ depolarization, ET induced slow response action potentials. These observations suggest that the effect of ET is closely associated with an increase in the Ca^{2+} influx through the voltage-dependent Ca^{2+}

channels of the myocardial cells.

ET also induces a dose-dependent positive chronotropic effect on spontaneously beating guinea pig right atria [26]. The EC_{50} value was also in the range of 10^{-9} M. Interestingly, the chronotropic response to ET exhibited a marked tachyphylaxis.

Stimulation of atrial natriuretic peptide (ANP) secretion. Another cardiac action of ET is the stimulatory effect of ANP secretion on cultured neonatal rat atrial cardiocytes [27]. ET stimulated the secretion of immunoreactive ANP in a dose-dependent manner (10^{-10}–10^{-7} M) with an EC_{50} value of about 2×10^{-10} M. The effect of ET was attenuated by nicardipine. Further, the secretagogue action of ET was synergistically potentiated by tetradecanoyl phorbol acetate (TPA) but not by the dihydropyridine Ca^{2+}-channel agonist Bay K 8644. These findings are compatible with the idea that this effect of ET also involves the increase in the Ca^{2+} influx via the Ca^{2+} channels.

Renal effects. In isolated rat glomerular preparations, ET has been demonstrated to inhibit both basal and isoproterenol-stimulated renin release [28]. In contrast, ET did not inhibit the renin release stimulated by nifedipine. In these systems, the renin release from juxtaglomerular (JG) cells is stimulated by an increase in the cellular cyclic AMP levels (e.g. β-adrenergic agonists) and inhibited by an increase in the intracellular free Ca^{2+} concentrations. The observations above are consistent with the idea that ET may increase intracellular Ca^{2+} through a dihydropyridine-sensitive pathway and/or lower the cellular cyclic AMP levels in the JG cells.

ET injected into the renal artery of anesthetized dogs *in vivo* caused a marked, long-lasting decrease in the renal blood flow, accompanied by a significant decrease in glomerular filtration rate, urine volume, and urinary sodium and potassium excretion [29]. These findings suggest that ET may be involved in the regulation of renal function and also indicate that the actual *in vivo* effect of ET on renal renin secretion is stimulated due to the constriction of renal vasculature induced by ET. However, the above-mentioned inhibition of renin release by ET at the cellular level could be functioning in the extrarenal renin-angiotensin pathways.

Proliferation of vascular smooth muscle cells. ET stimulates [^{3}H]hymidine incorporation into cultured rat aortic smooth muscle cells and Swiss 3T3 fibroblasts deprived of serum [30, 31]. ET also induces the expression of the cell-cycle-sensitive proto-oncogenes such as c-*fos* and c-*myc* in these cells. The demonstration that ET possesses not only a constrictor effect but also a trophic effect on vascular smooth muscle cells has important implications for the physiologic and pathologic role of ET in the pathogenesis of atherosclerosis as well as in vascular hypertrophy under hypertensive conditions and under increased blood flow (see below).

Possible cellular mechanisms of action. The pharmacological characteristics for the activities of ET listed above are consistent with the idea that the action of ET involves Ca^{2+} influx through the dihydropyridine-sensitive Ca^{2+} channels of the target cells. Whatever the mechanisms of the activation of the Ca^{2+} channels by ET, ET action seems to be dependent on the presence of extracellular Ca^{2+} at least in some system. For example, the constrictor action of ET in porcine coronary artery seems to be mediated by the activation of the Ca^{2+} channels [32, 33].

However, it was also reported that ET can mobilize Ca^{2+} from intracellular storage sites [34]. Sarafotoxins, an ET-related peptide from snake venom (see above), induced hydrolysis of phosphoinositides in rat heart and brain and that the toxins thereby mobilized intracellular Ca^{2+} [15]. If sarafotoxins and ET (as expected) share a common cellular receptor for their actions, it is likely that ET also activates phosphoinositide turnover, at least in certain target cells. Actually, it was reported recently that ET stimulates phospholipase C in cultured vascular smooth muscle cells [35, 36] and fibroblasts [31]. Further study is required to elucidate whether the cellular action of ET is associated with both of the above-mentioned mechanisms, or involves yet another intracellular signaling pathway.

