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Candesartan inhibits sinoaortic denervation-induced cardiovascular hypertrophy in rats[1]

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KEY WORDS candesartan; angiotensin receptors; left ventricular hypertrophy; heart; aorta; baroreflex; sinoaortic denervation; blood pressure variability

ABSTRACT

AIM: To study the effect of candesartan cilexetil (candesartan), a new AT_1 receptor antagonist, on sinoaortic denervation (SAD)-induced cardiovascular hypertrophy and its potential mechanisms in rats. **METHODS**: For long-term treatment, candesartan (6 mg·kg^{-1}·d^{-1}) was given in rat food for 16 weeks after SAD surgery, and for acute treatment, a single dose of candesartan (3 mg/kg) was administrated intragastrically at 30 d after SAD. **RESULTS**: The indexes of left ventricular and aortic hypertrophy in candesartan-treated SAD rats were decreased when compared with untreated SAD rats, and similar to or less than those in normal rats. SAD-induced cardiomyocyte hypertrophy, myocardial fibrosis, wall thickening of intramyocardial arterioles and aortae, and destruction of vascular internal elastin membrane were almost inhibited by candesartan. The plasma angiotensin II levels were markedly increased in treated SAD rats and negatively correlated with the indexes of hypertrophy. Both blood pressure and its variability were reduced by a single dose of candesartan during 3 h of observation period. **CONCLUSION**: Candesartan can efficiently inhibit SAD-induced cardiovascular hypertrophy. In addition to known mechanisms, upregulation of circulating angiotensin II and stabilization of blood pressure may be involved in this cardiovascular protection of candesartan.

INTRODUCTION

Arterial baroreflex plays a key role in the stabilization of blood pressure (BP). Interruption of arterial baroreflex by sinoaortic denervation (SAD) may lead to a substantial increase in blood pressure variability (BPV), and chronic SAD rat is considered as an experimental model of high BPV without sustained hypertension[1,2]. It has been found in our previous studies that chronic SAD can produce various forms of end-organ damage such as cardiac hypertrophy, vascular remodeling, and renal lesions, and the end-organ damage is associated with high BPV[2,3].

Candesartan is a new insurmountable AT_1 recep-

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tor antagonist to be approved as an antihypertensive drug[4-6]. The oral preparation is an inactive prodrug, candesartan cilexetil, which is hydrolyzed rapidly and completely during absorption in the gastrointestinal tract to an active compound, candesartan. Candesartan has a long duration of hypotensive action with a trough/peak ratio of more than 80 %[5]. It is reported that candesartan may reduce left ventricular hypertrophy in spontaneously hypertensive rats (SHR), stroke-prone SHR, transgenic hypertensive rats, and mild to moderate hypertensive patients, protect against myocardial ischemia-reperfusion injury in perfused Wistar rat hearts, decrease the incidence of stroke in stroke-prone SHR, and improve the renal function in hypertensive patients[6]. In the present study, the effects of candesartan on rat left ventricular and aortic hypertrophy produced by chronic SAD were studied. Also, the potential mechanisms involved in these effects were preliminarily explored.

MATERIALS AND METHODS

Preparation of SAD rats Male Sprague-Dawley rats were purchased from the Sino-British SIPPR/BK Lab Animal Ltd (Certificate № 02-25-3). At the age of 10 weeks, SAD was performed according to the procedure described by Krieger [7] with minor modification. Briefly, rats were anaesthetized with a mixture of ketamine (50 mg/kg, ip) and diazepam (5 mg/kg, ip) and were then medicated with atropine sulfate (0.5 mg/kg, ip) and procaine benzylpenicillin (60000 U, im). After a midline neck incision and bilateral isolation of the neck muscles, aortic baroreceptor denervation was carried out bilaterally by cutting the superior laryngeal nerves near the vagi, removing the superior cervical ganglia including a small section of the sympathetic trunk, and sectioning aortic depressor nerves. The carotid sinus baroreceptors were denervated bilaterally by stripping the carotid bifurcation and its branches followed by the application of 10 % phenol (in 95% ethanol) to the external, internal, and common carotid arteries and the occipital artery. In some rats, sham operation (Sham) was performed with the midline neck incision and bilateral isolation of the neck muscles. These rats were used as normal controls. After operation, rats were brought up with controlled temperature (23–25 ℃) and lighting (8:00–20:00 light, 20:00–8:00 dark) and with free access to rat chow and tap water.

