Calcineurin Is Activated in Rat Hearts With Physiological Left Ventricular Hypertrophy Induced by Voluntary Exercise Training

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Background—Calcineurin may play a pivotal role in the signaling of cardiac hypertrophy; since this hypothesis was first put forward, controversial reports have been published using various experimental models. This study was designed to compare the physiological left ventricular hypertrophy (LVH) induced by voluntary exercise with LVH induced by aortic constriction and to determine whether calcineurin participates in the signaling of exercise-induced LVH.

Methods and Results—Wistar rats were assigned to 1 of the following 5 groups: 10 weeks of voluntary exercise (EX), a sedentary regimen, a 1-week (AC1) or 4-week (AC4) ascending aortic constriction period, or a sham operation. EX rats ran 2.4 ± 0.7 km/day voluntarily in specially manufactured cages; this was associated with an increase of LV diastolic dimension and stroke volume. Myocardial calcineurin activity markedly increased in EX rats (46.4 ± 8.3 versus 18.4 ± 0.5 pmol/min·mg in sedentary rats; P < 0.001) and in AC1 rats (44.9 ± 6.7 versus 22.1 ± 3.7 pmol/min·mg in sham-operated rats; P < 0.001), but not in AC4 rats (29.0 ± 3.4 pmol/min·mg). Treatment with cyclosporin A completely inhibited the development of LVH in EX rats, but it only partially attenuated the development of LVH in AC4 rats.

Conclusions—Calcineurin was activated in exercise-induced physiological LVH and in the developing phase of LVH (AC1), but not in decompensated pressure-overload hypertrophy (AC4). Cyclosporin therapy for the prevention of LVH may be harmful because it does not block the development of pathological hypertrophy but rather that of favorable adaptive hypertrophy. (Circulation. 2000;101:2134-2137.)

Key Words: exercise hypertrophy signal transduction

Athlete’s heart, or left ventricular hypertrophy (LVH) without pathological change, results from properly designed endurance exercise training. In contrast, LVH induced by pathological hemodynamic overload can eventually result in maladaptive congestive heart failure (CHF). Indeed, LVH is a strong predictor of subsequent cardiovascular events. Elucidating the underlying molecular mechanisms that distinguish adaptive hypertrophy from pathological hypertrophy is essential for the development of therapies to prevent CHF.

Calcineurin participates in signal transduction leading to cardiac hypertrophy. It was initially thought to play a key role in the transition from adaptive hypertrophy to CHF. Several reports have been published regarding calcineurin activity during and the effects of calcineurin inhibitors on various forms of hemodynamic overload, with inconclusive results. Some groups reported that cyclosporin A (CsA) and FK506 prevented hypertrophy, but others failed to find any effects. Indeed, some groups even reported decreased or unchanged calcineurin activity in response to hemodynamic overload. Although most studies used animals with pathological hypertrophy, little is known about whether calcineurin participates in the signaling of physiological LVH.

The purposes of this study were (1) to compare the mechanical performance and structure of hearts from rats with voluntary exercise-induced physiological LVH with those in hearts from rats with aortic constriction-induced pathological LVH and (2) to determine whether calcineurin participates in the development of exercise-induced LVH.

Methods

Group Assignments
All animal experiments were approved by the Animal Experimentation Review Board of the University of Tokyo. Male Wistar rats...
were assigned to 1 of the following 5 groups: (1) 10-week voluntary exercise training (EX, n=9), (2) sedentary condition (SED, n=9), (3) 1-week (AC1, n=6) or (4) 4-week (AC4, n=7) ascending aortic constriction, or (5) sham operation (SHAM, n=10).

To determine whether CsA attenuated the development of LVH in EX or AC4 rats, we administered it (40 mg/kg, given subcutaneously over 2 days) to additional sets of EX and AC4 rats for 10 and 4 weeks, respectively.

Voluntary Exercise-Induced Physiological LVH
Seven-week-old rats were individually housed in a specially manufactured cage equipped with a controlled running wheel and a distance counter. Rats exercised at their favorite time, at a speed and duration of their choice. Age-matched sedentary control rats were housed in ordinary cages (SED). Ten weeks later, echocardiographic, hemodynamic, histological, and biochemical measurements were made.

