Hypotensive and sedative effects of clonidine injected into the rostral ventrolateral medulla of conscious rats

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Yamazato, Masanobu, Atsushi Sakima, Jun Nakazato, Shogo Sesoko, Hiromi Muratani, and Koshiro Fukiyama. Hypotensive and sedative effects of clonidine injected into the rostral ventrolateral medulla of conscious rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1868–R1876, 2001.—We examined the effects of clonidine injected unilaterally into the rostral ventrolateral medulla (RVLM) of conscious, unrestrained rats. We also examined whether the local α2-adrenoceptor mechanism contributed to the action of clonidine injected into the RVLM. Injection of clonidine but not vehicle solution significantly decreased the mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) in conscious, unrestrained rats as well as in propofol-anesthetized rats. The frequency of natural behavior was significantly lower after clonidine injection than after vehicle injection. The depressor and sympathoinhibitory responses were significantly larger after clonidine injection than after vehicle injection. Coinjection of a selective α2-adrenoceptor antagonist, 2-methoxydazoxan, with clonidine into the RVLM significantly attenuated the depressor, bradycardic, sympathoinhibitory, and sedative effects of clonidine injected alone. In conclusion, clonidine injected into the RVLM decreased MAP, HR, and RSNA and caused sedation in conscious, unrestrained rats. The action of clonidine in the RVLM was at least partly mediated by α2-adrenoceptor mechanisms.

The rostral ventrolateral medulla (RVLM) is a vasomotor center where cardiovascular sympathetic premotor neurons are located (7, 34). The neurons in the RVLM directly innervate sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord and also innervate to supramedullary brain regions (7). The neurons generate sympathetic tone and also participate in reflex control of the cardiovascular system. The RVLM neuron has been regarded as the main site of the hypotensive action of clonidine (34). Radioligand binding studies revealed clonidine-binding sites in the RVLM (13). Imidazoline receptors and α2-adrenoceptors exist on the RVLM neurons (13, 28). Microinjection of clonidine into the RVLM of anesthetized animals causes long-lasting hypotensive and sympathoinhibitory effects (9, 13, 23). Previous studies suggested that the sympathoinhibitory action of clonidine in the RVLM was mediated by the local α2-adrenoceptors (16) or novel I1-imidazoline receptors (14) or both (19). The functional predominance of either α2-adrenoceptors or I1-imidazoline receptors remains to be elucidated (27).

Cardiovascular, sympathetic, and behavioral responses to various drugs are influenced by anesthetics. For example, urethane attenuates cardiovascular responses of central α2-adrenoceptor stimulation (2), and pentobarbital sodium anesthesia enhances the cardiovascular effects of rilmenidine and clonidine in rats (31). In fact, in pentobarbital sodium-anesthetized rats, intracerebroventricular administration of clonidine decreased arterial pressure, whereas in conscious rats, intracerebroventricular administration of clonidine increased arterial pressure (21). To the best of our knowledge, all previous microinjection studies of clonidine in the RVLM were conducted on anesthetized animals.

We examined the cardiovascular, sympathetic, and behavioral effects of clonidine microinjected unilaterally into the RVLM of conscious, unrestrained rats. We also examined whether the local α2-adrenoceptors contributed to the action of clonidine microinjected into the RVLM.
METHODS

Animals. Male Sprague-Dawley rats (340–550 g) were purchased from Charles River Japan and fed standard laboratory rat chow and tap water ad libitum. The rats were kept in a room maintained at constant temperature (24 ± 2°C) and humidity (55 ± 10%) under a 12-h light period between 0800 and 2000. After 7 days of adaptation to these conditions, the experimental procedures were performed. All procedures were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The protocol was approved by the Animal Care and Use Committee, University of the Ryukyus.

