# Effect of valsartan and fosinopril on catecholamine-induced cardiac hypertrophy

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## ABSTRACT

**AIM**: To study the influence of angiotensin (Ang) Ⅱ receptor antagonist ($AT_1$) valsartan and angiotensin-converting enzyme (ACE) inhibitor fosinopril on the cardiac hypertrophy induced by catecholamine. **METHODS**: A cardiac hypertrophy model was produced by ip norepinephrine (NE) $1.5$ $mg \cdot kg^{-1} \cdot d^{-1} \times 15$ d. The animals were divided into four groups: 1) control (sodium chloride), 2) NE, 3) NE + fosinopril, 4) NE + valsartan. Fosinopril ig $15$ $mg \cdot kg^{-1} \cdot d^{-1} \times 15$ d, valsartan ig $30$ $mg \cdot kg^{-1} \cdot d^{-1} \times 15$ d. **RESULTS**: valsartan ig $30$ $mg \cdot kg^{-1} \cdot d^{-1} \times 15$ d and fosinopril ig $15$ $mg \cdot kg^{-1} \cdot d^{-1} \times 15$ d prevented left ventricular hypertrophy induced by NE and decreased the content of collagen in myocardium; valsartan and fosinopril both elevated the myosin ATPase activity, $Na^+$, $K^+$-ATPase activity in membrane, and $Ca^{2+}$-ATPase activity in mitochondrias. Apoptosis was induced in cardiomyocytes by catecholamine. Valsartan and fosinopril both inhibited apoptosis, and no significant differences were found in the apoptotic index between the two treatment groups. **CONCLUSION**: Valsartan and fosinopril prevent the remodeling of cardiac hypertrophy induced by norepinephrine. Cardiac myocyte apoptosis may play a key role in the heart remodeling.

## INTRODUCTION

In spite of intensive investigations, the signal linked increased workload of the heart with the development of cardiac hypertrophy is still a subject of controversy. Many authors consider that the catecholamine norepinephrine (NE) to play an important role in this context. In a number of pathophysiological conditions leading to cardiac hypertrophy, the activity of the sympathetic nervous system is enhanced, resulting in increased release of NE from the sympathetic nerve endings within the myocardium. Several studies have associated myocardial dysfunction with reduced myocardial $Na^+$, $K^+$-pump concentration. However whether impaired $Na^+$, $K^+$-pump capacity is a pathogenetic factor or an epiphenomenon accompanying cardiac hypertrophy is not yet established. Apoptosis is an active, tightly regulated, energy-requiring process in which cell death follows a programmed sequence of events. It is now increasingly evident that cell growth and apoptosis are in fact two linked processes. In this paper we tested the efficacy of angiotensin (Ang) Ⅱ receptor antagonist ($AT_1$) valsartan and angiotensin-converting enzyme (ACE) inhibitor fosinopril by measuring the cardiac mass, the content of collagen in myocardium and the presence of the apoptosis in the development of ventricular hypertrophy using NE-induced hypertrophic model, and the myosin ATPase activity, $Na^+$, $K^+$-ATPase activity in membrane, and $Ca^{2+}$-ATPase activity in mitochondria. The goal of this study was to determine the effects of valsartan and fosinopril in a model of cardiac hypertrophy on myocardial remodeling.

## MATERIALS AND METHODS

**Animals**   Sprague-Dawley (SD) rats of either sex weighing 180－220 g, were provided by the Experimental Animal Center of Nanjing Medical University (Grade Ⅱ, Certificate № 970001). The rats were housed under constant temperature and humidity conditions. Before and during the experimental period, all rats had free access to a standard rat chow and tap water.

**Chemicals**   Norepinephrine (Shanghai Hefeng Pharmaceutical Factory), valsartan (Novartis Pharmaceutical Factory, Switzerland), fosinopril (Squibb Pharmaceutical company, USA), ATP-$Na_2$(Shanghai Biochemical company), $KH_2PO_4$(Nanjing Chemical Agent Factory). *In Situ* Cell Death Detection Kit, alkaline phos-

phatase was from Boehringer Mannheim. Other chemicals were of analytical grade.

