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Research Article

Endothelium-dependent Vascular Responses

Mediators and Mechanisms

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The nonthrombogenic and high solute and water permeability properties of the vascular endothelium have long been recognized as essential components of normal microvascular integrity. In addition to the more recent recognition of other hemostatic as well as proproliferative and cytoadhesive properties, the past decade has witnessed a growing awareness that this endothelial monolayer is also capable of intrinsic modulation of vascular tone by elaborating several potent vasoactive substances. Two impressive vasorelaxants are prostacyclin and endothelium-derived relaxation factor (EDRF),[1] the latter at least in part composed of nitric oxide (NO). In addition, vascular endothelium has been shown recently to synthesize a novel polypeptide, termed endothelin, which exhibits potent vasoconstrictor properties. Prostacyclin, a major metabolite of the cyclooxygenase pathway in vessel walls and the subject of intense study for more than a decade, has recently been reviewed (1). Due to space constraints, this brief review[2] will therefore focus on other recently described endothelium-dependent vasoactive mediators, particularly NO and endothelin.

Endothelium-dependent vasorelaxation

Vasodilator drugs containing oxides of nitrogen (termed "nitrovasodilators" by Murad [2]), including sodium nitroprusside, glyceryl trinitrate, and various inorganic and organic nitrates have been widely used in the clinical management of angina pectoris and systemic hypertension. In vascular smooth muscle (VSM) cells, these agents activate soluble guanylate cyclase to generate intracellular cGMP (2). Chemically pure NO also activates soluble guanylate cyclase in VSM and induces marked but transient cGMP accumulation and concurrent relaxation of bovine coronary arteries. These responses are blunted either by heme-containing proteins (which bind NO with high affinity) or methylene blue (an inhibitor of soluble guanylate cyclase) (3). Activation of soluble guanylate cyclase and cGMP accumulation occurs promptly in response to submicromolar concentrations of NO, effects that are rapidly reversible after reduction/oxidation of NO (4). In isolated strips of rat aorta, sodium nitroprusside–induced cGMP accumulation serves to activate cGMP-dependent protein kinase, with subsequent phosphorylation/dephosphorylation of several smooth muscle proteins including myosin light chains (5, 6).

The recognition that NO is capable of directly inducing relaxation of vascular smooth muscle was followed by the important discovery in 1980 by Furchgott and Zawadski, and subsequently others, that potent vasorelaxant substances such as acetylcholine, bradykinin, histamine, serotonin, substance P, ADP, ATP, AA, thrombin, and the ionophore A23187 require the integrity of an intact endothelium (reviewed in reference 7). Many of these substances bind to specific endothelial cell surface receptors (Fig. 1) and ultimately induce the formation and liberation of a labile (3–5-s $t_{1/2}$) factor(s) generically termed endothelium-dependent relaxation factor (EDRF). Bioassay studies convincingly demonstrated labile EDRF to be chemically distinct from prostacyclin (and not inhibited by indomethacin) but capable of directly activating soluble guanylate cyclase (8, 9).

Endothelium-dependent relaxation has been demonstrated in large and small arteries and veins (7) and in microvessels (10). In cultured endothelial cells from the bovine renal glomerulus, a prototypical microvascular unit, EDRF is released in response to bradykinin, thrombin, platelet-activating factor, and ATP, effects closely correlated with concentration-dependent agonist-induced increases in intracellular calcium (10). Simultaneously cocultured bovine glomerular mesangial cells, analogous to contractile VSM cells in larger vessels, respond by liberating cGMP, the latter effect blocked by methylene blue, augmented by SOD (which impedes breakdown of NO) and mimicked by A23187, thus providing strong evidence for specific production of NO by endothelial cells, i.e., endothelium-derived NO, E-NO (10). In addition to the aforementioned chemical agonists, hypoxia and high fluid flow rates (shear stress) also augment vascular relaxation by stimulating EDRF release. By contrast, atrial peptides, beta-adrenergic agonists, prostacyclin, and nitrovasodilators induce vasorelaxation by acting directly on VSM, i.e., by endothelium-independent mechanisms.

Given the pharmacological and chemical similarities between NO and EDRF, Moncada and Ignarro and their respective associates (11–13) soon presented compelling evidence to indicate that E-NO is the major, if not the sole, chemical form

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1. *Abbreviations used in this paper:* ANP, atrial natriuretic peptide; Ca_i^{2+}, intracellular (cytosolic) calcium ion concentration; cGMP, cyclic guanosine monophosphate; EDCF, endothelium-dependent constriction factor; EDRF, endothelium-dependent relaxation factor; E-NO, endothelium-derived nitric oxide; ET, endothelin; GFR, glomerular filtration rate; L-NMMA, N^{ω}-monomethyl L-arginine; NO, nitric oxide; RPF, renal plasma flow rate; VSM, vascular smooth muscle.