Implantation of guide cannula. Each rat was anesthetized with an intraperitoneal injection of 50 mg/kg of pentobarbital sodium, and the rats were placed on a stereotaxic frame (Narishige Scientific Instruments, Tokyo, Japan) in a prone position. An incisor bar was set at 4 mm below the interaural line. The skin overlying the midline of the skull was incised, and a small hole was drilled to the dorsal surface of the cranium according to the following coordinates: 2.0 mm lateral to the midline and 3.5 mm posterior to the lambdoid suture. Two screws were fixed to the cranium beside the hole. A 25-gauge stainless steel guide cannula was lowered 6 mm vertically from the skull surface, and the tip was placed 4 mm above the left RVLM according to the coordinates of Paxinos and Watson (25). The guide cannula was fixed to the skull with screws, and the screws were hardened to the skull using cyanoacrylate adhesive (Aron Alpha: Toa Gosei Chemical Industries, Tokyo, Japan) and secured with dental cement. Before and after surgery, each rat received an intramuscular injection of 20,000 U/kg body wt of penicillin G for prophylaxis.

Implantation of arterial and venous catheters and the renal nerve electrode. At least 7 days after implantation of the guide cannula, rats were reanesthetized with pentobarbital sodium, and vascular catheters (PE-10 fused with PE-50) were inserted through the right femoral artery and vein for blood pressure recording and drug administration, respectively. The left renal nerves were exposed through a retroperitoneal approach. A branch of the nerves was separated from surrounding connective tissue, and a bipolar silver wire electrode (no. 7855; A-M Systems, Carlsborg, WA) was placed under the nerve branch. When an optimal neurogram was obtained, the nerve and electrode were embedded in silicone gel (Semicosil 932; Wacker, Munich, Germany) and allowed to harden. Catheters and lead wires from the electrode were exteriorized at the interscapular region through a subcutaneous tunnel and fixed to the skin. After surgery, each rat received an intramuscular injection of 40,000 U/kg body wt of penicillin G for prophylaxis.

Microinjection procedure. Drugs were microinjected unilaterally into the RVLM. The injection volume was always 200 nl on the basis of previous studies of conscious rats (15, 30). A 33-gauge stainless steel injector needle glued with a 27-gauge stainless steel connector was used in the experiment. The injector was connected via a polyethylene tube (both ends of PE-10 were fused with PE-20) to a Hamilton microsyringe (5 μl). The injections were delivered by hand, and the injection volume was measured by observing the movement of the fluid meniscus in the PE-10 tube marked with a scale of every 200-nl volume.

Unilateral injection of clonidine into the RVLM of conscious, unrestrained rats. At least 24 h after the implantation of the arterial and venous catheters and the renal nerve electrode, the rat was placed in an 18-cm-diameter plastic bowl and was allowed to move freely. During the recording period, acoustic disturbances were avoided, and the room was kept at constant temperature and with a moderate degree of illumination. After a stabilization period of at least 30 min, arterial catheter and lead wires from the electrode were connected to a pressure transducer (P10EZ; Spectramed, Tokyo, Japan) and biophysical amplifier (DPA-100E; Dia Medical System, Tokyo, Japan), respectively. The original renal nerve signals were amplified and filtered between 100 and 1,000 Hz. The amplified nerve pulses were counted with a spike counter (DSE-325A; Dia Medical System). The number of nerve spikes per second was continuously displayed on a chart recorder (RJG-4128; Nihon Kohden, Tokyo, Japan) together with pulsatile pressure, mean arterial pressure (MAP), and heart rate (HR), which was derived from blood pressure signals. The number of spikes per second after intravenous administration of 40 mg/kg of hexamethonium was determined as background noise level. Changes in renal sympathetic nerve activity (RSNA) were expressed as percent changes from baseline spike counts. After recording resting MAP, HR, and RSNA for at least 30 min, a 33-gauge stainless steel injector needle was inserted through each guide cannula previously fixed to the skull of the rat. The injector needle was extended 4 mm beyond the tip of the guide cannula. Blood pressure, HR, and RSNA were then allowed to stabilize for at least 20 min before the first injection was made. The RVLM was identified by the response to a unilateral injection of 2 nmol of L-glutamate on the basis of the modified criteria outlined previously (37): 1) the latency of the onset of changes in MAP was no more than 5 s, 2) the response plateau occurred within 20 s, and 3) the change in MAP was at least 25 mmHg. After identification of the RVLM, the injector needle was replaced by another containing clonidine solution or the vehicle solution. After a 20-min period for restabilization, 3 nmol of clonidine or vehicle solution was then injected unilaterally into the RVLM of the conscious, unrestrained rat. The injector needle was removed 3 min after the injection. MAP, HR, and RSNA were continuously recorded for 60 min. During the recording period, the onset and the end of each natural behavior, grooming and exploring, that lasted more than 30 s were marked on the chart to examine frequency and duration of the behavior. Grooming was defined as the animal scratching, licking, or rubbing any part of its body, and exploring was defined as the animal sniffing and standing on its hindlimbs to look outside the bowl (36). The preparation and microinjection procedures of conscious, unrestrained rats have been described in detail elsewhere (30).

