Salt-Dependency of Endothelin-Induced, Chronic Hypertension in Conscious Rats

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The effect of salt intake on the hypertensive response to long-term infusion of endothelin-1 was investigated. Chronically instrumented male Sprague-Dawley rats (325–375 g) were used in a 15-day protocol that included 3 control days followed by 7 days of endothelin-1 infusion at 5.0 pmol · kg⁻¹ · min⁻¹ and 5 days of recovery. Rats were maintained on either a normal sodium chloride intake (2.0 meq Na⁺ per day; normal sodium) or a high sodium chloride intake (6.0 meq Na⁺ per day; high sodium) throughout the protocol. Control rats received normal or high sodium intakes but not endothelin-1. In high-sodium rats, endothelin-1 produced a significant increase in mean arterial pressure and total peripheral resistance; a significant bradycardia was observed only on the first day after the start of the endothelin-1 infusion. Cardiac output, stroke volume, water balance, and urinary sodium and potassium excretion remained unchanged. Termination of endothelin-1 infusion resulted in rapid normalization of both arterial pressure and peripheral resistance. In contrast, normal sodium rats exhibited no alteration in mean arterial pressure, heart rate, total peripheral resistance, stroke volume, water balance, or urinary sodium and potassium excretion throughout the endothelin-1 infusion protocol. The hypertension produced by endothelin-1 infusion cannot be explained by alterations in salt or water balance since endothelin-1 infusion in high sodium animals produced significant increases in mean arterial pressure with no observable changes in water or electrolyte balance. These results indicate that endothelin-induced hypertension in conscious rats is a salt-dependent model of hypertension. (Hypertension 1992;19:549–554)

KEY WORDS • endothelins • sodium-dependent hypertension • rat studies • sodium chloride

Endothelin (ET-1) is a potent vasoactive peptide that is synthesized in many tissues, including vascular smooth muscle endothelial cells. Since the isolation of ET-1 by Yanagisawa et al in 1988, this 21-amino acid peptide has been shown to have both relaxing and constricting effects on vascular smooth muscle via the release of relaxing factors such as prostacyclin, atrial natriuretic factor, and endothelium-derived relaxing factor and via the mobilization of intracellular and extracellular calcium, respectively. Substantial data also exist that describe ET-1 as a constrictor of nonvascular smooth muscle, a secretagogue for the release of other hormones and circulating factors such as aldosterone and adrenocorticotropic hormone, a suppressant of the antidiuretic action of arginine vasopressin (AVP) in the kidney, a modulator of adrenergic neuroeffector transmission, a potent mitogen of vascular smooth muscle cells, and a regulator of neuronal activity in specific brain regions.

In agreement with these observations, autoradiographical studies using iodine-125-labeled ET-1 have revealed that this peptide has specific high affinity binding sites within the cardiovascular system (including cardiac tissue as well as the vasculature), the kidney, the adrenal medulla, the brain (including areas within and outside of the blood–brain barrier), and the spinal cord. These data and others suggest that ET-1 may be involved in numerous and diverse physiological processes. However, a role for ET-1 in blood pressure regulation seems particularly likely in view of the peptide’s localization within endothelial cells and its potent actions both on cardiovascular tissues and on certain brain regions involved in blood pressure homeostasis. Endothelin probably acts primarily in a paracrine fashion, especially in the vasculature, but also has been shown to circulate in blood and to be excreted in urine. A hormonal function for ET-1 is therefore a possibility as significantly altered circulating concentrations of endothelin have been reported in both clinical and experimental hypertension. Furthermore, two patients with an endothelin-secreting hemangioendothelioma exhibited hypertension in association with increased circulating concentrations of endothelin; blood pressure and plasma endothelin concentrations both fell after excision of the tumor.

We recently reported that continuous 7-day intravenous infusion of ET-1 produces a sustained and reversible hypertension in conscious, unrestrained rats, supporting a possible contribution of circulating ET-1 to the development of hypertension. The rats used in this earlier study were maintained on a high sodium intake since salt is known to potentiate hypertension development in other experimental models of hypertension. Subsequent demonstrations of the potent action of ET-1 on renal function led us to speculate that endothelin-induced hypertension might also require a high sodium intake for full expression. Thus, the purpose of
this investigation was to compare the cardiovascular responses to infusions of ET-1 in rats on normal or high sodium intakes.

