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Sympathoexcitation of moxonidine in the caudal ventrolateral medulla is dependent on I_1-imidazoline receptors in anesthetized rats

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Abstract

Moxonidine is a second-generation centrally acting antihypertensive drug that has a high affinity for I_1-imidazoline receptors (I_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 $48 \pm 5\%$ without modifying heart rate. Microinjection of the I_1R/α_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 18 ± 2 mmHg, $P < 0.01$) and RSNA (3 ± 2 versus $45 \pm 10\%$, $P < 0.01$) evoked by subsequent injection of moxonidine (4 nmol). However, prior injection of yohimbine (500 pmol), a selective antagonist of α_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 $42 \pm 7\%$). The current data suggest that moxonidine injection into the CVLM has an excitatory effect on cardiovascular activity, which is mediated by an I_1R dependent mechanism.

Keywords: Centrally acting drugs; α_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_1-imidazoline receptor (I_1R) but only a weak tendency to interact with α_2-adrenoceptors (α_2AR) [5,8]. Despite some controversies, moxonidine is generally described as a selective I_1R agonist that lowers blood pressure (BP) by decreasing sympathetic activity through I_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 solitary (NTS) and send a GABAergic projection connected to the RVLM [4,1,2,16]. The CVLM expresses the acting receptors (I_1R and α_2AR) 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_2AR and I_1R, the exact mechanism by which I_1R in the CVLM contributes to the effects of centrally acting drugs is undefined. Previous studies suggest that, in addition to α_2AR, the I_1R in the CVLM exerts an important physiological significance on cardiovascular regulation. For example, blockade of the selective α_2AR does not completely abolish the effects of local injection of clonidine into the CVLM [24]. Antagonism of the CVLM I_1R significantly attenuates the central transmission of arterial baroreflex [30]. However, there is no direct evidence showing the cardiovascular effects of I_1R activation within the CVLM. Therefore, the present study was undertaken to investigate whether CVLM microinjection of the selective I_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

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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, ip). 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 PaO_2 at approximately 100 mmHg and $PaCO_2$ 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), imported to a computer system with other parameters. Respective noise levels were subtracted from the nerve recording data before percentage changes from baseline were calculated. Integrated RSNA was normalized as 100% baseline in the control period.

Microinjections were made from multi-barrel micropipettes with total tip diameters of 20–50 μm and performed by a four-channel pressure injector (PM2000B, World Precision Instruments). The injections were made over 10–20 s and 50 nl injection volume was measured by observing the movement of the fluid meniscus on a reticule in a microscope. The coordinates for CVLM were 0–0.5 mm rostral to the calamus scriptorius, 1.7–2.0 mm lateral to the midline, and 2.6–3.0 mm below the dorsal surface of the medulla. The injection drugs were dissolved in an artificial cerebrospinal fluid (aCSF, pH 7.4), and 50 nl of aCSF was served as vehicle control. The doses for drug microinjections were based on previous studies [13,29,30,25]. Chemical identification of the CVLM was based on obtaining a depressor response elicited by microinjection of L-glutamate (2 nmol) at the beginning of each experiment. At the end of the experiments, 50 nl of 2% Pontamine sky blue was injected for marking the injection sites for histological identification. The rat was given a lethal injection of pentobarbital (100 mg/kg, iv) and perfused with 10% formaldehyde solution (100 ml) intracardially. The brain stem was then quickly removed and fixed in 10% buffered Formalin. Frozen 50-μm coronal sections were made on a freezing microtome and mounted on slides. The dye spots for injection sites were identified and plotted on standardized sections according to the atlas of Paxinos and Watson [18]. Fig. 1 shows the centers of the microinjection sites in the brainstem. Histological analysis indicated that the injection sites were located in the periambigual area, just dorsal to the lateral reticular nucleus. This area of the ventrolateral medulla has been suggested to contain the cell bodies of sympathoinhibitory interneurons [15].

All values were expressed as mean ± S.E. The changes in integrated RSNA after treatments were evaluated as percentage changes from control because of the variability in baseline RSNA in each animal. Student's *t* test (paired or unpaired) was used for comparing the baseline data and the difference between pre- and post-injections. Statistical comparisons between different groups were made by repeated-measures analysis of variance (ANOVA) followed by Student-Newman–Keuls test (SigmaStat 3.5). Differences were considered to be significant at $P < 0.05$.

A total of 19 rats (three groups, $n = 5$–8 rats each) were used to determine the cardiovascular effects of local microinjection of moxonidine into the CVLM. The baseline values for BP and HR for each group are shown in Table 1. Fig. 2A shows original tracings of BP, HR, and RSNA response to moxonidine (4 nmol) microinjected unilaterally into the CVLM. Unilateral injection of two doses (0.4 nmol, $n = 6$ and 4 nmol, $n = 8$) of moxonidine into the CVLM caused a significant elevation in BP (8 ± 2 and 18 ± 2 mmHg, $P < 0.05$) and RSNA (19 ± 3 and 48 ± 5%, $P < 0.05$) in a dose-dependent manner. However, HR was not

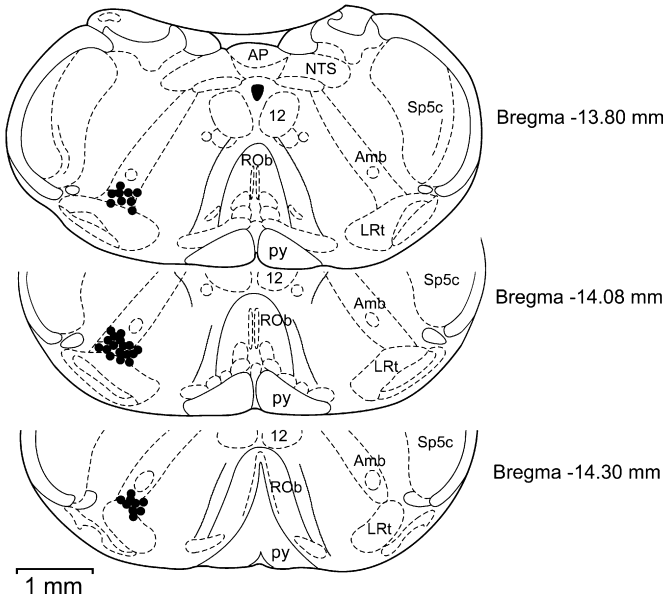

Fig. 1. Histological localization of drug microinjection sites (●) in the brainstem. 12, Hypoglossal nucleus; Amb, nucleus ambiguus; AP, area postrema; LRt, lateral reticular nucleus; Rob, raphe obscurus nucleus; py, pyramidal tract; NTS, nucleus solitary tract; Sp5C, spinal trigemina nucleus, caudal.