Effect of a Chronic Infusion of Atrial Natriuretic Peptide on Vascular Reactivity in Normotensive and Renal Hypertensive Rats (43146)

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Abstract. **In a previous study, we found that a long-term infusion of atrial natriuretic peptide (ANP) produced a sustained reduction of mean arterial pressure and peripheral vascular resistance in two-kidney, one-clip (2K-1C) hypertensive rats, whereas in control rats it had only a transient effect on cardiac output. However, plasma levels of ANP were actually 3-fold higher in normotensive than in hypertensive rats. Previous studies suggested that plasma ANP levels might modulate the vascular reactivity to the peptide. The present study examined whether the lack of chronic hemodynamic effects of ANP in control rats was due to changes in vascular reactivity to the peptide. In control rats, vascular reactivity to ANP was reduced 50% by a chronic infusion of ANP. However, in 2K-1C hypertensive rats, a long-term infusion of ANP had no effect on the vascular reactivity to ANP. The results of the present study indicate that the lack of persistent hemodynamic effects of a chronic infusion of ANP in control rats may be due to a decrease in the vascular reactivity to the peptide. The sustained hypotensive and vasodilatory effects of a long-term infusion of ANP in 2K-1C hypertensive rats are associated with no changes in the vascular reactivity to ANP.** [P.S.E.B.M. 1990, Vol 195]

It has been shown that long-term atrial natriuretic peptide (ANP) treatment produces a sustained fall in blood pressure in renin-dependent models of hypertension (1–3). In previous studies (1), we found that chronic ANP infusions elevated plasma ANP (pANP) levels in two-kidney, one-clip (2K-1C) rats, and the sustained hypotensive effect obtained is mediated through a decrease in total peripheral resistance. However, the same dose of ANP did not induce long-term hemodynamic changes when infused chronically to normotensive rats, despite pANP levels 3-fold higher in control than in hypertensive animals (1).

Previous investigations have shown that vascular reactivity to ANP is decreased in various models of experimental hypertension, compared with normotensive animals (4, 5), and this effect is associated with elevated pANP levels (5). In addition, it has been reported that the ANP-induced down-regulation of ANP receptors on cultured smooth muscle cells is dose dependent (6, 7). Thus, it appears that plasma levels of ANP can modulate the vascular responsiveness to the hormone through changes in the number of ANP receptors. However, at present it remains unknown whether the chronic hemodynamic effects of ANP can be modulated by alterations in the vascular reactivity to the peptide.

The purpose of this study was to investigate whether the lack of sustained hemodynamic effects of a chronic infusion of ANP in control rats is mediated through changes in vascular reactivity to the peptide.

Materials and Methods

Male Wistar rats weighing 270–300 g were used in this study. Two-kidney, one-clip hypertension (2K-1C) was produced in young male rats (200–220 g) by constriction of the left renal artery with a silver clip (0.2-mm i.d.); the contralateral kidney was left untouched.

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Received January 18, 1990. [P.S.E.B.M. 1990, Vol 195]
Accepted June 26, 1990.

0037-9727/90/1952-270$2.00/0

Hypertensive rats with mean blood pressure higher than 150 mm Hg were used 4 weeks after surgical induction of hypertension. Rats were anesthetized with sodium pentobarbital (40 mg/kg ip), after which catheters were placed in their left femoral artery for blood pressure determination (Tygon microbore tubing S-54-HL) and also in the left femoral vein for infusions (Silastic, 1-mm o.d., 0.5-mm i.d.; Dow Corning). Then, the two catheters were brought through the skin at the dorsal side of the neck. Finally, the distal ends of these lines were threaded through a lightweight flexible spring connected to a hydraulic swivel (Instech Laboratories). All surgical procedures were performed under aseptic techniques. Rats were placed in plastic cages with the swivels mounted above, allowing complete freedom of movement and free access to standard chow and tap water. Experiments began 4 days after surgery and continued through 4 days.

Experimental Protocols. *Normotensive rats.* Four days after surgery, either ANP (101-126; Peninsula Laboratories) (0.5 μg/hr, $n = 6$) or saline ($n = 6$) was infused, through 4 consecutive days, using a Watson-Marlow peristaltic pump (1 ml/24 hr). In pilot studies, this dose of ANP was the highest that did not alter mean arterial pressure in normotensive animals. Four days after the beginning of infusions, rats were sacrificed for vascular reactivity determinations.

