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

Intracellular regulation mechanisms of the changes in L-type Ca^{2+} current induced by α_1-adrenoceptor stimulation in the presence of a β-adrenoceptor agonist

We have previously shown that α_1-adrenoceptor (AR) stimulation alone potentiates L-type Ca^{2+} current (I_{Ca}) through α_{1A}-AR-Gq-phospholipase C (PLC)—protein kinase C (PKC)—calcium/calmodulin dependent protein kinase (CaMK) II pathway. However, the interaction of α_1- and β-AR signaling on I_{Ca} has not been fully clarified. This year, we examined the effect of α_1-AR stimulation on L-type Ca^{2+} current (I_{Ca}) when β-AR is stimulated in rat ventricular myocytes using a perforated patch-clamp technique. We found that α_{1A}-AR stimulation inhibits I_{Ca} in the presence of a β-AR agonist, which is opposite to the effect observed in the absence of a β-AR agonist. We also confirmed that tyrosine kinase, which might be activated by α_{1A}-AR-Gq signaling, inhibits β-AR signaling at the receptor site (or Gs protein). Thus, I_{Ca} is inhibited. This novel signaling pathway by α_{1A}-AR stimulation could serve as a regulatory feedback mechanism when the catecholamine concentration increases under pathophysiological conditions for protecting cardiomyocytes from Ca^{2+} overload.

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-PKC-CaMKII 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-AR 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-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 for the increase in PKA-dependent RyR phosphorylation.

Single sarcomere imaging in cardiac muscle

Skinned cardiac fibers exhibit spontaneous oscillatory contractions (SPOCs) over a broad range of intermediate activating conditions, namely, at a pCa of 6.0 to 5.0 (Ca-SPOC), or in the presence of MgADP and Pi under relaxing conditions (ADP-SPOC). We have reported that the period of sarcomeric oscillations in fibers correlates with that of the resting heartbeat in various animal species. The present study was performed to analyze SPOCs in single cardiomyocytes of the rat. To enhance the quality of sarcomere length measurement, we used quantum dots conjugated with an antibody against α-actinin to visualize the Z-line position during SPOC in a single sarcomere. We measured the period and amplitude of ADP- SPOC and Ca-SPOC at various sarcomere lengths and found that the period of sarcomeric oscillations is similar to that observed at the fiber level. We also measured sarcomere lengths in intact cardiomyocytes with quantum dots at various stimulation frequencies. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during contraction simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (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 skinned myocytes. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to myocardial beating *in vivo*.

Pathophysiology of cardiac muscle in dilated cardiomyopathy

We were supplied with a dilated cardiomyopathy model mouse by Kyushu University (knock-in of dilated cardiomyopathy troponin in mouse heart). The myocardial contractile proteins of the model mouse showed a decrease in Ca^{2+} sensitivity, which was proven by measuring the pCa-tension relation of the skinned preparations.

Pathophysiology of skeletal muscle

1. Disuse-induced changes in fatigability in skeletal muscle

We have reported that long-term hindlimb immobilization (6 weeks) lowers the expres-

sion of the giant protein titin in the soleus muscle of the rat, resulting in a decrease in active force production via abnormal sarcomeric organization. In the present study, we investigated how immobilization affects fatigability by using Triton X-100-treated single fibers taken from the same animal model. The intracellular concentrations of inorganic phosphate (P_i) and H^+ increase in skeletal muscle during intense exercise, resulting in a fall in active force. Therefore, we examined the effects of changes in pH and the inorganic phosphate (P_i) concentration on maximal Ca^{2+}-activated force in control fibers and immobilized fibers. We found that lowering the pH from 7.0 to 6.2 decreased the maximal force in both muscles, with the magnitude significantly greater in immobilized fibers. Likewise, the inhibitory effect of P_i up to 20 mM was more pronounced in immobilized fibers. These results suggest that fatigability is enhanced in immobilized muscle and that the mechanism includes a decrease in the fraction of force-generating cross-bridges coupled and the abnormal sarcomeric organization.

Publications

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Reviews and Books

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