Does Endothelin-1 Raise or Lower Blood Pressure in Humans?

Ernesto L. Schiffrin

Hypertension and Vascular Research Unit, Lady Davis Institute for Medical Research and Department of Medicine, Sir Mortimer B. Davis-Jewish General Hospital, McGill University, Montreal, QC, Canada

A comment on Gupta et al: A genetic variant associated with five vascular diseases is a distal regulator of endothelin-1 gene expression. *Cell* 2017;170:522–533.

Keywords

Arterial hypertension · Endothelin · Endothelium · Gene polymorphism

Abstract

Subject of Review: Gupta et al. [*Cell* 2017;170:522–533] have shown that a genetic variant associated with the 5 vascular diseases is a distal regulator of endothelin (ET)-1 gene (*EDN1*) expression. A common single nucleotide polymorphism (SNP) in the third intron of the *PHACTR1* gene, rs9349379, is the potential variant responsible for increased risk of coronary artery disease, and lower risk of migraine, carotid dissection, fibromuscular dysplasia, and hypertension. Since ET-1 acts through ET type B receptors (ET_BR) on endothelial cells to stimulate the production of nitric oxide and prostacyclin and induce vasodilation, this may contribute to these findings. **Second Opinion:** However, ET-1 has been demonstrated to play a role in experimental and human hypertension. How can enhanced transcription of *EDN1* and translation leading to increased production of ET-1 by endothelial cells play a role in hypertension despite the availability of data that suggest a hypotensive action of ET-1? This could depend on the genetic background of individuals. In some humans, the increased ET-1 vasoconstrictor

action on ET_AR and ET_BR in vascular smooth muscle might predominate over the vasodilator effects exerted via endothelial ET_BR, thus resulting in elevated blood pressure. **Conclusion:** Alternatively, hypertension could be attributed to renal actions of ET-1. Either of these pathophysiological actions may explain a hypertensive role of ET-1 despite a lower risk of hypertension associated with the G allele at rs9349379, the common SNP in the *PHACTR1* gene that is a distal regulator of *EDN1* and leads to an increased expression of ET-1.

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Endothelin-1 (ET-1) is a 21-aminoacid peptide produced by endothelial cells in blood vessels and also in many other cells of the body [1]. It acts in a paracrine manner on underlying vascular smooth muscle cells (VSMC) to produce a powerful vasoconstrictor effect mediated via VSMC ET subtype A receptors (ET_AR) and ET subtype B receptors (ET_BR) [2]. It also acts in an autocrine manner on endothelial cells via ET_BR to induce the release of nitric oxide (NO) and prostacyclin, which in turn results in vasodilatation. Studies of deletion of the ET-1 gene (*Edn1*) [3] or crosses of mice heterozygous for deletion of the ET_BR gene with mice homozygous for the

piebald mutation [4], both of which demonstrate an elevation of blood pressure (BP), have suggested that under physiological conditions, the vasodilating action of ET-1 may predominate [3, 4], whereas under pathophysiological conditions, ET-1 may behave as a vasoconstrictor. Indeed, several experimental hypertensive models such as deoxycorticosterone-salt hypertensive rats [5], Dahl salt-sensitive rats [6], and stroke prone spontaneously hypertensive rats (SHR-sp) [7] will not only express enhanced ET-1 but also respond with BP lowering to ET antagonists [8]. Furthermore, it has been shown that in the hypertensive models in which ET-1 plays a role, BP elevation is generally severe, and vascular remodeling is hypertrophic [9]. In humans with stage 2 (severe) hypertension, ET-1 expression in the endothelium is enhanced [10]. Hypertensive humans respond to non-selective ET_AR and ET_BR blockers such as bosentan [11] as well as selective ET_AR antagonists such as darusentan [12, 13] with a reduction in BP. In atherosclerosis, ET-1 expression is enhanced [14] and may contribute not only to the progression of atherosclerosis [15] but also to the development of aortic aneurysms [16]. We generated a mouse that overexpressed human ET-1 constitutively selectively in the endothelium, and it had hypertrophic vascular remodeling and endothelial dysfunction without much elevation of BP [17]. More recently, we generated an inducible human ET-1 overexpressing mouse that exhibited BP elevation [18], and after some months with sustained hypertension developed stiff arteries and endothelial dysfunction and mild renal hypoperfusion [19]. Thus, it has been evident for a long time from the literature, including recent publications, that vascular disease and hypertension are associated with the action of ET-1, and that ET-1 plays a role in the pathophysiology of BP and vascular diseases.

