Role of Endogenous Atrial Natriuretic Peptide in Regulating Sodium Excretion in Spontaneously Hypertensive Rats

Effects of Neutral Endopeptidase Inhibition


To explore whether pathophysiological plasma levels of atrial natriuretic peptide (ANP) actually involve sodium excretion in spontaneously hypertensive rats (SHR), we examined the in vivo and ex vivo effects of ANP and an endopeptidase inhibitor, thiorphan, on urinary sodium excretion and the elimination rate of ANP. We found the following: 1) The basal plasma ANP level was higher in 16-week-old SHR than in Wistar-Kyoto (WKY) rats (109±10 [SEM] versus 63±4 pg/ml, p<0.001). Thiorphan (30 mg/kg i.v.) significantly increased plasma ANP by 60% in both SHR and WKY rats. However, increases in urinary sodium excretion (+290% versus +130%, p<0.05) and cyclic GMP (+160% versus +60%, p<0.05) were greater in SHR than in WKY rats. Urinary excretion of ANP was markedly increased by thiorphan, and its increase was greater in SHR than in WKY rats. 2) The thiorphan-induced natriuresis was substantially attenuated by antiserum for ANP but not by a bradykinin receptor antagonist. 3) Isolated SHR kidneys excreted 50% less sodium than WKY rat kidneys at perfusion pressures of 100 and 160 mm Hg (p<0.05). Urinary sodium excretion was increased at the perfusate ANP level of 100 pg/ml, a concentration similar to the SHR plasma ANP (+70% at 160 mm Hg). 4) After bolus administration of ANP to the isolated kidney, the ANP concentration of the recirculating perfusate decreased rapidly in a log-linear fashion. Pretreatment with thiorphan significantly prolonged the elimination half-life of ANP to a greater degree in the SHR kidney than in the WKY rat kidney (+75% versus +36%, p<0.05). Thus, endogenous ANP may keep the pressure-natriuresis curve to the left and consequently facilitate sodium excretion in SHR. Moreover, the enzymatic degradation of ANP seems to be increased in SHR kidneys.

It has been well established that spontaneously hypertensive rats (SHR) show elevated plasma concentrations of atrial natriuretic peptide (ANP). However, it is unclear whether the pathophysiological level of ANP in SHR actually exerts renal effects, because the density of ANP receptors in the SHR kidney also has been reported to be reduced. The kidney is not only a main target organ of ANP but also a major site for ANP metabolism. It has been suggested that ANP is inactivated through receptor- and enzyme-mediated processes. In this regard, ANP metabolism may be altered particularly in the SHR kidney because of the dense distribution of so-called clearance receptors and endopeptidase.

To explore the roles of endogenous ANP in sodium homeostasis in SHR, we examined whether or not ANP at a concentration comparable to that in the SHR plasma increased urinary sodium excretion in the isolated perfused kidney and whether or not enhancement of the biological activities of endogenous ANP by a neutral endopeptidase inhibitor, thiorphan, brought about a natriuretic effect.

**Methods**

**Plasma Atrial Natriuretic Peptide Concentration and Effects of Thiorphan in Spontaneously Hypertensive and Wistar-Kyoto Rats**

Male 16-week-old SHR and Wistar-Kyoto (WKY) rats weighing approximately 300 g were anesthetized...
with Inactin (100 mg/kg i.p.). The trachea, right carotid artery, and jugular vein were cannulated. A PE-50 tube also was inserted into the bladder. Physiological saline was infused throughout the study into the venous line at a rate of 1.2 ml/hr using an infusion pump (IVAC 700, IVAC Corp., San Diego, Calif.). After rats recovered from the surgery, the first urine collection was followed by a bolus injection of thiorphan (CIBA-GEIGY Corp., Summit, NJ.). Urine collections were repeated every 15 minutes for 60 minutes in preweighed tubes. Blood pressure was monitored through the arterial line with a Statham pressure transducer (Gould Instruments, Cleveland, Ohio). Two milliliters of blood was collected before and 30 and 60 minutes after the thiorphan injection and immediately was replaced with the same volume of blood from donor rats. Separated plasma was stored in tubes containing EDTA and aprotinin at −80°C until the assay of ANP and cyclic guanosine 3':5' monophosphate (cGMP). Urine samples also were stored for the measurement of sodium, ANP, and cGMP. Thiorphan was dissolved in 0.25N sodium bicarbonate, and 10, 10, and 0 (0.25N sodium bicarbonate) mg/kg thiorphan was administered to 17 SHR and 16 WKY rats.

