Endothelins[1]

G. M. RUBANYI[2] AND L. H. PARKER BOTELHO
Berlex Laboratories, Inc., Cedar Knolls, New Jersey 07927, USA

ABSTRACT **The discovery of endothelium-derived relaxing (prostacyclin, EDRF) and contracting factors (EDCF) in the past decade opened up new vistas not only for basic and clinical research, but revolutionized our thinking about regulation and control of the cardiovascular system in health and disease. One of the most exciting developments in recent years was the discovery of a peptidergic EDCF and its isolation and identification as a unique 21-amino-acid peptide, endothelin (ET). This review summarizes the state-of-the-art in some areas of this fast-moving field, including the biosynthesis, tissue-specific expression, and binding of ET isoforms. Recent information about the nature of endothelin-converting enzyme (ECE) and the cloning, sequencing, and expression of ET receptor subtypes will be discussed. Based on current knowledge of the wide variety of biological actions of ETs, working hypotheses are presented about the possible autocrine, paracrine, and humoral actions of ETs and their potential role in modulating cardiovascular functions. In addition, the proposed significance of ETs in human cardiovascular diseases is summarized. In spite of the abundance of studies generated over the past 3 years and the postulated working hypotheses based on these findings, the true significance of ETs in short- and long-term regulation/modulation of tissue function remains to be determined. This will be the task of future investigations, using more sensitive detection methods and selective inhibitors of the biosynthesis and actions of ETs.— Rubanyi, G. M.; Parker Botelho, L. H. Endothelins. *FASEB J.* 5: 2713–2720; 1991.**

Key Words: aldosterone • atrial natriuretic peptide • big endothelin • cardiovascular diseases • EDRF • endothelium • endothelin-converting-enzyme • endothelium-derived vasoactive factors • endothelin receptors • gene expression • heart • kidney • nervous system • platelets • prostacyclin • renin-angiotensin system • vascular smooth muscle

DISCOVERY OF ENDOTHELIN AND ITS ISOFORMS

The discovery that endothelial cells synthesize and release substances that cause vasorelaxation or vasoconstriction revealed a novel role of the endothelium in the control of cardiovascular homeostasis in health and disease. The first endothelium-derived vasoactive factor, prostacyclin, was isolated and identified in 1976 (1). In 1980 the existence of a second potent vasorelaxant substance, nonprostanoid in nature, was discovered (2). This substance, which mediates endothelium-dependent relaxation to a variety of agents, was named endothelium-derived relaxing factor or EDRF.[3]

To bioassay EDRF in conditioned medium from cultured bovine aortic endothelial cells, it was discovered by Hickey et al. (3) that addition of the conditioned medium caused slowly developing and long-lasting vasoconstriction in isolated rings of canine, porcine, or bovine coronary arteries. Preliminary characterization revealed that the vasoconstrictor was a peptide, and analogous to EDRF it was named endothelium-derived contracting factor or EDCF (3). This peptidergic EDCF was isolated, purified, and identified as a 21-amino-acid unique peptide, termed endothelin (ET) by Yanagisawa et al. (4). The peptide contains two intrachain disulfide bonds and is homologous to Sarafotoxin S6b (5).

The corresponding cDNA was also isolated and sequenced, which implied that the biologically active 21-amino-acid mature polypeptide was synthesized as a much larger (203 amino acids) preproprotein precursor that required biochemical processing (4). One of the most remarkable steps in the progress of endothelin research after the initial publications (3, 4) was the discovery of isoforms of ET. Low hybridization stringency Southern blot analysis of human genomic DNA revealed the existence of three distinct genes, which encode three distinct ET peptides (6) termed endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3). A fourth isoform, deduced from the gene sequence of mouse genome, expressed exclusively in the intestine (7), was named endothelin-β or vasoactive intestinal-constricting peptide (VIC). Since its discovery 3 years ago, many laboratories have studied the molecular biology, diverse biological actions, and potential physiological and pathological significance of ET. Because of limitations of space and some recent excellent reviews (8–10), we can discuss only specific areas of this rapidly developing field including tissue-specific expression and biosynthesis, receptor subtypes and proposed autocrine, and paracrine and endocrine function of the ET isopeptides. Data regarding the potential physiological and pathological significance of this new family of homologous peptides are summarized, and working hypotheses for future research are presented.

PROTEIN STRUCTURE OF PROENDOTHELIN AND ENDOTHELIN ISOFORMS

The amino acid sequences and proposed disulfide bonding structure of the four known isoforms of ET (4, 6, 7) are shown in **Fig. 1.** Human and procine ET-1 have identical sequences. Human and mouse (VIC) ET-2 differ from one another by one amino acid and differ from ET-1 by two and

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[2]To whom correspondence should be addressed, at: Schering AG, Research Center, Institute of Pharmacology, P.O. Box 65 03 11, 1000 Berlin 65, Germany.

