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Acute and chronic neutral endopeptidase inhibition and the natriuretic response to acute volume expansion

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Abstract

Neutral endopeptidase inhibition (NEPI) provides a potential avenue to modulate the actions of atrial natriuretic peptide (ANP). We tested the hypothesis that acute and chronic NEPI increased the renal responses at baseline and after acute volume expansion in rats. ANP plasma levels and cGMP excretion were significantly increased with acute NEPI by SQ 28.603, whereas chronic inhibition with SCH 34826 did not lead to any changes. The ratio of cGMP excretion per plasma ANP, however, was significantly increased (6.2 ± 0.9) by chronic treatment with SCH 34826 compared to chronic vehicle treatment (4.2 ± 0.7) indicating an activated renal ANP receptor system. Baseline diuresis and natriuresis were enhanced with acute but not with chronic treatment. After acute volume expansion, ANP increased five-fold with acute NEPI, whereas it only increased about 70% in chronically inhibited rats. The natriuretic (497 ± 62 vs. 329 ± 42 μmol/60 min with vehicle, $P < 0.05$) and diuretic responses were significantly enhanced with chronic treatment. Together with an increased cGMP/ANP ratio, these data suggest that chronic activation of the renal ANP system after long-term NEPI facilitated the excretion of an acute volume load. These findings may have therapeutic implications in patients with chronic sodium retention. © 1998 Elsevier Science B.V.

Keywords: ANP (Atrial natriuretic peptide); cGMP; Neutral endopeptidase inhibition; Natriuresis; Diuresis

1. Introduction

The natriuretic peptide system is an important regulator of salt and water homeostasis (De Zeeuw et al., 1992; Inagami, 1989; Koller and Goeddel, 1992; Awazu and Ichikawa, 1993). Atrial natriuretic peptide (ANP) is released from atrial myocytes upon stretch and increases after volume expansion in isolated perfused rat hearts (Kabayama et al., 1987) and in vivo (Garcia et al., 1987). After release, ANP exerts its actions via specific natriuretic peptide receptors. Thus far, three distinctive natriuretic peptide receptors have been described. The type A and type B natriuretic peptide receptors are coupled to the particulate guanylate cyclase, which results in the generation of the second messenger cGMP after activation. Both plasma and urinary cGMP correlate well with the biological activity of ANP (Hamet et al., 1986; Margulies et al., 1991a). Furthermore, the relationship between plasma ANP and urinary cGMP has been suggested as an indicator of the efficacy of the ANP system (Sagnella et al., 1990). A third receptor, termed natriuretic peptide receptor type C, is not coupled to guanylate cyclase and instead plays a role in the clearance of ANP (Maack et al., 1987). Apart from the clearance of ANP via type C receptors, the peptide is also inactivated by enzymatic cleavage. This degradation occurs through a specific metalloproteinase, the neutral endopeptidase 3.4.24.11, which is found almost ubiquitously, the major site of degradation, however, being the renal brush borders (Erdös and Skidgel, 1989; Koepke et al., 1989). Several neutral endopeptidase inhibitors have been developed to block this enzymatic degradation (Sybertz et al., 1990; Margulies et al., 1991b).

Contradictory data have been published on neutral endopeptidase inhibition (NEPI), the resultant ANP plasma levels and changes in renal function. Without a preactivated ANP system, some investigators showed unchanged ANP plasma levels after acute NEPI (Burnier et al., 1991; Seymour et al., 1990; Ura et al., 1994). Others reported

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elevated ANP plasma levels (Kukkonen et al., 1992) with enhanced diuretic and natriuretic responses (Danilewicz et al., 1989). We recently showed that acute NEPI increased ANP plasma levels without improving renal responses in rats with a stimulated ANP system, whereas the increase in ANP plasma levels in control rats led to enhanced diuresis and natriuresis (Willenbrock et al., 1996). Few studies have investigated the effect of chronic inhibition of the neutral endopeptidase. In hypertensive patients, ANP plasma levels were not increased after NEPI for five days (Richards et al., 1993) or four weeks (Favrat et al., 1995). However, cGMP excretion was elevated in both studies with only transient increases in natriuresis and diuresis. Similar results were observed after two weeks of NEPI in stroke-prone spontaneously hypertensive rats (Stasch et al., 1995). Another study in rats with myocardial infarction reported no improved diuretic effects after 6 days of inhibition of the neutral endopeptidase (Helin et al., 1991). In states without a stimulated endogenous ANP system, NEPI for four days showed no effects on diuresis (Richards et al., 1991). To our knowledge, the long-term effects of NEPI on the ANP system and the renal responses to acute volume expansion have not been studied. We therefore analyzed the effect of chronic NEPI on ANP and cGMP plasma levels and on renal sodium and water excretion in response to acute volume expansion and compared these effects to an acute inhibition of ANP degradation.

