关键词 长春西汀；钠通道；河鲀毒素；心肌；膜片箝技术；长春花生物碱类

目的：研究长春西汀对心肌细胞钠电流的作用. 方法：用全细胞膜片箝技术记录大鼠心肌细胞钠电流. 结果：长春西汀可逆性抑制心肌细胞钠电流的作用为剂量依赖性和电压依赖性，但未发现频率或使用依赖性. 长春西汀 10－80 μmol·L^{-1}，对钠电流的抑制作用为 13 %±2 %至 75 %±6 %. 半数抑制浓度 IC_{50}值(95 %可信限)为 36.4 (28.1－47.1) μmol·L^{-1}. 在膜电位以 10 mV 的间隔从－90 mV 阶梯状去极化至＋40 mV 时，抑制作用呈逐渐增加的趋势，约在 0 mV 左右达到最大抑制. 长春西汀对钠通道的稳态激活和失活过程的影响，可使钠窗电流(缓慢失活的钠电流)减少. 结论：长春西汀抑制大鼠心肌细胞的钠电流.

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Effects of moxonidine injected into rostral ventrolateral medulla on blood pressure, heart rate, and renal sympathetic nerve activity in rats

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KEY WORDS moxonidine; medulla oblongata; sympathetic nervous system; blood pressure; heart rate; pressoreceptors

AIM: To examine the effects of moxonidine (Mox) injected into the rostral ventrolateral medulla (RVLM) on blood pressure (BP), heart rate (HR), and the renal sympathetic nerve activity (RSNA) in anesthetized normotensive rats. **METHODS**: BP, HR, and RSNA were simultaneously recorded after 1 μL Mox 1, 10, and 100 μmol·L^{-1} was injected into RVLM. **RESULTS**: Mox 1, 10, and 100 μmol·L^{-1} reduced BP from 13.9±1.0 kPa to 13.0±1.7 kPa ($P<0.05$), 13.8±1.8 kPa to 11.4±1.5 kPa ($P<0.01$), and 13.9±1.9 kPa to 9.4±1.7 kPa ($P<0.01$), respectively. Mox did not influence HR. RSNA varied with the doses: Mox 1 μmol·L^{-1} increased RSNA by 50 % ($P<0.05$), 10 μmol·L^{-1} insignificantly influenced RSNA ($P>0.05$), and 100 μmol·L^{-1} reduced RSNA by 23 % ($P<0.05$). In sinoaortic barodenervated rats, Mox 10 μmol·L^{-1} inhibited RSNA by 50 % ($P<0.05$), which substantially differed from that in buffer nerve intact rats ($P<0.01$). **CONCLUSION**: Mox injected into RVLM decreased BP, but did not influence HR. The changes of RSNA did not parallel with the depressor effect of Mox.

Moxonidine (Mox), an imidazoline, represents a new generation of clonidine-like centrally acting antihypertensive drugs, which acts via I_1-imidazoline receptor[1]. Mox injected into the rostral ventrolateral medulla (RVLM) decreased blood pressure (BP) and heart rate (HR) in conscious or anesthetized hypertensive rats[2-4]. Neurons in RVLM send excitatory input directly to the spinal sympathetic preganglionic neurons. It is assumed that Mox produces its depressor effect by decreasing peripheral sympathetic tone. But there was no report about the effect of Mox directly applied into the RVLM on sympathetic nerve outflow. The renal sympathetic nerve activity (RSNA) is mostly used to evaluate peripheral sympathetic tone. Intravenous injection of Mox in the doses inducing hypotension inhibited RSNA in anesthetized cats[5] and in conscious rabbits[6], but it was thought that the decrease of RSNA induced by iv Mox was due to its central action as well as peripheral presynaptic inhibition. The aim of the present study was to investigate the effects of injection of Mox into RVLM on BP, HR, and RSNA in anesthetized

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normotensive rats as well as effects of Mox in some sinoaortic barodenervated rats.

MATERIALS AND METHODS

Rats Sprague-Dawley rats, ♂, weighing 315 ± s 30 g were anesthetized with ip sodium pentobarbital 60 mg · kg^{-1}, additional doses when required. The femoral artery and vein were cannulated for recording BP by a pressure transducer (MPU-0.5) and for saline infusion or drug administration, respectively. HR was monitored by a Heart Rate Counter (AT-601G).