Unilateral injection of clonidine into the RVLM of propofol-anesthetized rats. To compare the cardiovascular and sympathetic effects of clonidine injected into the RVLM between the conscious rat and the anesthetized rat, a separate group of rats with the guide cannula fixed to the skull 7 days previously was anesthetized with propofol. In these experiments, anesthesia was induced with an intraperitoneal injection of pentobarbital sodium. Right femoral artery and bilateral femoral veins were cannulated for monitoring arterial pressure and drug administration, respectively, and the electrode was placed under the renal nerve branch. As the effects of the pentobarbital sodium began to disappear, propofol infusion was started. After the surgical procedures, continuous infusion of propofol was reduced at a rate of 20 mg·kg⁻¹·h⁻¹, and a stabilization period of at least 30 min was taken. Baseline MAP, HR, and RSNA were then recorded. Drugs were unilaterally injected into the RVLM by using a 33-gauge stainless steel injector needle. The surgical preparation and microinjection procedures were the same as in the conscious rats. The RVLM was identified by injection of 2 nmol of

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l-glutamate on the basis of the same criteria listed above. MAP, HR, and RSNA were continuously recorded for 60 min after injection of 3 nmol of clonidine or vehicle.

Coinjection of clonidine and 2-methoxyidazoxan unilaterally into the RVLM of conscious, unrestrained rats. To clarify whether the effects of clonidine injected into the RVLM of conscious, unrestrained rats were mediated by the local α2-adrenoceptors, a selective α2-adrenoceptor antagonist was coinjected with clonidine into the RVLM. The dose of each drug was chosen on the basis of previous studies (18, 20). After identification of the RVLM, 2 nmol of clonidine alone or 2 nmol of 2-methoxyidazoxan together with 2 nmol of clonidine was injected unilaterally into the RVLM of conscious, unrestrained rats. MAP, HR, and RSNA were continuously recorded for 60 min. Onset and end of natural behaviors were marked on the chart.

Unilateral blockade of α2-adrenoceptors in the RVLM of conscious, unrestrained rats. To clarify whether the α2-adrenoceptors in the RVLM contributed to the ongoing levels of MAP, HR, RSNA, and natural behaviors of conscious, unrestrained rats, 2-methoxyidazoxan was injected into the RVLM. After identification of the RVLM, 2 nmol of 2-methoxyidazoxan or vehicle was injected unilaterally into the RVLM of conscious, unrestrained rats. MAP, HR, and RSNA were continuously recorded for 60 min. The onset and the end of natural behaviors were marked on the chart.

Drugs. Drugs were dissolved in artificial cerebrospinal fluid (aCSF; 133.3 mol/l sodium chloride, 3.4 mmol/l potassium chloride, 1.3 mmol/l calcium chloride, 1.2 mmol/l magnesium chloride, 0.6 mmol/l sodium dihydrogen orthophosphate, 32.0 mmol/l sodium bicarbonate, and 3.4 mmol/l glucose). Concerning the coinjection of 2-methoxyidazoxan and clonidine and the injection of 2-methoxyidazoxan alone, 5% glucose solution was used as the vehicle. Mixed drugs for coinjection were always prepared just before the microinjection. Clonidine was purchased from Research Biochemicals International (Natick, MA). 2-Methoxyidazoxan was purchased from Sigma Chemical (St. Louis, MO).

