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Evidence supporting a physiological role for proANP-(1–30) in the regulation of renal excretion

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Dietz, John R., Dionne Y. Scott, Carol S. Landon, and Stanley J. Nazian. Evidence supporting a physiological role for proANP-(1–30) in the regulation of renal excretion. Am J Physiol Regulatory Integrative Comp Physiol 280: R1510–R1517, 2001.—The experiments, performed in pentobarbital sodium-anesthetized rats, consisted of a 1-h equilibration period followed by two 30-min control periods. Subsequently, synthetic rat pro atrial natriuretic peptide (ANP) [proANP-(1–30)] (n = 8) was given as a bolus of 10 μg in 1 ml of 0.9% saline followed by an infusion at 30 ng/min (20 μl/min) for six additional periods. Control rats (n = 6) received only 0.45% saline in the appropriate volumes. Mean arterial pressure, renal blood flow, and glomerular filtration rate did not change significantly in either group during the proANP-(1–30) infusion. Urine flow and potassium excretion increased approximately 50% in the proANP-(1–30)-infused group only (P < 0.05). Sodium excretion and fractional excretion of sodium, expressed as the change from their own baselines, were significantly increased by the proANP-(1–30) infusion (P < 0.05), whereas cGMP excretion was similar in both groups. These results suggest that the rat sequence of proANP-(1–30) produces a natriuresis in the rat independent of changes in hemodynamics and renal cGMP production. In a second study, rats (n = 8) were prepared as above and pretreated with 0.4 ml iv of rabbit serum containing an antibody directed against proANP-(1–30) (anti-proANP group). The rats were volume expanded with 3 ml of 6% albumin in Krebs and observed for 3 h to determine if the anti-proANP would attenuate the responses to volume expansion. Control rats (n = 7) received 0.4 ml of normal rabbit serum. The elevation in potassium excretion in response to volume expansion was significantly attenuated in the anti-proANP group (P < 0.05). Sodium excretion and urine flow responses also tended to be reduced but not significantly. These results suggest that in the rat, proANP-(1–30) plays a physiological role in regulating renal excretion.

ATRIAL NATRIURETIC PEPTIDE (ANP) is known to play an important role in the regulation of blood pressure and sodium balance. ANP is secreted by the atria in response to mechanical stretch (7) produced by changes in blood volume or arterial pressure (8). ANP increases renal sodium and water output and decreases blood pressure (6). Antibodies directed against ANP have been shown to attenuate the renal responses to acute volume expansion (18, 19) and to exacerbate volume-dependent forms of hypertension (25). ANP transgenic mice with increased plasma ANP levels show chronic hypotension (14), whereas ANP gene knockout mice exhibit fluid retention and hypertension (20, 21, 28). Thus introduction of the ANP gene, to increase the endogenous plasma levels, has been used to treat experimental forms of hypertension (23).

ANP is stored in granules in the atria as a 126-amino acid (aa) prohormone of which ANP constitutes the 99–126 aa portion (3, 9, 27). Evidence has accumulated suggesting that, in addition to ANP, several peptides derived from the ANP prohormone play a role in the regulation of sodium excretion. ProANP-(1–30), -(31–67), and -(79–98) are secreted from the heart (12), have been shown to circulate in plasma (31), and possess diuretic, natriuretic, and/or vasodilator properties. The human forms of proANP-(1–30) and -(31–67) have been shown to increase sodium and water excretion in several species including humans (1, 2, 11, 13, 17, 24, 29), and binding sites for these peptides have been found in the proximal tubules and collecting ducts (26) of the kidneys. However, one study failed to show any effect of these peptides or to find specific renal receptors in a rat cortical membrane preparation (30).

