Ultrastructure of Cardiac Myocytes in the Greater Bandicoot Rat (*Bandicota Indica*)

Hideki ENDO, Seishi MAEDA¹⁾, Junpei KIMURA²⁾, Junzo YAMADA³⁾, Worawut RERKAMNUAYCHOKE⁴⁾, Masamichi KUROHMARU¹⁾, Yoshihiro HAYASHI¹⁾, and Takao NISHIDA⁵⁾

Department of Zoology, National Science Museum, Shinjuku-ku, Tokyo 169, ¹⁾Department of Veterinary Anatomy, The University of Tokyo, Bunkyo-ku, Tokyo 113, ²⁾Department of Veterinary Anatomy, Nihon University, Fujisawa, Kanagawa 252, ³⁾Department of Veterinary Anatomy, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080, Japan, ⁴⁾Department of Veterinary Anatomy, Kasetsart University, Bangkok 10900, Thailand, and ⁵⁾Laboratory of Anatomy and Physiology, Nihon University, Fujisawa, Kanagawa 252, Japan

(Received 25 November 1994/Accepted 11 January 1995)

ABSTRACT. Cardiac myocytes in the left ventricle and atrium of the greater bandicoot rat (*Bandicota indica*) were examined by transmission electron microscopy. The fine structure showed typical features of mammalian cardiac myocytes. In atrial myocytes, however, the areas occupied by mitochondria were much smaller than that filled with myofibrils. The decrease in mitochondria and the abundance of myofibrils are thought to be an ultrastructural adaptation to the large body size of this species. Many conducting myocytes were observed in both atrium and ventricle. The atrial conducting myocytes were ultrastructurally different from the Purkinje fibers of the ventricle. We suggest that the abundance and the ultrastructural variation of conducting myocytes are related to the physiological aspects, such as body size, of this animal.—**KEY WORDS:** bandicoot rat, cardiac myocyte, conducting system, ultrastructure.

J. Vet. Med. Sci. 57(3): 389–393, 1995

Body weight of the greater bandicoot rat ranges from 500 g to 1,500 g in the adult [17]. This animal is much larger than ordinary myomorph rodents in the Old World. Thus, examination of this species effectively reveals morphological adaptations of various organs to large body size in myomorph rodents. In some mammals such as dog, rat, mouse, hamster and fruit bat, it has been reported that ventricular myocytes change in volume density of myofibrils and mitochondria according to body size [10, 11, 15]; however, ultrastructural changes in myofibrils and mitochondria have not been examined in the greater bandicoot rat. Moreover, the degree in development of conducting myocytes is expected to change morphologically in the greater bandicoot rat because of its large body size, but the conducting system of this species has not been examined ultrastructurally. In this study, therefore, we observed the general and conducting myocytes by electron microscopy and discussed adaptational changes from fine structural data in the greater bandicoot rat.

MATERIALS AND METHODS

Three greater bandicoot rats, weighing from 550 g to 660 g, were captured in Thailand. The animals were sacrificed under deep anesthesia with sodium pentobarbital. The perfusion was carried out with physiological saline and followed by 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) through the abdominal artery. Then, fixed specimens were dissected from the left atrium and ventricle. They were cut into small pieces and immersed in the same fixative for 2 hr at 4°C. After washing with phosphate buffer, they were postfixed in 1% osmium tetroxide for 90 min at 4°C. After dehydration in ethanol and infiltration in propylene oxide, they were embedded in Epon 812. Thick sections were cut at 1 µm and stained

with toluidine blue for light microscopy. Ultrathin sections were prepared with an LKB 4801A Ultratome. They were stained with uranyl acetate and lead citrate and examined with a JEM-1200 EX transmission electron microscope at 80 KV.

RESULTS

Both atrial and ventricular myocytes of the greater bandicoot rat revealed an ultrastructure typical of mammalian cardiac myocytes (Figs. 1–3). Well-developed myofibrils occupied the cytoplasm to a greater degree. Mitochondria with closely-arranged cristae were present among myofibrils. Couplings of the T-tubule system and sarcoplasmic reticulum occurred in Z band regions (Fig. 4). The area occupied by mitochondria was much smaller than that filled with myofibrils in the atrial myocytes (Fig. 1), although these features were not found in the ventricular myocytes. The area of myofibrils was enlarged in the cytoplasm of atrial myocytes. Many glycogen particles were dispersed in the cytoplasm of atrial tissues (Fig. 1). Intercalated discs developed to a degree. Atrial granules (AGs) were detected in the perinuclear and marginal regions of atrial myocytes (Figs. 4, 5) but were not discerned in ventricular myocytes. A few lipofuscin granules were found in the cytoplasm (Figs. 3, 4).

