

Intracerebroventricular Treatment with Vasopressin V₁-Receptor Antagonist Inhibits Centrally-Mediated Pressor Response to Clonidine in Conscious Rats

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ABSTRACT—The effect of intracerebroventricular (i.c.v.) treatment with a vasopressin (AVP) V₁-receptor antagonist on the pressor response to i.c.v. injection of clonidine was investigated in conscious rats with chronic i.c.v. guide cannulas and an arterial catheter. In conscious rats, i.c.v.-injected clonidine (5 and 10 μ g) dose-dependently produced a pressor response with a decrease in heart rate. No significant depressor response was induced by clonidine. The i.c.v. pretreatment with an AVP V₁-receptor antagonist ([d(CH₂)₅-Tyr(Me)]-AVP) at 0.5–2.5 μ g dose-dependently inhibited the pressor response to 10 μ g of i.c.v.-injected clonidine, while systemic (i.v.) pretreatment with the antagonist (2.5 μ g) had no effect. In pentobarbital-anesthetized rats, 10 μ g of i.c.v.-injected clonidine caused a marked depressor response associated with bradycardia. However, i.c.v. pretreatment with the antagonist (2.5 μ g) did not affect cardiovascular responses to i.c.v.-injected clonidine. These results suggest that endogenous AVP in the brain is involved in the mechanisms underlying the pressor response to i.c.v.-injected clonidine in conscious rats.

Keywords: Clonidine, Central pressor response, Vasopressin V₁-receptor antagonist

It is well documented that brain α -adrenoceptors, especially the α_2 -adrenoceptor subtype, play an important role in central control of blood pressure. Clonidine, an α_2 -adrenoceptor agonist and an antihypertensive drug, has been shown to decrease the arterial blood pressure when administered centrally in anesthetized rats (1). However, we have shown that intracerebroventricular (i.c.v.) injection of clonidine causes only a long-lasting pressor response in freely moving, conscious rats, while the drug produces a depressor response in rats anesthetized with pentobarbital (2–4). This pressor response to i.c.v.-injected clonidine in conscious rats is abolished by central (i.c.v.) pretreatment with yohimbine, an α_2 -adrenoceptor antagonist, indicating that the pressor response to i.c.v.-injected clonidine is mediated by central α_2 -adrenoceptors (2, 4).

On the other hand, we have reported that the decrease in body fluid volumes by water deprivation or a diuretic (furosemide) abolishes the pressor response to i.c.v.-injected clonidine even in conscious rats (5, 6). These findings suggest that endogenous arginine-vasopressin (AVP)

is involved in mechanisms underlying the centrally-mediated pressor response to clonidine, because water deprivation leads to loss of brain AVP (7). AVP has been shown to act through AVP V₁- and V₂-receptors, and only AVP V₁-receptors exist in the brain (8). In the present study, therefore, we investigated the effect of pretreatment with an AVP V₁-receptor antagonist on the pressor response to i.c.v.-injected clonidine in conscious rats and the possible involvement of endogenous brain AVP in mechanisms underlying the clonidine-induced pressor response.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats, weighing 300–350 g, were used in this study. The animals were given food and water ad libitum. They were housed in the Experimental Animal Center of Miyazaki Medical College at a controlled ambient temperature of 22°C with 50 \pm 10% relative humidity and a 12-hr light/dark cycle (light on at 7 : 30 a.m.). After surgery for chronic catheter implantation, the animals were transferred to individual cages.

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Surgical procedures

Under pentobarbital-anesthesia (50 mg/kg, i.p.), stainless-steel guide cannulas (0.7 mm in outer diameter) were bilaterally implanted into the lateral cerebroventricle as described previously (2). Briefly, the tip of the cannula was positioned 2.0 mm dorsal to the intended site of drug injection using the following coordinates: 0.0 mm anterior-posterior to the bregma, 1.5 mm lateral to the bregma and 2.2 mm below the surface of the dura mater. A stainless-steel bipolar electrode was also placed above the frontal cortex to measure electroencephalogram (EEG) activity. The animals were allowed to recover for 10 days before the surgery for chronic catheter implantation.

