Dissecting the Complex Physiology of Endothelin
New Lessons From Genetic Models

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It is an understatement to say that blood pressure regulation is complex, especially in terms of long-term control. Redundancy abounds among the intricate balance of vascular, neural, and renal components. For the past 30 years, a major focus has been on elucidating how the endothelium contributes to vascular physiology and pathophysiology, with many investigators suggesting a link between endothelial dysfunction and hypertension. After Yanagisawa et al first described the endothelial-derived constricting factor, endothelin (ET) 1, many investigators assumed that the most potent vasoconstrictor thus far identified would play a critical role in maintaining systemic vascular resistance and, therefore, contribute to blood pressure regulation. However, 20 years later, we are still only beginning to understand its very complex yet significant role in this realm. The difficulty in understanding the ET system is attributed to numerous factors, including the following: (1) the opposing roles of ETA and ETB receptors to produce vasoconstriction and vasodilation, respectively, along with the occasional ETB-dependent vasoconstriction; (2) the irreversible nature of ligand binding to the ET receptors; (3) the localized nature of ET action such that plasma levels do not necessarily reflect synthesis but, rather, the balance of synthesis and clearance; (4) global knockout models for ET and its receptors result in lethal phenotypes; and (5) the relatively underinvestigated function of ET receptors in the peripheral nervous system that can influence vascular tone and blood pressure.

Breakthrough evidence for the ET system in the control of blood pressure came from studies by Gariepy and collaborators, who published several key articles revealing that the ETB receptor prevents development of the enteric nervous tissue, and so they do not develop intestinal agangliosis but retain mutated, nonfunctional ETB receptors in the vasculature and kidney. As adults, these transgenic ETB receptor-deficient rats have slightly elevated basal blood pressures when measured by telemetry, but, more interestingly, a high-salt diet produces a profound hypertension providing solid evidence for ETB control of sodium excretion.

Fortunately, we also have available a wide range of highly specific and selective pharmacological tools for studying the ET system by way of receptor antagonists. Long-term administration of ETB receptor–specific antagonists produces hypertension that is salt dependent, similar to that observed with ETB receptor deficiency. The elevation in blood pressure because of ETB blockade can be reversed with an ETA receptor antagonist, indicating that the ETB receptor may function primarily to protect from ETA-dependent effects, through clearance of ET-1 as well as vasodilation. However, this does not explain the salt sensitivity of hypertension produced by ETB blockade or deficiency.

Renal collecting duct–specific knockout mice developed by Kohan’s laboratory have clearly identified a role for the intrarenal ET-1/ETB receptor pathway in blood pressure regulation and facilitating sodium excretion. Similar to the global loss of ETB receptor function, the collecting duct–specific ET-1 and ETB receptor knockout mice display elevated basal blood pressures that are exacerbated by a high-salt diet. The latter studies provide definitive evidence for the renal collecting duct ET system as an important control system for blood pressure.

ET-1 and ETA receptor–deficient mice, on the other hand, have developmental defects in the craniofacial region and do not survive beyond birth. Until now, our knowledge of how the ETA receptor may contribute to blood pressure regulation has been limited to pharmacological studies (although there has yet to be an ETA selective agonist developed). ETA receptor blockade can lower blood pressure in both animals and humans with hypertension, particularly in those that are salt dependent, but the physiological role of the ETA receptor has been more difficult to discern, because there are mixed reports of whether ETA selective antagonists can or cannot lower blood pressure in normotensive animals or humans. Much of this uncertainty is likely because of variability in the methods, unknown in vivo selectivity of antagonists, and the genetic background of the model being tested.

In the present issue of Hypertension, Kisanuki et al describe a newly developed mouse strain that represents a significant advance in our understanding of the physiological role of endothelial-derived ET-1. Using the Cre/loxP recombinase approach, these investigators created a mouse strain that does not express the prepro–ET-1 gene in vascular...
endothelial cells (ET-1\textsuperscript{floxed/floxed}, Tie2-Cre) in an effort to discern the physiological role of endothelial-derived ET-1 on blood pressure. These mice have lower blood pressures than intact genetic controls, thus providing the best evidence to date that the balance of the physiological actions of endothelial ET-1 is to maintain a degree of elevated vascular tone. Although the potency of ET-1 to cause vasoconstriction may have led some to believe that this would be a foregone conclusion, the opposing actions of ET\textsubscript{A} and ET\textsubscript{B} receptors have not allowed one to fully understand the net result of endogenous ET-1 activity. Furthermore, the effect of specific ET\textsubscript{A} versus ET\textsubscript{B} blockade, at least in a short-term setting, suggests that the predominant action of endothelial ET-1 would be via the ET\textsubscript{B} receptor. In other words, ET\textsubscript{B} blockade causes more dramatic increases in blood pressure compared with the blood pressure–lowering effects of ET\textsubscript{A} antagonists. The difficulty in understanding this balance is because removing ET\textsubscript{A} actions not only results in loss of endothelial vasodilatory pathways but also allows endogenous ET-1 to act on the ET\textsubscript{B} receptor unopposed.