2. Material and methods

2.1. Animals

Male Wistar rats from Moellegaard Animal Farms, Schoenwalde, Germany, weighing 300–350 g were used for all studies. The animals were fed normal rat chow and were allowed free access to tap water. The rats were housed in an approved facility with a 12-h light/dark cycle. All experiments were performed between 0700 and 1200 h. The studies were approved by the Council of Animal Care and performed according to the guidelines of the American Physiological Society.

2.2. Neutral endopeptidase inhibition

Experiments were performed in four groups of rats. In the first two groups, the animals received either acute neutral endopeptidase inhibitor by intravenous administration of SQ 28.603 (30 mg/kg) or vehicle ($NaHCO_3$ 0.83%) immediately before the beginning of the baseline period. In the last two groups of rats, the chronic neutral endopeptidase inhibitor SCH 34826 (30 mg/kg) or vehicle (carboxymethylcellulose 1.25%) was given twice daily (0800 h and 2000 h) as gavage for 28 days. In the chronically-treated groups, rats received their last medication approximately 12 h before the volume expansion began. Both neutral endopeptidase inhibitors have been described to effectively and selectively inhibit the enzyme with a K_i in the nanomolar range. Their chemical structure and binding activities to the neutral endopeptidase were published previously (Sybertz et al., 1990; Margulies et al., 1991b). The dosages which we used were reported not to have induced any hemodynamic effects (Monopoli et al., 1992; Cavero et al., 1990).

2.3. Hemodynamic measurements and acute volume load

All studies were performed under chloral hydrate (400 mg/kg i.p.) anesthesia. The animals were not fasting before starting the experiment. In all four groups of rats, a PE-50 catheter was advanced into the right carotid artery for measurement of blood pressure and heart rate via a Statham P23XL transducer and a Gould AMP 4600 amplifier and for withdrawal of blood for determinations of ANP and cGMP. Another PE-50 catheter was inserted through the right jugular vein into the superior vena cava for baseline infusion (1.5 ml/h of 0.9% NaCl) and acute volume load. For determination of diuresis, natriuresis and cGMP excretion, a PE-50 catheter was inserted into the bladder and urine was collected at 20-min intervals. Only in the acutely-treated groups of rats, an additional PE-50 catheter was inserted into the right femoral vein for administration of either acute vehicle or SQ 28.603. After surgery, a 20-min equilibration period was added which was followed by a 20-min baseline period (b). In this period, baseline renal parameters were obtained over the whole period of 20 min, and hemodynamic parameters were assessed at the end of the period. Acute volume load was then performed with infusion of 5 ml of hyperoncotic solution (Hydroxyethyl starch HAES 10%, Braun Melsungen, Germany) within 5 min. For the remaining 15 min, urine collections were continued to obtain a whole 20-min interval (t_1). Two additional collection periods of 20 min each were added (t_2 and t_3).

2.4. Determination of ANP and cGMP

Blood samples for ANP (500 μl) and cGMP (200 μl) were withdrawn at the end of each collection period in NaEDTA-preloaded and prechilled tubes (final concentration of NaEDTA 7 mmol/l). Degradation of ANP and cGMP was prevented with addition of phenylmethylsulfonyl fluoride (final concentration 10 μmol/l), pepstatin (3 μmol/l) and isobutylmethylxanthine (1 mmol/l). Immediately after withdrawal, the blood was centrifuged at 4°C and $2000 \times g$ for 10 min, and the plasma was kept at −80°C until extraction. The blood withdrawn was replaced with the same amount of blood from donor animals. ANP plasma samples were extracted with C18 Sep-Pak columns that had been activated with acetonitrile and ammonium acetate (0.2%, pH 4.0). After addition of the plasma, the columns were washed again with ammonium

