Contribution of $\alpha_2$-adrenoceptors in caudal ventrolateral medulla to cardiovascular regulation in rat

SHOGO SESOKO, HIROMI MURATANI, MASANOBU YAMAZATO, HIROSHI TERUYA, SHUICHI TAKISHITA, AND KOSHIRO FUKIYAMA

Third Department of Internal Medicine, University of The Ryukyu
School of Medicine, Nishihara, Okinawa 903-01, Japan

Contribution of $\alpha_2$-adrenoceptors in caudal ventrolateral medulla to cardiovascular regulation in rat. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1119–R1124, 1998.—The inhibitory action of $\alpha_2$-agonists on the cardiovascular neurons has been elucidated in the rostral ventrolateral medulla (RVLM) but not in the caudal ventrolateral medulla (CVLM). Our study aimed to clarify whether microinjection of clonidine into the CVLM elicits any cardiovascular effect and whether endogenous $\alpha_2$-adrenoceptor-mediated mechanisms contribute to the tonic activity of the CVLM neurons. In male Sprague-Dawley rats (7–9 wk old, 270–320 g) anesthetized with urethan, unilateral microinjection of 2 nmol of SKF-86466, a selective blocker of the HR) remained unaltered. Unilateral microinjection of 2 nmol of SKF-86466 into the CVLM (n = 10) increased mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) by 12.1 (P < 0.01 for each). Artificial cerebrospinal fluid caused a decrease in MAP, HR, and RSNA (6). Although imidazole receptor; clonidine; blood pressure; renal sympathetic nerve activity

METHODS

Animal preparation. Male Sprague-Dawley rats (7–8 wk of age, 270–320 g), purchased from Charles River, were anesthetized with urethan (1.0 g/kg ip). The right femoral artery and vein distal to the left adrenal vein; it was then cannulated for measurement of arterial pressure and administration of drugs. Body temperature was maintained within 37 ± 1°C with the use of a heat pad. Arterial blood gases and pH measured at the end of the experiments were within normal limits (mean PO2 = 103 ± 13.4 mmHg, mean PCO2 = 33.9 ± 1.3 mmHg, mean pH = 7.44 ± 0.02).

Anesthetized rats were fixed on a stereotaxic frame (Narishige Scientific Instruments, Tokyo, Japan) in a supine position. The trachea and esophagus were transected in the lower neck and reflected rostrally. The distal trachea was cannulated for artificial ventilation (Rodent Ventilator model 683; Harvard, South Natick, MA). After retraction of the bilateral longus capitis muscles, the inferior occipital bone was removed and the dura was incised and retracted to expose the ventral surface of the medulla, which was kept moist by endogenous cerebrospinal fluid. Particular care was taken not to damage the aortic depressor and the carotid sinus nerves.

The left kidney was exposed via transperitoneal approach. A branch of renal nerves was identified around the renal artery and vein distal to the left adrenal vein; it was then separated from surrounding tissues and placed on a bipolar silver wire electrode (no. 7855, A-M Systems). When an optimal neurogram was obtained, the nerve and the electrode were embedded in silicone gel (Siligel 604; Wacker, Munich,
Original renal nerve signals were amplified and filtered between 30 and 1,000 Hz (DPA-100E; Dia Medical System, Tokyo, Japan). The amplified nerve pulses were counted using a spike counter (DSE-325A, Dia Medical System).

Microinjection procedure Four-barrel micropipettes with tip diameters of 20–50 µm, made from calibrated microbore capillary glass tubing (Accu-Fill 90; Clay Adams, Parsippany, NJ), were used for the microinjections. Tips were drawn on a glass micropipette puller (type PE-2, Narishige Scientific Instruments). The injections were made over a 30-s period with a handheld syringe. The injected volume was measured using a spike counter. Tips were drawn on a glass micropipette puller (type PE-2, Narishige Scientific Instruments). The injections were made over a 30-s period with a handheld syringe. The injected volume was measured using a spike counter.

The CVLM and RVLM were identified by injection of 2 nmol of L-glutamate based on the criteria of our previous studies (16, 17, 23). The CVLM corresponds to the injection sites located between the second and third rootlet of the hypoglossal nerve, 1.9–2.1 mm lateral to the midline and 0.7–0.9 mm below the ventral surface, and the RVLM was located 0.6–1.0 mm rostral to the most rostral rootlet of the hypoglossal nerve, 1.7–1.9 mm lateral to the midline, and 0.5–0.8 mm below the surface (17, 23).

