Department of Cell Physiology

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General Summary

The main research interest of our department is the physiology of cardiac muscle contraction.

Research Activities

Depressed Frank-Starling mechanism in left ventricular muscle of the knock-in mouse model of dilated cardiomyopathy with troponin T deletion mutation ΔK210

We have demonstrated that the Frank-Starling mechanism is coordinately regulated in cardiac muscle via thin-filament "on-off" switching and titin-based changes in interfilament lattice spacing. In the present study, we investigated how the sarcomere length-dependence of active force production is altered in a knock-in mouse model of inherited dilated cardiomyopathy (DCM) with a deletion mutation ΔK210 in the cardiac troponin T gene. Confocal imaging revealed that the cardiomyocytes were significantly enlarged, especially in the longitudinal direction, in the hearts of ΔK210 knock-in mice, with striation patterns similar to those in wild-type hearts, suggesting that the number of sarcomeres is increased but their length remains unaltered. For analysis of the sarcomere length-dependence of active force, skinned muscles were prepared from the left ventricles of wild-type and ΔK210 mice. An increase in sarcomere length from 1.9 to 2.2 μm shifted the midpoint (pCa_{50}) of the force-pCa curve leftward by 0.21 pCa units in wild-type preparations. In ΔK210 muscles, Ca^{2+} sensitivity was lower by 0.37 pCa units, and the sarcomere length-dependent shift of pCa_{50}, i.e., ΔpCa_{50}, was less pronounced (0.11 pCa units), with and without treatment with protein kinase A. The rate of active force redevelopment was lower in ΔK210 preparations than in wild-type preparations, showing blunted thin-filament cooperative activation. An increase in thin-filament cooperative activation upon an increase in the fraction of strongly bound cross-bridges by MgADP increased ΔpCa_{50} to 0.21 pCa units. We therefore conclude that the depressed Frank-Starling mechanism in the hearts of ΔK210 knock-in mice is the result of a reduction in thin-filament cooperative activation.

Real-time measurement of sarcomere length in the mouse heart in vivo by means of α-actinin-green fluorescent protein

Despite numerous studies performed under various experimental settings, the molecular mechanisms of contraction and relaxation of cardiomyocytes remain elusive *in vivo*. In the present study, we expressed green fluorescent protein (GFP) at sarcomeric Z-disks (α-actinin) by means of an adenovirus vector system in adult mice and performed real-

time imaging of the movement of single sarcomeres in cardiomyocytes in the left ventricle under fluorescence microscopy at 10-nm precision (at 100 fps). First, we found that sarcomere length was 2.0 μm in the isolated heart when perfused with 30 mM 2,3-butanedione monoxime (hence, at rest). This value is close to values previously obtained in rats by other investigators with various experimental techniques. When perfused with Tyrode's solution containing 1 mM Ca^{2+}, the heart started to beat with diastolic and systolic sarcomere lengths of 2.2 and 1.7 μm, respectively, in the left ventricle. Finally, we attempted to visualize single sarcomeres *in vivo* in open-chest mice under anesthesia. We found that sarcomere length was 2.0 and 1.7 μm during diastole and systole, respectively, but varied by 0.3 μm even in the same left ventricular cell. We also found that sarcomere contraction occurred at the T-wave endpoint on electrocardiograms, followed by an increase in left ventricular pressure.

Microscopic analysis of spontaneous sarcomeric oscillations in neonatal cardiomyocytes
We have previously demonstrated that cardiac myocytes shows rhythmic, spontaneous sarcomeric oscillations (SPOCs) under partial activation states, namely, at pCa=6.0 (Ca-SPOC) or in the presence of MgADP and Pi under relaxing conditions (ADP-SPOC). We have reported that the period of SPOC (both Ca-SPOC and ADP-SPOC) in skinned myocardium correlates with the period of the resting heart rate in various animal species. To fully understand the molecular mechanisms of SPOC, in the present study we used neonatal rat cardiomyocytes expressing α-actinin-GFP and visualized the motions of sarcomeres. First, we successfully induced SPOCs in neonatal myocytes with an intact inner-membrane system following treatment with ionomycin by controlling cytoplasmic Ca^{2+} concentrations. We have termed this phenomenon Cell-SPOC. The measurement of intracellular Ca^{2+} with fluo-4 confirmed that Ca^{2+} oscillations did not occur under our experimental conditions. As found in adult cardiomyocytes, the sarcomeric oscillations consisted of quick lengthening and slow shortening during Cell-SPOC. We also found in untreated neonatal myocytes that an increase in the frequency of electrical stimuli to the physiological level (i.e., 3 to 5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in a waveform similar to that observed during Cell-SPOC in ionomycin-treated cardiomyocytes. These results suggest that the auto-oscillatory properties of cardiac sarcomeres are involved in the regulation of heartbeat.

