Prevention of Cardiac Hypertrophy by a Sub-Antihypertensive Dose of the α_1 -Adrenergic Antagonist Bunazosin in Dahl Salt-Sensitive Rats

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To assess the protective effect of an α_1 -blocker on the development of cardiac hypertrophy, the selective α_1 -receptor antagonist bunazosin (2 mg/kg/d, by oral gavage and in drinking water) was given to male Dahl salt-sensitive rats fed a 4% NaCl diet for 7 weeks. Control animals received water only by the same method. Treatment with bunazosin was started when the animals were 7 weeks of age. Blood pressure, pulse rates, and body weight were measured every week during the experiment. Urine was collected for 24 h on the final day of the experiment. All animals were killed by decapitation, blood was collected, and the heart was removed. In both the treated and control groups, time-dependent increases in blood pressure and body weight were observed, and there were no significant differences between the groups in blood pressure or body weight during the experiment. Pulse rate remained unchanged in both groups throughout the experiment. The left ventricular weight/body weight ratio and the left ventricular tissue DNA content were significantly lower in the rats receiving bunazosin than in the control rats. Plasma renin activity, plasma aldosterone, and plasma atrial natriuretic peptide did not differ significantly between the two groups. No significant differences in glomerular filtration rate, urine volume, sodium excretion, urinary metanephrine excretion, and urinary normetanephrine excretion were noted between the two groups. The results indicate that a sub-antihypertensive dose of bunazosin can inhibit the development of cardiac hypertrophy without suppression of the pressure load, suggesting an important role of α_1 -adrenergic receptors in the pathogenesis of cardiac hypertrophy following the development of hypertension. (*Hypertens Res* 1996; 19: 147-150)

Key Words: cardiac hypertrophy, α_1 -adrenergic receptor, bunazosin, Dahl salt-sensitive rat

It has been established that cardiac hypertrophy in chronic hypertension is an adaptive structural response of the ventricle to long-term hemodynamic overload. Several findings have suggested that hemodynamics are a major determinant of myocardial hypertrophy (1, 2). However, clinical and experimental studies have implicated several factors other than pressure load due to sustained high blood pressure in the pathogenesis of cardiac hypertrophy (3, 4). Reduction of blood pressure by α -methyl-dopa decreased the ventricular weight, whereas hydralazine and minoxidil lowered the blood pressure to normotensive levels without preventing ventricular hypertrophy in spontaneously hypertensive rats (SHR) (5, 6). Clinical observations have also revealed discrepancies between blood pressure control and the degree of cardiac hypertrophy (7).

Current evidence indicates that ACE inhibitors not only lower high blood pressure but also promote the regression of cardiac hypertrophy because of their suppressive effect on the direct action of

angiotensin II in the development of myocyte hypertrophy (8, 9). On the other hand, catecholamines may stimulate the development of cardiac hypertrophy, and norepinephrine-stimulated cardiac hypertrophy is thought to be mediated by α_1 -adrenergic receptors (10). Several α -adrenergic antagonists have shown anti-hypertrophic properties associated with reduction of blood pressure in animal experiments (11, 12) and clinical studies (13).

We examined whether or not a sub-antihypertensive dose of the α_1 -blocker bunazosin inhibited the progression of cardiac hypertrophy without suppression of the development of hypertension in young Dahl salt-sensitive rats fed a high salt diet.

Materials and Methods

Dahl Iwai salt-sensitive rats (Dahl-SS rats) were obtained from Eisai Supply, Tsukuba, Japan. The animals were divided into two groups. One group ($n = 7$) received bunazosin (Eisai Co., Tokyo, Japan) by oral gavage (weekdays) or in drinking

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Fig. 1. Changes in systolic blood pressure (upper graph) and pulse (lower graph) in bunazosin-treated and control rats. Results are expressed as the means $\pm$ SE. * $p < 0.01$, compared with the baseline blood pressure of each group at week 0

water (weekends) at a dose of 2 mg/kg/d for 7 weeks, from 7 to 14 weeks of age. The other group ($n = 7$) received the vehicle only by the same method and served as control. All animals were fed a 4% NaCl diet during the experiment. Blood pressure and pulse rate were measured by the tail-cuff method every week. Body weight was also measured every week. All animals were housed in metabolic cages for final 2 days of the experiment, and urine was collected during the final 24 h. After urine collection, all rats were killed by decapitation, blood was collected, and the heart was removed and rinsed with physiological saline solution. The heart was blotted and weighed. The atria and right ventricle were then carefully removed, and the remainder of the heart was weighed as the left ventricle (LVW). The left ventricle was homogenized with water and its tissue DNA content was measured with a modification of the method described by Burton (14). The plasma atrial natriuretic peptide (ANP), plasma renin activity (PRA), and plasma aldosterone were determined by radioimmunoassay (Shionoria ANP kit, Shionogi, Osaka, Japan; RENIN RIA-BEAD, Dainabot, Tokyo, Japan; ALDOSTERONE RIA-KIT II, Dainabot, Tokyo, Japan). The serum and urinary creatinine levels were estimated with an autoanalyzer. The urinary sodium was measured with an ion-selective electrode system. The urinary metanephrine and normetanephrine were determined with the use of