acetate, and ANP was eluted with acetonitrile (60%) and ammonium acetate (40%) following a previously described protocol (Gutkowska et al., 1986). ANP was measured by radioimmunoassay performed with antibodies kindly provided by Dr. G. Thibault and Dr. J. Gutkowska, Montreal, Canada. The cGMP plasma samples were extracted with alumina (AG 7) and Dowex (AG 50W-X8) columns before assessment by radioimmunoassay. Urinary cGMP was measured directly using a specific radioimmunoassay (Richman et al., 1980). Antibodies were generously donated by Dr. P. Hamet, Montreal, Canada.

2.5. Data analysis

The responses to acute volume load were compared using two-way analysis of variance. The differences between the groups were evaluated with the corrected unpaired Student's *t*-test and the Wilcoxon rank-sum test where appropriate. The significance level was set at $P < 0.05$. All data are expressed as mean ± SEM (standard error of the mean).

3. Results

3.1. Effect of acute and chronic NEPI on heart and body weight and on hemodynamics

Chronic inhibition of neutral endopeptidase did not change either body nor heart weight compared to acute or chronic administration of vehicle or acute administration of SQ 28.603 (data not shown). Similarly, no changes were found in heart rate and mean blood pressure after chronic NEPI (68 ± 11 mmHg) compared to acute vehicle (64 ± 6 mm Hg), chronic vehicle (65 ± 8 mmHg) or acute NEPI (66 ± 10 mmHg).

3.2. ANP and cGMP plasma levels after acute NEPI

ANP plasma levels in acutely vehicle-treated rats increased from 266 ± 59 to 918 ± 141 pmol/l ($P < 0.05$ vs. baseline) after acute volume expansion (t_1), as shown in Fig. 1a. This increase in ANP levels was reflected by a five-fold increase in plasma cGMP, as depicted in Fig. 1b. After acute administration of the neutral endopeptidase inhibitor SQ 28.603, ANP plasma levels at baseline were significantly elevated (499 ± 89 vs. 266 ± 59 pmol/l, $P < 0.05$, Fig. 1a). Acute volume load further increased ANP plasma levels up to a maximum of 2572 ± 538 vs. 918 ± 141 pmol/l in vehicle-treated rats ($P < 0.05$). cGMP plasma levels at baseline (6.9 ± 1.8 vs. 2.1 ± 0.4 nmol/l, $P < 0.05$) and after acute volume expansion (25.7 ± 1.6 vs. 10.2 ± 1.4 nmol/l, $P < 0.001$) were significantly elevated with acute NEPI compared to acute vehicle treatment (Fig. 1b).

