Captopril Prevents Chronic Hypertension Produced by Infusion of Endothelin-1 in Rats

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Endothelin-1 (ET-1), a potent vasoconstrictor peptide synthesized by the vascular smooth muscle endothelium, has been previously shown to produce a sustained, salt-sensitive elevation in mean arterial pressure when chronically infused over a 7-day period into male Sprague-Dawley rats. In addition to other physiological actions, ET-1 has been shown to have potent effects on various renal functions, including renin production. Activation of the renin-angiotensin system, therefore, may contribute to the pressor response induced by ET-1. In this investigation, captopril ([2S]-1-[3-mercaptop-2-methylpropionyl]-1-proline), a sulfhydryl-containing angiotensin I converting enzyme inhibitor, was chronically administered to endothelin-infused rats to elucidate the role of the renin-angiotensin system in this animal model of hypertension. Rats were catheterized, housed in metabolic cages, and maintained on a fixed 6.0 meq · day⁻¹ sodium intake throughout the experiment, with daily measurements taken of mean arterial pressure, heart rate, water intake, urine output, and urinary sodium and potassium excretions. Infusion of ET-1 alone at a rate of 5.0 pmol · kg⁻¹ · min⁻¹ for 7 days was associated with a significant and sustained increase in mean arterial pressure; concomitant chronic administration of captopril in another group of rats at a rate of 1.0 mg · kg⁻¹ · hr⁻¹ prevented the ET-1-induced hypertension. In an additional study, however, increases in plasma angiotensin II concentration were not observed in rats administered ET-1 alone at 5.0 pmol · kg⁻¹ · min⁻¹. These results indicate that endothelin-induced hypertension may involve stimulation of the renin-angiotensin system but not an increase in circulating angiotensin II concentration.

KEY WORDS • endothelin • captopril • renin-angiotensin system • chronic hypertension • rat studies

Since the isolation of the endothelial-produced, vasoactive peptide endothelin in 1988, extensive investigations into its biochemical and physiological nature have been performed. Presently, although the biochemical nature of this family of peptides has been extensively described in the available literature, much of the physiology of the endothelins remains to be elucidated. Likewise, the relevance of circulating or endothelial-derived endothelin in cardiovascular physiology or pathophysiology has not been firmly established. Considerable evidence exists, however, that suggests a possible pathophysiological role for circulating endothelin. For example, recent reports implicate the endothelin-1 (ET-1) isoform in several diseases, including cerebral ischemia, myocardial infarction, congestive heart failure, hypertension, and hemangiendothelioma (endothelin-secreting tumor), as these clinical situations were associated with significantly elevated plasma levels of endothelin. Alternatively, because this vasoactive peptide is produced ubiquitously throughout the vascular endothelium and other cardiovascular structures, there is also reason to suggest possible paracrine functions for ET-1 not only in pathophysiological states but in normal physiology as well, where ET-1 has been proposed to participate in the normal maintenance of vascular smooth muscle tone.

A role for ET-1 in the etiology of hypertension would most likely involve a chronic increase in ET-1 production by endothelial cells or other key tissues. Because it is not currently possible to produce a controlled stimulation of ET-1 release in vivo, a model using chronic infusion of exogenous ET-1 was developed to evaluate the chronic cardiovascular response to the peptide. In this model, it was shown that ET-1 produced a sustained, reversible, and salt-dependent hypertension when infused into normal, conscious rats at a rate of 5.0 pmol · kg⁻¹ · min⁻¹ over a 7-day period. This hypertension resulted primarily from a sustained increase in total peripheral resistance; long-term alterations in other hemodynamic parameters such as cardiac output, heart rate, or stroke volume did not appear to contribute. Renal sodium and water-handling ability did not seem to be influenced by ET-1, because there was no evidence of an antinatriuretic or antidiuretic effect. Other investigators, however, have demonstrated that higher doses of ET-1 are capable of decreasing renal blood flow and urinary sodium excretion. One investigation suggests that some of these renal effects may be due to stimulation of the renin-angiotensin system, because they were attenuated by administration of the angiotensin I converting enzyme (ACE) inhibitor captopril. Furthermore, infusion of ET-1 into intact ani-
mals in several different studies caused an increase in plasma renin activity. Finally, in two recent investigations, ET-1 directly stimulated ACE in cultured pulmonary artery cells.

Because of the above observations, this study was designed to further elucidate the role of the renin-angiotensin system, particularly the generation of angiotensin II (Ang II), in ET-1-induced hypertension and thereby aid in the characterization of a potential interaction between ET-1 and the renin-angiotensin system.