RSNA The left kidney was exposed via a retroperitoneal approach. One branch of the renal sympathetic nerves around the renal vessels was clamped distally to eliminate the afferent activity. The nerve was placed on a bipolar platinum electrode for potential recording, and immersed in liquid paraffin. The nerve activity was amplified by a biophysical amplifier (AB-620G, Nihon Kohden). The amplified signals were integrated with an integrator (EI-600G), with integrated time 5 s. The integrated RSNA was recorded along with BP and HR on a polygraph system (RM 6000. Nihon Kohden). Upon the end of the experiment, the proximal end of the nerve was clamped to get the noise level of RSNA, the data were expressed as % change from values before injection.

Injection Anesthetized rats were fixed on a stereotaxic frame (Model IC, Jiangwan) in supine position. The trachea and esophagus were transected in the lower neck and reflected rostrally. The distal trachea was cannulated for ventilation. After retraction of the bilateral longus capitis muscles, the basilar portion of the occipital bone was removed. The ventral surface of the medulla was exposed by incising the dura and the arachnoid.

An outer stainless steel tube (diameter 1 mm) was fixed on stereotaxic frame, an inner tube (diameter 0.5 mm) filled with sodium glutamate 10 mmol·L^{-1} was connected by polyethylene tubing to a microsyringe (10 μL). According to coordinates[7], 1 μL sodium glutamate was slowly injected into RVLM (2.8 – 3.6 mm caudal to the interaural line, 1.2 – 2.0 mm lateral to the midline, 0.5 – 1.0 mm dorsal to the ventral surface) to identify the maximal pressor zone, *ie*, glutamate sensitive area. Another inner tube filled with saline or Mox was used for injection in 1 min.

Bilateral buffer nerve section Carotid sinus areas were fully exposed. Sternohyoideus muscles and superior laryngeal nerves were cut. The aortic depressor nerves and the carotid sinus nerves were sectioned under microscope. The superior cervical sympathetic trunk and recurrent laryngeal nerves were also cut. Completeness of barodenervation was ensured by the absence of decrease in HR when hypertension was initiated by iv injection of phenylephrine.

Protocols Rats were divided in five groups, one of which received 1 μL saline and three received one of 3 doses of Mox (1, 10, and 100 μmol·L^{-1}). BP, HR, and RSNA were simultaneously recorded for 60 min. Only one dose was tested in each rat. To study the effects of Mox in sinoaortic denervated rats, one group were injected Mox 10 μmol · L^{-1} at least 2 h after sinoaortic denervation, by which time BP, HR and RSNA had returned to the baseline levels.

Drugs Mox (purity 99 %, Kali-Chemie, Hannover, Germany) was dissolved in saline. Sodium glutamate (Sigma) was also dissolved in saline.

Statistics Data were expressed as $\bar{x} \pm s$ and compared with t test, while the difference between before and after medication was done with paired t test. The effect of drug on RSNA was analyzed by Wilcoxon test.

RESULTS

BP and HR The glutamate sensitive area was identified just caudal to the trapezoid bodies, lateral to the pyramids, and rostral to the rootlets of the XII th nerve. Applying 1 μL glutamate 10 mmol·L^{-1} into this area increased BP by 3.7 ± 0.8 kPa ($P < 0.01$) and RSNA by 40 % ± 6 % ($P < 0.05$) in 6 rats.

BP and HR showed no change after injection of 1 μL saline into RVLM.

The injection of Mox into RVLM dose-dependently decreased BP, starting within 1 min, reaching its nadir within 10 – 15 min, and lasting 30 – 60 min. The nadir depressor responses of Mox 1, 10, and 100 μmol·L^{-1} were from 13.9 ± 1.0 kPa to 13.0 ± 1.7 kPa ($P < 0.05$), 13.8 ± 1.8 kPa to 11.4 ± 1.5 kPa ($P < 0.01$), and 13.9 ± 1.9 kPa to 9.4 ± 1.7 kPa ($P < 0.01$), respectively. There was no substantial change of HR before and after injection of Mox 1, 10, and 100 μmol·L^{-1} ($P > 0.05$) (Tab 1).

RSNA Injection of 1 μL saline into RVLM had no effect on RSNA. Mox 1 μmol · L^{-1} increased RSNA by 51 % ($P < 0.05$), 10 μmol·L^{-1} insignificantly affected RSNA ($P > 0.05$), while, 100 μmol · L^{-1} reduced RSNA by 23 % ($P < 0.05$) (Tab 1).

