Possible Mechanisms Underlying the Hypertensive Response to Clonidine in Freely Moving, Normotensive Rats

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Abstract—Possible mechanisms underlying the hypertensive response to intracerebroventricular (i.c.v.) or intravenous (i.v.) injection of clonidine were investigated in freely moving, normotensive rats. In conscious rats, clonidine (2–20 µg) injected i.c.v. caused a dose-dependent and long-lasting pressor response associated with bradycardia. A similarly long-lasting pressor response was induced following an initial rapid rise in mean blood pressure after i.v. bolus injections of clonidine (5–50 µg/kg). In pentobarbital-anesthetized rats, the prolonged pressor responses to i.v. and i.c.v. injected clonidine at high doses were significantly smaller than those in conscious rats. Low doses of clonidine caused only depressor responses which developed gradually. No significant changes in concentrations of plasma norepinephrine and epinephrine were found during the pressor period after i.c.v. injection of clonidine (20 µg). Systemic (2 mg/kg, i.v.) or central (100 µg, i.c.v.) pretreatment with phentolamine abolished only the prolonged pressor response to both i.c.v. (20 µg) and i.v. (50 µg/kg) injected clonidine. The prolonged pressor response to clonidine (20 µg, i.c.v.) was enhanced by pretreatment with hexamethonium (25 mg/kg, i.v.), methylatropine (1 mg/kg, i.v.) or atropine (1 mg/kg, i.v.) and it was not affected by pretreatment with saralasin (300 µg/kg and 25 µg/kg/min, i.v.), d(CH₂)₅Tyr(Me)-arginine-vasopressin, a vasopressin antagonist (50 µg/kg, i.v.) or naloxone (1 mg/kg, i.v.). Neither adrenalectomy nor adrenal demedullation had an effect on the pressor response to clonidine (20 µg, i.c.v.). In adrenalectomized rats, systemic pretreatment with hexamethonium (25 mg/kg, i.v.) caused a potentiation of the pressor response to clonidine (20 µg, i.c.v.).
These results suggest that clonidine induces the pressor response through activation of central α-adrenoceptors, probably the α₂ subtype, without an increase in sympathetic-adrenomedullary activity. It is speculated that the response may be mediated by vasoactive humoral substance(s).

It is well-known that clonidine decreases blood pressure and heart rate (HR), which has been demonstrated mostly in anesthetized animals. The mechanisms underlying the cardiovascular effects of clonidine have been postulated to be due mainly to its effects on autonomic pathways of the central nervous system (1–3). In addition to the hypotensive effect, clonidine has been demonstrated to have a hypertensive effect (1, 2), which became more pronounced at higher doses (1, 4). The hypertensive effect of clonidine has generally been considered to be due to a direct stimulation of peripheral α-adrenoceptors by the drug, because the pressor response is blocked by α-adrenoceptor antagonists (1, 5).
On the other hand, several investigators have reported that clonidine injected peripherally or centrally has only a hypertensive effect in conscious, normotensive rats, whereas the drug produces a hypotensive response in rats anesthetized with urethane and pentobarbital (6, 7). These results would suggest that the hypertensive effect of clonidine may be mediated, in part, by a
central pressor mechanism. However, little is known about this pressor effect by clonidine except that a transient increase in blood pressure is seen after intravenous (i.v.) injection of the drug.

There is evidence that centrally administered norepinephrine (NE) elicits a pressor response in conscious animals including the cat (8), monkey (9) and rat (10), which is also attenuated by anesthesia. Thus, these findings indicate that central α-adrenoceptors may be involved in the central pressor mechanisms and that anesthesia interferes with these mechanisms.

The present study was designed, therefore, to investigate the possible mechanism underlying the pressor response to centrally and peripherally administered clonidine in conscious rats. Furthermore, attention was directed toward a possible centrally-mediated hypertensive effect of clonidine.

Materials and Methods

Animals: Male Wistar rats, weighing 320–350 g at the beginning of the experiments, were used. All animals were given food and water ad libitum and housed in an air-conditioned room with a 12 hr light/dark cycle (light on at 7:30 A.M.). After the chronic implantation of catheters, the animals were transferred to individual cages.