ProANP-(31–67) appears to act in the kidney and the medullary collecting duct by inhibiting the Na⁺-K⁺ pump through a prostaglandin-dependent mechanism (15, 16). Although ANP also acts on the medullary collecting duct, its action involves an inhibition of sodium channels on the apical membrane mediated by cGMP. ProANP-(31–67) clearly has no effect on renal cGMP production (13). The mechanism of action of proANP-(1–30) is not known. The purpose of the present study was to determine if proANP-(1–30) infusion in physiologically relevant doses can increase sodium excretion. Second, are such increases in renal excretion accompanied by changes in renal hemodynamics or cGMP excretion? Finally, we wished to determine if a specific rat proANP-(1–30) antibody could...
attenuate the renal responses to acute blood volume expansion.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 314–375 g (mean 350 g) were anesthetized with pentobarbital sodium (50 mg/kg) with supplemental doses given as needed. They were placed on a surgical tray with a heating blanket to maintain body temperature between 37 and 38°C. After tracheal cannulation, indwelling catheters were placed in a jugular vein (PE-50), right carotid artery (PE-50), and a femoral vein (PE-50). Both ureters were cannulated (PE-10) for urine collections. An ultrasonic blood flow probe (Transonic Systems, Ithaca, NY) was placed on a renal artery. Therefore, the renal blood flow measurement in Figs. 2 and 6 represent flow to one kidney only. Arterial pressure was measured throughout the experiment via the carotid catheter using a pressure transducer (Gould Instrument Systems, Valley View, OH), and arterial pressure and renal blood flow were recorded and displayed continuously using WINDAQ analysis software (DATAQ Instruments, Akron, OH).

Effects of rat proANP-(1–30) on renal function. A bolus of [3H]inulin in 1.5 ml of 0.45% saline followed by a constant infusion was initiated at the beginning of a 1-h equilibration period. The infusion was continued, and data were collected over eight 30-min urine collection periods (240 min total). At the end of the second urine collection period, the experimental group received a bolus of 1 μg of the rat sequence of proANP-(1–30) in 0.5 ml of 0.45% saline followed by a constant infusion of 10 pg/min at a rate of 10 μl/min. The control group received a bolus and constant infusion of equal volumes of 0.45% saline. Arterial blood samples of 300 μl each were drawn at the midpoint of each urine collection period for measurement of inulin clearance, which was used as an index of glomerular filtration rate (GFR). Each blood sample was replaced simultaneously by an equal volume of 6% albumin in saline given intravenously to avoid acute changes in blood volume during blood sampling. A final arterial blood sample of 5 ml was also taken at the end of the experiment (240 min), and plasma samples were obtained by centrifugation (3,000 rpm for 15 min at 4°C). These plasma samples were then used to determine the steady-state plasma levels of ANP and proANP-(1–30).

Effects of proANP-(1–30) antibody on the renal response to acute volume expansion. The animals, anesthesia, and surgical preparation for these groups were the same as employed in the first experiment. The experimental group received 0.4 ml of rabbit serum containing a specific antibody to proANP-(1–30) at 60 min before the start of urine collections, whereas the control group received 0.4 ml of normal rabbit serum. After 2 baseline 30-min urine collections, both groups received 3 ml of 6% albumin in Krebs saline, given over ~3 min as an acute blood volume expansion. The volume expansion was calculated as ~15% of blood volume based on an estimated blood volume of 6% of body wt. Inulin was not infused as part of this experimental protocol.

Antibody production and radioimmunoassays. Rat proANP-(1–30) was synthesized and purified by Biosynthesis (Lewisville, TX). A portion of the purified product was conjugated to keyhole limpet hemocyanin by the same company. This conjugated compound was emulsified with Freund’s complete (first immunization) or incomplete (subsequent immunizations) adjuvant and administered subcutaneously to New Zealand White rabbits. Animals received booster immunizations every 3 wk and were bled 1 wk after the immunization. On the basis of its ability to bind radioiodinated rat proANP-(1–30), the antiserum collected from one of these rabbits was selected for use in the experiments reported here. A number of proANP-related peptides was examined for their ability to prevent the binding of radioiodinated rat proANP-(1–30) to this antiserum. Peptides tested included human proANP-(1–30), human ANP, rat proANP-(1–30), rat ANP-(31–67), rat proANP-(68–78), and rat ANP. Only rat proANP-(1–30) produced a significant inhibition of binding.

