Central α₂-Adrenoceptor-Mediated Pressor Response to Clonidine in Conscious, Spontaneously Hypertensive Rats

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ABSTRACT — Pressor responses to intracerebroventricular (i.c.v.) injection of clonidine were investigated in conscious spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY). Clonidine (1–10 μg, i.c.v.) caused a dose-dependent pressor response and decrease in heart rate in both SHR and WKY. In SHR, low doses (1, 2.5 μg) but not high doses (5, 10 μg) of i.c.v.-clonidine induced a depressor response following the pressor response. Both pressor and depressor responses to i.c.v.-clonidine were significantly greater in SHR than in WKY. In both SHR and WKY, pressor responses to i.c.v.-clonidine were abolished by pentobarbital anesthesia, pretreatment with i.v.-furosemide (5 mg/kg), 24-hr water deprivation and pretreatment with i.c.v.-yohimbine (100 μg), but not by pretreatment with i.v.-yohimbine (100 μg) and i.c.v.-prazosin (10 μg). On the 1st day after surgery for arterial catheter implantation, SHR reduced their water intake, and i.c.v.-clonidine (5 μg) caused a slight pressor response, whereas the same dose of clonidine on the 7th day after surgery resulted in a marked pressor response. These results suggest that clonidine caused a central α₂-adrenoceptor-mediated pressor response, which is greater in SHR than in WKY and is sensitive to body fluid volume changes and anesthesia.

Keywords: Clonidine, Central pressor response, Spontaneously hypertensive rat, Normotensive Wistar Kyoto rat, Central α₂-adrenoceptors

Previous studies have shown that intracerebroventricular (i.c.v.)-injection of clonidine, a centrally acting antihypertensive drug, causes a pressor response, but only causes a slight depressor response in conscious normotensive rats (1–3). However, i.c.v.-clonidine causes a marked depressor response and a slight pressor response in anesthetized rats (1–3). Furthermore, we have reported that the pressor response to centrally injected clonidine was abolished by central pretreatment with the α₂-adrenoceptor antagonist yohimbine, but not with the α₁-adrenoceptor antagonist prazosin. Therefore, we have proposed that the pressor response is mediated by central α₂-adrenoceptors (1, 3). Recently, we have reported that a reduction in body fluid volume, which was caused by water deprivation for 48 hr or by the diuretic, furosemide, abolishes the clonidine-induced pressor response and potentiated the clonidine-induced depressor response; and this suggested that the pressor response is influenced by body fluid volumes (4, 5). We have proposed that the response to clonidine has a centrally-mediated pressor component, which opposes the hypotensive effect, and that the pressor component of the response to clonidine is sensitive to the diuretic and to anesthesia. However, the precise mechanism of this pressor response to i.c.v. clonidine remains unclear.

Since all of these results were obtained from normotensive rats, it is not known whether centrally administered clonidine has a similar effect in hypertensive rats. In early reports using the conscious spontaneously hypertensive rat (SHR), centrally injected clonidine has been shown to result in only the hypotensive response (6, 7). This suggests that the central pressor component of the response to clonidine is absent in SHR. Therefore, the present study was designed to investigate the pressor response to centrally administered clonidine in SHR and in the normotensive Wistar Kyoto rat (WKY). Furthermore, we examined the effect of furosemide, a diuretic, on the pressor response to clonidine, since the combination of clonidine with a diuretic potentiates the antihypertensive effect (8).
MATERIALS AND METHODS

Experimental animals

Age-matched (15 week-old) male SHR of the Oka-moto strain and normotensive WKY, purchased from Charles River (Shizuoka, Japan), were used in this study. The animals were given food and water ad libitum and housed in the Experimental Animal Center of Miyazaki Medical College at a controlled ambient temperature of 22 ± 1°C with 50 ± 10% relative humidity and a 12 hr light/dark cycle (light on 7:30 a.m.). After surgery for chronic catheter implantation, the animals were transferred to individual cages.

Surgical procedures

The animals were anesthetized with pentobarbital-Na (50 mg/kg, i.p.), and stainless-steel guide cannulas (0.7 mm outer diameter) were implanted bilaterally into the lateral cerebroventricles as described previously (1). A stainless-steel bipolar electrode was also placed above the frontal cortex to measure the electroencephalogram (EEG). Guide cannulas and an electrode were fixed to the skull with dental cement and screws. A stylet which extended just to the tip of the guide cannula was locked inside each guide cannula. The animals were allowed to recover for 10 days before the surgery for chronic catheter implantation.