Many Purkinje fibers were seen in the ventricular wall, while a large number of conducting myocytes with many glycogen particles were present in the atrium. Irregularly-oriented myofibrils were scattered in the cytoplasm of these conducting myocytes (Figs. 6–8). Mitochondria were smaller in conducting myocytes of the atrium than in those of general myocytes (Fig. 7). The area without myofibrils was enlarged in the cytoplasm where glycogen particles were densely distributed in atrial conducting myocytes

Fig. 1. Cardiac myocytes of left atrium showing myofibrils (MF) and mitochondria (MT). The area occupied by mitochondria is small. $\times 7,000$.

Fig. 2. Cardiac myocytes of left ventricle. Myofibrils and mitochondria develop in the cytoplasm. Arrows indicate intercalated discs. $\times 7,500$.

Fig. 3. Perinuclear region of left ventricular myocyte. A lipofuscin granule can be seen (arrow). Capillary (C) and nerve endings (arrowhead) are present in the interstitial space. $\times 6,000$.

Fig. 4. Cardiac myocyte of left atrium. Longitudinal sections of T-tubules and sarcoplasmic reticulum are demonstrated in Z bands (small arrows). Atrial granules are seen in the marginal region (large arrows). A few lipofuscin granules are found (arrowhead). $\times 12,000$.

Fig. 5. Cardiac myocyte of left atrium. Many atrial granules are distributed in the perinuclear region (arrows). N, nucleus. $\times 6,000$.

Fig. 6. Conducting myocyte of left atrium (asterisk). Irregularly oriented myofibrils are dispersed in the cytoplasm where a few mitochondria are seen. Conducting cell attaches to general cardiac myocytes with junctional complex (arrows). $\times 9,500$.

Fig. 7. Conducting myocyte of left atrium. Small mitochondria are distributed in the cytoplasm (arrows) where myofibrils are dispersed. Many glycogen particles are seen in the cytoplasm. $\times 8,000$.

Fig. 8. Purkinje fiber of left ventricle. Irregularly oriented myofibrils are dispersed (arrows). The clear cytoplasm is enlarged. $\times 5,000$.

Fig. 9. Connection between a conducting cell and a general cardiac myocyte in left atrium. Intermediate junctions (small arrow), desmosome (large arrow) and gap junction (arrowhead) are well-developed. $\times 14,000$.

Fig. 10. Varicosity portion of nerves in the left atrial tissue. Cholinergic nerve endings with small non-cored vesicles are seen. Endothelial cell of capillary (C) is observed. N, nucleus of Schwann cell. $\times 13,000$.

(Fig. 7), and a clear area was seen in Purkinje fibers of ventricle (Fig. 8). Intercalated discs, including an intermediate junction, desmosome and gap junction were well-developed between conducting cells and general myocytes (Fig. 9).

Capillaries were distributed in the interstitial space (Figs. 3, 10). Many varicosity portions of nerve endings with small non-cored vesicles were encountered in the atrial tissues. Nerve endings were adjacent to myocytes at a distance of 100 nm (Fig. 10). Figure 10 shows that the Schwann cell coexists with the varicosity portion of nerves.

DISCUSSION

Ultrastructurally cardiac myocytes in the greater bandicoot rat contained typical components found in these of other mammals. The greater bandicoot rat examined in the present study was characterized by that the area occupied by myofibrils in left atrial myocytes was conspicuously large, whereas mitochondria occupied only a small area. The volume density of these two organelles in ventricular myocytes has been reported to have a strong relationship with the body size in other mammals [10, 11, 15]. So, it is suggested that these features may be explained as an adaptation to the large body size of this animal. It will be necessary to compare the volume density of organelles in atrial myocytes between bandicoot rat and smaller rodents as Norwegian rat. While, the characteris-

tic increase/decrease of these two organelles was not found in ventricular myocytes of the greater bandicoot rat in this observation. However, we suggest that the quantitative data of volume density in this species may be different from that in smaller rodents because of the functional adaptation to the large body size. So, a morphometric study will be needed to ascertain the ultrastructural adaptation of ventricular myocytes in this animal and various mammals with different body size. It also must be noted that the area occupied by mitochondria increased in the myocyte cytoplasm of aged animals [14]. Because a few lipofuscin granules were distributed in the cytoplasm, aging influences must be taken into account for the increase of mitochondria area. Ages were unknown for the three animals used in this study.

The abundance of conducting myocytes in the left atrium and ventricle was the most noteworthy. We suggest that the conducting tissue may be developed because of the physiological needs of large body. Morphological differences between general myocytes and these conducting myocytes were obvious. The Purkinje fibers in the left ventricle had an enlarged clear cytoplasm among irregularly arranged myofibrils and mitochondria, while the conducting myocytes in left atrium possessed a large number of glycogen particles among myofibrils. The Purkinje fibers with large clear cytoplasm in the left ventricle have been described in various mammals [1-4, 6-9], and conducting cells with many glycogen particles in the left atrium are consistent with those of the dark cells

described by Hayashi [4]. The latter cells may be the transitional type from general myocytes to typical conducting cells [4]. The occurrence of conducting myocytes similar to the dark cells has not been reported in the atrial wall of other mammals. Such an ultrastructural variation of conducting myocytes may be interpreted as an adaptation to the large body size and lower heart rate of this species.

