An Electron Microscopic Study on Adenosinetriphosphatase (ATPase) Activity in the Heart Muscle

SEITOKU MIZUNO, SHIGERU TAKAMATSU, YUICHI TAMADA*, KOHEI ARAYA,
AKIHIKO TAKAHASHI, AND RYOHEI SATO**

Adenosinetriphosphatase (ATPase) activity of the rat heart muscle was studied electron-microscopically. The heart muscle was prefixed in buffered aldehydes, blendorized, incubated in the Wachstein-Meisel ATPase medium, postfixed in osmium tetroxide, dehydrated in graded ethanol solutions, embedded in Epon, cut into thin sections, electron stained and observed on the electron microscope.

ATPase activity was found in the sarcolemma, the myofibril, the mitochondria, the sarcoplasmic reticulum, the nucleus of the muscle cell, the endothel cell of the blood capillary and the blood cell in the capillary. Activity of the myofibril was demonstrated in the A band, showing periodicity suggesting a relationship between the site of activity and the cross bridge.

IN RECENT years the combined technique of the histochemistry and the electron microscopy has resulted in a successful demonstration of end products of some types of enzymatic activities including esterases,¹⁻³ oxydative enzymes^{1,2,4} and phosphatases⁵⁻¹⁷ at a fine structural level. In cases of phosphatases enzymatic activities release phosphate ions from substrates. If lead ions are present as capture reagents in the incubation medium, released phosphate ions are combined lead ions to lead phosphate, which is water insoluble and electron dense, precipitates at the site of enzymatic activity.

The source of the energy of the contraction in the heart muscle is enzymatic hydrolysis of adenosinetriphosphate (ATP) to adenosinediphosphate and phosphate ion. Presence of the adenosinetriphosphatase (ATPase) in the heart muscle has been demonstrated chemically already. We tried to demonstrate ATPase activity in the heart muscle morphologically at the electron microscopic level.

EXPERIMENTALS

A rat heart was excised under ether anesthesia

and immersed immediately in ice cold 6% glutaraldehyde or 4% formaldehyde buffered to pH 7.2 by 0.05 M cacodylate containing 0.4 M sucrose. Then the heart was trimmed, the left ventricular muscle was cut into approximate 5 mm³ blocks. These blocks of heart muscle were prefixed in the above mentioned ice cold glutaraldehyde solutions for 1 hour or in the ice cold formaldehyde solutions for 4 hours. Then blocks were washed several times in the buffer with sucrose and stored overnight in the buffer at 4°C. After overnight storage heart muscle blocks were blendorized for 10 minutes and sedimented in the buffer with sucrose. All these preparations were carried out at ice cold. The sediment of myofibril pellets was suspended in a 5 ml volume of fresh isolation medium containing 0.04 M sodium borate, 0.025 M KCl and 0.4 M sucrose adjusted pH to 7.1 with dilute HCl. A few drops of this thick myofibril suspension were added to the WACHSTEIN-MEISEL ATPase medium.¹⁶ The WACHSTEIN-MEISEL ATPase medium consisted of 8.3×10^{-4} M ATP (disodium salt), 0.04 M Tris-maleate buffer (pH 7.2), 3.6×10^{-3} M Pb(NO₃)₂ and 0.01 M MgSO₄, these were added together in the above order, stirred for

(Received for Publication, June 24, 1968)

* Institute of Cerebrovascular Diseases, Hirosaki University School of Medicine, Hirosaki City, Japan

** Department of Medicine, Hirosaki University School of Medicine, Hirosaki City, Japan

several minutes at room temperature, then filtered. Sucrose was not added. Myofibrils added to the WACHSTEIN-MEISEL ATPase medium were incubated from 30 minutes to 60 minutes at 37°C. After the incubation, myofibrils were sedimented and postfixed in 1% osmium tetroxide adjusted pH to 7.2 with veronal acetate buffer at ice cold for 1 hour. Fixed myofibrils were sedimented and washed in the buffer, then dehydrated in graded ethanol solutions. Dehydrated specimens were embedded in Epon as usual.

Control specimens were prepared by following each of the procedures as outlined above with the exception that ATP was omitted from the incubation medium.

Specimens embedded in Epon were cut into ultrathin sections with glass knives on the Porter-Blum MT-2 ultramicrotome. Ultrathin sections were mounted on collodion filmed copper grids. The electron staining was carried out by floating grids on the saturated uranylacetate solution for 1 hour and then on the lead tartrate stain for 10 minutes. Sections were examined on the Hitachi HU-11A electron microscope.

ATPase activity was shown as electron dense fine granules of lead phosphate.

RESULTS

In control specimens, final deposits of ATPase activity were not seen. Non-specific contaminations from stains were occasionally seen, but they distinctly differed in their shapes and locations from final deposits of ATPase activity.