Surgery: Under anesthesia with sodium pentobarbital (50 mg/kg. i.p.), the animal’s head was fixed to a stereotaxic instrument. Stainless-steel guide cannulas (0.7 mm in outer diameter) were bilaterally implanted into the lateral cerebroventricle, and the tip of the guide cannula was positioned 2.0 mm dorsal to the intended site of drug injection using the following co-ordinates: 0.0 mm anterior-posterior to the bregma, 1.5 mm lateral to the bregma, 2.2 mm below the surface of the dura mater. Each electrode was fixed to the skull with dental cement and two screws. Each was then soldered to a connector socket which was also covered with dental cement. After surgery, all animals received a s.c. injection of procaine penicillin G (100,000 units. Takeda). The animals were allowed to recovery for a 10 day period before the surgery for chronic catheter implantation.

For the direct recording of blood pressure, the animals with chronic guide cannulas and electrodes were anesthetized with ether. An arterial catheter composed of polyethylene tubing (PE 10 and PE 20) was chronically implanted into the abdominal aorta via the left femoral artery as described in the previous report (12). At the same time, a catheter for i.v. administration of drugs was implanted chronically into the inferior vena cava via the left femoral vein. The remainder of each catheter was passed beneath the skin to emerge on the back of the neck and was plugged with a stainless-steel stopper. Both catheters were previously filled with sterile heparinized-0.9% saline (500 units/ml) and were flushed every 2 days. After surgery, the animal received procaine penicillin G (100,000 units, s.c.). A recovery period of one week after surgery was allowed before commencing the experiment.

In some rats, bilateral adrenalectomy, adrenal demedullation or sham operation was performed under ether anesthesia through a lumbar incision. Adrenalectomy involved isolation, ligation and removal of the entire adrenal gland. Adrenal demedullation involved making a small incision in the adrenal cortex with subsequent expulsion of the adrenal medulla. After surgery, the animals were allowed free access to food and water except for the adrenalectomized animals which were given 1% saline as drinking water. The experiments were done 5–6 days after the surgery.

Recording: On the day of the experiment, the catheter’s stopper was removed and attached into an extension polyethylene tubing (composed of PE 20, PE 120 and PE 190). The tubing had been previously filled
with sterile heparinized-0.9% saline (500 units/ml) and was brought out of a sound-proof box where it was connected to a pressure transducer (P231D, Statham). Arterial blood pressure was recorded on a polygraph (RM-6000, Nihon Kohden). HR was counted by a cardiotachograph (AT-600G, Nihon Kohden) triggered by arterial pulses and was recorded on the polygraph.

For recording of the electroencephalogram (EEG) and the electrical stimulation of the MRF, a lead wire was connected to the connector socket on the animal's head. EEG was monitored and recorded on the polygraph. Electrical stimulation of the MRF was carried out with an electronic stimulator (MSE-3R, Nihon Kohden) through an isolator (SS-101J, Nihon Kohden). Square wave pulses (50 Hz, 0.5 msec, 50–150 μA) were given for 5 sec when required.

After connecting the extension tubing and lead wire, the animal was moved to an open-topped cylindrical Plexiglas cage (30 cm in diameter) which was placed in a shielded, sound-proof box. The arterial blood pressure (pulsatile and mean pressure), HR and EEG were measured simultaneously while behavior was observed through a window of the sound-proof box. Both mean blood pressure (MBP) and HR were digitized by using a minicomputer (ATAC 450, Nihon Kohden) and printed out at 1-min intervals.

**Intracerebroventricular (i.c.v.) or i.v. injection of drugs:** For i.c.v. injection of drugs, the stylet was removed and an injection cannula composed of stainless-steel tubing (0.35 mm in outer diameter) was lowered into the lateral cerebroventricle to a depth of 2 mm beyond the guide cannula. The injection cannula was attached with an extension polyethylene tubing (PE 20) which was filled previously with the drug and was brought out of the sound-proof box and connected to a Hamilton microsyringe (2 μl or 25 μl). The venous catheter was connected to a piece of polyethylene tubing, approximately 60 cm in length (PE 20, 0.2 ml in inner volume), which was previously filled with sterile 0.9% saline. The other end of this tubing was led to the outside of the sound-proof 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 waves) was recorded for over 5 min, i.c.v. or i.v. injection of the drug was carried out. Clonidine or an equivalent amount of 0.9% saline was injected in a volume of 1 μl through the right guide cannula over a period of 10–20 sec by means of a microinjector.