2. The literature search for this review was concluded on 31 May 1989.

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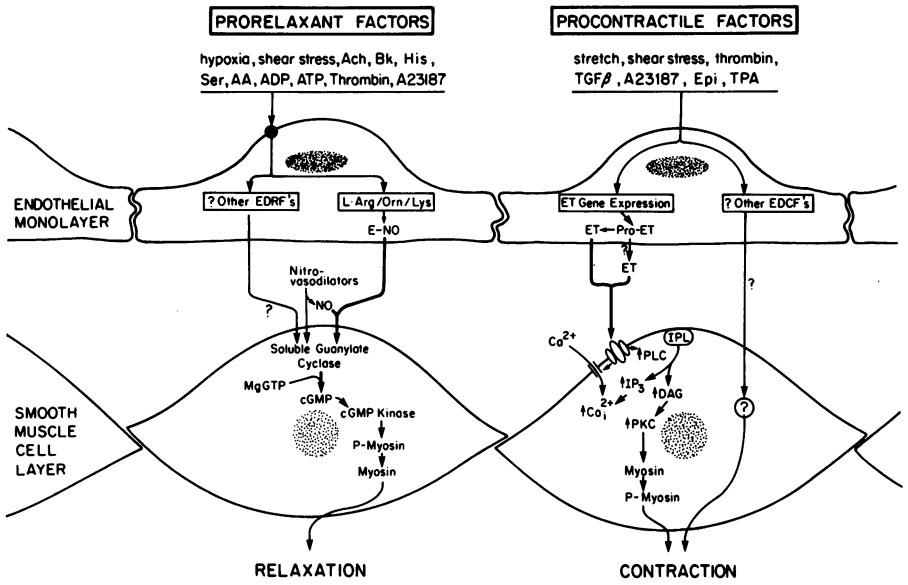

Figure 1. Proposed models of endothelium-dependent vascular smooth muscle relaxation and contraction. (*Left*) Several prorelaxant factors including hypoxia, increased shear stress, acetylcholine (Ach), bradykinin (Bk), histamine (His), serotonin (Ser), AA, ADP, and ATP, thrombin and the calcium ionophore A23187 are believed to generate EDRF, including E-NO, in endothelium, the latter from amino groups of L-arginine (L-arg), L-ornithine (L-orn) and L-lysine (L-lys). Nitrovasodilators, NO derived from endothelium (E-NO) or directly from nitrovasodilators, and possibly other EDRFs, stimulate soluble guanylate cyclase in vascular smooth muscle cells to convert MgGTP to cGMP. cGMP activates cGMP-dependent protein kinase, and leads ultimately to dephosphorylation of myosin light chains and muscle cell relaxation. (*Right*) Several procontractile factors including vessel wall stretch, increased shear stress, thrombin, TGFβ, A23187, epinephrine (Epi), and phorbol esters (TPA) are known to stimulate gene expression and release of ET and possibly other as yet uncharacterized EDCFs. Mature ET binds to smooth muscle cell surface receptors and eventually induces opening of voltage-sensitive plasma membrane calcium channels and activation of phospholipase C to liberate inositol trisphosphate (IP_3) and diacylglycerol (DAG) from plasma membrane inositol phospholipids (IPL). IP_3 and DAG in turn promote Ca^{2+} release from intracellular stores and activation of protein kinase C (PKC), respectively. Calmodulin and myosin light chain kinase (not shown) also aid in the phosphorylation of myosin light chains, a precondition for smooth muscle cell contraction.

of EDRF. In well-oxygenated systems, NO tends to be highly unstable, becoming rapidly transformed to the free radicals NO_2^- and NO_3^-. This chemical lability in physiological solutions makes it highly unlikely that E-NO functions as a circulating mediator, instead diffusing from sites of local generation to immediately adjacent smooth muscle cell targets within vessel walls. NO is highly lipophilic and readily permeates plasma membranes, where it combines with the heme moieties of reduced heme-containing proteins, including soluble guanylate cyclase, to produce an NO-heme adduct that facilitates the conversion of MgGTP to cGMP (14). cGMP-dependent protein kinase is activated in turn, leading to dephosphorylation of myosin light chains and eventual vasorelaxation (Fig. 1). Similar biochemical events are also believed to take place in platelets where NO is capable of inhibiting platelet aggregation (15), an action also shared by prostacyclin. Unlike prostacyclin, however, NO is also capable of impeding platelet adhesion to endothelial surfaces (16).