Histological examinations. At the end of the experiments, rats were anesthetized with pentobarbital sodium and received an injection of 200 nl of Alcian blue dye to mark the injection site. Rats were then perfused transcardially with 50 ml of 0.9% sodium chloride followed by 100 ml of 10% phosphate-buffered formalin. The brain stem was removed and stored in 10% phosphate-buffered formalin. The day before sectioning of the brain tissue, the brain stem was transferred to a fixative containing 20% succrose. Frozen brain tissues were sectioned in the coronal plane (50 μm) and stained with neutral red. Microinjection sites were identified by the deposition of Alcian blue dye and referred to standard anatomic structures of the rat medulla oblongata according to the atlas of Paxinos and Watson (25). We excluded those findings from further analysis when the Alcian blue dye penetrated to the ventral surface or apparent bleeding was observed.

Statistical analysis. Values are expressed as means ± SE. Differences among the groups were tested by two-way ANOVA with or without repeated measures. Subsequent analysis for significant difference was performed using Scheffe’s F test. Differences between two groups were tested by unpaired Student’s t-test. A value of P < 0.05 was considered significant.

RESULTS

Effects of clonidine injected unilaterally into the RVLM of conscious, unrestrained rats. The microinjection of 3 nmol of clonidine into the RVLM was performed in eight conscious, unrestrained rats. After insertion of the 33-gauge injector needle toward the RVLM, slight pressor and bradycardiac changes were typically observed. Mostly within 20 min, the rat became undisturbed and calm inside the bowl, and both MAP and HR became stable. Preinjection values of MAP and HR and maximal changes in values of MAP, HR, and RSNA after injection of the drugs are shown in Table 1. Typical traces of pulsatile arterial pressure, MAP, HR, and RSNA recorded for 60 min after clonidine injection into the RVLM of a conscious rat and the marked periods of natural behaviors that occurred after clonidine injection are shown in Fig. 1. Those traces and marked periods of natural behavior after aCSF injection are shown in Fig. 2. Unilateral injection of 3 nmol of clonidine but not aCSF into the RVLM gradually decreased MAP, HR, and RSNA in conscious, unrestrained rats. The depressor response peaked at 17 ± 3 min and returned to preinjection values within 45–60 min in five rats, and the response of the other three rats continued for >60 min. The time course of the MAP, HR, and RSNA responses to clonidine injection is shown in Fig. 3. The frequency of natural behaviors was significantly (P < 0.01) lower after clonidine injection than after vehicle injection. The duration of each behavior and changes of MAP, HR, and RSNA in response to natural behaviors were similar between clonidine-injected rats and vehicle-injected rats (Table 2).

Table 1. Preinjection values of MAP and HR, and maximal changes in the values of MAP, HR, and RSNA after injection of drugs

<table>
<thead>
<tr>
<th>Preinjection</th>
<th>Maximal Changes After Injection of Drugs</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>HR, beats/min</td>
</tr>
<tr>
<td>Conscious rats</td>
<td>Clonidine (n = 8)</td>
</tr>
<tr>
<td></td>
<td>aCSF (n = 8)</td>
</tr>
<tr>
<td>Propofol-anesthetized rats</td>
<td>Clonidine (n = 5)</td>
</tr>
<tr>
<td></td>
<td>aCSF (n = 5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of rats. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; aCSF, artificial cerebrospinal fluid. *P < 0.05 propofol vs. conscious; †P < 0.05 conscious + aCSF vs. conscious + clonidine; ‡P < 0.05 propofol + aCSF vs. propofol + clonidine; §P < 0.05 conscious + clonidine vs. propofol + clonidine.
Effects of clonidine microinjected unilaterally into the RVLM of propofol-anesthetized rats. The microinjection of 3 nmol of clonidine into the RVLM was performed in five propofol-anesthetized rats. Preinjection MAP was significantly ($P < 0.05$) lower in the propofol-anesthetized rats than that in the conscious rats, whereas HR was similar in the propofol-anesthetized rats and the conscious rats (Table 1). The unilateral injection of 3 nmol of clonidine into the RVLM decreased MAP, HR, and RSNA in the propofol-anesthetized rats. The depressor response peaked at 18 ± 2 min and returned to preinjection levels within 55 min in one rat. The depressor response continued for more than 60 min in four rats. The time course of the MAP, HR, and RSNA responses to clonidine injection is shown in Fig. 3. The depressor and sympathoinhibitory responses to clonidine injection were significantly larger in the propofol-anesthetized rats than in the conscious rats, whereas the bradycardiac response was similar in the conscious rats and the propofol-anesthetized rats (Table 1).

