The Endothelin System
A New Target for Therapeutic Intervention

Masashi Yanagisawa, MD, PhD

Since its identification in 1988, endothelin has attracted intensive interdisciplinary research interest because of its unique profile as an endothelium-derived vasoactive factor with a powerful and characteristically long-lasting vasopressor activity. Indeed, in terms of cellular mechanisms of action, endothelin appears to be quite similar to many classic vasoconstrictor substances such as angiotensin II and norepinephrine. However, the slow time course of the regulatory mechanisms of its biosynthesis and secretion more resembles that of inflammatory cytokines, giving this family of small peptides a unique position within the realm of intercellular mediators with cardiovascular relevance.

See p 1203

The endothelins are a family of 21-residue peptides consisting of three structurally related isoforms called endothelin (ET)-1, ET-2, and ET-3. These mature, active forms are produced from the corresponding prepropeptides that are encoded by three separate genes. These prepropeptides are first processed by a furin-like processing protease into biologically inactive intermediates called big ET-1, -2, and -3. Big endothelins are then proteolytically activated via cleavage at the common Trp21 residue by a highly specific endopeptidase(s) called endothelin-converting enzyme (ECE). The production of endothelins is tightly regulated at the level of mRNA transcription. In vascular endothelial cells, ET-1, once synthesized, is secreted via the constitutive pathway of secretion without further regulation at the level of exocytosis. Endothelins are expressed on proper stimulation in various cell types, although vascular endothelial cells appear to be the most abundant source of ET-1 in vivo under healthy conditions, and the endothelial cells produce only ET-1. It seems reasonable to consider that endothelins act primarily as local, paracrine/autocrine hormones, because (1) the "target" cell types that have endothelin receptors can always be found in the vicinity of the "producer" cell types within the same tissue; (2) circulating plasma levels of endothelins are generally much lower than the threshold concentrations for pharmacological activities; and (3) circulating endothelins are rapidly and efficiently cleared by the lungs and other organs, and even if they reach a threshold concentration, they first stimulate endothelial endothelin receptors of ETβ subtype (see below), leading to the formation of physiological antagonists such as nitric oxide and prostacyclin. These considerations indicate that systemic plasma concentrations may correlate only poorly with the local levels of production.

Endothelins act on two pharmacologically and molecularly distinct subtypes of the heptahelial superfamilies of receptors called ETα and ETβ receptors. Both receptor subtypes are expressed in a wide variety of cell types, with distinct but partially overlapping tissue distributions. On binding of endothelins, both ETα and ETβ receptors trigger a similar set of intracellular signaling systems, including the phospholipase C-β pathway via the activation of heterotrimeric G proteins. This leads to a variety of cellular actions depending on the target cell types, eg, contraction and proliferation of vascular smooth muscle cells. In contrast, the two receptor subtypes have highly distinct isopeptide specificities: although the ETα subtype has an affinity rank order of ET-1 ≥ ET-2 > ET-3 (ET-3 often acts only as a partial agonist), the ETβ receptor accepts all three isopeptides equally. The molecular subdomains of the receptors that determine this ligand selectivity have also been delineated.

Because of the way in which these receptor subtypes were initially discovered, in the past it was believed that the ETα and ETβ receptors have clearly demarcated anatomic and functional territories in vascular tissues: the smooth muscle ETα receptors mediate direct vasoconstrictor actions of endothelins, whereas the endothelial ETβ receptors produce vasodilator effects via the endothelin-induced release of nitric oxide. However, this simple model does not hold anymore in view of the accumulating lines of recent evidence in both experimental animals and humans.

The report in this issue of Circulation by Seo and colleagues provides the first definitive evidence that both ETα and ETβ receptors are involved in the vasoconstrictor action of endothelins in human blood vessels, further emphasizing the complexity of the vascular roles for endothelin receptor subtypes. The consensus in the field has become that the endothelium-dependent relaxation induced by endothelins, if it is at all observed, is always mediated by the ETβ receptor, regardless of species and vascular regions; and that relative contributions of the ETα and ETβ subtypes to the vasopressor responses vary greatly depending on both species and the vasculature in question.
The species-related variation is indeed striking. For example, in rabbits, the renal vasoconstrictor response is mediated entirely by the ETₐ receptor.¹⁰ In contrast, the ET₈ subtype contributes at least equally to the endothelin-induced renal vasoconstriction in rats.¹¹,¹² Although no information is currently available regarding the relative functional importance of the receptor subtypes in human renal vasculature, quantitative radioligand binding studies show that the ET₈ subtype predominates over ETₐ receptors in human kidney by approximately 2:1.¹³ As an additional example, the endothelin-induced constriction of pulmonary arteries is mediated entirely by the ETₐ receptors in dogs¹⁴ and humans,¹⁵ but in rabbits, it is governed wholly by the ET₈ receptors.¹⁶

Even within the same species, the relative contribution of endothelin receptor subtypes to the constrictor responses varies greatly from vasculature to vasculature. For example, in rabbits, the constriction of carotid arteries is mainly due to the ETₐ activation, whereas in the jugular veins, it occurs entirely via the ET₈ receptors.¹⁷ In canine coronary vasculature, the contraction of conduit arteries is mediated purely by the ET₈ subtype, whereas the ET₈ receptors are significantly involved in the constriction of the resistance arteries.¹⁸,¹⁹ Interestingly, the situation in the coronary vasculature appears to be the opposite in humans. Human proximal coronary arteries contain constrictor ET₈ receptors, whereas the constriction of the distal, prerenal arteries occurs purely via the ETₐ receptors.²⁰ Seo et al now report with solid lines of evidence that both ETₐ and ET₈ receptors are involved in the endothelin-induced contraction of human internal mammary arteries and veins. Especially, ET₈ receptors appear to be more important in the vasoconstriction induced by low concentrations of endothelins, which may be relevant to the actual pathophysiological situation.

