

Atrial Natriuretic Peptide Regulation of Noradrenaline Release in the Anterior Hypothalamic Area of Spontaneously Hypertensive Rats

Ning Peng,* Suzanne Oparil,† Qing C. Meng,‡ and J. Michael Wyss**

*Department of Cell Biology and †the Vascular Biology and Hypertension Program of the Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama 35294

Abstract

In spontaneously hypertensive rats (SHR), high NaCl diets increase arterial pressure and sympathetic nervous system activity by decreasing noradrenaline release in the anterior hypothalamic area (AHA), thereby reducing the activation of sympathoinhibitory neurons in AHA. Atrial natriuretic peptide (ANP) can inhibit the release of noradrenaline, and ANP concentration is elevated in the AHA of SHR. The present study tests the hypothesis that in SHR, local ANP inhibits noradrenaline release from nerve terminals in AHA. Male SHR fed a basal or high NaCl diet for 2 wk and normotensive Wistar Kyoto rats (WKY) fed a basal NaCl diet were studied. In SHR on the basal diet, microperfusion of exogenous ANP into the AHA elicited a dose-related decrease in the concentration of the major noradrenaline metabolite 3-methoxy-4-hydroxy-phenylglycol (MOPEG) in the AHA; this effect was attenuated in the other two groups. In a subsequent study, the ANP-C (clearance) receptor agonist c-ANP was microperfused into the AHA to increase extracellular concentration of endogenous ANP in AHA. c-ANP reduced AHA MOPEG concentration in SHR on the basal NaCl diet but not in the other two groups. These data support the hypothesis that local ANP inhibits noradrenaline release in the AHA and thereby contributes to NaCl-sensitive hypertension in SHR. (*J. Clin. Invest.* 1996; 98:2060–2065.) Key words: baroreflex • hypertension • hypothalamus • salt sensitivity • sympathetic nervous system • neuromodulation

Introduction

Circulating atrial natriuretic peptide (ANP)¹ plays an important role in the regulation of arterial pressure and fluid and electrolyte homeostasis (1, 2). Further, neuronally produced ANP can act directly on neurons in the brain to modify arterial

pressure and cardiovascular responses (3–5). Altered synthesis and/or release of ANP in critical brain nuclei appears to contribute to hypertension in spontaneously hypertensive rats (SHR; 6, 7) and in Dahl-NaCl sensitive rats (8). Alterations in ANP have been described in the anterior hypothalamic area (AHA) and the nucleus of the solitary tract (NTS) of SHR (6, 7, 9). Several lines of evidence suggest that an increase in ANP in the AHA may contribute importantly to the elevation of arterial pressure in SHR fed a high NaCl diet.

The AHA primarily subserves a sympathoinhibitory role, i.e., stimulation of the neurons in this nucleus causes a decrease in sympathetic nervous system activity and a resultant decrease in arterial pressure and heart rate (10, 11). Further, an increase in the release of noradrenaline in the AHA causes a decrease in arterial pressure due to sympathoinhibition (12). While SHR genetically develop hypertension irrespective of diet, in most substrains of SHR, diets high in NaCl exacerbate hypertension (by 20–25 mm Hg), at least in part, by selectively decreasing noradrenaline release in the AHA (12, 13). Noradrenaline release from nerve terminals in other brain regions is not altered in the SHR consuming a high NaCl diet, and AHA noradrenaline release is not altered in response to dietary NaCl supplementation in NaCl-resistant rodent strains, e.g., Wistar Kyoto rats (WKY). Since SHR have a severely blunted cardiopulmonary baroreflex response (14) and the baroreflex activates noradrenergic neurons in the brainstem (15, 16), we initially speculated that the reduction in noradrenaline release in the AHA of SHR fed a high NaCl diet resulted from blunted baroreflex activation of noradrenergic neurons in the brainstem that projected to the AHA. However, the vast majority of noradrenergic neurons that send axons to the AHA also project to other surrounding nuclei (i.e., the lateral and posterior hypothalamic areas) that do not display a decrease in extracellular noradrenaline in high NaCl fed SHR (unpublished data from our group). This suggests that some other mechanism regulates noradrenaline release in this model. Another mechanism that could account for the selective reduction in noradrenaline release in AHA of SHR on a high NaCl diet is the local action of inhibitory neuromodulator(s) on noradrenaline release from axon terminals in AHA. ANP is a neuromodulator that inhibits the release of noradrenaline from neurons in the hypothalamus in vitro (10). Previous studies in our laboratory have shown that the ANP content of the AHA in SHR is higher than that of normotensive WKY (6). We have further demonstrated that blockade of local ANP in the AHA by microinjection of a monoclonal antibody to ANP causes a greater hypotensive response in SHR than in WKY (7) and that direct microinfusion of ANP into the AHA increases arterial pressure more in SHR than in WKY (unpublished data). Therefore, we have hypothesized that endogenous ANP in the AHA inhibits noradrenaline release in the AHA of SHR and facilitates the development of NaCl-sensitive hypertension in this model.

Address correspondence to Dr. J. Michael Wyss, Ph.D., Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294-0019. Phone: 205-934-6086; FAX: 205-934-7029.

Received for publication 30 May 1996 and accepted in revised form 5 September 1996.