**Methods**

Male Sprague-Dawley rats (325–375 g) (Sasco-King, Madison, Wis.) were anesthetized with halothane (1.0% in O₂, 1.0 l·min⁻¹). A midline thoracotomy was performed for implantation of an aortic directional pulsed-Doppler flow probe (Crystal Biotech, Holliston, Mass.). Probe leads were directed into a subcutaneous pocket in the animal’s back, and the rats were allowed 5 days for recovery. After this recovery period, rats were anesthetized with sodium pentobarbital (50.0 mg·kg⁻¹ i.v.). Polyvinyl chloride catheters with silicone rubber tips were surgically implanted into the abdominal aorta and a femoral vein via the left femoral vessels and were exteriorized with the previously implanted flow probe leads through the rostral portion of the cranium. The catheters and probe leads were then threaded through a steel spring housing with one end of the spring attached to the cranium with dental acrylic and the other end attached to a plastic swivel that allowed the animal free movement within a metabolic cage. A minimum of 3 days recovery from this surgical procedure was allowed before any experimentation. Rats were allowed free access to distilled water from calibrated drinking tubes and to sodium-deficient rat chow (Teklad, Madison, Wis.). Rats were then placed on either a 2.0 meq·day⁻¹ sodium intake and urinary output. Hemodynamic and fluid measurements were obtained daily.

**Results**

Cardiovascular data from these experiments are summarized in Figure 1. Continuous, 7-day, intravenous infusion of ET-1 at a rate of 5.0 pmol·kg⁻¹·min⁻¹ produced significant, sustained, and reversible increases (—25.1%) in MAP in high sodium rats (n = 12). Maximal increases in MAP were attained within 24 hours after initiation of ET-1 infusion; tachyphylaxis was not observed throughout the ET-1 infusion period. In agreement with previous findings, the increase in MAP was primarily the result of an increased TPR (—17.8%) during ET-1 infusion, since SV and CO were not significantly affected. The increased MAP in high sodium rats receiving ET-1 was also associated with transient bradycardia; likewise, the fall in MAP on termination of the ET-1 infusion produced transient tachycardia. Infusion of ET-1 in high sodium rats produced no significant changes in WB, U₉N,V, or U₉V (Figure 2). Plasma electrolytes (control values: P₉N, 143.6±4.7; P₉K, 4.12±0.14) also were not affected by ET-1 infusion (data not shown). In contrast, infusion of ET-1 at 5.0 pmol·kg⁻¹·min⁻¹ into normal sodium rats (n = 7) produced no significant changes in any cardiovascular (Figure 1) or urinary (Figure 2) variable. Both normal sodium (n = 5) and high sodium (n = 7) rats not receiving ET-1 (controls) exhibited stable values for all measured variables throughout the 15-day protocol. No significant differences between the control normal sodium and high sodium groups were found for any variable except urinary U₉nV (a reflection of sodium intake).

**Discussion**

The data from this study indicate that endothelin hypertension is salt-sensitive. When infused intravenously for 7 days into conscious rats at 5.0 pmol·kg⁻¹·min⁻¹, ET-1 produced a significant, sustained, and reversible elevation in MAP when rats were
maintained on a fixed salt intake of 6.0 meq/day, but not when they were maintained on a salt intake of 2.0 meq/day. This hypertensive response was attributable to an increase in TPR, CO, SV, and HR were not consistently or significantly influenced by the ET-1/high sodium infusion protocol.

There are several potential mechanisms by which a high sodium/ET-1 combination could produce this observed increase in arterial pressure. An obvious possibility is the direct vasoconstrictor action of ET-1. Endothelins are known potent vasoconstrictors both in vivo and in vitro. This constriction has been shown to occur secondary to an increase in intracellular calcium concentrations derived from an increase in transmembrane calcium flux, a release of intracellular stored calcium, or both. Two considerations, however, suggest that a direct vasoconstrictor mechanism may not be the only means by which ET-1 infusion produces an elevated MAP and TPR in this study: first, the infusion rate of ET-1 used in this study has been previously shown to produce only a very small increase in MAP during acute (1 hour) infusions; second, if direct vascular constriction was the main action of ET-1, it is not clear why an increase in MAP and TPR occurred only in ET-1-infused rats on the higher salt intake.