[3]Abbreviations: ACE, angiotensin-converting enzyme; EDRF, endothelium-derived relaxing factor; EDCF, endothelium-derived contracting factor; ET, endothelin; ECE, endothelin-converting enzyme; VIC, vasoactive intestinal constricting peptide; TGFβ, transforming growth factor β; IL 1, interleukin 1; irET, immunoreactive ET; SHR, spontaneously hypertensive rats; GFR, glomerular filtration rate; RIA, radioimmunossay.

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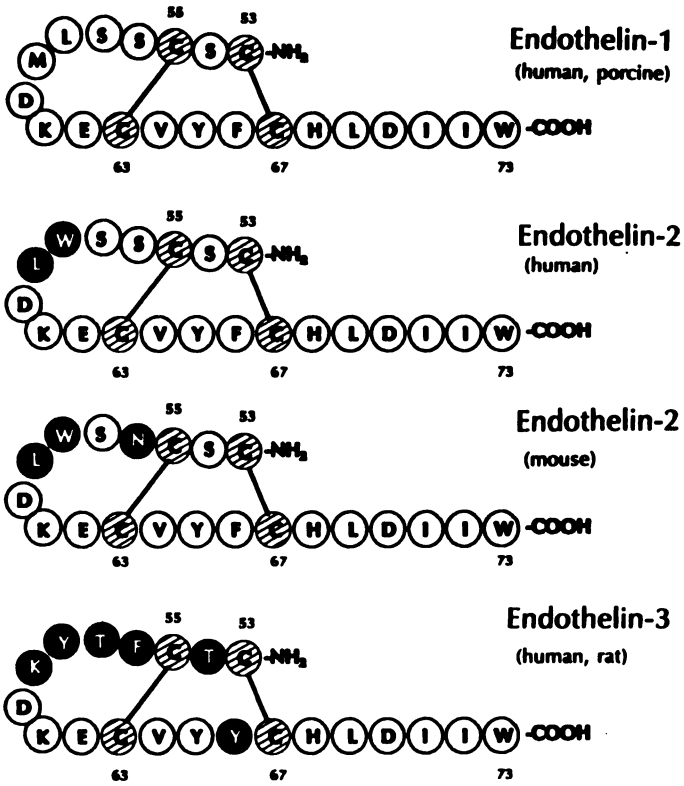

Figure 1. The amino acid sequences of the three isoforms of endothelin from different species. Cysteines believed to form disulfide bonds are represented by cross-hatched circles. The filled circles represent amino acid sequence changes compared with the endothelin-1 sequence.

three amino acids, respectively. Human and rat ET-3 have identical sequences, but differ from ET-1 by six amino acids.

The corresponding amino acid sequences and disulfide bonding structures for the four known isoforms of the immediate biological precursor of ETs, referred to as proendothelins or big endothelins (big ET), are shown in **Fig. 2.** The sequence of the big ET-1 isoforms from the human gene vs. the porcine gene vary by two amino acids. The porcine isoform contains 39 amino acids whereas the human isoform contains 38 amino acids with one additional amino acid substitution in the common 38 residues. The big ET-2 isoform from the human gene contains 37 residues with eight amino acid changes compared with human big ET-1. The big ET-3 isoform from both human and dog are identical, but somewhat larger than the other three big ET isoforms, containing 41 amino acids. There are in addition 12 amino acid substitutions within the first 38 amino acids of big ET-3 compared with human big ET-1. The most interesting difference among the sequences for the various big ET isoforms is that the proposed processing sites are different, with a Trp^{21}-Ile^{22} dipeptide in big ET-3 vs. a Trp^{21}-Val^{22} dipeptide for the others.

ENDOTHELIN GENE EXPRESSION

Endothelin gene expression and translation into an active protein product has been studied most extensively in cultured endothelial cells. Little or no mature or big ET has been found intracellularly and the rates of release into the cell culture media in the absence of serum over a 24-h period are linear, which suggests that the release is constitutive. The total amount of big ET-1 and ET-1 as well as the ratio of the two vary among different cell preparations from different species and tissue sources (for review, see refs 11, 12). In addition, the total amount of protein products and conversion of the proform of the final product also may vary depending on the confluency of the endothelial cells, the composition, and pH of the cell culture media (11). However, a cultured cell system might not accurately reflect the in vivo situation, and the constitutive release of ET may only represent release from activated cells or endothelial cells which lack their physiological environment (e.g., presence of smooth muscle, extracellular matrix, etc.).

The induction of ET-1 mRNA and the rate of peptide release have been found to be increased by a growing number of agents or mechanical stimuli, including thrombin, transforming growth factor β (TGFβ), angiotension II, vasopressin, hemodynamic shear stress, interleukin 1 (IL 1), phorbol esters, and calcium ionophores (11). Many reported inducers of ET production are known to promote intracellular calcium accumulation and/or protein kinase C activation, which may act at the level of transcription and/or translation. The increase in mRNA induced by agents such as phorbol esters and TGFβ has been suggested to result from increased transcription of the ET-1 gene possibly via interaction of intracellular mediators with cis-nucleotide sequences (9).