1. Abbreviations used in this paper: ACSF, artificial cerebral spinal fluid; AHA, anterior hypothalamic area; ANP, atrial natriuretic peptide; MOPEG, 3-methoxy-4-hydroxy-phenylglycol; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/96/11/2060/06 \$2.00

Volume 98, Number 9, November 1996, 2060–2065

The present study tested the hypothesis that elevation of local extracellular ANP reduces the release of noradrenaline from axon terminals in the AHA, and that interaction between ANP and noradrenaline is blunted in the SHR that have been maintained on a high NaCl diet. A second experiment tested more directly the role of endogenous ANP in this model by infusing into the AHA c-ANP, (ANP₄₋₂₃), a peptide fragment of ANP that binds to the ANP clearance receptor (ANP-C) and thereby decreases the ability of the ANP-C receptor to bind to and take up endogenously released ANP. This results in an elevation of extracellular ANP in the AHA. Together the results indicate that endogenous ANP in the AHA may contribute to NaCl-sensitive hypertension in the SHR.

Methods

All experiments were performed in conscious, freely moving, male SHR and normotensive WKY (Harlan Sprague Dawley, Inc., Indianapolis, IN). 2 wk before each experiment, one group of 7-wk-old male SHR ($n = 10$) was placed on a high (8%) NaCl diet (ICN Biochemicals, Inc., Costa Mesa, CA), while a second group of SHR and a group of WKY remained on a basal (1%) NaCl diet ($n = 10$ /group; diet 5001; Ralston Purina Co., St. Louis, MO). All animals were maintained on a 12/12 h light/dark cycle (light from 0600–1800) at a constant temperature ($24 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$), and they were housed three rats per cage before surgery. Body weights were measured weekly before the experiment.

2 wk after initiation of the diets, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and a stainless steel guide cannula (24 gauge) fitted with a removable 31-gauge obturator (which extended 0.5 mm past the tip of the outer cannula) was stereotactically implanted above the AHA (the tip of the guide cannula was positioned so that it would be 0.6 mm dorsal to the AHA [the stereotaxic coordinates measured from bregma were anterior -1.6 mm, lateral $+0.7$ mm, ventral -8.1 mm]; 17). The cannula was secured in place with acrylic cement that was anchored to the skull by three stainless steel screws. After recovery from anesthesia, all animals were placed into individual cages and maintained on their diet until the time of study. 5 d after implantation of the guide cannula, animals were anesthetized with sodium methohexital (60 mg/kg, i.p.). Catheters (PE-10 fused to PE-50 tubing; Becton Dickinson Co., Parsippany, NJ) were implanted into the abdominal aorta through the right femoral artery for measurement of arterial pressure and into the right femoral vein for intravenous infusion of saline. The free ends of the catheters were externalized between the scapulae and secured in place with dental acrylic. All animals were allowed to recover for at least 2 h after regaining consciousness. Approximately 60% of the rats in each group were allowed to recover overnight prior to being tested. The resulting arterial pressure and biochemical baselines and responses were not significantly different between the rats that had short (2–3 h) compared to long (15–16 h) recovery. The relatively small standard errors of the mean also suggest that postoperative recovery did not affect the results of the study.

On the day of the experiment, each animal was placed in a small cage which allowed free movement, and the arterial line was attached to a pressure transducer for recording blood pressure. After adaptation to these conditions (as evidenced by a stable blood pressure signal and resting posture; ~ 30 min), the push–pull assembly was inserted into the guide cannula, so that its tip extended 1 mm beyond the guide cannula. Polyethylene tubing (PE-20) was connected to the inflow and outflow of the push–pull assembly and to two identically calibrated Rabbit pumps (Rainin Instrument Co. Inc., Woburn, MA). Microperfusion with artificial cerebral spinal fluid (ACSF; NaCl 119 mmol/liter, KCl 3.3 mmol/liter, CaCl_2 1.3 mmol/liter, MgCl_2 1.2 mmol/liter, Na_2HPO_4 0.5 mmol/liter, NaHCO_3 21.0 mmol/liter, and glucose 3.4 mmol/liter; adjusted to pH 7.4 with 1N HCl) was then be-

gun. ACSF was pushed through the inner cannula and drawn up between the inner cannula and guide cannula at a rate of 10 $\mu\text{l}/\text{min}$. After a 30-min equilibration period, during which perfusate was discarded, perfusate was collected in 10 min fractions in plastic tubes containing 25 μl of a 0.5 N perchloric acid-EDTA solution (0°C) and subsequently stored at -20°C .

Experiment 1. After a 90-min baseline collection period, ANP (10^{-5} – 10^{-9} M in ACSF; Peninsula Laboratories, Inc., Belmont, CA) was microperfused through the push–pull cannula for 20 min. Perfusate samples were collected and mean arterial pressure (MAP) and heart rate were monitored continuously during the baseline, ANP infusion, and subsequent 90-min recovery periods. At the conclusion of the experiment, 0.3% pontamine sky-blue dye in ACSF was perfused through the push–pull cannula for 3 min, and the animal was killed with an overdose of ether. The brain was removed and sectioned for histologic verification of cannula placement by an investigator who was blind to the group designation of the sections examined.