**Heart hypertrophy**    Norepinephrine 1.5 mg · $kg^{-1} \cdot d^{-1}$ was injected intraperitoneally in ascorbic acid saline twice daily for 15 d, this treatment schedule produces hypertrophy without necrosis[1]. The animals were divided into four groups: 1) control (sodium chloride), 2) NE, 3) NE + fosinopril, 4) NE + valsartan. Fosinopril ig 15 mg · $kg^{-1} \cdot d^{-1} \times 15$ d, valsartan ig 30 mg · $kg^{-1} \cdot d^{-1} \times 15$ d. Immediately after the rats had been killed with excess ether, their hearts were removed and cleaned, the free wall of the right ventricle was separated from the intraventricular septum, which remained as a part of the left ventricle (LV). Left and right ventricular masses thus obtained were expressed as the ratio of ventricular weight to body weight (mg/g). The LV tissue was frozen immediately and stored at −70 ℃ until analyzed biochemically for the content of collagen, myosin ATPase activity, membrane $Na^+$, $K^+$-ATPase activity, and $Ca^{2+}$-ATPase activity in mitochondria.

***In situ* detection of apoptosis**    A complete cross section of LV was used for the morphometic analysis, each block was cut serially at 5 μm. The procedure was performed according to the manufacturer's instructions. The method is based on the preferential binding of terminal deoxynucleotidyl transferase (TdT) to the 3'-hydroxyl ends of DNA. Apoptotic bodies stain black. Tissue sections from each myocardial specimen were examined microscopically at × 40 magnification, and at least 200 cardiomyocytes were counted in a minimum of five high-power fields. The percentage of apoptotic cells was determined with an apoptotic index, the apoptotic index was calculated by dividing the number of positive-staining cardiomyocyte nuclei by the total number of cardiomyocyte nuclei and multiplying by 100.

**Isolation of mitochondria and membrane**    A portion of the left ventricle tissue was placed in homogenizing solution. After being homogenized, tubes were centrifuged at $750 \times g$ for 15 min. Supernatant was centrifuged again at $9000 \times g$ for 30 min and the pellet obtained was mitchondria, then the supernatant was centrifuged once again at $40\,000 \times g$ for 45 min and the pellet obtained was membrane redissolved in the homogenizing solution respectively. The whole schedule was processed under 4 ℃.

**Analytical methods**    In order to analyze the collagen content in myocardium, the hydroxyproline content was measured using the method of Sugihara *et al*[2]. The amount of hydroxyproline was expressed in mg/g tissue. The myosin ATPase activity, $Na^+$, $K^+$-ATPase activity in membrane, and $Ca^{2+}$-ATPase activity in mitochondria was measured by colorimetric method[3,4]. $Na^+$, $K^+$-ATPase activity was defined as the difference between enzymatic activities measured in the presence and the absence of ouabain. $Ca^{2+}$-ATPase activity was calculated by subtracting the activity measured in the absence of $CaCl_2$ from activity measured in the presence of $CaCl_2$. Absorbance was determined at 660 nm. The ATPase activity is expressed as micromoles of inorganic phosphate released from ATP per miligram of protein per hour at 37 ℃. All ATPase assays were performed in duplicate.

**Statistical analysis**    Data were expressed as $\bar{x} \pm s$ and analyzed by $t$ test.

# RESULTS

**Ventricular mass**    The weight of left ventricle expressed as ventricular index was increased in hypertrophic heart treated by NE compared to normal and was decreased by treatment with valsartan and fosinopril ( $P <$ 0.05) (Tab 1).

**Tab 1. Cardiac weight index after treatment with vehicle (control), valsartan, and fosinopril in rats. BW: body weight; LVW: left ventricular weight; RVW: right ventricular weight. $\bar{x} \pm s$. $^b P < 0.05$ vs control. $^e P < 0.05$ vs untreated hypertrophic group.**

| Groups | Rats | LVW/BW mg/g | RVW/BW mg/g |
|---|---|---|---|
| Normal | 8 | 1.79 ± 0.13 | 0.54 ± 0.08 |
| Untreated hypertrophic | 7 | 2.96 ± 0.31$^b$ | 0.59 ± 0.07 |
| Fosinopril | 7 | 2.11 ± 0.13$^e$ | 0.55 ± 0.04 |
| Valsartan | 8 | 2.06 ± 0.19$^e$ | 0.56 ± 0.07 |

**Collagen content**    The hydroxyproline content was measured in all groups. It was ( $7.0 \pm 0.3$ ) mg/g dry weight in hypertrophy untreated group, which was more as compared with control group ( $P < 0.01$ ). In fosinopril and valsartan treated groups, it was ( $3.9 \pm 0.2$ ), ( $3.8 \pm 0.7$ ) mg/g dry weight, which was much lower than that of hypertrophy untreated group.