In the experiments using the α-adrenoceptor antagonist, systemic (i.v.) or central (i.c.v.) pretreatment with the antagonist was made 10–15 min before i.c.v. and i.v. injection of clonidine. The central administration of the antagonist or an equivalent amount of vehicle was carried out over a period of 60 sec in a volume of 10 μl through the left guide cannula. The i.v. administration of the drug was performed over 30–60 sec through the extension tubing. After every i.v. injection, the tubing was flushed with 0.2 ml of sterile 0.9% saline.

In the experiments using the anesthetic, the animals with the chronic guide cannulas and catheters were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) 20 min before the drug administration.

At the end of each experiment, the catheters were sealed with stoppers, stylets were inserted into the guide cannulas, and then the animals were returned to their home cages. At 1-week interval, the animals were subjected to i.c.v. or i.v. injection of various drugs. Each animal received only one dose of clonidine and one pretreatment with an antagonist.

**Determination of plasma catecholamines:** When the animal was calm and a drowsy EEG pattern lasted for over 5 min, a blood sample (1 ml) was withdrawn through the arterial catheter as a pre-drug control. After a 20 min equilibration period, i.c.v. injection of clonidine or 0.9% saline was carried out. One ml blood samples were collected 10 and 60 min after the administration. After each collection, blood was replaced by an equivalent amount of sterile 0.9% saline via the venous catheter. Plasma was immediately separated by centrifugation (5000 rpm, 4°C, 20 min) and stored at below −20°C until assayed. Because of the limited amount of plasma obtained from each animal, plasma
samples from two rats (0.5 ml each) were pooled as one sample. According to a modification of the method of Yui et al. (13), NE and epinephrine (Ep) in plasma (1 ml) were purified with alumina and separated by high performance liquid chromatography (LC-3A and LC-1, Shimadzu Seisakusho). The concentrations of NE and Ep were determined spectrofluorometrically using the trihydroxyindole method by means of a spectrofluorophotometer (RF-500 LCA, Shimadzu Seisakusho).

Histology: After completion of the experiment, the animals were anesthetized with large doses of pentobarbital. To determine the site of the injection cannula placement, 5 μl of 0.5% thionine was injected and the brain was removed and tested. The correct guide cannula placement was verified by the diffusion of dye in the ventricle.

Statistical analysis: All values are expressed as the mean±S.E.M. Statistical analysis was performed using Student's t-test for paired and unpaired data. A P value less than 0.05 was considered to be statistically significant.

Drugs: The following drugs were used: angiotensin II (Sigma Chemical Co.), arginine vasopressin (AVP) (Sigma Chemical Co.), atropine methyl nitrate (Sigma Chemical Co.), atropine sulfate (Sigma Chemical Co.), clonidine hydrochloride (a gift from Nippon C.H. Boehringer Sohn Co.), d(CH2)5Tyr(Me)-AVP (kindly provided by Dr. M. Manning of the Department of Biochemistry, Medical College of Ohio, Toledo, OH, U.S.A.), hexamethonium bromide (Sigma Chemical Co.), naloxone hydrochloride (Sigma Chemical Co.), phentolamine mesylate (a gift from Japan Ciba-Geigy, Ltd.), [Sar^1-Ala^8]-angiotensin II (saralasin) (Sigma Chemical Co.), and sodium pentobarbital (Nembutal, Abbott Laboratories).

Clonidine and phentolamine were dissolved in sterile 0.9% saline, and the pH was adjusted to 7.4 when injected into the cerebroventricle. All other drugs were also dissolved in sterile 0.9% saline. All doses of drugs used are given in terms of the salts.

Results

Cardiovascular responses to i.c.v. or i.v. injected clonidine in conscious rats: The i.c.v. injected clonidine (2–20 μg) in conscious rats produced a dose-dependent rise in MBP. The pressor response was initiated within 10 sec; reached a maximum at 3–5 min after the administration and lasted for 30–60 min. However, a significant fall in MBP was not observed for up to 90 min after i.c.v. injection of clonidine at any of the doses used (Figs. 1A and 2). A dose-dependent decrease in HR was produced by i.c.v. injection of clonidine. Bradycardia reached a maximum at 10–15 min after clonidine administration, and HR returned to pre-drug levels 90 min after the administration.