RESULTS

The proANP-(1–30) infusion was delivered at a rate designed to simulate a physiological increase in the plasma levels, similar to that which occurs with acute blood volume expansion (11) or a high-salt diet (12). The infusion resulted in a final proANP-(1–30) plasma concentration of 1,816 ± 398 pg/ml in the infused group, which was 60% greater than the proANP-(1–30) level in the control group (Fig. 1, P < 0.01). Plasma ANP levels were not significantly different (NS) in the two groups (Fig. 1, 112 ± 39 vs. 116 ± 30 pg/ml, NS). Mean arterial pressure (MAP), expressed as a change from the baseline period [control 119 ± 12 mmHg; proANP-(1–30) 123 ± 13], is shown in Fig. 2. There was no significant effect of the proANP-(1–30) infusion on MAP. MAP tended to decrease ~10 mmHg in both groups, but the change was similar and not statistically significant in either group. Renal blood flow (RBF) and GFR were stable throughout the experiment, with no significant within-group or between-group differences (Fig. 2). Urine flow (Fig. 3) increased significantly only in the group receiving the ANP-(1–30) infusion. Urine flow reached values that were ~60% greater than the control group at 180 and 210 min of the experiment (P < 0.05). Absolute sodium excretion and fractional excretion of sodium (Fig. 3) increased significantly only in the group receiving the ANP-(1–30) infusion. Changes in potassium excretion were not statistically significant compared with control animals. However, when expressed as a change from their baseline values (Fig. 5), both sodium excretion and fractional sodium excretion increased significantly in the proANP-(1–30)-infused group compared with the control group (P < 0.05). Potassium excretion was also slightly but significantly greater in the proANP-(1–30)-infused group (P < 0.05), but fractional potassium excretion was not significantly different (Fig. 4). The excretion rate of cGMP increased in both groups compared with their own baseline periods (30 and 60 min, P < 0.05, Fig. 5), but
there were no significant differences between the groups.

Acute blood volume expansion resulted in a transient decrease in MAP (Fig. 6, $P < 0.05$) in the group receiving the anti-ANP-(1–30) compared with the group receiving normal rabbit serum (NRS). RBF tended to increase in both groups during volume expansion (Fig. 6), but there were no significant differences between the groups. Urine flow and the rates of excretion of sodium and potassium (Fig. 7) all increased significantly ($P < 0.05$) during volume expansion compared with their own baseline values (0–60 min). The increases in urine flow appeared to be attenuated in the proANP-(1–30) antibody group compared with the control group ($P = 0.08$ for time 150 min; $P = 0.06$ for time 180 min). Sodium and potassium excretion rates also appeared to be attenuated in the group receiving the proANP-(1–30) antibody, with potassium excretion being significantly attenuated during the 180-min urine collection period ($P < 0.05$).

An additional group of rats was treated with three times the volume of proANP-(1–30) antibody (1.2 ml) and volume expanded. There was no further attenuation of the renal responses to volume expansion with the higher dose of antibody (data not shown). However, the antibody significantly increased MAP $\sim$8–12 mmHg compared with the control (NRS) rats ($P < 0.05$, Fig. 8). This included a significantly greater MAP during the prevolume expansion periods ($P < 0.05$, 0–60 min).

**DISCUSSION**

The results show that the rat sequence of proANP-(1–30) is diuretic, natriuretic, and kaliuretic at physiological concentrations. The responses to proANP-(1–30) do not appear to be mediated by changes in renal
hemodynamic or cGMP. Second, the antibody injection experiments suggest that proANP-(1–30) plays a modest but significant role in the renal response to acute volume expansion. Finally, larger doses of the proANP-(1–30) antibody increased MAP, suggesting a role for this peptide in regulating arterial blood pressure.

There is clear evidence that ANP [proANP-(99–126)] plays a physiological role in the regulation of fluid balance and blood pressure. This hormone is released in response to a physiologically appropriate stimulus, atrial distension (7), and modest changes in the plasma concentration produce diuretic and natriuretic effects (5). Antibodies directed against ANP have been shown to attenuate the renal responses to volume expansion (18, 19) and exacerbate volume-dependent hypertension (25). Evidence has now accumulated suggesting a similar physiological role for several peptides derived from the NH₂-terminal of the proANP molecule. The NH₂-terminal prohormone peptides proANP-(1–30) and (31–67) have been reported to possess diuretic and natriuretic properties (24). These peptides are secreted by the heart along with ANP in response to atrial stretch (9, 10) and circulate in the plasma at several times the concentration of ANP (31). Also, specific binding sites for these peptides have been identified in the renal tubule (26). The natriuretic properties of the human sequence of proANP-(31–67) have been demonstrated in intact animals, such as rats (13, 24), dogs (17), monkeys (1, 2), and humans (29). In addition, proANP-(31–67) has been shown to inhibit sodium transport in cell culture (15, 16). A natriuretic effect has also been suggested for the human sequence of proANP-(1–30) in rats (24) and humans (29) but only

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**Fig. 3.** The effects of a 3-h (60–240 min) intravenous infusion of rat proANP-(1–30) on Na⁺ excretion and fractional Na⁺ excretion (FENa⁺,%), in anesthetized rats. Values are means ± SE. *Significant differences (P < 0.05) between the proANP-(1–30) and control (saline infusion) groups (t-test).