Experiment 2. Three additional groups of animals (SHR 1% and 8% NaCl diet, and WKY 1% NaCl diet) were tested according to the above protocol, except that 10^{-7} M c-ANP (the 4–23 fragment of ANP; Peninsula Laboratories, Inc.) was microperfused as the challenge. Otherwise, the procedures were identical to those in Experiment 1.

Quantification and statistics. Monamines and metabolites in the perfusate were measured using high performance liquid chromatography with electrochemical detection (HPLC-EC) as described previously (16). Quantitation of compounds of interest in each HPLC-EC sample was achieved by comparing peak heights to those obtained after injection of known quantities of the compound (standard). The elution profile of a standard preparation (250 pg in 25 μl) of monoamines and their metabolites clearly resolved the peak for MOPEG, the major extracellular metabolite of noradrenaline in the brain. In addition to MOPEG, dopamine, serotonin and their metabolites (3,4 dihydroxyphenylacetic acid and 5-hydroxy indoleacetic acid) also were consistently detected by this technique, but noradrenaline was typically undetectable.

The results are expressed as means \pm SEM. The data were analyzed by analysis of variance with appropriate post hoc tests (Newman-Keuls) to determine the source of main effects and interactions (18).

Results

Experiment 1. In SHR on the basal NaCl diet, microperfusion of the AHA with ANP at a concentration of 10^{-7} M caused a fall in the extracellular concentration of MOPEG in the AHA perfusate (Fig. 1 A) and a rise in arterial pressure (Fig. 1 B). Both responses began during the initial 10 min of the microperfusion. The peak reduction in AHA MOPEG was -72 ± 12 pg/10 min ($-26 \pm 4\%$), and AHA MOPEG remained significantly reduced below baseline levels for over 20 min following the termination of the ANP perfusion. Further, the 10^{-7} M concentration of ANP elevated arterial pressure significantly above baseline levels for the duration of the experiment. Although Fig. 1 suggests that the arterial pressure response begins prior to the MOPEG response, the experimental procedure introduces a delay of about 7 min in the MOPEG response, due to the dead space in the collection tubing.

In SHR on the basal NaCl diet, microperfusion of the AHA with ANP elicited a concentration-dependent reduction of AHA MOPEG ($r^2 = 0.84$; Fig. 2). Both the amplitude and duration of the MOPEG responses were dependent on the concentration of the ANP infused. Responses to the lowest concentration of ANP (10^{-8} M) lasted only slightly longer than the perfusion, while responses to the higher concentrations

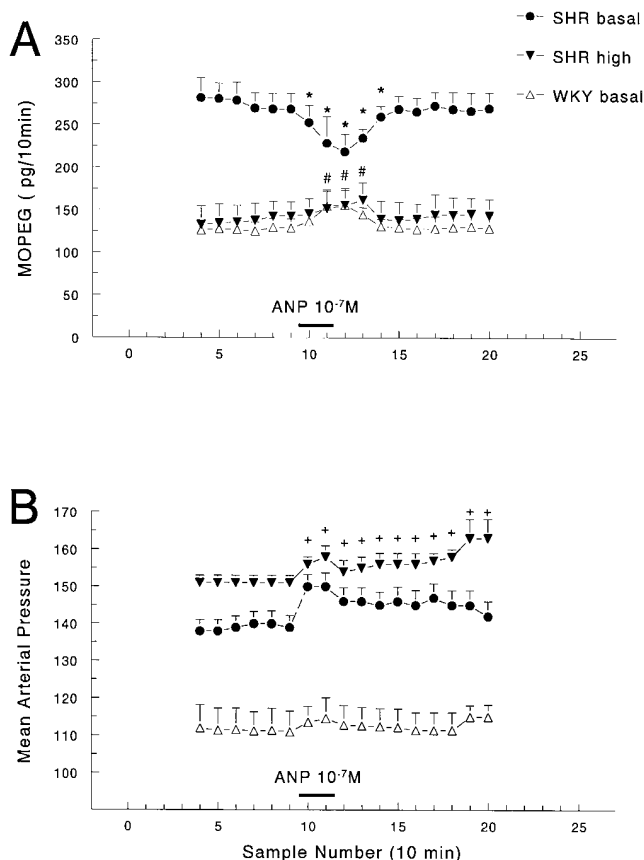


Figure 1. These line graphs demonstrate AHA MOPEG (A) and arterial pressure (B) responses to the microperfusion of the AHA with 10^{-7} M ANP in SHR on a basal or a high (for 2 wk) NaCl diet and in WKY on a basal NaCl diet. * $P < 0.05$ compared to preinfusion baseline for SHR on a basal NaCl diet. *# $P < 0.05$ compared to baseline for SHR on a high NaCl diet and WKY on a basal NaCl diet. + $P < 0.05$ compared to baseline for both SHR groups but not for WKY.

persisted for over 80 min. In contrast, arterial pressure responses to the microperfusion of the AHA with ANP were not significantly concentration-related (not shown). Because the 10^{-7} M concentration of ANP elicited consistent responses in the SHR and was in linear portion of the concentration-response curve (Fig. 2), this concentration was employed in the studies of the NaCl supplemented SHR and WKY rats in Experiment 1.