Ca^{2+}-independent on-off regulation of cardiomyocytes by microscopic heat pulses
Laser irradiation is a novel technique of noninvasive stimulation in cardiac and neural tissues. However, physical parameters for the laser irradiation-induced cardiac contractions have not been clarified, because various physicochemical reactions, such as photochemical and photothermal effects, are triggered in this process. Here we studied the effects of laser-induced local temperature changes on the functions of isolated cardiomyocytes. We have demonstrated previously that a microscopic heat pulse (ΔT=0.2°C for 2 seconds) induces a Ca^{2+} burst in cancer cells (HeLa cells) at body temperature, with a mechanism similar to that of rapid cooling contracture in skeletal and cardiac muscles. In the present study, we generated microscopic heat pulses by focusing infrared

laser light in the extracellular solution near adult rat cardiomyocytes. We found that a microscopic heat pulse (ΔT=5°C for 0.5 second) induces contractions at a basal temperature of 36°C. At 25°C, a larger ΔT was required to induce contractions. When 2.5-Hz heat pulses were repeatedly applied, we observed oscillatory contractions of cardiomyocytes. Unlike contractions induced by electric stimulation, these contractions were not accompanied by Ca^{2+} transients. Likewise, heat pulses induced contractions of skinned cardiomyocytes in a Ca^{2+}-free solution in the presence of ATP. These results demonstrate that heat pulses can regulate cardiac contractions without any involvement of Ca^{2+} dynamics, by directly activating the actomyosin interaction. Hence, our microheating technique may be useful for stimulating the beating of failing hearts without causing abnormal Ca^{2+} dynamics.

Ca^{2+} handling and contraction in cardiac papillary muscles with interstitial fibrosis

Cardiac fibrosis is a maladaptive response to pathophysiological conditions, such as in cardiac hypertrophy and ischemic heart diseases. However, the effects of interstitial fibrosis on Ca^{2+} handling and contraction in myocardium remain unclear. We prepared pulmonary artery banding (PAB) rats as a model of cardiac hypertrophy. Four weeks after the operation, the right ventricular weight of PAB rats was significantly greater than that of control rats, indicating right ventricular hypertrophy. Right ventricular papillary muscles of the PAB rats were divided into an interstitial fibrosis group and a nonfibrosis group by using Masson trichrome staining for comparison with those of the control group. To measure tension with intracellular Ca^{2+} transients, we used the aequorin method. The peak Ca^{2+} in both the interstitial fibrosis and nonfibrosis groups was significantly higher than that in the control group. However, peak tension in the interstitial fibrosis group was significantly less than that in the nonfibrosis and control groups. The time to peak Ca^{2+} in the interstitial fibrosis group was significantly longer than that in nonfibrosis and control groups. Immunohistochemical staining showed that connexin 43 accumulation in the intercalated disks was less in the interstitial fibrosis group than in the nonfibrosis and control groups. Depressed tension development of myocardium with interstitial fibrosis is likely due to: 1) lowering of myofibrillar Ca^{2+} sensitivity, 2) decreases in Ca^{2+} release, and 3) asynchronous activation of cardiomyocytes via impaired cell-to-cell communication.

Pathophysiologic study of the heart in collaboration with the Division of Cardiology, Department of Internal Medicine

We have investigated the role of Ca^{2+} handling in cardiac contractility under physiological and pathophysiological conditions, such as cardiac hypertrophy and heart failure. In the present study, we investigated the significance of the renin-angiotensin system in the pathogenesis of DCM in mice with troponin T deletion mutation ΔK210. We used an angiotensin-receptor blocker and a direct renin inhibitor to block the renin-angiotensin system *in vivo*. We found that both the angiotensin-receptor blocker and the direct renin inhibitor were effective for treating cardiac contractility in DCM mice, with, presumably, different mechanisms of action.

Publications

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