Role of Angiotensin II Receptors in Pressure Overload-induced Left Ventricular Hypertrophy

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ZHANG ET AL.: ***Role of Angiotensin II Receptors in Pressure Overload-induced Left Ventricular Hypertrophy.*** ***Objective:*** *To investigate the role of angiotensin receptors(ATRs) in pressure overload-induced left ventricular hypertrophy (LVH).* ***Methods:*** *The model of rat abdominal aortic constriction was adapted. At 10th week after operating, Ang II in myocardium was measured by radioimmunoassay, tissue ATRs and its subtype were analysed by radioligand binding assay.* ***Results:*** *The content of Ang II in the operated group was significantly more than that in the control group. Left ventricular mass index (LVMI) was positively correlated with Ang II (r=0.8066, P<0.01). The maximal binding capacity of ATRs in the operated group was significantly higher than that in the controls (P< 0.01). However, the dissociation constant (kd) and ratio of AT_1 to AT_2 receptor between the two groups were not significantly different. Left ventricular hypertrophy was markedly reduced by Irbesartan, an AT_1R antagonist, and was not influenced by CGP42112A, an AT_2R antagonist.* ***Conclusions:*** *These results suggest that left ventricular ATRs were upregulated during the pressure overload. The left ventricular hypertrophy induced by Ang II is mainly due to AT_1R pathway. (J HK Coll Cardiol 2001;9:41-44)*

Angiotensin II, angiotensin receptors, left ventricular hypertrophy, receptor

摘要

目的：探討血管緊張素II受體(ATRs)在壓力超負荷致左室肥大中的作用。方法：採用大鼠腹主動脉縮窄模型，術後10周通過放免法測心肌組織血管緊張素II(Ang II)含量，放射性配基結合分析法檢測心肌組織ATRs及其亞型的變化。結果：手術組Ang II含量顯著增高，與左室重量指數(LVMI)呈正相關(r=0.8066，P<0.01)。ATRs最大結合容量(Bmax)較對照組顯著增高(P<0.01)，但兩組之間的平衡解離常數(kd)、血管緊張素II1型受體(AT_1R)和血管緊張素II$_2$型受體(AT_2R)之間的比例无顯著差異。非肽類AT_1R拮抗劑Irbesartan可顯著抑制Ang II的升高和左室肥大，非肽類AT_2R拮抗劑CGP42112A則无此作用。結論：壓力超負荷時心肌組織ATRs上調，Ang II致左室肥大的作用主要由AT_1R介導。

關鍵詞：血管緊張素II　血管緊張素受體　左室肥大　受體

Left ventricular hypertrophy (LVH) is an independent, major risk factor for cardiovascular morbidity and mortality. Several studies suggested that the heart could secreate angiotensin II (Ang II) in an autocrine and paracrine fashion, and Ang II is involved in the development of LVH.[1] There is also evidence indicating that Ang II is a growth-promoting factor and its effect is mediated by angiotensin receptors (ATRs). However, there has been no agreement on the alteration and role of ATRs in LVH. The present study was designed to investigate the role of ATRs in LVH.

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Methods

Materials

Angiotensin II, Phenglmenthyl sulfongl fluride (PMSF, America), ^{125}I-Ang II (1200 μi/μg, China),

Ang II Radioimmunoassy (China), Losartan (Dup753, America), Irbesartan (France), CGP42112A (America).

Preparation of Animals

Male Wistar rats (body weight 150-180 g) were divided randomly into four goups: sham-operated group (n=15). Operated group (n=15). Operated group with Irbesartan (10 mg/kg/d, n=12). Operated group with CGP42112A (20 mg/kg/d, n=12), Animals were anesthetized with an intraperitoneal injection of pentobarbtal sodium (30 mg/kg). Aortic constriction was created via placement of stainless steal tip (0.7 mm ID) on the abdominal aorta. Irbesartan (an angiotensin II type 1 receptor (AT_1R) antagonist) and CGP42112A (an angiotensin II type 2 receptor (AT_2R) antagonist) were fed at the first day after operating and all rats were used for experimentation 10th week after the constriction.

