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Effect of moxonidine on carotid sinus baroreflex in anesthetized rats

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KEY WORDS moxonidine; carotid sinus; baroreflex; blood pressure; antihypertensive agents; adrenergic alpha-antagonists; efaroxan

ABSTRACT

AIM: To study the effect of moxonidine (Mox) on carotid sinus baroreflex. **METHODS**: By perfusing the carotid sinus in anesthetized rats, the functional parameters of baroreflex were measured. The femoral artery was perfused with constant flow and the change of perfusing pressure was recorded to determine the effect of Mox on vascular tone. **RESULTS**: Mox 32 and 100 $\mu mol \cdot L^{-1}$ shifted the functional curve of carotid sinus baroreflex to the right and upward, with the reduction in peak slope and in reflex decrease of mean arterial pressure, suggesting that Mox produced an inhibitory effect on baroreflex. The effect of Mox 100 $\mu mol \cdot L^{-1}$ on baroreflex was completely blocked by efaroxan 100 $\mu mol \cdot L^{-1}$. Mox increased vascular resistance. **CONCLUSION**: Mox inhibits carotid baroreflex *via* its constrictive action on sinus wall.

INTRODUCTION

Since the concept of "imidazoline receptors" was proposed[1], the mechanism underlying antihypertensive action of clonidine-like drugs has been the subject of much attention[2]. I_1-imidazoline receptors were responsible for the depressor effect of clonidine-like agents, whereas α_2-adrenoceptors might account for their side effects such as dryness of mouth and sedation[2], although it was still somewhat controversial[3]. Moxonidine (Mox) bound more selectively to I_1-imidazoline than to α_2-receptors and produced less side effects[4], so it represented a new generation of centrally acting antihypertensive agents. Our electrophysiologic experiments showed that Mox centrally decreased the renal sympathetic nerve activity[5]. Mox also exerted a hypotensive action by peripheral imidazoline receptors, such as inhibition of catecholamine release from the adrenal medulla and tubular sodium reabsorption[4]. Whether Mox may affect the arterial baroreceptor function remains unknown. This study was to observe the effect of Mox on baroreflex by perfusing the carotid sinus and the effect of efaroxan (Efa), a high selective antagonist for I_1-imidazoline receptor over α_2-receptor[6], on the action of Mox in anesthetized rats.

MATERIALS AND METHODS

Sprague-Dawley rats (♂, 330 ± 20 g, $n = 113$, Grade Ⅱ, Certificate № 04036) obtained from the Experimental Animal Center of Hebei Province. Rats were anesthetized with ip urethane 500 $mg \cdot kg^{-1}$ and α-chloralose 50 $mg \cdot kg^{-1}$. The trachea was cannulated for ventilation. The femoral artery was cannulated for recording blood pressure (BP) with a transducer (MPU-0.5).

Perfusion of left carotid sinus The perfusion of isolated carotid sinus area was carried out with a method modified by our laboratory[7]. Carotid sinus areas were fully exposed by turning rostrally the trachea and esophagus. Sternohyoideus muscles and superior laryngeal nerves were cut. The bilateral aortic nerves, right carotid sinus nerve, cervical sympathetic nerves, and recurrent laryngeal nerves were all cut. All branches emerging from the left carotid sinus bifurcation except for the external carotid artery and the common carotid artery were ligated, while painstakingly leaving

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the left carotid sinus nerve undisturbed. Plastic catheters were inserted into the left common carotid artery in the anterograde way (served as inlet tube) and the external carotid artery in the retrograde way (served as outlet tube). The carotid sinus was then perfused with warm oxygenated modified Krebs-Henseleit (K-H) solution (NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6 mmol·L^{-1}, pH 7.4). The intrasinus pressure (ISP) was monitored by a pressure transducer (MPU-0.5A, Nihon Kohden) connected with inlet tube. ISP was controlled by a peristaltic pump (1210, Harvard).

After perfusion of the left carotid sinus, ISP was kept at 13.3 kPa for 30 min and then was lowered to 0 rapidly, from which ISP was elevated to 29.3 kPa in the form of pulsatile ramp by regulating the speed of peristaltic pump, which was automatically controlled by a program designed by our laboratory. It took 2.5 – 3.0 min for ISP to be increased from 0 to 29.3 kPa. ISP and BP were simultaneously recorded on a polygraph (RM-6200). This process was repeated with an interval of 10 min to check the stability in the baroreflex. Intactness of the experimental preparation was documented by the repeatable presence of a drop of BP in response to the increase of ISP.

