Editorial Comment

The Endothelin Explosion
A Pathophysiological Reality or a Biological Curiosity?

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The ability of vascular endothelial cells to synthesize and release potent vasoconstrictor substances has focused much attention on the role of such mediators in the regulation of vascular tone. The discovery of prostacyclin and of endothelium-derived relaxing factor (EDRF), characterized as nitric oxide (NO) biosynthesized from L-arginine, has led to extensive investigation of the involvement of these potent vasoconstrictors in cardiovascular physiology and pathology. Until recently, however, less attention has been paid to vasoconstrictor factors derived from the endothelium. Studies in canine isolated arteries and veins have demonstrated endothelium-dependent contractions induced by a variety of stimuli including arachidonic acid, the actions of which were mediated by the release of vasoconstrictor eicosanoids. It soon became apparent, however, that such substances could not account for the contractile activity observed in other situations.

In the mid-1980s, two independent groups reported that endothelial cells in culture produced a peptide-like material with vasoconstrictor properties. The interest in endothelium-derived con-

tractile factors was heightened in 1988, when Yanagisawa and colleagues reported their classic description of the isolation and purification from cultured porcine endothelial cells of a 21-amino-acid peptide with potent vasoconstrictor properties both in vitro and in vivo. This peptide was termed endothelin (ET) and later demonstrated to be one member of a family of which the original form, derived from porcine or human endothelial cells, was designated ET-1.

Since the first report on ET-1, there has been a literary explosion with more than 450 citations published during the ensuing 20 months. Of these, 59 are full proceedings papers from the First William Harvey Workshop on Endothelin, which has held in London, England, within 8 months of the publication of the original paper. The potent biological properties of the endothelins, coupled with the rapid commercial availability and the comparatively low cost of the peptides, have no doubt been responsible for the phenomenal growth of interest in these novel vasoconstrictor mediators.

Detailed cardiovascular studies on ET-1 in a wide range of in vitro and in vivo preparations have confirmed its vasoconstrictor actions in most vascular beds. It has also become clear that ET-1 has additional actions on many organs and tissues, including lung, stomach, kidney, liver, nonvascular smooth muscle, and nervous tissue. Furthermore, ET-1 can alter the levels of circulating hormones such as renin and atrial natriuretic peptides; in some instances, it can induce an initial vasodilation that may be related to the release of other mediators.

The article by Miyauchi and colleagues published in this issue of Circulation describes the constrictor actions of ET-1 on segments of human mesenteric arteries. The authors report that the contractile responses were substantially inhibited by nicardipine, suggesting an action on calcium influx closely associated with voltage-dependent channels. This conclusion may, however, prove controversial because many groups have failed to antagonize ET-induced contractions of a variety of isolated vascular tissues using a selection of calcium channel antagonists. Furthermore, while high-affinity binding sites for ET-1 can be demonstrated on animal or human vascular and cardiac smooth muscle cells, such binding was not displaced by calcium channel antagonists. Thus, although extracellular calcium appears to be an important determinant in the vascular contractions induced by ET-1, it is unlikely that direct activation of voltage-sensitive channels is the sole triggering event. More complex mechanisms involving actions on intracellular mobilization or sequestration of calcium may be of greater importance. Moreover, ET-1 activates phospholipase C, leading to increased inositol trisphosphate and diacylglycerol synthesis with subsequent stimulation of protein kinase C. These events have been implicated in the initial rise in intracellular calcium and phosphorylation of myosin light chains that underlie the initiation of the vascular contraction by ET-1.

The formation and release of ET-1 by endothelial cells in culture, including those from the microvas-
culation, have been confirmed by several groups using a variety of assay procedures. Studies using in situ hybridization have localized ET—messenger (m)RNA in close proximity to ET-1 binding sites in rat lung, kidney, intestine, and eye. The present study by Miyachi and colleagues,
using novel immunohistochemical techniques, elegantly demonstrates the presence of ET-1-like immunoreactivity in endothelial cells of human mesenteric arteries. This vascular localization provides an important piece of evidence to support a physiological or pathological role of endogenous ET-1 in cardiovascular biology.