Receptor of endothelin

In cultured rat aortic smooth muscle cells, a single class of saturable, high-affinity binding sites for [^{125}I]Tyr^{13}-ET has been described [37]. The apparent dissociation constant (2–4×10^{-10} M) was comparable to the EC_{50} values for the ET-induced contraction of arterial strips. The binding was not affected by known vasoconstrictors (angiotensin II, arginine-vasopressin, neuropeptide Y, noradrenaline, serotonine, histamine), peptide neurotoxins (apamin, ω-conotoxin), or Ca^{2+}-channel blockers (nicardipine, diltiazem, verapamil), suggesting that ET binds to its novel receptor. The dissociation of the radiolabeled ET from the cells was extremely slow; 85% of the initial cell-bound radioactivity remained even after 2 hr. Although to what extent this was due to the internalization of the peptide is unknown, the slow dissociation from the putative receptor may partly explain the characteristically long-lasting vasoconstrictor activity of ET.

Autoradiographic studies of rat tissues with [^{125}I]Tyr^{13}-ET demonstrated the specific binding sites of ET, as expected, in the media of the blood vessels of various sizes. Moreover, the results showed that the binding sites were widely distributed not only in the blood vessels but also in the parencyma of other organs including the heart, kidneys, lungs, intestine, adrenal glands and brain [38]. A radioligand binding assay of the microsomal fractions from the ventricular myocardium and brain stem confirmed the existence in these tissues of the specific, single-class, high-affinity ($K_d = 4$–5×10^{-10} M) binding sites for ET. The binding was not displaced by angiotensin II, ANP, parathormone, ω-conotoxin, apamin, verapamil or nicardipine. The affinity of ET binding in the myocardium was comparable to the above-mentioned EC_{50} values of the cardiac effects of ET.

Regulation of production

Northern blot analysis of porcine tissues with cloned preproET cDNA as a probe showed that the mRNA was basally expressed not only in the cultured endothelial cells but also in the aortic endothelium *in vivo*. The cerebral cortex, atrium, lung or kidney

did not contain a detectable amount of preproET mRNA, suggesting that little ET is produced by parenchymal cells of these tissues. The fact that exogenously applied ET has a potent *in vivo* pressor activity suggests that ET is active also at the level of resistant arterioles. Whether ET is produced in the microvascular endothelium within the tissues or is synthesized chiefly in larger vessels and transported to the resistant vessels as a circulating hormone is unknown. Wherever the locations of ET production are, the basal tonus of vascular beds *in vivo* could be influenced by the constitutive production of ET by the endothelial cells *in vivo*.

PreproET mRNA is not only constitutively expressed but also markedly up-regulated in cultured endothelial cells by various chemical and mechanical stimuli including thrombin [7], Ca^{2+} ionophores [7], phorbol esters [39], transforming growth factor-β (TGF-β) [39, 40], and fluid-mechanical shear stress [40]. Vascular endothelial cells have very few secretory granules, in which the peptide can be stored and rapidly released in response to stimuli. Therefore, the regulation of the *release* of ET is very unlikely functioning in the endothelial cells. The production of ET may be regulated chiefly at the level of the expression and/or translation of the mRNA.

Concluding remarks—what are the physiological roles of endothelin?

The demonstration of the activities of ET other than the constriction of vascular smooth muscles, together with the existence of the receptor for ET in various non-vascular tissues including the brain, strongly suggests that ET mediates the regulatory function of a wide variety of tissues, both within and outside of the cardiovascular system. ET could possibly control the systemic arterial pressure and local blood distribution, the formation and composition of urine, the release of circulating hormones (e.g. renin, ANP, adrenaline) from kidneys, atria and adrenal glands, the tonus of airway smooth muscles, the movements of the intestinal tract, and various central nervous system functions including the autonomic regulation and higher functionalities.