Recently, Gupta et al. [20] published an interesting paper suggesting that a genetic variant associated with 5 vascular diseases is a distal regulator of ET-1 gene (*EDN1*) expression. Genome-wide association studies have implicated the *PHACTR1* locus (6p24) in the risk associated with 5 vascular diseases: coronary artery disease, migraine headache, cervical artery dissection, fibromuscular dysplasia, and hypertension. By performing genetic fine mapping, Gupta et al. [20] examined a common single nucleotide polymorphism (SNP) in the third intron of the *PHACTR1* gene, rs9349379, as the potential variant responsible for the association. Using epigenomic data from human tissue, they characterized an enhancer at rs9349379 exclusively in aorta, which suggested that this SNP could have a regulatory function on the vasculature. Endothelial-like

cells derived from stem cells and edited with CRISPR/cas9 demonstrated that rs9349379 could regulate the expression of *EDN1* that is located 600 kb upstream of *PHACTR1*. The authors concluded that the known physiological actions of *EDN1* on the vasculature could help explain that *EDN1* was associated with the risk for causing the 5 mentioned diseases. Furthermore, the authors believe that their data demonstrate how bringing together genetic, phenotypic, and epigenetic analyses helps identify biologic mechanisms, whereby a common, non-coding variant can regulate distally a gene and thus participate in the pathophysiology of several vascular conditions.

Understanding how enhanced *EDN1* resulting from regulation by an enhancer at rs9349379, a common SNP in the third intron of the *PHACTR1* gene, could result in a role in coronary artery disease, migraine headache, cervical artery dissection, fibromuscular dysplasia, and hypertension is important. Gupta et al. [20] demonstrate that the cis-regulatory action of the G allele at rs9349379 is associated with higher *EDN1* expression and higher ET-1 secreted peptide, and that the G allele at rs9349379 is associated with increased risk of coronary artery disease but lower risk of migraine headache, cervical artery dissection, fibromuscular dysplasia, and hypertension. Of these conditions, coronary artery disease has an atherogenic basis and a vasoconstrictor component. ET-1 is proatherogenic [14–16]. The coronary circulation has virtually no ET_BR in endothelial cells [21] to mediate vasodilation, and thus increased ET-1 would be expected to induce coronary vasospasm.

Fibromuscular dysplasia, carotid dissection and migraine are often associated [22]. Fibromuscular dysplasia could result from abnormalities in growth of VSMC at multiple sites, including coronary and renal arteries, and this could be corrected in part by the action of enhanced ET-1 promoting smooth muscle motility, thus reducing the risk of development of this condition. The mechanisms of carotid dissection are unclear, but the weakened vascular wall could contribute to dissection, and ET-1 growth-promoting effects on VSMC could reduce the risk of development of dissection. Migraine headaches result in part from vasodilation and improve under the action of vasoconstrictors such as ergot derivatives, although impaired vasodilation due to deficiency in endothelial NO synthase that could be genetic [23] has also been implicated. In the case of migraine headaches, we should not forget the not infrequent association of migraine with coronary artery spasm [24]. Migraines are also not infrequently associated with signs and symptoms of brain ischemia in complicated migraine attacks. ET-1

is both a vasodilator via endothelial ET_BR and a vasoconstrictor via ET_AR and ET_BR on VSMC. Thus, it would not be impossible to conceive that ET-1 could participate in migraine by dilating cerebral blood vessels and selectively contracting other blood vessels both in the brain and elsewhere in the body. Finally, there is evidence of association of migraine and an ET_AR (*ETA*-231a/g) gene polymorphism [25]. Accordingly, it is possible that some of these actions explain the reduced risk of migraine in the face of enhanced expression of *EDN1* via its vasoconstrictor effects.