The growing recognition of indistinguishable biological actions of EDRF and NO has led recently to the identification of L-arginine as the likely precursor for E-NO, both in vitro and in vivo (17, 18). L-Arginine depletion diminishes, whereas highly basic, large molecular weight polymers of L-arginine, L-lysine, or L-ornithine enhance, endothelium-dependent vasorelaxation and cGMP accumulation in precontracted rings of bovine pulmonary artery or vein, presumably by directly modifying the substrate availability for the enzyme system that catalyzes the conversion of basic amino groups to E-NO in endothelial cells (19). Thus far, a soluble NADPH-dependent enzyme in vascular endothelium capable of converting L-arginine to L-citrulline (and presumably NO) has been identified (20). The L-arginine analogue N^{ω}-monomethyl L-arginine (L-NMMA) competitively inhibits this reaction and subsequent endothelium-dependent relaxation of rabbit aortic rings in response to acetylcholine, A23187, substance P, or L-arginine without affecting endothelium-independent vasorelaxation induced by glyceryl trinitrate or sodium nitroprusside (21). Finally, in the first in vivo study reported to date (18), intravenously administered L-NMMA, but not its D-enantiomer, induced a concentration-dependent (3–100 mg/kg) and long-lasting (15–90 min) increase in mean arterial blood pressure in anesthetized rabbits. At the highest dose tested, L-NMMA also prevented the hypotensive effects of acetylcholine; both actions were reversed by excess L-arginine. The finding that L-NMMA inhibits E-NO release in vivo and in vitro and thereby augments vascular tone thus suggests that the vascular endothelium plays an important role in blood pressure homeostasis. This concept is supported by the finding that vessels from hypertensive rats exhibit impaired endothelium-dependent relaxation (22) and reduced accumulation of cGMP (23) in response to acetylcholine or A23187. Of interest, exposure of rabbit aorta to low density lipoproteins in vitro impairs endothelium-dependent but not endothelium-independent relaxation (24). Similarly, atherosclerotic aortas and coronary arteries of animals and humans exhibit diminished EDRF release, endothelium-dependent vasorelaxation and cGMP formation (25, 26), presumably reflecting impaired generation of E-NO. Such defects in E-NO production might also favor platelet aggregation and adhesiveness and, ultimately, vasoocclusion. Likewise, the prolonged tendency to local cerebral vasospasm seen in patients after subarachnoid hemorrhage might reflect the binding and bioinactivation of E-NO by free hemoglobin from lysed red blood cells (27). Whether other regional vascular beds are similarly influenced by deficits in E-NO and whether supplementation of NO-donor substrates such as L-arginine offer potential benefits to patients with vasospasm, thromboocclusive disorders, and hypertension are but two of the many important questions that remain to be addressed.

Endothelium-dependent vasoconstriction

Humoral, neural, and mechanical stimuli are capable of inducing endothelium-dependent vasoconstriction, with signal transduction mediated at least in part by soluble factor(s) released from endothelial cells (generically termed endothelium-dependent constriction factor, EDCF). The only such factors fully characterized to date are 21 amino acid peptides termed endothelins (Fig. 2). The first of these peptides, isolated and purified from porcine aortic endothelial cells and now termed endothelin-1 (ET-1), elicits a marked and prolonged pressor response when infused intravenously (28). ET-1 release is stimulated by thrombin (29) suggesting that ET-1 may play a role in local hemostasis. ET-1 gene expression is also augmented by shear stress (30), implying that local endothelial cell injury may be associated with enhanced ET release. A role for ET in systemic disorders associated with endothelial damage, including accelerated hypertension, preeclampsia/eclampsia, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura, may also be likely.

Southern genomic analysis has demonstrated that porcine ET-1 is encoded by a single gene (28). Porcine preproET-1 contains 203 amino acids, as deduced from a full-length cDNA constructed from poly (A)mRNA initially extracted from porcine aortic endothelial cells (28). A human placental cDNA library revealed marked conservation of both coding and noncoding regions with that constructed from porcine aorta, including 100% homology in the region encoding for the mature ET-1 (residues 53–73) (31). The amino acid sequence of preproET-1 contains a signal peptide sequence that is probably cleaved intracellularly shortly after translation. The resulting proET-1 is much less vasoconstrictive in vitro than in vivo, suggesting the presence of an ET-1 converting enzyme within vessel walls (32).

Since the initial identification of ET-1, it has become evident that two other endothelins and a group of highly homologous scorpion and snake venom toxins all belong to the same evolutionary supergene family (33). ET-2 and ET-3 are also 21 amino acids long and have highly homologous amino acid sequences to ET-1, including four cysteine residues connected by two internal disulfide bridges (Fig. 2). They are encoded by three distinct ET genes and all are expressed in rat, pig, and human (33). Endothelins share significant structural homologies with snake venom sarafotoxins (34), a group of 21 amino acid peptides that also contain the two intramolecular disulfide bridges, a group of charged residues that form a sharp bend in the molecule, and a long hydrophobic COOH-terminal alpha helix (Fig. 2). Tryptophan is a common carboxy-terminal residue and appears particularly important for the slow-onset of the vasoconstriction induced by these agents (35). Substitution of D- for L-tryptophan markedly reduces ET-1 biological activity as does disruption of the disulfide bridges. Differences in amino terminus residues between ET-1 and sarafotoxin S6b also contribute to observed differences in vasoconstrictor potency. Differences in the potency of ET-1, ET-2, and ET-3 in eliciting VSM contraction have also been demonstrated (33, 36). Half-maximal vasoconstrictor responses to ET-1 and ET-2 were similar, but half-maximal responses to ET-3 required concentrations one order of magnitude greater than ET-1. In addition, the maximal response elicited by ET-3 was markedly less than that achieved by ET-1, whereas the maximal contractile force in response to ET-2 was greater than that for ET-1 (36). These findings suggest differences in the affinity of ET isoforms for ET receptors and/or more than one ET receptor subtype in VSM.

