Clonidine and Morphine Increase Atrial Natriuretic Peptide Secretion in Anesthetized Rats (42924)

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Abstract. **In order to determine whether the activity of central α_2-adrenergic and opioid receptors influence plasma atrial natriuretic peptide (ANP) levels, clonidine and morphine were infused into the lateral cerebral ventricle for 45 min in anesthetized Sprague-Dawley rats. The central administration of a low dose of clonidine (10 ng/min) caused a significant increase in plasma ANP without changing arterial blood pressure or central venous pressure. Pretreatment with yohimbine (5 μg/min) completely blocked the effect of clonidine. Central infusion of morphine (100 ng/min) also elevated plasma ANP levels and naloxone (5 μg/min) blunted this effect. Intravenous infusion of the same dose of clonidine or morphine did not affect plasma ANP levels. Moreover, the effect of clonidine on plasma ANP was partially blocked by pretreatment with naloxone (5 μg/min). These results suggest that central α_2-adrenergic and opioid receptors may be involved in ANP secretion.** [P.S.E.B.M. 1989, Vol 191]

Atrial natriuretic peptides (ANP) which are released from secretory granules located in atria have been shown to induce natriuresis and diuresis and to inhibit contraction of vascular smooth muscle and secretion of aldosterone and renin (1). The major stimulus for ANP release appears to be direct atrial stretch; stimuli which cause an increase in atrial pressure have been reported to increase the release of ANP (2, 3). However, there is also evidence that suggests neuronal influences on ANP release (4–7).

Clonidine, an antihypertensive agent which acts primarily on central α_2-adrenergic receptors to inhibit sympathetic tone (8), causes diuresis and natriuresis (9, 10). The natriuretic effect of clonidine cannot be explained simply by its inhibitory effect on antidiuretic hormone secretion (11). Administration of a high dose of clonidine causes an increase in plasma ANP in hydrated rats, which suggests that clonidine may affect renal function by modulation of ANP release (4). Moreover, it has also been shown that morphine causes a diuresis and natriuresis (12) and increases plasma ANP concentrations in rats (13). These results imply a possibility of neuronal and hormonal factors regulating ANP secretion.

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Since there is some evidence of a relatinoship between the α_2-adrenergic and endogenous opiate systems in the CNS (14–16), it is possible that there is an interaction between the α_2-adrenergic system and endogenous opiate system regarding the effect on ANP secretion. This study was designed to determine whether intracerebroventricular administration of clonidine and morphine influences plasma ANP concentration in anesthetized rats.

Materials and Methods

Animal Preparation. Male Sprague-Dawley rats, weighing 200–300 g, received a diet of standard laboratory Purina chow and tap water *ad libitum* until the day of the experiment. The rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally. For cannula placement, the rat was placed in a stereotaxic apparatus (Kopf). A hole was made in the skull at 0.6-mm anterior and 1.4-mm lateral to the bregma. The cannula was lowered into the cerebroventricle while infusing artificial cerebrospinal fluid (ACSF) and recording pressure. Pressure rose as the brain tissue was pierced and fell precipitously as the cannula passed into the cerebroventricle (ACSF flowed into the cerebroventricle). The cannula was fixed to the skull in this position with dental acrylic. At the end of the experiment, its position was checked by injecting methylene blue through the cannula and oberving blue staining of the ventricular surface. An infusion pump was connected with the cannula via PE-50 tubing in order to

infuse drugs intracerebroventricularly. The femoral artery, femoral vein, and external jugular vein were catheterized with PE-50 tubing in order to monitor mean blood pressure (MBP), central venous pressure (CVP), and to serve as an intravenous infusion route, respectively. MBP and CVP were recorded continuously on a Grass polygraph (model 79B). Average values for MBP and CVP were calculated for each 15-min period. Seven observations were taken at 2-min intervals to provide a mean value. Mean values from three 15-min control periods were averaged to provide a single control value.

Experimental Protocols. Rats were randomly divided into 12 groups. Fifteen minutes after completion of the surgical procedures, rats of all groups were infused intracerebroventricularly with ACSF for 45 min using an infusion pump. The intracerebroventricular infusion rate was 1.25 μl/min. After a 45-min equilibration period, one of the following protocols was performed.