For the direct recording of blood pressure, the animals with chronic guide cannulas and electrode were anesthetized with ether. Polyethylene catheters (PE10 and PE20) were chronically implanted into the abdominal aorta via the left femoral artery and into the vena cava via the left femoral vein, respectively, as described previously (2, 9). The remainder of each catheter was passed beneath the skin to emerge on the back of the neck. Both catheters were pre-filled with the sterile heparinized 0.9% saline (500 I.U./ml) and were flushed every 2 days. A recovery period of 1 week after surgery was allowed before starting the experiment.

Recording

On the day of the experiment, the arterial catheter was attached to a polyethylene tubing which was connected to a pressure transducer (P23ID; Statham, Cleveland, OH, USA). The arterial blood pressure (pulsatile and mean), heart rate (HR) triggered by arterial pulses, and EEG activity were measured simultaneously while behavior was observed, and they were recorded on a polygraph (RM-6000; Nihon Kohden, Tokyo). Both mean blood pressure (MBP) and HR were digitized by a minicomputer (ATAC 450, Nihon Kohden) and printed out at 1-min intervals.

Experimental protocols

For i.c.v. injection of drugs, a stainless-steel cannula (0.35 mm in outer diameter) was lowered into the lateral cerebroventricle through the guide cannula. The injection cannula was attached with an extension polyethylene tube (PE20), which was connected to a 25- μ l Hamilton microsyringe. The venous catheter was connected to a piece of polyethylene tube approximately 60-cm-long (PE20, containing 0.2 ml of saline).

When the animal was completely relaxed and a drowsy EEG pattern (i.e., high voltage and slow waves) was recorded for over 5 min, the drugs were injected. Using a microinjector, a 5- μ l volume of clonidine or 0.9% saline was injected i.c.v. through the right guide cannula. There

was no significant change in MBP and HR after i.c.v. injection of saline. The i.c.v. administration of 10 μ l of the AVP receptor antagonist [d(CH₂)₅Tyr(Me)]-AVP (V₁-receptor antagonist) or an equivalent amount of vehicle (0.9% saline) was carried out over a period of 30 sec through the left guide cannula at 10 min before clonidine administration. The i.v. administration of the antagonist in the same volume (10 μ l) was performed through the extension tubing 10 min before i.c.v. injection of clonidine. After every i.v. injection, the tubing was flushed for 60 sec with 0.3 ml of sterile saline.

In the experiments using an anesthetic, the animals with chronic guide cannulas and catheters were anesthetized with pentobarbital (50 mg/kg, i.p.) 15 to 20 min before i.c.v. injection of vehicle or the antagonist.

Each animal received only one dose of antagonist pretreatment followed by i.c.v. clonidine at a 1-week interval on each occasion. At the end of each experiment, the catheters were sealed with stoppers, and then the animals were returned to their home cages.

After completion of the experiment the animals were anesthetized with large doses of pentobarbital. To determine the site of injection cannula placement, 5 μ l of dye was injected, and the brain was removed. Correct guide cannula placement was indicated by the diffusion of dye in the ventricle.

Statistical analyses

All values are expressed as mean \pm S.E.M. Statistical significance was determined by one way-analysis of variance followed by Dunnett's test. A P value less than 0.05 was considered statistically significant.

Drugs

Clonidine HCl was purchased from Nippon C.H. Boehringer Sohn (Kawanishi) and [d(CH₂)₅Tyr(Me)]-AVP was purchased from Peptide Institute (Osaka). Both drugs were dissolved in sterile 0.9% saline.