Experimental protocol In long-term treatment experiment, candesartan cilexetil (donated by Dr Peter MORSING at AstraZeneca, Molndal, Sweden) was mixed into the rat chow. The rat chow containing a certain content of drug was prepared according to the previously determined food consumption and the theoretically estimated drug dose. Immediately after SAD surgery, one group of SAD rats was fed with rat chow containing the drug, and the other two control groups, ie, untreated SAD rats and Sham rats, received normal rat chow without the drug. The average dose of candesartan cilexetil was about 6 mg·kg^{-1}·d^{-1}, which was calculated from the practically ingested food during 16 weeks of treatment. At the end of experiment, morphology of left ventricles and aortae were examined, and plasma angiotensin II concentrations were measured. In acute treatment experiment, a single dose of candesartan cilexetil (3 mg/kg) was given intragastrically at 30 d after SAD, and its effects on haemodynamics were studied in conscious unstrained rats.

Morphological examination After 16 weeks of treatment, the animal was weighed and killed by decapitation. The thoracic and peritoneal cavities were immediately opened. The aorta and heart were excised and rinsed in cold physiological saline. The left ventricle was isolated, blotted, and weighed. At the same time, the aorta was cleaned of adhering fat and connective tissue. Just below the branch of the left subclavicular artery, a 22-mm-long segment of thoracic aorta was harvested, blotted, and weighed. As an index of left ventricular hypertrophy, the ratio of left ventricular weight to body weight was determined, and as an index of aortic hypertrophy, the ratio of aortic weight to length was calculated[8]. Histopathological observation was also carried out with our conventional method[2]. Briefly, immediately after gross detection, all samples of left ventricles in 2- to 3-mm-thick slices and aortae were immersed in formalin solution for more than 1 week,

dehydrated in ethanol, cleared in dimethylbenzene, and embedded in paraffin. Then the 5-μm-thick sections were prepared and stained with hematoxylin and eosin, and Victoria blue and Van Gieson for light microscopic evaluation.

Measurement of plasma angiotensin Ⅱ concentration In rats used for long-term treatment study, immediately after decapitation, blood sample (2 mL) was collected into prechilled tube containing sodium edetic acid 2 g/L, cooled in ice-water bath, and centrifuged at 4 ℃. The plasma was removed, mixed with enzyme inhibitors, and stored at –80 ℃ before assay. Angiotensin II concentration was determined using the radioimmunoassay kit provided by China Institute of Atomic Energy[2].

Haemodynamic monitoring in conscious unstrained rats At 27 d after SAD, the rat was anaesthetized as described above. For drug administration, a polyethylene catheter (PE50) was directly inserted into the stomach, at the middle of the greater curvature of stomach. For measurement of BP and heart period (HP), another polyethylene catheter (PE-10 connected to PE-50) was placed into the lower abdominal aorta via the left femoral artery. Both catheters were tunneled subcutaneously, exteriorized between the scapulae, and fixed on the saddle. After 2 d of recovery, the rat was placed in a cylindrical cage (diameter: 300 mm; height: 240 mm), and the aortic catheter was connected to a BP transducer *via* a rotating swivel that allowed the rat to move freely. The rat was habituated for more than 14 h before the experiment was started at 10 o' clock next day. After BP was recorded for 1 h, a single dose of candesartan cilexetil (3 mg/kg) or the same volume of vehicle control was given through the gastric catherter, and immediately after drug administration, BP was measured for another 3 h. In this study, BP was continuously recorded with a computerized technique[9]. Briefly, the BP signals, transmitted to the electric signals by a transducer, were digitized and processed by a computer, which calculated on line the BP and HP. These values were sampled beat-to-beat and stored on hard disk. In off-line analysis, the means and standard deviations of BP and HP over the 1-h period were calculated. The mean of 1-h BP was used as an index of BP, and the standard deviation of 1-h BP as an index of BPV[9,10]. The same method was used for calculation of HP and HP variability.

Statistical analysis Statistical analysis was performed using statistical program SAS. Data are reported as mean±SD. The differences among three groups were evaluated using analysis of variance (ANOVA) followed by unpaired *t* test. The haemodynamic data before and after drug administration were compared by paired *t* test. The relationship between the plasma angiotensin II levels and the indexes of cardiovascular hypertrophy was assessed by linear regression analysis. Statistical significance was judged at $P<0.05$.