To directly record blood pressure, animals with chronic guide cannulas and an EEG electrode were anesthetized with ether. One thin polyethylene catheter (PE 10 and PE 20) was chronically implanted in the abdominal aorta via left femoral artery, and another was placed in the inferior vena cava via the left femoral vein, as described previously (1, 9). The remainder of each catheter was passed beneath the skin to emerge on the back of the neck, and the end of the catheter was plugged with a stainless-steel stopper. Both catheters were prefilled with sterile heparinized 0.9% saline (500 I.U./ml), and they were flushed every 2 days. The animals were allowed to recover for 10 days before the experiment began.

Recording

On the day of the experiment, the arterial catheter was attached into an extension polyethylene tubing, which was prefilled with sterile heparinized saline (500 I.U./ml), and this was then connected to a pressure transducer (P23ID, Gould, Cleveland, OH). The arterial blood pressure (pulsatile and mean) was recorded on a polygraph (RM-6000, Nihon Kohden, Tokyo, Japan). The heart rate (HR) was measured with a cardiotachograph (AT-600G, Nihon Kohden) triggered by the arterial pulses and was recorded on the polygraph. To record EEG activity, a lead wire was connected to the electrode. EEG was monitored and recorded on the polygraph. After the extension tubing and lead wire were connected, the animal was moved to an open-topped cylindrical Plexiglass cage, which was placed in a shielded, soundproof box. The arterial blood pressure, HR and EEG were measured simultaneously while behavior was observed. Both mean blood pressure (MBP) and HR were digitized with a minicomputer (ATAC 450, Nihon Kohden) and printed out at 1-min intervals.

Experimental protocols

For i.c.v.-injection of drugs, the stylet was removed, and a stainless-steel injection cannula (0.35 mm outer diameter) was lowered into the lateral cerebroventricle through the guide cannula. The injection cannula was attached to an extension polyethylene tubing (PE 20), which was prefilled with the drug, and was brought out of the soundproof box and connected to a Hamilton microsyringe (25 μl). The venous catheter was connected to a piece of saline-filled polyethylene tubing approximately 60 cm long (PE 20, 0.2 ml inner volume). The other end of this tubing was led to the outside of the soundproof box and connected to a 1-ml syringe.

When the animal was completely relaxed in the cage and a drowsy EEG pattern (i.e., high voltage and slow wave) was recorded for over 5 min, i.c.v. and intravenous (i.v.) administration of the drug began. Clonidine or an equivalent amount of vehicle was injected i.c.v. with a microinjector in a volume of 5 μl through the right guide cannula. There were no significant changes in MBP or HR of SHR or WKY after i.c.v.-injection of the vehicle.

The α-adrenoceptor antagonist or an equivalent amount of vehicle was injected i.c.v. over 30 sec in a volume of 10 μl through the left guide cannula, and this was done 15 min before clonidine was given through the right guide cannula. Furosemide or an equivalent amount of vehicle (saline) was given i.v. through the extension tubing 60 min before i.c.v.-injection of clonidine. After i.v.-injection, the tubing was flushed for 60 sec with 0.3 ml of sterile saline.

In experiments using an anesthetic, the animals with chronic guide cannulas and catheters were anesthetized with pentobarbital-Na 50 mg/kg, i.p. at 15 to 20 min before i.c.v.-injection of clonidine.

In another series of experiments, daily water intake before and after the surgery for implantation of chronic catheters was measured in both WKY and SHR with chronic i.c.v. guide cannulas, and cardiovascular responses to clonidine (5 μg, i.c.v.) were studied on the 1st and 7th day after the operation. In some experi-
ments, the animals were deprived of drinking water for 24 hours before i.c.v.-injection of clonidine.

At the end of each experiment, the catheters were sealed with stoppers, syllets were inserted into the guide cannulas, and then the animals were returned to their home cages. At 1-week intervals, the animals were subjected to i.c.v.-injection. Each animal received only one dose of clonidine and one pretreatment with the drug or an equivalent amount of vehicle.

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

*Statistical analysis*

All values are expressed as means ± S.E.M. Statistical analysis was performed using the unpaired Student’s t-test between two means and one-way analysis of variance followed by Dunnett’s test between different doses. A value of P < 0.05 was considered indicate a statistically significant difference between values.