Another important mouse model was developed by Bagnall et al\textsuperscript{12} and involved endothelial-specific deletion of the ET\textsubscript{B} receptor. Similar to the ET\textsubscript{B}-deficient rat, endothelial cell–specific deletion of the ET\textsubscript{B} receptor increased plasma ET-1 concentrations consistent with a “clearance” function of the ET\textsubscript{B} receptor. Furthermore, these mice developed endothelial dysfunction, as defined by an attenuated ability of isolated aorta to relax in response to acetylcholine and other stimuli. Importantly, these investigators observed that the loss of endothelial ET\textsubscript{B} receptors had no effect on blood pressure or the blood pressure response to changes in salt intake. The limiting feature of this study is that these animals were developed on a salt-sensitive background, and so they need to be rederived on a salt-resistant background to discern whether salt sensitivity is truly unrelated to endothelial ET\textsubscript{B} receptors. Nonetheless, the findings of Bagnall et al\textsuperscript{12} would indicate that the hypertension produced by global ET\textsubscript{B} blockade or genetic deficiency is most likely attributed to renal tubular ET\textsubscript{B} function and that collecting duct–derived ET-1 targets renal tubular ET\textsubscript{B} receptors and not endothelial ET\textsubscript{B} receptors.

Amiri and colleagues\textsuperscript{13–15} have published a series of articles where ET-1 has been overexpressed in vascular endothelium. Overexpression of endothelial ET-1 in mice results in hypertrophic remodeling, oxidant stress, and vascular inflammation but has no effect on blood pressure. These effects are attenuated and exacerbated by ET\textsubscript{A} and ET\textsubscript{B} receptor blockade, respectively. Thus, it appears as though the ET\textsubscript{B} receptor functions to protect the vasculature from the injurious effects of ET\textsubscript{A} receptor activity but that elevated ET-1 production in and of itself is insufficient to raise blood pressure. Such findings are supported by reports demonstrating that chronic ET-1 infusion does not always produce hypertension, at least in rats on a normal salt diet.\textsuperscript{16,17}

The study by Kisanuki et al\textsuperscript{11} using their newly developed endothelial cell–specific ET-1 knockout mice also addressed the interaction between ET-1 and other vasoactive systems. The endothelial cell ET-1 knockout mice had similar blood pressure responses to angiotensin II, norepinephrine, bradykinin, and the NO synthase inhibitor A\textsuperscript{6}-nitro-L-arginine methyl ester as genetic controls. Therefore, it appears that endothelial cell ET-1 has little influence on these systems, at least in terms of the acute vascular responsiveness. Additional studies that produce chronic changes in these systems are still needed to help discern whether the balance of these factors is influenced by endothelial cell ET-1.

In conclusion, the development of endothelial cell ET-1 knockout mice represents another significant advance in our efforts to understand the physiological role of ET-1 in the control of blood pressure. On balance, we can be confident that endothelial cell–derived ET-1 functions to maintain a higher level of vascular tone as a means of maintaining blood pressure and, therefore, appears to contribute in a vasconstrictor capacity to maintain blood pressure in its capacity as a major participant in the complex scheme of blood pressure control and fluid-volume balance. Furthermore, the endothelial ET\textsubscript{B} receptor appears to buffer ET\textsubscript{A} receptor activity both in terms of blood pressure and the mitogenic and proinflammatory actions of ET-1, as well as serving a permissive role in terms of endothelial-dependent relaxation (Figure). What is not clear, however, is how endothelial ET-1 actually produces the desired level of blood pressure, because increases in peripheral resistance alone do not necessitate a long-term change in blood pressure without an influence on body-fluid homeostasis. What is the influence of endothelial ET-1 on kidney function? Is there a relationship between the vascular and renal tubular ET-1 systems? To what extent might the endothelial ET-1 system contribute to hypertension? What factors distinguish the physiological from the pathophysiological actions of ET-1? Does the neural ET-1 system operate independent of the vascular and renal systems? We can look...
forward to answers to these and many other questions in the coming years.

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