Department of Cell Physiology

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General Summary

The main research interests of our department are the physiology of muscle contraction and related subjects.

Research Activities

Depressed length-dependent activation in left ventricular muscles from a mouse model of dilated cardiomyopathy

Cardiac sarcomeres produce greater active force in response to stretch, forming the basis of the Frank-Starling mechanism of the heart. We have demonstrated that length-dependent activation is coordinately regulated via thin filament "on-off" switching and titin-based lattice spacing reduction. In the present study, we investigated how length-dependent activation is altered in a knock-in (KI) mouse model of inherited dilated cardiomyopathy (DCM) with a deletion mutation ΔK210 in the cardiac troponin T gene (*TNNT2*). Skinned muscle preparations with a diameter of ～100 μm were obtained from left ventricular papillary muscles of KI and wild-type (WT) 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～0.2 pCa units in WT muscles. In KI muscles, Ca^{2+} sensitivity was lower (as in *Circulatory Research*, 2007), and the sarcomere length-dependent shift of pCa_{50}, i.e., ΔpCa_{50}, was less pronounced, with a mean value of ～0.1 pCa units. The ΔpCa_{50}, as well as Ca^{2+} sensitivity, became insignificant between WT and KI muscles following thin filament reconstitution with the identical troponin complex (extracted from rabbit fast skeletal muscle). It was also found that at a similar level of protein kinase A-dependent phosphorylation of sarcomere proteins (e.g., troponin I), length-dependent activation was still less pronounced in KI muscles than in WT muscles. The finding that only N2B titin is expressed in both WT and KI muscles suggests that the depressed length-dependent activation in KI muscles is coupled with a change in thin filament "on-off" regulation.

Microscopic analysis of spontaneous sarcomeric oscillations in neonatal cardiomyocytes

We have previously demonstrated that cardiac sarcomeres show spontaneous rhythmic oscillations (SPOCs) under partial activation states, namely, at pCa ～6.0 (Ca-SPOC), or at the coexistence of MgADP and Pi under the relaxing conditions (ADP-SPOC). We have reported that the period of SPOCs (both Ca-SPOC and ADP-SPOC) in skinned myocardium correlates with that of the resting heart rate in various animal species (Sasaki, D. et al., 2005 and 2006). In the present study, we analyzed the SPOC properties in rat neonatal cardiomyocytes expressing α-actinin-green fluorescent protein (GFP)

in the Z-lines. We found that Ca-SPOC occurred at pCa ～6.0 with a frequency of ～3 Hz after treatment with ionomycin. 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 Ca-SPOC. It was also found in untreated neonatal myocytes that an increase in the frequency of electrical stimuli to the physiological level (i.e., 3–5 Hz) caused a phase shift of shortening and relengthening owing to enhancement of the relengthening speed, resulting in the waveform being similar to that observed during SPOC in ionomycin-treated cardiomyocytes. These results suggest that the auto-oscillatory properties of cardiac sarcomeres may be involved in the regulation of cardiac beat.

Real-time imaging of single sarcomeres in the rodent heart

Numerous studies have been performed in tissues and cells to clarify the molecular mechanisms of myocardial contraction. However, because of a number of differences between *in vitro* and *in vivo* conditions, the dynamics of myocardial sarcomere contractions in living animals is not yet understood. We developed a novel system allowing us to conduct real-time imaging of single sarcomeres in the beating heart *in viv*o. First, to estimate the magnitude of regional myocardial movement, we performed imaging of fluorescent microspheres (diameter, ～1 μm) on the left ventricle of the beating heart of the anesthetized rat. We found that the movement of fluorescent microspheres on the heart took the form of an ellipse, with the major axis of 100 to 200 μm, when blood pressure and heart rate were within the normal range. Then, we caused green fluorescent protein to be expressed at sarcomeric Z-discs (α-actinin) by using the adenovirus vector system in adult rats and mice and performed real-time imaging of the movement of single sarcomeres at a video rate (30 frames per second) under fluorescence microscopy. Sarcomere length was found to be ～2.00 μm in the isolated heart of the rat when perfused with Tyrode's solution containing 30 mM 2,3-butanedione monoxime (hence, during diastole). This value is close to what was obtained previously by others under similar experimental conditions using various experimental techniques, i.e., X-ray diffraction and two-photon imaging. We also found that sarcomere length was ～1.7 and ～2.2 μm during contraction and relaxation, respectively, in the isolated heart of the mouse. Experiments are now underway to visualize the movement of single sarcomeres in the beating heart of the anesthetized mouse.

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

Cardiac fibrosis is a maladaptive response to pathophysiological conditions, such as cardiac hypertrophy and ischemic heart diseases. However, the changes in Ca^{2+} handling and contraction in the cardiac muscle with interstitial fibrosis remains unclear. We created pulmonary artery banding (PAB) rats for a model of cardiac hypertrophy. Four weeks after 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 the interstitial fibrosis group and the non-fibrosis group, to compare with the control group using Masson Trichrome stain. To measure tension with the intracellular Ca^{2+} transients, we used the aequorin method. The

peak Ca^{2+} in both the interstitial fibrosis group and the nonfibrosis group was significantly higher than that in the control group. However, peak tension in the interstitial fibrosis group was significantly smaller than that in the nonfibrosis group or the control group. The time to peak Ca^{2+} in the interstitial fibrosis group was significantly longer than that in the nonfibrosis group or the control group. The impairment of tension development in cardiac muscle with interstitial fibrosis is believed to be due to a decrease in the Ca^{2+} sensitivity and a disturbance of the Ca^{2+} release mechanism. However, asynchronous activation of each cardiac myocyte in the fibrotic preparation, due to scarce cell-to-cell communication, might be another mechanism.

Collaborative works with the Department of Cardiology

We attempted to observe the effects of endothelin-1on L-type Ca^{2+} channels and its mechanisms. Endothelin-1 expresses its effects through ET_A receptors, and protein kinase-C and Ca^{2+}/calmodulin kinase II are involved in this signal transduction. The Ca^{2+} leak from sarcoplasmic reticulum (SR) is enhanced by stimulation of the sympathetic nervous system through the protein kinase A-dependent phosphorylation of ryanodine receptors. However, FKBP12.6, which modifies ryanodine receptors, is not involved in this process.

The mechanism of dilated cardiac myopathy was investigated, and an angiotensin type 1 antagonist improved the prognosis and cardiac functions. However, the molecular mechanisms have not been clarified, although phospholamban and the phosphorylation of ryanodine receptors were not directly related.

Publications

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