The i.v. bolus injection of clonidine at low doses (5 μg/kg) caused a short-lasting rise in MBP. However, high doses (20 and 50 μg/kg) of clonidine produced a long-lasting pressor response following an initial rapid rise in MBP (Figs. 1B and 3). No significant fall in MBP was observed for up to 90 min after the i.v. administration of clonidine at any of the doses used. All doses of clonidine induced a reflex bradycardia followed by a prolonged decrease in HR which lasted for 30–60 min after the i.v. injection (Fig. 3).

Following i.c.v. or i.v. injection of clonidine, all animals manifested marked sedation with an exophthalmus, and a drowsy EEG pattern was observed for over 90 min. In high doses, urination was always observed during the next 60 min after both i.c.v. and i.v. injection of clonidine.

Effects of repeated i.c.v. injection of clonidine in conscious rats: As shown in Table 1, when the pressor response to the first i.c.v. injection of clonidine (20 μg) subsided 60 min after the injection, the repeated injection of clonidine at the same dose caused a marked pressor response accompanied by bradycardia. There was no tachyphylaxis to clonidine.

When i.c.v. injection (20 μg) of clonidine was repeated at 1-week intervals, each injection of clonidine produced a pressor response of similar magnitude. The peak times and maximum responses for injections at weeks 2–5 were similar to those in the responses to the first injection (Table 1).

Effect of anesthesia on cardiovascular responses to i.c.v. or i.v. injected clonidine:
In pentobarbital-anesthetized rats, i.c.v. injection of clonidine at doses of 2–10 μg produced only a long-lasting fall in MBP concomitant with a decrease in HR (Fig. 2). A modest pressor response followed by a depressor response was induced by a high dose (20 μg) of clonidine. The prolonged pressor response to clonidine at a high dose in anesthetized rats was significantly smaller than that observed in conscious rats.

Fig. 1. Cardiovascular responses to i.c.v. (A) and i.v. (B) injected clonidine in the freely moving, normotensive rat. SMA, spontaneous motor activity measured by a MT-pick up (Nihon Kohden); ECO, electrocorticogram; HR, heart rate; BP, pulsatile blood pressure; MBP, mean blood pressure.

Table 1. Changes in mean blood pressure (MBP) and heart rate (HR) following repeated administration of clonidine (20 μg, i.c.v.) in conscious rats

| i.c.v. Injection | MBP before clonidine | Maximum increase in MBP | Peak time | HR before clonidine | Maximum decrease in HR | Peak time |
|------------------|-----------------------|-------------------------|-----------|---------------------|------------------------|-----------|
| 60 min-intervals |                       |                         |           |                     |                        |           |
| 1st              | 98.8±4.9              | 48.6±2.4                | 2.6±0.2   | 283.6±18.9          | 72.4±8.7               | 10.2±1.2  |
| 2nd              | 106.0±4.7             | 50.8±3.5                | 3.4±0.2   | 294.6±24.5          | 92.6±11.3              | 10.6±0.9  |
| 1 week-intervals |                       |                         |           |                     |                        |           |
| 1st              | 97.4±2.8              | 49.4±4.2                | 4.2±0.7   | 284.6±4.8           | 60.8±5.5               | 8.8±0.7   |
| 2nd              | 92.4±1.3              | 54.8±1.5*               | 2.8±0.4   | 284.8±7.7           | 61.4±5.9               | 10.6±2.2  |
| 3rd              | 99.4±3.6              | 51.2±1.8                | 3.8±0.9   | 276.7±4.4           | 78.2±9.1               | 7.2±1.2   |
| 4th              | 95.8±1.4              | 53.8±2.2                | 2.8±0.5   | 279.4±11.1          | 69.6±10.2              | 7.7±3.8   |
| 5th              | 99.2±2.9              | 48.2±3.0                | 4.2±0.8   | 288.4±12.8          | 76.8±9.1               | 6.0±2.0   |

*P < 0.05 compared with the 1st injection (paired t-test). Data from 5 experiments in each group.
Fig. 2. Changes in mean blood pressure (MBP) and heart rate (HR) after i.c.v. injection of clonidine (CL) in freely moving (conscious, left) and anesthetized (right) rats. The maximal pressor response induced by the 20 μg dose in the conscious rats was significantly greater than that in the anesthetized rat (P<0.01, unpaired t-test). Numbers in parentheses indicate the number of experiments in each group.

