Department of Molecular Physiology

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General Summary

Our efforts have been concentrated on elucidating mechanisms for achieving biological function through the cooperative interaction of water and proteins.

Research Activities

Effect of exercise on ingested polyamine accumulation in various tissues of rats

Polyamines, such as putrescine, are polycation molecules indispensable for cell proliferation. Polyamines are also reported to modulate cardiac excitability through the gating of ion channels and to modulate cell viability through autophagy induction. We have recently found that exercise suppresses putrescine accumulation in the heart and serum of rats ingesting putrescine. Here, we examined the effects of exercise on ingested putrescine accumulation in other tissues. Six-week-old female Wistar rats were fed with 1 mg/ml drinking water for 9 weeks with or without free access to a wheel for spontaneous running. The skeletal muscle, liver, lung, spleen, and fat of these rats were then dissected for polyamine content analysis with high-performance liquid chromatography. Urine adsorbed on filter papers covering the cage bottom was also analyzed for polyamine metabolites. Exercise significantly suppressed putrescine accumulation after ingestion in other tissues tested. Although tissue levels of spermidine did not differ significantly in rats whether ingesting putrescine or exercising, we suspect that the ingested putrescine might be rapidly turned over because the spermidine, which is synthesized from putrescine in cells, increased in the urine. Because exercise might affect the metabolism of putrescine and its turnover, more comprehensive analysis of metabolites and stable-isotope tracer analysis by mass spectrometry are needed.

Functional analysis of skeletal muscle type ryanodine receptor carrying malignant hyperthermia associated mutations

In excitable cells, membrane depolarization is translated into intracellular Ca^{2+} signals, and ryanodine receptors (RYRs), located in the sarcoplasmic/endoplasmic reticulum membrane, play a key role in intracellular Ca^{2+} release. There are 3 isoforms of RYRs, of which RYR type 1 (RYR1) is dominantly expressed in skeletal muscle. Mutations of the ryanodine receptor 1 gene (*RYR1*) cause severe muscle diseases, such as malignant hyperthermia, which is a disorder of Ca^{2+}-induced Ca^{2+} release via RYR1 in skeletal muscle. Thus far, more than 300 mutations of *RYR1* have been reported in patients with malignant hyperthermia, and most mutations have been found in 3 “hotspot” regions. However, because the structure-function relationship of mutant RYR1 has not been comprehensively analysed, the mechanism remains largely unknown.

Here, we combined functional studies and molecular dynamics simulation of RYR1 bearing disease-associated mutations at the N-terminal region. When expressed in HEK293 cells, the mutant RYR1 caused abnormalities in Ca^{2+} homeostasis. Molecular dynamics simulation of the mutant RYR1 revealed that alterations of hydrogen bonds/salt bridges between N-terminal subdomains strongly correlate with the channel function of RYR1. In particular, mutations of R402, which play a key role in connecting the 3 subdomains (A, B, and C) of the N-terminal region, cause clockwise rotations of B and C subdomains with respect to the A subdomain. This movement might increase the probability the channel is open and provides the structural basis of malignant hyperthermia in R402 mutants.

Differential scanning calorimetry measurement of water components in skinned skeletal muscles

Magnetic resonance images reflect water content and water states in tissue. By taking advantage of well-organized skeletal muscle, we have recently clarified that magnetic resonance can be used to distinguish localized water clusters of 5 states. Although each water state is considered to reflect protein-water interactions mutually restricting their motional freedom, the nature of each water state has not been clarified in detail.

To obtain information about water states from different aspects, extra heat absorption of skeletal muscle fibers was measured with differential scanning calorimetry to detect the "melting" of ice; the motional freedom of fibers would be restored at a characteristic temperature absorbing extra heat.

With differential scanning calorimetry, skinned fibers of sartorius muscle from *Rana catesbeiana* at rigor condition showed extra heat absorption at −24˚C, −21˚C, 0˚C, 46˚C, and 65˚C. The peak at 46˚C and 65˚C would represent denaturation of myosin and actin filament, respectively, because selective removal of myosin or actin filaments diminished the corresponding peaks, and the temperature values are close to those reported for the denaturation of corresponding proteins from rabbit psoas.

The denaturation and selective removal of myosin and actin filaments differentially affected the peaks at −24˚C and −21˚C. The peak at −24˚C was affected mainly by the manipulation of actin filaments, and the peak at −21˚C was affected by both myosin filaments and actin filaments.

Finally, we confirmed the difference of the thermal stability of skinned fibers between rigor and relaxed states, because experiments conducted so far have been performed exclusively in the rigor state. As a result of heating for 60 minutes at 30˚C in each condition, the contractile force of the skinned fiber in the both the rigor and relaxed states was reduced to 80% before heating. These findings suggest that trends similar to those of the rigor state can be obtained in the relax state.

Publications

***Sato C*[1], *Yamazawa T, Ohtani A*[2], *Maruyama Y*[1], *Memtily N*[3], *Sato M*[1], *Hatano Y*[1], *Shiga T*[2], *Ebihara T*[1] (*[1]AIST, [2]Tsukuba Univ, [3]Xinjiang med Univ).** Primary cultured neuronal networks and type 2 diabetes model mouse fatty liver tissues in aqueous liquid observed by atmospheric SEM (ASEM): Staining preferences of metal solutions. *Micron*. 2019 Mar; **118**: 9-21.