Published in final edited form as:
Hypertension. 2010 July ; 56(1): 31–33. doi:10.1161/HYPERTENSIONAHA.109.139758.

Dissecting the complex physiology of endothelin:

New lessons from genetic models

David M. Pollock
Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912-2500

Keywords

ET-1; ET_A receptor; ET_B receptor; blood pressure; hypertension

It is an understatement to say that blood pressure regulation is complex, especially in terms of long-term control. Redundancy abounds between 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,[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 due to numerous factors including 1) the opposing roles of ET_A and ET_B receptors to produce vasoconstriction and vasodilation, respectively, along with the occasional ET_B 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 under-investigated function of ET receptors in the peripheral nervous system that can influence vascular tone and blood pressure.

Break-through evidence for the ET system in the control of blood pressure came from studies by Gariepy and collaborators who published several key papers revealing that genetic deficiency in ET_B receptor function results in elevated blood pressure and salt-dependent hypertension.[2,3] Mutation of the ET_B receptor prevents development of the enteric nervous system and so animals homozygous for this trait develop megacolon and die at about the time of weaning. This certainly makes these animals a poor model for studying hypertension. However, these investigators came up with the clever idea of creating a transgenic rat that expresses a functional, non-mutated ET_B receptor only in adrenergic tissues and so they do not develop intestinal agangliosis, but retain mutated, non-functional ET_B receptors in the vasculature and kidney. As adults, these transgenic ET_B 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 ET_B control of sodium excretion.

Correspondence: David M. Pollock Ph.D, Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912-2500 U.S.A., Phone: 706-721-8517, FAX: 706-721-9799, dpollock@mcg.edu.

Disclosures
None.

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.[4] Long-term administration of ET_B receptor specific antagonists produces hypertension that is salt-dependent similar to that observed with ET_B receptor deficiency.[5] The elevation in blood pressure due to ET_B blockade can be reversed with an ET_A receptor antagonist indicating that the ET_B receptor may function primarily to protect from ET_A dependent effects, through clearance of ET-1 as well as vasodilation. However, this does not explain the salt sensitivity of hypertension produced by ET_B blockade or deficiency.

Renal collecting duct specific knockout mice developed by Kohan's lab have clearly identified a role for the intrarenal ET-1/ET_B receptor pathway in blood pressure regulation and facilitating sodium excretion.[6,7] Similar to the global loss of ET_B receptor function, the collecting duct specific ET-1 and ET_B 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 ET_A receptor deficient mice, on the other hand, have developmental defects in the craniofacial region and do not survive beyond birth.[8,9] Until now, our knowledge of how the ET_A receptor may contribute to blood pressure regulation has been limited to pharmacological studies (even though there has yet to be an ET_A selective agonist developed). ET_A receptor blockade can lower blood pressure in both animals and humans with hypertension, particularly in those that are salt-dependent,[10] but the physiological role of the ET_A receptor has been more difficult to discern because there are mixed reports of whether ET_A selective antagonists can or cannot lower blood pressure in normotensive animals or humans. Much of this uncertainty is likely due to variability in the methods, unknown *in vivo* selectivity of antagonists, and the genetic background of the model being tested.

In the current 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.[11] Using the *Cre/loxP* recombinase approach, these investigators created a mouse strain that does not express the preproET-1 gene in vascular endothelial cells (*ET-1$^{flox/flox}$;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. While 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_A and ET_B receptors have not allowed one to fully understand the net result of endogenous ET-1 activity. Furthermore, the effect of specific ET_A versus ET_B blockade, at least in a short-term setting, suggests that the predominant action of endothelial ET-1 would be via the ET_B receptor. In other words, ET_B blockade causes more dramatic increases in blood pressure compared to the blood pressure lowering effects of ET_A antagonists. The difficulty in understanding this balance is because removing ET_B actions not only results in loss of endothelial vasodilatory pathways, but also allows endogenous ET-1 to act on the ET_A receptor unopposed.

