Hemodynamic and Renal Effects of $ProANF_{31-67}$ in Hypertensive Rats (44399)

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Abstract. **It has been demonstrated previously that the atrial natriuretic factor prohormone fragment 31–67 ($ProANF_{31-67}$) circulates in animals and possesses natriuretic and vasodilating actions. Although the plasma levels of the peptide are reportedly elevated in patients with high blood pressure, its role and actions in hypertension are unknown. In the present study, synthetic human $ProANF_{31-67}$ was infused intravenously at doses of 0, 10, 30, and 100 ng/kg/min into respective groups of anesthetized normotensive and spontaneously hypertensive rats. Mean arterial pressure (MAP), urine flow rate (UV), and sodium excretion ($U_{Na}V$) were measured during two consecutive 30-min periods. In both strains of rats, reductions in MAP with $ProANF_{31-67}$ were similar in magnitude and dose-related. Sodium excretion responses to the peptide infusions also were remarkably similar in both normotensive and hypertensive rats, and the responses demonstrated 3- to 5-fold ($P < 0.05$) increments compared to control at the doses of 10 and 30 ng/kg/min. However, in the two strains of rats, attenuation of natriuresis occurred with the highest infusion dose of 100 ng/kg/min and was probably related to the large decreases in MAP of 17–23 mmHg at this dose of the peptide. The present results indicate the $ProANF_{31-67}$ has important hemodynamic and renal effects in hypertension and may represent one compensatory mechanism involved in this disease.** [P.S.E.B.M. 1999, Vol 221]

In the past decade, evidence has accumulated demonstrating that atrial natriuretic factor (ANF) is stored in granules as the cardiac prohormone of 126 amino-acid residues $ProANF_{1-126}$. It is likely that the prohormone is subsequently cleaved by nonmyocyte cardiac cells (1) into the C-terminal 28 amino-acid peptide ANF_{99-126} and the 98 amino acid N-terminal peptide $ProANF_{1-98}$. $ProANF_{1-98}$ is further proteolytically cleaved in the heart or in plasma to render distinct peptide fragments consisting of amino acids 1–30, 31–67, and 79–98 (2, 3). These ProANF peptides circulate in several animal species, including rats, dogs, and humans, and appear to have biological actions similar to ANF_{99-126} (4–7). In particular, it has been demonstrated previously that $ProANF_{31-67}$ circulates at levels 10- to 20-fold higher compared to ANF_{99-126}, and pharmacological studies have reported that it possesses important effects for the enhancement of urinary sodium excretion in the rat (6), dog (7), and human (8). At least in the kidney, and in contrast to ANF_{99-126}, the actions of $ProANF_{31-67}$ are probably cyclic GMP independent (7, 9). Its natriuretic effect appears to be related, at least in part, to an increase in prostaglandin E_2 (PGE_2) production, which in turn, reduces tubular sodium transport by inhibiting Na^+-K^+ ATPase activity in the renal medullary collecting duct (9).

Although these initial observations suggest an involvement of $ProANF_{31-67}$ in the control of body fluid volume and pressures, the effects of this peptide are not well characterized in pathophysiological situations, particularly in hypertension. The present study was designed to examine the hemodynamic and renal excretory effects of graded infusions of synthetic human $ProANF_{31-67}$ in normotensive and genetic hypertensive rats.

Materials and Methods

Animal Models. Sprague-Dawley (SDR) and Spontaneously Hypertensive (SHR) male rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) with body weights between 250 and 350 g were housed in individual cages and main-

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tained on a regular rat chow (Purina, St. Louis, MO) for at least 7 days before the study. Tap water was available *ad libitum*. All the experiments were performed in the postabsorptive state, at least 18 hr after feeding. All animal care provided during the conduct of these studies met institutional guidelines. Anesthesia was induced with Inactin (100 mg/kg ip, Andrew Lockwood & Associates, Sturtevant, WI). A tracheotomy was performed, and polyethylene catheters (PE-50) were inserted into the carotid artery and jugular vein. The arterial catheter was connected by a Statham P23Db strain gauge pressure transducer (Oxnard, CA) to a Hewlett Packard 7714–041 A recorder (St. Louis, MO) for continuous mean arterial blood pressure (MAP) monitoring. The urinary bladder was exteriorized and cannulated. Immediately after the surgical procedures, a sustained intravenous infusion of isotonic saline at a rate of 25 μl/min was started through a jugular catheter *via* an infusion pump (Sage Instruments, Boston, MA).