*Drugs*

The following drugs were used: clonidine HCl (Nippon C.H. Boehringer Sohn, Kawanishi, Japan), furosemide (Lasix inj., Hoechst Japan, Tokyo, Japan), prazosin HCl (Taito Pfizer, Tokyo, Japan), sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL, USA), and yohimbine HCl (Sigma Chemical Co., St. Louis, MO, USA). Prazosin and yohimbine were dissolved in sterile 5% dextrose. All other drugs were dissolved in sterile 0.9% saline.

**RESULTS**

*Cardiovascular responses to i.c.v.-clonidine in conscious SHR and WKY*

The baseline MBP and HR before i.c.v.-clonidine in conscious SHR and WKY are summarized in Table 1. In conscious SHR and WKY, i.c.v.-clonidine caused a dose-dependent increase in blood pressure (Figs. 1A, 2 and 3A). The pressor effect of clonidine began 10 to 15 sec after injection, reached a maximum at 5 to 7 min, and lasted for more than 30 min at higher doses (Fig. 2). This pressor response was significantly greater in SHR than in WKY at 2.5, 5 and 10 μg, but not at 1 μg (Fig. 3A).

Lower doses of clonidine (1 and 2.5 μg) caused a fall in MBP after the pressor response, especially in SHR (Figs. 2 and 3B). The depressor response to clonidine began 7 to 10 min after injection, reached a maximum at 15 to 20 min, and lasted for 30 min. This depressor response was also significantly greater in SHR than in WKY (Fig. 3B).

In both WKY and SHR, i.c.v.-clonidine induced a dose-dependent decrease in HR (Figs. 1A, 2 and 3C). There was no significant difference in the magnitude of this effect between SHR and WKY (Fig. 3C).

The i.c.v.-clonidine caused a marked EEG drowsy pattern and, the animals were sedated for more than 60 min (data not shown).

*Cardiovascular responses to i.c.v.-clonidine in anesthetized SHR and WKY*

The baseline MBP and HR before i.c.v. clonidine in anesthetized SHR and WKY are summarized in Table 1. In pentobarbital (50 mg/kg, i.p.)-anesthetized SHR, i.c.v.-clonidine (5 and 10 μg) produced a slight and short-lasting pressor response, following by a marked

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Fig. 1. Typical recordings of the cardiovascular responses to i.c.v.-clonidine (10 μg) in the conscious (A) and pentobarbital-anesthetized (B) SHR. The same SHR was anesthetized and given clonidine i.c.v. 1 week after the experiment performed while the animal was conscious. HR, heart rate; BP, pulsatile blood pressure; MBP, mean blood pressure.

Fig. 2. Changes in mean blood pressure and heart rate following i.c.v.-saline and clonidine in conscious WKY (left) and SHR (right). ○ saline, 5 μl (n = 6); ● clonidine, 2.5 μg (n = 7 and 6); ▲ clonidine, 10 μg (n = 8).
depressor response, and a decrease in HR (Figs. 1B and 4). The pressor response to i.c.v.-clonidine in anesthetized SHR was significantly smaller than that in conscious SHR (Fig. 4). Also, the pressor effect of clonidine in anesthetized WKY was significantly smaller than the effect in conscious WKY (Fig. 4).

The depressor response to i.c.v.-clonidine (5 and 10 μg) was not observed in conscious rats, but was marked seen in anesthetized rats, especially in SHR (Figs. 1B and 4). The depressor effect was greater in SHR than in WKY (Fig. 4). The decrease in HR by clonidine was also greater after anesthesia in SHR, but not in WKY (Fig. 4).

Effects of i.c.v.-treatment with yohimbine and prazosin in conscious SHR and WKY

The baseline MBP and HR before i.c.v. clonidine in conscious SHR and WKY treated with i.c.v. vehicle, yohimbine or prazosin are summarized in Table 2. As shown in Fig. 5, central pretreatment (i.c.v.) with yohimbine at 50 and 100 μg dose-dependently inhibited the pressor response to i.c.v.-clonidine (10 μg) in con-
scious SHR and WKY; a dose of 100 μg almost abolished the pressor response. When given systemically (i.v.), 100 μg of yohimbine did not abolish the pressor response to 10 μg i.c.v.-clonidine (SHR, 42 ± 4 mmHg, n = 3; WKY, 38 ± 2 mmHg, n = 4). However, i.c.v.-yohimbine treatment did not inhibit the bradycardiac effect of clonidine, and it significantly potentiated the depressor response to clonidine in both SHR and WKY (Fig. 5).