Measurement

Left Ventricular Weight (LVW), Left Ventricular Mass Index (LVMI)

Heart of rats were removed and placed in ice-cold saline, pericardia, vessels, atria, right ventricular wall and fat were removed. Weighted LVW and caculated LVMI. LVMI=LVW/body weight (mg/g).

Diameter of Cardiac Myofibril (ID)

The mid myocardium was cut at 0.5 cm thickness at cross section and was fixed in 10% formalin solution, embedded in paraffin. The tissue were sliced and these sections were dyed with HE, Diameter of myofibril was measured by pathological examination combined with computed processing. 5 sections of each animal were observed by light electroscopy.

Measurement of Ang II Content

The heart was removed, left ventricle was immediately frozen on dry ice, weighed and stored at -80ºC until assay. On the day of extraction, the tissues were thawed and homogenized by Polytron in 0.1 N HCl. Homogenate was centrifuged at 20,000×g for 30 min at 4ºC, and the supernatant was applied to an octadecasilyl-silica cartridge. After washing with 10 ml of 0.1 N HCl, Ang II were eluted with a mixture of methanol:distilled water:trifluoroacetic acid (TFA) (80: 19.9:0.1, vol ratio). The eluate was evaporated to dryness in a vacuum centrifuge and resuspended in 0.1 M Tris-acetate buffer containing 2.6 mM EDTA, 1 mM PMSF, and 0.1% bovine serum albumin (BSA) pH 7.4, for radioimmunoassay of Ang II according to the method of Mizuno.[2]

Binding Assay[3]

Left ventricular tissue was homogenized. The homogenate was centrifuged at 4ºC for 10 minutes at 2000×g. The supernatant was removed and centrifuged at 4ºC for 20 minutes at 27,000×g. The supernatant was removed and discarded while the pellet was resuspended in 50 mM Tris buffer (pH 7.4). Membrane protein concentration was adjused to 0.75 mg/ml.

Binding studies were performed by incubation of membrane protein in binding buffer (composed of 50 mM Tris , 150 nM Nacl, 0.4 mM PMSF, 0.1% BSA. 0.1% bacitracin; 10 μM 1,10-phenanethroline; at pH 7.4) with various concentrations of ^{125}I-Ang II (0.2-6 nmol/L) for 120 minutes at 22ºC. After incubation, bound and free radioactivity were seperated by filtration through glass fiber filters, and the radioactivity of each filter was counted in a gamma counter. Nonspecific binding was determined by the addition of 2 μmol/L unlabed Ang II. The maximum binding capacity and the dissociation constant were determined by use of the scatchard equation.

Ang II receptor subtypes in LVH and control (n=4 in each group) hearts were characterized by the presence or absence of unlabeled Ang II or Losartan (an AT_1R antagonist) or CGP42112A.

Finally, Crossover inhibition experiments were performed in a modified fashion with 5nM ^{125}I-Ang II by determing competitive binding by Losartan or CGP42112A in the presence of 1 μM receptor antagonist. The half-maximal inhibitory concentration (IC50) and inhibitory constant (ki) were then calculated.

Statistical Analysis

All data were presented as mean±SD. Statistical analysis was done by using student's *t* test and correlation analysis. Statistical significance was defined as $P<0.05$.

Results

Changes of LVW, LVMI and ID (Table 1)

Compared with control, LVW, LVMI and ID in the operated group increased significantly ($P<0.05$,

P<0.01). These parameters in the Irbesartan-treated group were decreased significantly when compared to the operated group (P<0.05, P<0.01), but there were no significant difference between CGP42112A-treated and operated group (P>0.05).