Experimental protocols. To determine cardiovascular and sympathetic effects of clonidine or endogenous α2-adrenoceptor-mediated mechanisms in the CVLM, clonidine (8 nmol/50 nl) or SKF-86466 (2 nmol/50 nl), a selective α2-adrenoceptor blocker, was injected through multibarrel micropipettes into the CVLM unilaterally. The dose of each drug was chosen based on previous studies (10, 20). The area of CVLM was identified by administration of L-glutamate (2 nmol/50 nl). In other rats, injection of clonidine was performed 10 min after a prior injection of SKF-86466 into the CVLM to determine whether the effects of clonidine in the CVLM were mediated by the local α2-adrenoceptors. To clarify whether the RVLM transmits actions of clonidine in the CVLM, clonidine was injected into the CVLM unilaterally 5 min after bilateral administration of muscimol (500 pmol/100 nl), a GABAₐ agonist, into the RVLM. Muscimol was injected bilaterally, because the projection from the CVLM to the contralateral RVLM was documented (14).

Each drug was dissolved in artificial cerebrospinal fluid (aCSF; in mM: 133.3 NaCl, 3.4 KCl, 1.3 CaCl₂, 1.2 MgCl₂, 0.6 NaH₂PO₄, 32.0 NaHCO₃, and 3.4 glucose). Therefore, 50 nl of aCSF was injected as a control. At the end of each experiment, 10 nl of an emulsion of Alcian blue dye was used to mark the site of injection and hexamethonium (40 mg/kg) was administered intravenously to estimate noise level for RSNA.

Statistical analysis. Data were expressed as means ± SE. Unpaired t-tests were used to compare the effects of clonidine or SKF-86466 with those of aCSF on mean arterial pressure (MAP), heart rate (HR), and RSNA. P values < 0.05 were considered to be statistically significant.

RESULTS

Baseline values of MAP and HR were 101.3 ± 2.7 mmHg and 419 ± 16 beats/min in the rats injected with clonidine (n = 10) and 101.9 ± 3.6 mmHg and 424 ± 17 beats/min in the rats injected with SKF-86466 (n = 10). In the rats treated with aCSF (n = 6), baseline values were 100.9 ± 4.7 mmHg and 425 ± 25 beats/min, respectively.

Microinjection of clonidine and SKF-86466 into the CVLM. Unilateral microinjection of clonidine into the CVLM (n = 10) increased MAP and RSNA, whereas HR remained unaltered (Fig. 1). In contrast, microinjection of SKF-86466 into the CVLM (n = 10) decreased MAP, RSNA, and HR (Fig. 2). The responses occurred within 20 s of microinjection of clonidine or SKF-86466 and lasted at least 5 min. Table 1 summarizes onset times and peak latencies of the blood pressure to clonidine and SKF-86466 and also maximal changes in MAP, RSNA, and HR evoked by the microinjection of clonidine and SKF-86466. aCSF (n = 6) caused neither a cardiovascular effect nor a sympathetic response. The dose of clonidine used in this study had no local anesthetic effect because the depressor response to

![Graph](image-url)
L-glutamate remained unaltered at the peak of hypertensive action of the drug (before clonidine: \(-33.8 \pm 3.1\) mmHg, after clonidine: \(-35.5 \pm 2.1\) mmHg). Also, SKF-86466 in the dose used had no effect on pressor response to glycine (10 nmol/50 nl) at the peak of hypertensive action of the drug (before SKF-86466: \(11.8 \pm 2.4\) mmHg, after SKF-86466: \(12.2 \pm 1.5\) mmHg).

Effect of prior injection of selective \(\alpha_2\)-blockade in the CVLM on the clonidine-induced pressor and sympathoexcitatory effects. The prior injection of SKF-86466 into the CVLM \((n = 6)\) significantly attenuated but did not totally abolish the increases in MAP and RSNA evoked by clonidine (Figs. 1 and 3). In this series of experiments, clonidine injection was carried out after the recovery of the decreased MAP and RSNA to the prevailing levels.

Effect of suppression of the RVLM on the clonidine-induced pressor and sympathoexcitatory effects. Figure 4 shows that the pressor and sympathoexcitatory effects of clonidine injected into the CVLM were abolished after the microinjection of muscimol into bilateral RVLM. Bilateral microinjection of muscimol into the RVLM \((n = 5)\) decreased MAP and RSNA to \(40.4 \pm 3.1\) mmHg and \(60.9 \pm 7.7\%\) of the baseline activity, respectively. In these rats, microinjection of clonidine into the CVLM before the pretreatment of the RVLM with muscimol caused increases in MAP and RSNA by \(14.4 \pm 1.8\) mmHg and \(34.7 \pm 6.0\%\), respectively. However, microinjection of clonidine into the CVLM following the muscimol injection into the RVLM did not alter MAP, RSNA, or HR (Figs. 4 and 5). Likewise, the depressor and sympathoinhibitory effects of SKF-86466 injected into the CVLM were abolished after the muscimol injection into the RVLM (Figs. 4 and 5).