Then, are vascular endothelial cells the only source of ET, a peptide that possibly exists within all these tissues? Sarafotoxins, a group of peptide toxins evolutionarily related to ET, are found in the exocrine venomous gland of snakes, an organ apparently unrelated to the vascular endothelium. This may indicate that a member(s) of the ET family is expressed in mammalian organs other than vascular endothelium. In the brain, especially, intravenously applied radioligand distributed *in vivo* only to the limited regions which lack well-developed blood–brain barriers (e.g. the median eminence of the hypothalamus) despite the fact that the specific binding sites for ET were widely distributed over the slices of the brain [38]. This may indicate that ET-1, ET-2 and/or ET-3 is produced within the brain, possibly by neurons, and acts as a neuropeptide. The fact that sarafotoxins also specifically bind to rat brain membranes and activate the phosphoinositide hydrolysis may support this view.

Wherever the sources of ET are, the production of ET within the mammalian body must be meticulously regulated, since ET is a highly lethal endogenous toxin as mentioned above. Here we propose two distinct modes of the regulation of ET production *in vivo*, in relation to its possible roles in cardiovascular control. First, as suggested above, ET could be a signaling molecule that maintains the basal homeostasis of systemic and/or local circulation under healthy conditions, like many vasoactive hormones such as catecholamines, angiotensin II, vasopressin and ANP. The question as to whether ET acts as a local autacoid or as a circulating hormone may be answered by using a highly sensitive and specific assay system for the measurement of blood and tissue levels of ET under various conditions. The time course of the activities of ET suggests that ET could be involved in a long-term regulation (in hours to days) of the cardiovascular system. The down-regulation of the renin-angiotensin pathway by ET at the cellular level, as well as the induction by ET of the secretion of a potent vasorelaxant ANP, may represent interesting examples of negative feedback interactions between ET and other hormonal systems.

Alternatively, ET may be produced locally in significant amounts only in an emergency and/or in defensive events such as primary hemostasis, wound repair, and inflammation, to mediate more or less irreversible processes. Blood coagulation/fibrinolysis factors, cytokines from various sources, kinines, and certain growth factors may be included in this category of biologically active substances. The fact that ET is induced by thrombin and TGF-β, which are known to be produced at the site of various tissue injuries, may support this idea. In contrast, the possibility that ET acts as a neuromodulator in the central nervous system, and the interaction of ET and other circulating hormones, implies that ET contributes to the basal regulation as discussed above.

It is tempting to suppose that the breakdown of the regulatory mechanisms of ET production could lead to various pathological conditions such as coronary and cerebral vasospasm, bronchospasm, atherosclerosis, and hypertension. The basal expression of the preproET mRNA in the cultured porcine aortic endothelial cells seems to be much higher than that in the aortic endothelial cells *in vivo*. It has been generally accepted that cultured endothelial cells approximate to a state of injured endothelium *in vivo* [41]. Furthermore, it seems reasonable to assume that the factors that stimulate the expression of ET (e.g. thrombin, TGF-β, increased shear stress) would accumulate at the site of endothelial injury. We speculate that ET is produced more actively around the site of endothelial damage and, having both contractile and proliferative effects on vascular smooth muscle cells, contributes to the pathogenesis of atherosclerosis as well as the vasospasm which is preferentially seen in sclerotic lesions.

A complete definition of the physiological roles of ET would require a specific antagonist for the ET receptor, the development of which may require much time and effort. However, the availability of the synthetic peptides, specific antibodies and cloned

DNA probes will help us to answer several of the above points in the near future.

Acknowledgements—The authors thank Drs Katsutoshi Goto and Sadao Kimura for helpful discussions.