2K-1C hypertensive rats. Four weeks after surgical induction of hypertension, rats with mean arterial pressure higher than 150 mm Hg were infused with ANP ($n = 6$) or saline ($n = 6$) following the same experimental protocol as that used with normotensive rats.

Response of Aortic Rings to ANP. After 4 days of infusion with either ANP or vehicle, the rats were sacrificed, the thoracic aorta was rapidly excised, and adherent connective tissue was removed. The aorta was kept at 37°C in a modified Kreb's solution of the following composition (mM): NaCl, 128; KCl, 4.7; $NaHCO_3$, 12.5; KH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.5; glucose, 11.1; and Na_2EDTA, 0.01, aerated with 95% O_2 and 5% CO_2. From each aorta, a 5-mm-length ring was cut and mounted in a 1.2-ml chamber at the resting tension of 8 g, as previously described by our group (8), between two stainless steel hooks connected to a myographic force transducer (Hewlett-Packard). Before each experiment, the rings were equilibrated for 2 hr with the buffer, with flushing of the chamber every 20 min. Then, a concentration-response relationship was performed with norepinephrine (0.1 nM–1 μM). After relaxation and a 30-min equilibration period, the rings were contracted with norepinephrine (0.1 μM), which produced a near maximal (>80% maximal response) contraction. After a steady-state period, cumulative concentration-response relationships of ANP were obtained. Increasing concentrations of ANP were added to the bath (0.1 nM, 0.5 nM, 1 nM, 5 nM, 10 nM, 50 nM, and 0.1 μM) at 3-min intervals and the decrease of developed tension was recorded. The relaxation obtained was expressed as a percentage of previous contraction.

Statistics. Statistical comparisons between the means of two different groups were performed by unpaired t test. Differences were considered statistically significant at $P < 0.05$. Results are expressed as mean ± SE.

Results

The results presented in Figure 1 show the cumulative concentration-response curves to ANP (0.1 nM–μM) in aortic rings precontracted with norepinephrine (0.1 μM) for normotensive (Fig. 1A) and 2K-1C hypertensive rats (Fig. 1B). The chronic infusion of ANP had no significant effect on the vascular reactivity to norepinephrine in control rats. However, the relaxation obtained in aortic rings from ANP-treated control rats was significantly lower than that observed in animals infused with saline with all the doses of ANP tested (Fig. 1A). After the higher dose of ANP (10^{-7} M), the relaxation was 50.04 ± 5.3% in the aortic rings from control rats infused with ANP and 99.1 ± 0.6% in control rats infused with vehicle ($P < 0.001$). After the

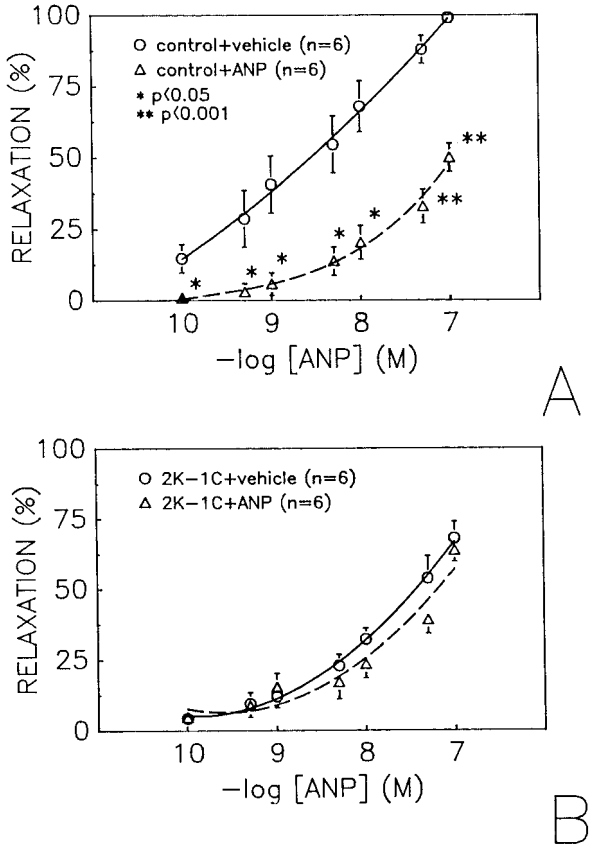

Figure 1. (A) Vascular reactivity to ANP in normotensive rats infused with ANP (0.5 μg/hr) or saline during 4 consecutive days. (B) Vascular reactivity to ANP in 2K-1C hypertensive rats infused with ANP (0.5 μg/hr) or saline during 4 consecutive days.

lower dose of ANP (10^{-10} M), the relaxation obtained was 0.94 ± 0.6% in control ANP-treated rats and 14.7 ± 5% in control animals infused with saline. Thus, the dose-response curves obtained with aortic rings from control rats infused with ANP were shifted to the right in comparison with those from control rats infused with saline. However, the relaxation observed in ANP-treated 2K-1C hypertensive rats was not significantly different from that in hypertensive rats infused with saline, indicating that in hypertensive animals the vascular reactivity was not significantly altered by ANP infusion.

