Chronic Hypertension Produced by Infusion of Endothelin in Rats

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Endothelin, a potent vasoconstrictor peptide synthesized by the vascular smooth muscle endothelium, was chronically infused into male Sprague-Dawley rats to determine whether a long-term increase in circulating endothelin levels would cause a sustained elevation in mean arterial pressure. Rats were catheterized, housed in metabolic cages, and maintained on a fixed 6 meq/day sodium intake throughout the experiment with daily measurements including mean arterial pressure, heart rate, water intake, urine output, urinary sodium excretion, urinary potassium excretion, cardiac output, total peripheral resistance, and stroke volume. Infusion of endothelin-1 (ET-1) at rates of 3, 5, or 7.5 pmol/kg/min for 7 days was associated with significant, sustained, and dose-dependent increases in mean arterial pressure and smaller less consistent elevations in total peripheral resistance. Other parameters were unaffected. Similar results were observed in rats receiving endothelin-3 (ET-3), except that a higher dose of ET-3 was required. These results indicate that elevated blood levels of endothelin could produce a maintained hypertension without sodium or water retention and that the hemodynamic basis for the increased mean arterial pressure is similar to that seen in most other forms of experimental and clinical hypertension. (Hypertension 1990;15:729–733)

The endothelin family (e.g., ET-1, ET-2, and ET-3) is composed of 21 residue peptides that have potent and sustained vasoconstrictive effects on vascular smooth muscle both in vivo1,2 and in vitro.3-4 This laboratory5 and others1,6 have shown that when administered intravenously as picomolar boluses, these agents produce a characteristic biphasic blood pressure response, that is, an initial short-lived vasodilation followed by a secondary sustained vasoconstriction.

Presently, only the hemodynamic events surrounding bolus injections5,7 and short-term infusions5,8,9 of endothelin have been investigated. A characteristic increase in mean arterial pressure (MAP) caused by an increased total peripheral resistance (TPR) is generally accepted as the mechanism for the pressor activity of endothelin. Numerous other pressor substances, however, cause similar acute rises in MAP or TPR, yet fail to produce a sustained hypertensive response when administered for more prolonged periods of time, that is, for days to weeks.8,10,11 This failure might be related to tachyphylaxis or tissue receptor down-regulation, or to the activation of other cardiovascular homeostatic mechanisms such as vascular autoregulation, arterial and cardiopulmonary baroreceptor reflexes, renal fluid loss, or any of these mechanisms together. If either circulating or locally released endothelins serve a vasoconstrictor function in hypertension, then they should be capable of producing a long-term pressor action not effectively countered by the mechanisms listed above.

It was the purpose of this study, therefore, to investigate the hemodynamic responses to endothelin administered as an infusion over a period of 7 days to allow observation of the effects of activation of homeostatic pressure regulatory mechanisms. Also, previous investigations12,13 have shown that differences might exist in the biological activity or metabolic fate of ET-1 and ET-3, which differ in six amino acid residues. Thus, an additional purpose of this study was to compare the hemodynamic responses to these two homologous peptides.

Methods

Male Sprague-Dawley rats (340–380 g) were anesthetized with halothane (1.0 vol% in O2 at 1.0 l/min). A midline thoracotomy was performed for implantation of an aortic directional pulsed Doppler flow probe. Probe leads were directed into a subcutaneous pocket in the animal's back, and the rats were allowed 5 days of recovery. After this recovery period, rats were anesthetized with pentobarbital sodium (50.0 mg/kg i.v.). Polyvinyl silicone catheters were surgically implanted into the abdominal aorta and a femoral vein through 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 rat free movement within a metabolic cage. A minimum of 3 days' recovery from this surgical procedure was allowed before any experimentation. Rats were then placed on a 6.0 meq/day (5.0 ml/day) sodium chloride infusion, which was maintained throughout the experiment. Rats were allowed free access to distilled water from calibrated drinking tubes and to sodium-deficient rat chow (Teklad Test Diets, A Harlan Sprague Dawley, Inc., Co., Madison, Wisconsin). The infusion protocol consisted of 3 control days during which only sodium chloride solution was infused, 7 days of ET-1 or ET-3 infusion at 3.0, 5.0, or 7.5 pmol/kg/min (dissolved in sodium chloride solution), and 5 days of recovery during which only sodium chloride solution was infused.