RESULTS

Cardiovascular responses to i.c.v. injection of clonidine in conscious rats

The i.c.v. injection of 5 and 10 μ g clonidine resulted in a long-lasting and dose-dependent increase in MBP (pressor response) associated with a decrease in HR in conscious rats (Fig. 1 and Table 1). After i.c.v. injection, the pressor response initiated within 10 sec, reached a maximum at 3 to 4 min and lasted for over 30 min. However, no significant fall in MBP (depressor response) was observed for 60 min (Table 1). After clonidine injection, the animal manifested a marked sedation, and a pronounced drowsy EEG pattern (high voltage and slow waves) was

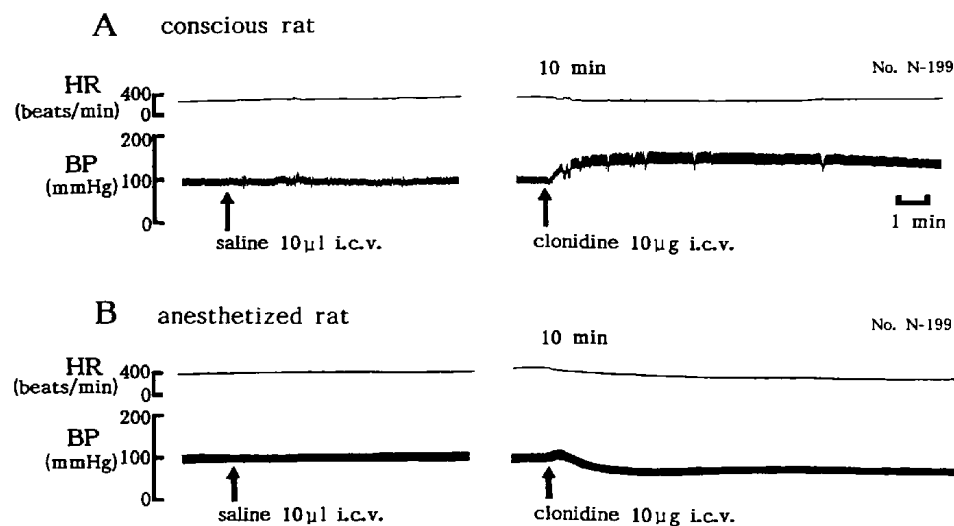


Fig. 1. Typical records showing cardiovascular responses to i.c.v. injection of saline (10 μ l) and clonidine (10 μ g) in the conscious rat (A) and pentobarbital (50 mg/kg, i.p.)-anesthetized rat (B). The experiment was done at a 1-week interval in the same animal. HR, heart rate; BP, blood pressure.

Table 1. Cardiovascular responses to i.c.v.-injected clonidine and effects of i.c.v. and i.v. pretreatment with the AVP V_1 -receptor antagonist on cardiovascular responses to i.c.v.-injected clonidine (10 μ g) in conscious rats

	n	MBP (mmHg)			HR (beats/min)	
		baseline MBP	maximum increase in MBP	maximum decrease in MBP	baseline HR	maximum decrease in HR
Control						
Clon, 5 μ g, i.c.v.	5	96 \pm 2	23 \pm 4	-5 \pm 2	307 \pm 14	-63 \pm 8
Clon, 10 μ g, i.c.v.	15	100 \pm 2	37 \pm 2	-3 \pm 1	321 \pm 8	-90 \pm 10
AVP-ant + Clon, 10 μ g, i.c.v.						
AVP-ant, 0.5 μ g, i.c.v.	5	97 \pm 4	36 \pm 2	-6 \pm 3	315 \pm 14	-90 \pm 18
AVP-ant, 1 μ g, i.c.v.	8	94 \pm 2	9 \pm 1**	-10 \pm 2**	325 \pm 8	-55 \pm 8
AVP-ant, 2.5 μ g, i.c.v.	12	103 \pm 3	8 \pm 2**	-8 \pm 2	319 \pm 11	-41 \pm 9**
AVP-ant, 2.5 μ g, i.v.	8	99 \pm 2	34 \pm 1	-3 \pm 1	304 \pm 6	-59 \pm 5

Values are means \pm S.E.M. n, number of experiments. MBP, mean blood pressure; HR, heart rate; Clon, clonidine; AVP-ant, AVP V_1 -receptor antagonist. ** P < 0.01, compared with 10 μ g Clon.

observed for over 60 min (Fig. 2A).