**Fig. 4.** The effects of a 3-h (60–240 min) intravenous infusion of rat proANP-(1–30) on K⁺ excretion and fractional K⁺ excretion (FEK⁺,%), in anesthetized rats. Values are means ± SE. *Significant differences (P < 0.05) between the proANP-(1–30) and control (saline infusion) groups (t-test).
with very high doses of this peptide. In contrast, one study failed to show any renal effects in the rat using either the human or rat sequences of proANP-(1–30) or -(31–67) or any specific binding in rat cortical preparations for these peptides (30).

The present results clearly demonstrate a significant increase in urine output (50%) and sodium (100%) and potassium (50%) excretions (Figs. 3–5) in response to a proANP-(1–30) infusion that increased the plasma concentration by 60% compared with the control infusion (Fig. 1). In two previous studies from our laboratory, acute volume expansion in rats using whole blood was estimated to increase blood volume by 20% and resulted in an increase in plasma proANP-(1–30) of 30% (11). A high-salt diet for 7 days resulted in a 60% higher plasma level of proANP-(1–30) compared with rats on a low-salt diet (12). Thus plasma levels achieved in the present study with synthetic rat proANP-(1–30) correspond closely to the plasma levels we observed with physiological perturbations. It is unlikely that these effects were mediated by changes in the plasma concentration of ANP, because plasma ANP was not different in the two groups at the end of the infusion (Fig. 1). It should also be pointed that the plasma concentrations of proANP-(1–30) produced in the present study are far below the levels seen in pathophysiological conditions such as heart failure (31).

The mechanism by which proANP-(1–30) exerts its effects is unknown. The effects of ANP on both the renal tubule and the vasculature are mediated by cGMP (3). In contrast, proANP-(31–67) appears to exert its renal tubular actions by inhibiting Na\(^+\)-K\(^+\)-ATPase in the medullary collecting duct (15, 16). The inhibition of sodium transport in the tubule appears to be mediated by an increase in prostaglandin synthesis (15). ProANP-(1–30) appears to increase sodium and water excretion by a tubular effect as suggested by the increase in fractional sodium excretion (Fig. 5). This increase in sodium excretion does not appear to involve

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Fig. 5. The effects of a 3-h (60–240 min) intravenous infusion of rat proANP-(1–30) on the change in Na\(^+\) excretion (\(\Delta\text{Na}^+\)), the \(\Delta\text{FENa}^+\)\%, and cGMP excretion in anesthetized rats. Values are means ± SE. *Significant differences (\(P < 0.05\)) between the proANP-(1–30) and control (saline infusion) groups (t-test).

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Fig. 6. The effects of a specific proANP-(1–30) antibody (injected 1 h before the start of the experiment) on MAP and renal blood flow (1 kidney only) during a 3-ml blood volume expansion (6% albumin in Krebs) performed at 60 min. Values are means ± SE. *Significant differences (\(P < 0.05\)) between the group receiving the proANP-(1–30) antibody and control group that received normal rabbit serum (t-test).
increased renal cGMP synthesis, because cGMP excretion increased in a similar fashion in both control and proANP-(1–30)-infused rats (Fig. 5). One study suggested that proANP-(1–30) inhibits Na⁺-K⁺-ATPase in rat cortical slices in a manner similar to proANP-(31–67) (4). This might suggest that the effects of proANP-(1–30) are also mediated by renal prostaglandins, but additional studies will be needed to determine if that is the case.

The differences between the present study and a study by Weir and colleagues (30) is unclear. They infused both the rat and human sequences of proANP-(1–30) and -(31–67) into anesthetized rats and found no effects. They also failed to find receptors or specific binding sites for these NH₂-terminal ANP prohormone peptides in rat cortical preparations (30). A likely difference is the greater volumes infused in the present study, where even in the control group there was a significant increase in sodium excretion over time. The actions of ANP on the kidney are markedly increased under conditions of volume expansion (3). The volume-expanded state would lead to renal vasodilatation, inhibition of the renin-angiotensin-aldosterone system, and stimulation of ANP secretion, all of which promote an increase in salt and water excretion. Thus a modest increase in the plasma levels of proANP-(1–30) may have been more effective in this volume-expanded state. This would suggest a possible synergism between ANP and proANP-(1–30) to increase sodium and water output. A synergistic relationship has already been demonstrated for ANP and proANP-(31–67) in the monkey (2).

