Calcineurin Inhibition Attenuates Mineralocorticoid-Induced Cardiac Hypertrophy

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Background—It remains unclear how mineralocorticoids induce cardiac hypertrophy and fibrosis. Recently, activation of the calcium-dependent phosphatase, calcineurin, has been shown to induce cardiac hypertrophy. In the present study, we examine the role of calcineurin in mineralocorticoid-induced cardiac hypertrophy and fibrosis.

Methods and Results—Uninephrectomized Wistar-Kyoto rats were placed on a 1.0% NaCl diet and treated with aldosterone (0.75 μg · h⁻¹) for 6 weeks with or without the calcineurin inhibitors, FK506 (0.5 mg · kg⁻¹ · d⁻¹) or cyclosporine A (10 mg · kg⁻¹ · d⁻¹). The effect of the angiotensin II type 1 receptor antagonist, losartan (10 mg · kg⁻¹ · d⁻¹) on aldosterone-induced cardiac hypertrophy was also studied. Treatment with aldosterone increased the heart weight/body weight ratio, cardiomyocyte size, and collagen amount. The expression of mRNA of both type-III collagen and atrial natriuretic peptide in the heart were increased by aldosterone administration. Both calcineurin activity and its mRNA expression were also increased in aldosterone-induced hypertrophic heart. Treatment with losartan, FK506, or cyclosporine partially prevented aldosterone-induced cardiac hypertrophy and fibrosis.

Conclusion—These results suggest that calcineurin is involved in the development of cardiac hypertrophy and fibrosis induced by mineralocorticoid excess. Inhibition of calcineurin may therefore prevent cardiac hypertrophy and fibrosis in mineralocorticoid hypertension. (Circulation. 2002;105:677-679.)

Key Words: calcineurin ■ aldosterone ■ heart ■ hypertrophy ■ fibrosis

Cardiac hypertrophy is regulated by ventricular pressure and volume loading, both of which can be increased by effector hormones of the renin-angiotensin-aldosterone system. These effectors also increase perivascular fibrous tissue synthesis. In addition, the fibrosis that follows myocyte necrosis is associated with increased plasma angiotensin II (Ang II) concentration and myocardial K⁺ depletion accompanying chronic mineralocorticoid excess,1 underlining the importance of the study of mineralocorticoids such as aldosterone in cardiac hypertrophy and fibrosis.

Aldosterone plays an important role in the pathogenesis of cardiovascular disease that is independent of Ang II. For example, patients with primary aldosteronism, in which Ang II levels are usually very low, have a higher incidence of left ventricular hypertrophy and stroke than do patients with essential hypertension. A recent study performed in patients classified with New York Heart Association class III and IV cardiac failure showed a 30% reduction in morbidity and mortality with the addition of the aldosterone antagonist spironolactone to conventional therapy, which included ACE inhibitors, loop diuretics, and digoxin.2 This decrease occurred with an average dose of spironolactone that did not have significant hemodynamic effects. Experimental animal data support a role for aldosterone in mediating cardiovascular injury. In the stroke-prone spontaneously hypertensive rat (SHRSP), administration of spironolactone, greatly attenuated cardiac hypertrophy.3 An important pathological effect of aldosterone in the heart has been reported in experimental models of mineralocorticoid hypertension.4 In these studies prolonged exposure to aldosterone was associated with the development of myocardiopathy and fibrosis.

Many reports have indicated that intracellular Ca²⁺ plays an important role in gene expression and growth in a variety of cell types including cardiomyocytes. However, it is unknown how Ca²⁺ regulates these events in cardiomyocytes. Recently, Molkentin et al5 have demonstrated the importance of the Ca²⁺-dependent phosphatase, calcineurin, and its downstream transcription factor NF-AT3 in the development of cardiac hypertrophy. Transgenic mice expressing activated calcineurin or NF-AT3 in the heart showed marked cardiac hypertrophy, and some of them progressed to dilated cardiomyopathy with interstitial fibrosis and congestive heart failure. The calcineurin inhibitors, cyclosporine A (CysA) and FK506, prevented cardiac hypertrophy in activated calcineurin transgenic mice and humoral factor–induced hypertrophy of cultured cardiomyocytes of neonatal rats. In addition, Sussman et al6 reported that several transgenic mouse models with hypertrophic cardiomyopathy could be success-
fully treated with CysA. These results suggest that calcineurin plays a pivotal role in the development of cardiac hypertrophy induced by various causes. We performed the present study to determine whether calcineurin could play a role in the cardiac hypertrophy and fibrosis induced by mineralocorticoid excess.

Methods

Animals
Male Wistar-Kyoto (WKY/Izm) rats (weight: 200 to 220 g) were housed in metabolic cages with free access to water and normal rat chow (0.1 mmol/g sodium and 0.24 mmol/g potassium; Nippon Charles River).

Experimental Protocol
All experiments were performed according to the guidelines for the use of experimental animals of the Animal Research Committee of Kanazawa University. Uninephrectomized rats were divided into 5 experimental groups. Group 1 (n = 8) was given aldosterone (0.75 μg · h⁻¹) via an implanted osmotic minipump for 6 weeks as previously described. Aldosterone (Sigma) was dissolved in 0.154 mol/L NaCl + 0.5% ethanol. Rats were fed ad libitum and given 0.17 mol/L NaCl, 40 mmol/L KCl solution as drinking water. Group 2 (n = 8) received daily oral administration of FK506 (Fujisawa) solubilized in 0.5% Arabic gum solution at a dose of 0.5 mg · kg⁻¹ · d⁻¹ and aldosterone (0.75 μg · h⁻¹) for 6 weeks. Group 3 (n = 8) received daily subcutaneous injections of CysA (Sandoz Ltd) solubilized in olive oil at a dose of 10 mg/kg and aldosterone (0.75 μg · h⁻¹) for 6 weeks. Group 4 (n = 8) received aldosterone and losartan (Merck) (10 mg · kg⁻¹ · d⁻¹) infused by osmotic minipump. Uninephrectomized sham-operated rats were implanted with osmotic minipumps containing vehicle for aldosterone (0.154 mol/L NaCl + 0.5% ethanol) and received no salt in the drinking water (group 5).