Experimental Design. The experimental protocol consisted of four separate groups of SDR and SHR, respectively (n = 8 for each group). At the end of a 30-min equilibration period postsurgery, baseline MAP was recorded. Immediately after, the SDR and SHR randomly received intravenously either a constant infusion of synthetic human $ProANF_{31-67}$ (Peninsula Lab, Belmont, CA) at 0, 10, 30, or 100 ng/kg/min. Based on preliminary experiments, this range of doses was selected to produce minimal to maximal hypotensive and natriuretic responses in normal rats. Following an additional 15 min of equilibration with the peptide infusions, two 30-min urine collection periods were obtained for determination of volume and sodium concentration. At the end of each period, MAP was recorded again. Finally, arterial blood (3.0 ml) was obtained at the end of the second experimental period and assayed for plasma renin activity (PRA). It is relevant to point out that although the amino-acid sequence homology between rat and human $ProANF_{31-67}$ is ≈ 75% (10), the biological effects of the synthetic human form have been demonstrated repeatedly in the rat (6, 11); and specific binding sites to human $ProANF_{31-67}$ have been localized in the rat distal tubule and collecting duct (12).

Assay Methodology. Electrolytes were determined by flame photometer. PRA was determined by radioimmunoassay as described previously (13).

Data Analysis. Group data were expressed as a mean ± SEM. Within SDR and SHR groups, data were analyzed using analysis of variance (ANOVA) with either a two-factor mixed design (MAP, urine volume, and urine sodium) or completely randomized design (PRA). Least significant difference (Duncan's) was used as a *posthoc* test (14). Differences of $P < 0.05$ were considered statistically significant.

Results

In the SDR vehicle-control group (0 dose), MAP and renal excretory function remained stable throughout the experimental protocol (Figs. 1–3). The hemodynamic responses to graded infusions of $ProANF_{31-67}$ in the groups of normotensive SDR are shown in Figure 1 (upper panel). There were significant reductions of ≈ 4–6 mmHg in MAP from their respective baselines ($P < 0.05$) during the administration of $ProANF_{31-67}$ at a dose of 10 and 30 ng/kg/min. A greater reduction in MAP from its baseline was observed with the 100 ng/kg/min infusion of the peptide ($P < 0.05$), with a peak decrement of 17 mmHg. The renal excretory responses to $ProANF_{31-67}$ in the SDR are shown in Figures 2 and 3. Compared to the vehicle-control group, a 3-fold increase in urinary sodium excretion was observed with $ProANF_{31-67}$ at the 10 ng/kg/min infusion dose during the second experimental period ($P < 0.05$), from a mean of 246 ± 30–704 ± 140 nEq/min; and this natriuretic effect was 5-fold higher compared to the vehicle control group with the 30 ng/kg/min dose ($P < 0.05$), from a mean of 246 ± 30–1590 ± 367 nEq/min (Fig. 2, upper panel). Sodium excretion also increased significantly compared to the vehicle control group in the SDR infused with the dose of 100 ng/kg/min, from a mean of 246 ± 30–1230 ± 216 nEq/min. Interestingly, however, there was a significant ($P < 0.05$) attenuation of the natriuretic response with this infusion dose of $ProANF_{31-67}$ when compared to the 30 ng/kg/min dose of ≈ 360 nEq/min, but it is pertinent to point out that this effect was associated with an average reduction in MAP of 17 mmHg. Similarly, with the three doses of $ProANF_{31-67}$, the urinary flow rate response followed the pattern of sodium excretion (Fig. 3, upper panel). Lastly, PRA remained unchanged from the control group with the three different infusion doses of the peptide (Table I).