In SHR maintained on the high (compared to basal) NaCl diet for 2 wk, basal levels of MOPEG in the AHA were $\sim 50\%$ lower and arterial pressure was ~ 15 mmHg higher, as previously reported (17; Fig. 1 A). The ANP (10^{-7} M) microperfusion of the AHA elicited no significant decrease in AHA MOPEG in the SHR on the high NaCl diet, and in fact caused a slight increase in MOPEG levels ($+24 \pm 6$ pg/10 min). This contrasts with the large decrease in AHA MOPEG (-72 ± 12 pg/10 min) elicited in SHR on the basal NaCl by ANP (10^{-7} M; Fig. 1 A). The ANP microperfusion caused a smaller rise in arterial pressure in the SHR on the high ($5 \pm 1\%$) compared to basal ($10 \pm 2\%$) NaCl diet.

In WKY, the ANP microperfusion elicited a small rise ($+36 \pm 10$ pg/10 min) in extracellular MOPEG concentration in AHA (Fig. 1 A), but no change in arterial pressure (Fig. 1

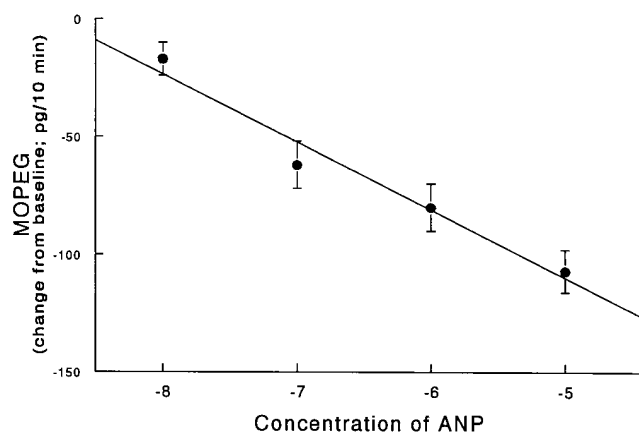


Figure 2. This line graph demonstrates that there is a linear relationship (solid line) between AHA MOPEG and the concentration of ANP microperfused through the AHA in SHR on a basal NaCl diet.

B). In both WKY on a basal NaCl diet and SHR on a high NaCl diet, none of the concentrations of ANP (10^{-8} to 10^{-5} M) elicited a decrease in extracellular MOPEG concentration in the AHA.

Control microperfusion of ACSF alone for 180 min did not alter either AHA MOPEG or arterial pressure in any of the groups tested. Further, the experiment was repeated on consecutive days in a few animals (data not shown). These animals displayed similar baselines and responses to ANP microperfusion on both days of testing.

In SHR, maintenance on the 8% (compared to the 1%) NaCl diet for 2 wk significantly elevated MAP (Fig. 1 B). WKY on the basal NaCl diet were normotensive (Fig. 1 B). Resting heart rate was similar in all three groups (SHR basal NaCl diet = 371 ± 8 bpm; SHR high NaCl diet = 378 ± 15 bpm; WKY = 397 ± 19 bpm). The SHR on the basal compared to high NaCl diet were slightly heavier (256 ± 6 g, 1% NaCl diet; 240 ± 4 g, 8% NaCl diet), and the WKY were slightly lighter than either SHR group (214 ± 6 g). The ANP infusion did not cause a significant alteration in heart rate in any group (the average response for all groups was $+17 \pm 13$ bpm), despite the ANP-induced rise in arterial pressure in the SHR (Fig. 1 B).

Experiment 2. In SHR on the basal NaCl diet, microperfusion of the AHA with c-ANP (10^{-7} M) caused a rapid decrease in the extracellular MOPEG concentration in the AHA that persisted for the duration of the experiment (Fig. 3 A). The peak reduction in MOPEG concentration in AHA perfusate was -77 ± 10 pg/10 min ($-33 \pm 4\%$; Fig. 3 A), which was comparable to the effect of perfusing exogenous ANP at the same concentration in Experiment 1. In SHR fed the basal NaCl diet, c-ANP microperfusion reduced the MOPEG concentration in the AHA perfusate to approximately the preperfusion AHA MOPEG level observed in the high NaCl fed SHR. Infusion of c-ANP was also associated with a delayed increase in arterial pressure that began more than 20 min after termination of the c-ANP microperfusion in basal NaCl-fed SHR (Fig. 3 B).

In SHR fed a high NaCl diet, c-ANP microperfusion did not significantly alter either AHA MOPEG concentration or arterial pressure (Fig. 3, A and B). In WKY, the c-ANP microperfusion elicited a small increase ($+17 \pm 10$ pg/10 min) in