REFERENCES

1. Moncada S and Vane JR, Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol Rev* **30**: 293–331, 1979.
2. Furchgott RF and Zawadzki JV, The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**: 373–376, 1980.
3. Furchgott RF, The role of endothelium in the responses of vascular smooth muscle to drugs. *Annu Rev Pharmacol Toxicol* **24**: 175–197, 1984.
4. Moncada S, Radomski MW and Palmer RMJ, Endothelium-dervied relaxing factor: Identification as nitric oxide and role in the control of vascular tone and platelet function. *Biochem Pharmacol* **37**: 2495–2501, 1988.
5. Rubanyi GM, Endothelium-derived vasoconstrictor factors. In: *Endothelial Cells* (Ed. Ryan US), Vol. 3, pp. 61–74. CRC Press, Boca Raton, FL, 1988.
6. Vanhoutte PM and Katusic ZS, Endothelium-derived contracting factor: Endothelin and/or superoxide anion? *Trends Pharmacol Sci* **9**: 229–230, 1988.
7. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K and Masaki T, A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**: 411–415, 1988.
8. Hickey KA, Rubanyi G, Paul RJ and Highsmith RF, Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am J Physiol* **248**: C550–556, 1985.
9. Gillespie MN, Owasoyo JO, McMurtry IF and O'Brien RF, Sustained coronary vasoconstriction provoked by a peptidergic substance released from endothelial cells in culture. *J Pharmacol Exp Ther* **236**: 339–343, 1986.
10. O'Brien RF, Robbins RJ and McMurtry IF, Endothelial cells in culture produce a vasoconstrictor substance. *J Cell Physiol* **132**: 263–270, 1987.
11. Kumagaye S, Kuroda H, Nakajima K, Watanabe TX, Kimura T, Masaki T and Sakakibara S, Synthesis and secondary structure determination of porcine endothelin: An endothelium-derived vasoconstricting peptide. *Int J Pept Protein Res* **32**: 519–526, 1988.
12. Itoh Y, Yanagisawa M, Ohkubo S, Kimura C, Kosaka T, Inoue A, Ishida N, Mitsui Y, Onda H, Fujino M and Masaki T, Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: Identity of human and porcine endothelin. *FEBS Lett* **231**: 440–444, 1988.
13. Kimura S, Kasuya Y, Sawamura T, Shinmi O, Sugita Y, Yanagisawa M, Goto K and Masaki T, Conversion of big endothelin to 21-residuc endothelin is essential for expression of full vasoconstrictor activity: Structure–activity relationships of big endothelin. *J Cardiovasc Pharmacol*, in press.
14. Takasaki C, Yanagisawa M, Kimura S, Goto K and Masaki T, Similarity of endothelin to snake venom toxin. *Nature* **335**: 303, 1988.
15. Kloog Y, Ambar I, Sokolovsky M, Kochva E, Wollberg Z and Bdolah A, Sarafotoxin, a novel vasoconstrictor peptide: Phosphoinositide hydrolysis in rat heart and brain. *Science* **242**: 268–270, 1988.
16. Takasaki C, Tamiya N, Bdolah A, Wollberg Z and Kochva E, Sarafotoxins S6: Several isotoxins from *Atractaspis engaddensis* (burrowing asp) venom that affect the heart. *Toxicon* **26**: 543–548, 1988.
17. Lee SY, Lee CY, Chen YM and Kochva E, Coronary vasospasm as the primary cause of death due to the venom of the burrowing asp *Atractaspis engaddensis*. *Toxicon* **24**: 285–291, 1986.
18. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K and Masaki T, The human endothelin family: Three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA*, in press.
19. Yanagisawa M, Inoue A, Ishikawa T, Kasuya Y, Kimura S, Kumagaye S, Nakajima K, Watanabe TX, Sakakibara S, Goto K and Masaki T, Primary structure, synthesis and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. *Proc Natl Acad Sci USA* **85**: 6964–6967, 1988.
20. Kimura S, Kasuya Y, Sawamura T, Shinmi O, Sugita Y, Yanagisawa M, Goto K and Masaki T, Structure–activity relationships of endothelin: Importance of the C-terminal moiety. *Biochem Biophys Res Commun* **156**: 1182–1186, 1988.
21. de Nucci G, Thomas R, D'Orleans-Juste P, Antunes E, Walder C, Warner TD and Vane JR, Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* **85**: 9797–9800, 1988.
22. Tomobe Y, Miyauchi T, Saito A, Yanagisawa M, Kimura S, Goto K and Masaki T, Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. *Eur J Pharmacol* **152**: 373–374, 1988.
23. Wright CE and Fozard JR, Regional vasodilatation is a prominent feature of the haemodynamic response to endothelin in anaesthetized, spontaneously hypertensive rats. *Eur J Pharmacol* **155**: 201–203, 1988.
24. Uchida Y, Ninomiya H, Saotome M, Nomura A, Ohtsuka M, Yanagisawa M, Goto K, Masaki T and Hasegawa S, Endothelin, a novel vasoconstrictor peptide, as potent bronchoconstrictor. *Eur J Pharmacol* **154**: 227–228, 1988.
25. Ishikawa T, Yanagisawa M, Kimura S, Goto K and Masaki T, Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. *Am J Physiol* **255**: H970–H973, 1988.
26. Ishikawa T, Yanagisawa M, Kimura S, Goto K and Masaki T, Positive chronotropic effects of endothelin, a novel endothelium-derived vasoconstrictor peptide. *Pflugers Arch* **413**: 108–110, 1988.
27. Fukuda Y, Hirata Y, Yoshimi H, Takatsugu K, Kobayashi Y, Yanagisawa M and Masaki T, Endothelin is a potent secretagogue for atrial natriuretic peptide in cultured rat atrial myocytes. *Biochem Biophys Res Commun* **155**: 167–172, 1988.
28. Rakugi H, Nakamaru M, Saito H, Higaki J and Ogihara T, Endothelin inhibits renin release from isolated rat glomeruli. *Biochem Biophys Res Commun* **155**: 1244–1247, 1988.
29. Yukimura T, Yamashita Y, Shichino K, Skimmen T, Saito M, Miura K, Okumura M and Yamamoto K, Effects of endothelin on renal hemodynamics and urine formation in anesthetized dogs. In: *Biosignalling in cardiac and Vascular Systems* (Eds. Fujiwara M, Narumiya S, Taniguchi T and Miwa S). Pergamon Press, Oxford, in press.
30. Komuro I, Kurihara H, Sugiyama T, Takaku F and Yazaki Y, Endothelin stimulates c-*fos* and c-*myc* expression and proliferation of vascular smooth muscle cells. *FEBS Lett* **238**: 249–252, 1988.
31. Takuwa N, Takuwa Y, Yanagisawa M, Yamashita K and Masaki T, A novel vasoactive peptide endothelin stimulates mitogenesis through inositol lipid turnover in Swiss 3T3 fibroblasts. *J Biol Chem*, in press.