Effect of pretreatment with AVP V_1 -receptor antagonist in conscious rats

The i.c.v. injection of 0.5 and 1 μ g AVP V_1 -receptor antagonist did not cause behavioral changes. However, in 5 out of 12 rats, i.c.v. injection of 2.5 μ g antagonist caused a characteristic behavior, so called "barrel rotation", in which the animal rotated about its longitudinal axis. This behavior lasted for 5 to 7 min. During the behavior, the blood pressure slightly increased. The basal MBP and HR before i.c.v. injection of clonidine and the values after i.c.v. injection of the antagonist are summarized in Table

1.

As shown in Fig. 2B, Fig. 3 and Table 1, i.c.v. pretreatment with the AVP V_1 -receptor antagonist significantly inhibited the pressor response to i.c.v.-injected clonidine (10 μ g) in a dose-dependent manner. The central pretreatment with AVP V_1 -receptor antagonist potentiated depressor responses to i.c.v.-injected clonidine (Table 1). However, the clonidine-induced sedation and drowsy EEG pattern were not affected by treatment with the AVP V_1 -receptor antagonist (Fig. 2B). The decrease in HR induced by i.c.v. clonidine was significantly inhibited by i.c.v. treatment with 2.5 μ g of the AVP V_1 -receptor antagonist (Table 1).