Methods

All animals used in this investigation were surgically prepared and chronically maintained according to protocols approved by the Michigan State University Committee for Animal Use and Care. Male Sprague-Dawley rats (340–400 g) were anesthetized with pentobarbital sodium (45.0 mg · kg⁻¹ · i.p.). One arterial and two venous polyvinyl silicone catheters were surgically implanted into the left femoral vessels, passed subcutaneously, and exteriorized through the cranial dermis overlaying bregma. The catheters were then threaded through an 8-in. steel spring housing. One end of the spring was attached to the cranial bone with dental acrylic, and the other end was attached to a plastic swivel allowing 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 Premier Laboratory Diets, Madison, Wis.). All rats were placed on two chronic intravenous infusions consisting of a 6.0 meq · day⁻¹ sodium chloride solution (used as a vehicle for ET-1 infusion at 5.0 pmol · kg⁻¹ · min⁻¹) and a 5.0% dextrose solution (used as a vehicle for continuous infusion of captopril, [25]-1-[3-mercapto-2-methylpropionyl]-L-proline [Sigma Chemical Co., St. Louis, Mo.], at a rate of 0.0 [control] or 1.0 mg · kg⁻¹ · hr⁻¹). These infusions were maintained throughout the experimental protocol. All protocols were 15 days in length and consisted of 3 control days (C₁–C₃) followed by 7 ET-1 infusion days (E₁–E₇) and 5 days of recovery (R₁–R₅).

Daily hemodynamic measurements included mean arterial pressure and heart rate, which were obtained with a pressure transducer (model PS50, Gould Instruments, Cleveland, Ohio) attached to a Stemtech blood pressure monitor (model BP2) and a polygraph (model 7B, Grass Instrument Co., Quincy, Mass.). Urinary sodium and potassium content and plasma sodium and potassium content were analyzed with a flame photometer (model 943, Instrumentation Laboratories, Lexington, Mass.). Daily urinary sodium excretion and potassium excretion were calculated by multiplying electrolyte content by the daily urine volume. Water balance was calculated as the difference of water intake and urinary output. All hemodynamic and fluid measurements were obtained daily between 8 AM and 11 AM.

Radioimmunoassay

To determine the effects of ET-1 infusion on plasma Ang II generation, plasma samples were obtained on days C₁, E₁, E₇, and R₅ in a separate group of rats in which ET-1 was infused at 5.0 pmol · kg⁻¹ · min⁻¹. The Ang II radioimmunoassay was performed on collected blood samples using a method described previously. Briefly, after extraction with ethanol, plasma samples were reconstituted in 0.2 ml assay buffer containing 0.05 M tris(hydroxymethyl)aminomethane, 0.3% bovine serum albumin, and 0.2% neomycin sulfate, at pH 7.4. The Ang II assay was performed using iodine-125-labeled Ang II (New England Nuclear, Boston; final concentration, 200,000 cpm/ml) and Ang II antiserum (Arnel, Inc., New York; final dilution, 1,500). Samples were then incubated for 24 hours at 4°C. The bound fraction (supernatant) was separated by addition of dextran-coated charcoal and centrifugation at 4°C and 3,000g. The supernatant was then counted for 1 minute with a Micromedic Plus automatic gamma counter.

Statistical Analysis

Results are expressed as mean±SEM. For all data, within- and between-groups differences were analyzed by use of mixed-design analysis of variance. Individual post hoc comparisons of means within a variable were also performed by use of the Newman-Keuls test and the t test. Probability levels of less than 0.05 (p<0.05) were considered significant.

Results

Figure 1 consists of four graphs depicting mean arterial pressure, heart rate, water intake, water balance, urinary sodium excretion, and urinary potassium excretion. These data demonstrate that infusion of ET-1 alone at 5.0 pmol · kg⁻¹ · min⁻¹ produced a significant increase in mean arterial pressure that was sustained throughout the ET-1 infusion period (days E₁–E₇) and was reversible on cessation of ET-1 infusion (day R₅). The onset (day E₁) and regression (day R₅) of ET-1 hypertension were accompanied by brief and statistically insignificant reflex bradycardia and tachycardia, respectively. Sodium and water retention were not observed in this hypertensive model, as water balance and electrolyte excretion were maintained at a steady state throughout the protocol.

Addition of captopril to the chronic infusion regimen completely inhibited the development of endothelin hypertension. This group of animals did not exhibit the transient heart rate changes observed in the animals administered ET-1 alone. The captopril-treated animals also demonstrated no significant changes in fluid or electrolyte balance throughout the protocol.

In another group of animals, infusion of ET-1 at 5.0 pmol · kg⁻¹ · min⁻¹ (Figure 2) produced a significant and sustained rise in mean arterial pressure but no statistically significant alterations in plasma Ang II levels.

