Enhancing Effect of Hypergravity on Development of Cardiac and Skeletal Muscles of Chick Embryos

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Abstract Fertilized eggs of White Leghorn chickens were incubated for 11 days at 37°C in normal condition and then for 7 days under hypergravity (2G) induced by centrifugation. Then the morphologies of muscles isolated from their heart ventricle and femur were examined. The cardiac and skeletal muscle cells of the 2G-group were more densely packed and regularly organized than those of the control group. Collagen and lattice fibers were also more abundant in stroma of the 2G-group. Electron microscopical studies showed that after hypergravity treatment, muscle cells contained a larger number of myofibrils forming larger bundles with clear electron dense Z-bands in the cytoplasm. These results show that hypergravity enhances the development of muscle cells.

Introduction

Alteration of gravity causes many functional and metabolic changes in muscle. Morphological studies have shown that orbital flight for 7 days causes muscle atrophy (Il'ina-Kakueva, 1987). Head-down bed rest causes myofibrillar disorganization and decreases the size of human skeletal muscle fibers (Hikida, et al, 1989). A human model of unilateral lower limb unloading, which simulates microgravity, was also found to reduce the mass and strength of muscle (Berg, et al, 1991). All these findings indicate that microgravity greatly affects the metabolism of muscle. Little attention has, however, been paid to the effect of hypergravity on muscles. In addition, the effect of gravity on the development of embryonic muscle has not been examined. We have reported that the protein content of muscle tissue from young cockerels is

increased by hypergravity (Takahashi, et al, 1989). For further analysis of this enhancing effect and for observing the changes of embryonic muscles, in this study we examined the morphologies of cardiac and skeletal muscles of chick embryos exposed to hypergravity (2G).

Materials and Methods

Material

Forty fertilized eggs of White Leghorn chickens were incubated for 11 days at 37°C and then put into an incubator box controlled at 37°C and centrifuged for 7 days at 2G in a machine designed in this laboratory (Takahashi, et al., 1989a,b). The hypergravity was applied from the rounded to the pointed end of the eggs. All embryos survived and 5 of them were used for this study. Control eggs were incubated in the same type of incubator at 37°C without centrifugation. After centrifugation the radiographs of the eggs were taken with a computed radiography to estimate the direction of hypergravity applied to the skeletal muscles. Radiographs showed that the hypergravity was applied from the proximal to the distal end of the femur of the

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embryos, which coincides with the direction of gravity loaded on the skeletal muscle of chickens when they stand on the ground.

Methods

Light Microscopic Examination

Muscles isolated from the heart ventricle and femur, respectively, were fixed in 10% neutral formalin and embedded in paraffin. Serial sections of 5 μ m thickness were prepared and stained with hematoxylin-eosin, and Masson

trichrome and by Watanabe's silver impregnation.

Electron Microscopic Examination

Immediately after their isolations, cardiac and skeletal muscles were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer of pH 7.4, and post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer of pH 7.4. The muscles were then dehydrated and embedded in Epon. Ultrathin sections stained with uranyl acetate and lead citrate were observed with a transmission electron microscope (JEOL, JEM 1200 EX).

Fig. 1 Effects of hypergravity on cardiac and skeletal muscle cells. Serial sections from control (A) and 2G-treated (B) cardiac muscle were stained by Watanabe's silver impregnation (magnification, $\times 300$). Muscle cells grown under hypergravity (2G) are densely packed and arranged in parallel with many lattice fibers. Serial sections from control (C) and 2G-treated (D) skeletal muscles were stained with Masson trichrome (magnification, $\times 150$). Increases in fibrils of large diameter in the parenchyma and collagen fibers in the stroma were observed in 2G-treated muscle.

Results and Discussion

Two morphological differences were observed between the cardiac muscles of the control group and 2G group. One was that the muscle cells were more densely and regularly organized in the 2G-group (Fig 1A, B). Electron microscopy also showed larger numbers of regularly arranged myofibrils, forming large bundles with clear electron-dense Z bands in the cytoplasm of 2G-treated cells (Fig. 2A, B). The second difference was that lattice fibers were more abundant in the stroma of 2G-treated muscle (Fig. 1A, B). The regular organization of 2G-treated muscle cells might have resulted from dislocation of the cells caused by cen-

trifugation. But this was not the case because centrifugation enhanced myofibril formation (Fig. 2A, B), indicating that hypergravity actually affected the function of embryonic muscle cells. Cardiac muscle cells were affected by hypergravity, possibly because in conditions of hypergravity, stronger muscular activity was necessary for circulation of blood throughout the body. Hypergravity had essentially the same effects on skeletal and cardiac muscle; namely it resulted in denser fibrils of larger diameter in the parenchyma and increase in collagen fibers in the stroma (Fig. 1C, D). The above morphological changes all indicate that hypergravity enhanced the development of cardiac and skeletal muscles in chick embryos.