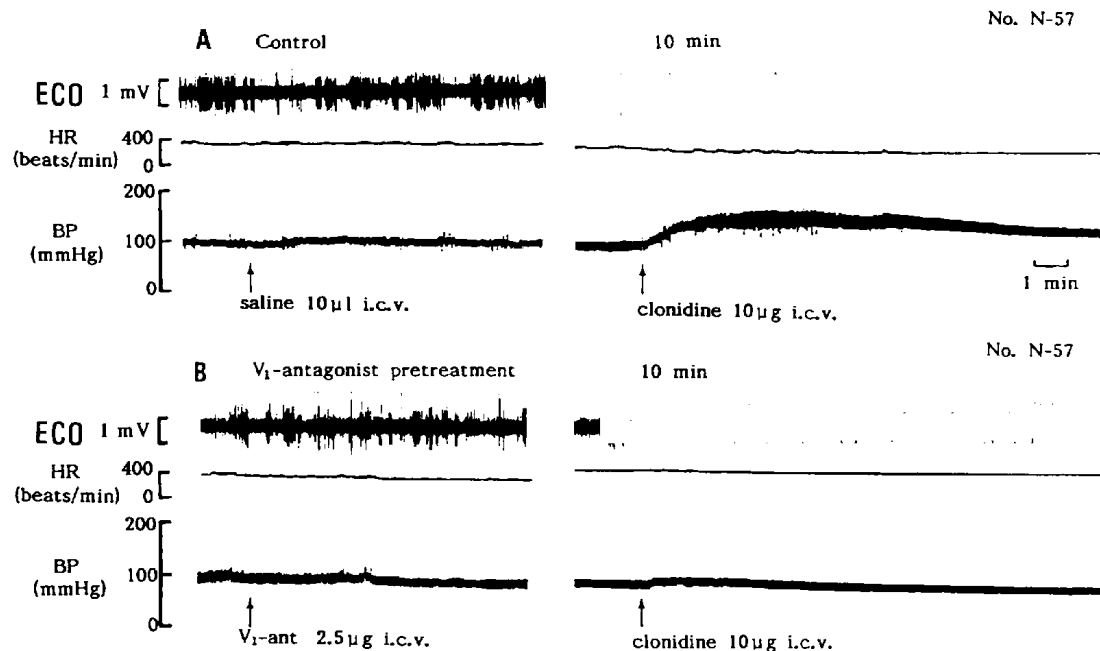


Fig. 2. Typical records showing changes in blood pressure, heart rate and electrocorticogram induced by i.c.v.-injected clonidine (10 µg) after i.c.v. pretreatment with vehicle (saline 10 µl, A) and the AVP V₁-receptor antagonist (V₁-ant, 2.5 µg, B) in conscious rats. The experiment was done at a 1-week interval in the same animal. ECO, electrocorticogram; HR, heart rate; BP, blood pressure.

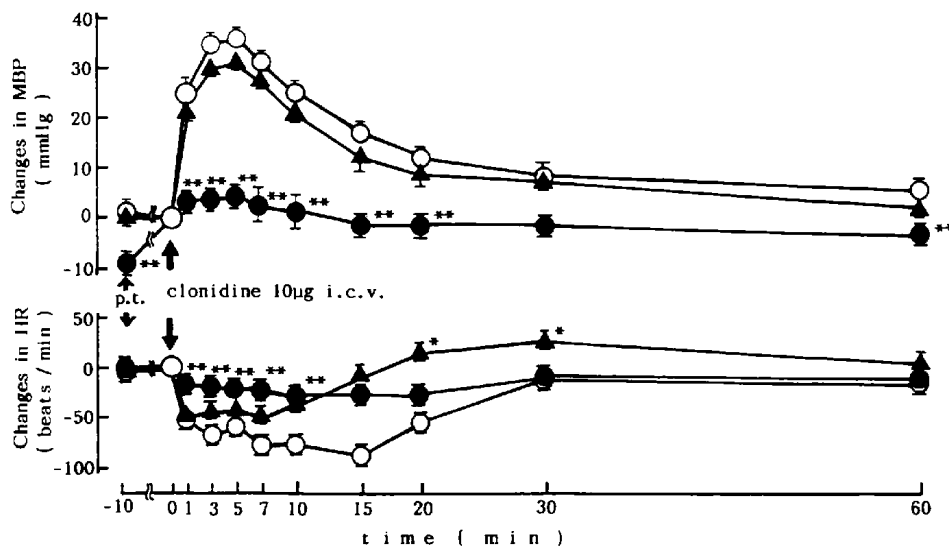


Fig. 3. Effects of central (i.c.v.) and systemic (i.v.) pretreatment with vehicle and the AVP V₁-receptor antagonist on cardiovascular responses induced by i.c.v.-injected clonidine (10 µg) in conscious rats. (○) i.c.v. pretreatment with vehicle (10 µl, n=15); (●) i.c.v. pretreatment with the AVP V₁-receptor antagonist (V₁-ant, 2.5 µg, n=8); (▲) i.v. pretreatment with the AVP V₁-receptor antagonist (V₁-ant, 2.5 µg, n=8). MBP, mean blood pressure; HR, heart rate. * $P < 0.05$, ** $P < 0.01$, compared with the vehicle (saline) control.

Systemic (i.v.) pretreatment with the AVP V₁-receptor antagonist (2.5 µg) had no effect on the pressor response to i.c.v.-injected clonidine (Fig. 3 and Table 1). However,

the decrease in HR at 10 to 30 min after clonidine injection was significantly less than that in the saline control.