With respect to hypertension, the associations demonstrated in genome-wide association studies have been with endothelial mediators including *EDN3* [26], although some older studies have shown association with some polymorphisms of *EDN1* [27]. Gupta et al. [20] pointed out the association of the rs9349379 variant with lower risk of hypertension. How can we understand this? Hypertension is a complex disease in which genes associated with high BP usually contribute 1 or at most a few mm Hg each [26]. BP elevation, of which ≈40% is genetic, presumably results from the combined effect of many hypotensive and hypertensive genetic variants. Accordingly, the influence of each genetic variant may depend on the genetic background of the subject as well as a significant environmental influence, in part exerting its effects via epigenetic transcript regulation. If *EDN1* transcription is enhanced and ET-1 is being expressed in increased amounts by endothelial cells, it appears that physiologically the predominant effects of ET-1 on BP may be vasodilation via the ET_BR endothelial receptors producing NO and prostacyclin, resulting in reduced BP, as demonstrated in mice with the deletion of *Edn1* [3] or crosses of mice heterozygous for deletion of the ET_BR gene with mice homozygous for the piebald mutation [4], which results in elevated BP, as mentioned earlier. This could explain the association of rs9349379 and *EDN1* with lower risk of hypertension. However, if the genetic background of the individual is hypertensive, the vasoconstrictor effect of ET-1 secreted by the endothelium acting via ET_AR and ET_BR on underlying VSMC could predominate and result in elevated BP. Alternatively, elevated BP induced by increased ET-1 may depend on renal actions of the peptide on renal blood flow, intrarenal hemodynamics, or immune infiltration of the kidney stimulated by ET-1 [19, 28]. This would explain the evidence from experimental models [1, 5–9] and humans [10–13] that ET-1 participates in BP elevation and hypertension. Thus, a hypertensive effect of ET-1 is possible in susceptible individuals despite the association of the G allele at rs9349379 with higher *EDN1*

expression and higher ET-1 secreted peptide, and the finding that the G allele at rs9349379 is associated with lower risk of hypertension.

In conclusion, ET-1 exerts only vasoconstrictor effects in the coronary circulation virtually devoid of endothelial vasorelaxing ET_BR, as well as proatherogenic effects, and thus enhanced *EDN1* expression associated with the G allele at rs9349379 increases the risk of coronary artery disease. Mainly through ET_AR and ET_BR vasoconstrictor actions on the cerebral vasculature and growth and motility-promoting effects on VSMC enhanced *EDN1* expression reduces risk of migraine headaches, carotid dissection, and fibromuscular dysplasia. With respect to hypertension, the physiological vasodilating effects of ET-1 are responsible for the lower risk of hypertension associated with the enhanced expression of *EDN1* resulting from the G allele at rs9349379. However, in 40% of adults in whom a prohypertensive phenotype is genetically present, or as a result of environmental in part epigenetically mediated effects, the vasoconstrictor actions of ET-1 predominate mediated by ET_AR and ET_BR in VSMC leading to a hypertensive effect of increased endothelial expression of *EDN1*. Alternatively, renal actions of ET-1 could also explain the BP elevation.

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Disclosure Statement

The author declares that there are no conflicts of interest to disclose.

References

- 1 Rautureau Y, Schiffrin EL: Endothelin in hypertension: an update. *Curr Opin Nephrol Hypertens* 2012;21:128–136.
- 2 Sharifi AM, Schiffrin EL: Endothelin receptors mediating vasoconstriction in rat pressurized small arteries. *Can J Physiol Pharmacol* 1996;74:934–939.
- 3 Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, Oda H, Kuwaki T, Cao WH, Kamada N, Jishaga K, Ouchi Y, Azuma S, Toyoda Y, Ishikawa T, Kumada M, Yazaki Y: Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. *Nature* 1994;368:703–710.