Fig. 3. Changes in mean blood pressure (MBP) and heart rate (HR) after i.v. injection of clonidine (CL) in conscious (left) and anesthetized (right) rats. The initial pressor response induced by the 20 μg/kg dose in the anesthetized rats was significantly greater than that in the conscious rat (P<0.05, unpaired t-test). Numbers in parentheses indicate the number of experiments in each group.
When clonidine was intravenously injected in anesthetized rats, an initial rapid rise in MBP was somewhat greater than that in conscious rats as shown in Fig. 3. A prolonged depressor response concomitant with a decrease in HR was induced 1–5 min (5–20 μg/kg) and 20–30 min (50 μg/kg) after clonidine administration (Fig. 3).

Effect of i.c.v. injected clonidine on plasma NE and Ep in conscious rats: Plasma levels of NE and Ep during the pressor period induced by i.c.v. injection of clonidine (20 μg) decreased up to 75% and 45% of the pre-drug control, respectively (Fig. 4). However, there was no statistically significant difference from the pre-drug level and/or 0.9% saline control.

Effect of systemic pretreatment with phentolamine on pressor response to i.v. and i.c.v. injected clonidine in conscious rats: Systemic administration of phentolamine at a dose of 2 mg/kg, i.v., produced a transient fall (20.5±4.5 mmHg, n=12) in MBP associated with a reflex tachycardia (183.3 ±14.0 beats/min, n=12). After a 10 min equilibration period, i.c.v. injection of clonidine at a dose of 20 μg failed to induce a pressor response (Fig. 5A). The initial pressor phase of the response to i.v. injected clonidine at a dose of 50 μg/kg was markedly reduced, but not abolished by phentolamine treatment. The subsequent long-lasting pressor response to clonidine did not occur (Figs. 5B and 7B). A decrease in HR was induced by i.v. or i.c.v. injection of clonidine even when the long-lasting pressor response to clonidine was abolished by systemic pretreatment with phentolamine.

Effect of central pretreatment with phentolamine on pressor response to i.v. or i.c.v. injected clonidine in conscious rats: Central administration of phentolamine at doses of 50 and 100 μg produced a transient fall in MBP (3.8±1.7 mmHg, n=4 and 6.3±2.0 mmHg, n=10, respectively) followed by a sustained elevation of MBP (13.0±3.8 mmHg, n=4 and 14.0±2.0 mmHg, n=10, respectively) and an increase in HR (52.5±15.6 beats/min, n=4 and 78.9±13.6 beats/min, n=10, respectively). After a 15 min equilibration period, the pressor response to i.c.v. injected clonidine (20 μg) was dose-dependently inhibited (Fig. 6A). The initial phase of pressor response to i.v. injection of clonidine (50 μg/kg) was slightly but significantly suppressed, and the subsequent long-lasting pressor response was abolished after central administration of phentolamine (Figs. 6B and 7D). The same dose of phentolamine, when administered intravenously, inhibited slightly but significantly the prolonged pressor response to i.v. injected clonidine without affecting the initial rapid rise in MBP (Fig. 6B). The decrease in HR induced by i.c.v. injection of clonidine was significantly reduced, but not abolished by central pretreatment with phentolamine.

Effects of various drugs and treatments on pressor response to centrally administered clonidine: Tables 2 and 3 summarize the effects of systemic pretreatment with anti-cholinergic drugs, a ganglionic blocker, a vasopressin antagonist, an angiotensin II
antagonist and an opiate antagonist and the effects of adrenalectomy or adrenal de-demullation on the long-lasting pressor response to clonidine (20 μg, i.c.v.) in conscious rats.

The pressor response to clonidine was enhanced by pretreatment with atropine (1 mg/kg, i.v.) or with methylatropine (1 mg/kg, i.v.). Hexamethonium (25 mg/kg, i.v.), in a dose which significantly inhibited the pressor response to stimulation of the MRF (control pressor response, 44.8±1.9 mmHg; pressor response after hexamethonium, 7.8±2.1 mmHg, n=4, P<0.001), significantly potentiated the pressor response to clonidine, whereas the response was abolished by combined pretreatment with hexamethonium and phentolamine (2 mg/kg, i.v.).

The vasopressin antagonist (50 μg/kg, i.v.), in a dose which significantly reduced the pressor response to AVP (0.2 μg/kg, i.v.; control pressor response, 46.7±2.9 mmHg; pressor response after the antagonist, 6.3±7.1 mmHg, n=3, P<0.001), had no effect on the pressor response to clonidine.