Another important mouse model was developed by Bagnall and colleagues and involved endothelial-specific deletion of the ET_B receptor.[12] Similar to the ET_B deficient rat, endothelial cell specific deletion of the ET_B receptor increased plasma ET-1 concentrations consistent with a "clearance" function of the ET_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_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 re-derived on a salt-resistant background in order to discern whether salt-sensitivity truly is unrelated to endothelial ET_B receptors. Nonetheless, the findings of Bagnall et al. would indicate that the hypertension produced by global ET_B blockade or genetic deficiency is most likely due to renal tubular ET_B function and that collecting duct derived ET-1 targets renal tubular ET_B receptors and not endothelial ET_B receptors.

Amiri and colleagues have published a series of papers where ET-1 has been over-expressed in vascular endothelium.[13–15] Over-expression of endothelial ET-1 in mice results in hypertrophic remodeling, oxidant stress, and vascular inflammation, but no effect on blood pressure. These effects are attenuated and exacerbated by ET_A and ET_B receptor blockade, respectively. Thus it appears as though the ET_B receptor functions to protect the vasculature from the injurious effects of ET_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.[16,17]

The study by Kisanuki et al. using their newly developed endothelial cell specific ET-1 knockout mice also addressed the interaction between ET-1 and other vasoactive systems.[11] The endothelial cell ET-1 knockout mice had similar blood pressure responses to angiotensin II, norepinephrine, bradykinin, and the NOS inhibitor, L-NAME, 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.

The lack of endothelial cell ET-1 clearly results in lower arterial pressure as demonstrated by both tail cuff and telemetry methods in the mouse. Acute administration of an ET_A selective antagonist, FR139317, to a wild-type mouse also lowered blood pressure consistent with endogenous ET-1/ET_A dependent tone. However, it is interesting to note that FR139317 lowered blood pressure to a similar degree in the control and endothelial cell ET-1 knockout mice even though the latter change was not statistically significant, most likely due to the low number of mice studied (n=5). In any event, the authors concluded that there remains some degree of ET_A dependent vascular tone in endothelial cell ET-1 knockout mice. Since there is a wide range of cell types that synthesize ET-1, this may not be a surprising finding. Indeed, while endothelial cell ET-1 knockout mice have reduced ET-1 in heart, lung, kidney and brain, there remains measurable, albeit reduced, ET-1 in the plasma.

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 vasoconstrictor 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_B receptor appears to buffer ET_A receptor activity both in terms of blood pressure and the mitogenic and pro-inflammatory actions of ET-1 as well as serving a permissive role in terms of endothelial-dependent relaxation. What is not clear, however, is how endothelial ET-1 actually produces the desired level of blood pressure since 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.

Acknowledgments

Sources of Funding

The author is supported by grants from the NIH (HL69999 and HL60653) and a grant from the Medical College of Georgia Cardiovascular Discovery Institute.

