Evidence for the Implication of Endogenous Vasopressin in Cardiovascular Response to Central α-adrenoceptor Stimulation

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To determine the role of endogenous vasopressin (AVP) in cardiovascular response to central α-adrenoceptor stimulation, α₁-agonist methoxamine or α₂-agonist clonidine was administered intracerebroventricularly (ICV) to conscious Long-Evans (LE) rats as well as Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI). In LE rats, ICV methoxamine increased blood pressure (BP) and decreased heart rate (HR), while ICV clonidine caused initial hypertension associated with bradycardia followed by prolonged hypotension with tachycardia. In DI rats, however, ICV methoxamine had no detectable effect on BP and HR, whereas ICV clonidine produced greater hypotension than in LE rats together with less initial bradycardia. Plasma levels of AVP increased 5-15 fold by methoxamine but did not change by clonidine. The intravenous (IV) but not ICV pretreatment with AVP vascular receptor antagonist d(C₈)₅ Tyr(Me) AVP significantly attenuated the cardiovascular effects of methoxamine in LE rat, while neither IV nor ICV pretreatment with AVP antagonist modulated the cardiovascular effects of clonidine. These results provide the evidence for the implication of endogenous AVP in the cardiovascular response to central stimulation of α-adrenoceptors.

(RECENT) neuroanatomical studies have demonstrated extensive and reciprocal pathways between the hypothalamic vasopressinergic and the medullary catecholaminergic systems in the brain. The hypothalamic paraventricular (PVN) and supraoptic nuclei (SON), where vasopressin (AVP) is synthesized, receive dense noradrenergic nerve terminals arising from medullary cardiovascular centers including the nucleus tractus solitarius (NTS), the locus coeruleus (LC) and the cell groups of A1, A5 and A7. Moreover, adrenergic nerve endings have also been found in the PVN and SON. Recently, descending vasopressinergic neural projections from the PVN and SON have been reported to terminate in medullary regions involved in cardiovascular regulation such as the NTS, the dorsal motor nucleus of vagus (DVN), the anteroventral region of the third ventricle (AV3V) and so on.

These anatomical findings strongly suggest a functional interaction between the catecholaminergic and the vasopressinergic systems in the central regulation of cardiovascular function. In the present study, therefore, we have examined the cardiovascular responses to central stimulation of α-adrenoceptors in conscious Long-Evans (LE) rats and compared them to those in Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI). For the further determination of the role of AVP, we have not only measured plasma AVP levels in LE rats but also made use of a specific antagonist to the pressor effect of AVP.

Key Words:
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Japanese Circulation Journal Vol. 30, November 1986 1149
LE Rat

![Graph showing cardiovascular effects of intracerebroventricular administration of two substances, methoxamine and clonidine, in LE rats.](image)

Fig. 1 Cardiovascular effects of intracerebroventricular administration of $\alpha_1$-agonist methoxamine ($10 \mu g/kg$, filled circles) and $\alpha_2$-agonist clonidine ($30 \mu g/kg$, open circles) in conscious Long-Evans (LE) rats. $n = \text{Number of animals. } \Delta \text{MAP} = \text{Changes in mean arterial pressure. } \Delta \text{HR} = \text{Changes in heart rate.}$

**METHODS**

Male homozygous DI rats and LE rats weighing 240-380g were used. Lateral ventricular cannulae (PE 20 polyethylene tubing) were implanted under alphaxalone anesthesia (Glaxo, 9 mg/kg) with co-ordinates of 1.0 mm posterior, 1.5 mm lateral and 5.0 mm deep with respect to bregma. Seven days after ventricular cannulation, the femoral artery and vein were catheterized using polyethylene tubing under ether anesthesia. The catheters were tunneled subcutaneously and exteriorized on the neck. Rats were allowed at least 24 hr to recover from the surgery.

