Effects of Natriuretic Peptides on the Centrally Mediated Pressor Response to Clonidine in Freely Moving Rats

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ABSTRACT—Effects of intracerebroventricular (i.c.v.) treatment with atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) on the pressor response to i.c.v.-injected clonidine were investigated in conscious rats. I.c.v.-pretreatment with ANP (10 μg) or BNP (10 μg) inhibited the pressor response to i.c.v.-injected clonidine (10 μg). Systemic (i.v.) treatment with ANP and BNP had no such effect. These results suggest that natriuretic peptides in the brain modulate the centrally mediated pressor response to clonidine.

Intracerebroventricular (i.c.v.) injection of clonidine, an α2-adrenoceptor agonist and an antihypertensive drug, causes a pressor response in conscious rats, but not in anesthetized rats (1, 2). Central pretreatment with yohimbine, an α2-adrenoceptor antagonist, and a reduction in body fluid by water deprivation for 48 hr abolished the pressor response to i.c.v.-injected clonidine, suggesting that the pressor response is mediated by central α2-adrenoceptors (1, 2) and is influenced by body fluid volumes (3). However, the precise mechanism of this pressor response remains unknown.

Recently, the clonidine-induced pressor response has been shown to be widely distributed in the central nervous system, especially in the hypothalamus and medulla oblongata that are involved in cardiovascular regulation (7). Therefore, in the present study, we investigated the effects of ANP and BNP on the pressor response to i.c.v.-injected clonidine in conscious rats.

Male Wistar rats, weighing 300–350 g, were anesthetized with pentobarbital-Na (50 mg/kg, i.p.). Stainless-steel guide cannulas and a bipolar electrode were chronically implanted into the lateral cerebroventricle and above the frontal cortex, respectively, as described previously (1). Ten days after surgery, the animals were again anesthetized with ether, and thin polyethylene catheters were chronically implanted into the abdominal aorta via the left femoral artery and into the inferior vena cava via the left femoral vein as described previously (1). A recovery period of 1 week after surgery was allowed before starting the experiment. The arterial blood pressure was measured via the arterial catheter connected to a pressure transducer (P23ID, Statham). The
arterial blood pressure, heart rate (HR) triggered by arterial pulses, and electroencephalogram (EEG) were measured simultaneously while behavior was observed, and they were recorded on a polygraph (RM-6000, Nihon Kohden). Clonidine was injected i.c.v. in a volume of 5 μl via a stainless-steel injection cannula through the right guide cannula. ANP, BNP or an equivalent amount of vehicle (0.9% saline) was injected i.c.v. in a volume of 10 μl via an injection cannula through the left guide cannula. This was done 10 min before i.c.v.-injection of clonidine. There was no significant changes in MBP and HR after i.c.v.-injection of the vehicle. The i.v.-administration of ANP, BNP or vehicle was performed via the venous catheter 10 min before i.c.v.-injection of clonidine. All values are expressed as means ± S.E.M. Statistical analysis was performed using the unpaired Student’s t-test and one-way analysis of variance; A P value less than 0.05 was considered statistically significant. Clonidine HCl (Boehringer Sohn), α-rat ANP (Peptide Institute), and porcine BNP (Peptide Institute) were dissolved in sterile saline.

In conscious rats, i.c.v.-injected clonidine (10 μg) produced a long-lasting pressor response concomitant with a decrease in HR (Fig. 1). No depressor response was observed for 60 min after clonidine injection (Fig. 1). I.c.v.-injection of 10 μg ANP or 10 μg BNP did not cause behavioral changes and slightly increased MBP and HR. After i.c.v.-injection, the basal MBP and HR before i.c.v.-injection of clonidine were 99 ± 3 mmHg and 313 ± 10 beats/min (control), 102 ± 9 mmHg and 324 ± 13 beats/min (10 μg ANP), and 104 ± 2 mmHg and 318 ± 12 beats/min (10 μg BNP), respectively. There was no significant differences in the basal MBP and HR between the control and ANP or BNP. As shown in Fig. 1 and Fig. 2, i.c.v.-treatment with 10 μg ANP or 10 μg BNP significantly inhibited the clonidine-induced pressor response. The degree of magnitude of inhibition was greater in

![Figure 1](image_url)
BNP treatment than in ANP treatment. However, both ANP and BNP at the low dose of 5 μg had little effect on the clonidine-induced pressor response (data not shown). Systemic (i.v.) treatment with 10 μg BNP or 10 μg ANP did not affect the pressor response to i.c.v.-injected clonidine (Fig. 2). This indicates that the interaction occurred within the brain. In the animals treated with 10 μg BNP or 10 μg ANP, clonidine caused a long-lasting depressor response. Since the depressor response to clonidine is well-known to be produced by activation of α2-adrenoceptors in the brain stem (8), it is unlikely that the inhibitory effect of ANP and BNP on the clonidine-induced pressor response is due to antagonism of central α2-adrenoceptors. The decrease in heart rate for 30 min after clonidine injection was not significantly altered by ANP or BNP treatment. This suggests that the mechanism of the pressor response is different from that of the clonidine-induced bradycardia and depressor response.

We have reported that water deprivation abolishes the pressor response to i.c.v.-injected clonidine (3). Since water deprivation leads to loss of brain arginine-vasopressin (AVP) (9), endogenous brain AVP may play a role in this response. I.c.v.-injected ANP as well as BNP has been shown to inhibit AVP release (10, 11). Furthermore, specific binding sites for ANP have been observed in the paraventricular nuclei (PVN) of the hypothalamus (10), which contains AVP-containing magnocellular cells (12), and in the nucleus tractus solitarius (NTS) that are involved in cardiovascular regulation. Electrical stimulation of the PVN causes a pressor response and increases the AVP content in the NTS region, but not in the circulation (13). Microinjection of norepinephrine into the PVN produces a pressor response through activation of α2-adrenoceptors (14). The preliminary study showed that i.c.v.-treatment with an AVP V1-receptor antagonist markedly inhibits the pressor response to i.c.v.-injected clonidine (15). Taken together, these results suggest that extra-hypothalamic AVP may participate in the pressor mechanism mediated by central α2-adrenoceptors and that both ANP and BNP inhibit the clonidine-induced pressor response, probably by decreasing the release of extra-hypothalamic AVP. The inhibitory effect of BNP on AVP release has been shown to be a similar magnitude to that of ANP (11). Therefore, it is of interest that BNP has a more potent inhibitory effect on the clonidine-induced pressor response than ANP. Further studies are needed to clarify the difference between the effect of ANP and BNP.

In conclusion, the present results suggest that natriuretic peptides in the brain modulate the pressor response mediated by central α2-adrenoceptors.
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REFERENCES