Protocol 1. Rats in Group 1 (control group) were infused with ACSF intracerebroventricular for 45 min. Group 2 rats received a 45-min intracerebroventricular infusion of clonidine at a dose of 10 ng/min. Group 3 was infused intravenously with the same dose of clonidine as Group 2 for 45 min. In Group 4, rats received a 10-min intracerebroventricular infusion of yohimbine (5 μg/min) followed by 45-min intracerebroventricular infusion of yohimbine (5 μg/min) plus clonidine (10 ng/min). Group 5 received a 10-min intracerebroventricular infusion of naloxone (5 μg/min) followed by naloxone (5 μg/min) plus clonidine (10 ng/min) intracerebroventricularly for 45 min.

Protocol 2. A second control group (Group 6) was used for comparing this protocol. Groups 7 and 8 were infused with morphine (100 ng/min) intracerebroventricularly and intravenously for 45 min, respectively. Group 9 received an intracerebroventricular infusion of naloxone, at a dose of 5 μg/min, for 5 min followed by a 45-min infusion of naloxone (5 μg/min) plus morphine (100 ng/min). CVP was not measured in these groups. At the end of the infusion, rats were decapitated to collect blood samples for plasma immunoreactive ANP analysis.

Protocol 3. After the equilibration period, three groups of rats received a 45-min icv infusion of ACSF, yohimbine (5 μg/min), and naloxone (5 μg/min), respectively. At 15 min after the beginning of intracerebroventricular administrations, these three groups were infused intravenously with isooncotic bovine albumin dissolved in physiologic saline solution (25% of estimated blood volume) over 15 min, as described by Veress and Sonnenberg (17). Fifteen minutes after the volume load, the rats were decapitated to collect blood samples for plasma immunoreactive ANP analysis.

ANP Assay. Each blood sample was collected in a precooled glass tube containing 7.2 mg of EDTA, immediately centrifugated at 4°C, and then stored at −70°C until analysis. Plasma samples were extracted through C_{18} octadecylsilane cartridges (Sep-Pak; Waters Associates, Milford, MA). Plasma ANP concentrations were measured by radioimmunoassay (18).

Drugs. The drugs used were clonidine HCl, yohimbine HCl, morphine, and naloxone HCl (Sigma). ACSF was prepared by the Pharmacy Laboratory, University of Michigan Medical Center. Drugs were freshly made before each experiment and were dissolved in ACSF except yohimbine which was dissolved in 0.1% sodium metabisulphite solution in ACSF.

Statistics. Values are expressed as means ± SE. Comparisons were made using Student's *t* test for nonpaired data. The data from BP and CVP measurements were compared with basal data using analysis of variance for repeated measures.

Results

Figure 1 illustrates that the central administration of clonidine caused a significant increase in plasma ANP compared with the control group, but an intravenous infusion of the same dose of clonidine failed to alter plasma ANP values. Pretreatment with yohimbine completely blocked the effect of clonidine on ANP. The mean ANP value for the naloxone plus clonidine group was intermediate between those of the control and clonidine intracerebroventricular groups and was not significantly different from either.

Figure 2 shows the results obtained with infusion of morphine. The intracerebroventricular infusion of morphine increased plasma ANP levels significantly with a dose which was ineffective when given intravenously. Administration of naloxone intracerebroventricularly prevented the stimulatory effect of morphine on plasma ANP.

Volume expansion markedly elevated the plasma ANP level. Neither yohimbine intracerebroventricu-

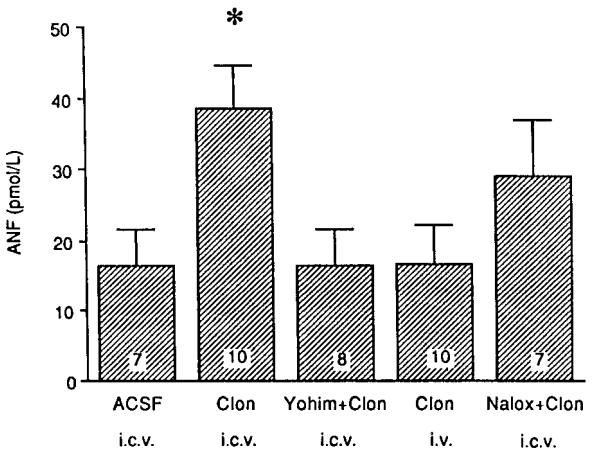

Figure 1. Effects of intracerebroventricular (icv) and intravenous (iv) clonidine and pretreatment with yohimbine or naloxone on plasma ANP. *$P < 0.05$, compared with control group. Numbers within columns indicate number of animals. Clon, clonidine; Yohim, yohimbine; Nalox, naloxone.