Saralasin (300 μg/kg, i.v.) did not affect the pressor response to clonidine, whereas saralasin at the dose used markedly inhibited the pressor response to angiotensin II (0.1 μg/kg, i.v.; control pressor response, 49.7±3.8 mmHg; pressor response after saralasin, 9.3±2.0 mmHg, n=3, P<0.001). Furthermore, the hypertensive effect of clonidine was not affected by a continuous infusion of saralasin (25 μg/kg/min, i.v.). Also, naloxone (1 mg/kg, i.v.) had no effect on the prolonged pressor response to clonidine.

In either the adrenalectomized or adrenal demedullated rats, clonidine produced a marked pressor response. In addition, pretreatment with hexamethonium (25 mg/kg, i.v.) in the adrenalectomized rat caused a significant potentiation of the pressor response to clonidine.

Discussion

The present experiments demonstrated that clonidine, when administered centrally,
Fig. 6. Cardiovascular responses to i.c.v. (20 μg) (A) or i.v. (50 μg/kg) (B) injected clonidine after central pretreatment with phentolamine (50 and 100 μg, i.c.v.) in freely moving rats. *P<0.05, **P<0.01, ***P<0.001, compared with vehicle treatment. Numbers in parentheses indicate the number of experiments in each group. Phent, phentolamine.

Fig. 7. Typical records of effects of systemic (2 mg/kg, i.v.) (left) and central (100 μg, i.c.v.) (right) pretreatment with phentolamine on the cardiovascular and EEG effects induced by i.v. injected clonidine (50 μg/kg) in free moving rats. The systemic and central treatments with phentolamine were carried out at one week after the vehicle treatment in the same animal.
induced a long-lasting pressor response associated with a marked bradycardia in freely moving, normotensive rats. The prolonged hypertensive effect induced by centrally administered clonidine was highly reproducible and no tachyphylactic effect was observed. When clonidine was injected systemically in conscious rats, a similar pattern of pressor response was observed after an initial rapid rise in MBP. However, in anesthetized rats, a pressor response to i.c.v. injected clonidine was markedly attenuated and instead, a depressor response was produced by clonidine, especially in low doses. Furthermore, pentobarbital anesthesia abolished the secondary phase of pressor response to i.v. injected clonidine, whereas the initial rapid rise in MBP was somewhat enhanced under anesthesia. The results observed here are consistent with observations by Trolin (6) and Correa et al. (7), who reported that i.v. or i.c.v. injected clonidine caused only a hypertensive response in conscious rats, whereas anesthetized rats showed a hypotensive response to the low dose of clonidine. In addition, systemic administration of clonidine produces a hypotensive effect in conscious rats with decerebration below the inferior colliculus (6). Because pentobarbital has no effect on the peripheral α-adrenoceptors (14), taken together, the present findings suggest that the long-lasting pressor response to systemically or centrally administered clonidine is central.

Table 2. Effects of systemic pretreatment with various drugs on pressor response to clonidine (20 μg, i.c.v.) in conscious rats

| Pretreatment | Dose | n | MBP before clonidine | Maximum increase in MBP | HR before clonidine | Maximum decrease in HR |
|--------------|------|---|----------------------|------------------------|--------------------|-----------------------|
| **Vehicle**  | 1 ml/kg | 7 | 96.7±4.3 | 54.3±3.5 | 280.0±9.5 | 83.3±5.3 |
| Atropine     | 1 mg/kg | 5 | 101.6±2.7 | 58.0±2.1 | 334.8±12.4** | 70.2±4.2 |
| Methylatropine| 1 mg/kg | 5 | 100.6±2.7 | 63.2±2.6** | 384.2±9.4*** | 96.0±9.1 |
| Hexamethonium| 25 mg/kg | 5 | 82.6±2.0* | 68.0±2.1* | 307.4±4.2* | 83.4±7.5 |
| Hexamethonium + Phentolamine | 2 mg/kg | 3 | 95.7±5.4 | 9.3±2.7*** | 306.0±22.7 | 33.7±8.2*** |
| Saralasin    | 300 μg/kg | 3 | 94.0±1.5 | 49.7±0.3 | 271.0±4.4 | 92.3±15.6 |
| Saralasin    | 25 μg/kg/min | 3 | 85.7±2.7 | 56.7±2.0 | 277.7±6.4 | 98.7±14.9 |
| AVP Ant 50 μg/kg | 4 | 88.0±3.1 | 53.3±2.7 | 298.3±14.3 | 98.0±13.1 |
| Naloxone     | 1 mg/kg | 5 | 90.4±3.1 | 50.6±1.5 | 276.6±5.8 | 71.4±7.1 |

*P<0.05, **P<0.01, ***P<0.001, compared with the vehicle control (unpaired t-test). n, number of experiments in each group. MBP, mean blood pressure; HR, heart rate; AVP Ant, arginine vasopressin antagonist.