RESULTS

Effects of candesartan on SAD-induced left ventricular and aortic hypertrophy Before SAD or sham operation, the body weights were not different among three groups (Tab 1). After 16 weeks of experiment, the body weights in SAD rats were reduced, when compared with those in sham rats. However, there were no significant differences in body weights between candesartan-treated and untreated SAD rats. SAD rats exhibited left ventricular and aortic hypertrophy, as evidenced by increases in the normalized weights of

Tab 1. Effects of long-term treatment of candesartan on SAD-induced left ventricular and aortic hypertrophy in rats. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* Sham. $^{d}P>0.05$, $^{f}P<0.01$ *vs* SAD.

	Sham (n=6)	SAD (n=7)	SAD+Can (n=6)
Initial BW (g)	301±6	298±5	298±8
Final BW (g)	518±37	473±29 ^{b}	465±21 ^{bd}
LVW/BW (mg/g)	1.91±0.17	2.17±0.15 ^{c}	1.86±0.07 ^{f}
AW/length (mg/mm)	1.12±0.07	1.25±0.08 ^{c}	0.96±0.10 ^{cf}

Sham indicates sham operation; SAD, sinoaortic denervation; Can, candesartan cilexetil; BW, body weight; LVW, left ventricular weight; and AW, aortic weight.

left ventricles and aortae, when compared with sham controls. In candesartan-treated SAD rats, the normalized weights of left ventricles and aortae were decreased compared with untreated SAD rats, and similar to or less than the levels in Sham rats. Under microscope, left ventricular and aortic tissues from untreated SAD rats demonstrated obvious pathological changes, including cardiomyocyte hypertrophy, myocardial interstitial and perivascular fibrosis, wall thickening of intramyocardial arterioles and aortae, and destruction of vascular internal elastin membrane (Fig 1). These characteristic changes induced by chronic SAD were markedly inhibited by long-term treatment of candesartan.

Effects of candesartan on plasma angiotensin II levels in SAD rats There existed no differences in plasma angiotensin II levels between untreated Sham and SAD groups. In candesartan-treated SAD group, the plasma angiotensin II levels were increased, 6.7 times higher than the levels in untreated groups (Fig 2). Furthermore, the linear regression analysis showed that the plasma angiotensin II levels were significantly and negatively correlated with the indexes of left ventricular and aortic hypertrophy (Fig 3).

Effects of candesartan on haemodynamics in SAD rats Tab 2 summarizes the haemodynamic data before and after candesartan administration. Both BP and BPV were decreased by a single dose of candesartan, and these effects were maintained during 3 h of experimental period. The extents of decreases in BP and BPV were not different among 1st, 2nd, and 3rd h post candesartan administration. HP was increased, whereas HP variability remained unchanged after administration of candesartan. In vehicle control study, the haemodynamic data after vehicle administration were not different from those before vehicle administration (data not shown).

Tab 2. Effects of candesartan (ig) on haemodynamics in sinoaortic-denervated rats. n=6. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* Pre-Can.

	Pre-Can	Post-Can		
		1st h	2nd h	3rd h
	(10:00–11:00)	(11:00–12:00)	(12:00–13:00)	(13:00–14:00)
Systolic BP/kPa	16.4±1.2	12.7±1.1^{c}	12.8±1.5^{c}	12.4±1.1^{c}
Diastolic BP/kPa	11.2±1.7	7.3±1.9^{c}	7.5±2.1^{c}	7.2±1.7^{c}
HP/ms	146±24	150±22^{b}	153±19^{b}	155±18^{b}
Systolic BPV/kPa	1.8±1.0	1.4±0.5	1.4±0.7^{b}	1.4±0.7^{b}
Diastolic BPV/kPa	1.7±0.8	1.2±0.4^{b}	1.2±0.6^{c}	1.2±0.5^{b}
HP variability/ms	18±4	20±7	20±6	20±6

Can indicates candesartan cilexetil; BP, blood pressure; HP, heart period; and BPV, blood pressure variability.

