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ORIGINAL RESEARCH

Central Pressor Response by Olmesartan in the Rostral Ventrolateral Medulla of Spontaneously Hypertensive Rats

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Abstract

Objectives: The major aims of the present study were to evaluate the effect of chronic oral treatment with olmesartan on cardiovascular response to exogenous angiotensin II (Ang II), and its relationship with the AT_1 receptor (AT_1R) mRNA in rostral ventrolateral medulla (RVLM) of spontaneously hypertensive rats (SHR).

Methods: SHR (12 weeks old) were treated with olmesartan, 30 mg/(kg·day), in drinking water. 4 weeks later, Ang II (100 *pmol*) was microinjected into the RVLM, and arterial pressure and the heart rate were observed.

Results: The blood pressure of all SHR-olme rats reach near normal level during breeding period. Ang IIresulted in an increase in mean arterial pressure (MAP) of each group. While, the increase of MAP in olmesartan-treated SHR was significantly smaller than that in untreated SHR(26.3 ± 0.75 mmHg vs. 47.2 ± 1.41 mmHg, n = 10, $P < 0.01$); however, it was still greater than that in WKY rats(26.3 ± 0.75 mmHg vs. 21.5 ± 0.72 mmHg, n = 10, $P < 0.05$). The heart rate (HR) of the each group was also increased, but there was no statistical significance among groups. In addition, AT_1R mRNA in RVLM was measured by RT-PCR and analyzed with optic density. It demonstrated that the mean optic density (MOD) of AT_1R mRNA in olmesartan-treated SHR was significantly reduced compared with untreated SHR(0.94 ± 0.41 vs. 2.41 ± 0.37, n = 10, $P < 0.01$); however, it was still higher than that in WKY rats(0.94 ± 0.41 vs. 0.81 ± 0.22, n = 10, $P < 0.05$).

Conclusions: These results demonstrated that chronic oral treatment with olmesartan could obviously attenuate the exaggerated pressor response to exogenous Ang II and inhibit the excessive expression of AT_1R in RVLM of SHR, so we presume olmesartan may attenuate central pressor response by reducing AT_1R in RVLM of SHR.

Keywords: olmesartan, rostral ventrolateral medulla, AT_1 receptor, spontaneously hypertensive rats

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Introduction

RVLM plays a major role in neural control of the circulation.[1] It receives various cardiovascular messages from both senior cardiovascular centers above medulla oblongata and peripheral nervous system, changes the excitatory and inhibitory state of vasculomotor neurons, maintains sympathetic vasomotor tone and mediates neurally mediated cardiovascular reflexes.

Studies have reported that significantly higher blood pressure was increased by electrical or chemical stimulation of the RVLM in many forms of experimental hypertension.[2,3] Many subsequent studies have also showed similar results.[4] It is proposed that the disordered function in RVLM is closely associated with hypertension. Although the precise mechanism of this disorder in RVLM remains unclear, the pathological changes of AT_1R in this region has been suggested to play an important role in the accentuation of sympathetic vasomotor function in RVLM of hypertension. Using immunofluorescence image technique, a much higher concentration of angiotensin receptors, predominantly of AT_1R subtype, is found on the vasomotor neuron membrane in RVLM of SHR compared with normotensive rats.[5] Thus, the high expression of AT_1R, at least in part, contributes to the enhanced function of RVLM. In addition, recent studies report injection of AT_1R antagonists into RVLM could attenuate the cardiovascular response to exogenous Ang II by blocking AT_1R in RVLM of SHR.[6] However, the effects of chronic oral treatment with AT_1R antagonist on the cardiovascular response and the AT_1R gene expression and protein expression in RVLM of SHR scarcely be investigated. The purpose of the present study is to test the hypothesis that oral treatment with olmesartan, a new type of AT_1R antagonists, could attenuate the pressor response to exogenous Ang II by inhibiting the expression of AT_1R in RVLM of SHR.

Method

Animals

Adult male SHR and Wistar–Kyoto (WKY) rats, weighing 350 to 500 g, were obtained from Vital River Laboratory Animal Technology Co. Ltd. All of the experiments were approved by the institutional animal care and use committee of the China Medical University and complied with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. SHR were randomly divided into treatment group and control group. Treatment group rats (n = 20) were treated with olmesartan, 30 mg. (kg·day), dissolved in drinking water, while control group (n = 20) and WKY rats (n = 20) did not receive any medications throughout the experimental period. Food was freely available. All of the rats were fed for 4 weeks. Systolic pressure was measured twice weekly by the tail-cuff method.