Fig. 2 Effect of hypergravity on the electron microscopic appearance of cardiac muscle cells. Ultrathin sections from control (A) and 2G-treated cells (B) were stained with uranyl acetate and lead citrate and observed by transmission electron microscopy. Bars in A and B indicate 1 μ m (magnification, $\times 15,000$).

Hypergravity generated by centrifugation enhances the proliferation of mammalian cells (Tschopp & Cogoli, 1983) and cultured osteoblast-like cells (Miwa, et al, 1991). Kumei et al. demonstrated that the proliferation of HeLa cells induced by hypergravity was mainly due to reduction of the G1 phase (Kumei, et al., 1989). Mack et al. (Mack, et al, 1967) reported that the bone mineral of astronauts decreased during orbital flight, indicating that alteration of gravity affects bone metabolism. We found previously that hypergravity stimulates the growth of cockerels (Takahashi, et al., 1989b) and enhances their bone strength, as estimated by the force and energy necessary to break their bones (Takahashi, et al., 1989a). Thus, gravitational alteration brings about many biological changes in cells and tissues. It is noteworthy that the control of bone and muscle tissues by gravity are parallel, because the balance of bone and muscle tissues is important for maintaining skeletal tissue. We found previously that the collagen content in the skin of tree frogs was decreased after orbit in the Russian space station MIR (Yamazaki, et al., 1991), indicating that microgravity disturbs collagen synthesis. Conversely, the stimulation of myofibril formation in 2G-treated cells suggests that hypergravity stimulates collagen synthesis in muscle cells. To confirm the enhancing effects of hypergravity on muscle tissue, we are now examining its effects on muscle components such as collagen.

References

- Berg, H. E., Dudley, G. A., Haggmark, T., Ohsen, H., and Tesch, P. A. 1991. *J. Appl. Physiol.* 70: 1882-1885.
- Hikida, R.S., Gollnick, P.D., Dudley, G.A., Convertino, V.A., and Buchanan, P. 1989. *Aviat. Space Environ. Med.* 60: 664-670.
- Il'ina-Kakueva, E. I. 1987. *Kosm. Biol. Aviakosm. Med.* 21: 31-35.
- Kumei, Y., Nakajima, T., Sato, A., Kamata, N., and Enomoto, S. 1989. *J. Cell Sci.* : 93, 221-226.
- Mack, P. B., Lachance, P. A., Vose, G. P., and Vogt, F. B. 1967. *Am. J. Roent.* : 100, 503-511
- Miwa, M., Kozawa, O., Tokuda, H., Kawakubo, A., Yoneda, M., Oiso, Y., and Takatsuki, K. 1991. *Bone Mineral*: 14, 15-25.
- Takahashi, S., Shioya, M., Ito, H., Taira, T., Yamazaki, J., Ogawa, K., Ezawa, I., Kita, F., and Sudoh, M. 1989a. *Jpn. J. Aerospace Environ. Med.* 27: 29-34
- Takahashi, S., Shioya, M., Ito, H., Taira, T., Yamazaki, J., Ogawa, K., Ezawa, I., Kita, F., and Sudoh, M. 1989b. *Jpn. J. Aerospace Environ. Med.* 26: 41-46.
- Tschopp, A., and Cogoli, A., 1983. *Experientia.* 39: 1323-1329.
- Yamazaki, H., Kita, F., Ikuma, K., Ohnaka, H., Koike, K., Takahashi, S., Shiraishi, A., and Ohashi, S. 1991. *Biol. Sci. Space.* 5: 202-207.

和文抄録

鶏胚の筋肉の発達に対する重力の影響について調べた。

孵卵 11 日の鶏胚を、遠心により重力 (2G) を負荷しながら、さらに、7 日間、37℃で孵卵を続け、その後、筋肉を採取、組織学的検索を行った。

顕微鏡的に見ると、心筋、骨格筋とも、重力負荷群は細胞の配列が規則正しく、整然としており、また、細胞密度も対照群より高かった。細胞間質には、コラーゲン繊維、格子繊維が対照群より豊富に存在した。顕微鏡的に見ると、重力負荷群では、筋原繊維が多く見られ、また、電子密度の高い Z 帯の形状も明瞭であった。

これらの事実は、重力負荷が、鶏胚の筋肉の発達を増強していることを示すものである。

キーワード：重力、筋肉、コラーゲン繊維、格子繊維、筋原繊維