In specimens prefixed in buffered glutaraldehyde, ATPase activity was found in the sarcolemma, in the triad of the sarcoplasmic reticulum, in the myofibril, in the nucleus of the muscle cell. The intercellular junction also showed positive activity. Final deposits of ATPase activity were found inside the plasma membrane at the cellular junction. The tight junction type showed the most intense activity. The cytoplasm and the nucleus of the endothel cell of the blood capillary, the surface of the erythrocyte, the nucleus and the cytoplasm of the leucocyte which were found occasionally in the blood capillary also showed ATPase activity (Figs. 1, 2, and 3). No activity was found in the mitochondria of the muscle cell in the glutaraldehyde prefixed specimen. In myofibrils, the A band showed definite ATPase activity, although, the Z line and the I line no activity (Fig. 4). The location of activity in the myofibril was identical with the location of the thick myofilament (myosin filament). In the high-

er magnification, the myosinfilament itself was not reactive as well as the thin filament (actin filament) (Fig. 5). Final deposits were arranged along the myosinfilament and showed some periodicity. They were arranged with the fixed distance around 330 Å on lines which were parallel to the Z line, perpendicular of the direction of the myofilament (Fig. 4).

In specimens prefixed in buffered formaldehyde, ATPase activity was found in the sarcolemma, in the triad of the sarcoplasmic reticulum, in the myofibril, in the mitochondria, in the nucleus of the muscle cell, in the cytoplasm and the nucleus of the blood capillary endothel. Red and white blood cells also showed activity as in the glutaraldehyde prefixed specimen. The most distinct finding in this specimen was positive activity in the mitochondria which showed no activity in the glutaraldehyde prefixed specimen. Sometimes intense activity was found in some mitochondriae, although less or no activity was found in other mitochondriae in one field. Deposits showing activity were demonstrated both on the surface of the mitochondria and on the crista of the mitochondria, chiefly on the crista.

DISCUSSION

In histochemical studies of enzymatic activity, the preservation of activity is the most important. In the light microscopic work, the use of the frozen sections yield the satisfactory information. Although, in the electron microscopy, the serious ultrastructural deterioration is seen in frozen sections. The usual aldehyde fixation inhibits enzymatic activity almost entirely.

TICE and BARNETT⁵ and SABATINI, MILLER and BARNETT² showed that the brief prefixation in some aldehydes and the post-fixation in osmium tetroxide fairly retained enzymatic activity. They also showed that intense ATPase activity were observed after the formaldehyde prefixation and that moderate ATPase activity were retained after the glutaraldehyde prefixation. PADYKULA and GAUTHIER¹⁷ reported sensitivity of the myosin ATPase to formaldehyde. Author's results showed that the prefixation in buffered formaldehyde retained myosin and mitochondrial ATPase activity, but the preservation of the ultrastructure was a little poor, and that the brief prefixation in buffered glutaraldehyde allowed the demonstration of myosin ATPase activity satisfactory and gave the good preservation of the fine structure. Both prefixation should be tried for the observation of a relation between enzy-

matic activity and the ultrastructure.

TICE and BARNETT⁵ demonstrated activity of the myofibrillar ATPase at a fine structural level. They found ATPase activity in the A band, but no activity in the H, I, and Z line, and deposits of the final product frequently exhibited an axial periodicity and bore a close relationship to filaments within the A band. We also found ATPase activity in the A band, which corresponds to the localization of the myosin filament. Although, the myosin filament itself showed no activity. Final products of ATP activity were arranged on parallel lines to the Z line, and these lines showed some periodicity. TICE and BARNETT⁵ reported the periodicity near 365 Å. These findings suggest the close relationship between the site of myosin ATPase activity and cross bridges reported by HUXLEY¹⁸ which are present between thick filaments and thin filaments. It is reported that the heavy meromyosin in thick filaments have a function of the ATPase. Positive ATPase activity of the myosin filament itself including processes of heavy meromyosin heads seems probable. Although, it is difficult to confirm morphologically from our finding, because a large amount of activity is inhibited by fixation, and the histochemical procedure does not reveal all enzymatic activity.

ROSENTHAL, MOSES, BEAVER and SCHUFFMAN¹³ reported that the transport related sodium plus potassium dependent ATPase of the plasma membrane was completely inhibited by fixation even for brief period at 0°C, and deposits found on the plasmamembrane were not final products of ATPase activity but products of the nonezymatic hydrolysis of adenosinetriphosphate. They also reported that this nonezymatic hydrolysis could be prevented by increasing ATP or reducing lead nitrate in the incubation medium. We failed to demonstrate sodium and potassium dependent membrane ATPase activity in the fixed tissue by replacing magnesium sulfate with sodium chloride and potassium chloride in the incubation medium. We also tried to reduce the content of lead nitrate to half of the original. Deposits at the cell membrane decreased a little, but prevention was not complete.

SUMMARY

ATPase activity of the heart muscle was dem-

onstrated histochemically on the electron microscope.