Table 2. Effects of i.c.v. pretreatment with saline and the AVP V_1 -receptor antagonist on cardiovascular responses to i.c.v.-injected clonidine (10 μ g) in anesthetized rats

Pretreatment	n	Baseline value	Changes in MBP and HR after i.c.v. injection of 10 μ g clonidine					
			1 min	3 min	5 min	10 min	30 min	60 min
Blood pressure (mmHg)								
Saline, 10 μ l, i.c.v.	12	94 \pm 2	7 \pm 3	1 \pm 4	-3 \pm 4	-8 \pm 3	-14 \pm 2	-4 \pm 4
AVP-ant, 2.5 μ g, i.c.v.	7	94 \pm 2	4 \pm 3	3 \pm 4	1 \pm 5	-2 \pm 6	-15 \pm 2	-13 \pm 3
Heart rate (beats/min)								
Saline, 10 μ l, i.c.v.	12	380 \pm 11	-47 \pm 6	-75 \pm 7	-79 \pm 7	-88 \pm 7	-68 \pm 11	-40 \pm 17
AVP-ant, 2.5 μ g, i.c.v.	7	369 \pm 10	-37 \pm 12	-66 \pm 14	-70 \pm 13	-87 \pm 16	-94 \pm 8	-63 \pm 21

Values are means \pm S.E.M. n, number of experiments. MBP, mean blood pressure; HR, heart rate; AVP-ant, AVP V_1 -receptor antagonist.

Effect of pretreatment with AVP V_1 -receptor antagonist on cardiovascular responses to i.c.v. injection of clonidine in anesthetized rats

As shown in Fig. 1B and Table 2, i.c.v. injection of 10 μ g clonidine caused a small pressor response followed by a long-lasting fall in MBP concomitant with a marked decrease in HR in pentobarbital-anesthetized rats. The depressor response reached a maximum at 30 min and lasted for the observed 60 min.

In pentobarbital-anesthetized rats, i.c.v. injection of the AVP V_1 -receptor antagonist did not cause changes in MBP and HR. In vehicle-treated rats, i.c.v.-injected clonidine (10 μ g) resulted in a slight pressor response and a long-lasting depressor response associated with a marked decrease in HR. The i.c.v. pretreatment with 2.5 μ g of the AVP V_1 -receptor antagonist had no effect on the clonidine-induced depressor response and decrease in HR (Table 2).

DISCUSSION

The present and previous studies showed that i.c.v. administered clonidine produces a long-lasting pressor response in conscious rats but not in pentobarbital-anesthetized rats (2). This pressor response is abolished by central (i.c.v.) pretreatment with yohimbine, a preferential α_2 -adrenoceptor antagonist, but not by systemic (i.v.) pretreatment with yohimbine (2, 4). Additionally, the pressor response to i.c.v.-injected clonidine is less inhibited by i.c.v. or i.v. pretreatment with prazosin, a selective α_1 -adrenoceptor antagonist. Therefore, we suggest that central α_2 -adrenoceptors are mainly involved in the long-lasting pressor response to i.c.v.-injected clonidine in conscious rats (2, 4).

In the present study, we demonstrated that i.c.v. pretreatment with the AVP V_1 -receptor antagonist [d(CH₂)₅Tyr(Me)]-AVP markedly inhibits the pressor response to i.c.v.-injected clonidine in conscious rats,

while systemic (i.v.) pretreatment with the same dose (2.5 μ g) of this AVP V_1 -receptor antagonist has no such effect. These results clearly indicate that the interaction occurred within the brain and the clonidine-induced pressor response originates in the brain but not in the peripheral site.

The central pretreatment with 2.5 μ g of the AVP V_1 -receptor antagonist significantly inhibited but did not abolish the decrease in HR induced by clonidine, while it abolished the pressor response. Therefore, this inhibition seems to be due mainly to a reduced baroreflex as a result of the decreased pressor response. Furthermore, both sedation and the drowsy EEG pattern induced by clonidine, which are mediated by central α_2 -adrenoceptors (10), were not affected by the antagonist. Therefore, it is unlikely that brain AVP is involved in the decrease in HR, sedation and EEG effect induced by clonidine.