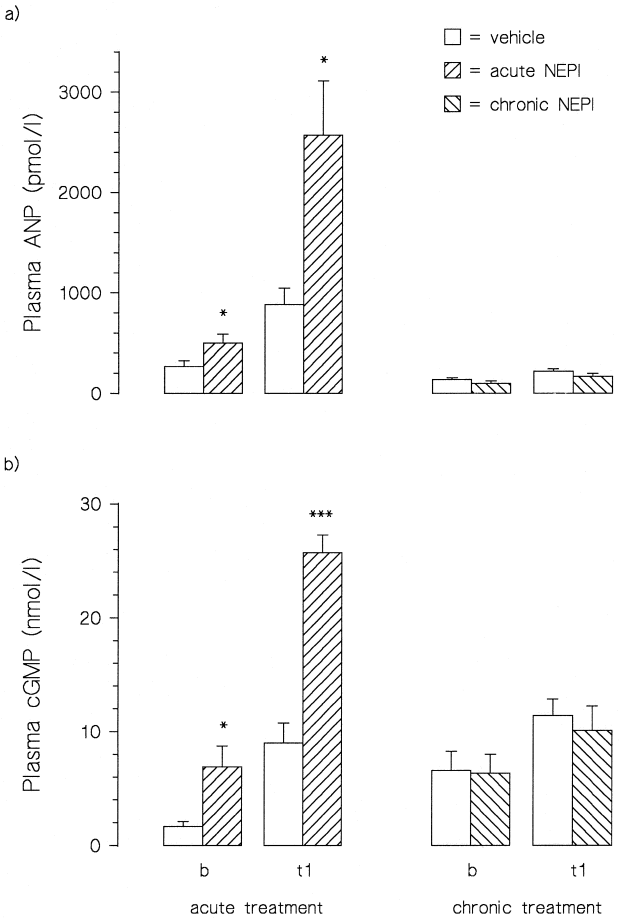

Fig. 1. The ANP plasma levels (a) and cGMP plasma levels (b) are shown at baseline (*b*) and after acute volume expansion (t_1) in rats treated with acute and chronic neutral endopeptidase inhibition (NEPI) or vehicle. Baseline as well as t_1 were 20 min each with acute volume expansion within the first 5 min of t_1. Data are expressed as mean ± SEM, $n = 9$ rats in each group. ∗∗ = $P < 0.01$, ∗∗∗ = $P < 0.001$ vs. vehicle-treated rats.

3.3. cGMP excretion after acute NEPI

The excretion of the second messenger cGMP increased after acute volume load from 0.3 ± 0.1 mmol/20 min at baseline to 2.1 ± 0.2 mmol/60 min ($P < 0.05$, Fig. 2) in animals given vehicle acutely. Acute NEPI induced a significant, threefold increase in cGMP excretion at baseline compared to vehicle-treated animals. After acute volume load, cGMP excretion increased further from 1.4 ± 0.3 mmol/20 min at baseline to 13.1 ± 2.0 mmol/60 min ($P < 0.001$ vs. vehicle, Fig. 2).

3.4. Renal responses after acute NEPI

To determine the renal responses after acute NEPI, we measured diuresis and natriuresis at baseline and after acute volume expansion and compared the effects to acutely vehicle-treated rats. Acute NEPI significantly increased

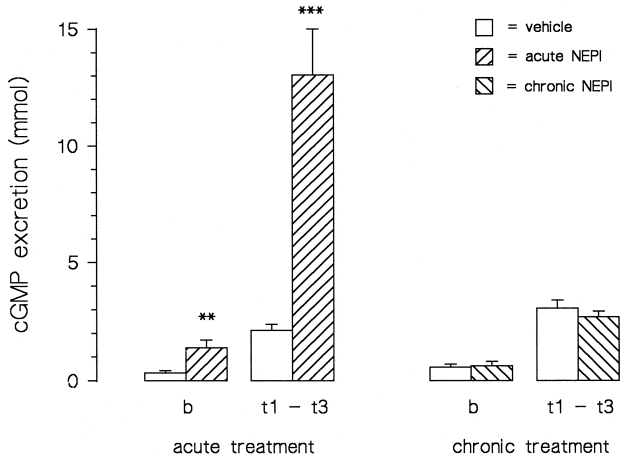

Fig. 2. The excretion of the second messenger cGMP is shown at baseline (b) and after acute volume expansion (t_1–t_3) in rats treated with acute and chronic NEPI or vehicle. Data are expressed as mean ± SEM, $n = 9$ rats in each group. $* * = P < 0.01$, $* * * = P < 0.001$ vs. vehicle-treated rats.

diuresis (Fig. 3a) and natriuresis (Fig. 4a) at baseline. The diuretic response after acute volume expansion was enhanced compared to acutely vehicle-treated rats (3965 ± 217 vs. 2626 ± 230 μl/60 min, $P < 0.001$). Fig. 3b demonstrates the additional diuresis induced by acute volume load starting from baseline values with a significant increase in urinary output compared to vehicle-treated animals (3250 ± 186 vs. 2463 ± 175 μl, $P < 0.001$). Similarly, the natriuretic response after acute volume load was increased by acute NEPI (Fig. 4a and b).

3.5. Effect of chronic NEPI on ANP and cGMP plasma levels

While acute treatment induced a rise in plasma ANP, ANP levels were not elevated at neither baseline period nor after acute volume expansion with chronic neutral endopeptidase inhibitor treatment (Fig. 1a). Similarly, cGMP plasma levels were unchanged with chronic NEPI at either baseline and after acute volume expansion compared to vehicle-treated rats (Fig. 1b).