In the SHR vehicle control group (0 dose), both renal

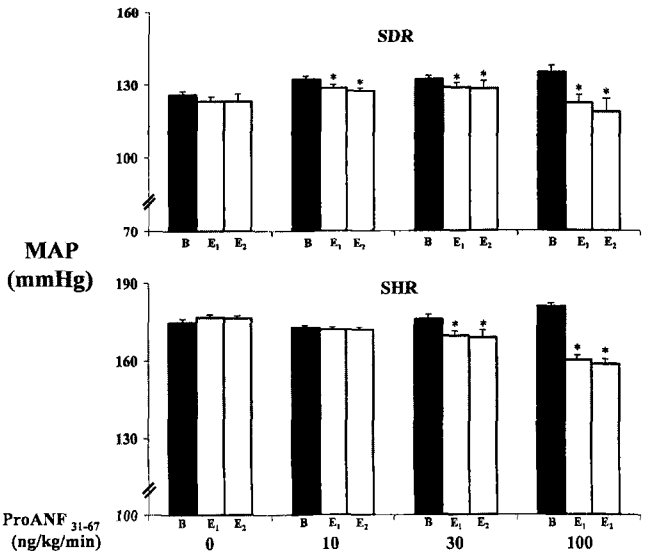

Figure 1. Effects of $ProANF_{31-67}$ at doses of 0 (vehicle control), 10, 30, and 100 ng/kg/min in four separate groups of Sprague-Dawley Rats, (SDR, n = 8 per group, upper panel) and four separate groups of Spontaneously Hypertensive Rats (SHR, n = 8 per group, lower panel). Values are mean ± SEM. MAP, mean arterial pressure; B ■, baseline values immediately before $ProANF_{31-67}$ infusion; E_1, E_2 □, Experimental periods. *$P < 0.05$ compared to baseline (B).

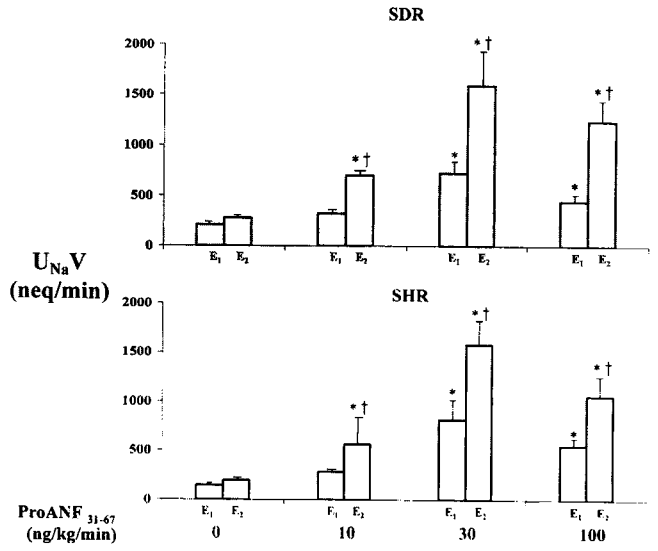

Figure 2. Effects of $ProANF_{31-67}$ at doses of 0 (vehicle control), 10, 30, and 100 ng/kg/min in four separate groups of Sprague-Dawley Rats (SDR, $n = 8$ per group, upper panel) and four separate groups of Spontaneously Hypertensive Rats (SHR, $n = 8$ per group, lower panel). Values are mean ± SEM. Other abbreviations as in Figure 1. $^*P < 0.05$ compared to corresponding experimental periods (E_1, E_2) of the respective vehicle control group; $\dagger P < 0.05$ compared to E_1.

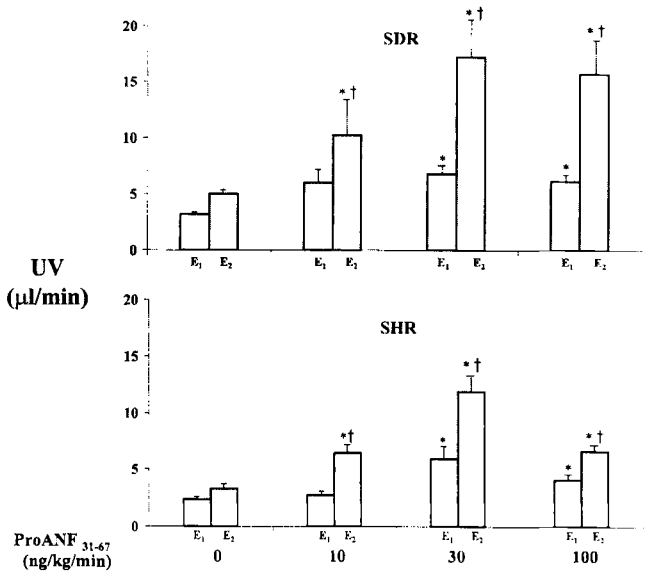

Figure 3. Effects of $ProANF_{31-67}$ at doses of 0 (vehicle control), 10, 30, and 100 ng/kg/min in four separate groups of Sprague-Dawley Rats (SDR, $n = 8$ per group, upper panel) and four separate groups of Spontaneously Hypertensive Rats (SHR, $n = 8$ per group, lower panel). Values are mean ± SEM; UV, urine flow rate. Other abbreviations as in Figure 1. $^*P < 0.05$ compared to corresponding experimental periods (E_1, E_2) of the respective vehicle control group $\dagger P < 0.05$ compared to E_1.