Surgery and general procedures

4 weeks later, 30 rats randomly taken from three groups were anesthetized with intraperitoneal injection of urethane (1.5 g/kg), Then, placed in a stereotaxic apparatus in a supine position (Narishige Co., Japan). Animals were tracheotomized and artificially ventilated (10~12 ml/kg, 60~70 strokes/min). Polyethylene catheters (PE50) filled with heparinized saline were inserted into the right femoral artery and vein for arterial pressure recording and anesthesia maintenance (1 g/kg), respectively. MAP was continuously monitored by a pressure transducer (P50; NEC Sanei Co., Tokyo, Japan) and HR was determined by the arterial pressure waves. to expose the ventral surface of the medulla oblongata by surgical procedures. In the whole experiment process, electric blanket was used to keep rectal temperature around 37 °C.

RVLM orientation and microinjection

The position of RVLM was orientated according to the Paxinos and Watson rat brain atlas, which is 2.0 mm rostral to the caudal tip of the area postrema, 1.9 mm lateral to midline, 3.0 mm below the dorsal surface of the medulla. After the RVLM was identified, Ang II (100 pmol, Sigma Co., USA) was microinjected into unilateral RVLM with a microsyringe pushed by motor-driven micromanipulator (IM-1, Narishige, Japan) at a constant rate of 500 nL/h. The volume injected was measured by observing the displacement of the fluid meniscus in the pipette with respect to a horizontal grid in a microscope. At the same time, blood pressure and HR were consecutively monitored by transducer. Ang II was dissolved in standard artificial cerebrospinal fluid. At the end of the experiment, 100 nL of 2% Evans blue dye was injected into

the microinjection site. Rats were sacrificed by an overdose of urethane. The animals were perfused through left cardiac ventricle with 150 mL of 0.9% NaCl followed by 250 mL of 4% paraformaldehyde solution, and the brain stem was rapidly removed and fixed in 4% paraformaldehyde solution. The medulla oblongata was sectioned and the microinjection site was verified (Fig. 1). Only the data's of rats whose microinjection sites were in the region of the RVLM were used for analysis.

Measurement of AT_1R mRNA in RVLM

The last 30 rats were sacrificed, the brain tissue was rapidly removed and frozen in liquid nitrogen. The medulla oblongatas were cut into 500 μm thick coronal sections, and the brain tissue of RVLM was punctured with a 15-gauge needle. Thereafter, the level of AT_1R mRNA in RVLM was measured by RT-PCR. In brief, total RNA from 200 mg of brain tissue was isolated and extracted with Trizol Reagent Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Then, the concentration of the RNA was determined by spectrophotometer. The extracted RNA (5 μL) was subjected to first-strand cDNA synthesis. 2 μL of reaction product was taken to perform PCR , which carried out as follows: 94 °C 1 min, 94 °C 30 s, 57 °C 30 s, 72 °C 1 min, after exponentially increasing for 30 cycles, extended for 4 min at 72 °C. The PCR products (8 μL)were finally separated on 2% agarose gel electrophoresis. The bands were quantified with an image analyzer (Sunnyvale, CA, USA) and expressed as the ratio to β-actin mRNA product.

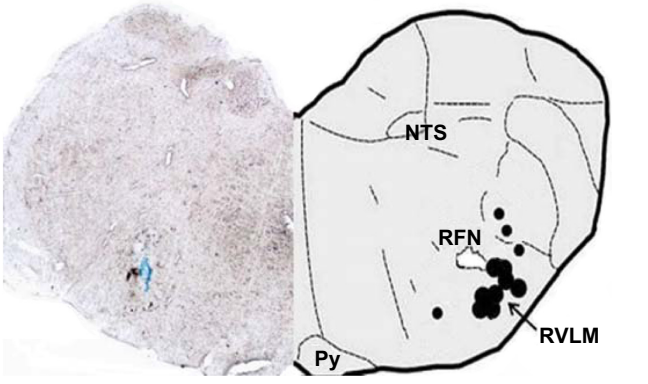

Figure 1. Typical microinjection site in the RVLM evaluated by 100 nL of Evans blue diffusion (left), and a schematic representation (right).The black stains represent the microinjection site.
Abbreviations: NTS, nucleus of the tractus solitarii; RFN, retrofacial nucleus; Py, pyramidal tract.

