To determine whether angiotensin II participates in the pathogenesis of cardiac hypertrophy and impairs coronary circulation in DOCA/salt hypertension, DOCA hypertensive rats were treated with candesartan cilexetil for 8 wk. DOCA/salt hypertension was induced in Wistar rats by removing the right kidney and subcutaneously injecting deoxycorticosterone acetate once a week. Control rats were given subcutaneous injections of saline and maintained on a normal diet. After 4 wk of observation, the angiotensin II receptor antagonist candesartan cilexetil was administered by oral gavage for 8 wk to 14 rats. Systolic blood pressure was measured weekly with the tail-cuff method. After 12 wk, the rats were killed and prepared. The isolated hearts were perfused by a Langendorff apparatus at constant flow. Perfusion pressure was measured by a small-volume transducer, and perfusion flow was recorded by a drop counter. Development of hypertension was not prevented by candesartan cilexetil treatment, but development of cardiac hypertrophy was inhibited. Minimum coronary vascular resistance (MCVR) obtained upon infusing adenosine into the isolated hearts was significantly higher in DOCA/salt hypertensive rats than in sham-operated controls. The elevated MCVR in DOCA/salt hypertensive rats was decreased by the administration of candesartan cilexetil for 8 wk. Thus, candesartan cilexetil regressed cardiac hypertrophy and improved coronary vascular resistance without affecting high blood pressure. These findings suggest that angiotensin II plays an important role in the pathogenesis of cardiac hypertrophy in DOCA/salt hypertension and that cardiac hypertrophy increases coronary vascular resistance. (Hypertens Res 1997; 20: 263-267)

Key Words: DOCA/salt hypertension, rat, cardiac hypertrophy, coronary perfusion resistance, angiotensin II receptor antagonist, angiotensin
Material and Methods

Twenty-eight male Wistar rats weighing 100 to 120 g were purchased from Simizu Experimental Animal Care Co. Ltd. (Kyoto, Japan). All experiments conformed with the guidelines for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985), and were approved by the Committee for Animal Research, Kyoto Prefectural University of Medicine. Under ether anesthesia, the right kidney was removed in all rats. In 14 of the rats, deoxycorticosterone acetate (40 mg/kg) was injected once a week subcutaneously and regular chow was replaced with a high-salt diet (8% NaCl, Oriental Bio Co. Ltd., Tokyo, Japan). The other 14 rats, serving as controls, received weekly subcutaneous injections of saline and were maintained on a normal diet. In each group, 7 rats were treated with an angiotensin II receptor antagonist (candesartan cilexetil) given by daily gavage for 8 wk, commencing 4 wk after DOCA and saline injections. Candesartan cilexetil (Takeda Chemical Industrial, Ltd.) was dissolved in gum arabic and given to the rats once a day (10 mg/kg). Systolic blood pressure was measured once a week by tail-cuff plethysmography (UR 5000, Ueda Ltd., Japan). After 12 wk, the rats were anesthetized with sodium pentobarbitate (50 mg/kg, i.p.) and given heparin (500 units). Then, a thoracotomy was performed, and the hearts were isolated. Each isolated heart was retrogradely perfused at a constant flow rate (12 ml/min) with the use of a peristaltic pump (MP-3B, Eyela, Japan) in a Langendorff apparatus containing modified Krebs-Henselte solution of the following composition (nmol/l): NaCl 118.0, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, CaCl2 3.0, Na2EDTA 0.5, and glucose 11.0. The solution was aerated with 95% O2 and 5% CO2, and the pH was adjusted to 7.3. Perfusion pressure was monitored by a transducer (MPV, NEC-Sanei, Japan), and perfusion flow was measured by a drop counter (F 360A, Unipulse, Japan). The perfusion solution was collected in a beaker.

Isolated hearts were paced electrically at 300 beats/min by a stimulator (Nihon Koden, Japan). After stabilization, adenosine (10−3 M) was added by means of a syringe pump (A-99, Eazel, Japan) to obtain the maximal coronary vasodilatation. Minimal coronary vascular resistance is expressed as perfusion pressure during adenosine infusion/perfusion flow/100 g heart wet weight. At the end of the experiment, 2% ethylenediaminetetraacetic acid di-sodium salt dehydrate was perfused to obtain the diastolic phase of the heart. Hearts were then dipped in 10% formaldehyde solution. After removing the atria and aorta, the myocardium was weighed. The right ventricle was then removed, and the left ventricle was weighed.

Results are expressed as means ± SEM. Differences between groups were evaluated by one-way analysis of variance for repeated measures. To compare each group, Fisher protected least significant difference was used. Data processing was performed with the Stat View (Ver 4.5) software package (Abacus Concepts, Inc). Differences with p values less than 0.05 were considered statistically significant.