It is unlikely that a mediator as potent as ET-1, with long-lasting vasoconstrictor effects, would circulate freely or achieve high plasma levels in its active form under physiological conditions. One regulatory process may be its inactivation during passage through the pulmonary circulation. It is feasible, however, that endothelial compartmentalization and abluminal release of ET-1 are effective mechanisms to localize its actions to discrete areas of vascular smooth muscle. The determination of local levels of ET-1 in the microenvironment of the vessel wall would be of great interest, and an initial approach could be provided by the study of ET-1 turnover in vascular biopsies.

Recent reports indicate that circulating levels of immunoreactive ET-1 are extremely low (0.1–1 fmol·ml⁻¹) or nondetectable in plasma from healthy volunteers or animals. Detectable increases in plasma levels of immunoreactive ET-1, in the low femtomoles per milliliter range, have been demonstrated in patients with uremia undergoing hemodialysis and in patients in cardiogenic shock but not in those with stable congestive heart failure. Furthermore, preliminary studies in patients with acute myocardial infarction or acute renal failure have also reported increases in plasma immunoreactive ET-1 in this concentration range, but whether these are secondary to endothelial cell injury is not known. It is noteworthy that local infusion of ET-1 into the brachial artery at the comparatively high dose of 5 pmol·min⁻¹ was required to induce a 40% fall in forearm blood flow in healthy volunteers. Thus, the relation between the plasma levels of ET-1 and the etiology of such diseases is not clear, although the possibility that there is an increased vascular sensitivity to ET-1 under these conditions should be investigated. Elevated plasma levels of ET-1 have also been detected in preliminary studies in patients with aneurysmal subarachnoid hemorrhage. Whether ET-1 contributes to the associated cerebral vasospasm in such patients and whether levels of this peptide are also elevated in the cerebrospinal fluid have yet to be determined.

Other biological properties of ET-1 may also have importance in the cardiovascular system. Indeed, ET-1 can induce proliferation in cultured vascular smooth muscle, as evidenced by enhanced DNA synthesis and expression of mRNA for the proto-oncogenes c-myc and c-fos. This effect, which has also been observed in glomerular mesangial cells and fibroblasts, may involve inositol lipid turnover. Furthermore, ET-1 interacts synergistically with epidermal growth factor and with transforming growth factor α but only minimally with platelet-derived growth factor in these systems. It is therefore possible that proliferation and migration of smooth muscle cells to a subintimal location after damage to endothelial cells may involve the local release of ET-1. This could suggest a role for ET-1 in the formation of atherosclerotic plaques and possibly in other reactions of the vessel wall to injury.

The formation by endothelial cells of ET-1, as well as prostacyclin and NO, has further established the vascular endothelium as an organ having important metabolic and regulatory functions. The role of prostacyclin as an endogenous vasodilator is still unclear, however, because inhibition of the synthesis of this autacoid does not result in substantial effects on systemic arterial blood pressure or on blood flow in many organs. In contrast, profound increase in vascular tone and blood pressure has been observed in experimental animals or humans after inhibition of NO synthesis. This has led to the proposal that the cardiovascular system under physiological conditions is constantly under the influence of an NO-dependent vasodilator tone that ensures adequate blood supply and regulates blood pressure. It is, therefore, reasonable to envisage pathological conditions in which increased ET-1 release from the endothelium may counteract this normal vasodilator tone, particularly under conditions of reduced NO biosynthesis. Although little is known at present about the mechanisms that modulate the respective biosynthesis of ET-1 or NO within the endothelium, it is interesting that each of these mediators can be released by shear stress.

Moreover, it is intriguing that in contrast to ET-1, nitrovasodilators that release NO have been shown to inhibit mitogenesis in vascular smooth muscle cells. This could, therefore, represent a sensitive regulatory system in the endothelial cell with the ability to up- or down-regulate vascular cell proliferation. Hence, a local imbalance between these factors could be one of the initiating events in the development of atherosclerotic lesions as well as in vasospastic episodes.

Further understanding of the biological relevance of ET-1 and related products will be achieved by the availability of selective antagonists or specific antibodies as well as by the use of inhibitors of its biosynthesis. These studies will determine whether ET-1 is indeed an important biological mediator or merely a curiosity from an obscure phylogenic past, related to the highly toxic snake-venom sarafotoxins. As with many novel endogenous mediators, the relevance of ET-1 may only become clear after considered analysis, which is now following on from the initial enthusiasm for broad pharmacological profiling.
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(Circulation 1990;81:2022–2025)
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doi: 10.1161/01.CIR.81.6.2022
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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