Histological analysis. Figure 6A is a composite of the locations where clonidine, SKF-86466, or aCSF was injected. Sites in which hypertensive responses of clonidine or hypotensive responses of SKF-86466 occurred were restricted to a region ventral to the nucleus ambiguus and dorsal to the lateral reticular nucleus. Figure 6B is a composite of the locations where muscimol was injected; the locations are in the dorsolateral aspect of the lateral paragigantocellular nucleus. According to the atlas of Paxinos and Watson (19), these regions represent medullary sections extended from 3.30 mm to 5.30 mm caudal to interaural line.

Table 1. Maximal changes, onset times, and peak latencies in cardiovascular parameters evoked by the microinjection of clonidine and SKF-86466

<table>
<thead>
<tr>
<th></th>
<th>Onset Time, s</th>
<th>Peak Latency, s</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>RSNA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine ((n = 10))</td>
<td>15 (\pm 1.7)</td>
<td>32.4 (\pm 2.4)</td>
<td>12.1 (\pm 1.8)*</td>
<td>5.0 (\pm 2.7)</td>
<td>25.8 (\pm 4.8)*</td>
</tr>
<tr>
<td>SKF-86466 ((n = 10))</td>
<td>16.5 (\pm 3.3)</td>
<td>44.7 (\pm 4.0)</td>
<td>4.8 (\pm 23.2)</td>
<td>(-10 \pm 20)</td>
<td>10.3 (\pm 62.2)</td>
</tr>
<tr>
<td>aCSF ((n = 6))</td>
<td>7.2 (\pm 28.8)</td>
<td>21.6 (\pm 57.6)</td>
<td>-11.4 (\pm 3.6)*</td>
<td>(-25 \pm 6.8)*</td>
<td>-15.2 (\pm 1.7)*</td>
</tr>
</tbody>
</table>

Values are means \(\pm SE\), with range given in parentheses; \(n = \) no. of rats studied. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; aCSF, artificial cerebrospinal fluid. *\(P < 0.005\) post- vs. preinjection level.
in a region where the specific $[^3H]$ para-aminodoni
dine binding was shown (25).

The pressor and sympathoexcitatory actions of the
drug injected into the CVLM demonstrated in this
study might be independent of its systemically hy
tensive action. However, the design of this study is not
appropriate to address the question of whether the
hypoventive effect of systemically administrated cloni
dine is buffered by an opposing effect in the CVLM.

Our results are in contrast, however, to the findings
of McAuley et al. (15) that 10 and 20 nmol of clonidine
dissolved in 100 nl of saline caused long-lasting depre
ssor effects when injected into either the CVLM or the
RVLM of Wistar-Kyoto rats. The data of McAuley et al.
(15) suggest that clonidine act as a neuroinhibitory
agent in the RVLM and as a neuroexcitatory agent in
the CVLM to cause depressor response in both sites.
However, an important aspect of our study differs from
the study of McAuley et al. For microinjection, we used
multibarrel glass micropipettes with tip diameters of
20–50 µm to enable injections to be made without
causing substantial disruption to tissue, and also we
verified the injection sites by typical depressor and
sympathoinhibitory responses to L-glutamate and the
deposition of Alcian blue dye. In contrast, McAuley et
al. (15) utilized a 30-gauge needle, which might have
damaged the medullary tissue. Furthermore they did
not verify the injection sites by the typical responses to
L-glutamate injection (15). Orer et al. (18) microin
jected 1 nmol of clonidine dissolved in 100 nl of 0.9%
saline into four sites in the CVLM in cats. Although the
power of the 10-Hz rhythms in sympathetic discharge
was almost eliminated by the clonidine injection, the
total power of sympathetic nerve discharge and MAP
was not significantly altered. Clonidine might have a
different influence on the CVLM neurons in rat and cat.
Further studies are definitely needed to clarify the
species difference in the responsiveness of the CVLM
neurons.

Clonidine binds to both $\alpha_2$-adrenoceptors and imidaz
one receptors in the RVLM (9). There is a controversy
concerning which receptors are primarily responsible
for the hyotensive effects of clonidine or clonidine
analogs. In the RVLM, the fall in MAP elicited by
microinjection of clonidine analogs significantly corre
lated with their affinities for imidazole receptors but

Fig. 3. Cardiovascular changes by clonidine after administration of SKF-86466 in the CVLM. Administration of
SKF-86466 significantly attenuated the effects of clonidine in MAP and RSNA. ** $P < 0.005$ clonidine vs.
SKF-86466 + clonidine.

