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 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), and the pulmonary vein. In addition, we developed an *in vivo* nanoimaging system to observe sarcomere contraction in the ventricles of small animals, such as rats and mice.

Research Activities

Development and pathogenesis of the great arteries

1. Molecular mechanism of closure of the DA

The DA is a mysterious artery that attracts considerable research interest. The DA is an essential artery that connects the main pulmonary artery and the descending aorta in a fetus. The DA closes immediately after birth in accordance with its smooth muscle contraction and vascular remodeling. We are investigating molecular mechanisms of DA closure after birth.

Decreased elastogenesis is a hallmark of structural change in the DA 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 signaling of the prostaglandin E receptor EP4 promotes degradation of the mature lysyl oxidase protein that is a cross-linking enzyme for elastic fibers in the DA, leading to decreased elastogenesis. Both the avian DA and the mammalian DA close after birth. Although the avian DA has characteristics of vascular structure that differ from those of the mammalian DA, the poor elastogenesis is similar. We are examining the chicken DA to elucidate the molecular mechanisms by which elastogenesis is impaired in this specific artery.

2. Causal factors of aortic coarctation

Aortic coarctation is a congenital heart disease in which the descending aorta is narrow, usually in the area where the DA connects. In some cases, re-narrowing of the aorta develops after definitive operation. We found that DA smooth muscle cells were straying into aortic smooth muscle cells of the narrowed area. We are now elucidating the molecular mechanisms of aortic coarctation. We are collaborating in this study with Hyogo Prefectural Kobe Children's Hospital.

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 regulation of the sarcoplasmic reticulum Ca^{2+}-ATPase and Ca^{2+} homeostasis in the sarcoplasmic reticulum. We generated sarcolipin-Cre knockin mice in which Cre recombinase is inserted in the sarcolipin-coding region. Although sarcolipin homozygous deletion exhibited an increase in Ca^{2+}-ATPase activity in the atria, heterozygous deletion of sarcolipin generally had no phenotype. Therefore, sarcolipin-Cre knockin mice could be used to generate an atrium-specific gene deletion mouse, because sarcolipin is specifically expressed in the mouse atria.

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. The main cardiac metabolism is known to switch from fatty acid oxidation to glycolysis when the heart is exposed to stress. Vitamin B1 (thiamine) deficiency causes beriberi, which is characterized by peripheral sensory and motor neuropathy and congestive heart failure. Dr. Kanehiro Takaki, the founder of The Jikei University, eliminated beriberi from the Imperial Japanese Navy by improving its food supply (thiamine supplementation). We hypothesized that vitamin B1 derivative products (thiamine pyrophosphate) would protect the heart against ischemia/reperfusion injury. We found that pretreatment with vitamin B1 preserves cardiac function in cases of ischemia/reperfusion injury. We are now investigating the mechanism of these effects.

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. Using DNA microarray analyses, we found that fibroblast growth factor 23, which is known to play a role in the regulation of osteogenesis, was up-regulated in the interstitial fibrosis group. We are now investigating the role of fibroblast growth factor 23 in the development of cardiac fibrosis.

Mechanism of sarcomere contraction in cardiac muscle

1. 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 spa-

tial 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. 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 the sarcomere lengthening speed to be markedly underestimated owing to the superposition of different timings for lengthening between sequentially connected sarcomeres. 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.

2. 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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