DISCUSSION

Left ventricular and aortic hypertrophy are the typical pathological changes following chronic SAD, as shown by our lab and others[2,3,10,11]. In the present study, rats 16 weeks after SAD exhibited hypertrophy and other damage in left ventricles and aortae. These results are consistent with our previous data[2,3]. To quantify the cardiovascular hypertrophy, we use the indexes of left ventricular and aortic hypertrophy, which are commonly used in the literature[8,11,12]. It was found that the indexes of left ventricular and aortic hypertrophy in candesartan-treated SAD rats were lower than those in untreated SAD rats, and similar to or less than those in normal rats. In addition, microscopic observations showed that SAD-induced myocardial hypertrophy and fibrosis and vascular wall thickening were markedly inhibited by 16-week treatment of candesartan. These results demonstrate that candesartan can efficiently prevent the SAD-induced cardiovascular hypertrophy.

The cardiovascular protection of candesartan may come from several mechanisms. The known mechanisms are hypotensive effect and cardiovascular growth inhibition induced by AT_1 receptor blockade, which are extensively studied[13-16]. In addition, our preliminary results from the present study indicate that upregulation of circulating angiotensin II and stabilization of BP may be involved in this cardiovascular protection.

Angiotensin II mainly acts through 2 receptor subtypes, AT_1 and AT_2. It is well known that most effects of angiotensin II, including vasoconstriction, aldosterone and vasopressin release, renal salt and water