Table 3. Effects of adrenalectomy and adrenal demedullation on pressor response to clonidine (20 μg, i.c.v.) in conscious rats

| Treatments                  | n | MBP before clonidine | Maximum increase in MBP | HR before clonidine | Maximum decrease in HR |
|-----------------------------|---|----------------------|------------------------|--------------------|-----------------------|
| **Sham**                    | 5 | 94.0±3.2 | 52.2±3.5 | 265.0±13.1 | 68.4±8.6 |
| Adrenal demedullation       | 4 | 92.0±2.2 | 51.0±6.2 | 269.3±6.3 | 76.3±3.9 |
| Adrenalectomy               | 6 | 83.0±3.5* | 49.3±2.5 | 325.7±9.5** | 88.0±16.8 |
| Adrenalectomy + Hexamethonium (25 mg/kg, i.v.) | 5 | 50.8±6.1*** | 77.4±5.2*** | 275.4±13.0 | 37.4±7.0* |

*P<0.05, **P<0.01, ***P<0.001, compared with sham-operated rats (unpaired t-test). n, number of experiments in each group. MBP, mean blood pressure; HR, heart rate.
in origin.

The pressor response to centrally administered clonidine was abolished by central pretreatment with phentolamine, a mixed $\alpha_1$- and $\alpha_2$-adrenoceptor antagonist, suggesting that the response may be mediated by central $\alpha$-adrenoceptors. Additionally, it should be noted that central pretreatment with phentolamine abolished only the secondary phase of the pressor response to systemically administered clonidine. Because systemic pretreatment with phentolamine at the same dose was less effective in reducing the long-lasting pressor response to i.v. injected clonidine, the possibility of some leakage of phentolamine into the systemic circulation is unlikely. This implies that the interaction seems to occur within the brain. Because clonidine readily penetrates through the blood-brain barrier (15), it appears that central $\alpha$-adrenoceptors may be involved in the prolonged pressor response to systemically administered clonidine except for the initial rapid rise in MBP, which results from a direct stimulant action of the drug on peripheral postsynaptic $\alpha$-adrenoceptors (1).

Systemic pretreatment with phentolamine completely inhibited the long-lasting pressor response both to i.v. and i.c.v. injected clonidine, but not the initial rapid rise in MBP after i.v. injection of the drug, showing that the pressor response to i.c.v. injected clonidine was more sensitive to phentolamine. This is in agreement with the results reported by Correa et al. (16), who demonstrated that i.v. treatment with phenoxybenzamine induces a greater inhibition of the pressor response to centrally administered NE than that to systemically administered NE. Phentolamine has been shown to cross the blood-brain barrier (16). Therefore, the blockade by systemically administered phentolamine may indicate an action at a central level after crossing the blood-brain barrier, but not an action at the peripheral level.

In the present study, hexamethonium, a ganglionic blocker, failed to inhibit the prolonged pressor response to centrally administered clonidine. In fact, a potentiation occurred. Because hexamethonium at the dosage used abolished the hypertensive response to the brain stimulation, contribution of increased sympathetic outflow to the prolonged pressor response induced by centrally and systemically administered clonidine is unlikely. This contention is strongly supported by several of the present findings. No increase in plasma catecholamines was found during the pressor period after i.c.v. injection of clonidine. Additionally, the pressor response to centrally administered clonidine was not affected by either adrenalectomy or adrenal demedullation or even by pretreatment with hexamethonium in the adrenalectomized rat. Therefore, these results give rise to speculation that the long-lasting pressor response induced by clonidine may be mediated by vasoactive humoral substance(s). In fact, Correa et al. (16) reported that the pressor response to i.c.v. injected NE was not blocked by a ganglionic blocker but inhibited by a vasopressin antagonist, suggesting an involvement of a vasoactive substance, probably the increased vasopressin release. The enhanced pressor response to centrally administered clonidine seen after treatment with the ganglionic blocker seems to be due to the decreased cardiovascular reflex and inhibition of the decreased central sympathetic outflow by clonidine.