MAP was monitored with a Gould pressure transducer (model P50, Gould-Statham, Oxnard, California) attached to a Grass polygraph (model 7B, Grass Instr. Co., Quincy, Massachusetts). In some rats, cardiac output (CO) was estimated as blood flow velocity (in kHz) in the aortic root and recorded on the polygraph by using a directional pulsed Doppler monitor (model 545C-3, University of Iowa, Iowa City, Iowa) attached to the implanted flow probe (Crystal Biotech, Holliston, Massachusetts). The flow probe was implanted at least 7 days before the beginning of flow measurements to ensure firm attachment of the relatively rigid probe to the aorta. Thus, aortic diameter changes under the probe were minimized and flow velocity should have closely paralleled absolute changes in cardiac output (minus coronary flow). Such proportionality between volume flow and Doppler velocities has been shown previously. TPR was calculated by using the following formula: $\text{TPR} (\text{mm Hg}/\text{kHz}) = \frac{\text{MAP}}{\text{CO}}$. Heart rate (HR) was measured directly from the pulse wave of the blood pressure recordings. Stroke volume index (SV) was calculated by using the following formula: $\text{SV} (\text{kHz} \cdot \text{min}/\text{beat}) = \frac{\text{CO}}{\text{HR}}$. Urine samples were measured daily for sodium and potassium content (meq/l) with an E2A electrolyte analyzer (Beckman Instruments, Inc., Schiller Park, Illinois). Water balance was calculated as the difference of water intake and urinary output. Hemodynamic and fluid measurements were obtained daily.

**Statistical Analysis**

Results were expressed as mean±SEM. The hemodynamic responses to infusions of ET-1 and ET-3 were analyzed separately by using a repeated measures analysis of variance with each factor representing a change in a variable within each rat over time. Individual post hoc comparisons of means within a variable for MAP, CO, HR, urine sodium and potassium, and water balance were performed by using a protected least significant difference test. Similar comparisons for TPR and SV were performed with Friedman's test. Probability levels of less than 0.05 ($p<0.05$) were considered significant.

**Results**

Initial experiments were performed in rats without aortic flow probes to determine the long-term ET-1/arterial pressure dose-response relation. Figure 1 illustrates the results of these experiments. Chronic infusion of ET-1 produced dose-dependent increases in MAP that were readily reversible on termination of the infusion. The infusion of ET-1 at 7.5 pmol/kg/min caused a 60% mortality within a few days; thus, higher rates were not used. Subsequent studies with ET-1 were performed by using an infusion rate of 5 pmol/kg/min. Figures 2 and 3 present the hemodynamic and body fluid responses to a 15-day infusion regimen that included 7 days of intravenous infusion of ET-1 at a rate of 5.0 pmol/kg/min ($n=7$). Infusion of ET-1 at this rate produced a statistically significant increase in MAP (26%) ($p<0.05$). This appeared to be caused by statistically insignificant increases in TPR (16%) and CO (11%), whereas SV, urine sodium and potassium content, and water balance remained unchanged throughout the infusion period. HR was significantly decreased on the first day after starting the ET-1 infusion, and a significant tachycardia occurred after termination of the infusion. Increases in MAP reached a plateau by the end of the first day of ET-1 infusion and returned to baseline levels only after the infusion was discontinued. We observed no tachyphylaxis at any time during the infusion period.

Figure 4 presents the hemodynamic responses from similar experiments using ET-3. The peptide was infused for a 7-day period at a rate of 7.5 pmol/kg/min. Compared with values obtained during infusion of ET-1, ET-3 infusion at this higher molar...
FIGURE 2. Plottings showing infusion of endothelin-1 at 5.0 pmol/kg/min, which produced significant (*p<0.05) increases in mean arterial pressure (MAP) as compared with control values, which remained elevated throughout the infusion period. Insignificant increases in total peripheral resistance (TPR) and cardiac output (CO) appear to contribute to the pressor effect of endothelin-1. Transient alterations in heart rate (HR) might be due to baroreceptor reflex responses to initiation and termination of endothelin infusion. Stroke volume (SV) was not significantly altered by ET-1 infusion.

dose produced only a 15.8% increase in MAP (p<0.05), which appeared to be primarily caused by a statistically insignificant increase in TPR (12.4%). CO actually decreased 3% during the infusion period. ET-3 infusion did not alter HR, SV, urine sodium and potassium content, or water balance (data not shown). Increases in MAP reached their maximum levels after the second day of ET-3 infusion. Again, tachyphylaxis was not noted at any time during the infusion period.