TISSUE-SPECIFIC EXPRESSION OF ENDOTHELINS

The cellular sites of synthesis of the proendothelin and ET isoforms have been assessed by several different techniques in cultured cells, tissue samples, and whole animals. Endothelin-1 mRNA was detected in porcine aortic endothelial cells (4) and in human umbilical vein endothelial cells (6). These same cells were specifically probed for ET-3 mRNA and were found to be negative. To date, measurable

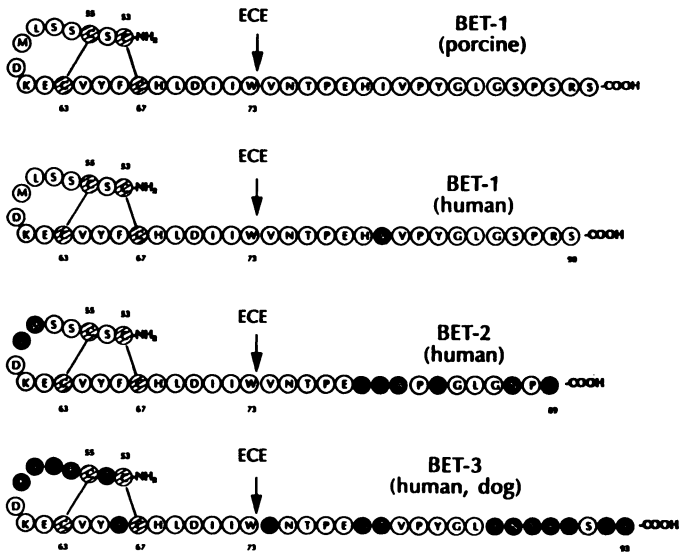

Figure 2. Amino acid sequences of the three isoforms of the immediate biological precursor of endothelin (BET) from different species. Cysteines believed to form disulfide bonds are represented by cross-hatched circles. The filled circles represent amino acid sequence changes compared with the big endothelin-1 sequence. The arrow indicates the unusual proteolytic cleavage site (by endothelin-converting enzyme; ECE, see also Fig. 3) responsible for production of the biologically active mature endothelins.

mRNA levels have been detected in cultured cells only for preproendothelin-1 but not for preproendothelin-2 and -3. Neuronal cell bodies of the human spinal cord, human dorsal root ganglia (13), and porcine paraventricular nuclear neurons (14) have also been found to contain measurable ET-1 mRNA.

In contrast, measurable levels of ET-1 or ET-3 mRNA have been detected by Northern blot analysis and in situ hybridization in many tissues from various species (12). When studying mRNA levels in adult vs. fetal tissues, both ET-1 and ET-3 mRNA have been detected in a number of human fetal, but not adult, tissues (such as fetal lung, spleen, and pancreas), which implies that perhaps mRNA degradation is slower or that the ET gene is more freely expressed in developing tissue (15). Endothelin-2 (VIC) gene expression has been detected by Northern blot analysis only in the mouse intestine and not in other tissues (7).

It is also possible to detect and quantitate the protein products of endothelin gene expression in cultured cells and tissue homogenates. Many cell lines have been investigated for ET isoform synthesis and have been found not to have detectable levels of mRNA or immunologically reactive peptides (12). Currently, the ET-1 isoform, and in some cases its protein precursor big ET-1, have been detected in the culture media of endothelial and epithelial cells derived from different tissues and organs from several species (for review see ref 12). Only one cell type, monkey Cos-7 kidney cells, has been found to produce an endothelin-2-like peptide (16). There are no data available on the biological precursor of ET-2. Similarly, immunologically reactive ET-3 has been quantitated thus far exclusively in culture media from canine tracheal epithelial cells (17), and again there are no data available on the presence or absence of the precursor big ET-3.

There are many reports of immunoreactive ET (irET) in tissue homogenates (e.g., refs 15, 18, 19), but these data could represent cellular production from a mixture of many cell types, including endothelial cells derived from capillaries and/or small arteries or venules remaining during preparation of tissues. In addition, the levels of irET in tissue homogenates (e.g., lung) may represent peptide bound to or taken up by cells as opposed to synthesis by resident cells.

The predominant form of ET detected in tissues has been reported as ET-1 but most commercially available radioimmunossay (RIA) kits do not adequately distinguish between ET-1 and ET-2, so the data could be misleading. The levels reported for ET-1 vary from 0.02 pmol/g tissue in rat pituitary to 2.48 pmol/g tissue in porcine renal inner medulla (for review, see ref 12). In almost all tissues tested, ET-3, which can be distinguished from ET-1 and ET-2 if present in high enough concentrations, was detected at lower levels compared with ET-1 or not at all. This is not the case in rat pituitary, where ET-3 levels were fourfold higher than that of ET-1 (19); in rat inner medulla, where equal amounts of ET-1 and ET-3 were detected (18); and in rat intestine, where ET-3 levels were approximately half that of the ET-1 levels (19).