When a higher dose (more than 1.0 μ g) of the AVP V_1 -receptor antagonist was injected into the cerebroventricle, half of the animals injected manifested a barrel-rolling behavior. This rotative behavior might affect the pressor response to i.c.v. injected clonidine. However, a lower dose of the V_1 -receptor antagonist (1.0 μ g), which did not cause the barrel rolling behavior, still inhibits the clonidine-induced pressor response. This indicates that the inhibitory effect of the AVP V_1 -receptor antagonist on the clonidine-induced pressor response is not related to barrel rolling behaviors.

Recent reports have shown that reduction of body fluid volumes induced by water deprivation for 48 hr or furosemide, a diuretic, abolishes the pressor response to i.c.v.-injected clonidine even in conscious rats (5, 6). Water deprivation has been shown to cause loss of brain AVP due to the reduction of body fluid (7). Furthermore, we have reported that i.c.v. pretreatment with atrial natriuretic peptide, which inhibits AVP release (11), attenuates the clonidine-induced pressor response (12). Central pretreatment with the AVP V_1 -receptor antagonist

abolishes the pressor response to i.c.v. clonidine. Taken together, these results strongly suggest that endogenous brain AVP plays an important role in mechanisms underlying the pressor response induced by centrally administered clonidine.

In the studies using conscious Brattleboro rats that are hereditarily deficient in endogenous AVP, i.c.v.-injected clonidine results in no pressor response, but induces a depressor response associated with a decrease in HR. However, when AVP is preadministered in Brattleboro rats, i.c.v. clonidine causes the pressor response (13). Furthermore, centrally administered noradrenaline, a mixed α_1 - and α_2 -adrenoceptor agonist, induces no increase in blood pressure in conscious Brattleboro rats (14). These reports also support the present findings that endogenous brain AVP is responsible for the central α_2 -adrenoceptor-mediated pressor response.

An extensive network of AVP-containing neurons has been identified within the central nervous system. Electrophysiological (15, 16) and retrograde tracer studies using immunocytochemical techniques showed that immunoreactive neurons for AVP project from the hypothalamic paraventricular nucleus (PVN) to brain areas involved in the regulation of systemic blood pressure (17–20). Electrical stimulation of PVN in rats causes a pressor response and increases AVP content in the extra-hypothalamic area (21). Microinjection of noradrenaline into the PVN causes a pressor response through activation of α_2 -adrenoceptors (14). The preliminary study showed that microinjection of clonidine into the PVN of conscious rat produced a long-lasting pressor response, which mimicked the pressor response to i.c.v.-injected clonidine and was sensitive to anesthesia and yohimbine (22). Because systemic pretreatment with AVP V_1 -receptor antagonist failed to inhibit the i.c.v. clonidine-induced pressor response, extra-hypothalamic AVP in the brain may participate in the pressor mechanism mediated by central α_2 -adrenoceptors.

On the other hand, the depressor response to i.c.v.-injected clonidine was always seen in anesthetized rats. Since the i.c.v. clonidine-induced depressor response under pentobarbital-anesthesia is blocked by α_2 -adrenoceptor antagonists and the sympathetic outflow from the brain is inhibited by centrally injected clonidine (1), clonidine is considered to exert the hypotensive effect through activation of central α_2 -adrenoceptors within the central nervous system, mainly at structures in the medulla oblongata. However, it has recently been proposed that the hypotensive effect of clonidine is mediated not only by α_2 -adrenoceptors but also by imidazoline-preferring receptors (23). In the present study, i.c.v. pretreatment with the AVP V_1 -receptor antagonist failed to inhibit the depressor response to i.c.v.-injected clonidine not only in

anesthetized rats but also in conscious rats. This suggests that endogenous brain AVP does not contribute to the depressor mechanism mediated by α_2 -adrenoceptors or imidazoline-preferring receptors.

In conclusion, the present results suggest that endogenous AVP in the brain, via AVP V_1 -receptors, is involved in the mechanisms underlying the pressor response to i.c.v.-injected clonidine in conscious rats.

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