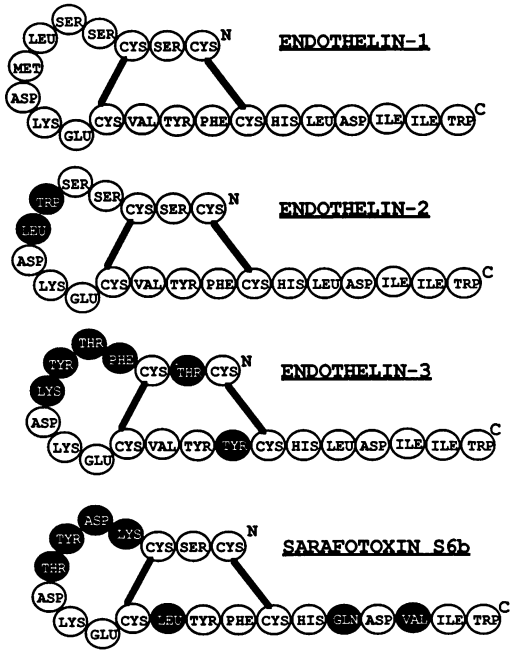

Figure 2. Amino acid sequences of ET-1, ET-2, ET-3, and the structurally similar snake venom sarafotoxin S6b. Residues differing from those in ET-1 are highlighted, as are NH^2- and COOH-termini.

PreproET-1 mRNA expression in porcine endothelial cells is enhanced by A23187, thrombin, epinephrine (28), transforming growth factor β (TGF-β) (37, 38), enhanced shear stress (30), and in bovine glomerular endothelial cells by bradykinin (29). With the exception of TGF-β and shear stress, these agents all activate phospholipase C (Fig. 1). Furthermore, in human umbilical vein endothelial cells, phorbol esters also enhance preproET-1 gene expression (39), suggesting regulation of this gene by a protein kinase C–dependent mechanism. Stimulation of ET-1 gene expression by thrombin and TGF-β may indicate that ET-1 release is stimulated at the site of platelet aggregation and clot formation during endothelial cell injury. Also, enhanced ET-1 gene expression in response to shear stress could indicate that the peptide participates in local autoregulatory control of blood flow.

To date, studies of cellular ET actions have been limited mainly to those performed with ET-1. Cellular responses to ET-1 make up a cascade of events triggered by ET-1 binding to cell-surface receptors. Radioligand binding and autoradiographic studies indicate that a large number of tissues express ET receptors. Among these are VSM (40, 41), adrenal glomerulosa (42), and renal glomeruli, medullary vascular bundles, and papilla (43, 44). ET receptors are also found in coronary and intrasplenic arteries, pulmonary vessels, airways smooth muscle and peripheral nerves (45, 46). In the central nervous system, ET binding sites have been identified in the basal ganglia and brain stem (46). ET binding sites are of high affinity, with equilibrium dissociation constants in the picomolar to nanomolar range (41, 42). Because cellular responses to ET can be elicited at similar concentrations, it is likely that binding sites represent true receptors. The exceedingly high affinity of the peptide for its receptor is also reflected in the observation that [^{125}I]ET binding is difficult to reverse (43). This find-

ing may explain the prolonged vasoconstriction seen following a single bolus of ET in rats (28, 47) despite an estimated plasma $t_{1/2}$ of < 1 min (48). Scatchard analysis of ET binding indicates that ET receptors are of uniform affinity (41, 43), although pharmacologic studies using vascular strips suggest more than one ET receptor subtype. That ET receptors and voltage-dependent calcium channels are not identical proteins is suggested by the finding that dihydropyridine calcium channel agonists and antagonists fail to compete for ET binding sites (40, 43).

In VSM and glomerular mesangial cells ET-1 activates phospholipase C to generate inositol trisphosphate and diacylglycerol; the former in turn mobilizes cytosolic calcium (Ca_i^{2+}) from intracellular stores (40, 49–51) (Fig. 1). The finding that the initial rise in Ca_i^{2+} in response to ET is not dependent on the presence of extracellular Ca^{2+} is consistent with this formulation (40). This early rise in Ca_i^{2+} in response to ET-1 is probably involved in the initiation of cell contraction. In addition, Ca_i^{2+} concentrations remain elevated above baseline values for prolonged periods after exposure to ET-1. These prolonged increments in Ca_i^{2+} are dependent on extracellular Ca^{2+} and are thought to reflect Ca^{2+} entry through voltage-sensitive plasma membrane Ca^{2+} channels. Diacylglycerol accumulation following activation of phospholipase C in turn activates protein kinase C and subsequent phosphorylation of myosin light chains (40, 49–51) (Fig. 1). These early signaling pathways are generally similar to those involved in the action of other vasoconstrictor peptides including angiotensin II and vasopressin (via its V_1 receptor). However, the more prolonged and poorly reversible contractile responses seen with ET-1 relative to these other peptide agonists suggests that other "late" signaling mechanisms and pathways may be involved.