Estimates of ET isoform synthesis in vivo has been evaluated by quantitating circulating levels of irET in plasma, excreted levels in urine, and levels in body fluids such as cerebrospinal fluid (20, 21). Compared with plasma levels it was found that concentrations of irET were on average sixfold higher in human urine samples (20) and about sevenfold higher in normal human cerebrospinal fluid (21). Studies measuring circulating ET levels in plasma have reported exclusively values of ET-1 (0.1–25 pM) [e.g., 22] with the exception of dog plasma, which we found to contain a significantly higher concentration of ET-3 (213 pM) compared with ET-1 (25 pM) (12). The relatively high circulating centrations of ET-3 in dog plasma were unexpected as the production of ET-3 has been detected so far only in dog tracheal epithelial cells (17).

BIOSYNTHESIS OF MATURE ENDOTHELIN-1: THE NATURE OF ENDOTHELIN-CONVERTING ENZYME

The amino acid sequences of prepro-, pro(big)-, and mature ET-1 predicted a hypothetical biosynthetic pathway for production of the 21-amino-acid mature ET-1 (4) (**Fig. 3**). The 203-residue prepro-form is initially processed by dibasic pair-specific endopeptidases and carboxypeptidases to yield a 39-amino-acid intermediate (big ET-1), and the big ET-1 is converted to the mature form via an unusual proteolytic processing between Trp^{21} and Val^{22} by a putative enzyme, named endothelin-converting enzyme (ECE) (4). As the vasoconstrictor activity of big ET-1 is about 100-fold lower than that of ET-1 (23), this step in the biosynthesis appears to be important for the biological significance of ET-1. This hypothesis was supported by the presence of big ET-1 and its carboxyl (COOH)-terminal fragment, big ET-1 (*22–39*), in the conditioned medium of endothelial cells (24). Intravenous injection of big ET-1 in rats evoked a pressor effect with a potency similar to ET-1 (25), suggesting a rapid and efficient conversion of exogenous big ET-1 to biologically active ET-1 in vivo.

Characterization of the conversion of big ET-1 to ET-1 in cultured bovine carotid artery (26) and porcine aortic endothelial cells (27) revealed that ECE may be a membrane-bound neutral metalloproteinase, as production of ET-1 by endothelial cells or conversion of exogenously added big ET-1 to ET-1 had a narrow pH optimum (at pH 7.1) and could be selectively inhibited by phosphoramidon and EDTA, but not by various inhibitors of aspartic, serine, or cysteine proteases. Although a good sequence homology exists around the cleavage site of big ET-1 and big ET-3, this

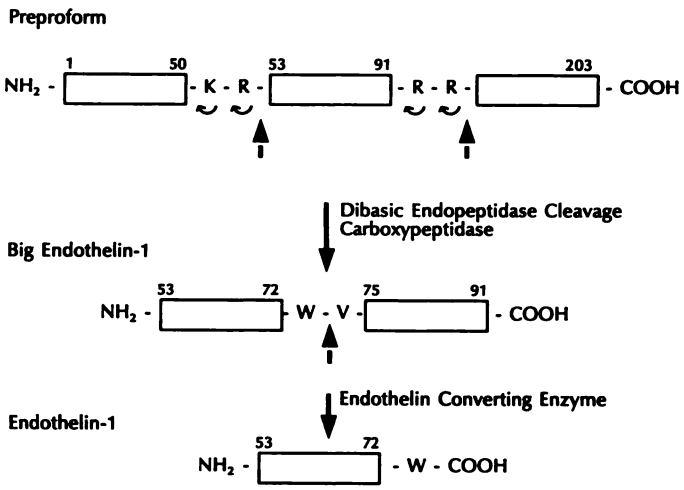

Figure 3. The proposed proteolytic processing pathway for conversion of preproendothelin to endothelin. The preproform of porcine/human endothelin-1 which contains 203 amino acids is believed to be converted to the 39-amino-acid form referred to as big endothelin-1 by dibasic endopeptidases and carboxypeptidases (small curved arrows) as shown. Big endothelin-1 is then cleaved at the Trp^{73}-Val^{74} bond by a specific endopeptidase referred to as endothelin-converting enzyme. The final product is the 21-amino-acid peptide, endothelin, containing amino acids Cys^{53} to Trp^{73}.

enzyme converted big ET-3 poorly (26), suggesting the existence of other (several) types of enzymes converting the various isoforms of big ETs. Indeed, a pepstatin-sensitive, cathepsin D-like aspartic protease was found to convert big ET-1 to ET-1 at low pH (3.0) in extracts of porcine aortic endothelial cells (28). However, the physiological importance of this enzyme is questionable (26, 27), as the pressor response evoked by big ET-1 injected to rats could be selectively inhibited by phosphoramidon but not by pepstatin-A (29).