Perfusion of hindlimb vessels The constant flow perfusion method was adopted to measure the hindlimb vascular resistance. A catheter was inserted into common carotid artery, from which blood was perfused with a peristaltic pump into femoral artery by constant flow. The perfusing pressure was adjusted to be similar to BP. Various doses of Mox or saline (50 μL) were injected into femoral artery and the perfusing pressure was recorded.

Protocol By perfusing left carotid sinus with K-H solution, ISP was increased, the functional curve for the ISP-BP relation might be constructed, and the functional parameters of baroreflex such as threshold pressure (TP), saturation pressure (SP), equilibrium pressure (EP), peak slope (PS), reflex decrease of BP (RD), and operating range (OR) were determined. TP was the ISP at which BP began to decrease in response to the increase of ISP. SP was the ISP at which BP just showed no further decrease reflexly with the increase in ISP. EP was the ISP which equalled to systemic BP. OR was calculated as the difference of SP minus TP. ISP was then fixed at 13.3 kPa for 20 min, and K-H solution containing Mox (10, 32, and 100 μmol·L^{-1}) was then used to perfuse for 30 min, followed by measurements of parameters again. Finally the carotid sinus was perfused with K-H solution as postcontrols.

The effect of Efa on the response to Mox was examined. After the control parameters of carotid sinus baroreflex were obtained, the carotid sinus was perfused with K-H solution containing Efa 100 μmol·L^{-1} for 10 min, and parameters were measured. Then Mox 100 μmol·L^{-1} was added to perfusate. The parameters were measured for 30 min, then drugs were washed out with K-H solution.

Drugs Mox (purity 99 %, Kali-Chemie, Hannover, Germany) and Efa (Sigma) were dissolved in saline. Data were expressed as $\bar{x} \pm s$ and compared with paired t test.

RESULTS

Effects of Mox on left carotid sinus baroreflex By perfusing left carotid sinus with K-H solution and elevating ISP from 0 to 29.3 kPa, BP was reflexly decreased. There was no difference in baroreflex parameters among controls. Mox induced obvious changes in baroreflex parameters, which appeared within 10 min after perfusing isolated carotid sinus with K-H solution containing Mox and reached the peak at 20 – 30 min, and disappeared 1 – 2 h after washout. Mox 100 μmol·L^{-1} decreased RD ($P < 0.01$) and PS ($P < 0.01$), and increased TP ($P < 0.01$), EP ($P < 0.05$), and SP ($P < 0.01$) (Fig 1, Tab 1).

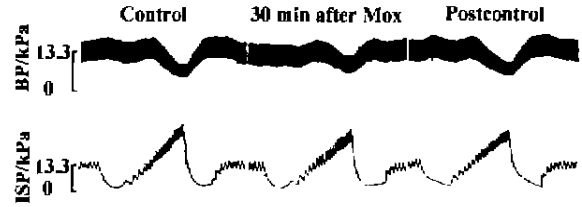

Fig 1. Reflex responses of blood pressure (BP) to the increase in intrasinus pressure (ISP).

Mox 32 μmol·L^{-1} also reduced RD ($P < 0.01$) and PS ($P < 0.01$), and increased SP ($P < 0.05$), while the decrease in RD was smaller than that induced by Mox 100 μmol·L^{-1} ($P < 0.05$). Mox 10 μmol·

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Tab 1. Effects of Mox on carotid sinus baroreceptor function in rats. $\bar{x} \pm s$. $^{a}P > 0.05$, $^{b}P < 0.05$, $^{c}P < 0.01$ *vs* control. $^{d}P > 0.05$, $^{e}P < 0.05$ *vs* Mox 100 μmol·L^{-1}. $^{g}P > 0.05$, $^{h}P < 0.05$ *vs* Mox 32 μmol·L^{-1}.