Fig. 4. Effects of clonidine and SKF-86466 after
suppression of bilateral rostral ventrolateral me
dulla (RVLM) by muscimol. Injection of muscimol
into the RVLM decreased the basal level of MAP
and RSNA. Injection of clonidine and SKF-86466
into the CVLM after treatment of muscimol in
the bilateral RVLM did not alter MAP, HR, or
RSNA.
not with their affinities for α2-adrenoceptors (7), and the depressor effects of locally injected clonidine were attenuated by imidazole receptor antagonists but not by α2-selective adrenoceptor antagonists (8, 10) and were mimicked by substances with imidazole structures (3). However, α-methylnorepinephrine, an agent with similar affinities as clonidine for the α2-adrenoceptors but not for imidazole receptors, exerted only negligible effects in ventrolateral medulla of cats (3). These papers (3, 7, 8, 10) suggest functional predominance of imidazole receptors in the RVLM. On the other hand, iontophoretic application of α-methylnorepinephrine exerted a similar degree of inhibition of the vasomotor neurons in the RVLM as does clonidine (1). Intravenous administration of α2-selective or nonselective adrenoceptor antagonists reversed or antagonized the sympathoinhibitory and hypotensive effects of clonidine and/orLevamiline (1, 5, 12, 21). Furthermore, the hypotensive response to α2-adrenoceptor agonists was lost in mice with a point mutation in the gene of the α2a-adrenoceptor subtype (13). These papers (1, 5, 13, 12, 21) suggest that α2-adrenoceptors, especially of α2a-subtype, seem to play a principal role in the hypotensive effect of clonidine or clonidine-like substances. In the present study, the attenuation of the pressor and sympathoexcitatory effects of clonidine in the CVLM by a prior injection of SKF-86466 strongly suggests an involvement of local α2-adrenoceptors. However, a slight but significant pressor and sympathoexcitatory effect was observed in response to the microinjection of clonidine performed after the pretreatment with SKF-86466 (Figs. 3 and 4). Therefore, the possibility that imidazole receptors in the CVLM (22) also participate in the pressor and sympathoexcitatory effects of locally injected clonidine could not be excluded, although rilmenidine, having higher selectivity for imidazole receptors compared with clonidine, had no effect on arterial pressure or HR when injected into the CVLM (10).

Another important finding in the present study was that microinjection of either clonidine or SKF-86466 into the CVLM after bilateral administration of muscimol into the RVLM did not alter MAP, RSNA, and HR. These findings underscore the requirement of vasomotor...
tor neurons of the RVLM for the action of clonidine and the \( \alpha_2 \)-adrenoceptor antagonist in the CVLM. Because the cardiovascular neurons in the CVLM send tonically inhibitory inputs, which are GABAergic, to the vasomotor neurons in the RVLM (4), it is likely that clonidine diminishes and SKF-86466 enhances the inhibitory input to the RVLM.

In summary, we show that clonidine administered into the CVLM increasesMAP and RSNA mainly by acting on local \( \alpha_2 \)-adrenoceptors. These pressor and sympathoexcitatory effects suggest a neuroinhibitory action of clonidine on the CVLM neurons. In addition, SKF-86466, an \( \alpha_2 \)-adrenoceptor blocker, microinjected into the CVLM elicits depressor and sympathoinhibitory effects. These results indicate that activation of \( \alpha_2 \)-adrenoceptors by endogenous ligand inhibits CVLM neurons. The cardiovascular and sympathetic effects of clonidine and \( \alpha_2 \)-antagonist injected into the CVLM require integrity of the vasomotor neurons in the RVLM.

The authors thank Rijiko Matayoshi for her technical assistance. SKF-86466 was a kind gift of SmithKline Beecham Pharmaceuticals. This work was supported in part by Grants in Aids for Scientific Research (05670825 and 03670646) from the Ministry of Education, Science and Culture of Japan. Additional experiments were supported by a research grant from Ministry of Health and Welfare (9A-1).

Present address of H. Teruya and S. Takishita: National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565, J. Japan.

Address for reprint requests: S. Sesoko, Third Dept. of Internal Medicine, Univ. Of The Ryukyus School of Medicine, 207 Uehara, Nishihara, Okinawa 903-01, J. Japan.

Received 3 June 1997; accepted in final form 25 December 1997.

REFERENCES