ENDOTHELIN RECEPTOR SUBTYPES

The binding of ET to cell-surface receptors is rapid, specific, and saturable, but the dissociation rate is slow, and in most cases does not reach 100%. The discovery of various ET isoforms and the diverse biological function exerted by them (for review, see refs 8–10) predicted the existence of more than one ET receptor.

Partial isolation and purification studies (e.g., ref 30) also suggested that at least two receptor subtypes exist. At the recent 2nd International Conference on Endothelin (Tsukuba, Japan; December 9–12, 1990), two groups independently reported the cloning and sequencing of two different ET receptors (31, 32). Both receptors have a transmembrane topology (seven turns) similar to other G protein-coupled receptors, and their activation by ET-1 in animal cells transfected with the cloned cDNA leads to increased inositol phosphate production and elevation of intracellular free Ca^{2+}. The receptor subtype isolated from rat lung by Sakurai et al. (31) does not discriminate between the three ET isotypes, and is probably of endothelial origin (classified as ET_B receptor) (33). The receptor cloned by Arai et al. (32) from bovine tissues has higher selectivity to ET-1 than ET-3, and probably represents the vascular smooth muscle ET receptor (classified as ET_A) (33).

CELLULAR SYNTHESIS VS. CELLULAR BINDING: POSSIBLE AUTOCRINE, PARACRINE, AND ENDOCRINE FUNCTIONS OF ENDOTHELINS

A possible autocrine function of ET has been reported for endothelial cells. Vigne et al. (34) demonstrated the presence of high-affinity surface binding sites for ET-1 linked to a mitogenic response in endothelial cells from rat brain microvessels. Similar data were obtained for human vascular endothelial cells where endogenously produced ET-1 was found to be involved in the modulation of endothelial cell proliferation (35). Stimulation of the synthesis/release of endothelium-derived vasorelaxing and antiplatelet mediators (PGI_2, EDRF) (36), and modulation of ACE-activity (localized on endothelial cells) (37) may also represent autocrine actions of ET-1 (**Fig. 4**).

Endothelin production in endothelial cells that line the blood vessels (3, 4) and potent receptor-induced signaling mechanisms and actions (contraction, mitogenesis) on the underlying vascular smooth muscle cells (9) imply a paracrine function for the peptide. A similar situation may exist in the bronchopulmonary bed where ETs synthesized by the epithelial cells interact with specific, functional receptors on the underlying smooth muscle cells. It has been suggested that ET released from nerve terminals in the pituitary may locally modulate release of neurosecretory hormones such as vasopressin and oxytocin (14, 38, 39). Because of these autocrine and paracrine actions, and the fact that circulating ETs are effectively and rapidly cleared from the blood by the lung and kidney (40), it is suggested that ETs are most likely local mediators.

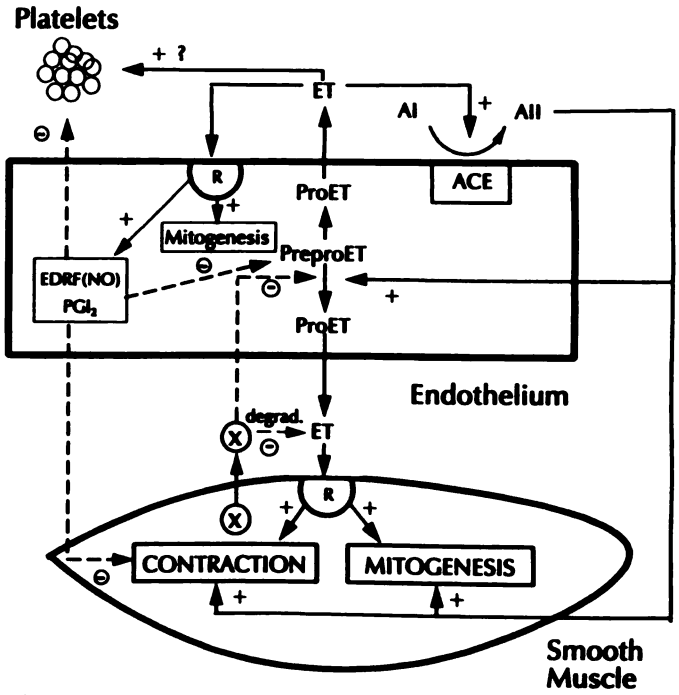

Figure 4. Autocrine and paracrine actions and modulation of biosynthesis/action of endothelin (ET) in the vascular wall. ET can act in an autocrine fashion on some endothelial cells by stimulating production of endothelium-derived relaxing factors (PGI_2, EDRF), activating angiotensin-converting enzyme (ACE), and promoting gene expression and mitogenesis. In a paracrine fashion, ET binds to underlying vascular smooth muscle, triggers vasoconstriction, and may facilitate mitogenesis (growth/proliferation). The release of prostacyclin (PGI_2) and endothelium-derived relaxing factor (EDRF) by ET from endothelial cells can mediate vasodilation and inhibition of platelet aggregation (---->). Facilitated production of angiotension II (AII), on the other hand, acts synergistically with ET (——>). The biosynthesis of ET is modulated (suppressed) by EDRF (NO) and by a still unknown factor or (factors) (X) released from smooth muscle cells. Proteolytic enzymes at or near the smooth muscle surface can degrade ET-1, thereby modulating its bioactivity.