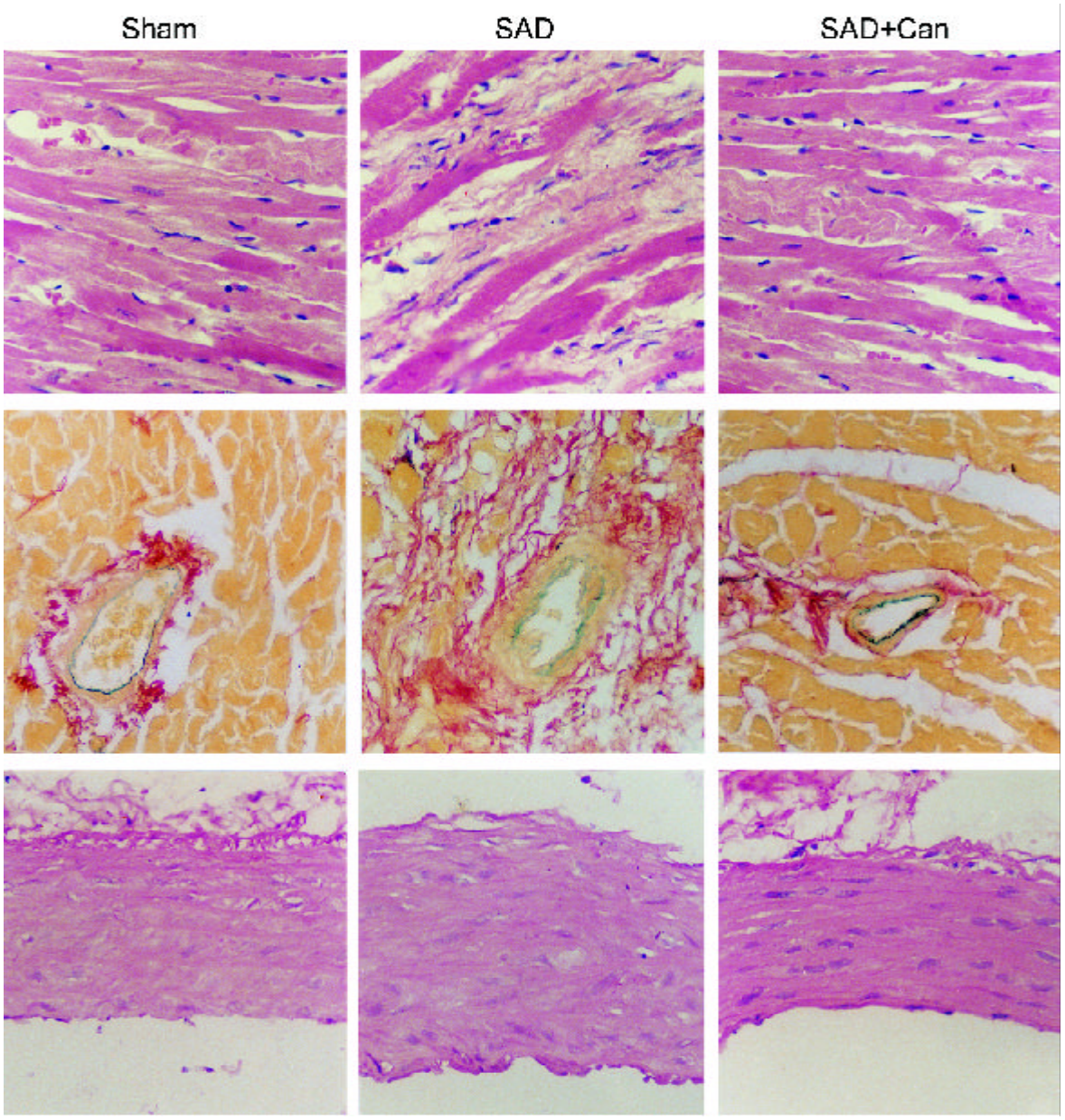

Fig 1. Effects of long-term treatment of candesartan on SAD-induced histopathological changes in rat left ventricles and aortae. Sham indicates sham operation; SAD, sinoaortic denervation; and Can, candesartan cilexetil. Top row: left ventricular tissues with hematoxylin and eosin stain, 3.3×20; Middle row: left ventricular tissues with Victoria blue and Van Gieson stain, 3.3×10; Bottom row: aortic tissues with hematoxylin and eosin stain, 3.3×20. There existed cardiomyocyte hypertrophy, myocardial interstitial and perivascular fibrosis, wall thickening of intramyocardial arterioles and aortae, and destruction of vascular internal elastin membrane in untreated SAD group. These pathological changes were efficiently inhibited by long-term treatment of candesartan.

retention, sympathetic facilitation, and cell growth are mediated by AT_1 receptor. Recently, the function of AT_2 receptor has been explored. It is found that the actions of AT_2 receptor appear to be opposite in some cases to those of AT_1 receptor. For example, AT_2 receptors are associated with antiproliferation, apoptosis, and vasodilation[5,17]. AT_1 receptor antagonists effectively block the AT_1 receptor, and leave the AT_2 receptor unopposed. Moreover, blockade of AT_1 receptor-mediated feedback inhibition of rennin release from the kidney results in increased plasma rennin activity and, consequently, in increased plasma angiotensin II

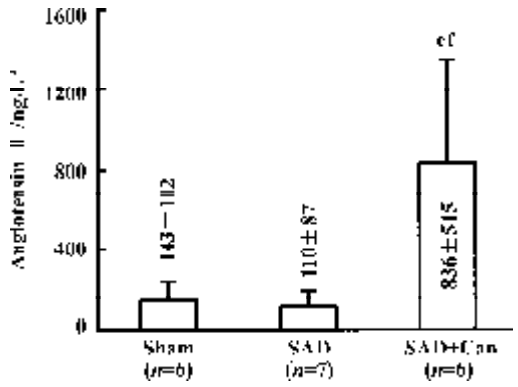

Fig 2. Effects of candesartan on plasma angiotensin II levels in SAD rats. Sham indicates sham operation; SAD, sinoaortic denervation; and Can, candesartan cilexetil. Mean±SD. $^{c}P<0.01$ *vs* Sham. $^{f}P<0.01$ *vs* SAD.