References

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–415. [PubMed: 2451132]
2. Gariepy CE, Williams SC, Richardson JA, Hammer RE, Yanagisawa H. Transgenic expression of the endothelin-B receptor prevents congenital intestinal aganglionosis in a rat model of Hirschsprung disease. J Clin Invest 1998;102:1092–1101. [PubMed: 9739043]
3. Gariepy CE, Cass DT, Yanagisawa M. Null mutation of endothelin receptor type B gene in spotting lethal rats causes aganglionic megacolon and white coat color. Proc Natl Acad Sci U S A 1996;93:867–872. [PubMed: 8570650]
4. Kirkby NS, Hadoke PW, Bagnall AJ, Webb DJ. The endothelin system as a therapeutic target in cardiovascular disease: great expectations or bleak house? Br J Pharmacol 2008;153:1105–1119. [PubMed: 17965745]
5. Pollock DM, Pollock JS. Evidence for endothelin involvement in the response to high salt. Am J Physiol Renal Physiol 2001;281:F144–150. [PubMed: 11399655]
6. Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE. Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. J Clin Invest 2004;114:504–511. [PubMed: 15314687]
7. Ge Y, Bagnall A, Stricklett PK, Strait K, Webb DJ, Kotelevtsev Y, Kohan DE. Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention. Am J Physiol Renal Physiol 2006;291:F1274–1280. [PubMed: 16868309]
8. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, Kumada M, Hammer RE, Yanagisawa M. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. Development 1998;125:813–824. [PubMed: 9449664]
9. Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, Oda H, Kuwaki T, Cao W, Kamada N, Hishage K, Ouchi Y, Azuma S, Toyada Y, Ishikawa T, Kumada M, Yazaki Y. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. Nature 1994;368:703–710. [PubMed: 8152482]
10. Schneider MP, Boesen EI, Pollock DM. Contrasting actions of endothelin ET_A and ET_B receptors in cardiovascular disease. Annu Rev Pharmacol Toxicol 2007;47:731–759. [PubMed: 17002597]
11. Kisanuki YY, Emoto N, Ohuchi T, Kedzierski RM, Hammer RE, Yanagisawa H, Williams SC, Richardson JA, Suzuki T, Yanagisawa M. Low blood pressure in endothelial cell-specific endothelin-1 knockout mice. Hypertension. 2010 in press.
12. Bagnall AJ, Kelland NF, Gulliver-Sloan F, Davenport AP, Gray GA, Yanagisawa M, Webb DJ, Kotelevtsev YV. Deletion of endothelial cell endothelin B receptors does not affect blood pressure or sensitivity to salt. Hypertension 2006;48:286–293. [PubMed: 16801484]
13. Amiri F, Paradis P, Reudelhuber TL, Schiffrin EL. Vascular inflammation in absence of blood pressure elevation in transgenic murine model overexpressing endothelin-1 in endothelial cells. J Hypertens 2008;26:1102–1109. [PubMed: 18475147]

14. Amiri F, Virdis A, Neves MF, Iglarz M, Seidah NG, Touyz RM, Reudelhuber TL, Schiffrin EL. Endothelium-restricted overexpression of human endothelin-1 causes vascular remodeling and endothelial dysfunction. Circulation 2004;110:2233–2240. [PubMed: 15466627]

15. Amiri F, Ko EA, Javeshghani D, Reudelhuber TL, Schiffrin EL. Deleterious combined effects of salt-loading and endothelial cell restricted endothelin-1 overexpression on blood pressure and vascular function in mice. J Hypertens.

16. Mortensen LH, Fink GD. Salt-dependency of endothelin-induced, chronic hypertension in conscious rats. Hypertension 1992;19(6 Pt 1):549–554. [PubMed: 1592449]

17. Elmarakby AA, Loomis ED, Pollock JS, Pollock DM. NADPH oxidase inhibition attenuates oxidative stress but not hypertension produced by chronic ET-1. Hypertension 2005;45:283–287. [PubMed: 15623539]

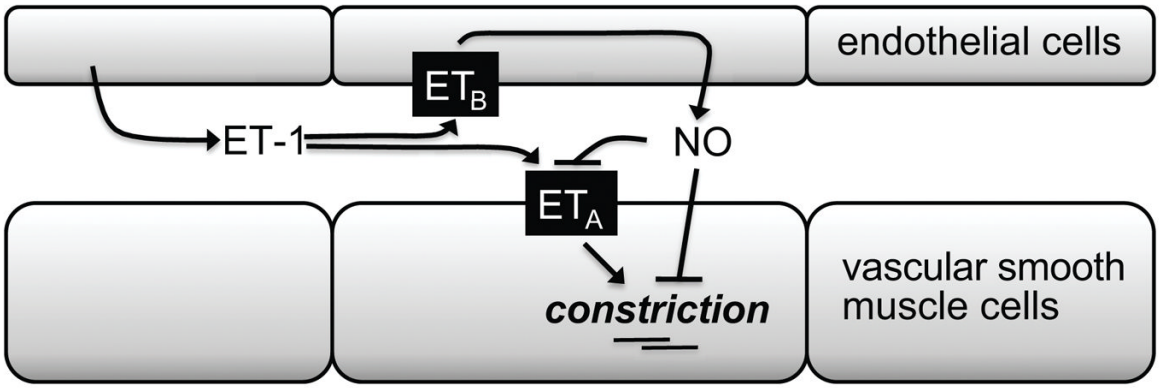

Figure 1.
Hypothetical scheme depicting the balance of ET_A and ET_B receptors in the vascular wall.