Central pretreatment with prazosin (10 μg) slightly but significantly inhibited the pressor response to i.c.v.-clonidine in both SHR and WKY (Fig. 5). However, prazosin did not affect the depressor response to i.c.v.-clonidine (Fig. 5).

Central pretreatment with yohimbine (100 μg) but not prazosin (10 μg) inhibited the drowsy EEG pattern induced by i.c.v.-clonidine (10 μg) (data not shown).

Effect of furosemide treatment in conscious SHR and WKY

After furosemide administration, the animals showed a marked urination: it caused 2 to 3% loss of body weight. The baseline MBP and HR before i.c.v.-clonidine in conscious SHR and WKY treated with i.v.-vehicle or -furosemide are summarized in Table 2.

As shown in Fig. 6, systemic (i.v.) pretreatment with furosemide (5 mg/kg) markedly inhibited the pressor response to i.c.v.-clonidine (5 and 10 μg) in both SHR and WKY. The depressor response to i.c.v.-clonidine was significantly greater after furosemide treatment. However, furosemide treatment did not affect the drowsy EEG pattern or the sedation induced by clonidine (data not shown). The clonidine-induced bradycardia was also potentiated by furosemide treatment in SHR but not in WKY (Fig. 6).

Effect of 24-hr water deprivation in conscious WKY and SHR

The baseline MBP and HR before i.c.v.-clonidine in conscious SHR and WKY dehydrated for 24 hr are summarized in Table 1. As shown in Fig. 7, pressor responses to i.c.v.-clonidine (5 and 10 μg) was significantly smaller in dehydrated WKY and SHR than in hydrated (control) WKY and SHR. Depressor responses to i.c.v.-clonidine was also greater after water deprivation. The clonidine-induced bradycardia was not altered by water deprivation except for 10 μg clonidine in WKY and 5 μg clonidine in SHR (Fig. 7).

| Table 2. Baseline mean blood pressure (MBP) and heart rate (HR) before various treatments and injection of clonidine following various treatments in SHR and WKY |
|---|---|---|---|---|
| Effect of furosemide treatment in conscious SHR and WKY |
| n | MBP before MBP before HR before HR before treatment (mmHg) treatment (mmHg) treatment (beats/min) treatment (beats/min) |
| SHR | | | | |
| Saline, i.c.v. | 10 μl | 9 | 143 ± 4 | 139 ± 5 | 310 ± 10 | 302 ± 7 |
| Yohimbine, i.c.v. | 50 μg | 8 | 157 ± 8 | 168 ± 8 | 297 ± 10 | 330 ± 11 |
| | 100 μg | 4 | 147 ± 6 | 167 ± 3 | 279 ± 9 | 324 ± 20 |
| Prazosin, i.c.v. | 10 μg | 6 | 151 ± 5 | 137 ± 3 | 286 ± 14 | 320 ± 18 |
| Saline, i.v. | 1 ml/kg | 8 | 144 ± 6 | 151 ± 4 | 292 ± 7 | 291 ± 10 |
| Furosemide, i.v. | 5 ml/kg | 8 | 163 ± 5 | 170 ± 5 | 286 ± 7 | 302 ± 5 |
| WKY | | | | |
| Saline, i.c.v. | 10 μl | 11 | 101 ± 2 | 101 ± 2 | 283 ± 5 | 277 ± 6 |
| Yohimbine, i.c.v. | 50 μg | 8 | 108 ± 3 | 119 ± 3 | 299 ± 10 | 334 ± 11 |
| | 100 μg | 4 | 104 ± 5 | 118 ± 2 | 291 ± 17 | 359 ± 12 |
| Prazosin, i.c.v. | 10 μg | 5 | 103 ± 2 | 100 ± 2 | 283 ± 7 | 360 ± 12 |
| Saline, i.v. | 1 ml/kg | 8 | 102 ± 3 | 104 ± 5 | 281 ± 2 | 274 ± 6 |
| Furosemide, i.v. | 5 mg/kg | 8 | 103 ± 6 | 108 ± 2 | 272 ± 5 | 302 ± 5 |
Water intake and blood pressure response to i.c.v.-clonidine after the surgery to implant chronic catheters

The baseline MBP and HR before i.c.v.-clonidine on the 1st day and 7th day after surgery from catheter implantation in conscious SHR and WKY are summarized in Table 1. As shown in Fig. 8, in SHR, i.c.v. injection of 5 μg clonidine on the 1st day after surgery produced a small pressor response followed by a marked depressor response, whereas the same dose of clonidine on the 7th day after the operation caused a marked pressor response and a smaller depressor response.