- 4 Ohuchi T, Kuwaki T, Ling GY, DeWit D, Ju KH, Onodera M, Cao WH, Yanagisawa M, Kumada M: Elevation of blood pressure by genetic and pharmacological disruption of the ETB receptor in mice. *Am J Physiol* 1999; 276:R1071–R1077.
- 5 Larivière R, Thibault G, Schiffrin EL: Increased endothelin-1 content in blood vessels of deoxycorticosterone acetate-salt hypertensive but not in spontaneously hypertensive rats. *Hypertension* 1993;21:294–300.
- 6 d'Uscio LV, Barton M, Shaw S, Moreau P, Lüscher TF: Structure and function of small arteries in salt-induced hypertension: effects of chronic endothelin-subtype-A-receptor blockade. *Hypertension* 1997;30:905–911.
- 7 Sharifi AM, He G, Touyz RM, Schiffrin EL: Vascular endothelin-1 expression and effect of an endothelin ETA antagonist on structure and function of small arteries from stroke-prone spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1998;31(suppl 1):S309–S312.
- 8 Li JS, Larivière R, Schiffrin EL: Effect of a non-selective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats. Evidence for a role of endothelin in vascular hypertrophy. *Hypertension* 1994;24:183–188.
- 9 Schiffrin EL, Larivière R, Li JS, Sventek P: Enhanced expression of the endothelin-1 gene in blood vessels of DOCA-salt hypertensive rats: correlation with vascular structure. *J Vasc Res* 1996;33:235–248.
- 10 Schiffrin EL, Deng LY, Sventek P, Day R: Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. *J Hypertens* 1997;15:57–63.
- 11 Krum H, Viskoper RJ, Lacourciere Y, Budde M, Charlon V: The effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. *Bosentan Hypertension Investigators. N Engl J Med.* 1998;338:784–790.
- 12 Nakov R, Pfarr E, Eberle S: HEAT Investigators: Darusentan: an effective endothelinA receptor antagonist for treatment of hypertension. *Am J Hypertens* 2002;15:583–589.
- 13 Bakris GL, Lindholm LH, Black HR, Krum H, Linas S, Linseman JV, Arterburn S, Sager P, Weber M: Divergent results using clinic and ambulatory blood pressures: report of a darusentan-resistant hypertension trial. *Hypertension* 2010;56:824–830.
- 14 Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr: Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med* 1991; 325:997–1001.
- 15 Simeone SM, Li MW, Paradis P, Schiffrin EL: Vascular gene expression in mice overexpressing human endothelin-1 targeted to the endothelium. *Physiol Genomics* 2011;43: 148–160.
- 16 Li MW, Mian MO, Barhoumi T, Rehman A, Mann K, Paradis P, Schiffrin EL: Endothelin-1 overexpression exacerbates atherosclerosis and induces aortic aneurysms in apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol* 2013;33:2306–2315.
- 17 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.
- 18 Rautureau Y, Coelho SC, Fraulob-Aquino JC, Huo KG, Rehman A, Offermanns S, Paradis P, Schiffrin EL: Inducible human endothelin-1 overexpression in endothelium raises blood pressure via endothelin type A receptors. *Hypertension* 2015;66:347–355.
- 19 Coelho SC, Berillo O, Ouerd S, Fraulob-Aquino JC, Caillon A, Barhoumi T, Offermanns S, Paradis P, Schiffrin EL: Three-month endothelial human endothelin-1 overexpression causes blood pressure elevation and vascular and kidney injury. *Hypertension* 2018;71: 208–216.
- 20 Gupta RM, Hadaya J, Trehan A, Zekavat SM, Roselli C, Klarin D, Emdin CA, Hilvering CRE, Bianchi V, Mueller C, Khera AV, Ryan RJH, Engreitz JM, Issner R, Shores N, Epstein CB, de Laat W, Brown JD, Schnabel RB, Bernstein BE, Kathiresan S: A genetic variant associated with five vascular diseases is a distal regulator of endothelin-1 gene expression. *Cell* 2017;170:522–533.
- 21 Russell FD, Skepper JN, Davenport AP: Detection of endothelin receptors in human coronary artery vascular smooth muscle cells but not endothelial cells by using electron microscope autoradiography. *J Cardiovasc Pharmacol* 1997;29:820–826.
- 22 Olin JW, Gornik HL, Bacharach JM, Biller J, Fine LJ, Gray BH, Gray WA, Gupta R, Hamburg NM, Katzen BT, Lookstein RA, Lumsden AB, Newburger JW, Rundek T, Sperati CJ, Stanley JC: Fibromuscular dysplasia: state of the science and critical unanswered questions: a scientific statement from the American Heart Association. *Circulation* 2014;129: 1048–1078.
- 23 Griffiths LR, Nyholt DR, Curtain RP, Goadsby PJ, Brimage PJ: Migraine association and linkage studies of an endothelial nitric oxide synthase (NOS3) gene polymorphism. *Neurology* 1997;49:614–617.
- 24 Kurth T, Gaziano JM, Cook NR, Logroscino G, Diener HC, Buring JE: Migraine and risk of cardiovascular disease in women. *JAMA* 2006;296:283–291.
- 25 Tzourio C, El Amrani M, Poirier O, Nicaud V, Bousser MG, Alperovitch A: Association between migraine and endothelin type a receptor (ETA -231 A/G) gene polymorphism. *Neurology* 2001;56:1273–1277.
- 26 Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM: Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; 41:677–687.
- 27 Tiret L, Poirier O, Hallet V, McDonagh TA, Morrison C, McMurray JJ, Dargie HJ, Arveiler D, Ruidavets JB, Luc G, Evans A, Cambien F: The Lys198Asn polymorphism in the endothelin-1 gene is associated with blood pressure in overweight people. *Hypertension* 1999;33:1169–1174.
- 28 Kohan DE, Rossi NF, Inscho EW, Pollock DM: Regulation of blood pressure and salt homeostasis by endothelin. *Physiol Rev* 2011;91: 1–77.