In the glutaraldehyde prefixed specimen, ATPase activity was demonstrated in the sarcolemma, the myofibril, the sarcoplasmic reticulum, the endothel cell and blood cell. In the formaldehyde prefixed specimen, ATPase activity in the mitochondria was also demonstrated. In the myofibril, ATPase activity was found on the A band.

The effect of the fixation on ATPase activity and a relationship between the location of activity and the ultrastructure were discussed.

REFERENCES

1. SABATINI, D. D., BENSCH, K., & BARNETT, R. J.: *J. Cell Biol.* 17: 19, 1963.
2. SABATINI, D. D., MILLER, F., & BARNETT, R. J.: *J. Histochem. Cytochem.* 12: 57, 1964.
3. VATTER, A. E., REISS, O. K., NEWMAN, J. K., LINDQUIST, K., & GROENBOER, E.: *J. Cell Biol.* 38: 80, 1968.
4. SELIGMAN, A. M., KARNOVSKI, M. J., WASSERKRUG, H. L., & HANKER, J. S.: *J. Cell Biol.* 38: 1, 1968.
5. TICE, L. W. & BARNETT, R. J.: *J. Cell Biol.* 15: 401, 1962.
6. TORACK, R. M. & BARNETT, R. J.: *J. Histochem. Cytochem.* 11: 763, 1963.
7. GOLDFISCHER, S., ESSMER, E., & NOVIKOFF, A. B.: *J. Histochem. Cytochem.* 12: 72, 1963.
8. TORACK, R. M. & BARNETT, R. J.: *J. Neuro-path. Exsp. Neurol.* 23: 46, 1964.
9. MARCHESI, V. T. & BARNETT, R. J.: *J. Ultrastr. Res.* 10: 103, 1964.
10. TORACK, R. M.: *J. Histochem. Cytochem.* 13: 191, 1965.
11. NAKAI, Y. & HILDING, D.: *Acta Otolary.* 64: 477, 1967.
12. GROSSMAN, I. W. & HEITKAMP, D. H.: *J. Histochem. Cytochem.* 16: 645, 1968.
13. ROSENTHAL, A. S., MOSES, H. L., BEAVER, D. L., & SHUFFMAN, S. S.: *J. Histochem. Cytochem.* 14: 698, 1966.
14. MOSES, H. L. & ROSENTHAL, A. S.: *J. Histochem. Cytochem.* 16: 530, 1968.
15. TICE, L. W. & BARNETT, R. J.: *J. Histochem. and Cytochem.* 10: 754, 1962.
16. WACHSTEIN, M. & MEISEL, B.: *Amer. J. Clin. Path.* 27: 13, 1957.
17. PADYKULA, H. A. & GAUTHIER, G. F.: *J. Cell Biol.* 18: 87, 1963.
18. HUXLEY, H. E.: *J. Biophysic. and Biochem. Cytol.* 3: 631, 1957.

LEGENDS FOR FIGURES

Fig. 1. Prefixed in glutaraldehyde buffered with cacodylate and incubated in the Wachstein-Meisel ATPase medium. The sarcoplasmic reticulum (SR) and the A band (A) are reactive, the Z line (Z) and the I line (I) are not reactive. Stained with uranyl acetate and lead tartrate.
Original magnification $\times 10,000$

Fig. 2. Prefixed in glutaraldehyde buffered with cacodylate and incubated in the Wachstein-Meisel ATPase medium. The myofibril (Mf), endothel cell (Ed) of the blood capillary and erythrocyte (Er) show positive reaction. Mitochondria (Mi) shows no reaction. Stained with uranyl acetate and lead tartrate.
Original magnification $\times 10,000$

Fig. 3. Prefixed in glutaraldehyde buffered with cacodylate and incubated in the Wachstein-Meisel ATPase medium. The intercalated disc (arrow) shows positive activity. Stained with uranyl acetate and lead tartrate.
Original magnification $\times 10,000$

Fig. 4. Prefixed in glutaraldehyde buffered with cacodylate the incubated in the Wachstein-Meisel ATPase medium. Activity is seen in the A band (A). The M line (M) is reactive, the Z line (Z) and the I line (I) are not reactive. The arrangement of final deposits shows some periodicity.
Original magnification $\times 20,000$

Fig. 5. Prefixed in glutaraldehyde buffered with cacodylate the incubated in the Wachstein-Meisel ATPase medium. The myofilament itself shows no activity. Stained with uranyl acetate and lead tartrate.
Original magnification $\times 40,000$

Fig. 6. Prefixed in formaldehyde buffered with cacodylate and incubated in the Wachstein-Meisel ATPase medium. Activities in the mitochondria (Mi), the sarcolemma (Sl) and the myofibril (Mf) are shown. Some mitochondriae are intensely reactive, others are less or not reactive. Stained with uranyl acetate and lead tartrate.
Original magnification $\times 10,000$

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.