Water intake of SHR on the 1st day after the operation was significantly less than that on the day before the operation (Fig. 9, upper panel). The daily water intake returned to the pre-operation level the 4th day after the operation. As shown in Fig. 9 (lower panel), on the 1st day after the operation, the pressor response to i.c.v.-clonidine was significantly less in SHR than in WKY, but the depressor response to clonidine was significantly greater in SHR than in WKY. However, on the 7th day after the operation, the pressor response to i.c.v.-clonidine in both WKY and SHR was significantly greater than the response on the 1st day, and the depressor responses in SHR was significantly less than the response on the 1st day.

DISCUSSION

The present study performed in conscious SHR and WKY shows that i.c.v.-clonidine causes a dose-dependent and long-lasting pressor response, which is greater in SHR than in WKY. In SHR and WKY, higher doses
of clonidine did not lower blood pressure. These findings are in accord with previous reports that i.c.v.-clonidine causes a pressor response only in conscious rats (1–3). In both SHR and WKY, the pressor response to i.c.v.-clonidine was abolished by pentobarbital anesthesia, in accord with our previous reports (1, 3). Furthermore, this pressor response was abolished by i.c.v.-pretreatment with the α2-adrenoceptor antagonist yohimbine, but was not abolished by the α1-antagonist prazosin. Inasmuch as systemic (i.v.) treatment with yohimbine at the same dose had no such effect and pentobarbital does not affect α2-adrenoceptors (10), it is very likely that the pressor response to i.c.v.-clonidine in SHR and WKY is of central origin and is mediated by central α2-adrenoceptors.

In conscious rats, pressor responses to i.c.v.-clonidine (2.5–10 μg) are significantly greater in SHR than in WKY. Additionally, in conscious and anesthetized rats, depressor responses to i.c.v.-clonidine, which are well-known to result from activation of central α2-adrenoceptors, are much greater in SHR than in WKY. Therefore, it seems likely that the enhanced pressor response to i.c.v.-clonidine in conscious SHR is due to the increased sensitivity of central α2-adrenoceptors.

Recent studies have proposed that the hypotensive effect of clonidine is mediated not only by α2-adrenoceptors but also by non-alpha adrenergic imidazoline-preferring receptors (11, 12). Since yohimbine selectively binds to α2-adrenoceptors but not to imidazoline-preferring receptors (13–15), central α2-adrenoceptors are mainly responsible for the pressor response to i.c.v. clonidine. The present results, however, show that the depressor response to i.c.v.-clonidine in WKY, and especially in SHR, was not inhibited, but was increased, by i.c.v.-treatment with yohimbine at a dose that abolished the pressor response to i.c.v.-clonidine. This may indicate that non-alpha adrenergic imidazoline-preferring receptors are involved in the depressor response to i.c.v.-clonidine. In SHR, lower doses of clonidine (1 and 2.5 μg) resulted in greater depressor responses, while there

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**Fig. 7.** Effect of water deprivation for 24 hours on the pressor response (top), depressor response (middle) and decrease in heart rate (bottom) induced by i.c.v.-clonidine (10 μg) in conscious WKY (left) and SHR (right). Numbers in parentheses indicate the number of experiments in each group. □ control, ■ 24 hr water deprivation.
Fig. 8. Representative records of cardiovascular responses to i.c.v.-injected clonidine (5 μg) in conscious SHR on the 1st day (A) and 7th day (B) after the surgery for implantation of chronic catheters. The experiments were done in the same animal. HR, heart rate. BP, pulsatile blood pressure. MBP, mean blood pressure.

Fig. 9. Daily water intake (upper panel) and the pressor response and depressor response to i.c.v.-clonidine (5 μg) in WKY and SHR on the 1st day and 7th day after the surgery for implantation of chronic catheters (lower panel). *P < 0.05, **P < 0.01, compared with the water intake on the day before the surgery and with the response on the 1st day after surgery, respectively. ○ □ WKY (n = 6), ● ■ SHR (n = 9).
was a small depressor response in WKY. Thus, the non-alpha adrenergic imidazoline-preferring receptors in SHR may be sensitive.