In sinoaortic denervated rats, the baselines of BP and HR were similar to those in buffer nerve intact rats. Mox 10 μmol·L^{-1} inhibited RSNA to 50 % ($P < 0.05$), which differed from that in buffer nerve intact rats ($P < 0.01$). However, BP and HR after injection of Mox were not different from those in buffer nerve intact rats ($P > 0.05$) (Tab 2).

Tab 1. Effects of 1 μL Mox injected into RVLM on BP, HR, and RSNA in anesthetized normotensive rats. $n=8$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* before; $^dP>0.05$ *vs* Mox 1 μmol·L^{-1}; $^eP<0.05$ *vs* Mox 10 μmol·L^{-1}.

	Saline	Moxonidine/μmol·L^{-1} 1	10	100
BP/kPa				
Before	13.4±1.2	13.9±1.0	13.8±1.8	13.9±1.9
After	13.5±1.1^a	13.0±1.7^b	11.4±1.5^{cd}	9.4±1.7^{ch}
HR/beats·min^{-1}				
Before	340±34	350±21	340±46	350±33
After	344±40^a	350±34^a	340±51^a	351±40^a
RSNA/%				
Before	100±0	100±0	100±0	100±0
After	110±31^a	150±58^b	120±55^a	77±23^b

Tab 2. Effects of 1 μL Mox 10 μmol·L^{-1} inject into RVLM on BP, HR, and RSNA in buffer nerve intact (A, $n=8$) and barodenervated (B, $n=6$) rats. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* before; $^dP>0.05$, $^eP<0.05$ *vs* A.

		A	B
BP/kPa	Before	13.8±1.8	14.3±3.8^d
	After	11.4±1.5^c	11.0±3.0^{cd}
HR/beats·min^{-1}	Before	340±46	340±14^d
	After	340±51^a	340±16^{ad}
RSNA/%	Before	100±0	100±0
	After	120±55^a	50±10^{be}

DISCUSSION

It was well known that RVLM was main target site for clonidine-like antihypertensive drugs[8]. The results of the present study showed that Mox injected into the pressor area of RVLM elicited a significant depressor effect in anesthetized normotensive rats. This is accordance with the results that Mox injected into RVLM effectively normalized the blood pressure in hypertensive rats[3,4]. For the first time, this study demonstrated effects of Mox directly administrated into RVLM on RSNA. The results showed that RSNA varied with the doses of Mox although Mox dose-dependently decreased BP. Mox 1 μmol·L^{-1} induced hypotensive action accompanied with increase in RSNA, 100 μmol·L^{-1} with decrease in RSNA, 10 μmol·L^{-1} did not influence RSNA. These were obviously different from the previous results that RSNA was constantly inhibited by iv Mox[5,6]. Here, there are two possibilities concerning the effects of moxonidine on RSNA in our study. One is its direct inhibitory effect on the neurons of RVLM, another is indirect excitatory effect secondary to baroreflex. In sinoaortic denervated rats, RSNA was significantly reduced as compared with that in buffer nerve intact animals, suggesting that sinoaortic baroreflex was acting so as to mask its direct action. The result that Mox 100 μmol·L^{-1} inhibited RSNA indicated the direct effect of Mox overrode the buffering action of baroreflex. Also, the results showed that the depressor effect of Mox 1, 10 μmol·L^{-1} was not due to its inhibition on RSNA. What is the underlying mechanism responsive for hypotension? There was evidence that different regional sympathetic outflows varied in their sensitivity to the sympathoinhibitory actions of some centrally acting hypotensive drugs[5]. So, it is possible that RSNA is not sensitive to sympathoinhibition of Mox and the depressor effect of Mox 1, 10 μmol·L^{-1} may mainly result from inhibition of other sympathetic nerves.

The study indicated that hypotensive doses of Mox did not decrease heart rate, an effect which was different from studies in hypertensive animals[3,4]. It is generally believed that bradycardia produced by clonidine-like drugs is a consequence of α_2-adrenoceptor stimulation[9]. Mox has highly selective affinity for I_1-imidazoline receptor over α_2-adrenoceptor[10]. So, the fact that Mox had no influence on heart rate in anesthetized normotensive rats might be attributed to weak α_2-adrenoceptor activation of Mox.