Drugs, μmol·L^{-1}	Rats	TP, kPa	EP, kPa	SP, kPa	OR, kPa	PS, kPa/kPa	RD, kPa
Control	8	7.3±0.6	12.0±0.9	22.2±0.8	15.1±0.6	0.41±0.06	6.0±0.9
Mox (100)	8	10.4±0.9^{c}	12.9±1.0^{b}	25.7±1.3^{c}	15.3±1.6^{a}	0.22±0.07^{c}	3.1±0.8^{c}
Control	8	7.4±0.4	12.5±0.9	22.5±0.4	15.1±0.5	0.43±0.08	6.2±1.2
Mox (32)	8	9.4±1.2^{cd}	12.7±0.9^{ad}	24.0±1.3^{be}	14.5±0.8^{ad}	0.32±0.06^{ce}	4.4±0.9^{ce}
Control	8	7.4±0.8	11.8±0.9	22.1±0.5	14.7±0.8	0.43±0.07	6.2±0.9
Mox (10)	8	8.1±1.4^{ag}	12.1±0.9^{ag}	22.8±0.9^{ag}	13.6±1.2^{ag}	0.39±0.06^{bg}	5.6±1.0^{bh}
Control	6	7.2±0.4	12.2±0.4	22.4±0.5	15.1±0.5	0.44±0.03	6.5±0.6
Efa (100)	6	7.4±0.8^{a}	12.4±0.5^{a}	22.5±0.8^{a}	15.1±1.0^{a}	0.43±0.04^{a}	6.6±0.4^{a}
+ Mox (100)	6	7.5±0.6^{a}	12.5±0.8^{a}	22.4±0.6^{a}	14.8±0.4^{a}	0.43±0.06^{a}	6.2±1.2^{a}

EP: equilibrium pressure. OR: operating range. PS: peak slope. RD: reflex decrease of blood pressure. SP: saturation pressure. TP: threshold pressure.

L^{-1} only induced the decrease of RD ($P < 0.05$, Tab 1).

Mox shifted the functional curve of the baroreflex to the right and upward in a dose-dependent manner (Fig 2).

Effect of Efa on Mox responses Efa 100 μmol·L^{-1} *per se* did not induce any change in functional parameters of baroreflex, but completely blocked the effects of Mox 100 μmol·L^{-1} (Tab 1).

Effects of Mox on hindlimb vascular resistance Mox increased perfusing pressure of hindlimb (Tab 2).

Tab 2. Effect of Mox on the perfusing pressure in femoral artery. $n = 4$ rats. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* before. $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* control.

Mox/ μg·kg^{-1}	Perfusing pressure /kPa Before	After
0	11.2±0.6	11.2±0.6
16	11.2±0.6	12.7±0.6^{ce}
40	11.5±1.1	14.0±0.8^{ce}
80	11.2±1.4	15.8±2.1^{ce}
143	10.9±0.7	20.5±1.1^{cf}

When the dose of Mox was fixed at 140 μg·kg^{-1},

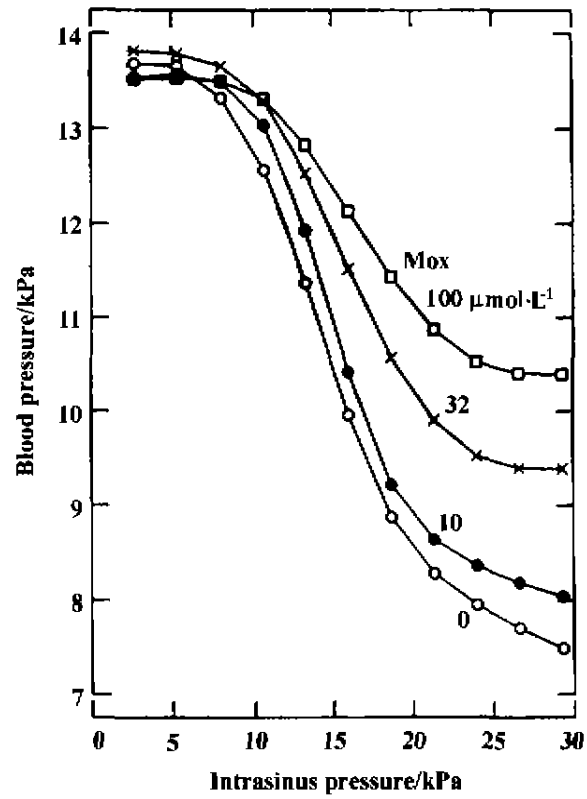

Fig 2. Effects of Mox on the functional curve of carotid sinus baroreflex in rats.

Efa was injected prior to Mox and the dose of Efa was gradually increased, the increment of perfusing pressure

was reduced by 67 % (Fig 3).