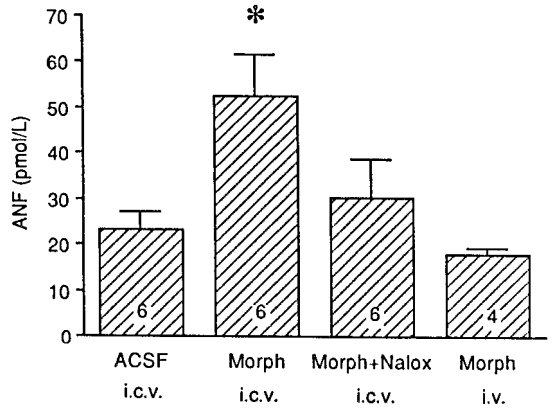

Figure 2. Effects of intracerebroventricular (icv) and intravenous (iv) morphine and pretreatment with naloxone on plasma ANP. *$P < 0.05$, compared with control group. Numbers within columns indicate number of animals. Morph, morphine.

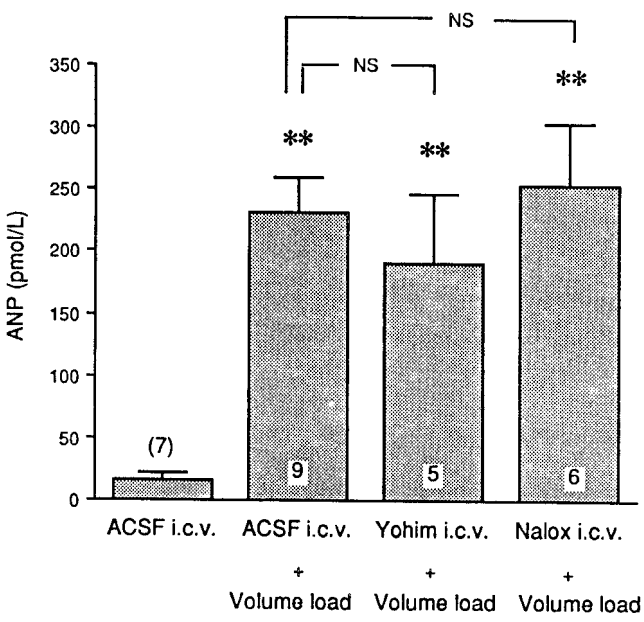

Figure 3. Effects of volume load and pretreatment with yohimbine or naloxone on plasma ANP. **$P < 0.01$, compared with control group. Numbers indicate number of animals used for each group.

larly nor naloxone intracerebroventricularly attenuated the rise in plasma ANP levels associated with volume expansion (Fig. 3).

For all groups, MBP did not change significantly during the experimental periods. Infusions of clonidine intracerebroventricularly or intravenously did not alter CVP. Volume expansion significantly increased CVP in the groups with or without yohimbine intracerebroventricularly (Table I).

Discussion

This study demonstrates that the central administration of clonidine caused an increase in plasma ANP levels in anesthetized rats. The increased plasma ANP was not attributable to any change in arterial blood pressure or central venous pressure.

Although clonidine is efficacious at both presynaptic and postsynaptic receptor sites, it is generally thought to be more potent as a presynaptic adrenoceptor agonist (19). Clonidine acts on central α_2-adrenergic receptors at various levels of the brain to diminish the sympathetic outflow from the CNS, which translates into decreasing arterial blood pressure (8). Administration of the selective α_2-adrenoceptor antagonist yohimbine reduces significantly the hypotensive effect of clonidine (20). In the present study, the effect of clonidine on plasma ANP appears to result from the activation of central α_2-adrenergic receptors, as it could be blocked by pretreatment with yohimbine.