However, some data support a potential endocrine function of these peptides (38). In situ hybridization studies found that in certain tissues ET-1 binding sites were present in the absence of ET-1 mRNA, suggesting that ET-1 may act distant from its site of synthesis (41). Certainly ET has a wide range of actions on cardiovascular, renal, and several endocrine systems (see below), which taken together with the observed circulating plasma levels, cerebrospinal fluid levels, and the excreted urinary levels of ET-1 may be consistent with an endocrine function of the peptide.

POTENTIAL CARDIOVASCULAR SIGNIFICANCE OF ENDOTHELINS

Some potential biological functions of ETs (predominantly of ET-1) proposed to date are summarized in **Fig. 5.** However, most are still hypothetical, as they are based (with few exceptions) on data obtained with exogenously administered synthetic peptides, and not by detection of local changes in endogenous ET production or by the use of selective inhibitors of ET biosynthesis or action. Another danger of making conclusions based on data obtained with synthetic ET is that most effects described are highly dependent on the dose of ET administered.

LOCAL CONTROL OF VASCULAR TONE

The mechanism of maintenance of basal vascular tone (i.e., the tone observed after all known neurohumoral control mechanisms have been inhibited) is still unknown. It is believed to be caused by stretch-induced activation of smooth muscle cells, but localization and signal transduction of the stretch-sensor are still unknown. Endothelial cells can respond to changes in shear stress and transmural pressure by altering the synthesis/release of endothelium-derived relaxing and contracting factors (42). Because the response to both the onset and release of a quick stretch occurs rapidly, it is unlikely to involve ET, as this peptide is not released abruptly from endothelial cells, and when released it causes a sustained contraction of the smooth muscle both in vitro and in vivo (4). However, these very properties make ET a unique candidate for being the mediator of long-term modulation of vascular tone (43) either under physiological conditions or during disease states such as vasospasm or hypertension. Regulation (modulation) of gene expression by ETs could contribute to long-term events such as mitogenesis and vascular remodeling in pathological conditions (9).

MODULATION OF ENDOTHELIN PRODUCTION AND ACTION IN THE VESSEL WALL

An important feature of ET-induced vascular contraction is that it can be inhibited by most known vasodilator agents and by the potent endogenous vasodilator endothelium-derived relaxing factor (36, 44). Endothelin can stimulate release of vasodilator prostanoids, EDRF and tPA (36, 45) from the endothelium, which may mediate the vasodilator, antiplatelet, and anticoagulant actions of the peptide (Fig. 4). In addition, there seems to be a link between EDRF and ET synthesis: EDRF (nitric oxide) was reported to inhibit the synthesis/release of ET-1 in porcine aorta (46) (Fig. 4). Smooth muscle cells in the vessel wall media may also modulate ET production/bioactivity. A recent study (47) showed that the presence of smooth muscle cells inhibits the accumulation of ET-1 produced by endothelial cells in a coculture setup. The smooth muscle cells appear to modify ET-1 accumulation by two different mechanisms: *1*) protelytic enzymes on the surface of smooth muscle cells degrade ET-1, and *2*) smooth muscle cells release a diffusible substance (or substances) that suppresses the biosynthesis of ET-1 in endothelial cells (Fig. 4).

Recent reports indicate a role for blood/blood cell-endothelium interactions in the regulation/modulation of ET synthesis. Increased shear forces during high blood flow velocity (48), thrombin generated during procoagulant states facilitated by activated/aggregating platelets (4), and cytokines released from activated monocytes or lymphocytes (49) can all activate endothelial cells and trigger increased expression and release of ET.

PRESSOR AND DEPRESSOR ACTIONS IN INTACT ANIMALS

Intravenous bolus injection of ET to anesthetized, chemically denervated, or intact normotensive (WKY) and spontaneously hypertensive rats (SHR) triggers a biphasic blood pressure response: an initial rapid and transient decrease (depressor response) is followed by a sustained rise (pressor response) in systemic arterial blood pressure (50, 51). The initial transient depressor response probably results from the release of vasodilator mediators (prostacyclin and EDRF) from the vascular endothelium (36). ET-3 is more potent