**Myosin ATPase activity**    The myosin ATPase activity in hypertrophic untreated group was much lower than that in control group [ ( $0.39 \pm 0.06$ ) *vs* ( $1.0 \pm 0.9$ ) mmol · $min^{-1}$/g protein, $P < 0.01$ ]. It was increased in valsartan and fosinopril treated groups respec-

tively $[(0.92 \pm 0.2)$, $(0.88 \pm 0.09)$ mmol·min$^{-1}$/g protein] compared with that in hypertrophic untreated group ($P < 0.05$).

### Na$^+$, K$^+$-ATPase and Ca$^{2+}$-ATPase activity

Compared with control group, the Na$^+$, K$^+$-ATPase and Ca$^{2+}$-ATPase activity from hypertrophic untreated rats was decreased remarkably. In valsartan and fosinopril treated groups, Na$^+$, K$^+$-ATPase and Ca$^{2+}$-ATPase activity was increased compared with that in hypertrophic untreated group (Tab 2).

Tab 2. Effects of valsartan and fosinopril on the ATPase activity (mmol·h$^{-1}$/g protein) of myocardial cardiac hypertrophic rats induced by norepinephrine. $n$ is the number of rats. $\bar{x} \pm s$. $^bP < 0.05$ vs control. $^cP < 0.05$ vs untreated hypertrophic group.

| Group | $n$ | Na$^+$, K$^+$-ATPase membrane | mitochondrium | Ca$^{2+}$-ATPase mitochondrium |
|---|---|---|---|---|
| Control | 8 | $20.1 \pm 2.2$ | $13.9 \pm 2.3$ | $16.4 \pm 3.0$ |
| Untreated hypertrophic | 7 | $8.9 \pm 1.2^b$ | $9.1 \pm 2.0^b$ | $8.0 \pm 2.2^b$ |
| Fosinopril | 7 | $17.4 \pm 1.6^c$ | $15.1 \pm 2.4^c$ | $15.2 \pm 1.1^c$ |
| Valsartan | 8 | $19.0 \pm 3.4^c$ | $16.0 \pm 2.3^c$ | $15.9 \pm 2.3^c$ |

**Apoptosis of cardiomyocytes** The apoptotic index was significantly higher ($P < 0.01$) in hypertrophy ($1.97 \pm 0.29$) than normal group ($0.18 \pm 0.04$). The apoptotic index were ($0.70 \pm 0.03$) and ($0.53 \pm 0.04$) respectively in valsartan and fosinopril treated groups, which were significantly lower ($P < 0.05$) than hypertrophic group. No significant difference were found in the apoptotic index between the two treatment groups.

### DISCUSSION

In this *in vivo* study it has been shown that stimulation of myocardial β- and α-adrenergic receptors can result in the development of hypertrophy of the rat heart as an earlier report[1,5]. The rapid development of cardiac hypertrophy in the norepinephrine model seems to be directly mediated by stimulation of myocardial β- and α-receptors rather than by hemodynamic changes. However, we want to emphasize that this mechanism probably is limited to pathophysiological circumstances with increase levels of catecholamines.

It has been well established that ACE inhibitors improve cardiac function and remodeling. AT$_1$ receptor antagonists constitute an exciting and important new class of antihypertensive drugs. The present results demonstrate that valsartan and fosinopril both prevented left ventricular mass from increasing, and this was associated with a significant decrease in total collagen contents. Collagen is responsible for the functional integrity and structural matrix of the ventricle, which allows interdigitation and the transmission of force between contracting myocytes. In this model, apparently it is the ability of valsartan and fosinopril to prevent the NE-induced changes in systolic left ventricular and arterial BP that is responsible for its ability to attenuate the development of left ventricular hypertrophy[6]. These results also suggested that valsartan and fosinopril might improve the diastolic and systolic mechanical properties by decreasing the collagen content in ventricule tissue. The exact mechanism by which various drugs affect cardiac collagen metabolism is still not clear. Valsartan, a new angiotensin Ⅱ type 1 receptor antagonist offers many potential benefits in the treatment of many disease states where the RAS is involved[7,8]. Ang Ⅱ receptor antagonist appears to be as effective as angiotensin-converting enzyme inhibition in the treatment of cardiac hypertrophy and other pathologic processes[7].