Certain agonists (thrombin, A23187) and other stimuli (e.g., shear stress) are capable of augmenting both E-NO and ET production (Fig. 1) but it is not yet clear which vascular effect predominates in a given circumstance. Indeed, time course considerations (thrombin induces E-NO production more rapidly than ET release) imply sequential vasomotor responses. Furthermore, as ET has recently been shown to augment prostacyclin and E-NO production (52, 53), the potential for effective molecular antagonism of vascular responses also exists.

ET-1 in the presence of insulin or low concentrations of fetal bovine serum stimulates [^{3}H]thymidine incorporation into quiescent cultures of VSM (54, 55) and glomerular mesangial cells (50, 51), reflecting new DNA synthesis. ET-1 also augments the expression of the protooncogenes c-*fos* and c-*myc* (51, 55). The proteins encoded by these genes are presumably involved in regulating DNA replication. ET-1 also activates phospholipase A_2 to stimulate the production of cyclooxygenase products in a number of tissues. In isolated perfused rat lung, ET-1 stimulated prostacyclin and E-NO production (52); in rabbit spleen, ET-1 augmented $PGE_2 > PGI_2 > TxA_2$ production; and in rabbit kidney, ET-1 stimulated $PGI_2 > PGE_2$ but not TxA_2 synthesis (56). Also, ET-1 induced cyclooxygenase-dependent bronchoconstriction in guinea pigs (57). In rats, inhibition of cyclooxygenase activity antagonized the action of ET-1 on GFR, augmented the pressor response to ET-1 (52, 58), and potentiated its ulcerogenic effect in rat gastric mucosa (59). Clearly, ET-1-induced cellular actions can be modified by the concomitant release of prostaglandins.

ET-1 markedly inhibits renin release from isolated glomeruli and from dispersed juxtaglomerular cells (60), suggesting that ET-1 may suppress renin release in vivo, at least under conditions when renal perfusion is preserved. ET-1 also influences atrial natriuretic peptide (ANP) secretion, raising circulating ANP levels in dogs (61) and augmenting ANP release from cultured atrial myocytes (62), an effect partially inhibited by Ca^{2+} channel antagonists. Taken together with the finding that ANP markedly reduces the vasoconstrictor response to ET-1 (40), it is possible that ANP serves as a negative feedback regulator of ET-1 actions. Finally, in dogs, ET-1 infusion augments circulating aldosterone levels (61), a finding in vivo that accords well with in vitro evidence of direct stimulation of aldosterone release from adrenal glomerulosa cells (42).

Systemic infusions of ET-1 and ET-3 elicit transient reductions in mean arterial blood pressure followed by prolonged hypertension (28, 47). Cardiac index does not fall during the early hypotensive period (63), indicating an initial decrease in peripheral vascular resistance, possibly due to EDRF and ANP release. Systemic arterial pressure rises slowly in dog and rat after the initial vasodilatory response, with maximal effects noted at 10–20 min (28, 47). The hypertensive response, which is concentration dependent, is more profound after intraarterial vs. intravenous administration, consistent with evidence for loss of considerable ET-1 bioactivity in a single pass through the pulmonary circulation, an effect not altered by inhibition of angiotensin I converting enzyme (52). The systemic pressor response to ET-1 occurs despite bradycardia and a reduction in cardiac index, indicating increased peripheral vascular resistance (61, 63). In isolated cardiac muscle, ET-1 exerts a prolonged positive ionotropic effect, mediated at least in part by Ca^{2+} influx through voltage-sensitive Ca^{2+} channels (64). Although ET-1 infusion into coronary arteries induces local vasoconstriction (65), ET-1 reduces coronary blood flow only minimally in vivo (61), presumably due to the offsetting effects of the high perfusion pressure.

Coronary vasoconstriction, increased afterload and bradycardia all tend to reduce cardiac output, whereas the positive inotropic action of ET and possibly constriction of venous capacitance vessels tend to oppose these effects. Heterogeneity in the responses of different vascular beds to ET-1 has been noted, in that mesenteric and renal vessels appear particularly sensitive to the vasoconstrictor actions of ET-1 (50, 52, 54, 61). Furthermore, in vitro studies indicate that venous VSM is more sensitive to the constrictor effects of ET-1 than arterial VSM (54).

In rats, ET-1 infusion elicits a concentration-dependent increase in renal vascular resistance and reduction in renal plasma flow (RPF) (47). As with the systemic pressor response, the renal response to ET-1 is prolonged even when given as a single bolus injection. ET-1 also lowers GFR; however, at low doses (75 pM) the decline in GFR is less than proportional to that in RPF so that filtration fraction tends to rise (47). By contrast, after bolus infusion of 300 pM ET-1 RPF and GFR both decline markedly and proportionately (47). In dogs receiving a constant infusion of ET at 10 pmoles/min, RPF and GFR fall in parallel (61), and in isolated perfused rat kidneys, proportional changes in RPF and GFR are observed over a wide range of ET doses (66).