Comparisons between different sized animals should be undertaken among taxonomically close species to avoid the misunderstanding of functional convergence. However, it is difficult to compare various species of Asian myomorphs, because most of animals have a small body size. Comparison of the greater bandicoot rat with the other small species of myomorphs should resolve the problem of functional adaptation.

Nerve endings appeared adjacent to the myocytes and capillary endothelium. Ultrastructural examinations of enzymatic activity demonstrated the presence of nervous control at a neuromuscular distance of 200 to 400 nm in general cardiac myocytes [5] and of 110 to 170 nm in smooth muscle cells [12, 13]. A distance of 100 nm between varicosity portion of nerves and myocytes suggests nervous control in cardiac myocytes, but the junctions at a distance of 20 nm in the heart as described by Thaemert [16] were not discerned in this study. Because many small non-cored vesicles were observed in the varicosity portion of nerves, the nerves with varicosity are suggested to be cholinergic [10]. Navaratnam [10] classified nerve varicosities into three types and pointed out that cholinergic nerve endings are most frequent in the atrium of rats. The present study also demonstrated many cholinergic nerve endings in atrial tissues of the greater bandicoot rat.

Many AGs were detected in atrial myocytes. It becomes obvious that the left atrial myocytes of this species also synthesize, store and secrete the atrial natriuretic polypeptide (ANP) as those of other mammals.

ACKNOWLEDGEMENTS. We wish to thank Drs. Somchai Pongjunyakul, Narong Chungsamarnyart and Vanda Sujarit of the Faculty of Veterinary Medicine in Kasetsart University for their support and encouragement throughout this work. We are grateful to Mr. Iwao Tsugiyama of the Department of Veterinary Anatomy, The University of Tokyo for his valuable assistance with histology.

REFERENCES

1. Ayettey, A. S. and Navaratnam, V. 1978. The T-tubule system in the specialized and general myocardium of the rat. *J. Anat.* 127: 125-140.
2. Cheng, Y. 1971. The ultrastructure of the rat sino-atrial node. *Acta Anat. Nippon* 46: 339-358.
3. Colborn, G. L. and Carsey, E. 1972. Electron microscopy of the sinoatrial node of the squirrel monkey *Saimiri sciureus*. *J. Molec. Cellul. Cardiol.* 4: 525-536.
4. Hayashi, S. 1971. Electron microscopy of the heart conduction system of the dog. *Arch. Histol. Jpn.* 33: 67-86.
5. Hirano, H. and Ogawa, K. 1967. Ultrastructural localization of cholinesterase activity in nerve endings in the guinea pig heart. *J. Electron Microsc.* 16: 313-321.
6. James, T. N., Sherf, L., Fine, G., and Morales, A. R. 1966. Comparative ultrastructure of the sinus node in man and dog. *Circulation* 34: 139-163.
7. Kim, S. and Baba, N. 1971. Atrioventricular node and Purkinje fibers of the guinea pig heart. *Am. J. Anat.* 132: 339-354.
8. Melax, H. and Leeson, T. S. 1970. Fine structure of the impulse-conducting system in rat heart. *Can. J. Zool.* 48: 837-839.
9. Mochet, M., Moravec, J., Guillemot, H., and Hatt, P. Y. 1975. The ultrastructure of rat conducting tissue; an electron microscopic study of the atrioventricular node and the bundle of His. *J. Molec. Cellul. Cardiol.* 7: 879-889.
10. Navaratnam, V. 1987. *Heart Muscle: Ultrastructural Studies*. Cambridge Univ. Press, New York.
11. Navaratnam, V., Ayettey, A. S., Addae, F., Kesse, K., and Skepper, J. N. 1986. Ultrastructure of the ventricular myocardium of the bat. *Eidolon helvum. Acta Anat.* 126: 240-243.
12. Ohsawa, K., Nishida, T., Kurohmaru, M., and Hayashi, Y. 1990. Distribution of acetylcholinesterase in the nerve endings of chicken *m. retractor phalli cranialis*. *J. Anat.* 170: 63-71.
13. Robinson, P. M. 1969. A cholinergic component in the innervation of the longitudinal smooth muscle of the guinea pig vas deferens. The fine structural localization of acetylcholinesterase. *J. Cell Biol.* 41: 462-476.
14. Sachs, H. G., Colgan, J. A., and Lazarus, M. L. 1977. Ultrastructure of the ageing myocardium: A morphometric approach. *Am. J. Anat.* 150: 63-72.
15. Schaper, J., Meiser, E., and Stämmler, G. 1985. Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice, and from human hearts. *Circ. Res.* 56: 377-391.
16. Thaemert, J. C. 1969. Fine structure of neuromuscular relationships in mouse heart. *Anat. Rec.* 163: 575-585.
17. Walker, E. P. 1991. *Walker's Mammals of the World*, 5th ed. (Nowak, R. M. ed.), Johns Hopkins Univ. Press, Baltimore and London.