Sympathoexcitation of moxonidine in the caudal ventrolateral medulla is dependent on I\textsubscript{1}-imidazoline receptors in anesthetized rats

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Received 20 July 2007; received in revised form 25 August 2007; accepted 27 August 2007

Abstract

Moxonidine is a second-generation centrally acting antihypertensive drug that has a high affinity for I\textsubscript{1}-imidazoline receptors (I\textsubscript{1R}). The caudal ventrolateral medulla (CVLM), an important region involved in cardiovascular activity, contains binding sites for centrally acting drugs. Our study aimed to determine the effects of moxonidine injected into the CVLM on cardiovascular activity in anesthetized rats. Unilateral microinjection of moxonidine (0.4 and 4 nmol) into the CVLM dose-dependently increased blood pressure (BP) by 8 ± 2 and 18 ± 2 mmHg and renal sympathetic nerve activity (RSNA) by 19 ± 3 and 48 ± 5% without modifying heart rate. Microinjection of the I\textsubscript{1R}/α\textsubscript{2}-adrenoceptor antagonist efaroxan (4 nmol) into the CVLM produced significant decreases in baseline BP and RSNA, but also completely abolished the increases in BP (2 ± 1 versus 17 ± 3 mmHg, \(P < 0.01\)) and RSNA (3 ± 2 versus 45 ± 10%, \(P < 0.01\)) evoked by subsequent injection of moxonidine (4 nmol). However, prior injection of yohimbine (500 pmol), a selective antagonist of α\textsubscript{2}-adrenoceptors, into the CVLM had no significant (\(P > 0.05\)) effect on the moxonidine-induced increase in BP (18 ± 2 versus 17 ± 3 mmHg) and RSNA (45 ± 10 versus 42 ± 7%). The current data suggest that moxonidine injection into the CVLM has an excitatory effect on cardiovascular activity, which is mediated by an I\textsubscript{1R} dependent mechanism.

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Keywords: Centrally acting drugs; α\textsubscript{2}-Adrenoceptors; Blood pressure; Renal sympathetic nerve activity; Rostral ventrolateral medulla

The imidazoline-like drug moxonidine, a centrally acting antihypertensive agent, has high affinity for I\textsubscript{1}-imidazoline receptor (I\textsubscript{1R}) but only a weak tendency to interact with α\textsubscript{2}-adrenoceptors (α\textsubscript{2}AR) [5,8]. Despite some controversies, moxonidine is generally described as a selective I\textsubscript{1R} agonist that lowers blood pressure (BP) by decreasing sympathetic activity through I\textsubscript{1R} activation within the central nervous system [6,13]. It is well known that the rostral ventrolateral medulla (RVLM), a key region regulating cardiovascular function [4], has been demonstrated to mediate the mechanism responsible for hypotension and sympathoinhibition of centrally acting agents [13,19,20]. The major inhibitory source of the RVLM is the caudal ventrolateral medulla (CVLM), which has been suggested to be involved in controlling cardiovascular activity [4]. The CVLM neurons receive excitatory input from the nucleus of the tract solitarius (NTS) and send a GABAergic projection connected to the RVLM [4,1,2,16]. The CVLM expresses the acting receptors (I\textsubscript{1R} and α\textsubscript{2}AR) for centrally acting drugs [21,22]. It has been demonstrated that local application of the centrally acting drug clonidine within the CVLM modifies the cardiovascular effects, suggesting the involvement of the CVLM in clonidine actions [24,29]. Because clonidine is a mixed agonist for α\textsubscript{2}AR and I\textsubscript{1R}, the exact mechanism by which I\textsubscript{1R} in the CVLM contributes to the effects of centrally acting drugs is undefined. Previous studies suggest that, in addition to α\textsubscript{2}AR, the I\textsubscript{1R} in the CVLM exerts an important physiological significance on cardiovascular regulation. For example, blockade of the selective α\textsubscript{2}AR does not completely abolish the effects of local injection of clonidine into the CVLM [24]. Antagonism of the CVLM I\textsubscript{1R} significantly attenuates the central transmission of arterial baroreflex [30]. However, there is no direct evidence showing the cardiovascular effects of I\textsubscript{1R} activation within the CVLM. Therefore, the present study was undertaken to investigate whether CVLM microinjection of the selective I\textsubscript{1R} agonist moxonidine produces cardiovascular effects and, if so, determine the acting receptor mechanism responsible for the effects of CVLM moxonidine.