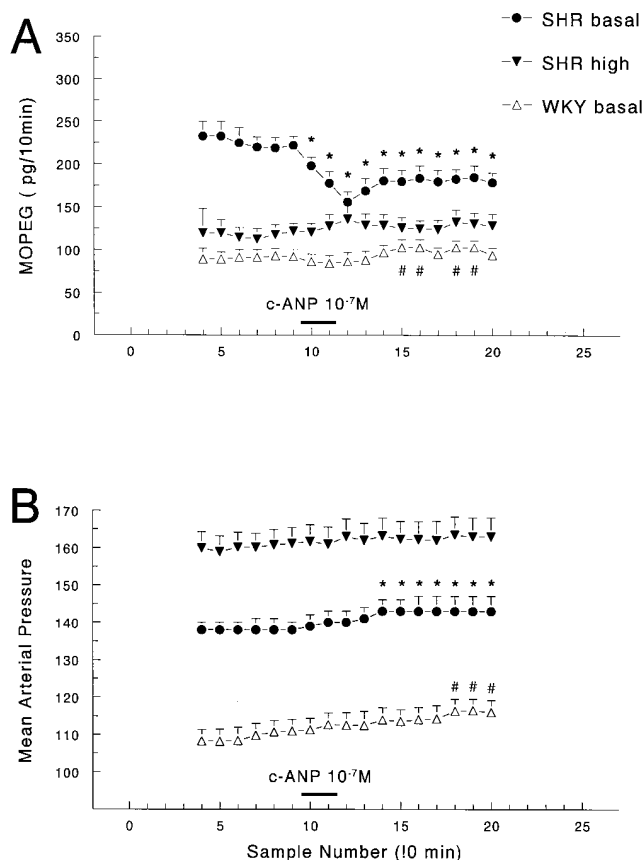


Figure 3. These line graphs demonstrate AHA MOPEG (*A*) and arterial pressure (*B*) responses to the microperfusion of the AHA with 10^{-7} M c-ANP in age-matched SHR on a basal NaCl diet, SHR on a high NaCl diet for 2 wk and WKY on a basal NaCl diet. * $P < 0.05$ compared to preinfusion baseline for SHR on a basal NaCl diet. # $P < 0.05$ compared to baseline for WKY on a basal NaCl diet.

AHA MOPEG concentration and a small elevation in arterial pressure. Both of these responses were delayed, beginning more than 20 min after termination of the c-ANP microperfusion (Fig. 3, *A* and *B*).

In SHR in Experiment 2, as in Experiment 1, maintenance on the 8% (compared to the 1%) NaCl diet for 2 wk was associated with a significant increase in arterial pressure (Fig. 3 *B*). WKY on the basal NaCl diet were normotensive (Fig. 3 *B*). Resting heart rate was similar in all three groups (SHR basal NaCl diet = 369 ± 7 bpm; high NaCl diet = 388 ± 10 bpm; WKY = 376 ± 5 bpm). SHR on the basal NaCl diet were slightly heavier than the SHR on the high NaCl diet (245 ± 3 g, 1% NaCl diet; 230 ± 3 g, 8% NaCl diet; $P < 0.05$), and the WKY were slightly lighter than either group of SHR (214 ± 4 g; $P < 0.05$).

Histological Analyses. Histological examination demonstrated that in both experiments nearly all cannula placements (54 of 60) were within the AHA. Typically, the area of the hypothalamus containing pontamine sky blue dye was centered in the AHA and had a diameter of ~ 1 mm (Fig. 4). Six cannula placements were outside of the AHA, and on that basis, these rats were eliminated from further analysis. 7 of the 54 rats with correct cannula placement were eliminated from the analysis because of inadequate push-pull microperfusion. Fol-

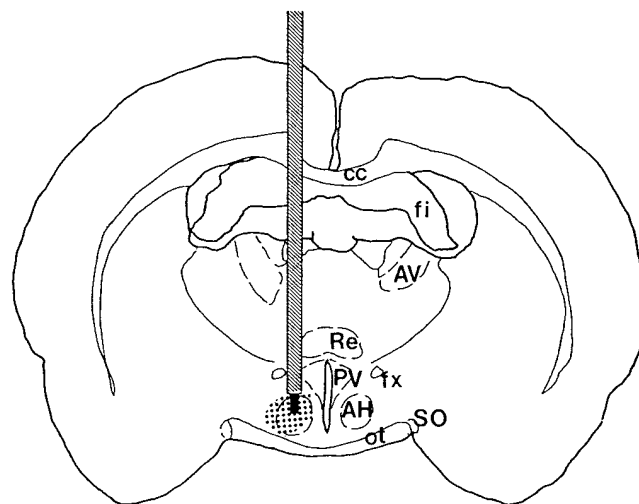


Figure 4. This camera lucida drawing of a coronal section through the anterior hypothalamus demonstrates the size and typical position of the push-pull outer (cross hatched) and inner (black) cannula and the size of the area to which the pontamine sky blue dye diffused (stippling). AV, antroventral thalamic nucleus; AH, anterior hypothalamic area; cc, corpus callosum; fi, fimbria; fx, fornix; ot, optic tract; Re, nucleus reuniens of the thalamus; PV, paraventricular nucleus of the hypothalamus; SO, supraoptic nucleus of the hypothalamus.

lowing these exclusions, at least six evaluable rats were left in each group.