Effects of coinjection of clonidine and 2-methoxyida-
zoxan unilaterally into the RVLM of conscious, unrestrained rats. To examine whether local $\alpha_2$-adrenoceptors contribute to the action of clonidine in the RVLM, coinjection of 2-methoxyidazoxan, a selective $\alpha_2$-adrenoceptor antagonist, with clonidine into the RVLM was performed in six conscious, unrestrained rats. Preinjection values of MAP and HR; changes in values of MAP, HR, and RSNA; and the frequency of natural behavior after injection of the drugs are shown in Table 3. The unilateral injection of 2 nmol of clonidine alone into the RVLM decreased MAP, HR, and RSNA. Coinjection of 2 nmol of 2-methoxyidazoxan with 2 nmol of clonidine into the RVLM of conscious rats significantly ($P < 0.05$) attenuated the depressor, bradycardiac, and sympathoinhibitory effects of clonidine injected alone. The frequency of the natural behaviors was significantly ($P < 0.01$) higher after coinjection of 2-methoxyidazoxan with clonidine injection than after clonidine injection alone.
Effects of unilateral blockade of $\alpha_2$-adrenoceptors in the RVLM of conscious, unrestrained rats. To clarify whether the $\alpha_2$-adrenoceptors in the RVLM contributed to the ongoing levels of MAP, HR, RSNA, and natural behaviors, microinjection of 2 nmol of 2-methoxyidazoxan into the RVLM was performed in six conscious, unrestrained rats. Preinjection values of MAP and HR; changes in values of MAP, HR, and RSNA; and the frequency of natural behavior after injection of the drugs are shown in Table 3. The unilateral injection of 2 nmol of 2-methoxyidazoxan or 5% glucose solution into the RVLM caused no significant change in MAP, HR, and RSNA. The frequency of the natural behaviors was similar after 2-methoxyidazoxan injection and after vehicle injection.

Histological analysis. A typical Alcian blue dye injection site of conscious rats is shown in Fig. 4, right. The injection sites were restricted to an area encompassing the dorsolateral aspect of the lateral paragangocellular nucleus (LPGi) and the region dorsolateral to the LPGi. This area lies at the caudal end of the facial nucleus, which is known as the pressor area of the RVLM.

In the present experiment, the percentage of failures or missing experiments was ~35%. Main causes of these failures were obstruction or bending of guide cannula, brain hemorrhage after insertion of the injector needle, or failure to meet our criteria of MAP responses of injected L-glutamate.

**DISCUSSION**

The principal observations in the present study were that clonidine injected unilaterally into the RVLM decreased MAP, HR, and RSNA in conscious, unrestrained rats as well as in propofol-anesthetized rats. Clonidine injection into the RVLM decreased the frequency of natural behaviors such as grooming and exploring. The actions of clonidine were attenuated by coinjection of 2-methoxyidazoxan, a selective $\alpha_2$-adrenoceptor antagonist.

Clonidine, an imidazoline receptor and $\alpha_2$-adrenoceptor agonist, elicits hypotensive and sedative effects by acting on the central nervous system. Intravenous administration of clonidine increases blood pressure transiently and then gradually decreases peripheral sympathetic tone and blood pressure (29, 35). The sympathoinhibitory and depressor effects of clonidine were mediated by the action within the central nervous system (29). The RVLM has been nominated as the main site of action of clonidine in the central nervous system (13, 29, 34). Blockade of $\alpha_2$-adrenoceptors/imi-

![Fig. 3. Time course of changes in MAP, HR, and RSNA after unilateral microinjection of clonidine (3 nmol/200 nl) into the RVLM of conscious and propofol-anesthetized rats. *$P < 0.05$, conscious rats vs. anesthetized rats.]