All experiments were performed on 37 adult male Sprague-Dawley rats weighing between 300 and 350 g and were approved...
by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and were carried out under the guidelines of the American Physiological Society and the National Institutes of Health Guide of the Care and Use of Laboratory Animals. The methods for general surgery, renal sympathetic nerve activity (RSNA) recording, and microinjection were described previously from our studies [30,27].

Rats were anesthetized with urethane (800 mg/kg, ip) and α-chloralose (40 mg/kg, iv). The trachea was cannulated, and the rats were paralyzed with pancuronium bromide (1 mg/kg iv, 0.1 mg/kg thereafter as needed) and ventilated artificially with room air supplemented with 100% oxygen. Ventilation parameters were adjusted to maintain PaO2 at approximately 100 mmHg and PaCO2 below 40 mmHg. The left common carotid artery was cannulated, and the blood pressure (BP) was measured with a pressure transducer (Model PT300, Grass Instruments) for measurement of mean arterial pressure (MAP). Heart rate (HR) was derived from the BP pulse using a Powerlab model 16S (AD instruments). The femoral vein was cannulated for intravenous injections. Rats were placed in a stereotaxic frame (Stoelting, Chicago, IL) and the dorsal surface of the medulla was surgically exposed by occipital craniotomy. Supplemental doses of α-chloralose (20 mg/kg, iv) were administered to maintain an appropriate level of anesthesia. Depth of anesthesia was gauged by the stability of BP and HR and the absence of a pressor response to paw pinch. Body temperature was maintained at about 37 °C by an animal temperature controller (ATC1000, World Precision Instruments).

The left renal sympathetic nerves were exposed, identified and dissected free of the surrounding connective tissue, and placed on a pair of platinum-iridium recording electrodes. Both the nerve and the electrodes were covered with a fast setting silicone (Wacker Sil-Gel). The signal was amplified (band pass 100–1000 Hz) with a preamplifier (Model P 18D, Grass Instruments). The amplified discharge was monitored on a storage oscilloscope (Model 121 N, Tektronix, Beaverton, OR) and the dorsal surface of the medulla was surgically exposed by occipital craniotomy. Supplemental doses of α-chloralose (20 mg/kg, iv) were administered to maintain an appropriate level of anesthesia. Depth of anesthesia was gauged by the stability of BP and HR and the absence of a pressor response to paw pinch. Body temperature was maintained at about 37 °C by an animal temperature controller (ATC1000, World Precision Instruments).

Fig. 1. Histological localization of drug microinjection sites (●) in the brainstem. 12, Hypoglossal nucleus; Amb, nucleus ambiguus; AP, area postrema; LRt, lateral reticular nucleus; Rob, raphe obscurus nucleus; py, pyramidal tract; NTS, nucleus solitary tract; Sp5C, spinal trigeminal nucleus, caudal.
Table 1
Baseline values of MAP and HR in experimental groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>5</td>
<td>90 ± 2</td>
<td>359 ± 17</td>
</tr>
<tr>
<td>0.4 nmol Moxonidine</td>
<td>6</td>
<td>94 ± 4</td>
<td>381 ± 13</td>
</tr>
<tr>
<td>4 nmol Moxonidine</td>
<td>8</td>
<td>92 ± 2</td>
<td>369 ± 14</td>
</tr>
<tr>
<td>Vehicle + moxonidine</td>
<td>5</td>
<td>92 ± 4</td>
<td>356 ± 12</td>
</tr>
<tr>
<td>Efaroxan + moxonidine</td>
<td>7</td>
<td>95 ± 4</td>
<td>362 ± 12</td>
</tr>
<tr>
<td>Yohimbine + moxonidine</td>
<td>6</td>
<td>93 ± 4</td>
<td>347 ± 15</td>
</tr>
</tbody>
</table>

n is the number of the rats in each group.