Evidence from recent studies, however, suggests that a high sodium intake may augment the vascular response of the whole animal to the acute pressor
Figure 2. Line plots show water balance (WB), sodium excretion (UNaV) and potassium excretion (UKV) in rats receiving infusion of endothelin-1 (ET-1) at 5.0 pmol·kg⁻¹·min⁻¹. *Significant (p<0.05) difference from control period values. HS, high sodium group (n=7); HS+ET-1, high sodium group receiving ET-1 (n=12); NS, normal sodium group (n=5); NS+ET-1, normal sodium group receiving ET-1 (n=7).

effects of ET-1. Thus, in the current study, it is possible that rats on the high sodium intake simply had greater direct vasoconstrictor responses to the infused ET-1. There remains the question, though, of why an increased salt intake would enhance the vascular responsiveness to ET-1. In the intact animal, chronic ET-1 infusion may, for example, produce higher plasma concentrations of ET-1 during high sodium versus normal sodium intake conditions. Currently, however, we have no information to either confirm or refute this possibility. Alternatively, a high sodium intake could alter physiological properties of vascular smooth muscle cells to enhance the contractile responsiveness to ET-1. An increased sensitivity to contractile agonists is one proposed action of putative ouabain-like, natriuretic factors released in response to a high sodium intake,30 and the sensitivity of aortic strips to ET-1 in vitro has been shown to be increased by ouabain.31 Thus, a simple increase in vascular responsiveness to ET-1 cannot be ruled out as an explanation for the increased MAP and TPR observed here during chronic infusion of ET-1 in rats on a high sodium intake.

A sustained increase in circulating concentrations of mineralocorticoids32 or angiotensin II33 has also been shown to produce a salt-sensitive hypertension in rats. Infusion of endothelins into intact animals for short periods has been reported to increase plasma renin activity34 and raise plasma aldosterone concentration.6 It is conceivable, therefore, that long-term infusion of ET-1 in this investigation produced a persistent elevation in plasma levels of angiotensin II or aldosterone, or both, thereby leading to a salt-sensitive hypertension. This possibility is currently under investigation.

There is now some evidence to implicate neural blood pressure control mechanisms in ET-1–induced hypertension. Binding sites (receptors?) for the endothelins have been found in brain nuclei known to participate in neural cardiovascular regulation,12,13 and blood-borne ET-1 has been shown to activate some of these neuronal populations.12 Endothelins administered selectively into
the brain at low doses elicit an increase in arterial pressure dependent on activation of the sympathetic nervous system. Endothelins also may facilitate the release of norepinephrine from sympathetic nerve terminals. ET-1 has also been reported to alter baroreceptor reflex function in some studies but not in others. It is not known, however, whether the central or peripheral neural actions of ET-1 are differentially affected by dietary salt intake. Thus, it is premature to speculate on a possible neurogenic basis for the chronic hypertensive action of ET-1.

Finally, it is well established that a variety of different experimental manipulations of the kidney, including reductions of renal mass or renal perfusion pressure and administration of sodium retaining hormones, produce a hypertension that is usually more severe in animals on a high sodium intake. Although the precise mechanism of these forms of hypertension is not well established, there is general agreement that the initiating event is an excessive sodium chloride intake relative to renal excretory capacity. This has been postulated to result in either 1) an initial increase in CO followed by autoregulatory increments in TPR or 2) release into the circulation of natriuretic factors that also have vasoconstrictor activity. It is notable, then, that the endothelins have been found to exert very potent effects on renal function, including reductions in renal blood flow, glomerular filtration rate, and urinary U_{Na}V.

In support, autoradiographic studies have identified high-density binding sites for ET-1 in glomeruli, vasa recta, and inner medullary structures of rat kidney. Circulating ET-1 is, therefore, likely to exert direct effects on the kidney, leading to reduced sodium chloride excretory ability. The results reported here, however, indicate that renal actions of ET-1 are not a primary cause of an increased MAP during chronic low dose infusion in rats, since daily collections of urine did not reveal a significant reduction in sodium or water excretion during ET-1 infusion in this or a previous study. An increase in CO also was not observed at any time during ET-1 infusion, as would have been predicted from at least one of the common theories concerning salt-sensitive hypertension. Although it might be reasonably argued that the rise in arterial pressure countered antinatriuretic effects of ET-1 in the rats on high sodium intake, this would not account for the failure to observe sodium retention in the rats on normal sodium intake in whom ET-1 did not increase arterial pressure. Thus, the renal effects of chronic ET-1 infusion in rats remain to be elucidated.

In summary, the results of the present investigation indicate that chronic low dose intravenous infusion of ET-1 into rats produces a sustained elevation in arterial pressure when the animals are placed on a fixed, high sodium intake; rats on a salt intake fixed at a level equivalent to that provided by normal laboratory rat chow failed to exhibit hypertension. Thus, hypertension produced by exogenous infusion of ET-1 into conscious rats is salt-dependent.

References


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