**Table 2. Frequency of natural behaviors, duration of each behavior, and changes in MAP, HR, and RSNA in response to natural behaviors**

<table>
<thead>
<tr>
<th></th>
<th>Frequency, times/h</th>
<th>Duration, min</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RSNA, %</th>
</tr>
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<tbody>
<tr>
<td>Clonidine (n = 8)</td>
<td>2.5 ± 0.3*</td>
<td>4.7 ± 4</td>
<td>12 ± 1</td>
<td>54 ± 7</td>
<td>169 ± 24</td>
</tr>
<tr>
<td>aCSF (n = 8)</td>
<td>4.1 ± 0.4</td>
<td>4.7 ± 4</td>
<td>15 ± 1</td>
<td>64 ± 6</td>
<td>147 ± 11</td>
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Values are means ± SE; n = no. of rats. *$P < 0.05$ aCSF vs. clonidine.

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azoline receptors in the RVLM eliminates the hypotensive effects of systemic clonidine (17). Radioligand binding studies revealed clonidine binding sites in the RVLM (13). Imidazoline receptors and \( \alpha_2 \)-adrenoceptors exist on the RVLM neurons (13, 28). \( \alpha_2 \)-Adrenoceptors have been detected in the RVLM by membrane binding studies (13), immunohistochemistry (28), in situ hybridization (39), and electrophysiology (1). Microinjection of clonidine and other \( \alpha_2 \)- and/or imidazoline receptor agonists into the RVLM of anesthetized animals either unilaterally or bilaterally caused long-lasting sympathoinhibitory, hypotensive, and bradycardiac effects (9, 13, 23). In the present study, we injected 3 nmol of clonidine unilaterally into the RVLM of conscious, unrestrained rats, a dose based on the findings of previous studies performed on anesthetized rats (9, 13). In conscious rats, the unilateral injection of 3 nmol of clonidine into the RVLM also caused sympathoinhibitory, hypotensive, and bradycardiac effects. The depressor and sympathoinhibitory responses to clonidine injection were significantly larger in the propofol-anesthetized rats than in the conscious rats. These findings suggested that propofol anesthesia enhanced the depressor and sympathoinhibitory action of clonidine injected into the RVLM of rats. We recently reported that urethane anesthesia reduced the magnitude of sympathetic responses to glutamate and glycine injected into the RVLM (30). Taken together, anesthetic agents may variously influence the sympathetic responses evoked by neuroexcitatory or inhibitory drugs injected into the RVLM.

Clonidine acts on the RVLM. Phenotypically identified catecholaminergic and noncatecholaminergic neurons in the RVLM were equally inhibited by systemically administered clonidine (32). Either \( \alpha_2 \)-adrenoceptors (16) or novel \( I_1 \)-imidazoline receptors (14) or both (19) may be responsible for the sympathoinhibitory action of clonidine in the RVLM. The central administration of an imidazoline receptor antagonist, either intracerebroventricularly (6) or by microinjection into the RVLM (26), blocked the antihypertensive actions of systemically administered clonidine and/or rilmenidine or moxonidine. A number of selective \( \alpha_2 \)-antagonists appeared to have weak or no blocking effects on the action of peripherally administered clonidine or its related compounds (24). These studies (6, 24, 26) suggested the functional dominance of the imidazoline receptors. However, discharges of neurons in the RVLM that expressed \( \alpha_2 \)-adrenoceptors were inhibited by systemic and/or iontophoretic application of either catecholamines or clonidine (1). Transgenic mice express-

| Clonidine (2 nM) | 6 | 105 ± 4 | 335 ± 21 | −12 ± 3 | −40 ± 12 | −79 ± 7 | 2.5 ± 0.4 |
| Clonidine (2 nM) + 2-MI (2 nM) | 6 | 98 ± 4 | 381 ± 29 | −4 ± 2* | 2 ± 13* | −20 ± 10* | 5.2 ± 0.5* |
| 2-MI (2 nM) | 6 | 99 ± 4 | 376 ± 10 | −1 ± 3 | −6 ± 7 | −4 ± 4 | 4.8 ± 0.9 |
| 5% Glucose (200 nl) | 6 | 109 ± 3 | 364 ± 19 | −2 ± 2 | −10 ± 22 | −5 ± 7 | 4.8 ± 0.5 |

Values are means ± SE; \( n = \) no. of rats. *\( P < 0.05 \) clonidine vs. clonidine + 2-methoxyidazoxan (2-MI).

