Department of Physiology (II)

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General Summary

The main research topics of our department are the physiology of cardiac and skeletal muscle contraction and other related subjects.

Research Activities

Physiology of cardiac muscles

Alpha$_1$ adrenoceptor (α_1-AR) signaling plays crucial roles in the regulation of physiological and pathophysiological cardiac cellular responses. We next investigated the subcellular localization of α_1-AR subtypes (α_{1A} and α_{1B}) and distinct signaling pathways in native cardiac cells. We compared the functional effects of subtype-selective α_1-AR stimulation on L-type Ca^{2+} current ($I_{Ca,L}$). We provided the direct evidence that cardiac α_1-AR signaling diverges at the level of α_1-AR subtype and G-protein. The α_{1A}-AR and α_{1B}-AR signaling pathways couple with different G-proteins, $G_{q/11}$ and G_o, respectively and produce different functional outcomes (an increase and decrease of $I_{Ca,L}$).

We investigated the relations among the uptake rate, content, release (Ca^{2+}-induced Ca^{2+} release [CICR]), and the leakage of Ca^{2+} in cardiac sarcoplasmic reticulum (SR). To modulate the Ca^{2+} uptake rate of SR, we used transgenic mice hearts that over-express sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a; SERCA-TG mice) or sarcolipin (SLN-TG mice), a SR-coupled protein to suppress Ca^{2+} uptake. We employed saponin-treated thin trabeculae to directly apply various solutions to SR. The Ca^{2+} remaining in the SR after various maneuvers modulating SR functions was released by caffeine (50 mM), and the released Ca^{2+} was measured with fluo-3. The Ca^{2+} uptake rate was estimated by measuring Ca^{2+} content in the SR after Ca^{2+} was loaded into the SR in the presence of ATP (4 mM) and various concentrations of Ca^{2+} for different periods. The Ca^{2+} leakage was estimated by measuring the Ca^{2+} remaining in the SR after it was washed with a solution containing EGTA for various durations (5 to 300 seconds) after Ca^{2+} loading. The CICR was estimated by measuring the Ca^{2+} remaining in the SR after CICR was induced by the application of solutions containing various concentrations of Ca^{2+} (pCa, 7.6-4.3) without ATP after Ca^{2+} loading. With a short Ca^{2+}-loading time, the Ca^{2+} content was larger in SERCA-TG mice (113.8%) and smaller in SLN-TG mice (71.6%) than in control non-transgenic mice. At pCa 6.6, the Ca^{2+} content was significantly larger in SERCA-TG mice (199.0%) and smaller in SLN-TG mice (69.8%) than that in non-transgenic mice. The maximal Ca^{2+} content, CIC, and Ca^{2+} leakage in SERCA-TG and SLN-TG mice did not differ from those in non-transgenic mice. Thus, Ca^{2+} leakage is independent of the rate of Ca^{2+} uptake in the SR, and the altered Ca^{2+} leakage in heart failure is mainly due to the dysfunction

of ryandine receptors.
Skinned cardiac fibers exhibit spontaneous oscillatory contraction (SPOC) over a broad range of intermediate activating conditions (Ca-SPOC) or in the coexistence of ADP and Pi under relaxing conditions (ADP-SPOC). We recently reported that the period of sarcomeric oscillations during SPOC correlates with that of the heartbeat in various animal species. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres (myofibrils) may contribute to myocardial beating *in vivo*.
We investigated the possible involvement of troponin in the length effect (leftward shift of pCa-tension relation at longer muscle length) in cardiac muscle. We used skinned porcine left ventricular muscle, and cardiac troponin was replaced with fast skeletal troponin (sTn; prepared from rabbit psoas muscle). The replacement of cardiac Tn with sTn markedly attenuated length-dependent activation. We also measured the rate constant of force re-development (k_{tr}) and found that k_{tr} increased at submaximal activation upon sTn reconstitution, suggesting a reduction in the fraction of detached cross-bridges that can potentially produce active force upon attachment to actin.

Mechanism of lower Ca^{2+} sensitivity observed in immobilized skeletal muscle
Immobilization of rat hind limb causes a marked reduction in the wet weight of soleus muscle. We found that in immobilized fibers, Ca^{2+} sensitivity was reduced with a reduction in maximal Ca^{2+}-activated force. To clarify the molecular mechanism of these mechanical changes, we measured interfilament lattice spacing with the small-angle X-ray diffraction method. The interfilament lattice spacing was expanded in immobilized fibers, which could explain the lower maximal tension and the lower Ca^{2+} sensitivity of immobilized skeletal muscle fibers.

L-type Ca^{2+} channel in secondary hyperparathyroidism
We investigated the effect of the extracellular Ca^{2+} concentration of the cell-culture medium on the depolarization-induced Ca^{2+} transients (with 150 mM K^+) in parathyroid cells isolated from patients with secondary hyperparathyroidism. The peak of the Ca^{2+} transients was dependent upon the Ca^{2+} concentration in the culture medium. Thus, the expression of the voltage-dependent Ca^{2+} channel is influenced by the Ca^{2+} concentration around the parathyroid cells.

Publications

Ishikawa T, Mochizuki S, Kurihara S. Cross-bridge-dependent change in Ca^{2+} sensitivity is involved in the negative inotropic effect of nifedipine in aequorin-injected ferret ventricular muscles. *Circ J* 2006; **70:** 489–94.
Fukuda N, Udaka J, Kurihara S. Research on molecular mechanisms of active force reduction and easy fatigability in atrophied skeletal muscle with the aim of developing new rehabilitation exercises(in Japanese). *Desanto Sports Kagaku* 2006; **27**(**Suppl**)**:** 164–70.
Sasaki D[1], Fukuda N, Ishiwata S[1] ([1]Waseda Univ). Myocardial sarcomeres spontaneously oscillate with the period of heartbeat under physiological conditions. *Biochem Biophys Res Commun* 2006; **343:** 1146–52.
Kusakari Y, Hongo K, Kawai M, Konishi M, Kurihara S. Use of the Ca-shortening curve to estimate the myofilament responsiveness to Ca^{2+} in tetanized rat ventricular myocytes. *J Physiol Sci* 2006; **56:** 219–26.
Hirano S, Kusakari Y, O-Uchi J, Morimoto S, Kawai M, Hongo K, Kurihara S. Intracellular mechanism of the negative inotropic effect in-

duced by α_1-adrenoceptor stimulation in mouse myocardium. *J Physiol Sci* 2006; **56:** 297–304. ***Mizuno J[1], Otsuji M[1], Takeda K[1], Yamada Y[1], Arita H[1], Hanaoka K[1], Hirano S[1], Kusakari Y[1], Kurihara S[1] ([1]Dept Anesthesiol, Faculty Med, Univ Tokyo).*** Superiror logistic model for decay of Ca^{2+} transient and isometric relaxation force curve in rabbit and mouse papillary muscles. *Int Heart J* 2007; **48:** 215–32.