To avoid the effect of anesthesia on hormonal and cardiovascular functions, all experiments were carried out in fully conscious and unrestrained rats. Blood pressure (BP) and pulse period were monitored continuously during the experiment and heart rate (HR) was calculated from the measured pulse period.

**Experiments were performed as follows:**

The dose-response relationships for the cardiovascular responses to intracerebroventricular (ICV) administration of $\alpha_1$-agonist methoxamine (Wellcome) and $\alpha_2$-agonist clonidine (Boehringer Ingelheim) were examined in LE and DI rats with a dose range of 3-30 $\mu g/kg$.

The specific antagonist to the pressor effect of vasopressin ($V_1$-antagonist), $\text{d (CH}_2\text{)}_6\text{Tyr (Me)}$ AVP (Peninsula Labs) was given either intravenously (IV) (10 $\mu g/kg$) or ICV (300 ng/kg) to LE rats, and cardiovascular effects of $\alpha_1$- or $\alpha_2$-agonist were examined 10 min after injection of vasopressin antagonist. Trunk blood of LE rat was collected by decapitation 10 min after ICV $\alpha_1$-agonist or 30 min after $\alpha_2$-agonist, and plasma level of AVP was measured according to the method described previously.

All the values reported are mean ± SEM. Statistical analysis was performed by one- or two-way analysis of variance for repeated measures.

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RESULTS

In LE rats, ICV $\alpha_1$-agonist methoxamine produced an increase in BP associated with a decrease in HR, while ICV $\alpha_2$-agonist clonidine caused a biphasic cardiovascular response, i.e. an initial increase in BP with bradycardia followed by a prolonged decrease in BP with tachycardia (Fig.1). All of these responses were dose-dependent within a dose rage of 3-30 $\mu$g/kg.

In contrast to LE rats, ICV methoxamine had no detectable effect on BP and HR in DI rats (Fig.2). ICV clonidine, however, caused a monophasic hypotensive response, which was significantly greater than in LE rats ($p < 0.001$), and slight initial bradycardia followed by tachycardia (Fig.2). The bradycardic response evoked by clonidine was significantly less in DI rats than in LE rats ($p < 0.05$).

ICV administration of $\alpha_1$-agonist methoxamine (10 $\mu$g/kg) increased plasma levels of AVP from 2.6 ± 0.4 to 22.4 ± 3.5 pg/ml ($p < 0.01$), whereas ICV $\alpha_2$-agonist clonidine with a dose up to 30 $\mu$g/kg had no effect on plasma AVP levels (Fig.3).

For the further evaluation of a role of endogenous AVP for the cardiovascular effects of ICV $\alpha$-agonists, LE rats were pretreated with a specific antagonist to the pressor effect of AVP. The AVP $V_1$-antagonist d(CH$_2$)$_2$Tyr(Me) AVP administered either IV or ICV did not affect the basal BP or HR in LE rats. IV pretreatment with AVP antagonist significantly, but not completely, attenuated the hypertensive and bradycardic effects of ICV methoxamine (10 $\mu$g/kg; $\Delta$MAP, 19.4 ± 2.3 to 9.8 ± 1.4 mmHg, $p < 0.01$; $\Delta$HR, −39.8 ± 4.1 to −11.8 ± 2.0 beats/min, $p < 0.01$, n = 5), while ICV pretreatment with AVP antagonist did not alter the cardiovascular effect of ICV methoxamine (Fig.4). Neither IV nor ICV pretreatment with AVP antagonist affected cardiovascular responses to ICV clonidine in LE rats.
DISCUSSION

In the present study, ICV administration of α₁-agonist methoxamine evoked the hypertensive and bradycardic responses in LE rats but had no detectable effect on BP and HR in DI rats. In contrast to α₁-agonist, ICV administration of α₂-agonist clonidine produced a greater decrease in BP with less initial bradycardic response in DI rats than in LE rats. These findings provide the evidence for significant contribution of endogenous AVP to the cardiovascular effects of central α-adrenoceptor stimulation.