Fig. 4. Clonidine injection sites of conscious and propofol-anesthetized rats. Right: typical Alcian blue dye injection site (arrow) of a conscious rat. Left: •, injection sites of conscious rats; ○, injection sites of propofol-anesthetized rats. Amb, nucleus ambiguus; NTS, nucleus of the solitary tract.
ing mutated α2A-adrenoceptor, with intact α2B- and α2C-
adrenoceptor subtypes of α2-adrenoceptor, were reported
to lack hypotensive responses to imidazoline analogs (22).
In the present study, we used 2-methoxyidazoxan as a
selective α2-adrenoceptor antagonist. This drug reported an
~200-fold higher affinity for α2-adrenoceptor than
imidazoline receptor (12) and was used as the α2-adreno-
ceptor antagonist (18, 19). The sympatholytic, hypoten-
sive, bradycardiac, and sedative effects of clonidine microinjected into the RVLM were attenuated by coinjection of 2-methoxyidazoxan in conscious, unrestrained rats. The results of the present study are quite different from those of Ernsberger et al. (13), who reported that in anesthetized rats the drug SKF-86466, a selective α2-adrenoceptor antagonist, had no effect on the hypotensive response to clonidine in the RVLM. Compared with SKF-86466, 2-methoxyidazoxan has 15 times higher affinity to the α2-adrenoceptor (12). We used 2 nmol of 2-methoxyidazoxane, whereas Ernsberger et al. (13) used an even smaller dose, namely 1 nmol, of SKF-86466. Furthermore, they used urethane as the anes-
thetic. Urethane is reported to attenuate the central α2-adrenoceptor-mediated response (2). We suppose that in the study of Ernsberger et al. (13), the use of SKF-
86466 in a smaller dose in urethane-anesthetized rats
masked the effect of the α2-adrenoceptor antagonist on
the hypotensive response to clonidine in the RVLM. The findings of the present and previous studies (1, 22) sup-
port the notion that α2-adrenoceptors in the RVLM, at
least in part, contribute to the action of clonidine in the
RVLM. Head et al. (19) proposed a hypothesis that imi-
dazoline receptors are presynaptic on noradrenergic ter-
minals, whereas α2-adrenoceptors lie downstream on cell bodies or other nonadrenergic terminals.

The magnitude of the depressor response evoked by clonidine in both the conscious and propofol-anesthe-
tized rats was less than in previous studies in which similar doses of clonidine were injected into the RVLM
of anesthetized rats (9, 13). Drolet et al. (9) used sponta-
neously hypertensive rats, whereas we used normo-
tensive Sprague-Dawley rats in the present study. The
difference in the baseline blood pressure levels could
have affected the magnitude of the depressor responses
evoked by clonidine. We performed unilateral injection
of clonidine into the RVLM, whereas Ernsberger et al.
(13) performed bilateral injection. Compared with uni-
ilateral injection, bilateral injection of clonidine is ex-
pected to cause more profound sympathoinhibition
that yields a larger depressor response.

We also examined whether the action of the α2-ad-
renoceptors in the RVLM contributed to the ongoing
levels of MAP, HR, RSNA, and natural behaviors. The
injection of 2 nmol of 2-methoxyidazoxan alone into the
RVLM caused negligible effects on MAP, HR, RSNA,
and natural behavior in conscious, unrestrained rats.
These findings do not support the significant role of the
local α2-adrenoceptors in maintaining the ongoing ac-
tivity of the RVLM neurons. In the present study, howev-
however, we only used one dose of the α2-antagonist.
We could not exclude the possibility that higher doses of 2-methoxyidazoxan blocked the ongoing activity of
the RVLM neurons, resulting in cardiovascular and
sympathetic responses. In urethane-anesthetized spontaneously hypertensive rats, microinjection of 4
nmol of efaroxan, an α2-antagonist with relatively
higher affinity for I1-imidazoline receptors, into the
bilateral RVLM increased MAP and HR, whereas 10
nmol of SKF-86466, a selective α2-antagonist, did not
change baseline MAP and HR (17). In the caudal ven-
trolateral medulla, microinjection of 2 nmol of SKF-
86466 decreased MAP and RSNA in urethane-anesthe-
tized rats (33). These previous studies (17, 33) sug-
gested that the imidazoline receptor with or without
the α2-adrenoceptor mechanism in the RVLM and in the caudal ventrolateral medulla tonically regulated
blood pressure in urethane-anesthetized rats.