3.6. Effect of chronic NEPI on cGMP excretion

As shown in Fig. 2, cGMP excretion at baseline and after acute volume expansion was unchanged by chronic NEPI compared to chronic vehicle administration. After acute volume expansion, cGMP excretion significantly increased in both chronically vehicle-treated rats and in rats with chronic neutral endopeptidase inhibitor (from 0.6 ± 0.2 mmol/20 min at baseline to 2.7 ± 0.2 mmol/60 min, $P < 0.05$).

3.7. Renal responses after chronic NEPI

Chronic NEPI changed neither diuresis (Fig. 3a) nor natriuresis (Fig. 4a) at baseline compared to chronically vehicle-treated rats. However, after acute volume expansion the diuretic response was significantly increased with chronic NEPI compared to vehicle (3338 ± 371 vs. 2500 ± 305 μl/60 min, $P < 0.05$). As depicted in Fig. 3b, the additional diuresis induced by acute volume expansion was significantly enhanced compared to vehicle-treated rats (3019 ± 301 vs. 2253 ± 291 μl, $P < 0.05$). Thus, chronic NEPI markedly improved the diuretic response to acute volume expansion without any changes in baseline diuresis, whereas acute NEPI changed both baseline diuresis and the diuretic response after volume expansion. Simi-

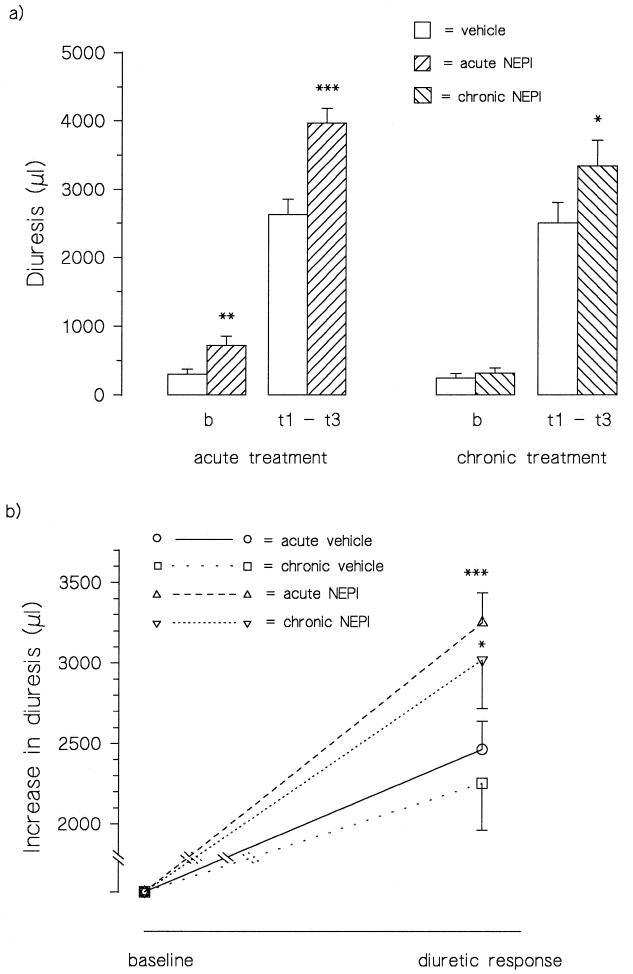

Fig. 3. The diuretic response (a) is shown at baseline (b) and as cumulative diuresis after acute volume expansion (t_1–t_3, 60 min) in rats treated with acute or chronic NEPI or vehicle. (b) shows the increase in urinary volume after acute volume expansion (diuretic response) from baseline values in each treatment group. Data are expressed as mean ± SEM; $n = 9$ rats in each treatment group. $* = P < 0.05$, $* * = P < 0.01$, $* * * = P < 0.001$ vs. vehicle-treated rats.

larly, the natriuretic response after acute volume load was increased in the chronically-treated rats (Fig. 4a) with a significant additional natriuresis induced by volume expansion (490 ± 60 vs. 308 ± 35 μmol in chronically vehicle-treated rats, $P < 0.05$), as shown in Fig. 4b.