Fig. 2. Bar graphs showing body weight, left ventricular weight/body weight (LVW/BW) and left ventricular DNA content (LV DNA) after 7 weeks of bunazosin treatment. Results are expressed as the means $\pm$ SE.

HPLC coupled with trihydroxyindole fluorimetric detection.

The changes in systolic blood pressure and pulse rate in the two groups during the experiment were compared by one-way analysis of variance (ANOVA). The differences between the groups in systolic blood pressure, pulse rate, and other variables were compared with unpaired t -tests. Values of $p < 0.05$ were considered to indicate statistical significance. All data are expressed as the means $\pm$ SE.

Results

The changes in systolic blood pressure (upper graph) and pulse rate (lower graph) measured weekly, are shown in Fig. 1. A time-dependent increase in blood pressure was observed in both groups. The absolute values of the blood pressure at week 0 (before treatment) were 144.3 ± 2.2 mmHg in the group that received bunazosin (bunazosin group) and 139 ± 2.1 mmHg in the control group. At week 7, the blood pressure in the treated group was 181.1 ± 5.9 mmHg and that in the control group was 190.5 ± 5.2 mmHg. There were no significant differences between the groups in blood pressure or pulse rate at any time during the experiment. ANOVA of pulse rate revealed no significant changes in either group during the experiment. At week 0 the pulse rate in the treated group and control group was 404.3 ± 7.9 beats/min and 390.6 ± 9.1 beats/min, respectively; at week 7 the pulse rate was 410.6 ± 13.4 beats/min and 400.6 ± 13.4 beats/min, respectively.

Changes in body weight, left ventricular weight/body weight ratio (LVW/BW), and total left ventricular DNA content are shown in Fig. 2. Body weight did not differ significantly between the control group and the bunazosin group (393.2 ± 4.8 g vs. 369.3 ± 9.6 g, control group vs. bunazosin group). LVW/BW in the treated group (2.09 ± 0.05 mg/g) was significantly lower than in the control group (2.33 ± 0.06 mg/g). The total left ventricular DNA content (1052.0 ± 42.1 μ g) in the treated group was also significantly lower than that in the control group (1307.0 ± 64.9 μ g).

The PRA, plasma aldosterone, and plasma ANP did not differ significantly between the groups.

Table 1. Urine Volume (UV), Urinary Sodium Excretion Volume (UNa), Glomerular Filtration Rate (GFR), Urinary Metanephrine Excretion Volume (U Metaneph) and Urinary Normetanephrine Excretion Volume (U Normeta). Results Are Expressed as the Means $\pm$ SE.

Group	UV (ml/d)	UNa (mEq/d)	GFR (ml/min/kg)	U Metaneph (μ g/d)	U Normeta (μ g/d)
Control	25.3 $\pm$ 1.3	3.8 $\pm$ 0.3	5.1 $\pm$ 0.4	0.68 $\pm$ 0.11	3.26 $\pm$ 0.36
Bunazosin	24.0 $\pm$ 3.1	3.3 $\pm$ 0.2	5.6 $\pm$ 0.3	0.49 $\pm$ 0.06	2.79 $\pm$ 0.21

There also were no significant differences between the groups in urine volume, urinary sodium excretion, and glomerular filtration rate (GFR) (Table 1).

There was a trend toward lower urinary excretion of metanephrine and of normetanephrine in the bunazosin group, but the differences with the control group were not significant (Table 1).

Discussion

Hemodynamic factors have been established to be major determinants of left ventricular structure in cardiac hypertrophy associated with chronic hypertension. Non-hemodynamic factors involved in the pathogenesis of cardiac hypertrophy following sustained hypertension have also been identified, and several antihypertensive agents have been reported to modulate or promote regression of cardiac hypertrophy associate with hypertension in addition to producing vasodilation. Many studies have demonstrated successful regression of, or protection against, hypertrophy, accompanying a reduction in blood pressure, with several antihypertensive agents. Only diltiazem has induced regression of hypertrophy without lowering the blood pressure in SHR (15). In SHR, 5 mg/kg/d (12) or 15 mg/kg/d (16) of bunazosin attenuated the development of both hypertension and cardiac hypertrophy. To determine the effect of a small dose of an α_1 -adrenergic receptor antagonist on cardiac hypertrophy associated with hypertension, 2 mg/kg/d of bunazosin, a dose producing no significant hemodynamic effect, was employed in the present study. This dose did not prevent the development of hypertension in Dahl-SS rats fed a high salt diet; therefore, other doses of bunazosin were not used.