32. Goto K, Kasuya Y, Matsuki N, Takuwa Y, Kurihara H, Ishikawa T, Kimura S, Yanagisawa M and Masaki T, Endothelin activates dihydropyridine-sensitive, voltage-dependant Ca^{2+} channels in vascular smooth muscle. *Proc Natl Acad Sci USA*, in press.
33. Kasuya Y, Ishikawa T, Yanagisawa M, Kimura S, Goto K and Masaki T, Mechanism of contraction to endothelin in the isolated porcine coronary artery. *Am J Physiol*, in press.
34. Miasiro N, Yamamoto H, Kanaide H and Nakamura M, Does endothelin mobilize calcium from intracellular store sites in rat aortic vascular smooth muscle cells in primary culture? *Biochem Biophys Res Commun* **156**: 312–317, 1988.
35. Van Renterghem C, Vigne P, Barhanin J, Schmid-Alliana A, Frelin C and Lazdunski M, Molecular mechanism of action of the vasoconstrictor peptide endothelin. *Biochem Biophys Res Commun* **157**: 977–985, 1988.
36. Resink TJ, Scott-Burden T and Buhler FR, Endothelin stimulates phospholipase C in cultured vascular smooth muscle cells. *Biochem Biophys Res Commun* **157**: 1360–1368, 1988.
37. Hirata Y, Yoshimi H, Takata S, Watanabe TX, Kumagai S, Nakajima K and Sakakibara S, Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. *Biochem Biophys Res Commun* **154**: 868–875, 1988.
38. Koseki C, Imai M, Hirata Y, Yanagisawa M and Masaki T, Autoradiographic distribution of binding sites for [^{125}I]-endothelin in rat tissues. *Am J Physiol*, in press.
39. Yanagisawa M, Inoue A, Takuwa, Y, Mitsui Y, Kobayashi M and Masaki T, The human preproendothelin gene: Possible regulation by endothelial phosphoinositide-turnover signaling. *J Cardiovasc Pharmacol*, in press.
40. Kurihara H, Yoshizumi M, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Hamaoki M, Kato H and Yazaki Y, Transforming growth factor-β stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochem Biophys Res Comm*, in press.
41. Ross R, The pathogenesis of atherosclerosis—An update. *N Engl J Med* **314**: 488–500, 1986.