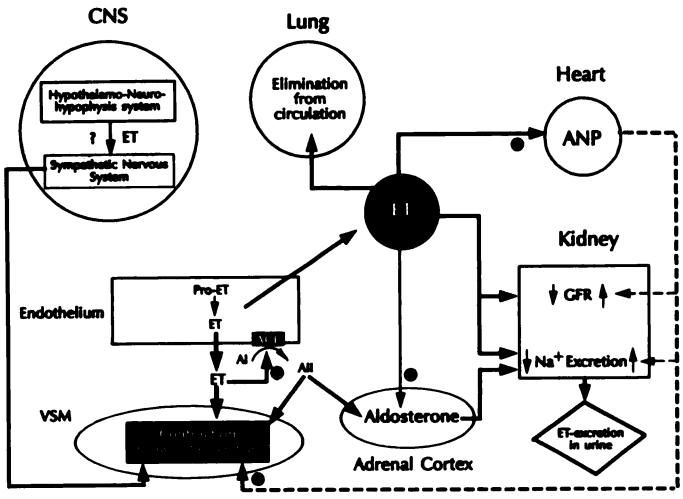

Figure 5. Hypothetical integrated cardiovascular actions of endothelin (ET). In addition to modulation of vascular tone/proliferation (see also Fig. 4), ET may modulate cardiovascular functions by acting on central cardiovascular centers, modulating baroreceptor functions and peripheral neuroeffector mechanisms. By complex interactions with various endocrine systems (renin-angiotensin; aldosterone, and atrial natriuretic peptide [ANP]), ET may modulate cardiovascular and renal function in an indirect way. Circulating ET (black circle in the middle of the figure) is removed by the lung and excreted by the kidney (for details see text). CNS, central nervous system; VSM, vascular smooth muscle; Pro-ET, precursor (or precursors) of ET (prepro ET; big-ET); ACE, angiotensin-converting enzyme; AI, angiotensin I; AII, angiotension II; ANP, atrial natriuretic peptide; GFR, glomerular filtration rate; ——> (+), stimulation; ---> (−), inhibition.

than ET-1 or ET-2 in eliciting vasodilation (52). The pressor response is caused primarily by direct vasoconstrictor action of the peptide. However, pentolinium attenuates the increase in hindlimb vascular resistance and also the bradycardia induced by ET-1 in anesthetized rats, suggesting that activation of the sympathetic nervous system may also contribute to the pressor response (53).

CARDIAC ACTIONS

High-affinity ET-binding sites are present in cardiac tissue (30). In isolated, electrically driven rat and human cardiac tissue, ET induces positive inotropic effects (54). Positive chronotopic effect was also observed in spontaneously beating guinea pig atria (55), which was absent in isolated rat atria (56). Intracoronary injection of ET-1 to anesthetized dogs decreases cardiac contractility (dp/dtmax), which presumably is secondary to the potent coronary vasoconstrictor effect of the peptide (57). Infusion of ET-1 to conscious rats decreases cardiac output, which could not be observed in denervated rats (58), indicating that the decrease in cardiac output may result from some indirect neuroendocrine mechanism (or mechanisms).

CENTRAL AND REFLEX CONTROL OF THE CIRCULATION

Chronic infusion of ET-1 into the cerebral ventricles of conscious rats evokes a progressive increase in arterial blood pressure accompanied by increased urinary excretion of norepinephrine, epinephrine, and vasopressin (59), probably reflecting activation of autonomic vasomotor centers. These observations suggest that ET produced in the central ner-

vous system may modulate the central control of circulation via an as yet unidentified mechanism (Fig. 5). Local release of endothelium-derived vasoactive factors at the site of the mechanoreceptors in the circulation, both in the carotid sinus and aortic arch, and also those in the heart, and lungs, could contribute to resetting of baroreceptors (60). That ET may be involved in this mechanism is supported by the demonstration that the peptide suppresses activity of the mechanoreceptors in the isolated canine carotid sinus during step increases in carotid sinus pressure (61).

HUMORAL CONTROL OF THE CIRCULATION AND KIDNEY FUNCTION

Aldosterone

High density of ET binding was found in the zona glomerulosa of the adrenal gland (53). Endothelin stimulates aldosterone biosynthesis in isolated zona glomerulosa cells (62) and stimulates release of aldosterone in vivo (63). The known effects of ET on renal function suggest a synergistic action (i.e., antinatriuresis) with aldosterone (Fig. 5). Exogenous administration of ET-1 causes a dose-dependent decrease in glomerular filtration rate (GFR) (63, 64). This may be achieved through contraction of mesangial cells leading to glomerular capillary vasoconstriction and a lower ultrafiltration coefficient, and also by a decrease in glomerular filtration pressure via potent afferent arterial vasoconstriction. Water deprivation leads to release of ET from hypothalamic neurons (14), suggesting a potential role of ET in volume homeostasis.

Renin-angiotensin system

Endothelin inhibits renin release in vitro (65). However, in vivo studies have demonstrated a significant rise in plasma renin activity after intravenous infusion of ET-1 (38, 63). In cultured bovine pulmonary artery endothelial cells, ET stimulates angiotensin-converting enzyme (ACE) activity (37). The increased renin release and activation of ACE may result in elevated angiotensin II levels. Angiotensin II then may act synergistically with ET in increasing vascular tone, promoting cell proliferation and growth (Fig. 4), and facilitating aldosterone biosynthesis (Fig. 5).