These findings have enormous implications in terms of the discovery and development of a novel modality of therapeutics that make use of endothelin receptor antagonists. Virtually all major players in the pharmaceutical industry are involved in this race of drug discovery. Now they face an interesting situation—thanks to the modern methods of rational molecular design and mass screening with cloned receptors, the discovery of antagonists with a favorite subtype selectivity (ie, ETₐ selective, ET₈ selective, or nonselective) itself is not a problem anymore. Indeed, a number of peptidic antagonists, which are extremely useful at least as research tools, have been discovered.²¹,²² More recently, Clozel and colleagues²³ reported the first orally active nonpeptidic antagonist. Rather, a real challenge in the field is to find the right animal models of potential target human disease and to match those models with antagonists having the right receptor subtype selectivity. As the aforementioned findings clearly dictate, there would be no benefit if a wrong model species or a drug with the wrong subtype selectivity were chosen. Also, it will be extremely difficult to predict the effects of a candidate drug in humans, especially in the current dearth of precise pharmacological studies in humans. However, an obvious lesson from these recent studies is that the initial hypothesis is invalid, where the ET₈ receptors were portrayed as bad guys and the ETₐ as saints. Research in the field clearly needs to address the pathophysiological roles of both receptor subtypes separately, without negligent a priori assumptions.

Thus, the situation is complicated due to the fact that the ET₈ subtype can act as either vasoconstrictor or vasodilator receptor depending on the vasculature and species. One must be extremely careful in designing and interpreting a study that involves ligands that are active on ET₈ receptors. The current lack of a good, published ET₈-selective antagonist makes it even more problematic. In this regard, a recent study by Warner and colleagues²⁴ suggests the possible existence of subtypes of ET₈ receptors; they postulated a dilator (or endothelial) ET₈ receptor that is sensitive to the hexapeptide ETA/ET₈ nonselective antagonist PD 142893 and a constrictor ET₈ subtype that is resistant to this class of compound. However, these organ-bath findings have to be substantiated by radioligand binding assays and molecular cloning studies before any definitive conclusion can be derived.

Because of the historical reasons described, only ETₐ-selective antagonists have been widely used for research until relatively recently. Despite this unfortunate shortcoming, the pathological significance of endogenous endothelins has become encouragingly convincing in a number of experimental models. For example, antiendothelin monoclonal antibodies as well as the receptor antagonists BQ-123 (ETₐ selective, peptidic) and Ro 46-2005 (nonselective, nonpeptidic) have been shown by a number of researchers to ameliorate ischemic²⁵,²⁶ and cyclosporine-induced²⁷,²⁸ acute renal failure. Chronic administration of FR 139317, another ETₐ-selective antagonist, was effective in preventing progressive proliferative renal disease and associated hypertension in a rat model of chronic glomerulonephropathy induced by surgical renal mass reduction.²⁹ The blockade of endothelin action by neutralizing antibodies or BQ-123 has been shown to reduce the extent of experimental acute myocardial infarction in rats,³¹ rabbits,³² and dogs.³³ The peptidic ETₐ-selective antagonist BQ-485 is effective in preventing delayed cerebrovascular spasm in a dog model of subarachnoid hemorrhage.³⁴ A nonpeptidic antagonist Ro 47-0203 reverses delayed vasospasm in a similar model in rabbits.³⁵ Furthermore, endothelin receptor antagonists lower basal blood pressure in spontaneously hypertensive and spontaneously hypertensive stroke-prone strains of rats³⁶,³⁷ as well as in sodium-depleted squirrel monkeys,³³ suggesting a possible role of endothelin in the maintenance of blood pressure under certain conditions and in the development of this class of genetically determined hypertension. The antagonist not only reduces blood pressure but also prevents secondary renal disease observed in DOCA/salt-treated spontaneously hypertensive rats with malignant hypertension.³⁸

Finally, it is important to note that the ECE represents another plausible target for pharmacological intervention to the endothelin system because ECE appears to be a novel metalloprotease that has a strict substrate specificity.³⁹ A selective inhibitor for the enzyme can presumably inhibit the production of active endothelins in a highly specific manner. Unfortunately, this avenue of research has been severely hampered in the past because of the elusive molecular nature of ECE itself. However, now that ECE has been apparently
purified to near-homogeneity,99 this major component of the endothelin system should soon be revealed at the molecular level.

We have increasingly better evidence for the causal role of endothelins in disease involving an abnormal vasoconstriction. Development in the near future of nonpeptidic receptor antagonists with even higher potency and specific ECE inhibitors should further facilitate the understanding, eventually in humans, of the biology of endothelins in health and disease. Those new biological insights should provide renewed promise for progress toward this novel target for therapeutic intervention, which may include disease where no effective drug treatment is currently available.

References


Key Words • endothelin • kidneys • infarction • Editorsials • hypertension
The endothelin system. A new target for therapeutic intervention.
M Yanagisawa

Circulation. 1994;89:1320-1322
doi: 10.1161/01.CIR.89.3.1320

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/3/1320.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/