excretory function and MAP remained stable throughout the experimental protocol (Figs. 1–3). The hemodynamic responses to graded infusions of $ProANF_{31-67}$ in the hypertensive rats are shown in Figure 1 (lower panel). In contrast to the SDR, $ProANF_{31-67}$ at a dose of 10 ng/kg/min had no significant effect on MAP. At higher infusion doses, MAP was reduced progressively from their respective baselines, with a maximal hypotensive response of 23 mmHg in the 100 ng/kg/min group. $ProANF_{31-67}$ also demonstrated a similar qualitative and quantitative effect on urinary sodium excretion and urine flow in the SHR when compared to the SDR (Figs. 2 and 3, lower panels). Indeed, progressive natriuresis and diuresis occurred with the low and intermediate doses of the peptide, but at the highest infusion dose of 100 ng/kg/min, and again concomitant with a 23-mmHg reduction in MAP, these renal excretory functions were attenuated by ≈ 30%–50% ($P < 0.05$) compared to the intermediate dose of 30 ng/kg/min. Also similar to the SDR, $ProANF_{31-67}$ infusions did not appear to modify PRA when compared to the vehicle control group (Table I).

Discussion

The results of the present study document, for the first time, the natriuretic and vasodepressor actions of synthetic human $ProANF_{31-67}$ in experimental genetic hypertension. It is important to observe that urinary sodium excretion increased similarly for both the hypertensive and normotensive groups but not in a dose-dependent fashion. The attenuated natriuresis with the highest compared to the intermediate dose of the peptide most likely resulted from the concomitant fall in MAP of ≈ 20 mmHg in both the SDR and SHR. Under these conditions, these attenuated renal responses are better explained by a reduction in perfusion pressure and a consequent alteration in the pressure-natriuresis relationship (15, 16). Alternatively, it is possible that receptor saturation occurred with increased infusion dose of the peptide. Also, in this context, it is of interest to point out that an involvement of an enhanced circulating renin-angiotensin-aldosterone system (RAAS) for antinatriuresis is unlikely, since the levels of PRA were not different from respective controls in either strain of rats infused at any dose of synthetic $ProANF_{31-67}$ (Table I). Since activation of the RAAS would have been predicted with this magnitude of pressure reduction and consequent renal hypoperfusion (17), the observed lack of stimulation of the RAAS in this study is consistent with previous information indicating that $ProANF_{31-67}$ can reduce renin secretion, at least in part by a macula densa mechanism (18). Figure 1 illustrates that $ProANF_{31-67}$-induced decrements in MAP were comparable in both SHR and SDR, and this could be interpreted to suggest a similar vascular reactivity to the peptide in normotensive and hypertensive animals. These observations were similar to previous studies using synthetic ANF_{99-126} in several models of hypertension in the rat (19), including SHR (20), in which the magnitude of reduction in arterial blood pressure was comparable to normotensive controls (19). Also in these earlier rat studies with ANF_{99-126} (19, 20), and in agreement with the present results, attenuation of the natriuretic effect was observed when the absolute magnitude of the peptide-induced MAP reduction exceeded 15 mmHg (19). These observations are in keeping with the concept that moderate reductions in perfusion pressure

Table I. Effects of Synthetic $ProANF_{31-67}$ on Plasma Renin Activity in Groups of Normotensive and Hypertensive Rats

	PRA (ng/ml/hr)			
	$ProANF_0$	$ProANF_{10}$	$ProANF_{30}$	$ProANF_{100}$
Sprague-Dawley Rats	3.49 ± 0.20	2.83 ± 0.27	3.46 ± 0.27	2.71 ± 0.29
Spontaneously Hypertensive Rats	6.62 ± 0.88	6.71 ± 0.75	7.15 ± 0.96	6.70 ± 0.44

Note. Values are mean ± SEM (n = 8 per group). PRA, plasma renin activity; 0 (vehicle control), 10, 30, and 100 are corresponding infusion doses of $ProANF_{31-67}$ (ng/kg/min).

translate into a marked diminution of the natriuresis produced by $ProANF_{31-67}$.