Discussion

The data from this study indicate that endothelin hypertension is prevented by the ACE inhibitor captopril. ET-1, when infused intravenously for 7 days into conscious rats at 5.0 pmol · kg⁻¹ · min⁻¹, produces a significant, sustained, and reversible elevation in mean arterial pressure when rats are maintained on a fixed salt intake of 6.0 meq · day⁻¹. We have demonstrated in previous studies that this hypertension is attributable only to an increase in total peripheral resistance, because cardiac output, stroke volume, heart rate, water...
intake, urine output, and sodium and potassium excretions were not changed. When this infusion protocol is combined with a concomitant intravenous infusion of captopril at a rate of 1.0 mg · kg⁻¹ · hr⁻¹, the pressor response is completely inhibited, with no observable variations in the measured hemodynamic or fluid and electrolyte balance parameters mentioned above.

Because of the various reported pharmacological properties of captopril, there are several potential mechanisms by which this agent could effectively prevent ET-1 hypertension. One possibility is the known property of captopril to inhibit ACE. The dose of captopril used in this investigation has been shown by other investigators to effectively inhibit Ang II formation in normal rats. It is well known that an increase in the circulating plasma level of Ang II produces a sustained, salt-dependent increase in mean arterial pressure. This form of hypertension has a hemodynamic profile similar to that seen in ET-1 hypertension, i.e., a sustained elevation in total peripheral resistance, with little effect on other hemodynamic parameters. An interaction between ET-1 and the renin-angiotensin system has been suggested in a study in which short-term infusion of ET-1 into intact animals increased plasma renin activity. In addition, two recent investigations by Kawaguchi et al demonstrated that ET-1
is a potent stimulant of ACE in cultured bovine pulmonary artery endothelial cells; maximum stimulation (a twofold increase) of ACE occurred at $1 \times 10^{-8}$ M ET-1. Therefore, in the current investigation, it is conceivable that ET-1 induced elevations in plasma Ang II levels sufficient to produce hypertension; administration of captopril would then prevent hypertension by inhibiting an increase in plasma Ang II concentrations. Direct measurements performed in the current study, however, indicate that ET-1 did not significantly increase plasma Ang II levels (Figure 2). These observations suggest that if Ang II is involved in ET-1 hypertension, it is probably at a local tissue level. An interaction between the renin-angiotensin system and ET-1 at the endothelial level is supported by Dohi et al., who recently demonstrated that Ang II directly stimulates endothelial ET-1 production in situ through an increase in the expression and transcription of ET-1 mRNA. There is currently no evidence, however, that ET-1 increases local Ang II formation by vascular tissue.

In addition to possible effects on the vascular renin-angiotensin system, the renal renin-angiotensin system could also be involved in the actions of ET-1. ET-1, like Ang II, has been found to exert very potent effects on renal function, including reductions in renal blood flow, glomerular filtration rate, and urinary sodium excretion. In addition, receptors for both peptides have been identified in the glomeruli, vasa recta, and medulla. Localized stimulation of renal Ang II formation by ET-1 therefore could contribute to the observed hypertension, which would be reversed by an action of captopril on renal ACE. In support, one recent investigation performed in anesthetized dogs and rats demonstrated that captopril attenuated ET-1-induced decreases in renal blood flow, urinary sodium excretion, and urine flow, while having little effect on the short-term pressor response to ET-1. Daily measurements of urine volume and electrolyte excretion in the current study, however, did not reveal any significant actions of ET-1 on renal function.

Although it is generally accepted that the antihypertensive mechanism of captopril is through inhibition of Ang II formation, several in vivo and in vitro studies suggest that captopril has vasodilator activity that is independent of the renin-angiotensin system and ACE inhibition. Investigations have shown that the unique sulfhydryl moiety (–SH) of captopril acts as a scavenger of free radicals such as superoxide anion ($O_2^-$). Vasoconstriction mediated by $O_2^-$ has been shown to occur through inactivation of endothelium-derived relaxing factor. Nagase et al. recently demonstrated that ET-1 enhances the release of oxygen radicals in vitro and in vivo. Likewise, ET-1 has been shown to be a potent secretagogue for endothelium-derived relaxing factor. Accordingly, it is possible that captopril administration in this study may have prevented ET-1 hypertension development through a scavenging action on ET-1-induced $O_2^-$ generation, allowing the vasodilator action of ET-1-induced endothelium-derived relaxing factor release to antagonize any ET-1-induced vasoconstriction. Other potential mechanisms for the antihypertensive effect of captopril in this model may be through the inhibition of kinin degradation or through the release of vasodilator prostanoids. These endogenous vasodilators and others are known to antagonize the vasoconstrictor effect of ET-1.

In conclusion, the results of this study suggest that endothelin hypertension may be caused by tissue-specific, renin-angiotensin system activation or possible superoxide generation, because captopril effectively attenuates hypertension development. Additional studies are required to further describe the potential interaction or interactions between the renin-angiotensin system and ET-1 in endothelin hypertension.

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