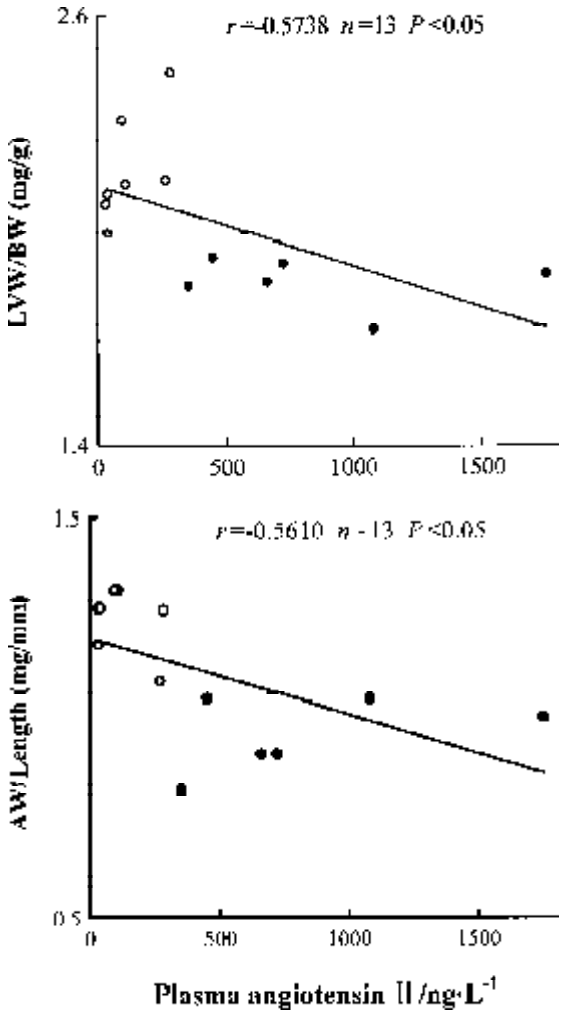

Fig 3. Scatter plots showing the relationship between the plasma angiotensin II levels and the indexes of left ventricular and aortic hypertrophy in untreated (○) and candesartan-treated (●) SAD rats.

levels[5,17,18]. Therefore, it has been proposed that high circulating levels of angiotensin II that occur after AT_1 receptor blockade may be beneficial in cardiovascular protection, through stimulating the unblocked AT_2 receptor to produce antiproliferation and apoptosis[17,18]. In deed, two *in vivo* studies using selective AT_2 receptor antagonists have shown that AT_2 receptor is involved in cardiovascular protection in SHR[19] and in myocardial ischemia pigs[20]. In the present study, the plasma angiotensin II levels were markedly increased after long-term blockade of AT_1 receptor with candesartan, and there existed a negative correlation between the plasma angiotensin II levels and the indexes of cardiovascular hypertrophy in untreated and candesartan-treated SAD rats. These provide the direct evidence for the first time that upregulation of circulating angiotensin II produced by AT_1 receptor blockade has a beneficial effect on cardiovascular protection.

BPV is a new concept in cardiovascular medicine, arising from the development of techniques designed for continuous BP monitoring[9]. In 1987, Parati *et al*[21] reported that among hypertensive patients with similar BP levels, the level of target organ damage was more advanced in those patients with higher levels of BPV. This is the first report regarding clinical importance of BPV. During the last 6 years, a series of experiments were carried out in our lab to study the relationship between BPV and organ damage. It has been demonstrated that high BPV is a risk factor involved in end-organ damage in SHR and SAD rats[2,3,22]. We have also proposed that decreasing BPV may be a new strategy for end-organ protection[3]. In the present study, a single dose of candesartan has a BP stabilization, ie, a BPV-lowering effect, in SAD rats. So it is deduced that the efficient inhibition of SAD-induced cardiovascular hypertrophy by long-term treatment of candesartan may also relate to its stabilization of BP. However, the hypothesis remains to be further confirmed in another chronic treatment study in SAD rats.

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坎替沙坦抑制大鼠去窦弓神经引起的心血管肥大[1]

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关键词 坎替沙坦；血管紧张素受体；左心室肥大；心脏；主动脉；压力感受性反射；去窦弓神经；血压波动性

目的： 研究新型AT_1受体拮抗剂坎替沙坦对大鼠去窦弓神经(SAD)引起的心血管肥大的作用及其可能机制．**方法：** 长期治疗时，大鼠SAD术后从食物中给予坎替沙坦(6 mg·kg^{-1}·d^{-1})16周．急性治疗时，大鼠SAD术后第30天经胃内单次给坎替沙坦3 mg/kg．**结果：** SAD大鼠坎替沙坦治疗组的左心室和主动脉肥厚指数明显低于未治疗组，相当于或低于正常水平．SAD引起的心肌肥大、纤维化、心肌内小动脉和主动脉管壁增厚以及血管内弹力膜破坏几乎被坎替沙坦完全抑制．治疗后血浆血管紧张素Ⅱ浓度明

显升高，与心血管肥厚指数呈负相关．坎替沙坦单次给药后 3 小时观察期内，SAD 大鼠血压及其波动性维持在较低水平．结论：坎替沙坦能有效抑制SAD引起的心血管肥厚．这种心血管保护作用除与已知机制有关外，还可能与其上调循环血管紧张素Ⅱ和稳定血压有关．

(责任编辑　朱倩蓉)