Administration of ET-1 to rats in vivo is associated with a marked decrement in the glomerular capillary ultrafiltration coefficient (K_f) (47, 50). Changes in K_f are elicited by a number of vasoactive hormones known to cause contraction of glo-

merular mesangial cells. ET-1 elicits contraction of glomerular mesangial cells in vitro, and mesangial cells, similar to VSM cells, respond to this agonist with increments in intracellular levels of inositol trisphosphate and Ca^{2+} (50, 51). Preliminary studies show that GFR, but not RPF, is preserved during ET-1 infusion when the cyclooxygenase inhibitor ibuprofen is administered simultaneously, suggesting that the action of ET-1 on K_f may be mediated by a vasoconstrictor cyclooxygenase product(s) (58). Micropuncture studies indicate that ET-1 induces a significant rise in the mean glomerular transcapillary hydraulic pressure gradient, due to greater efferent than afferent arteriolar vasoconstriction (47, 50).

Administration of ET-1 in doses that do not severely impair GFR has been shown to produce a modest natriuresis (47, 58), implying inhibition of renal tubule Na^+ reabsorption. Indeed, in freshly isolated inner medullary collecting duct cells picomolar concentrations of ET-1 inhibit ouabain-sensitive oxygen consumption (67), an effect shown to be due to inhibition of Na^+-K^+-ATPase activity. Ouabain-sensitive ^{86}Rb uptake, another measure of Na^+-K^+-ATPase activity, was also inhibited by ET-1. Because prostaglandin E_2 elicits similar responses and because ET-1-induced inhibition of Na^+-K^+-ATPase activity is abolished by ibuprofen, the ET-1-induced inhibition of Na^+ transport in these cells appears to be mediated by prostaglandins (67). In contrast to the ET-1-induced inhibition of Na^+-K^+-ATPase activity in inner medullary collecting duct cells, the peptide is reported to augment Na^+-K^+-ATPase activity in VSM cells, an effect attributed to increased Na^+ entry into cells via the $Na^+/K^+/2Cl^-$ cotransporter (68). As with the findings in inner medullary collecting duct cells, this effect is reported to be prostaglandin dependent. Because ET-1 also augments ANP secretion, the observed natriuresis might also reflect ANP's ability to block Na^+ channels in collecting duct epithelia, an effect shown to be important in mediating the natriuresis that attends ANP infusion (69). Of interest, intrarenal infusion of an anti-ET antibody into rats blunted the reduction in single nephron GFR observed 48 h after renal ischemia (70), suggesting that ET produced in the kidney plays a role in the pathophysiology of postischemic acute renal failure.

Although much remains to be learned, evidence already amassed since the reported discovery of ET-1 in March 1988 (28) suggests that ET plays an important role in normal as well as disordered regulation of microcirculatory hemodynamics, volume homeostasis, and blood pressure. Further clarification of these and other issues related to the physiology and pathobiology of ET will be aided greatly by the development of effective strategies for blockade of endogenous ET actions, whether by specific ET receptor antagonists or converting enzyme inhibitors.

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References

1. Gryglewski, R. J., R. M. Botting, and J. R. Vane. 1988. *Hypertension (Dallas).* 12:530–548.

2. Murad, F. 1986. *J. Clin. Invest.* 78:1–5.

3. Gruetter, C. A., B. K. Barry, D. B. McNamara, D. Y. Gruetter, P. J. Kadowitz, and L. J. Ignarro. 1979. *J. Cyclic Nucleotide Res.* 5:211–224.

4. Arnold, W. P., C. K. Mittal, S. Katsuki, and F. Murad. 1977. *Proc. Natl. Acad. Sci. USA.* 174:3203–3207.

5. Fiscus, R. R., R. M. Rapoport, and F. Murad. 1983. *J. Cyclic Nucleotide Protein Phosphorylation Res.* 9:415–424.

6. Rapoport, R. M., M. B. Draznin, and F. Murad. 1983. *Nature (Lond.).* 306:174–176.

7. Furchgott, R. F. 1984. *Annu. Rev. Pharmacol. Toxicol.* 24:175–197.

8. Gryglewski, R. J., S. Moncada, and R. M. J. Palmer. 1986. *Br. J. Pharmacol.* 87:685–694.

9. Ignarro, L. J., R. G. Harbison, K. S. Wood, and P. J. Kadowitz. 1986. *J. Pharmacol. Exp. Ther.* 237:893–900.

10. Marsden, P. A., T. A. Brock, and B. J. Ballermann. 1989. *Clin. Res.* 37:496*a*. (Abstr.)

11. Palmer, R. M. J., A. G. Ferrige, and S. Moncada. 1987. *Nature (Lond.).* 327:524–526.

12. Radomski, M. W., R. M. J. Palmer, and S. Moncada. 1987. *Br. J. Pharmacol.* 92:181–187.

13. Ignarro, L. J., G. M. Buga, K. S. Wood, R. E. Byrns, and G. Chaudhuri. 1987. *Proc. Natl. Acad. Sci. USA.* 84:9265–9269.