Statistical Analysis

All of the values are expressed as the means ± SEMs. Each variable was analyzed by *t* test or 1-way ANOVA with SPSS 11.0 software. Differences were considered significant at $P < 0.05$.

Results

The condition of blood pressure in each group

The systolic blood pressure (SBP) of olmesartan-treated SHR gradually reduced to 110~120 mmHg during the first week of treatment, then keep at a low level during the following weeks, whereas the untreated SHR and WKY rats showed no such a tendency in SBP (Fig. 2).

Cardiovascular effects of Ang II in the RVLM

Microinjection of 100 pmol Ang II into unilateral RVLM caused MAP increased in each group of rats at different degree with a peak at 2 min after administration (Table 2). The increase in MAP of untreated SHR was significantly greater than that of olmesartan-treated SHR and WKY rats. In contrast, the exaggerated pressor response was significantly attenuated in olmesartan-treated SHR; although it still showed a slightly increase in MAP compared with that in WKY rats (Fig. 3). The HR exhibited an increase but did not differ among the three groups.

Expression of AT_1R *mRNA* in RVLM

The level of AT_1R mRNA in RVLM corresponded to the intensity of the electrophoretic bands, which were treated with image analyzer and showed as

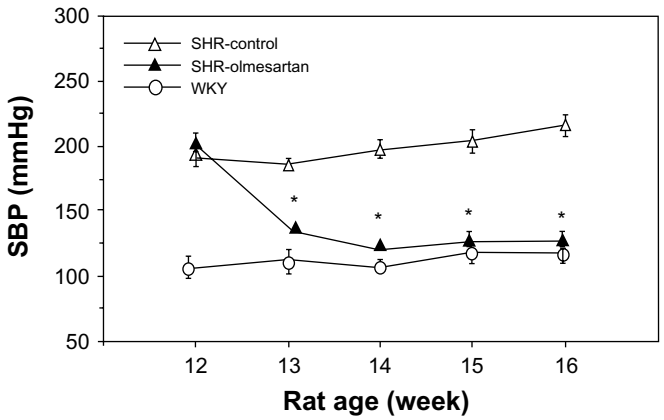

Figure 2. Shows the changes in SBP during the antihypertensive treatment.
Note: *$P < 0.05$ vs. SHR-control.

Table 1. RT-PCR primer sequence.

Product		Sequence	Product size
AT1R	forward primer	5'-TGGCAGGCACAGTTAC-3'	341 bp
	reverse primer	5'-TCATTGGGTGGACGAT-3'	
β-actin	forward primer	5'-ACCACAGTCCATGCCATCAC-3'	452 bp
	reverse primer	5'-TCCACCACCCTGTTGCTGTA-3'	

semi-quantitative results. High-level expression of AT_1R mRNA was detected in the RVLM of SHR. In contrast, the AT_1R mRNA level in RVLM significantly decreased in olmesartan-treated SHR compared with untreated SHR (0.94 ± 0.41 vs. 2.41 ± 0.37, $P < 0.01$, Fig. 3); although it was still higher in the level of AT_1R mRNA compared with that in the WKY rats (0.94 ± 0.41 vs. 0.81 ± 0.22, $P < 0.05$, Fig. 4).

Discussion

Specific activation of RVLM neurons causes an increase in arterial pressure mediated by an increase in peripheral resistance, cardiac output, and secretion of catecholamines. Previous studies have demonstrated that blood pressure regulation function and subcellular structures on the vasomotor neurons in RVLM are obviously damaged in hypertension.[7,8]

In the present study, we found that the cardiovascular response to Ang II and the AT_1R mRNA expression in RVLM were both significantly higher in SHR compared with that in WKY rats. which are consistent to previous studies. However, after a period of treatment with olmesartan, the cardiovascular response to Ang II was significantly attenuated and the AT_1R mRNA in RVLM was greatly reduced in SHR. These results extend previous studies and demonstrate chronic oral treatment with olmesartan could obviously attenuate the pressor response to exogenous Ang II by reducing the level of AT_1R in RVLM of SHR. The present study is the first research to prove the AT_1R in RVLM is responsible for olmesartan to induce partial normalization of the exaggerated blood pressure to Ang II in SHR.