The relationship of decreased cardiac mechanical performance with decreased myosin ATPase activity was reported in several models of cardiac hypertrophy[9-11]. This study demonstrate that valsartan and fosinopril can improve the cardiac contractility.

ATPases of cardiac cells are known to be among the most important enzymes to maintain the fluxes of vital cations by hydrolysis of the terminal high-energy phosphate of ATP. Our experiments indicate that the myocardial Na$^+$, K$^+$-ATPase and Ca$^{2+}$-ATPase activity from hypertrophic untreated rats is reduced, and this may reflect a decreased capacity for sodium-potassium pumping across the myocardial sarcolemma. The intracellular calcium overload may be partly due to the insufficient ATPase activities. Inhibition of the sodium pump would favor a rise in cell calcium[12], which in turn depresses Na$^+$, K$^+$-ATPase[13]. Mitochondria is an important organelle in regulating intracellular Ca$^{2+}$ eqilibrium by means of ATPases. Influx of Ca$^{2+}$ through the Ca$^{2+}$ channels is necessary for the initiation of myocyte contraction[14]. Moreover, phosphorylation of the Ca$^{2+}$ channels secondary to β-adrenergic receptor activation is one mechanism by which β-adrenergic stimulation increases myocyte contractility. Thus, changes in Ca$^{2+}$-ATPase activity will have important effects on overall contractility and β-adrenergic response. Because of its actions on the myocardial contractivity and electrical activi-

ties, the reduced myocardial $Na^+$, $K^+$-ATPase activity combined with an increased cytolic calcium level may contribute to the development of cardiac hypertrophy. The effects of valsartan and fosinopril on ATPase activites were in favour of improving intracellular calcium disorder and preventing cardiac remodeling.

It is now apparent that long-living, terminally differentiated cells of the heart retain the ability to die via the apoptotic mechanism[15]. The TdT assay is very sensitive and can detect even very low apoptosis. The *in situ* assay shows that cardiac myocyte apoptosis increases catecholamine-induced cardiac hypertrophy significantly in the left ventricle, valsartan and fosinopril both can inhibit cardiac apoptosis in this model. These findings suggest that cardiac myocyte apoptosis may play a key role in the heart remodeling. During the development of hypertrophy, cells within the ventricular wall undergo ischemic injury. We propose that part of the cell death occurring during hypertrophy is due to the apoptotic process. A number of experimental evidences indicate that exaggerated apoptosis may account for the loss of cardiomyocytes in the hypertensive left ventricle. Furthermore, some factors intrinsic and extrinsic to the cardiomyocytes emerge as potential candidates to trigger apoptosis. The elucidation of the possible interactions between these factors may be of major interest to prevent the progression of heart failure[16].

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## 缬沙坦与福辛普利对儿茶酚胺诱导的心肌肥厚的影响

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关键词 肥大性心肌病；去甲肾上腺素；缬沙坦；福辛普利；细胞凋亡

目的：研究血管紧张素 II 受体拮抗剂缬沙坦和新型血管紧张素转化酶抑制剂福辛普利对心肌肥厚的影响. 方法：SD 大鼠 ip 去甲肾上腺素 1.5 mg·kg$^{-1}$·d$^{-1}$×15 d，造成心肌肥厚模型. 缬沙坦 ig 15 mg·

$kg^{-1} \cdot d^{-1} \times 15$ d, 福辛普利 ig 30 mg·$kg^{-1} \cdot d^{-1} \times$ 15 d. 测量心重指数, 心肌胶原含量, 肌球蛋白 ATP 酶及细胞膜和线粒体 $Na^+$, $K^+$-ATP 酶, $Ca^{2+}$-ATP 酶的活性, 并检测此模型中心肌细胞的凋亡水平. **结果:** 缬沙坦和福辛普利均能阻止心肌肥厚的发生, 减少胶原合成, 提高肌球蛋白 ATP 酶及细胞膜和线粒体 $Na^+$, $K^+$-ATP 酶、$Ca^{2+}$-ATP 酶的活力, 抑制心肌细胞的凋亡. **结论:** 缬沙坦和福辛普利可防止儿茶酚胺诱导的心肌重塑, 心肌细胞凋亡可能在儿茶酚胺诱导的心肌重塑中起重要作用.

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