Change of Ang II Content in Myocardium (Table 1)

Ang II content in the operated group was significantly more than that in the controls (P<0.01). Compared with the operated group, Ang II content decreased obviously by administration of Irbesartan (P<0.01), but was not influenced by treament of CGP42112A (P>0.05).

Correlated Analysis between LVMI and Ang II Content

There was a significant positive correlation between LVMI and Ang II content (r=0.8066, P<0.01).

Measurment of ATRs in Myocardium

The scatchard plot analysis showed a single class of high-affinity Ang II binding sites in both operated group and control group, without significant difference in kd between them (0.93±0.17 vs 1.08±0.22 nM; P>0.05). However, The present study demonstrated that there was a significantly higher Bmax in LV tissue in the operated group than in the controls (34.13±3.25 vs 15.95±2.15 fmol/mg.pro; P<0.01). Competitive inhibition and crossover inhibition experiments indicated that AT_1R and AT_2R in the control group accounted for 51.18% and 45.15% Ang II binding sites respectively. In the operated group, they were 46.78% and 52.18%, respectively. There was no significant difference in the proportional binding site between the above two groups (P>0.05).

Ang II, Losartan and CGP42112A inhibited competivily the binding of ^{125}I-Ang II with ATR. Compared with controls, IC50 and ki in the operated group had no significant difference (P>0.05). (Table 2)

Discussion

The present study demonstrates the presence of specific high affinity, low binding capacity sites for Ang II receptor in rats in the left ventricular myocardium in the controls, and the presence of almost equal proportions of AT_1R and AT_2R. These findings were in accordance with that found by Schi et al,[4] where by autoradiography it was determined that AT_1R and AT_2R each accounted for ~50% of specific binding in normal rat hearts. At 10th week after operating, the Bmax of ATRs in the operated group was two times higher than that in the controls. However, there is no difference in ki and ratio of AT_1R to AT_2R between hypertrophied and control hearts, similar to the findings of Suzuki et al[5] and Fujii et al.[6] The former have reported AT_1R mRNA underwent upregulation during the development of cardiac hypertrophy in both spontaneous and renovascular hypertensive rats. The latter revealed that AT_1R and AT_2R were upregulated at the protein level in

Table 1. Changes of LVW, LVMI, ID and Ang II content (n=12, mean±SD)

	LVW (mg)	LVMI (mg/g)	D (μm)	Ang II (pg/g)
Control	777.88±124.38	2.27±0.18	9.85±1.08	126.50±2.33
Operated	1122.38±229.20*	3.15±0.59**	16.59±0.60**	252.90±4.03**
Irbesartan	790.25±109.14$^{\triangle\triangle}$	2.34±0.19$^{\triangle}$	11.27±0.18$^{\triangle\triangle}$	132.70±2.02$^{\triangle\triangle}$
CGP42112A	1079.63±242.88*	3.09±0.78*	15.97±0.77**	261.90±4.87**

vs control * P<0.05 ** P<0.01; vs operated $^{£G}$P<0.05 $^{£G£G}$P<0.01

Table 2. IC50 and ki of non-labled ligand binding competivily with ^{125}I-Ang II (n=4, nmol/L, mean±SD)

	Ang II		Losartan		CGP42112A	
	IC50	ki	IC50	ki	IC50	ki
Control	3.85±0.75	0.68±0.16	5.40±1.79	0.92±0.19	82.01±6.45	14.40±2.47
Operated	3.58±0.56	0.56±0.15	5.16±2.06	0.88±0.44	82.24±10.06	12.58±1.12

Tsukubia hypertensive mice model, and the presence of nearly equal proportion of these Ang II receptor subtypes in membrane of heart ventricles. In contrast, Lopez et al[7] indicated that AT_1R was downregulated at least at the protein level in the rat heart with chronic pressure-overload hypertrophy created by ascending aortic banding, and AT_2R is the major ATR subtype in hypertrophied heart. The reason for this discrepancy is not clear but it may be related to the difference in rat model, degree of hypertrophy and experimental conditions.