Discussion

The results of this study indicate that ET-1 and ET-3 produce a dose-dependent increase in arterial pressure when infused intravenously into normal rats for a period of 7 days. An important new finding is that acute pressor effects of the endothelins demonstrated previously by ourselves and others are well maintained during long-term peptide infusion. It appears that the normal cardiovascular homeostatic processes that prevent many acute pressor agents from inducing long-term increases in arterial pressure are not sufficiently engaged to effectively oppose the vasoconstrictor effects of the endothelins.

There is disagreement in the literature as to whether the vascular actions of the endothelins exhibit tachyphylaxis; however, no indication of such an effect was observed in the current experiments. Likewise, there is evidence that the endothelins alter baroreceptor reflex function; however, only transient bradycardia was observed during ET-1 infusion in this study, suggesting that at least the cardiac component of the baroreceptor reflex is appropriately reset during long-term endothelin infusion. Recent detailed studies by Knuepf et al also found no effect of acute endothelin infusion on the cardiac baroreceptor reflex in conscious rats. Increases in arterial pressure can also be effectively countered by elevated renal fluid excretion, that is, the pressure diuresis–natriuresis mechanism. Abundant data
suggest, however, that infused endothelins bring about pronounced reductions in renal blood flow, glomerular filtration rate, and sodium excretion.\textsuperscript{8-20,21} The failure of endothelin-induced hypertension to cause the expected sodium and water loss in this study might reflect the importance of these renal actions of endothelin. All these possibilities require additional detailed investigation.

The hemodynamic mechanism of the acute pressor action of the endothelins is a rise in vascular resistance.\textsuperscript{1-2,7}\textsuperscript{,}8\textsuperscript{-12} This study shows that long-term infusion of the endothelins also raises arterial pressure primarily by increases in TPR although, with the small number of rats used, the variability of this derived measure precluded demonstration of a statistically significant increase. An elevated CO during long-term endothelin infusion might have been expected as a result of the inotropic effects of the peptide\textsuperscript{22,23} or from volume expansion secondary to renal sodium and water retention.\textsuperscript{8,20} Neither of these factors is likely to have contributed to the modest increases in CO observed during ET-1 infusion, however, since renal fluid retention did not occur and estimated stroke volume did not increase. These results, though, do not rule out the possibility of important effects of circulating or local tissue-derived endothelin on cardiac function.

Three mature (21-residue) endothelin isoforms (ET-1, ET-2, and ET-3) are encoded in the human, rat, and pig genomes.\textsuperscript{24} Endothelial cells produce only ET-1, and it is this isoform that is detectable in the circulating blood of humans.\textsuperscript{25,26} Both ET-2 and ET-3 can be expressed in a tissue-specific manner; for example, ET-3 has been suggested to be the "neuronal" endothelin isoform.\textsuperscript{24} Multiple endothelin receptor subtypes also have been inferred based on a number of pharmacological differences between ET-1 and ET-3.\textsuperscript{24} Possible disparities in the cardiovascular responses to repeated ET-1 and ET-3 infusion, therefore, were explored in the current investigation. The effects of ET-3 on hemodynamic and body fluid variables were found to closely resemble those caused by infusion of ET-1; the only noteworthy differences were that the pressor action of ET-3 developed more slowly and required a higher infusion rate. Thus, these results do not suggest the existence of distinct receptor subtypes mediating the long-term cardiovascular effects of the endothelins.

Finally, although the contribution of the endothelins to the pathogenesis of hypertension is just beginning to be explored, the present experiments clearly indicate that long-term exposure of the vasculature to circulating endothelin is capable of producing a sustained elevation in arterial blood pressure.

References


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