Atrial natriuretic peptide (ANP)

Endothelin can release ANP, a potent natriuretic, diuretic, and vasodilator peptide, from rat atria (60). Some of the known physiological actions of ANP oppose those of ET-1. In the kidney, ET-1 has a potent antinatriuretic effect whereas ANP evokes natriuresis. Similarly, ET-1 and ANP have opposing effects on renal blood flow and on peripheral vascular resistance and blood pressure. However, the effects of both ET-1 and ANP are highly dose-dependent. ANP effectively antagonizes ET-induced vasoconstriction and decrease in sodium excretion (63). Although infusion of ET-1 increases plasma ANP levels in conscious animals (38, 63), it is still uncertain whether release of ANP represents a negative feedback regulatory mechanism (Fig. 5).

ENDOTHELIN IN HUMAN PLASMA: BIOLOGICAL CURIOSITY OR PATHOLOGICAL IMPORTANCE?

With the development of specific radioimmunoassays and sandwich-enzyme immunoassays that allow measurement of plasma concentrations of ET in human subjects (22), considerable information has been accumulated about changes in circulating plasma levels of irET in various human cardiovascular diseases.

RIAs using polyclonal and monoclonal antibodies of various origin and different extraction techniques revealed a relatively wide range (0.26–19.9 pg/ml) of plasma irET-1 in normal, healthy volunteers (22, 67–73). Postural changes (e.g., tilting) and acute volume expansion induce transient but significant elevation of irET-1 in normal subjects (67), suggesting a potential physiological role of ET-1 in hemodynamic regulation in humans.

Different laboratories reported significantly elevated (i.e., above the range found in normal subjects) irET-1 levels in patients with essential hypertension (68), vasospastic angina (69), acute myocardial infarction (70), renal failure (71), shock (72), and toxemia (73). In addition to these observations, studies with anti-ET-1 antibodies indicated some role of ET in certain cardiovascular diseases. Watanabe et al. (74) reported a significant increase in irET-1 level in the myocardium of rats after 60 min of coronary artery occlusion, and ET-1 monoclonal antibody significantly reduced the infarct size. Anti-endothelin-1 serum also prevented vasoconstriction of postischemic nephrones (75) and cyclosporin-induced renal damage (76).

However, the importance of observations with elevated plasma irET-1 levels must be interpreted cautiously. Circulating plasma levels of irET-1 (even when elevated in certain diseased states) are lower than the concentration required to induce most of the biological actions of ET-1. It is widely accepted that similar to other endothelium-derived vasoactive factors (e.g., PGI_2, EDRF), ET also probably acts as an autocrine/paracrine local substance rather than a circulating hormone. Being a local regulator produced by the vascular endothelium and acting on the underlying vascular smooth muscle, the concentration of ET-1 may be several orders of magnitude higher than that measured in the plasma. Thus, ET levels found in plasma may result from an *overflow* of locally produced peptide rather than from systemic secretion, representing production of ET at sites of endothelial injury (e.g., atherosclerosis, hypoxia/ischemia, etc.). The source (or sources) of local production of ETs are still unknown, as are the exact mechanisms regulating its synthesis/release. It is also possible that elevation of plasma ET level in most of these reported cases is caused by an acute phase reaction. Thus it is believed that in most cases elevated circulating irET is the consequence of increased local production of ET, and that with few exceptions (e.g., cyclosporin induced renal damage or vasospasm in the coronary and cerebral circulation) circulating ET does not contribute to the pathomechanism of these diseases. This is probably not the case with ET-1 producing malignant hemangioendotheliomas, which are associated with significantly elevated circulating irET-1 and hypertension (68). Surgical removal of the tumor leads to fall in blood pressure, which is elevated again after recurrence of the tumor (68).

SUMMARY AND CONCLUSION

The discovery of a peptidergic EDCF produced by cultured endothelial cells (3) and the determination of its structure and molecular biology (4) generated worldwide interest and exponentially growing research activity. Merely 3 years after the first publication on ET, several hundred papers appeared on this novel and unique peptide. This plethora of publications revealed important information about genes, gene expression, and potential biochemical processing of the ET fam-

ily of peptides. In addition, data about specific membrane receptors, signal transduction pathways, and the wide variety of biological actions of ETs are abundant and still growing. In contrast, little is known about the exact physiological and pathological significance of ETs. Based on data generated by in vivo binding and in situ hybridization studies, by administration of exogenous synthetic peptides, and by measuring changes in plasma levels of irET, some hypotheses have been proposed on the potential significance of ETs in health and disease. However, these techniques provided (at best) only circumstantial evidence. Elucidation of the true importance of ETs in health and disease must await development of new sensitive techniques which allow detection of local production of the peptides, and even more important, that of selective inhibitors of their biosynthesis and/or diverse biological actions. FJ

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