Another important observation of the present study
was the attenuation of the natural behaviors after the
clonidine injection into the RVLM. The suppression of
the frequency of natural behaviors was blocked by
coinjection of α2-adrenoceptor antagonist. We do not
have a clear explanation for the mechanism of this
observation. Decreasing arterial pressure in a normo-
tensive animal might reduce the occurrence of natural
behaviors. In our previous study (30), however, the
frequency of natural behaviors of normotensive rats
did not decrease during the depressor response by the
−16 ± 3 mmHg evoked by microinjection of glycine
into the RVLM. Thus the decrease of natural behaviors
in the present study was probably not caused by the
blood pressure reduction. Furthermore, Takishita et
al. (36) reported that in spontaneously hypertensive
rats, grooming behavior was equally observed both
before and after the intravenous administration of
manidipine, which decreased MAP by 13 ± 1 mmHg.
Another possibility was the involvement of the locus
ceruleus. Sedation is related to reduction of noradren-
nergic input to the thalamus and cerebral cortex (29).
Noradrenergic neurons in the locus ceruleus send ma-
jor efferent fibers to the thalamus and the cerebral
cortex (5). These noradrenergic neurons in the locus
ceruleus receive major afferent input from the RVLM
(4). In most cases, excitation of the RVLM neurons by
electrical stimulation mostly excites the activity of
locus ceruleus neurons (11). The excitatory input
cause by electrical stimulation of the RVLM neurons
to the locus ceruleus neurons appears to be mediated
by the excitatory amino acid pathway because the
excitatory response was blocked by kynurenic acid and
γ-D-glutamylglycine (10). Injection of kynurenic acid
into the locus ceruleus significantly decreased the
spontaneous discharge rate of the locus ceruleus neu-
rons (3). Taken together, it is possible that injection of
clonidine into the RVLM caused inhibitory effects on
the RVLM neurons, resulting in a decrease in the
excitatory input to the locus ceruleus, which sends
noradrenergic fibers to the forebrain sites. Further
studies are definitely needed to confirm this hypothe-
sis.

In summary, clonidine injected unilaterally into the
RVLM decreased MAP, HR, and RSNA in conscious,
unrestrained rats, as well as in propofol-anesthetized
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rats. Clonidine injection into the RVLM decreased the frequency of natural behaviors. The actions of clonidine were attenuated by coinjection of an α2-adrenoceptor antagonist, 2-methoxyidazoxan. These findings suggest that clonidine injected into the RVLM decreases MAP, HR, and RSNA in conscious, unrestrained rats, as well as in anesthetized rats, and causes sedation. The action of clonidine in the RVLM is at least partly mediated by the local α2-adrenoceptors.

Perspectives

There are various substances taking charge of neural control of blood pressure in the RVLM. In the present study, the depressor and sympathoinhibitory responses to clonidine injection into the RVLM were significantly larger in the propofol-anesthetized rats than in the conscious rats. These findings clearly demonstrated the influence of propofol anesthesia on the cardiovascular and sympathetic responses to α2-adrenoceptor stimulation in the RVLM. To know whether a substance endogenous to the RVLM contributes to the ongoing levels of sympathetic tone and blood pressure, microinjection of the receptor antagonist, or the anti-sense of the substance, into the RVLM would be an appropriate method. However, microinjection of α2-antagonist into the RVLM yielded results of an inconclusive nature about whether the α2-adrenoceptor in the RVLM contributed to the ongoing levels of blood pressure and sympathetic tone. To address this issue, bilateral injection of α2-antagonist into the RVLM would be helpful. Establishment of the technique of bilateral injection into the RVLM of conscious animals is the next step to be achieved in this field of study. Furthermore, the use of conscious animals enables us to observe the change in naturally occurring behavior. In the present study, microinjection of clonidine into the RVLM caused sedation of the rats. The use of naturally behaving conscious animals allowed us to have more insight into the role of neuroacting substances in the RVLM.

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