Discussion

These experiments demonstrate that in SHR on a basal NaCl diet, acute local microperfusion of the AHA with ANP causes a significant, concentration-related decrease in local extracellular MOPEG concentration, reflecting reduced release of noradrenaline from nerve terminals in AHA. This effect is accompanied by increases in arterial pressure that are less obviously related to the concentration of ANP administered. These data provide the first evidence that activation of ANP receptors in AHA can modulate local release of noradrenaline from nerve terminals. Further, the present results demonstrate that microperfusion of the ANP-C receptor agonist (c-ANP) into AHA causes a similar reduction in extracellular MOPEG in the AHA. This finding suggests that the endogenous ANP concentration in AHA can be elevated by binding the ANP-C receptors with exogenously administered ligand, thereby reducing the reuptake of ANP by the ANP-C receptor and resulting in an increase in local ANP inhibition of noradrenaline release in AHA. This study provides the first solid evidence that endogenous ANP in AHA can modulate local noradrenaline release. In contrast to the data from SHR on the basal diet, microperfusion of neither exogenous ANP nor the ANP-C receptor agonist reduced AHA MOPEG concentration in SHR on a high NaCl diet or in WKY on a basal NaCl diet, suggesting that in these rats, tonic inhibition of AHA noradrenaline release by ANP was already maximal.

In the interpretation of our data two further points should be considered. First, although the effect of the microperfused c-ANP is most likely due to the direct action on the ANP-C receptor and the resulting inhibition of ANP reuptake, it is possi-

ble that c-ANP stimulates the other classes of ANP receptors in unexpected ways or that the ANP-C receptor has actions other than simple clearance of ANP. Either possibility could lead to unexpected interactions between ANP and noradrenaline release. Second, as Inagami has demonstrated, in SHR compared to WKY there is an increased responsiveness to ANP in the brain and periphery (19), and Tremblay et al., have demonstrated that in peripheral tissues of SHR compared with WKY there is an overexpression of the ANP-A receptor and a related, exaggerated second messenger activation (20). Thus, increased responsiveness and/or overexpression of an ANP receptor in the AHA may contribute importantly to the increased responsiveness of SHR on the basal NaCl diet to exogenous ANP microinfusion in the AHA. Whether the difference in responses among the SHR and WKY groups in this study are the result of altered responsiveness of ANP receptor/second messenger systems or altered extracellular content of ANP awaits further study.

The current study provides strong evidence that ANP in the AHA of SHR is an inhibitory neuromodulator of noradrenaline release. The regulation of noradrenaline release by local neuromodulators has been described in several areas of the brain. Work by Marrocco and colleagues suggests that in the primary visual cortex of the cat and monkey, noradrenaline release is tightly regulated by the activity of lateral geniculate afferent terminals that end in this area of cortex; however, the neuromodulator responsible for this interaction in the visual cortex is unknown (21). Examples of neuromodulators that appear to regulate noradrenaline release include acetylcholine and α_2 adrenergic receptor agonists (22, 23).

ANP likely regulates noradrenaline release in the hypothalamus by acting as an inhibitory neuromodulator of the presynaptic release of noradrenaline (24–29). In PC-12 cells in vitro and peripheral nerve terminals in vivo, ANP has a significant, direct inhibitory effect on the release of noradrenaline (24–26). In humans, ANP inhibits sympathetic nervous system activity (27), and the depressor effect of ANP is related to the ability of circulating ANP to modify noradrenergic neurotransmission (28). In the rat hypothalamus, ANP regulates the pressor action of angiotensin II (29) and inhibits neuronal firing (30). The effect of ANP on extracellular noradrenaline concentration in brain is likely mediated by two direct actions of ANP on noradrenergic nerve terminals: (a) inhibition of noradrenaline release from nerve terminals, as documented above, and (b) enhancement of the reuptake of noradrenaline by nerve terminals (31, 32). Further, in SHR compared to WKY, ANP concentration is increased selectively in the AHA but not in other hypothalamic nuclei (33).

Local ANP in the brain appears to play an important role in the regulation of the cardiovascular system (34). Cerebrospinal fluid ANP is increased by acute volume load in rats (35). Conversely, hemorrhage increases plasma ANP but does not alter hypothalamic ANP (36). In at least two models of hypertension (i.e., SHR and Dahl salt sensitive), there is an alteration of the ANP system in the brain (8, 34). In the SHR (compared to WKY), ANP content is significantly lower in the nucleus of the solitary tract (37) and higher in the AHA (6). Whereas microinjection of ANP into AHA elicits a hypertensive response, ANP microinjection into the nucleus of the solitary tract causes hypotension and injection of an ANP antibody into the nucleus of the solitary tract causes hypertension (7, 9). These effects in the nucleus of the solitary tract appear

to relate to the ability of ANP to increase baroreflex responsiveness in SHR (38). The decrease in ANP in the nucleus of the solitary tract in SHR leads directly to a lack of activation of baroreflex feedback to the AHA, and thereby decreases the ability of AHA to inhibit the sympathetic nervous system. ANP concentration in the hypothalamus appears to be tonically inhibited by the cardiopulmonary baroreflex (7), and elevation of plasma osmolality increases the release of ANP in the brain (35).

Under most conditions, arterial pressure and the release of noradrenaline in the AHA are directly related (39). Thus, as arterial pressure rises, noradrenaline release in the AHA increases, resulting in the activation of AHA neurons, and leading to an increased inhibition of sympathetic nervous system activity (39). In contrast, in SHR both microperfusion of the AHA with ANP and intravenous infusion of hypertonic saline cause the opposite effect, an increase in arterial pressure and a decrease in AHA MOPEG, i.e., an inverse relationship (40). It is of interest in this regard that in the SHR groups on basal compared to high NaCl diets, the ANP microperfusion elicited differential MOPEG responses (decrease in the former and increase in the latter) but increases in arterial pressure in both groups. The c-ANP microperfusions caused a rapid change in AHA MOPEG but a delayed arterial pressure increase in SHR on a basal NaCl diet. These results suggest that the immediate rise in arterial pressure following ANP infusion may be, at least in part, independent of the change in AHA MOPEG. Further experiments are needed to clarify the relationship between AHA, ANP, and arterial pressure.

