Department of Cell Physiology

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General Summary

The aim of research in our laboratory is to understand the regulatory mechanism of the cardiovascular system. In particular, we are interested in the mechanics of sarcomere contraction, Ca^{2+} homeostasis in the cardiac sarcoplasmic reticulum, the pathophysiology of cardiac fibrosis, and development of the cardiovascular system. In 2012, Professor Minamisawa succeeded Professor Emeritus Kurihara as the department chair. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus. In addition, we developed an *in-vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as the rat and mouse.

Research Activities

Mechanism of sarcomere contraction in cardiac and skeletal muscles

1. 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 that their length remains unaltered. To analyze the sarcomere length-dependence of active force, skinned muscles were prepared from the left ventricles of wild-type and ΔK210 mice. We found 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.

2. 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 frames per second). We attempted to visualize single sarcomeres *in vivo* in open-chest mice under anesthe-

sia. 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 next found that sarcomere contraction occurred at the T-wave endpoint on electrocardiograms and was followed by an increase in left ventricular pressure. Finally, we successfully obtained Z-sectioning images (Z = 1 μm) of sarcomeres by means of a piezoelectric actuator and reconstructed the images to analyze changes in sarcomere length at nanometer precision during the cardiac cycle.

3. Real-time intracellular Ca^{2+} imaging in the heart

In the present study, we developed an experimental model for real-time imaging of intracellular Ca^{2+} in ventricular myocytes in the heart. Ca^{2+} waves were clearly observed at the cellular level in the isolated heart. Interestingly, randomly occurring Ca^{2+} waves or transients or both became synchronized by electric stimulation (～5 Hz). We also found that temperature control is highly important for intracellular Ca^{2+} imaging in the heart *in vivo*. Intracellular Ca^{2+} imaging in the heart will greatly enhance our understanding of the excitation-contraction coupling in health and disease.

4. Real-time nanoimaging of single sarcomere dynamics in rat neonatal cardiomyocytes via expression of actinin-*Aequorea coerulescens* GFP in Z-disks

A change in sarcomere length in cardiomyocytes causes a marked change in contractility. This intrinsic nature of sarcomere length-dependence of activation highlights the significance of simultaneous measurement of sarcomere length and intracellular Ca^{2+} concentration in localized areas of cardiomyocytes, at high spatial and temporal resolution. To directly visualize the motion of single sarcomeres at nanometer precision during excitation-contraction coupling, we applied cutting-edge nanoimaging technologies to primary cultured rat neonatal cardiomyocytes. We developed an experimental system for simultaneous nanoscale analysis of single-sarcomere dynamics and changes in intracellular Ca^{2+} concentration *in vivo* via expression of *Aequorea coerulescens* GFP in Z-disks. We first examined steady spontaneous sarcomeric oscillations at partial Ca^{2+} activation in primary-cultured rat neonatal cardiomyocytes. The present experimental system has a broad range of possible applications for unveiling single-sarcomere dynamics during excitation-contraction coupling in neonatal cardiomyocytes under various conditions.

Pathophysiological mechanisms of cardiac remodeling and 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 papillary muscles of PAB rats were dissected, and tension was measured with intracellular Ca^{2+} transients by using the photoprotein aequorin. On the basis of histological analysis, papillary muscles after PAB were clearly divided into 2 groups: an interstitial fibrosis group and a non-fibrosis with hypertrophy group. The peak Ca^{2+} in both interstitial fibrosis and non-fibrosis 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 non-fibrosis and control groups. The

time to peak Ca^{2+} in the interstitial fibrosis group was significantly longer than that in the non-fibrosis and control groups. Immunohistochemical staining showed that connexin 43 accumulation in the intercalated disks was less in the interstitial fibrosis group than in the non-fibrosis and control groups. These results indicate that impairment of tension development of the cardiac muscle with interstitial fibrosis is due to lower Ca^{2+} sensitivity and less cell-to-cell communication.

Regulation of cardiac sarcoplasmic reticulum ATPase activity

Impaired Ca^{2+} reuptake into the sarcoplasmic reticulum underlies a primary pathogenesis of heart failure in the aging heart. Sarcalumenin, a Ca^{2+}-binding glycoprotein located in the longitudinal sarcoplasmic reticulum, regulates Ca^{2+} reuptake by interacting with sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). We found that the expression levels of both sarcalumenin and SERCA2 proteins were significantly downregulated with age and that the downregulation of sarcalumenin protein preceded that of SERCA2 protein. Using senescent sarcalumenin knockout mice, we found that sarcalumenin plays a critical role in maintaining Ca^{2+} transport activity of SERCA2a and cardiac function in the senescent population.

Development and pathogenesis of the great arteries

Molecular mechanism of closure of the ductus arteriosus

The ductus arteriosus is a mysterious and interesting artery. The ductus arteriosus is an essential vascular shunt between the aortic arch and the pulmonary trunk during fetal development. The ductus arteriosus closes immediately after birth in accordance with its smooth muscle contraction and vascular remodelling. When the ductus arteriosus fails to close after birth, the condition is known as patent ductus arteriosus (PDA), which is a common problem in premature infants. Although cyclooxygenase inhibitors are often used to treat PDA, their efficacy is often limited. Because thromboxane A_2 (TXA_2) induces vascular contraction via the TXA_2 receptor, we hypothesized that TXA_2 receptor stimulation promotes ductus arteriosus closure. The selective TXA_2 receptor agonists U46619 and I-BOP caused constriction of the fetal ductus arteriosus in a dose-dependent manner on embryonic days 19 and 21. In addition, U46619 exerted a vasoconstrictive effect in 2 different postnatal PDA models: premature PDA and hypoxia-induced PDA. Furthermore, we found that U46619 at lower concentrations (up to 0.05 mg/g of body weight) had a minimal vasoconstrictive effect on other vessels and did not induce microthrombosis in the pulmonary capillary arteries. Therefore, we conclude that low-dose TXA_2 receptor stimulation constricts the ductus arteriosus with minimal adverse effects, at least in rat neonates, and our results could lead to an alternative potent vasoconstrictor for PDA.

Publications

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