Results

Effect of Candesartan Cilexetil on Systolic Blood Pressure and Body Weight in Sham-Operated and DOCA/Salt Hypertensive Rats

Systolic blood pressure increased progressively in DOCA/salt treated rats from 3 wk to 6 wk and was significantly higher than in sham-operated rats. The elevated blood pressure was sustained during the observation period. Candesartan cilexetil treatment did not alter the development of hypertension in DOCA/salt treated rats, and had no effect on systolic blood pressure in sham-operated rats (Fig. 1). Body weight gain was less in DOCA/salt treated rats than in sham-operated rats. Candesartan cilexetil treatment reduced body weight gain in
Effect of Candesartan Cilexetil on Cardiac Hypertrophy in DOCA/Salt Hypertensive Rats

After 12 wk of observation, the hearts were removed and weighed. Left ventricular weight/body weight (mg/100 g) was significantly greater in DOCA/salt rats than in sham-operated rats (481 ± 25 vs. 274 ± 13, p < 0.0001). However, LV weight was significantly less in DOCA/salt rats treated with candesartan cilexetil than in DOCA/salt rats not treated with candesartan cilexetil (374 ± 15 vs. 481 ± 25, p < 0.001). Candesartan cilexetil treatment did not affect LV weight in sham-operated rats as compared with DOCA/salt rats (289 ± 8 vs. 274 ± 13, n.s.) (Fig. 3).

Increased MCVR in DOCA/Salt Hypertensive Rats and Effect of Candesartan Cilexetil

MCVR obtained by infusion of adenosine was significantly greater in DOCA/salt hypertensive rats than in sham-operated rats (0.117 ± 0.006 vs. 0.074 ± 0.006, p < 0.0001). In DOCA/salt rats given candesartan cilexetil, MCVR was significantly lower than in DOCA/salt treated rats not given candesartan cilexetil (0.092 ± 0.009 vs. 0.117 ± 0.006, p < 0.05). Candesartan cilexetil did not change MCVR in sham-operated rats (0.068 ± 0.003 vs. 0.074 ± 0.006, n.s.) (Fig. 4).

Discussion

Our results show that in DOCA/salt hypertensive rats chronic treatment with the angiotensin II receptor antagonist, candesartan cilexetil, inhibited the development of cardiac hypertrophy without lowering high blood pressure and also reduced the elevated coronary vascular resistance.

Recently, activation of the renin-angiotensin system has been suggested to play a role in cardiovascular hypertrophy and remodeling. In volume-overload cardiac hypertrophy produced by an aortocaval shunt in rats, angiotensin II in the heart increases, and treatment with an ACE inhibitor prevents the development of cardiac hypertrophy and the increase in left ventricular end-diastolic pressure (22). In pressure-load cardiac hypertrophy produced by transverse aortic constriction, ACE inhibitors and angiotensin II receptor antagonists also prevent cardiac hypertrophy (23, 24). Cardiac hypertrophy is prevented by inhibition of the renin-angiotensin system in SHR (9, 14, 15, 25, 26), Dahl salt sensitive rats (27), and 2K1C hypertensive rats (28). The reduction in cardiac hypertrophy is not only due to lowering high blood pressure, because even small doses of ACE inhibitors with no antihypertensive effect can cause regression of cardiac hypertrophy in SHR (9, 29). ACE inhibitors also inhibit the development of cardiac hypertrophy in the low-renin hypertensive rat model (30). Thus, blockade of angiotensin inhibits cardiac hypertrophy not only in volume and pressure overload models, but also in hypertension. Our results show that even when the renin-angiotensin system does not contribute to the development of hypertension in DOCA/salt treated rats, the cardiac angiotensin system may still be involved in the development of cardiac hypertrophy. Moreover, our results also indicate that the renin-angiotensin system influences the development of cardiac hypertrophy, independent of any action on blood pressure level.

ACE inhibitors inhibit the increased fibrosis that occurs in cardiac hypertrophy (31). Candesartan cilexetil inhibits myocyte hypertrophy by mechanical stretching (26). Moreover, angiotensin II increases mRNA of skeletal α-actin, β myosin heavy chain, atrial natriuretic polypeptide, and fibronectin (15). These findings and our present results suggest that angiotensin II may play an important role in the development of cardiac hypertrophy.

The present results show that candesartan cilexetil reduced the increased MCVR in DOCA/salt hypertension with a reduction in cardiac hypertrophy. ACE inhibitors also reduce increased MCVR in the hypertensive heart (6). When ACE inhibitors cause regression of cardiac hypertrophy,
there is also a concomitant reduction in vascular wall thickness (32). This mechanism may improve coronary flow reserve (33). Angiotensin receptor antagonists also reduce medial hypertrophy of the vascular wall and decrease cardiac hypertrophy in hypertensive rats (34). Scieff er demonstrated that angiotensin receptor antagonists and ACE inhibitors equally reduce cardiac hypertrophy and improve coronary flow reserve with a reduction in myocardial fibrosis after cardiac infarction with cardiac hypertrophy in rats (35). Taken together these findings indicate that angiotensin receptor antagonists improve coronary vascular reserve by reducing coronary vascular hypertrophy and myocardial fibrosis. This effect may have played a part in our results.

We have previously demonstrated that candesartan cilexetil increases nitric oxide (NO) release in coronary arteries of the hypertensive heart while lowering high blood pressure in SHR (14). In addition, Tschudi et al. have demonstrated that ACE inhibitors, calcium antagonists, and angiotensin receptor antagonists equally increase NO release in coronary arteries of the hypertensive heart while exerting an antihypertensive effect (36). They concluded that the increased NO release resulted from a reduction in high blood pressure. Reduced release of endothelium-derived relaxing factor (NO) in the aorta has been demonstrated in DOCA hypertensive rats (37). However, the reduction in MCVR in our study may not have been caused the increase in NO release, because we found no reduction in high blood pressure in DOCA hypertensive rats treated with candesartan cilexetil. However, angiotensin receptor antagonist, also directly dilate coronary vessels, which may depend on NO release (38). Therefore, NO may have contributed to the improvement in MCVR in the present study.

References