3.8. Ratio of cGMP per ANP

An explanation for the enhanced diuretic and natriuretic responses after acute volume expansion in rats with chronic neutral endopeptidase inhibitor despite unchanged ANP plasma levels could be an improved efficacy in cGMP production. Therefore, we determined the ratio of excreted cGMP per plasma ANP, as shown in Table 1. In rats with chronic NEPI, this ratio was significantly increased at baseline (6.2 ± 0.9) compared to chronic vehicle (4.2 ± 0.7). Similarly, acute NEPI increased the ratio of cGMP per ANP (from 1.2 ± 0.2 to 2.8 ± 0.4). After acute volume expansion, the relationship between cGMP and ANP remained increased in chronic neutral endopeptidase inhibitor-treated rats compared to vehicle-treated control rats. Similarly, the ratio of plasma cGMP to plasma ANP at baseline was significantly increased by chronic NEPI (63.5 ± 7.8) compared to chronic vehicle (43.8 ± 4.8) and remained elevated after acute volume expansion.

Table 1
The ratio of urinary and plasma cGMP to plasma ANP

	Acute vehicle	Acute NEPI	Chronic vehicle	Chronic NEPI
Urinary cGMP / plasma ANP				
b	1.2 ± 0.2	2.8 ± 0.4[a]	4.2 ± 0.7	6.2 ± 0.9[a]
t_1	0.9 ± 0.1	1.5 ± 0.1	5.9 ± 0.8	7.4 ± 0.4[a]
Plasma cGMP / plasma ANP				
b	7.9 ± 0.7	13.8 ± 2.1[a]	43.8 ± 4.8	63.5 ± 7.8[a]
t_1	11.1 ± 1.0	10.0 ± 2.9	45.4 ± 5.8	59.5 ± 7.6[a]

Values are expressed as mean ± SEM. $n = 9$ rats in each treatments group. *b*, baseline; t_1, collecting period of 20 min after baseline with acute volume expansion within first 5 min of t_1.
The ratio was calculated as urinary as urinary cGMP/plasma ANP (μmol 20 min^{-1}/pmol×l^{-1}) and plasma cGMP/plasma ANP (pmol l^{-1}/pmol l^{-1}).
[a] $P < 0.05$ vs. vehicle-treated animals.

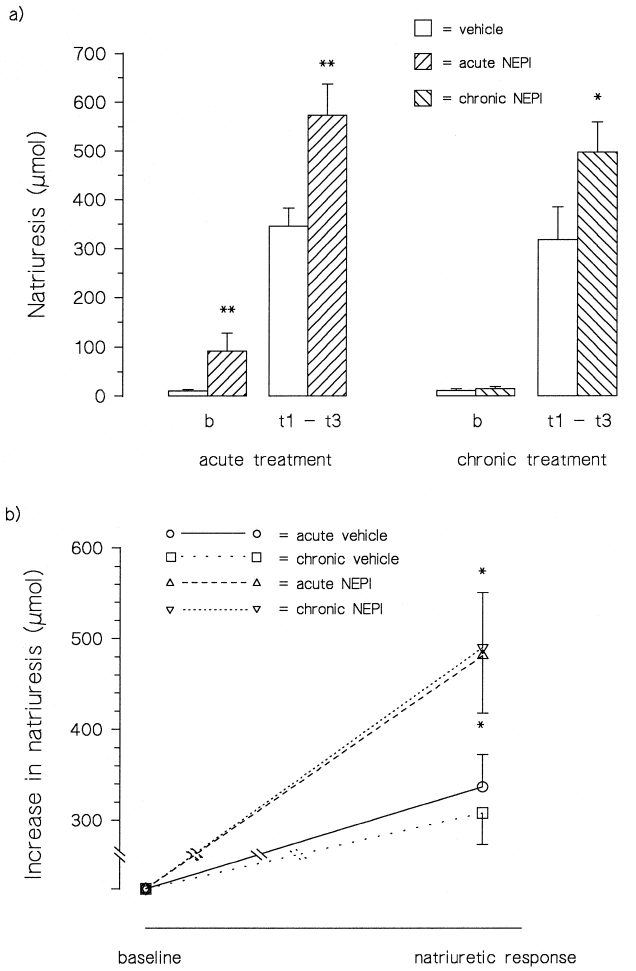

Fig. 4. The natriuretic response (a) is shown in rats treated with acute or chronic NEPI or vehicle at baseline (*b*) and after acute volume expansion (t_1–t_3, 60 min). (b) shows the increase in natriuresis after volume expansion (natriuretic response) from baseline values. Data are expressed as mean ± SEM; $n = 9$ rats in each treatment group. $* = P < 0.05$, $** = P < 0.01$ vs. vehicle-treated rats.