Salt intake is one factor that can induce cardiac hypertrophy independently of hemodynamic burden (17). The Dahl-SS rat may therefore be a good model of salt-induced cardiac hypertrophy, even if hemodynamic factors are involved. High salt intake also elevates sympathetic nervous activity (18) and increases catecholamine turnover in Dahl-SS rats, as compared with Dahl salt-resistant rats (19). Catecholamines rank with angiotensin II as important factors in cardiac hypertrophy, and catecholamine contractility has been suggested to be regulated by β -adrenergic receptors. Kupfer *et al.* identified α_1 -adrenergic receptors in cultured rat myocardial cells in radio ligand studies (20), and Simpson demonstrated that hypertrophy was stimulated by norepinephrine via α_1 -adrenergic receptors

in cultured neonatal rat heart muscle cells (21). Recently, these findings have been widely confirmed, and it is now accepted non-hemodynamic factors participate in the pathogenesis of cardiac hypertrophy. Alpha-adrenoreceptors are distributed in all cardiovascular tissues, including those of the heart and the entire vascular system. The reason why only the heart was affected by treatment with a low dose of the α_1 -blocker bunazosin remains unclear. Does this α_1 -blocker have a high affinity for cardiac tissue? Tamai *et al.* demonstrated an increase in cardiac α_1 -adrenoreceptor density after hemodynamic load before cardiac hypertrophy (22). The results of the present study can be explained based on the assumption that cardiac sensitivity to bunazosin was greater than that of other organs. Therefore, the low dose of bunazosin used affected only the cardiac tissue. It is of interest that chronic infusion of a subpressor dose of norepinephrine produced myocardial hypertrophy in dogs, suggesting that α_1 -receptors act independently of hemodynamic load (23). In addition, the same author postulated that in the pressure-overloaded heart, stress on the ventricular wall stimulated release of norepinephrine into the myocardium, which then initiated the hypertrophy process. This is a good example of the important role played by α_1 -adrenergic receptors in the pathogenesis of cardiac hypertrophy. Slight but significant inhibition of hypertrophy in young volume-expanded hypertensive rats in the developmental phase of hypertension does suggest the participation of α_1 -adrenergic receptors.

Total left ventricular DNA content decreased in parallel with the decrease in the left ventricular weight. Therefore, there was no difference between the groups in the ratio of total DNA content and left ventricular weight. Similar findings were observed by Morkin and Ashford in hypertrophic hearts following aortic coarctation in rats (24). They concluded that an increase in cardiac DNA by cardiac hypertrophy occurred mainly in the interstitial cells, not in myocytes. However, nuclear polyploidy in myocardial cells was associated with cardiomegaly in hypertensive rats (25), and an increase in left ventricular DNA content following hypertrophy can reflect not only interstitial cell DNA but also myocyte DNA (26). The distribution of changes in left ventricular DNA after treatment with bunazosin remains to be investigated.

No differences in sodium excretion, GFR, urine volume, urinary metanephrine, and urinary normetanephrine were found between the two groups in the present experiment. A reduction in renal func-

tion linked to activation of the sympathetic nervous system is an important mechanical factor in the development and maintenance of hypertension in Dahl-SS rats (18, 19). However, no differences in renal function and catecholamine release were observed between the two groups in this study. Therefore, a low dose of bunazosin apparently did not affect the sympathetic nervous system, which influences vasoconstriction and renal function, but did affect only the hypertrophic heart. In addition, the absence of changes in PRA, plasma aldosterone, and plasma ANP following treatment with a low dose of bunazosin produced no significant changes in homeostasis. However, doses of bunazosin substantially higher than the 2 mg/kg/d employed in this study may prevent the development of both cardiac hypertrophy and hypertension. At high doses, bunazosin is expected to influence renal function because α_1 -adrenergic receptors are distributed in the kidney and participate in the control of renal function (27). Higher doses of bunazosin also inhibit the development of hypertension by improving renal function.

In summary, 7 weeks' treatment with a sub-depressor dose of the selective α_1 -adrenergic receptor antagonist bunazosin inhibited the progression of cardiac hypertrophy during the development of hypertension in Dahl-SS rats fed a 4% NaCl diet. This effect was unassociated with a reduction in blood pressure or a change in renal function. These observations suggest that α_1 -adrenergic stimulation may be one factor involved in the development of hypertrophy independently of the hemodynamic burden associated with hypertension induced by volume expansion.

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