Central cholinergic stimulation by muscarinic agonists or acetylcholinesterase inhibitors elicits a hypertensive response in both conscious and anesthetized rats; an effect which is blocked by atropine but not by methylatropine (17). A recent report has revealed that in conscious rats, the pressor response to i.c.v. injection of carbachol, a cholinergic agonist, is involved in the increased sympathetic activity and vasopressin release (18). The present study demonstrated that the pressor response to centrally administered clonidine was not inhibited by the anticholinergic drugs, by a specific vasopressin antagonist or by an angiotensin II antagonist. Thus, an involvement of central cholinergic nerves is ruled out. Also, it is unlikely that an increase in the concentration of plasma vasopressin or angiotensin II contributed to the prolonged pressor response to clonidine. This is supported further by the reports that clonidine causes the suppression of vasopressin secretion (19) and renin secretion (20).
Naloxone, an opiate antagonist, has been shown to inhibit and reverse the fall in blood pressure induced by clonidine in humans and rats with hypertension (21), although a recent report proposed no contribution of endogenous opiates to the clonidine-induced hypotension (22). In the present experiments, naloxone was ineffective in antagonizing the pressor response to centrally administered clonidine. Thus, an involvement of endogenous opioid peptides and opiate receptors in the long-lasting pressor response to clonidine is excluded.

Clonidine is a preferential $\alpha_2$-adrenoceptors agonist, although it has an agonist action on $\alpha_1$-adrenoceptors (23). We have reported that in conscious rats, i.c.v. injection of BHT-920, a selective $\alpha_2$-adrenoceptor agonist, produced a dose-dependent pressor response, whereas i.c.v. injection of methoxamine, a selective $\alpha_1$-adrenoceptor agonist, caused a slight pressor response even at a dose of 100 $\mu$g (24). Furthermore, the pressor response to i.c.v. injected clonidine was antagonized by central pretreatment with yohimbine, an $\alpha_2$-adrenoceptor antagonist, but not with prazosin, a selective $\alpha_1$-adrenoceptor antagonist (24). Therefore, it appears that central $\alpha_2$-adrenoceptors are mainly responsible for the prolonged pressor response to clonidine. Thus, the activation of central $\alpha_2$-adrenoceptors may evoke the release of a vasoactive humoral substance(s), which appears to differ from well-known pressor substances such as angiotensin II, vasopressin and catecholamines.

A selective $\alpha_2$-adrenoceptor agonist such as clonidine and BHT-920, when injected systemically and/or centrally, has been shown to induce a decrease in arterial blood pressure, which has been demonstrated mostly in anesthetized animals. This effect is blocked by the $\alpha$-adrenoceptor antagonist (1–3). It generally has been accepted that the hypotensive effect of these agonists is exerted at sites within the central nervous system, probably at structures in the medulla oblongata, where they induce an inhibition of sympathetic outflow from the brain (1–3). The present finding that clonidine is hypotensive under anesthesia is in agreement with these reports, whereas the drug has only a hypertensive effect in conscious animals, which is in line with the reports of Trolin (6), Correa et al. (7) and Kawasaki and Takasaki (24). Although this apparent discrepancy is difficult to explain, some possible interpretations might be suggested. The activation of central $\alpha_2$-adrenoceptors induces not only a decrease in blood pressure by an inhibition of central sympathetic outflow but also a hypertensive response through the release of a vasoactive substance(s) without an activation of the sympathoadrenal system. Pentobarbital probably suppresses the central pressor factor by inhibiting a polysynaptic pathway which may mediate the vasoactive substance(s) release, and consequently, the reduction of central sympathetic outflow is predominant, leading to the fall in blood pressure. On the other hand, in conscious animals, this humoral factor is released. If the vasoactive substance(s) evokes postsynaptically vasoconstriction, the blood pressure should increase even though the central sympathetic outflow is being reduced by clonidine.

In conclusion, the present results suggest that clonidine produces the centrally-mediated hypertensive response through a stimulant action of central $\alpha_2$-adrenoceptors. It is also suggested that this hypertensive effect does not involve an increase in sympathoadrenal activity and may be mediated by a vasoactive humoral substance(s). Obviously, additional studies are needed to determine whether the vasoactive substance(s) is released by activation of central $\alpha_2$-adrenoceptors.

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