Traditionally, AT_1R antagonists as an antihypertensive treatment primarily affects on blocking the peripheral AT_1R. However, During recent years, more and more studies showed that AT_1R antagonists or angiotensin converting enzyme antagonists could attenuate central pressor response,[9,10] even some of them are able to decrease the AT_1R and mRNA in regions of the rat brain,[11] however, most of experiments are restricted in intracerebral injection or intraventricular infusion. In the present study, it is demonstrated that chronic oral treatment with olmesartan obviously attenuates the pressor response to exogenous Ang II by reducing the level of AT_1R in RVLM of SHR. The previous findings and the present study may support the idea that chronic treatment with AT_1R antagonist has some beneficial effects on cardiovascular regulation in the brain, which are likely to be independent of the effects of AT_1R antagonist on blood pressure. This may provide new insights into novel therapeutic concepts for hypertension.

Not all AT_1R antagonists chronic orally treated with are able to alter the central pressor response. In contrast to olmesartan, another AT_1R antagonist, losartan, has little effect on pressor response to intracerebroventricular injection Ang II by oral or intravenous adiministration.[12,13] Olmesartan is a novel AT_1R antagonist of imidazole. It has high selectivity and affinity to AT_1R and has powerful and persistent clinical effect in hypertension therapy. Our previous study has already demonstrated that chronic treatment with olmesartan could effectively inhibit the pressor response to glutamate in RVLM of SHR. This time

Table 2. The changes of MAP and HR to Ang II mean ± SEM.

	SHR-control	SHR-olmesartan	WKY
N	10	10	10
ΔMAP mmHg	47.2 ± 1.41	26.3 ± 0.75*	21.5 ± 0.72
ΔHR beat/min	29.3 ± 0.94	39.5 ± 1.66	38.7 ± 2.24

Notes: *$P < 0.01$ vs. SHR-control; *$P < 0.05$ vs. WKY.

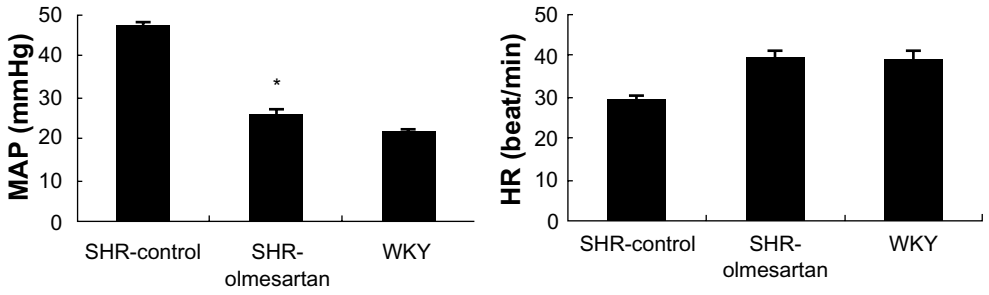

Figure 3. The changes in MAP(left) and HR(right) in response to bilateral microinjection of Ang II into the RVLM in SHR-control (n = 10), SHR-olmesartan (n = 10) and WKY rats (n = 10).
Notes: Values are means ± SE. **P* < 0.01 vs. SHR-control; **P* < 0.05 vs. WKY; *P* > 0.05 among groups in HR.

we once again prove olmesartan effectively attenuates central pressor response by reducing the level of AT_1R in RVLM. However, the major question remains is that olmesartan is difficult to penetrating blood-brain barrier in normal physiological condition, and how olmesartan affects AT_1R in central nervous system? The previous observation that AT_1R in the RVLM seems to have little impact on sympathetic drive under normal conditions but has prominent influence on sympathetic drive under conditions of stress. Further studies will be required to elucidate the mechanism of olmesartan penetrating into the central nervous system or by other methods influencing AT_1R in central nervous system.

