Department of Cell Physiology

Susumu Minamisawa, *Professor*
Norio Fukuda, *Associate Professor*
Masato Konishi, *Visiting Professor*
Yoichiro Kusakari, *Assistant Professor*

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 development of the cardiovascular system, the mechanics of sarcomere contraction, Ca^{2+} homeostasis in the cardiac sarcoplasmic reticulum, and the pathophysiology of cardiac fibrosis. We established an experimental system to investigate small fetal arteries, such as the rat fetal ductus arteriosus (DA). 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

Development and pathogenesis of the great arteries

1. Molecular mechanism of closure of the DA

The DA is a mysterious artery that is interesting to study. The DA is an essential vascular shunt between the aortic arch and the pulmonary trunk during fetal development. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. When the DA fails to close after birth, the condition is known as patent DA, which is a common problem in premature infants. Although cyclooxygenase inhibitors are often used to treat patent DA, their efficacy is often limited. Both vascular contraction and remodeling, i.e., intimal thickening, are required for complete anatomical closure of the DA. Decreased elastogenesis is a hallmark of DA remodeling and is thought to contribute to intimal thickening of the DA. However, the molecular mechanisms of decreased elastogenesis are not fully understood. We found that prostaglandin E_2 (PGE_2) receptor EP4 signaling promotes degradation of the mature lysyl oxidase protein, a cross-linking enzyme for elastic fibers, only in the DA, leading to decreased elastogenesis. In addition, using a fluorescence-activated cell sorter, we isolated endothelial cells from pooled tissues from the DA of fetal Wistar rats. We found several significant differences in transcriptional profiles between the DA and aortic endothelial cells. Newly identified DA endothelium-dominant genes may play an important role in DA-specific functional and morphologic characteristics.

2. Molecular mechanism of elastic fiber formation in the great arteries

Abdominal aortic aneurysm (AAA) is a common but life-threatening disease among the elderly. In collaboration with Yokohama City University, we found that selective blocking of PGE_2, in particular, EP4 prostanoid receptor signaling, attenuated the development of AAA. We are examining the molecular mechanisms using animal models, such as the calcium chloride-induced AAA rat model and Brown-Norway rats that exhibit irregular internal elastic laminae.

Regulation of cardiac sarcoplasmic reticulum ATPase activity

Impaired Ca^{2+} reuptake into the sarcoplasmic reticulum is thought to be a primary pathogenic mechanism of heart failure. We are interested in sarcolipin, a regulator of the sarcoplasmic reticulum Ca^{2+}-ATPase, that is specifically expressed in atrial muscle. We generated a knockout mouse to insert a gene-targeting vector containing the cassette of the Cre recombinase gene into an endogeneous sarcolipin locus by homologous recombination. This mouse enables us to generate atrium-specific gene targeting. We are characterizing the phenotypes of sarcolipin knockout mice.

Regulation of cardiac metabolism

Cardiac metabolism plays an essential role in maintaining cardiac function. The energy of cardiac muscle largely depends on fatty acid oxidation. Furthermore, the atrium and ventricle have chamber-specific functions, structures, gene expressions, and pathologies. The left ventricle works as a high-pressure chamber to pump blood toward the body, and its muscle wall is thicker than those of the other chambers, suggesting that energy utilization in each chamber should be different. Using capillary electrophoresis and mass spectrometry, we found that overall metabolic profiles, including nucleotides and amino acids, were similar between right and left ventricles. On the other hand, the atria exhibited a metabolic pattern distinct from those of the ventricles. Importantly, the high-energy phosphate pool (the total concentration of ATP, ADP, and AMP) was higher in both ventricles. Accordingly, the activities or expression levels or both of key enzymes were higher in the ventricles to produce more energy. Our findings provide a basis for understanding the chamber-specific metabolism underlying pathophysiology in the heart.

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 the PAB rats were dissected and their tension was measured with intracellular Ca^{2+} transients by means of the photoprotein aequorin. On the basis of histological analysis, papillary muscles after PAB were clearly divided into 2 groups: the interstitial fibrosis group and the nonfibrosis with hypertrophy group. 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 the 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. These results indicate that impairment of tension development of cardiac muscle with interstitial fibrosis is due to lower Ca^{2+} sensitivity and less cell-to-cell communication.

Mechanism of sarcomere contraction in cardiac muscle

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 investigated how the sarcomere length-dependence of active force production is altered in a knock-in mouse model of inherited dilated cardiomyopathy (DCM) with 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. To analyze the sarcomere length-dependence of active force, skinned muscles were prepared from the left ventricles of wild-type and ΔK210 mice. Accordingly, we found that the depressed Frank-Starling mechanism in the hearts of ΔK210 knock-in mice is the result of reduced thin-filament cooperative activation.

2. Sarcomere length nanometry in rat neonatal cardiomyocytes via expression of α-actinin-*Aequorea coerulescens* green fluorescent protein in Z-disks

In cardiac muscle, a change in sarcomere length by a mere 100 nm causes a dramatic change in contractility, indicating the need for the simultaneous measurement of sarcomere length and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cardiomyocytes at high spatial and temporal resolutions. To accurately analyze the motion of individual sarcomeres with nanometer precision during excitation-contraction coupling, we applied nanometry techniques to primary-cultured rat neonatal cardiomyocytes. First, we developed an experimental system for simultaneous nanoscale analysis of single sarcomere dynamics and $[Ca^{2+}]_i$ changes via the expression of *Aequorea coerulescens* green fluorescent protein in Z-discs. We found that the averaging of the lengths of sarcomeres along the myocyte, a method generally now used in myocardial research, caused marked underestimation of sarcomere lengthening speed due to the superposition of different timings for lengthening between sequentially connected sarcomeres. Then, we found that following treatment with ionomycin, neonatal myocytes exhibited spontaneous sarcomeric oscillations (Cell-SPOC) at partial activation with blockage of sarcoplasmic reticulum functions and that the waveform properties were indistinguishable from those obtained in electric field stimulation. The present experimental system has a broad range of possible applications for unveiling single sarcomere dynamics during excitation-contraction coupling in cardiomyocytes under various settings.

3. *In vivo* visualization of sarcomeric motions in the beating mouse heart

The Frank-Starling law predicts that a change in the length of myocardial sarcomeres by only 100 nm dramatically changes the heart's pump functions, indicating the importance of highly accurate measurements of cardiac sarcomere length displacement *in vivo*. We have developed a high-speed high-resolution *in vivo* cardiac imaging system in mice. This system enables 3-dimensional analysis of sarcomere dynamics during the cardiac cycle, simultaneously with electrocardiography and left ventricular pressure measurements. We demonstrated that the working range of sarcomere length exists on the shorter resting distribution side and that the developed pressure is a linear function of the sarcomere length change between diastole and systole at 100-nm levels.

Publications

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