Endothelin (ET) is an endothelial-derived peptide that exerts potent vasoconstrictive activity in vivo and in vitro. An increasing body of evidence suggests that ET may have a significant role in the pathophysiological processes leading to cardiovascular disease states. Although it is not certain at this point whether ET is primarily responsible for initiating these events, sufficient evidence exists to conclude that ET is involved as a mediator or cofactor in these disease states.

ET was first described by Yanagisawa et al. in 1988 \([1]\). The pre-propeptide is cleaved to form big ET (39 amino acids) and then is further cleaved by an endothelin-converting enzyme (ECE) to form the 21-amino acid active peptide. Three pharmacologically distinct isopeptides (ET-1, ET-2, and ET-3) exist, derived from three different pre-propeptides that are encoded by separate genes \([2]\). The ET-1 isopeptide is primarily responsible for the observed vasomotor effects. Two distinct ET receptors exist: ET-A and ET-B, the latter being associated with both vasoconstrictive and vasodilatory effects \([3]\). In addition to its vasoactive effects, ET has been shown to affect cellular proliferation.

Endothelial cells form a single-cell-thick layer that lines the entire vasculature. The continuous production and release of endothelin from the endothelial cells underscores the significant role of this peptide in the regulation of vascular tone and growth in both physiological and pathophysiological states. ET-1 has been implicated in the pathogenesis of numerous disease processes, particularly cardiovascular diseases. Significant ongoing research efforts are further delineating the mechanisms involved, which in turn may lead to the development of potential therapeutic agents.

ET has been implicated in the pathogenesis of atherosclerosis and is intimately involved in the cellular and humoral mechanisms responsible for its progression \([4]\). ET is released in response to vascular endothelial injury, which is the critical initiating event in vascular disease and atherosclerosis; functions as a strong chemoattractant for circulating monocytes; and activates macrophages. These cells in turn further injure the overlying endothelium and lead to recruitment of other proliferative and mitogenic factors. ET also has potent effects on smooth muscle cells \([5]\), fibroblasts, and the formation of extracellular matrix \([6]\), all of which are involved in the formation of atherosclerotic plaque. Elevated concentrations of ET-1 have also been demonstrated in patients with risk factors for atherosclerosis, such as hyperlipidemia \([7]\) and tobacco use \([8]\), even before the development of overt morphological changes in the vasculature. ET-1 has been implicated in acute coronary syndromes (unstable angina and acute myocardial infarction) \([9,10]\) and may play a role in neointima formation and restenosis of coronary arterial stenoses that have been treated by percutaneous transluminal coronary angioplasty (PTCA) \([11]\); interest-

ingly, blockade of ET receptors has been shown to decrease the incidence of neointima formation in animal models of PTCA \([12]\).

ET-1 has also been shown to be important in congestive heart failure; plasma ET-1 concentrations may be increased as much as fourfold above normal in affected patients and correlate with the severity of symptoms \([13]\). ET receptor antagonism in patients with severe heart failure has resulted in improvement of objective hemodynamic measurements \([14]\). ET has also been implicated in the pathogenesis of various other disease entities characterized by vasomotor impairment \([15]\), including renal failure, pulmonary hypertension, portal hypertension, and cerebral vasospasm occurring in the setting of subarachnoid hemorrhage.

Currently, plasma concentrations of ET-1 are measured by RIA techniques. However, such assays are hampered by cross-reactivity with other ET isopeptides and with the precursor big ET. The C-terminal portion of ET-1 may cross-react with ET-2 and ET-3, and the N-terminal fragment can cross-react with big ET-1 and possibly ET-2 \([16]\). Because big ET-1 has no significant intrinsic vasoconstrictor effect, its presence may lead to overestimation of the quantity of the active vasoconstrictive peptide by RIA. Sandwich enzyme immunoassays (EIAs), however, make use of two antibodies and thus have a higher degree of specificity than conventional RIAs. Sandwich EIAs for ET-1 and big ET-1 exist already \([16]\); however, antibodies in the former assay cross-react fully with ET-2, making this EIA therefore not of practical value for clinical research purposes.

In the current issue of Clinical Chemistry, Aubin et al. elegantly describe a novel sandwich EIA for big ET-1 in which an acetylcholinesterase label is used \([17]\) instead of the peroxidase label previously reported by Suzuki et al. \([18]\). Assaying sera from 22 young healthy subjects, Aubin et al. demonstrate that this method is extremely reliable, with high sensitivity as well as specificity, excellent reproducibility, and minimal cross-reactivity with ET isopeptides and isopropeptides. These authors also show that concentrations of big ET-1 are gender- and posture-independent. Before widespread acceptance of its applicability as a research or clinical tool in human subjects, however, the assay should be used to determine age-adjusted reference values for big ET-1 as well.

Such an assay has potential implications as a research tool, especially given suggestions that activity of the ET pathway may be better assessed by measuring big ET-1 rather than ET-1, because the former has a longer serum half-life and may thus more accurately represent the relative physiological effect present at any given time. The shorter half-life of ET-1 in circulation probably results both from redistribution into other tissues and from binding to high-affinity ET receptors \([19,20]\); therefore, serum assays for ET-1 may underestimate the physiolog-
tical effect of the peptide. Furthermore, it has been demonstrated that circulating ET-1 concentrations in normal and pathological states are much lower than the concentrations required to cause in vitro smooth muscle cell contraction; it has been suggested that in vivo, ET-1 binds stoichiometrically to its receptors [21], and that in this condition, most ET-1 is receptor-bound and unavailable for measurement in an assay. These findings support the potential advantages of measuring big ET-1 to indirectly, but perhaps more accurately, assess the net physiological effects of ET-1. ET immunoreactivity is also increased after the administration of ET-receptor antagonists [22]; the mechanism for this may be either displacement of ET-1 from ET receptors by the receptor blockers, or up-regulation of ET-1 production. An assay for big ET-1 may further delineate this mechanism. In addition, measurement of both big ET-1 and ET-1 may be of value in evaluating the activity of the ECEs; conceivably, the development of ECE inhibitors may have therapeutic value in the clinical setting.

In summary, the ET pathway appears to be critically important in the pathogenesis of various disease states, particularly of the cardiovascular system, although much remains to be elucidated regarding its role in initiating and modulating these processes. It is interesting to postulate that pharmacological blockade of the effects of ET may retard or even prevent the manifestations of these disease states; indeed, this ability has already been demonstrated to varying degrees in some conditions in which ET has been implicated. Assays for the ET pathway are a crucial component of ongoing research investigating the physiological effects of this peptide as well as measuring the therapeutic effects of potential ET-receptor blockers and ECE inhibitors. A reliable assay for big ET-1 that is reproducible, highly sensitive, and specific and shows low cross-reactivity may well prove to be an extremely useful research tool.

References


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