ICV α₁-agonist methoxamine increased plasma levels of AVP 5-15 fold in LE rats. Furthermore, IV pretreatment with a specific antagonist to AVP V₁ receptors significantly but not completely attenuated the hypertensive and bradycardic responses to ICV methoxamine in LE rats. These results suggest that the cardiovascular effect of α₁-agonist is partly mediated through the increased levels of circulating AVP. We have previously reported an important role of plasma AVP to the maintenance of BP during dehydration and hemorrhage.

However, other mechanisms than circulating AVP may also be involved in the cardiovascular response to ICV methoxamine because of incomplete attenuation of the response by IV pretreatment with AVP antagonist in LE rats and (2) lack of the response in DI rats. This contention is further supported by our previous findings that IV supplemental infusion of AVP to DI rats could not restore the cardiovascular response to ICV another α₁-agonist phenylephrine. In spite of a potent vasoconstrictor effect of AVP in vitro the pressor effect of this peptide is moderate in vivo because the autonomic nervous system blunts the pressor effect through the strong buffering action of baroreflex mechanisms. Plasma levels of AVP attained after ICV methoxamine in the present study, therefore, are not in the pressor range in normal rats or dogs. This suggests that baroreflex mechanisms may be blunted following ICV α₁-agonist in normal LE rats, as has been suggested by Huchet et al. and that central AVP may mediate or modulate baroreflex mechanisms. Although ICV pretreatment with AVP antagonist in LE rats had no modulatory effect on the

Fig. 4 Modulatory effects of intracerebroventricular (ICV) and intravenous (IV) pretreatment with vasopressin antagonist (VP-ANT) on the cardiovascular response to ICV methoxamine (10 μg/kg). Otherwise, the same as in Figs 1 and 3.

Japanese Circulation Journal Vol. 30, November 1986
cardiovascular response to ICV methoxamine, the implication of a central vasopressinergic mechanism is probable if the central site of action for AVP was difficult to access from lateral ventricle, or if the subtype of AVP receptor in the central nervous system was different from that in the periphery.

ICV $\alpha_2$-agonist clonidine produced initial hypertension with bradycardia in LE rats but not in DI rats. The centrally mediated hypertensive effect of clonidine has been suggested by several previous investigations. In LE rats, however, the time course of initial responses to ICV clonidine was similar to that observed after ICV methoxamine. Since the dose of clonidine applied in the present study was rather high, ICV clonidine may exert an $\alpha_1$-agonistic property in the central. The initial cardiovascular effect of ICV clonidine in LE rats, therefore, may be evoked by the central $\alpha_1$-adrenoceptor stimulation.

Plasma levels of AVP did not change after ICV clonidine in LE rats, and cardiovascular effects of ICV clonidine were not modulated by IV pretreatment with AVP antagonist. This, however, could not rule out the implication of circulating AVP in the cardiovascular responses to ICV clonidine because we have previously found that the baroreflex mechanism is modulated by circulating AVP. Present findings imply only that pressor component of circulating AVP has a less dominant contribution to the cardiovascular effects of ICV clonidine.

Clonidine has been shown to cause hypotension by acting upon the central depressor sites of vasomotor centers including the NTS. In the present study, the hypotensive response to ICV clonidine was significantly greater in DI rats than in LE rats. Although the ICV pretreatment with AVP antagonist in LE rats did not affect the cardiovascular effects of ICV clonidine, central AVP may potentiate the pressor effect of ICV clonidine (as discussed previously), which normally counteracts the hypotensive effect. Lack of central AVP in DI rats, in turn, may result in a greater fall in BP after ICV clonidine than in LE rats.

In summary, the present study has provided evidence for the implication of endogenous AVP in the cardiovascular responses to central stimulation of $\alpha_1$- and $\alpha_2$-adrenoceptors.

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