In Vivo Effects of Atrial Natriuretic Peptide Antiserum or Bradykinin Antagonist on Thiorphan-Induced Natriuresis

The influences of specific antagonists for bradykinin and ANP on the thiorphan-induced natriuresis were compared to examine the mechanism of renal effects of thiorphan. After baseline urine collections, either 50 μl of the antiserum raised against α-rat ANP or nonimmunized rabbit serum was intravenously injected. Fifteen minutes after the antiserum injection, 30 mg/kg thiorphan was administered to five SHR and five WKY rats. In a preliminary study, it was found that pretreatment with 50 μl antiserum abolished the natriuretic effects of 1 μg α-rat ANP in WKY rats.

In a separate group of SHR and WKY rats (n=5 each), a bradykinin receptor antagonist, [d-Arg⁶,Hyp⁷, Thr³,D-Phe⁶,Thr⁷]-bradykinin (Peptide Institute, Osaka, Japan), was continuously infused at 30 μg/kg/min throughout the study. After a 15-minute urine collection, the effects of 30 mg/kg thiorphan on blood pressure, urine volume, urinary sodium excretion (UₐV), plasma, and urinary cGMP were measured.

Effects of Atrial Natriuretic Peptide on Urinary Sodium Excretion in Isolated Perfused Kidneys

Rats were anesthetized with Inactin, and the kidneys from SHR (n=12) and WKY rats (n=12) were
Effects of Thiorphan on Atrial Natriuretic Peptide Metabolism in Isolated Kidneys

The perfusate concentration of exogenously administered ANP was measured in the presence or absence of thiorphan in SHR (n=17) and WKY (n=14) isolated kidneys. Under conditions similar to those just discussed, α-rat ANP (1,000 pg/ml) was injected as a bolus into the perfusate 5 minutes after vehicle or administration of 10^-4 M thiorphan. One milliliter perfusate was collected every 10 minutes from 5 to 55 minutes after ANP administration. Perfusion pressure was adjusted initially to 100 mm Hg, after which the perfusion flow was maintained constant. The elimination half-life of ANP (T1/2) was calculated from the linear regression line of time versus the natural logarithm of ANP concentration.12

Assays

Plasma, perfusate, and urinary concentrations of ANP were determined by radioimmunoassay as reported previously.13,14 Samples were extracted using Sep-Pak C18 columns (Waters Chromatography Div., Millipore Corp., Milford, Mass.) and were eluted

isolated and perfused according to the method of Nishiiitatsuji-Uwo et al.9 The perfusate was a Krebs-Henseleit buffer that contained 6.7 g/dl fraction V bovine serum albumin (Sigma Chemical Co., St. Louis), 20 amino acids,10 100 mg/dl glucose, and 100 mg/dl inulin. After the isolation, 120 ml perfusate was recirculated at 37°C and continuously oxygenated with 95% O2-5% CO2. Renal perfusion flow was measured using an in-line flowmeter (Float Meter S-2, Kusano Kagaku, Chiba, Japan), and perfusion pressure was monitored at the renal artery through a double lumen needle (18 gauge outside and 30 gauge inside).11 Urine was collected through a ureter catheter of a short PE-10 tube connected to a PE-50 tube. After an equilibrium period, the effects of vehicle and 100 and 1,000 pg/ml α-rat ANP (Protein Research Foundation, Osaka, Japan) on UrV were consecutively examined at a constant perfusion pressure of either 100 or 160 mm Hg. Urine collection was started 5 minutes after vehicle or ANP administration and continued for 5–10 minutes. UrV, renal perfusion pressure, renal perfusion flow, and glomerular filtration rate (inulin clearance) for each period were determined.

Effects of Thiorphan on Plasma Atrial Natriuretic Peptide (ANP) Concentration (top panel) and urinary cyclic GMP (cGMP) excretion (bottom panel) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats.

FIGURE 2. Line graphs showing effects of thiorphan on plasma atrial natriuretic peptide (ANP) concentration (top panel) and urinary cyclic GMP (cGMP) excretion (bottom panel) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats.
with 60% acetonitrile/0.1% trifluoroacetic acid. Plasma and urinary levels of cGMP also were determined after succinylation by radioimmunoassay.\textsuperscript{15}

### Statistical Analysis

Values are expressed as mean±SEM. Linear regression was calculated by the least-squares method. Differences between two groups were compared by the Student's t test. Effects of agents administered were assessed by analysis of variance for repeated measures. A value of $p<0.05$ was considered significant.