Pressure Overload–Induced Pathological LVH
Aortic constrictions were created as follows: 10-week-old rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). A ligature was placed around the ascending aorta, and an 18-gauge needle was set parallel to it. Then, the needle was quickly removed, leaving the aorta constricted to the diameter of the needle. At 1 or 4 weeks later, hemodynamic, histological, and biochemical assessments were performed.

Echocardiography
Echocardiography was performed using a LOGIC 500 echocardiograph (GE-Yokogawa) with a 5.5 MHz phased-array and a 6.5 MHz curved-array sector transducer. Rats were lightly anesthetized with diethylether and kept in the left-lateral decubitus position. LV dimensions were measured using the parasternal long-axis view. Pulsed-wave Doppler spectra of aortic ejection flow were recorded to calculate the velocity-time integral.

Hemodynamic Measurement
Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). A 3-French polyethylene catheter was advanced into the ascending aorta and LV via the right carotid artery. The pressure and ECG signals were obtained using a MacLab/400 (AD Instruments) data acquisition system.

Calcineurin Activity
Preparation of tissue extracts and assays of calcineurin activity were performed as previously described, with minor modifications. Briefly, calcineurin activity was defined as the difference in the amount of phosphate released from the 32 P-labeled cAMP-dependent protein kinase R-II subunit phosphopeptide (Peninsula Laboratories) in either the presence or absence of 50 μL of a specific calcineurin autoinhibitory peptide (Sigma). Myocardial samples were disrupted using a sonicator, and phosphate was isolated by Dowex AG 50W-X8 chromatography (Bio-Rad) and quantified by scintillation counting.

Histology
Transverse transmural myocardial sections were cut perpendicular to the apex-to-base axis and processed conventionally for histological examination. Random fields at a final magnification of ×200 were selected. Myocyte width was measured by a single observer who had no knowledge of other results. The transmural widths of randomly selected longitudinally oriented myocytes in circular midwall muscle bundles was measured with a calibrated microscope eyepiece reticle (10 cells for each sample).

Statistical Analyses
Differences among the groups were compared by ANOVA. Data were expressed as the mean±SEM. Pressure gradient indicates LV-to-ascending aorta pressure gradient; LVSPS, LV peak systolic pressure; LVEDP, LV end-diastolic pressure.

Results
EX rats ran 2.4±0.7 km/day; they had an increase in the LV diastolic dimension (7.2±0.5 versus 6.6±0.8 mm in SED rats; P=0.06) and velocity-time integral of the aortic ejection flow (4.6±0.3 versus 3.7±0.2 cm; P<0.05). Heart rate during the echocardiographic study was almost the same between the 2 groups. Exercise did not affect LV end-diastolic pressure. LV weight (LVW) and the LVW to body weight ratio (LVW/BW) increased in EX rats by 9.7% (P<0.01) and 33.1% (P<0.01), respectively, but those in AC1 rats did not differ from values in SHAM rats (Table).

LVW (0.79±0.05 g) and LVW/BW (1.92±0.10 g/kg) in CsA-treated EX rats were lower (P<0.01) than corresponding values in EX rats and were not different from those in SED rats. In AC rats, CsA treatment only partially attenuated the increase in LVW (0.96±0.04 g) and LVW/BW (2.40±0.12 g/kg).

Myocardial calcineurin activity markedly increased in EX rats (46.4±8.3 pmol·min⁻¹·mg⁻¹; P<0.001) compared with SED rats (18.4±0.5 pmol·min⁻¹·mg⁻¹; Figure 1). Calcineurin activity was also elevated in AC1 rats (44.9±6.7 pmol·min⁻¹·mg⁻¹; P<0.001) compared with SED rats (22.1±3.7 pmol·min⁻¹·mg⁻¹). Activity was not elevated in AC4 rats.
Myocyte width was larger in AC4 rats (24.0±2.2 μm; \(P<0.01\)) but not AC1 rats (20.9±1.7 μm) compared with SHAM rats (19.5±2.3 μm). Exercise resulted in a slight, but not significant, increase in myocyte width compared with SED rats (20.7±1.4 versus 17.9±3.2 μm; \(P=0.07\)) (Figure 2).