14. Wolin, M. S., K. S. Wood, and L. J. Ignarro. 1982. *J. Biol. Chem.* 257:13312–13320.

15. Mellion, B. T., L. J. Ignarro, E. H. Ohlstein, E. G. Pontecorvo, A. L. Hyman, and P. J. Kadowitz. 1981. *Blood.* 57:946–955.

16. Radomski, M. W., R. M. J. Palmer, and S. Moncada. 1987. *Lancet.* ii:1057–1058.

17. Palmer, R. M. J., D. S. Ashton, and S. Moncada. 1988. *Nature (Lond.).* 333:664–666.

18. Rees, D. D., R. M. J. Palmer, and S. Moncada. 1989. *Proc. Natl. Acad. Sci. USA.* 86:3375–3378.

19. Ignarro, L. J., M. E. Gold, G. M. Buga, R. E. Byrns, K. S. Wood, G. Chaudhuri, and G. Frank. 1989. *Circ. Res.* 64:315–329.

20. Palmer, R. M. J., and S. Moncada. 1989. *Biochem. Biophys. Res. Commun.* 158:348–352.

21. Rees, D. D., R. M. J. Palmer, H. F. Hodson, and S. Moncada. 1989. *Br. J. Pharmacol.* 96:418–424.

22. Lüscher, T. F., L. Raij, and P. M. Vanhoutte. 1987. *J. Hypertens.* 5(Suppl. 5):S153–S155.

23. Otsuka, Y., A. DiPiero, E. Hirt, B. Brennaman, and W. Lockette. 1988. *Am. J. Physiol.* 254:H163–H169.

24. Andrews, H. E., K. R. Bruckdorfer, R. C. Dunn, and M. Jacobs. 1987. *Nature (Lond.).* 327:237–239.

25. Bossaller, C., G. B. Habib, H. Yammamoto, C. Williams, W. Wells, and P. D. Henry. 1987. *J. Clin. Invest.* 79:170–174.

26. Shimokawa, H., and P. M. Vanhoutte. 1989. *Circ. Res.* 64:900–914.

27. Kanamaru, K., S. Waga, T. Kojima, K. Fujimoto, and S. Niwa. 1987. *Stroke.* 18:938–943.

28. Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, and T. Masaki. 1988. *Nature (Lond.).* 332:411–415.

29. Marsden, P. A., E. R. Martin, D. Dorfman, T. A. Brock, B. M. Brenner, T. Collins, and B. J. Ballermann. 1989. *Am. J. Hypertension.* 2:49A. (Abstr.)

30. Yoshizumi, M., H. Kurihara, T. Sugiyama, F. Takaku, M. Yanagisawa, T. Masaki, and Y. Yazaki. 1989. *Biochem. Biophys. Res. Commun.* 161:859–864.

31. Itoh, Y., M. Yanagisawa, S. Ohkubo, C. Kimura, T. Kosaka, A. Inoue, N. Ishiha, Y. Mitsui, H. Onda, M. Fujino, and T. Masaki. 1988. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 231:440–444.

32. Kimura, S., Y. Kasuya, T. Kawamura, O. Shinmi, Y. Sugita, M. Yanagisawa, K. Goto, and T. Masaki. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S5–S7.

33. Inoue, A., M. Yanagisawa, S. Kimura, Y. Kasuya, T. Miyauchi, K. Goto, and T. Masaki. 1989. *Proc. Natl. Acad. Sci. USA.* 86:2863–2867.

34. Kloog, Y., I. Ambar, M. Sokolovsky, E. Kochva, Z. Wollberg, and A. Bdolah. 1988. *Science (Wash. DC).* 242:268–270.

35. Kimura, S., Y. Kasuya, T. Sawamura, O. Shinmi, Y. Sugita, M. Yanagisawa, K. Goto, and T. Masaki. 1988. *Biochem. Biophys. Res. Commun.* 156:1182–1186.

36. Yanagisawa, M., A. Inoue, T. Ishikawa, T. Kasuya, S. Kimura, S.-I. Kumagaye, K. Nakajima, T. X. Watanabe, S. Kakakibara, K. Goto, and T. Masaki. 1988. *Proc. Natl. Acad. Sci. USA.* 85:6964–6967.

37. Kurihara, H., M. Yoshizumi, T. Sugiyama, F. Takaku, M. Yanagisawa, T. Masaki, M. Hamaoki, H. Kato, and Y. Yazaki. 1989. *Biochem. Biophys. Res. Commun.* 159:1435–1440.

38. Kurihara, H., M. Yoshizumi, F. Takaku, M. Yanagisawa, T. Masaki, and Y. Yazaki. 1988. *Circulation.* 78:II–336. (Abstr.)

39. Yanagisawa, M., A. Inoue, Y. Takuwa, Y. Mitsui, M. Kobayashi, and T. Masaki. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S13–S17.

40. Marsden, P. A., N. R. Danthuluri, B. M. Brenner, B. J. Ballermann, and T. A. Brock. 1989. *Biochem. Biophys. Res. Commun.* 158:86–93.

41. Hirata, Y., H. Yoshimi, S. Takaichi, M. Yanagisawa, and T. Masaki. 1988. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 239:13–17.