Our results showed that LVW, LVMI, ID and Ang II content in the operated group increased significantly. there was a positive correlation between LVMI and Ang II content, and binding capacity of ATR was increased. These suggest that increasing the binding of Ang II to ATRs maybe a mechanism of inducing LVH. It has been clearly demonstrated in the present study that LVW, LVMI, ID and Ang II content were reduced significantly by Irbesartan administration, but were not influenced by CGP42112A treatment. It infers that LVH induced by Ang II is mainly due to the AT_1R pathway. Although the exact mechanism remains unsettled, some studies indicated that the signal tranducing mechanism of AT_1R is mediated via: (1) Activation of phosphoinositide system; (2) Activation of mitogen activated protein kinase (MAPK) linkage; (3) activation of Janus kinase-signal transducer and activators of transcription (JAK-STAT) pathway;[8] (4) Expression of proto-oncogene. They act and influence each other, promoting transcripting of RNA and synthesis of protein in cardiocytes.

There is lack of the data on physiological implication of AT_2R. Some evidence supports AT_2R is abundantly and widely expressed in embryonic and fetal heart, but gradually decrease with development and growth. It suggests that expression of AT_2R is related to development of the heart.[9] Recently, the growth inhibitory action of AT_2R has been demonstrated that it can antagonize the growth promoting effects of AT_1R. Furthermore, it is suggested that AT_2R mediates growth inhibition,which is one of the mechanisms mediating apoptosis.[10,11] It is possible that AT_2R is involved in the development of LVH. However, our results indicate that although pathological hypertrophied myocardium posses some features of fetal heart, such as upregulation of AT_2R, AT_2R antagonist CGP42112A has no effect on LVH. This suggests that AT_2R has no significant role in mediating LVH. It is concluded that the action of AT_2R is different not only between fetal and adult heart but between fetal and diseased heart.

References

1. Sadoshima J, Xu Y, Slayter HS, et al. Autocrine release of angiotensin II mediates strech-induced hypertrophy of cardiac myocytes in vitro. Cell 1993;75:977.
2. Mizuno K, Nakamaru M, Higashimori K, et al. Local generation and release of angiotensin II in peripheral vascular tissue. Hyertension 1988;11:223
3. Chen XM, He GX, Wang GC. Role of angiotensin II receptor neointima hyperplasia after vascular balloon injury. Chin J Cardiol 1998;26:142.
4. Sechi LA, Griffin CA, Grady EF, et al. Characterization of angiotensin receptor subtypes in rat heart. Cir Res 1992;71: 1482.
5. Suzuki J, Matsubara H, Urabami M, et al. Rat angiotensin (type 1A) receptor mRNA regulation and subtype expression in the myocardial growth and hypertrophy. Cir Res 1993;73:439.
6. Fujii N, Tanaka M, Ohnishi J, et al. Alterations of angiotensin II receptor contents in hypertrophied hearts. Biochem Biophy Res Commun 1995;212:326.
7. Lopez JJ, Lorell BH, Ingelfinger JR, et al. Distribution and function of cardiac angiotensin AT_1- and AT_2- receptor subtypes in hypertrophied rat hearts. Am J Physiol 1994;267:H844.
8. Pan J, Fukuda K, Kodama H, et al. Role of angiotensin II in activation of the JAK/STAT pathway induced by acute pressure overload in the rat heart. Cir Res 1997;81:611.
9. Everett AD, Fisher A, Tufro-McReddie A, et al. Developmental regulation of angiotensin type 1 and 2 receptor gene expression and heart growth. J Mol Cell Cardial 1997;29:141.
10. Booz GW, Reisprish MJ, Baker KM. AT_2 receptor blockade augment angiotensin II-induced cardiomycyte hypertrophy. Hypertension 1995;25:547.
11. Yamada T, Horiuchi M, Dzau VJ. Angiotensin II type 2 receptor mediates programmed cell death. Pro Natl Acad Sci USA 1996; 93:156.