In summary, the study provided the evidence that sympathetic nerve outflow was modulated by imidazoline-agonist Mox applied into RVLM. Mox administrated into RVLM decreased blood pressure and did not influence heart rate. The depressor effect of Mox does not always parallel with the change of RSNA which may be masked by sinoaortic baroreflex in buffer nerve intact animals.

REFERENCES

1 Chrisp P, Faulds D. Moxonidine. A review of its pharmacology and therapeutic use in essential hypertension. Drugs 1992; 44: 993–1012.

2 Buccafusco JJ, Lapp CA, Westbrooks KL, Ernsberger P. Role of medullary I_1-imidazoline and α_2-adrenergic receptors in the antihypertensive responses evoked by central administration of clonidine analogs in conscious spontaneously hypertensive rats. J Pharmacol Exp Ther 1995; 273: 1162 – 71.

3 Haxhiu MA, Dreshaj I, Erokwu B, Schäfer SG, Christen MO, Ernsberger PR. Vasodepression elicited in hypertensive rats by the selective I_1-imidazoline agonist moxonidine administrated into the rostral ventrolateral medulla. J Cardiovasc Pharmacol 1992; 20 Suppl 4: S11 – S15.

4 Haxhiu MA, Dreshaj I, Schäfer SG, Ernsberger PR. Selective antihypertensive action of moxonidine is mediated mainly by I_1-imidazoline receptors in the rostral ventrolateral medulla. J Cardiovasc Pharmacol 1994; 24 Suppl 1: S1 – S8.

5 Ramage AG, Wilkinson SJ. Evidence that different regional sympathetic outflows vary in their sensitivity to the sympathoinhibitory actions of putative 5-HT_{1A} and α_2-adrenoceptor agonists in anaesthetized cats. Br J Pharmacol 1989; 98: 1157 – 64.

6 Urban R, Szabo B, Starke K. Involvement of peripheral presynaptic inhibition in the reduction of sympathetic tone by moxonidine, rilmenidine and UK 14304. Eur J Pharmacol 1995; 282: 29 – 37.

7 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 1st ed. Sydney: Academic Press, 1982: Fig 38 – 9.

8 Punnen S, Urbanski R, Krieger AJ, Sapru HN. Ventrolateral medullary pressor area: site of hypotensive action of clonidine. Brain Res 1987; 422: 336 – 46.

9 Deckert V, Lachaud V, Panni A, Elghozi JL. Contribution of alpha 2-adrenoceptors to the central cardiovascular effects of clonidine and S8350 in anaesthetized rats. Clin Exp Pharmacol Physiol 1991; 18: 401 – 8.

10 Ernsberger P, Graves ME, Graff LM, Zakieh N, Nguyen P, Collins LA, *et al* I_1-imidazoline receptors. Definition, characterization, distribution, and transmembrane signaling. Ann NY Acad Sci 1995; 763: 22 – 42.

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延髓腹外侧头端注射莫索尼定对大鼠血压、心率和肾交感神经放电的影响

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关键词 莫索尼定；延髓；交感神经系统；血压；心率；压力感受器

目的：观察延髓腹外侧头端（RVLM）注射莫索尼定（Mox）对麻醉大鼠血压（BP）、心率（HR）及肾交感神经放电（RSNA）的影响. **方法**：麻醉大鼠 RVLM 注射 1 μL Mox 1，10，100 μmol·L^{-1}，同步记录 BP，HR 及 RSNA. **结果**：Mox 1，10，100 μmol·L^{-1} 分别使 BP 从 13.9 ± 1.0 kPa 降至 13.0 ± 1.7 kPa（$P < 0.05$），13.8 ± 1.8 kPa 至 11.4 ± 1.5 kPa（$P < 0.01$），and 13.9 ± 1.9 kPa 至 9.4 ± 1.7 kPa（$P < 0.01$）. Mox 不影响 HR. Mox 1 μmol·L^{-1} 增加 RSNA 50 %（$P < 0.05$），10 μmol·L^{-1} 对 RSNA 无影响（$P > 0.05$），100 μmol·L^{-1} 则降低 RSNA 23 %（$P < 0.05$）. 在缓冲神经切断大鼠，Mox 10 μmol·L^{-1} 抑制 RSNA 50 %（$P < 0.05$），明显不同于缓冲神经完整的动物（$P < 0.01$）. **结论**：麻醉大鼠 RVLM 注射 Mox 可降低 BP，但不影响 HR，且 RSNA 变化与其降压作用并不平行.