To determine if a hormone plays an important physiological role, it is important to study conditions where the effects of that hormone can be negated. In the case of ANP, Hirth and colleagues (18, 19) showed that a specific antibody to ANP attenuated the diuresis and natriuresis to acute volume expansion. Their studies clearly showed that ANP plays a significant role in the response to volume expansion. We developed a specific polyclonal antibody to proANP-(1–30) that shows no cross-reactivity with ANP or any of the other ANP prohormone peptides. Injections of this peptide significantly attenuated the kaliuresis to acute volume expansion and also tended to attenuate the diuresis and natriuresis to volume loading (Fig. 7). To determine if a more effective blockade of proANP-(1–30) would further attenuate the renal responses to volume expansion, we performed additional experiments using a
threefold greater dose of anti-proANP-(1–30). Although this provided no further attenuation of the response to volume expansion, the higher dose of antibody significantly increased arterial pressure (Fig. 8). Taken together, these results suggest that proANP-(1–30) plays a modest role in the renal response to acute volume expansion and a significant role in regulating arterial blood pressure.

A previous study in humans found that proANP-(1–30) infusions significantly decreased arterial pressure (29), and it was concluded that proANP-(1–30) has a marked vasodilatory effect. We found no significant change in arterial pressure with proANP infusion in the present study (Fig. 2), although the doses of proANP-(1–30) employed and the plasma concentration achieved were much lower than in the previous study (29). However, we also found that the highest dose of the proANP-(1–30) antiserum significantly increased blood pressure. The antisera to proANP-(1–30) was injected 1 h before the start of the experiment, which suggests that blocking endogenous proANP-(1–30) contributed to both the elevation in baseline blood pressure and the later rise in pressure during volume expansion (Fig. 8). Therefore, the present results suggest that, similar to ANP (25), endogenous proANP-(1–30) also exerts a significant vasodilatory effect, but that much higher doses of exogenous proANP-(1–30) are required to lower arterial pressure.

In summary, the present studies suggest that in the rat, proANP-(1–30) plays a physiological role in the regulation of sodium, potassium, and water excretion and in the control of arterial pressure. We demonstrated that the rat sequence of proANP-(1–30) is diuretic and natriuretic at physiological concentrations. Furthermore, blocking the effects of endogenous proANP-(1–30) reduces potassium excretion significantly and increases arterial blood pressure.

**Perspectives**

*The cardiac hormone system.* The heart secretes a number of hormones that appear to be involved in the physiological regulation of blood volume, blood pressure, and kidney function. ANP, the COOH-terminal peptide of the ANP prohormone, was the first of these hormones to be discovered (6), and evidence supports its important function in volume homeostasis under both normal and pathophysiological conditions. ANP is secreted when the atria are stretched, as occurs in blood volume expansion (7). When the plasma levels of this hormone are reduced, arterial blood pressure is increased (20, 25) and the renal responses to acute volume are attenuated (18, 19). Two other related hormones, BNP and CNP, also have been identified in the heart. BNP appears to function in a similar fashion to ANP, whereas CNP may act as a local paracrine factor. These hormones, which are vasodilators, are released in response to ischemia and thus have a potential protective function to help preserve the coronary circulation. ANP and BNP are markedly elevated in heart failure and are an important early compensatory mechanism to prevent fluid retention in this disease state.

More recent evidence suggests that several peptides derived from the NH2-terminal portion of the ANP prohormone also possess diuretic, natriuretic, kaliuretic, and vasodilator properties (20). These ANP prohormone peptides are also released in response to atrial stretch (10), acute blood volume expansion (11), and high-salt diet (12). The present results show that proANP-(1–30) is diuretic and natriuretic in the rat at physiological plasma concentrations, and blocking the effects of this peptide with a specific antisera produces a modest attenuation of the renal responses to an acute volume expansion. Furthermore, larger doses of the antisera resulted in an elevation in arterial pressure, suggesting that the endogenous hormone has significant vasodilatory benefits.

Therefore, the ANP prohormone appears to function as a polypeptide, producing several circulating peptides with cardiovascular protective effects.

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