In conscious SHR and WKY treated by furosemide, i.c.v.-clonidine caused a slight pressor response and instead produced a marked depressor response. In addition, the present study shows that after 24 hr of water deprivation, the pressor response to i.c.v.-clonidine is abolished and a depressor response appears in both WKY and SHR. These results are consistent with our previous reports that the clonidine-induced pressor response was abolished by 48 hours of water deprivation (4) and by furosemide (5). Inasmuch as the inhibitory effect of furosemide was correlated with increased urine volume, we proposed that the inhibition is associated with loss of body fluid volume (5). The present finding that clonidine causes a marked depressor response in WKY and SHR pretreated with furosemide, and in those deprived of water, is also consistent with our previous reports of a depressor response to clonidine in water-deprived or furosemide-treated rats (4, 5). Taken together, these results suggest that clonidine has not only an \( \alpha_2 \)-adrenoceptor- and imidazoline-preferring receptor-mediated hypotensive effect but also an \( \alpha_2 \)-adrenoceptor-mediated hypertensive effect. It seems likely that the hypertensive effect masks the hypotensive effect and that reduced body fluid volume affects only the pressor component of the response.

Early studies which used conscious SHR with arterial catheters showed that centrally administered clonidine mainly results in a depressor response (6, 7, 16). However, these experiments were done within 24–48 hr after the surgery for implantation of the arterial catheter. As shown in Fig. 9, SHR markedly reduced their water intake from 24 to 48 hr after the surgery, and clonidine injection on that day mainly caused a depressor response, with a small pressor response. These results strongly indicate that experiments performed within 48 hr after the surgery can not reveal the centrally mediated pressor response to clonidine. The decreased pressor response is probably due to loss of body fluid volumes, which results not only from bleeding during the operation but also from reduced water intake after the operation, because water-deprivation (even for 24 hr) inhibits the clonidine-induced pressor response in SHR. These phenomena should be taken into consideration in the design of studies of the cardiovascular action of clonidine in conscious SHR with chronically implanted catheters. The present results indicate that the animals should be allowed to recover for at least 7 days after the arterial catheter is implanted.

Changes in sodium ion concentration in vitro (17) and in sodium balance in vivo (18) have been reported to modify the binding properties of central and peripheral \( \alpha_2 \)-adrenoceptor agonists. Therefore, it is possible that the inhibitory effect of furosemide, which causes electrolyte alterations, results from alterations in the binding activities of \( \alpha_2 \)-adrenoceptors. However, this probably did not happen in the present study, because furosemide did not inhibit either the hypotensive effect of the drowsy EEG pattern induced by clonidine, which are mediated by central \( \alpha_2 \)-adrenoceptors (19–21). Furthermore, we have recently reported that in conscious normotensive rats, 10 mg/kg, i.v. of furosemide does not affect the pressor response to i.v.-noradrenaline, a mixed \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptor agonist (5). Thus, it is likely that electrolyte alterations caused by furosemide at the dose used in this study (5 mg/kg) have little effect on the binding activities of central or peripheral \( \alpha_2 \)-adrenoceptors.

The decrease in extracellular fluid induced by water deprivation has been shown to lead to a loss of arginine-vasopressin (AVP) in the brain (22). The present and previous findings that the pressor response to i.c.v.-clonidine is inhibited by reduction of body fluid volumes raises the possibility that endogenous brain AVP may play a role in the clonidine-induced pressor response. This is supported by the preliminary result that central (i.c.v.), but not systemic (i.v.), pretreatment with an AVP \( V_1 \)-receptor antagonist markedly inhibits the pressor response to i.c.v. clonidine in conscious rats (23). In addition, central administration of AVP has been shown to increase blood pressure in conscious rats (24). Further studies are needed to clarify the mechanism of this effect.

In conclusion, the present study suggests that i.c.v.-clonidine causes a pressor response in WKY, and especially in SHR, and that reduction of body fluid volume inhibits the centrally-mediated pressor response to clonidine and leads to the clonidine-induced hypotensive effect. In a human clinical study, tolerance to the hypotensive effect of clonidine has been shown to result from salt and water retention (8, 25). It is conceivable that water retention potentiates the pressor response to clonidine, and this may oppose the hypotensive action. Therefore, we suggest that combined treatment with a diuretic increases the hypotensive efficacy of clonidine. In fact, in the clinical treatment of hypertension, combining clonidine with a diuretic has been shown to enhance the hypotensive effect (8, 26, 27).

Acknowledgment

Authors wish to thank Mrs. Keiko Kawabata for preparing the figures.
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