Department of Cell Physiology

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General Summary

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

Research Activities

Intracellular regulation mechanisms of the changes in L-type Ca^{2+} current induced by endothelin-1 stimulation

Endothelin-1 (ET-1) is a potent vasoconstrictive peptide. This peptide has a direct effect on cardiomyocytes which produces a positive inotropic effect through an increase in the intracellular Ca^{2+} transient. However, the intracellular mechanism of the positive inotropic effect remains unclear. Using a perforated patch-clamp and biochemical method, we found that ET-1 activated I_{Ca} through the ET_A-receptor-Gq-protein kinase (PK) C-calcium/calmodulin-dependent protein kinase (CaMK) pathway, as in the case of α_{1A}-AR signaling. We assume that ET-1 stimulation has a positive inotropic effect partly by increasing Ca^{2+} entry through L-type Ca^{2+} channels. The detailed molecular mechanism of the coupling of ET-1 stimulation and Ca^{2+} signaling will provide new insights into the functional roles of ET-1 signaling under physiological and pathophysiological conditions in cardiac muscle.

Intracellular mechanisms of the increase in Ca^{2+} leak from ryanodine receptor by β-adrenoceptor stimulation in mouse cardiac muscle

In heart failure, chronic catecholaminergic stimulation increases diastolic Ca^{2+} leakage from ryanodine receptors (RyRs) of the sarcoplasmic reticulum (SR), leading to arrhythmia and a decrease in contractility. The increased Ca^{2+} leakage from the SR by β-adrenoceptor (AR) stimulation might be due to the phosphorylation of RyRs through the activation of PKA or CaMKII or both. In the present study, we intended to identify which kinase activation is responsible for the enhanced Ca^{2+} leakage from the SR induced by β-AR stimulation using a saponin-skinned multicellular preparation. We examined the phosphorylation levels of RyR after β-AR stimulation by using commercially available antibodies against the PKA- and CaMKII-specific phosphorylation site of RyR. We found that the increase in Ca^{2+} leakage from the SR after β-AR stimulation is responsible, at least in part, for the increase in PKA-dependent RyR phosphorylation.

Pathophysiology of cardiac muscle contraction in dilated cardiomyopathy

We are investigating, in collaboration with Kyushu University, the pathophysiology of

cardiac muscle contraction in a knock-in mouse model of inherited dilated cardiomyopathy (DCM). The administration of an angiotensin II type 1 receptor blocker, candesartan, was found to improve the survival rate in DCM mice, owing, presumably, to amelioration of myocardial fibrosis and electrocardiographic abnormalities.

Molecular mechanism of length-dependent activation in cardiac muscle

Cardiac sarcomeres produce greater active force in response to stretch, forming the basis of the Frank-Starling mechanism of the heart. In the present study, we explored the molecular mechanism of length-dependent activation by using skinned porcine ventricular muscle. We found that MgADP increased the Ca^{2+} sensitivity of force and the rate of the rise of active force, consistent with the increase in thin filament cooperative activation. MgADP attenuated length-dependent activation, with and without thin-filament reconstitution with the fast skeletal troponin complex. Conversely, inorganic phosphate decreased the Ca^{2+} sensitivity of force and the rate of rise of active force, consistent with the decrease in thin filament cooperative activation. Inorganic phosphate enhanced length-dependent activation, with and without skeletal troponin reconstitution. Linear regression analysis revealed that the magnitude of length-dependent activation was inversely correlated with the rate of rise of active force. These results were quantitatively simulated by a model that incorporates the Ca^{2+}-dependent "on-off" switching of the thin filament state and interfilament lattice spacing modulation. Our model analysis revealed that the cooperativity of the thin filament "on-off" switching, but not the Ca^{2+}-binding ability, determines the magnitude of the Frank-Starling effect. These findings demonstrate that the Frank-Starling relation is strongly influenced by thin filament cooperative activation.

Role of spontaneous sarcomeric oscillations in cardiac beat

Cardiac sarcomeres exhibit spontaneous rhythmic oscillations (SPOC) under partial activation states, namely, at pCa ~6.0 (Ca-SPOC), or with the coexistence of MgADP and inorganic phosphate under relaxing conditions (ADP-SPOC). We have reported that the period of SPOC (both Ca-SPOC and ADP-SPOC) in skinned myocardium correlates with that of the resting heart rate in various animal species (2005, 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 the Ca-SPOC occurred at pCa ~6.0 with a frequency of ~3 Hz after treatment with ionomycin. As found in adult cardiomyocytes, the sarcomeric oscillations consisted of quick lengthening and slow shortening during Ca-SPOC. In untreated neonatal myocytes 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 due 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 intrinsic auto-oscillatory properties of sarcomeres may contribute to the regulation of cardiac beat *in vivo*.

Single sarcomere imaging in the heart in vivo

Numerous studies have been performed in tissues and cells to clarify the molecular mechanisms of myocardial contraction. However, because of differences between *in vitro* and *in vivo* conditions, the dynamics of myocardial sarcomere contractions in living animals is not understood. In the present study, we developed a novel system allowing for real-time imaging of single sarcomeres in the living heart. Male Wistar rats were anesthetized with pentobarbital sodium, and median sternotomy was performed under artificial ventilation. To visualize the Z-discs quantum dots (Qdots) were conjugated with anti-α-actinin antibodies and then transfected to the surface of the epicardium of the beating heart. An electron microscopic study confirmed the presence of Qdots in and around the T-tubules and Z-discs in the myocardial cells of the left ventricular wall. Consistent with this finding, a striated pattern of Qdots ($\sim$2-μm spacing) in the heart was observed with fluorescence microscopy. Furthermore, to visualize Z-discs at a higher resolution, we engineered GFP-α-actinin incorporated into adenoviral vectors. We are now performing real-time imaging of single sarcomeres by using Qdots and GFP in the beating heart of the rat.

Publications

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Reviews and Books

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