The results of the current study support the hypothesis that the inhibition of noradrenaline release in the AHA by locally released ANP reduces activity of sympathoinhibitory neurons in the AHA, leading to increased sympathetic nervous system activity and higher arterial pressure in the SHR on a high compared to basal NaCl diet. The precise mechanisms that link dietary NaCl to ANP release in the AHA and the role of hypothalamic ANP in human NaCl-sensitive hypertension await further study, but together the existing data clearly point to an important role for hypothalamic ANP in this experimental model of hypertension.

Acknowledgments

This study was supported, in part, by the National Institutes of Health grants HL-37722, HL-47081, HL-07457, and a grant from the American Heart Association, Alabama Affiliate.

References

1. de Bold, A.J., H.B. Borenstein, A.T. Veress, and H. Sonnenberg. 1981. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci.* 28:94–98.
2. Brenner, B.M., B.J. Ballermann, M.E. Gunning, and M.L. Zeidel. 1990. Diverse biological actions of atrial natriuretic peptide. *Physiol. Rev.* 70:665–699.
3. Casto, R., J. Hilbig, G. Schroeder, and G. Stock. 1987. Atrial natriuretic factor inhibits central angiotensin II pressor responses. *Hypertension.* 9:473–477.
4. Ermirio, R., P. Ruggeri, C.E. Cogo, C. Molinari, M. Bergaglio, and F.R. Calaresu. 1990. The role of ANF in functional properties of the central pathways of cardiovascular reflexes. *J. Auton. Nerv. Syst.* 30:S51–54.
5. Stepniakowski, K., A. Budzikowski, S. Lon, and E. Szczepanska-Sadowska. 1994. Central cardiovascular effects of AVP and ANP in normotensive and spontaneously hypertensive rats. *J. Auton. Nerv. Syst.* 47:33–43.
6. Jin, H., R. Yang, Y.F. Chen, J.M. Wyss, and S. Oparil. 1991. Altered stores of atrial natriuretic peptide in specific brain nuclei of NaCl-sensitive spontaneously hypertensive rats. *Am. J. Hypertens.* 4:449–455.