Recent studies found Mitogen-Activated Protein Kinase(MAPK) signaling pathways triggered by Ang II plays an important role in the excessive expression of AT_1R in the subfornical organ and the paraventricular nucleus of heart failure rats and rabbits. These studies report a 4-week intracerebroventricular (ICV) infusion of Ang II activates MAPK signaling

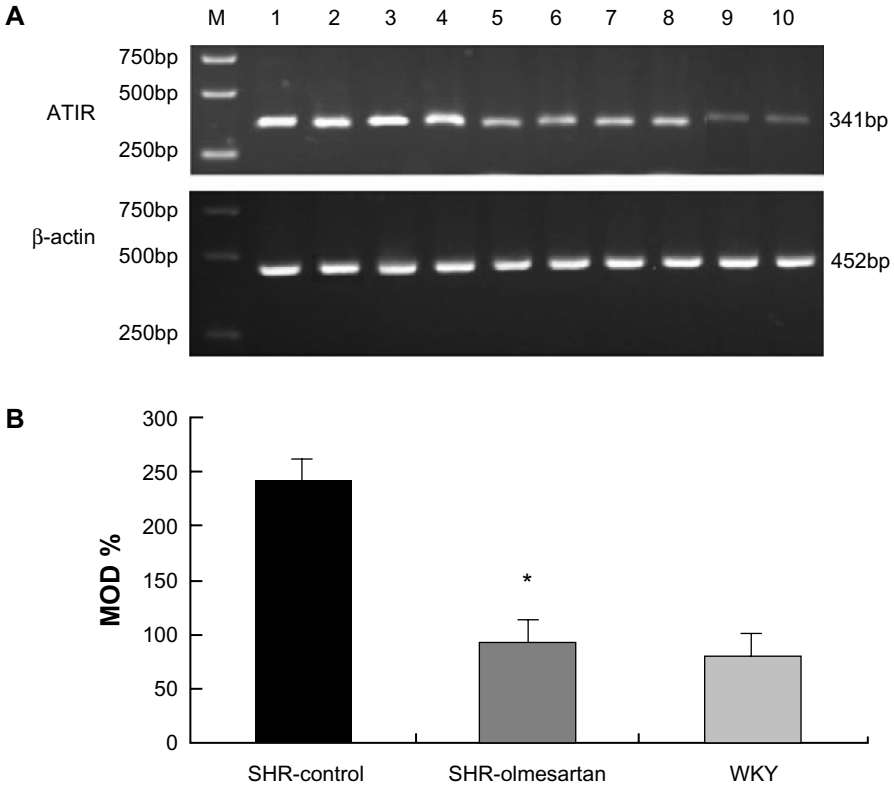

Figure 4. The effect of olmesartan on the expression of AT_1R mRNA in RVLM. **A**) Electropherogram of RT-PCR products. The optic density of electrophoresis bands represents the level of AT_1R mRNA. **B**) Bar graphs showing the MOD of AT_1R mRNA treated with image analyzer in RVLM of the SHR-control (n = 10), SHR-olmesartan (n = 10) and WKY rats (n = 10). β-actin was used as internal control.
Notes: M, RNA marker; 1–4, SHR-control; 5–8, SHR-olmesartan; 9,10, WKY rats. Values are means ± SE. **P* < 0.01 vs. SHR-control; **P* < 0.05 vs. WKY.

pathways and increases the expression of AT_1R mRNA and protein; a 4-week ICV infusion of the MAPK inhibitors (PD98059 or SP600125) prevent upregulation of AT_1R mRNA and protein; moreover, a 4-week ICV infusion of an AT_1R antagonist (losartan) reduces the expression of MAPK.[14,15] Hence, AT_1R antagonist infused through ICV may antagonizing intracephalic Ang II, then, inhibiting MAPK signaling pathways, produce a downregulation effect on AT_1R in the subfornical organ and the paraventricular nucleus.[16] Thus, we suppose olmesartan may alter AT_1R mRNA in the RVLM via this way if it could penetrate into the central nervous system in some way. Moreover, the feedback theory may reasonably explain this question if not penetrating into the central nervous system. The decreasing of blood pressure by olmesartan may contribute to a negative feedback system that inhibits the AT_1R expression in the RVLM of SHR. Certainly, these hypotheses will be tested in the following experiments performed *in vivo* and *in vitro* in the future.

In conclusion, chronic oral treatment with olmesartan could obviously attenuate the pressor response to exogenous Ang II and inhibit the excessive expression of AT_1R in RVLM of SHR. So ,we presume olmesartan may attenuate Central pressor response by reducing AT_1R in RVLM of SHR.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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