### Results

SHR exhibited a higher baseline plasma ANP concentration than WKY rats (109±10 versus 63±4 pg/ml, $p<0.001$). Figure 1 shows the effects of thiorphan on blood pressure and $U_{\text{Na}}^\text{V}$ in SHR and WKY rats. Thiorphan did not change blood pressure in either SHR or WKY rats, whereas it dose-dependently increased $U_{\text{Na}}^\text{V}$ in both rat strains. The increase in $U_{\text{Na}}^\text{V}$ was greater in SHR than in WKY rats (30 mg/kg: +1.2±0.5 versus +0.4±0.2 μeq/min/kg body wt, $p<0.05$). These natriuretic effects of thiorphan were associated with significant increases in plasma ANP concentration and urinary cGMP excretion (Figure 2). Plasma cGMP concentration also was increased by thiorphan (30 mg/kg: SHR, 11.2±1.9 versus 13.1±2.5 pmol/ml, $p<0.05$; WKY, 8.1±0.7 versus 11.0±0.9 pmol/ml, $p<0.05$). Compared with the changes in plasma ANP concentration, the urinary excretion of ANP was markedly increased by thiorphan. Furthermore, its increases were greater in SHR than in WKY rats (Figure 3).

Figure 4 presents the effects of pretreatment with a bradykinin antagonist or with antisera for ANP on thiorphan-induced natriuresis. Even in the presence of the bradykinin antagonist, thiorphan caused a significant increase in $U_{\text{Na}}^\text{V}$. This increase was comparable with that in the absence of the antagonist (Figure 1). In contrast, although thiorphan administration after antisera treatment still increased $U_{\text{Na}}^\text{V}$, the increase was significantly smaller compared with that in rats pretreated with normal rabbit serum.

Figure 5 shows the effects of ANP on $U_{\text{Na}}^\text{V}$ in isolated perfused kidneys. SHR kidneys excreted approximately 50% less sodium than WKY rat kidneys at either 100 or 160 mm Hg perfusion pressure. ANP dose-dependently increased $U_{\text{Na}}^\text{V}$ in SHR. Moreover, 100 pg/ml ANP, a concentration similar to that in the SHR plasma, significantly increased $U_{\text{Na}}^\text{V}$. ANP also increased renal perfusion flow (SHR, 25.1±0.8 to 26.2±1.2 ml/min/g kidney wt, $p<0.05$; WKY, 25.5±3.0 to 28.0±2.8 ml/min/g kidney wt, $p<0.02$ at 100 mm Hg by 100 pg/ml ANP) and glomerular filtration rate (SHR, 0.70±0.20 to 0.82±0.22 ml/min/g kidney wt, $p<0.05$; WKY, 0.92±0.13 to 1.08±0.17 ml/min/g kidney wt, $p<0.05$ at 100 mm Hg by 100 pg/ml ANP); however, the changes were not different between SHR and WKY rat kidneys.

As illustrated in Figure 6, the ANP concentration in the perfusate decreased rapidly in a log-linear fashion to 10% of the initial ANP concentration 55 minutes after administration of ANP. The $T_{1/2}$ of ANP in SHR and WKY rats was comparable (27±6 versus 25±3 minutes, respectively). Pretreatment with thiorphan significantly prolonged the $T_{1/2}$ in SHR by 75% (47±6 minutes, $p<0.05$); however, this effect of thiorphan was smaller in WKY rats (+36%, NS).
SHR showed approximately a twofold higher plasma concentration of ANP than WKY rats. However, it is unclear as to whether such an increase in ANP contributes to body fluid volume regulation. It is known that a low-dose infusion of ANP, which corresponds to the physiological elevation of plasma ANP concentration, increases U\textsubscript{NaV}, at least in the acute phase in human essential hypertension.\textsuperscript{16} However, this phenomenon has not been confirmed in SHR. In the present study, ANP at the perfusate concentration similar to the plasma level of SHR significantly increased U\textsubscript{NaV} in SHR isolated kidneys. The SHR kidney is known to show altered pressure-natriuresis.\textsuperscript{17} This has been considered to be crucial for maintaining blood pressure elevation.\textsuperscript{18} Therefore, it is highly possible that the pathophysiological level of plasma ANP in SHR may contribute to maintaining the renal function curve to the left, and consequently may facilitate sodium excretion.

An endopeptidase inhibitor, thiorphan, caused a substantial natriuretic effect. However, this enzyme is not specific for ANP. It cleaves many kinds of vasoactive peptides, including bradykinin. Ura et al\textsuperscript{19} suggested that another endopeptidase inhibitor, phosphoramidone, increased U\textsubscript{NaV} through inhibiting the intrarenal degradation of bradykinin. Smits et al\textsuperscript{20} have reported that a bradykinin antagonist abolished the augmentation of ANP-induced natriuresis by thiorphan and concluded that thiorphan exerts its biological effects through the potentiation of bradykinin activity. In the present study, pretreatment with the bradykinin antagonist did not influence the natriuretic effects of thiorphan alone. On the other hand, the antiserum for ANP significantly attenuated the natriuretic effects of thiorphan. Thus, although this study did not deny that mechanisms other than ANP may be involved, the effects of thiorphan seem to depend, at least in part, on the effects of ANP.