42. Cozza, E. N., M. C. Vila, C. E. Gomez-Sanchez, S. Chiou, and M. F. Foeking. 1988. *Clin. Res.* 37:11*a*. (Abstr.)

43. Martin, E. R., P. A. Marden, B. M. Brenner, and B. J. Ballermann. 1989. *Biochem. Biophys. Res. Commun.* 162:130–137.

44. Kohzuki, M., C. I. Johnston, S. Y. Chai, D. J. Casley, and F. A. O. Mendelsohn. 1989. *Eur. J. Pharmacol.* 160:193–194.

45. Power, R. F., J. Wharton, Y. Zhao, S. R. Bloom, and J. M. Polak. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S50–S56.

46. Davenport, A. P., D. J. Nunez, J. A. Hall, A. J. Kaumann, and M. J. Brown. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl.5):S166–S170.

47. King, A. J., B. M. Brenner, and S. Anderson. 1989. *Am. J. Physiol.* 256:F1051–F1058.

48. Anggard, E., S. Galton, G. Rae, R. Thomas, L. McLoughlin, G. DeNucci, and J. R. Vane. 1989. *J. Cardiovasc. Pharmacol.* 13 (Suppl. 5):S46–S49.

49. Resink, T. J., T. Scott-Burden, and F. R. Bühler. 1988. *Biochem. Biophys. Res. Commun.* 157:1360–1368.

50. Badr, K. F., J. J. Murray, M. D. Breyer, K. Takahashi, T. Inagami, R. C. Harris, M. Schwartzberg, and J. Ebert. 1989. *J. Clin. Invest.* 83:336–342.

51. Simonson, M. S., S. Wann, P. Mené, G. R. Dubyak, M. Kester, Y. Nakazato, J. R. Sedor, and M. J. Dunn. 1989. *J. Clin. Invest.* 83:708–712.

52. De Nucci, G., R. Thomas, P. D'Orleans-Juste, E. Antunes, C. Walder, T. D. Warner, and J. R. Vane. 1988. *Proc. Natl. Acad. Sci. USA.* 85:9797–9800.

53. Thiemermann, C., P. S. Lidbury, G. R. Thomas, and J. R. Vane. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S138–141.

54. Nakaki, T., M. Nakayama, S. Yamamoto, and R. Kato. 1989. *Biochem. Biophys. Res. Commun.* 158:880–883.

55. Komuro, I., H. Kurihara, T. Sugiyama, F. Takaku, and Y. Yazaki. 1988. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 238:249–252.

56. Rae, G. A., M. Trybulee, G. De Nucci, and J. R. Vane. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S89–S92.

57. Payne, A. N., and B. J. R. Whittle. 1988. *Eur. J. Pharmacol.* 158:303–304.

58. Munger, K. A., M. Sugiura, T. Inagami, K. Takahashi, and K. F. Badr. 1989. *Clin. Res.* 37:497*a*. (Abstr.)

59. MacNaughton, W. K., C. M. Keenan, G. W. McKnight, and J. L. Wallace. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S118–S122.

60. Tagaki, M., H. Matsuoka, K. Atarashi, and S. Yagi. 1988. *Biochem. Biophys. Res. Commun.* 157:1164–1168.

61. Miller, W. L., M. M. Redfield, and J. C. Burnett, Jr. 1989. *J. Clin. Invest.* 83:317–320.

62. Fukada, Y., Y. Hirata, H. Yoshima, T. Kojima, Y. Kobayashi, M. Yanagisawa, and T. Masaki. 1988. *Biochem. Biophys. Res. Commun.* 155:167–172.

63. King, A. J., J. Pfeffer, M. Pfeffer, and B. M. Brenner. 1989. *Am. J. Hypertension.* 2:48A (Abstr.)

64. Moravec, C. S., E. E. Reynolds, R. W. Stewart, and M. Bond. 1989. *Biochem. Biophys. Res. Commun.* 159:14–18.

65. Kurihara, H., K. Yamaoki, R. Ragai, M. Yoshizumi, F. Takaku, H. Saot, J. Inui, and Y. Yazaki. 1988. *Circulation.* 78:II-171. (Abstr.)

66. Firth, J. D., P. J. Ratcliffe, A. E. G. Raine, and J. G. G. Ledingham. 1988. *Lancet.* ii:1179–1182.

67. Zeidel, M. L., H. Brady, B. Kone, S. Gullans, and B. M. Brenner. 1989. *Am. J. Physiol.* In press.

68. Garay, R., C. Rosati, M. Braquet, P.-E. Chabrier, and P. Braquet. 1989. *J. Cardiovasc. Pharmacol.* 13(Suppl. 5):S213–S215.

69. Zeidel, M. L., D. Kikeri, P. Silva, M. Burrowes, and B. M. Brenner. 1988. *J. Clin. Invest.* 82:1067–1074.

70. Kon, V., T. Yoshioka, A. Fogo, and I. Ichikawa. 1989. *J. Clin. Invest.* 83:1762–1767.