Voluntary exercise training did not increase adrenal gland weight (3.7±3 mg in EX rats versus 3.5±2 mg in SED rats) or plasma atrial natriuretic factor concentrations (0.73±0.28 versus 0.59±0.26 ng/mL in EX versus SED rats). The concentration of atrial natriuretic factor increased in AC4 rats (1.48±0.30 ng/mL; \(P<0.01\)) and tended to increase in AC1 rats (1.12±0.29 ng/mL; \(P=0.11\)). These results, together with the hemodynamic data, suggest that voluntary exercise training induced physiological LVH without CHF.

**Discussion**

Voluntary exercise training induced LVH without impairing cardiac function, which is compatible with the athlete’s heart phenomenon. The LVW/BW, LV end-diastolic dimension, and velocity-time integral of aortic ejection flow increased, without an increase in cardiomyocytes width as previously reported.\(^{12,13}\)

This is the first report that shows the involvement of calcineurin in physiological adaptive hypertrophy. The role of calcineurin in cardiac hypertrophy had not yet been elucidated. In a previous study, transgenic mice overexpressing calcineurin developed cardiac hypertrophy and heart failure,\(^{3}\) indicating the central role of calcineurin in the development of pathological hypertrophy. Furthermore, calcineurin inhibition by CsA in these transgenic mice prevented hypertrophy.\(^{4}\) However, more recently, several researchers have investigated the effects of calcineurin inhibition on pressure-overload hypertrophy and found results that conflicted with this initial hypothesis.\(^{5–8}\)

We also observed a similar discrepancy between calcineurin activity and severity of LVH. Calcineurin activity

![Figure 1. Myocardial calcineurin phosphatase activity. *\(P<0.05\) compared with SED; †\(P<0.05\) compared with SHAM.](http://circ.ahajournals.org/)

![Figure 2. Hematoxylin and eosin-stained histological sections. Magnification ×200. A, EX rat; B, SED rat; C, AC1 rat; D, AC4 rat; and E, SHAM rat. Bar=100 μm.](http://circ.ahajournals.org/)
increased in AC1 rats, which did not develop LVH, but not in AC4 rats, which did develop LVH. One possible explanation is that calcineurin may be important mainly in the development of compensatory or adaptive hypertrophy. We showed that CsA blocked exercise-induced LVH but only partially attenuated aortic constriction–induced LVH, which was consistent with the results of a previous study showing the inability of CsA to completely block pressure overload–induced LVH. The inhibition of calcineurin by CsA in pressure-overload LVH could be deleterious, because it would attenuate the hypertrophy developing as a compensatory mechanism.

Our results differ from those of a recent study, which showed an activation of calcineurin in patients with heart failure. The differences may be explained as follows. The increased calcineurin activity in the heart samples from the patients involved in the previous study may have reflected the inhomogeneous nature of myocardium with ischemic cardiomyopathy and the beneficial effects of angiotensin-converting enzyme inhibitors in patients with idiopathic cardiomyopathy. Part of the myocardial samples analyzed may have been in the compensated phase of hypertrophy. The authors developed a coimmunoprecipitation assay that detected the association of calmodulin with calcineurin. A recent report indicated dramatic changes in the immunoreactivity of calcineurin during hypertrophy and failure, suggesting that the calcineurin isoform shifts. Further investigations are needed to evaluate the association of particular calcineurin isoforms with calmodulin. Consequently, we consider that, at present, phosphatase assays using calcineurin-specific substrates are more reliable indexes of myocardial calcineurin activity.

Limitations
In the present study, it was hard to obtain good-quality echocardiographic images and to accurately determine wall thickness in some rats with aortic constriction. One reason may be the development of tissue adhesions after surgery. During the echocardiographic study, EX rats did not exhibit bradycardia, as is usually observed in athletes. This was probably because of anesthesia with diethyl ether.

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