In the present study, thiorphan elevated the plasma ANP concentration by approximately 60% and increased U\textsubscript{NaV} by approximately 200%. Previous reports have shown that twofold to threefold increases in U\textsubscript{NaV} usually require at least a doubling of the plasma ANP concentrations.\textsuperscript{21,22} Therefore, the elevation of plasma ANP alone may not explain thiorphan-induced natriuresis. However, because the endopeptidase is distributed densely in the kidney, such as the proximal tubules and glomeruli, thiorphan may augment the intrarenal action of ANP through the inhibition of its inactivation. This is supported by the fact that thiorphan markedly increased the urinary excretion of ANP. ANP inhibits sodium reabsorption in the medullary collecting...
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**P<0.02
+ P<0.05
++ P<0.02

? vs baseline
} vs WKY

FIGURE 5. Bar graphs showing effects of atrial natriuretic peptide (ANP) on urinary sodium excretion (UNaV) in isolated kidney from spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats at 100 or 160 mm Hg perfusion pressure.

It is controversial as to whether or not endopeptidase inhibitors alone may elevate plasma ANP concentration. Trapani et al28 showed that the administration of thiorphan alone failed to raise plasma ANP, whereas coadministration of exogenous ANP with thiorphan potentiated the increase in plasma ANP. Similar results were observed in deoxycorticosterone acetate-salt hypertensive rats.29 On the other hand, several kinds of inhibitors of this enzyme increased plasma ANP, even in normal subjects and dogs without coadministration of ANP.30-32 Although such an inconsistency may be related to differences in the endopeptidase inhibitors administered, the inhibitors consistently increase the plasma ANP in heart failure or renal failure.31,33,34 These arguments suggest that the enzymatic degradation of ANP seems to be increased in cases with elevated ANP secretion. Koepke et al35 reported that thiorphan doubled the plasma ANP level in coadministration with an ANP clearance-receptor agonist, suggesting that the relative contribution of enzymatic degradation of ANP may be influenced by the function of the clearance-receptor.

In conclusion, the endogenous ANP in SHR may contribute to sodium homeostasis by keeping the

duct24; however, little is known as to whether ANP acts on the luminal side of the renal tubules. Sonnenberg et al25 previously showed that probenecid significantly attenuated the natriuretic effects of atrial extract, suggesting that the natriuretic effects of ANP, at least in part, require the transport of ANP from the proximal tubule to the distal tubule. Therefore, the accumulation of ANP in the extravascular compartment may be related to the effects of thiorphan. Further studies are necessary to elucidate the exact mechanisms for thiorphan-induced natriuresis.

SHR isolated kidneys excreted much less sodium than WKY rat kidneys at a given perfusion pressure. However, the differences in sodium excretion disappeared when compared with SHR kidneys at 160 mm Hg of perfusion pressure and WKY rat kidneys at 100 mm Hg. Furthermore, the natriuretic response to ANP was not attenuated in SHR kidneys. Therefore, when renal perfusion pressure to SHR kidneys is high enough, the basal sodium excretion and natriuretic response to ANP in SHR and WKY rats appear to be comparable in both in vivo and ex vivo conditions. SHR exhibited a greater natriuretic response to thiorphan than WKY rats. This may be related to the findings that ANP infusion causes exaggerated natriuresis in SHR, most likely because of higher renal perfusion pressure.26,27 Another possibility is that the reduced ANP receptors in SHR kidneys may be involved in the differences in the effects of thiorphan. It has been suggested that most plasma ANP is cleared by receptor-mediated mechanisms, such as internalization.5,6 Therefore, a reduction in ANP receptors may result in a relative increase in the enzymatic degradation of ANP. It also is likely that the neutral endopeptidase activity in SHR may be increased because of the elevated circulating ANP concentration. These hypotheses are supported by the findings that thiorphan delayed the elimination of ANP in SHR to a greater extent than WKY rats and that thiorphan caused a greater urinary excretion of ANP in SHR.

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In conclusion, the endogenous ANP in SHR may contribute to sodium homeostasis by keeping the
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pressure–natriuresis curve to the left. Furthermore, this role of ANP seems to be modified by alterations in ANP metabolism.

References


27. Gellai M, DeWolf RE, Kinter LB, Beeuwkes R III: The effect of atrial natriuretic factor on blood pressure, heart rate, and


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