Discussion

The results of the present study indicate that a long-term infusion of ANP (0.5 μg/hr during 4 days) decreased significantly the vascular reactivity to the peptide in normotensive rats. In a previous study (1), we found that this dose of ANP elevated the plasma ANP levels more that 20-fold. However, the hemodynamic effects were transient. Cardiac output decreased during the first 2 days of ANP treatment, but all hemodynamic parameters returned to control values on the fourth day of ANP infusion. These data suggest that chronic exogenously increased plasma ANP levels may produce a fall in the vascular responsiveness to the peptide in control rats. This is in accordance with the work of other authors (4, 5, 9), who hypothesized that chronically elevated levels of ANP in physiopathologic situations may elicit a down-regulation of ANP receptors and thereby, reduce the vascular reactivity to the peptide. This effect could account, at least in part, for the lack of chronic hemodynamic actions of a long term infusion of ANP observed in our study in control rats (1). Thus, our results indicate that, in control rats, chronic changes in the plasma levels of ANP may modulate the long-term hemodynamic effects of the hormone.

Our data also show that chronic ANP treatment did not modify the vascular responses to the peptide in 2K-1C hypertensive rats. The mechanisms mediating such lack of effect are unknown. Schiffrin *et al.* (4, 5) found that the development of several forms of hypertension (DOCA; one kidney, one clip) is associated with increased plasma levels of ANP and a decrease in the number of vascular ANP receptors. However, in 2K-1C hypertensive rats the density of ANP receptors was not different from controls, and they postulated that there might be a certain resistance to the down-regulating effect of ANP on its vascular receptors in this model of hypertension, but the mechanism mediating this effect is unknown. On the other hand, we previously found (1) that pANP levels were 3-fold lower in 2K-1C rats than in normotensive animals during the infusion of the same dose of ANP. We suggested that it could be due to an increased clearance of ANP in hypertensive rats, perhaps because the plasma levels of ANP are chronically elevated in these animals. In this regard, it has been reported that the ANP-induced down-regulation of ANP receptors on cultured smooth muscle cells is dose dependent (6, 7). Thus, the lower pANP reached during the infusion of the peptide in hypertensive compared with control rats could explain this absence of changes in vascular reactivity in hypertensive animals after the chronic ANP treatment.

It has been reported that basal vascular reactivity to ANP is reduced in 2K-1C rats (5) and that chronic ANP treatment decreased blood pressure to nearly normal values in 2K-1C rats (1–3). This sustained hypotensive effect was mediated through a decrease in peripheral resistance (1). The present study shows that such vasodilatory action is associated with no changes in the vascular reactivity to the peptide. It appears that the vascular reactivity to ANP is low in hypertensive rats, compared with control animals, and a pharmacologic increase in the plasma levels of the peptide cannot further decrease the vascular responsiveness to ANP in 2K-1C rats. However, the fact that the hypotensive effect of ANP is greater and more sustained in 2K-1C rats than in control rats cannot be attributed only to the changes in vascular reactivity observed after a chronic infusion of ANP. Because the two models differ (i.e., high renin and elevated peripheral resistance in 2K-1C rats), we cannot exclude a role for these variables, rather than vascular reactivity events, in the greater and sustained hypotensive effect of ANP in hypertensive over control rats.

The present study indicates that the lack of persistent hemodynamic effects of a chronic infusion of ANP in control rats may be due to a decrease in the vascular reactivity to the peptide. The sustained hypotensive and vasodilatory effects of a long-term infusion of ANP in 2K-1C hypertensive rats is associated with no changes in the vascular reactivity to the peptide.

This study was supported by the United States-Spain Joint Committee for Scientific and Technological Cooperation (CCA 85/10025) and by the Fondo de Investigaciones Sanitarias of the Spanish Ministry of Health (FISS no. 87/1529).

The authors wish to thank M. C. Pérez Peñalver for his technical assistance.

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