remarkably altered following CVLM injection of moxonidine. The degrees of the increases in BP and RSNA by the higher dose (4 nmol) of moxonidine injection were significantly (\( P < 0.05 \)) greater than those by the lower dose (0.4 nmol) of moxonidine. Unilateral injection of the same volume of vehicle (50 nl aCSF, \( n = 5 \)) into the CVLM had no effect on cardiovascular values. The maximal increases in BP and RSNA after CVLM moxonidine reached within 20 min, and gradually returned to preinjection values within 30–50 min. The peak changes in BP, HR, and RSNA evoked by CVLM moxonidine or aCSF are shown in Fig. 2B.
Other 18 rats (three groups, \( n = 5-7 \) rats each) were used to determine the receptor mechanism responsible for the effects of CVLM moxonidine. The baseline BP and HR are shown in Table 1. Fig. 3A shows original tracings of BP, HR, and RSNA response to subsequently injected moxonidine into the CVLM 10 min after pretreatment with the \( I_1R/\alpha_2AR \) antagonist efaroxan. Unilateral injection of efaroxan (4 nmol, \( n = 7 \)) into the CVLM produced a significant \((P < 0.05)\) reduction in BP by 14 ± 3 mmHg and RSNA by 26 ± 5% without affecting HR. The peak decrease in BP and RSNA by efaroxan was usually reached in 3–5 min and gradually returned to control level within about 10 min. Ten minutes after pretreatment with efaroxan, injection of moxonidine (4 nmol) into the CVLM failed to elicit the significant changes in BP and RSNA, which was similar to 50 nl (\( n = 5 \)) of aCSF injection (changes in BP and RSNA: -1.4 ± 2.2 mmHg and -2.5 ± 3.7%, respectively) after efaroxan treatment. These data indicate that pretreatment with efaroxan completely abolishes the effects of CVLM moxonidine. Although injection of the selective \( \alpha_2AR \) antagonist yohimbine (500 pmol, \( n = 6 \)) into the CVLM slightly reduced the baseline BP by 8 ± 3 mmHg and RSNA by 15 ± 4% without modifying HR, we found that, compared with pretreated vehicle (\( n = 5 \)), prior injection of yohimbine had no significant effect on the moxonidine-induced increase in BP (18 ± 2 versus

![Graph](image-url)
Moxonidine has been demonstrated to produce hypotensive and sympathoinhibitory effects through selectively stimulating I1R in the central nervous system [13,7]. The RVLM has been recognized as a specific target mediating the cardiovascular inhibition produced by moxonidine [13,3]. Moxonidine in the RVLM acts as an inhibitory agent for decreasing activity of vasomotor neurons and thereby lowering the sympathetic activity [13,3,11]. However, the significance of CVLM, a major inhibitory input to the RVLM, in mediating the centrally hypotensive mechanism has been not extensively studied. In the present study, we found that microinjection of moxonidine into the CVLM presented an excitatory effect on BP and RSNA. It appears that moxonidine also exhibits an inhibitory effect on the CVLM neurons as well as on the RVLM vasomotor neurons. Because the CVLM neurons have an inhibitory effect on driving the RVLM vasomotor neurons via a GABAergic projection, inhibition of the CVLM neurons would excite the RVLM neurons and then increase sympathetic activity. Therefore, it is logical to assume that the inhibitory effect of moxonidine on the CVLM neurons would increase sympathetic outflow and elevate BP. These specific observations that the cardiovascular excitation was elicited by CVLM moxonidine were very similar to the cardiovascular response to clonidine, a cardiovascular excitation was elicited by CVLM moxonidine actions described in previous studies [5,13,9,26], it is suggested that I1R in the ventrolateral medulla (rostral or caudal) is likely to be required for moxonidine action. Notably, blockade of I1R or α2AR in the CVLM significantly decreased the baseline BP and RSNA, suggesting that these two receptors in the CVLM are involved in generation of tonic cardiovascular activity. This data is consistent with previous studies showing the decrease in BP and sympathetic activity following injection of another I1R or α2AR antagonist idazoxan or SKF86466, respectively, into the CVLM [24,29]. In addition, bilateral injection of the I1R antagonist idazoxan into the CVLM significantly reduces the arterial baroreflex sensitivity, suggesting that the CVLM I1R mediates the central transmission of arterial baroreflex [30]. Therefore, the CVLM I1R and α2AR are suggested to exhibit the physiological significance in processing of cardiovascular regulation.

In summary, the present study demonstrated that injection of the centrally acting agent moxonidine into the CVLM dose-dependently increased BP and RSNA, which were completely abolished by the I1R/α2AR antagonist efaroxan but not by the selective α2AR antagonist yohimbine. It is suggested that local moxonidine in the CVLM stimulates cardiovascular excitation through an I1R dependent mechanism.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (30470636 and 30670759) and the Major State Basic Research Development Program of China (2006CB503807). This study was also funded by NIH grant RO-1 HL077691 and PO-1 HL62222, and a postdoctoral fellowship to Wei-Zhong Wang from the American Heart Association, Heartland Affiliate (no. 0720066Z).
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