4. Discussion

The important finding in this study was that chronic NEPI alters the relationship between circulating plasma ANP and second messenger production reflected by urinary and plasma cGMP. This alteration is of functional significance as reflected by a greater natriuresis and diuresis after volume expansion in rats with NEPI compared to vehicle. The effects on ANP release in response to acute volume expansion with chronic inhibition of the neutral endopeptidase were profound. While ANP increased with volume expansion three- to five-fold in rats receiving either acute vehicle or acute NEPI, plasma ANP levels barely increased at all in rats receiving chronic NEPI. Plasma and urinary cGMP responses to volume expansion were not different in rats receiving vehicle or chronic NEPI; nevertheless, diuresis and natriuresis were facilitated with chronic NEPI. Thus, our data underscore a fundamental alteration in the relationship between ANP and its receptor and post-receptor events with chronic NEPI. We believe that this alteration could have therapeutic implications.

In an earlier study, we examined the effects of chronic NEPI in rats with aortocaval shunts, which exhibit a blunted natriuresis in response to acute volume load. These rats have a chronically stimulated ANP system because of mild heart failure. In that study, we showed that chronic NEPI improved the blunted natriuretic responses after

acute volume loading (Willenbrock et al., 1996). Others reported only transiently increased natriuresis and diuresis or even no influence on diuresis with a preactivated ANP system due to hypertension (Richards et al., 1993; Favrat et al., 1995; Stasch et al., 1995) or myocardial infarction (Helin et al., 1991). The effect of chronic NEPI on the renal responses to acute volume expansion without a previously stimulated ANP system has not been studied. To our knowledge, this is the first report demonstrating that long-term NEPI enhances the efficacy of the ANP system and the renal response to an acute volume challenge.

In our study, acute NEPI significantly increased ANP plasma levels at baseline and potentiated the ANP increase after acute volume expansion. Contradictory effects of acute inhibition of the neutral endopeptidase on ANP plasma levels have been reported earlier. Acute NEPI was shown to increase ANP plasma levels, particularly with a preactivated ANP system (Kukkonen et al., 1992; Trippodo et al., 1991; Willenbrock et al., 1996). When the ANP system was not stimulated, ANP levels were found to be either increased (Danilewicz et al., 1989) or unchanged (Seymour et al., 1990; Burnier et al., 1991; Ura et al., 1994). The reason why elevated ANP plasma levels after acute NEPI were not observed in all studies is not entirely clear; however, differences in baseline infusion and a less effective inhibition of the enzyme may have contributed to these divergent reports. Along with increased ANP plasma levels, the natriuretic and diuretic responses we observed with acute NEPI were significantly enhanced at baseline. This observation is similar to previously published results on acute NEPI in mice (Danilewicz et al., 1989) with potentiation of ANP plasma levels leading to enhanced natriuresis and diuresis. In parallel to the effect of acute NEPI on ANP plasma levels after acute volume expansion in our study, the second messenger cGMP in plasma and urine was also increased. Accordingly, diuresis and natriuresis after volume expansion were significantly enhanced. These data confirm a recent finding with significantly increased renal responses to acute hypervolemia in rats acutely-treated with a neutral endopeptidase inhibitor (Scott et al., 1993).

In contrast to acute NEPI, chronic inhibition of this enzyme did not increase ANP plasma levels at baseline. These results differ from another study reporting increased ANP plasma levels with chronic NEPI in experimental heart failure (Helin et al., 1991). Other groups observed increases in ANP plasma levels only at the beginning of chronic NEPI (Richards et al., 1993) with a diminished increase in ANP due to reduced endogenous secretion already after 4 days of treatment. Since we did not measure ANP during the entire time course of chronic NEPI, we cannot exclude a transient increase that may have occurred during the first weeks. Recently, the regulation of neutral endopeptidase in endothelial cells was reported (Graf et al., 1995), and a decreased activity of the enzyme in pulmonary, but not in renal tissue in a rat model of heart failure was described (Abassi et al., 1995). However, it is unknown whether or not chronic NEPI might influence endogenous activity of the enzyme.