Systolic blood pressure was determined using the plethysmographic tail-cuff method. Before the animals were killed, they were anesthetized, decapitated, and the heart immediately removed. Quantification of calcineurin mRNA was performed using the competitive polymerase chain reaction (PCR) method as previously reported. Sequences for the sense and antisense primers for calcineurin have been reported previously. Quantification of AT₁ receptor mRNA was also performed using the competitive PCR method as previously reported.

Northern Blot Analyses of mRNA of Type-III Collagen and Atrial Natriuretic Peptide
Poly(A) RNAs (5 μg per lane) were separated by formaldehyde/agarose gel electrophoresis, transferred to a nylon membrane (Hybond-N⁺, Amersham Japan), and hybridized with 32P-labeled oligonucleotide probe specific for type-III collagen cDNA or atrial natriuretic peptide (ANP) cDNA probe, as previously reported. For quantification of relative levels of expression of the mRNA of type-III collagen or ANP, the autoradiographic signals were standardized to signals determined from β-actin mRNA in each preparation to control for amounts of RNA loaded per lane.

Histological Analysis
The transverse diameter of cardiomyocytes stained by hematoxylin and eosin was measured by micrometers (μm) in 50 different randomly chosen points from a cross section of LV free wall. The extent of LV fibrosis was measured in 10 fields randomly selected from a section of calculating the ratio of azan-stained fibrosis area divided by total myocardium area. Five sections of each heart were measured.

The Table summarizes the data of hemodynamics and parameters of cardiac hypertrophy and fibrosis. Aldosterone significantly increased blood pressure, the heart weight/body weight ratio, cardiac myocyte size, interstitial and perivascular fibrosis, and type-III collagen and ANP mRNA levels in the heart (P < 0.05). Treatment with losartan, FK506, or CysA significantly decreased both the heart weight/body weight ratio, cardiac myocyte size, cardiac fibrosis, and type-III collagen and ANP mRNA levels in the heart (P < 0.05). FK506, CysA, or losartan did not significantly decrease blood pressure. Calcineurin activity and the expression of calcineurin mRNA were significantly increased in rats treated with aldosterone (P < 0.05) (Figure, middle panel). Calcineurin activity and calcineurin mRNA levels did not increase if FK506 or CysA was administered with aldosterone. Treatment with losartan in the presence of aldosterone weakly decreased calcineurin activity (45% decrease, P < 0.05) and gene expression (56% decrease, P < 0.05).
Aldosterone also increased the expression of AT₁ receptor mRNA in the heart (2.8-fold increase).

Discussion

Mechanisms to explain how mineralocorticoid excess induces cardiac hypertrophy and fibrosis are still unknown. Previous results have shown that mineralocorticoids-induced cardiac hypertrophy and fibrosis may be prevented or reduced by several classes of inhibitors, making it difficult to postulate a simple mechanism of fibrotic or hypertrophic development. In this study, chronic infusion of aldosterone increased calcineurin activity and its mRNA expression in the heart. Treatment with FK506 or CysA, a calcineurin inhibitor, attenuated aldosterone-induced cardiac hypertrophy and fibrosis in rats. These results suggest that calcineurin plays a role in the development of cardiac hypertrophy and fibrosis induced by mineralocorticoid excess. Robert et al. have found that aldosterone increased cardiac AT₁ receptor expression and proposed that augmented Ang II action could contribute to cardiac hypertrophy and fibrosis. Murat et al. have reported that long-term elevation of Ang II caused cardiac hypertrophy and increased MAPK activation in the heart, which was blocked by CysA, a nonspecific calcineurin inhibitor. Robert et al. also reported that treatment with losartan at 10 mg · kg⁻¹ · d⁻¹ prevented aldosterone-induced cardiac hypertrophy and fibrosis without lowering blood pressure. Our results demonstrate that treatment with losartan resulted in approximately a 50% inhibition of the aldosterone-induced increase in heart weight/body weight ratio and type-III collagen levels. These results suggest that the increased AT₁ receptor expression caused by aldosterone excess contributes in part to the development of cardiac hypertrophy and fibrosis.

The calcineurin inhibitor, CysA and FK506, each prevented cardiac hypertrophy in Dahl salt-sensitive hypertensive rats and renovascular hypertensive rats. In contrast, CysA did not prevent cardiac hypertrophy due to hypertension in the spontaneously hypertensive rat. In this study, the ability of FK506 to prevent aldosterone-induced cardiac hypertrophy and fibrosis was incomplete, although the calcineurin activity was decreased to control levels. This suggests a calcineurin-independent pathway for regulating cardiomyocyte and fibroblast reactivity by mineralocorticoid excess. Bénitah and Vassort have reported the genomic regulation of cardiac IC₅₀ by aldosterone in cultured cardiomyocytes. Fiebeler et al. reported that AP-1 and the nuclear factor-κB are activated by aldosterone. Further study is therefore necessary to clarify the mechanism of cardiac hypertrophy and fibrosis induced by mineralocorticoid excess.

In summary, aldosterone directly or indirectly increases calcineurin activity in the heart, which may contribute to cardiac hypertrophy and fibrosis.

References