7. Yang, R.H., H. Jin, Y.F. Chen, and J.M. Wyss. 1990. Wyss. Blockade of endogenous anterior hypothalamic atrial natriuretic peptide with monoclonal antibody lowers blood pressure in spontaneously hypertensive rats. *J. Clin. Invest.* 86: 1985–1990.
8. John, A.E., and F.J. Morich. 1990. ANP concentration of atria, plasma and brain in Dahl S (DS) and Dahl R (DR) rats. *Clin. Exp. Hypertens. Part. A Theory Pract.* 12:191–204.
9. Yang, R.H., H. Jin, J.M. Wyss, Y.F. Chen, and S. Oparil. 1992. Pressor effects of blocking atrial natriuretic peptide in nucleus tractus solitarius. *Hypertension*. 19:198–205.
10. Folkow, B., B. Johansson, and B. Oberg. 1959. A hypothalamic structure with a marked inhibitory effect on tonic sympathetic activity. *Acta Physiol. Scand.* 47:262–270.
11. Spyer, K.M. 1972. Baroreceptor sensitive neurons in the anterior hypothalamus of the rat. *J. Physiol. (Lond.)*. 224:245–257.
12. Wyss, J.M., R. Yang, H. Jin, and S. Oparil. 1988. Hypothalamic microinjection of α_1 -adrenoceptor agonists causes greater sympathoinhibition in spontaneously hypertensive rats on high NaCl diets. *J. Hypertens.* 6:805–813.
13. Chen, C.W., Y.F. Chen, Q.C. Meng, J.M. Wyss, and S. Oparil. 1991. Decreased norepinephrine release in anterior hypothalamus of NaCl-sensitive spontaneously hypertensive rats during high NaCl intake. *Brain Res.* 565:135–141.
14. Thornton, R., J.M. Wyss, and S. Oparil. 1989. Impaired reflex response to volume expansion in NaCl-sensitive spontaneously hypertensive rats. *Hypertension*. 14:518–523.
15. Howe, P.R. 1985. Blood pressure control by neurotransmitters in the medulla oblongata and spinal cord. *J. Auton. Nerv. Syst.* 12:95–115.
16. Simon, O.R., B.N. Basuray, W.L. West, and R. Copeland. 1985. Interaction between the baroreflex and anterior hypothalamic stimulation. Demonstration of a noradrenergic involvement. *Neuropharmacology*. 24:665–675.
17. Paxinos, G., and C. Watson. 1986. *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York. 25–37.
18. Winer, B.J. 1971. *Statistical Principles in Experimental Design*. 2nd ed. McGraw-Hill, New York. 261–304.
19. Takayanagi, R., R.T. Grammer, and T. Inagami. 1986. Regional increase of cyclic GMP by atrial natriuretic factor in rat brain: markedly elevated response in spontaneously hypertensive rats. *Life Sci.* 39:573–580.
20. Tremblay, J., C. Hout, R. Willenbrock, F. Gossard, N. Fujio, C. Koch, O. Kuchel, W. Debinski, and P. Hamet. 1993. Increased cyclic guanosine monophosphate production and overexpression of atrial natriuretic peptide receptor mRNA in spontaneously hypertensive rats. *J. Clin. Invest.* 92:2499–2508.
21. Marrocco, R.T., R.F. Lane, J.W. McClurkin, C.D. Blaha, and M.F. Alkire. 1987. Release of cortical catecholamines by visual stimulation requires activity in thalamocortical afferents of monkey and cat. *J. Neurosci.* 7:2756–2767.
22. Cuadra, G., K. Summers, and E. Giacobini. 1994. Cholinesterase inhibitor effects on neurotransmitters in rat cortex in vivo. *J. Pharmacol. Exp. Ther.* 27:277–284.
23. Zsilla, G., T. Zelles, A. Mike, A. Kekes-Szabo, E. Milusheva, and E.S. Vizi. 1994. Differential changes in presynaptic modulation of transmitter release during aging. *Int. J. Dev. Neurosci.* 12:107–115.
24. Nakamaru, M., and T. Inagami. 1986. Atrial natriuretic factor inhibits norepinephrine release evoked by sympathetic nerve stimulation. *Eur. J. Pharmacol.* 123:459–461.
25. Drewett, J.G., G.J. Trachte, and G.R. Marchand. 1989. Atrial natriuretic factor inhibits adrenergic and purinergic neurotransmission in the rabbit isolated vas deferens. *J. Pharmacol. Exp. Ther.* 248:135–142.
26. Holtz, J., T. Muntzel, O. Sommer, and E. Bassenge. 1989. Sympathoadrenal inhibition by atrial natriuretic peptide during the development of congestive heart failure. *Circulation*. 80:1862–1869.
27. Floras, J.S. 1995. Inhibitory effect of atrial natriuretic factor on sympathetic ganglionic neurotransmission in humans. *Am. J. Physiol.* 269:R406–412.
28. Lang, C.C., A.M. Choy, D.J. Balfour, and A.D. Struthers. 1992. Prazosin attenuates the natriuretic response to atrial natriuretic factor in man. *Kidney Int.* 42:433–441.
29. Shibata, K., M. Sakimura, and T. Furukawa. 1993. Antagonism of central pressor response to angiotensin II by α -human atrial natriuretic polypeptide at the preoptic area and posterior hypothalamus in rats. *Neuropharmacology*. 32:175–184.
30. Yamashita, H., and H. Kannan. 1992. Inhibition of hypothalamic neurons by the atrial natriuretic peptide family. *News Physiol. Sci.* 7:75–79.
31. Fernandez, B.E., M.S. Vatta, and L.G. Bianciotti. 1993. Comparative effects of bradykinin and atrial natriuretic factor on neuronal and non-neuronal noradrenaline uptake in the central nervous system of the rat. *Arch. Int. Physiol. Biochim.* 10:337–340.
32. Vatta, M., M. Travagianti, L. Banciotti, C. Coll, J. Perazzo, and B. Fernandez. 1994. Atrial natriuretic factor effects on norepinephrine uptake in discrete telencephalic and diencephalic nuclei of the rat. *Brain Res.* 646:324–326.
33. Jin, H., R. Yang, Y.F. Chen, J.M. Wyss, and S. Oparil. 1991. Altered stores of atrial natriuretic peptide in specific brain nuclei of NaCl-sensitive spontaneously hypertensive rats. *Am. J. Hypertens.* 4:449–455.
34. Oparil, S., and J.M. Wyss. 1993. Atrial natriuretic factor in central cardiovascular control. *News Physiol. Sci.* 8:223–228.
35. Nakamura, K., S. Nakamura, A. Hojyo, and K. Nakanishi. 1992. Effect of saline loading on ANP in the cerebrospinal fluid of rats. *Nippon Jinzo Gak-kai Shi.* 34:27–31.
36. Phillips, M.I., B. Kimura, H. Wang, and W.E. Hoffman. 1989. Effect of vagotomy on brain and plasma atrial natriuretic peptide during hemorrhage. *Am. J. Physiol.* 257:R1393–R1399.
37. Jin, H., Y.F. Chen, R.H. Yang, and Q.C. Meng. 1988. Impaired release of atrial natriuretic factor in NaCl-loaded spontaneously hypertensive rats. *Hypertension*. 11:739–744.
38. Jin, H., R.H. Yang, D.A. Calhoun, J.M. Wyss, and S. Oparil. 1992. Atrial natriuretic peptide modulates baroreflex in spontaneously hypertensive rat. *Hypertension*. 20:374–379.
39. Peng, N., Q.C. Meng, K. King, S. Oparil, and J.M. Wyss. 1995. Acute hypertension increases noradrenaline release in the anterior hypothalamic area. *Hypertension*. 25:828–833.
40. Peng, N., C. Meng, S. Oparil, and J.M. Wyss. 1996. Acute saline infusion decreases noradrenaline release in the anterior hypothalamic area. *Hypertension*. 27:578–583.