Recent immunocytochemical studies in kidneys of rats (12) and humans (17) reportedly have localized binding sites of $ProANF_{31-67}$ in the subbrush border of the pars convoluta and pars recta of proximal tubules, as well as in the cortical collecting ducts, glomeruli, and peritubular and interstitial blood vessels. However, although these studies (11, 12) have characterized the distribution of binding sites of $ProANF_{31-67}$ along with the nephron, the mechanisms of $ProANF_{31-67}$-induced natriuresis and vasodilation are not completely understood. Nevertheless, data derived from earlier studies (7, 9, 18, 21) suggested that its precise renal actions are different from those of ANF_{99-126}. In several animal species, including rats (21) and dogs (7), synthetic $ProANF_{31-67}$ stimulated sodium excretion by a tubular effect that, in contrast to ANF_{99-126}, did not appear to be dependent on the renal generation of cGMP. In addition, previous studies in dogs, rats, and monkeys documented that synthetic $ProANF_{31-67}$-induced natriuresis is not associated with the enhancement of glomerular filtration rate or renal blood flow (7, 21, 22). This *in vivo* information is consonant with *in vitro* studies in medullary collecting duct cells, suggesting that $ProANF_{31-67}$ increased prostaglandin E_2 production, which in turn, reduced tubular sodium transport by inhibiting Na^+-K^+ ATPase (9). Moreover, recent investigations from this laboratory with indomethacin administration in normal dogs indicated that the renal excretory effects of $ProANF_{31-67}$ are dependent, at least in part, on the local synthesis of prostaglandins (18).

Similarly, the mechanisms involved in $ProANF_{31-67}$-induced vasodilation are not well elucidated. In recent studies in normal rats (21) and dogs (7), the peptide-induced reductions in arterial (7, 21) and central venous pressure (7) were not associated with increases in plasma cGMP (7, 21); these findings were in contrast to previous *in vitro* studies suggesting that the dilation of porcine aortic rings produced by synthetic $ProANF_{31-67}$ was cGMP-dependent (4). As an alternative cGMP-independent mechanism, it has been suggested that, similar to its renal actions, $ProANF_{31-67}$ could enhance the local production of vasodilatory prostaglandins, including PGE_2 (18). Indirect support for this concept is provided by studies in normal conscious dogs in which cyclooxygenase inhibition with indomethacin completely blocked the synthetic $ProANF_{31-67}$-induced reductions in arterial pressure and right atrial pressure (18).

In physiological and pathophysiological cardiovascular conditions, the potential significance of $ProANF_{31-67}$ has not been examined extensively. A recent longitudinal study in the canine model of high-output heart failure indicated progressive elevations in the plasma levels of the peptide, as well as a significant enhancement in renal sodium and water excretion during synthetic $ProANF_{31-67}$ infusions, an effect that is in contrast to the blunted natriuretic actions of synthetic ANF_{99-126} in this dog preparation of heart failure (7, 13). In human essential hypertension, earlier studies demonstrated elevated plasma levels of the N-terminal ANF prohormone 1–98 compared to normal subjects (23, 24). A subsequent investigation in patients with obesity-hypertension indicated that in addition to the entire prohormone 1–98, the circulating levels of $ProANF_{31-67}$ also were significantly increased compared to obese, nonhypertensive individuals (25). Interestingly, blood pressure reduction in these obese patients was associated with a parallel decrement of both ProANF peptides (25), and a strong correlation between MAP and the plasma levels of these two hormones was maintained throughout the 12-week length of the study (25).

The available information (23–25) and the present results with synthetic $ProANF_{31-67}$ infusions in the context of high blood pressure in humans are unclear. However, in view of the human longitudinal data discussed above (25) and the significant pharmacological hypotensive and natriuretic actions of the peptide in this study, it is possible to suggest that the circulating levels of $ProANF_{31-67}$ may represent one adaptive mechanism, which is independent of ANF_{99-126}, and is involved in the regulation of systemic hemodynamics and renal function in hypertension. It would be of interest to examine in future research if hypertensive humans exhibit renal and vascular responses to synthetic $ProANF_{31-67}$ comparable to rats with genetic hypertension. Further, in view of their different mechanism of action (18), it would also be important to investigate the potential additive effects of synthetic $ProANF_{31-67}$ and ANF_{99-126} in the pharmacological management of high blood pressure.

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