氏名	竹倉　宏明
学位の種類	博士（体育学）
学位記番号	第1号
学位授与の要件	学位規則第4条第2項該当
学位授与年月日	平成 19 年 6 月 21 日
学位論文題目	主論文 Differential contribution of skeletal and cardiac Ⅱ-Ⅲloop sequences to the assembly of dihydropyridine-receptor arrays in skeletal muscle 副論文1 Plasticity of the transverse tubules following denervation and subsequent reinnervation in rat slow and fast muscle fibres 副論文2 Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle
論文審査委員	主査　教授　浜岡　隆文 副査　教授　齋藤　和人 副査　教授　荻田　太

論　文　概　要

（主論文）

1. **Takekura, H., Paolini, C., Franzini-Armstrong, C., Kugler, G., Grabner, M. and Flucher, B.E. (2004) Differential contribution of skeletal and cardiac II-III loop sequences to the assembly of DHP-receptor arrays in skeletal muscle. Mol. Biol. Cell, 15, 5408-5419.**

The sarcolemmal dihydropyridine receptor (DHPR) is the voltage-sensor in skeletal muscle excitation-contraction (e-c) coupling. It activates calcium release from the sarcoplasmic reticulum via protein-protein interactions with the ryanodine receptor (RyR). To enable this interaction DHPRs are arranged in arrays of tetrads opposite RyRs. In the DHPR α_{1S} subunit the cytoplasmic loop connecting repeats II and III is a major determinant of skeletal-type e-c coupling. Whether the essential II-III loop sequence (L720-L764) also determines the skeletal-specific arrangement of DHPRs was examined in dysgenic (α_{1S}-null) myotubes reconstituted with distinct α_1 subunit isoforms and II-III loop chimeras. Freeze-fracture analysis showed that DHPRs with skeletal e-c coupling properties displayed the skeletal arrangement of DHPRs in arrays of tetrads, those with cardiac e-c coupling properties failed to form tetrads. However, a chimera with the *Musca* II-III loop produced tetrads but did not reconstitute skeletal e-c coupling. Thus, the organization of DHPRs in tetrads vis-à-vis the RyR is necessary but not sufficient for skeletal-type e-c coupling.

（副論文）

2. **Takekura, H., Tamaki, H., Nishizawa, T. and Kasuga, N. (2003) Plasticity of the transverse tubules following denervation and subsequent reinnervation in rat slow and fast muscle fibres. J. Muscle Res. Cell Motility, 24, 439-451.**

We have studied the effects of short term denervation followed by reinnervation on the ultrastructure of the membrane systems and on the content of and distribution of key proteins involved in calcium regulation of fast-twitch (FT) extensor digitorum longus (EDL) and slow-twitch (ST) soleus (SOL) muscle fibres. Ischiadic nerve freezing resulted in total lack of neuromuscular transmission for 3 days followed by a slow recovery, but no decline in twitch force elicited by direct stimulation. The latter measurements indicate no significant atrophy within this time frame. The membrane systems of skeletal muscle fibres were visualized using Ca^{2+}-$K_3Fe(CN)_6$-OsO_4 techniques and observed using a high voltage electron microscope. [^{3}H]nitrendipine binding was used to detect levels of dihydropyridine receptor (DHPR) expression. The Ca^{2+} pumping free sarcoplasmic reticulum domains were not affected by the denervation, but the Ca^{2+} release domains were dramatically increased, particularly in the

FT EDL muscle fibres. The increase is evidenced by a doubling up of the areas of contacts between SR and transverse (t-) tubules, so that in place of the normal triadic arrangement, pentadic and heptadic junctions, formed by multiple interacting layers of ST and t-tubules are seen. Frequency of pentads and heptads increases and declines in parallel to the denervation and reinnervation but with a delay. Immunofluorecence and electron microscopy observations show presence of DHPR and ryanodine receptor clusters at pentads and heptads junctions. A significant (P<0.01) positive correlation between the level of [^{3}H]nitrendipine binding component and the frequency pentads and heptads was observed in both the FT-EDL and ST-SOL muscle fibres indicating that overexpression of DHPRs accompanies the build up extra junctional contacts. The results indicate that denervation reversibly affects the domains of the membrane systems involved in excitation-contraction coupling.

（副論文）

3. **Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. and Kasuga, N. (2001) Eccentric exercise induced morphological changes the membrane systems involved in E-C coupling in rat muscle. J. Physiol. (Lond.), 533, 571-583.**

Physiological evidence suggests that excitation-contraction (E-C) coupling failure results from eccentric contraction-induced muscle injury because of structural and morphological damage to membrane systems directly associated with the E-C coupling processes within skeletal muscle fibres. In this study using rats, we observed the ultrastructural features of the membrane systems of fast-twitch (FT) and slow-twitch (ST) muscle fibres involved in E–C coupling following level and downhill running exercise. We set out to find out whether mechanically mediated events following eccentric exercise caused disorder in the membrane systems involved in E–C coupling, and how soon after exercise such disorders occurred. We also compared the morphological changes of the membrane systems between ST and FT muscle fibres within the same muscles. Single muscle fibres were dissected from triceps brachii muscles of male Fischer 344 rats after level or downhill (16^o decline) motor-driven treadmill running (18 m/min., 5 min. running with 2 min. rest interval, 18 bouts). All single-muscle fibres were histochemically classified into ST or FT fibres. The membrane systems were visualized using Ca^{2+}-$K_3Fe(CN)_6$-OsO_4 techniques, and observed by high voltage electron microscopy (120 - 200 KV). There were four obvious ultrastructural changes in the arrangement of the transverse (t) tubules and the disposition of triads after the downhill running exercise: (1) an increase in the numbers of longitudinal segments of the t-tubule network, (2) changes in the direction and disposition of triads, (3) the appearance of caveolar clusters, and (4) the appearance of pentads and heptads (close appositions of two or three t-tubule elements with three or four elements of terminal cisternae of the sarcoplasmic reticulum). The caveolar clusters appeared almost exclusively in the ST fibres immediately after downhill running exercise and again sixteen hours later. The pentads and heptads appeared almost exclusively in the FT fibres, and their numbers increased dramatically two to three days after the downhill running exercise. The eccentric exercise led to the formation of abnormal membrane systems involved in E-C coupling processes. These systems have unique morphological features, which differ between ST and FT fibres, even within the same skeletal muscle, and the damage appears to be concentrated in the FT fibres. These observations also support the idea that eccentric-exercise- induced E-C coupling failure is due to physical and chemical disruptions of the membrane systems involved in the E-C coupling process in skeletal muscle.

論 文 審 査 の 要 旨

骨格筋細胞収縮のメカニズムを明らかにするために、興奮収縮連関の構造様式と機能性特性の相互関係を検討する研究を行った。また、運動、不活動、発育・加齢などが骨格筋細胞の興奮収縮連関に及ぼす影響についても、筋細胞内膜系やCa^{2+}チャンネルの形態変化と機能的変化を指標として分子・細胞生物学的手法を用いて検討した。主論文は、興奮収縮連関機能を直接制御する２種類のCa^{2+}チャンネルの形態的特徴が形成されるメカニズムを分子生物学的手法を用いて明らかにし、副論文は、除神経に伴う不活動と下り走に伴う伸張性収縮が筋細胞内膜系とCa^{2+}チャンネルの構造様式に及ぼす影響を明らかにした。以上、これまでにない新知見が得られており、博士論文としてふさわしいと認められた。