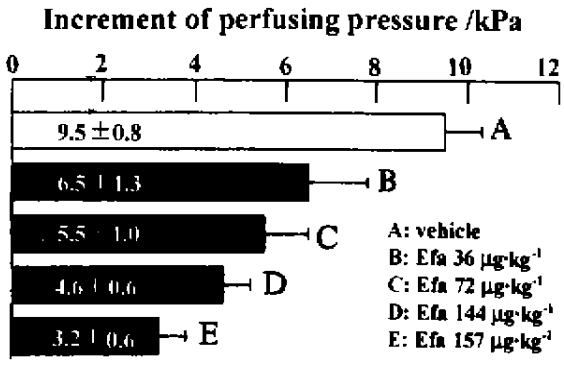

Fig 3. Effect of various doses of Efa on the response elicited by Mox 140 μg·kg^{-1}. $n = 3$. $\bar{x} \pm s$.

DISCUSSION

This study first presented the evidence that Mox was able to affect carotid sinus baroreflex function. In our experiment, the technique of perfusing carotid sinus was used and the activity of baroreceptors was altered by changing ISP. Under such condition, drugs in perfusate were restricted to local sinus area and the possible secondary actions to pressor or depressor effects of Mox were avoided. So the effects of Mox on baroreflex resulted from its direct action on carotid sinus baroreceptor. Mox shifted the functional curve of carotid sinus baroreflex to the right and upward, with reduction of PS and RD, thereby indicating that Mox inhibited carotid baroreflex associated with a resultant increase in BP in anesthetized rats. Our result is accordant with a study that aortic baroreceptors exerted a potent restraining influence on the centrally mediated depressor effect of clonidine[8], although this action of clonidine was attributed to its central effect by the author. The inhibitory effects of Mox on carotid baroreflex restrained its centrally mediated depressor responses and perhaps prevented BP from overdrop, and involved in the early pressor action of iv Mox. With regard to mechanisms underlying the inhibitory effect of Mox on carotid baroreflex, two possibilities may be proposed. One is the change in vascular tone of sinus wall, which regulates the sensitivity of baroreceptor. Another is the direct effect of Mox on sinus nerve terminals. From our results, the former may be mainly working mechanism, since Mox dose-dependently increases peripheral vascular resistance. What excites the baroreceptors is the deformation of their ending by tissue tension[9], ie, wall tension σ, which by the Laplace's relation is: $\sigma = P \cdot r/h$, where P is the distending pressure, r is the radius of the vessel lumen, and h is the wall thickness. Mox might directly induce arterial constriction and thus reduce r, so σ became smaller and the sensitivity of baroreceptor was decreased.

The type of receptor mediating inhibitory effect of Mox on baroreceptor remained to be defined. The present study showed that Efa completely blocked the effects of Mox on baroreflex and attenuated its effect on hindlimb vessels, implying that I_1-imidazoline receptor may be involved in the constrictive effect of Mox. To our knowledge, up to now it has not been reported that there are I_1-imidazoline receptors on arteries. Our study proposed that there were I_1-imidazoline receptors along with α_2-[10] and α_1-[11] adrenergic receptors on peripheral arterial tree, which mediated the vasoconstrictive action of Mox. The reason pertaining to partial antagonism of Efa on the constrictive effect of Mox on the hindlimb vessels merits further investigation.

In conclusion, Mox inhibits the carotid baroreflex and thereby exerts a buffering action on its depressor effect.

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莫索尼定对麻醉大鼠颈动脉窦压力感受器反射的影响

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关键词 莫索尼定；颈动脉窦；压力反射；血压；抗高血压药；肾上腺 α 拮抗剂；伊法克生

目的：观察莫索尼定对颈动脉窦压力感受器反射的影响. **方法**：利用灌流左颈动脉窦方法，观察莫索尼定对麻醉大鼠压力反射机能参数的影响. 恒流灌流股动脉，记录灌流压变化，确定莫索尼定对血管阻力的影响. **结果**：莫索尼定 32，100 μmol·L^{-1}使颈动脉窦压力反射机能曲线向右上移位，曲线最大斜率和反射性平均动脉压下降幅度均减小，提示莫索尼定对压力感受器反射有抑制作用. 伊法克生 100 μmol·L^{-1}完全阻断莫索尼定 100 μmol·L^{-1}的效应. 莫索尼定显著增加血管阻力. **结论**：莫索尼定对颈动脉窦压力反射有直接抑制作用，此作用可能在于其引起窦壁收缩所致.

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读者注意

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