There is accumulating evidence indicating an influence of the autonomic nervous system on ANP secretion. Since there are abundant catecholamine-containing vesicles in myocardium, it is possible that a change of the sympathetic nerve activity could affect ANP secretion. A study by Petterson *et al.* (6) showed that sympathetic denervation abolished the ANP release caused by volume expansion (6). *In vitro*, α- and β-adrenergic agonists stimulated ANP release from the isolated perfused rat heart or isolated rat atria (7, 21, 22). Volpe *et al.* (23, 24) demonstrated that carotid

Table I. CVP in Control and Treated Rats

Group	*n*	Control	CVP (CmH_2O)		
			Experimental period (min)		
			0–15	15–30	30–45
ACSF intracerebroventricularly	5	0.2 ± 0.7	0.3 ± 0.5	0.3 ± 0.5	0.8 ± 0.8
Clonidine intracerebroventricularly	5	0.4 ± 0.4	0.4 ± 0.5	0.4 ± 0.6	0.4 ± 0.6
Clonidine + yohimbine intracerebroventricularly	6	0.1 ± 0.5	0.8 ± 0.5	1.0 ± 0.4	0.3 ± 0.7
Clonidine intravenously	7	0.5 ± 0.6	0.6 ± 0.6	0.7 ± 0.6	0.4 ± 0.7
ACSF intracerebroventricularly + volume load	5	0.2 ± 1.4	2.4 ± 1.3	3.6 ± 0.7[a]	2.7 ± 1.5[a]
Yohimbine intracerebroventricularly + volume load	5	0.9 ± 0.6	2.0 ± 0.9	4.1 ± 0.8[a]	2.3 ± 0.6

[a] $P < 0.05$ compared with control.

baroreceptor unloading reduced ANP secretion independently of changes in atrial pressure. Furthermore, baroreceptor denervation decreased basal ANP levels as well as osmotically induced ANP release (25). However, a study by Arjamaa and Vuolteenaho (26) showed that norepinephrine did not change ANP release from statically incubated rat atria. The seemingly controversial results may be due to use of different techniques. In our experiments, the central infusion of a low dose of clonidine increased plasma ANP levels without changing blood pressure or central venous pressure, which suggests a direct role of the sympathetic nervous system in the modulation of ANP release. These results are consistent with the study of Baranowska *et al.* (4) in which the intravenous injection of a high dose of clonidine (50 μg) increased plasma ANP levels in hydrated rats.

These results also show that the central administration of morphine significantly elevated plasma ANP levels and that naloxone blunted this effect. There is evidence that α_2-adrenergic and opiate receptors are colocalized in some cerebral areas (27, 28). The finding of Mastrianni and Ingenito (14) indicates that the hypotensive action of clonidine is caused by α_2-adrenergic stimulation of brain, causing β-endorphine release and central opiate receptor activation. On the other hand, the sympathoinhibitory actions of methionine enkphalin can be enhanced by clonidine (15, 16). Our data show that the effect of clonidine on increasing plasma ANP was attenuated by the morphine antagonist naloxone. It is possible that the effect of clonidine on ANP release may be partially mediated by activity of opioid receptors. The results also suggest that the diuresis and hypotensive actions of clonidine and morphine may be secondary to the actions of ANP.

In this study, plasma ANP values in volume loaded rats were increased more than 10-fold compared with control values. Infusion intracerebroventricularly of yohimbine or naloxone did not reduce the response of ANP to volume load (Fig. 3). The results seem to be consistent with the finding of Ledsome *et al.* (29) that ANP secretion in response to atrial stretch does not depend on neural influences. However, since atrial stretch is a major stimulus for ANP release (2, 3), the strong stimulus applied in this study may have overwhelmed any physiologically significant modulation by central mechanisms.

Central infusions of clonidine and morphine increased plasma ANP levels without changing arterial blood pressure or central venous pressure. Central administration of α_2-adrenoreceptor antagonist and opioid receptor antagonist blocked these effects. The doses of clonidine and morphine were too low to affect ANP secretion when given intravenously. These results support the hypothesis that the activities of central α_2-adrenergic and opioid receptors may be involved in ANP secretion.

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