In our study, we observed that despite unchanged ANP plasma levels, renal responses to acute volume load were significantly enhanced with chronic NEPI. Enhanced natriuresis, along with unchanged ANP plasma levels, has been described after only 4 days of NEPI in healthy humans (Richards et al., 1991). Recently, we reported facilitated renal sodium excretion with chronic NEPI in rats with heart failure without any increases in ANP plasma levels (Willenbrock et al., 1996). These data suggest that renal rather than circulating ANP levels may be responsible for the natriuretic and diuretic effects of chronic NEPI. We did not measure urinary ANP; however, our data on facilitated renal sodium and water excretion after chronic inhibition of the neutral endopeptidase with a significantly increased ratio of cGMP excretion per plasma ANP levels support the importance of an enhanced renal ANP system. Since the description of cGMP as second messenger of the natriuretic peptides and mediator of their biological effects, several studies described parallel changes in ANP plasma levels and cGMP excretion (Lewis et al., 1988; Sagnella et al., 1990; Waldman et al., 1984). The coupling of ANP to cGMP was an important determinant of the short-term natriuretic response to acute NEPI in hypertensive patients (Sagnella et al., 1992). Since cGMP excretion reflects the renal actions of ANP (Hamet et al., 1984), the ratio of urinary cGMP per plasma ANP may indicate the degree of renal ANP receptor activation. In our study, the ratio of urinary cGMP per plasma ANP was significantly increased with chronic NEPI at baseline and after acute volume expansion. Recently, we described a similarly enhanced ratio of urinary cGMP per plasma ANP with chronic NEPI in rats with aortocaval shunt (Willenbrock et al., 1996).

Other factors, such as kinins or the nitric oxide system could have influenced renal cGMP generation and could thereby have led to an enhanced relationship of excreted cGMP per given ANP concentration (Siragy et al., 1994). Increase in cGMP plasma levels was shown to be specific for ANP (Roy et al., 1989; Tremblay et al., 1988), and the ratio of plasma cGMP per plasma ANP was also increased in chronically-treated rats. These results suggest an enhanced coupling of cGMP/ANP after long-term NEPI. Since a simultaneous inhibition of angiotensin converting enzyme is unlikely with the compound we used (Monopoli et al., 1992; Cavero et al., 1990), the improved renal function could not be attributed to an inhibited renin–angiotensin-system. Unexplored are the chronic effects of NEPI on natriuretic peptide receptor regulation. Down-regulation of the receptors may be expected due to either transient increases of ANP plasma levels or to possibly elevated renal ANP concentrations. Nevertheless, the natriuretic and diuretic responses to volume expansion were enhanced, suggesting that the biologically active receptors were not down-regulated. Perhaps down-regulation of the

type C natriuretic peptide receptor alters ANP kinetics in chronically neutral endopeptidase inhibited rats. Kinetic studies in such rats might elucidate this possibility.

We believe that our observations have therapeutic implications. Application of ANP therapeutically has thus far been disappointing. In treating acute renal failure (Allgren et al., 1997) or the hepatorenal syndrome (Aronoff et al., 1990), administration of ANP or its analogs proved not to be beneficial. However, acute application of ANP had a dose-dependent effect in patients with various chronic renal diseases, including chronic glomerulonephritis and chronic interstitial renal disease (DeNicola et al., 1997). In that study, the ANP-induced effect was of such a magnitude to suggest that the ANP system may be pivotal in maintaining sodium handling. Chronic ANP treatment is not practical, since the peptide cannot be given orally. Chronic blockade of ANP breakdown with neutral endopeptidase inhibition, however, could be a useful therapeutic tool. We suggest that chronic NEPI may alter the relationship between cGMP and ANP and facilitate the water and sodium excretion in such patients. Careful clinical trials will be necessary to address these issues.

Taken together, we showed that the renal sodium and water excretory responses to acute volume expansion were significantly enhanced with acute and chronic NEPI. Whereas acute inhibition of the neutral endopeptidase augmented the increase in ANP plasma levels after acute volume load up to fivefold, chronic inhibition of the enzyme did not change ANP plasma levels, but nevertheless enhanced renal responses to acute volume load. Thus, together with an enhanced cGMP generation per ANP, our results suggest that chronic NEPI activates the renal ANP receptors and renders the endogenous ANP system more effective with an improved ability to respond to acute volume load.

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