ATPASE ACTIVITY ON THE INTERCALATED DISC AND C_z BANDS OF MOUSE HEART MUSCLE

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In their histochemical study of ATPase activity in cardiac muscle tissue with the light microscope, Padykula and Herman (1) found a linear longitudinal arrangement of the precipitate, suggesting a localization in the myofibrils, and saw in some sections an inconstant blackening of certain cross-striations. Using electron microscopy, Tice and Barrnett (2) described the occurrence of calcium-activated ATPase activity in the A zone region of skeletal muscle. It is the purpose of the present note to discuss the occurrence of light- and electron-microscopically visible lead precipitate which is the result of the activity of Mg-activated ATPase in the cardiac muscle of the mouse.

MATERIALS AND METHODS

The experimental animals were F_1 hybrids of C_{57} black and O_{20} mice, ranging in age from 3 to 4 months, with an average weight of 25 gm. The mice received standard mouse food, wheat grains, and tap water *ad lib.* and were killed by luxation of the cervical vertebrae. Tissue samples were taken from the left ventricle within 3 to 4 minutes after death.

Preparation procedures were as follows:

Preparation Procedure I

For electron microscopy, tissue blocks of about 1 mm^3 were fixed for 1 hour in an isotonic solution of cold (4°C) 1 per cent osmium tetroxide (OsO_4), buffered at a pH of 7.2, with sucrose added to a final concentration of 4 per cent. After rinsing in distilled water and dehydration in alcohol, the tissue blocks were embedded in methacrylate. Thin sections were cut on Porter-Blum Servall microtomes. The sections were collected on grids covered with a carbon film on collodion, from which the collodion had been previously dissolved by means of amyl acetate. Sections were examined with a Siemens electron microscope (Elmiskop 1).

Preparation Procedure II

For the demonstration in the electron microscope of Mg-activated ATPase, the method described by Persijn *et al.* (3) was followed. Tissue blocks of about 1 mm^3 were prefixed for 3 to 4 minutes with a cold 1 per cent OsO_4 solution in a 0.05 M Tris-HCl buffer (pH 7.3) containing 100 mg sucrose per ml. After decantation of the fixative, the incubation mixture was added and changed several times during incubation (20 to 30 minutes at room temperature). The incubation medium contained: 0.05 M Tris-HCl buffer (pH 7.3), 1.3×10^{-3} M ATP (Boehringer, Mannheim, Germany) 0.18 M potassium sodium tartrate (Merck & Co., Rahway, New Jersey, *pro analysi*), 1×10^{-3} M magnesium chloride, and 5×10^{-3} M lead nitrate (B.D.H. analar quality). The mixture was prepared in the sequence indicated. The mean final pH value was 6.8 ± 0.2. Sucrose (60 mg per ml) was added because it had been found to improve the preservation of the fine structure. After incubation the procedure described under I was followed.

Preparation Procedure III

A. In the control experiments, the incubation media was identical with the medium described under procedure II, except that equimolar quantities of monophenyl phosphate (Brocades, Amsterdam) or adenosine-5′-monophosphate (AMP; Boehringer) were substituted for ATP. After incubation the procedure described under I was followed.

B. In addition, parachlormercuribenzoate (PCMB) was used as a sulfhydryl (-SH) inhibitor (1). PCMB was added to the incubation fluid (procedure II) to a final concentration of 0.003 M. It appeared that in this concentration PCMB was completely soluble in the incubation fluid at pH 6.8. The precipitate in the experiment on PCMB inhibition was compared with the precipitate formed in tissue blocks incubated at the same time and in the same medium, but in which PCMB was absent. The admixture of cysteine to the PCMB-containing medium was not used, since addition of this substance resulted in the formation of a heavy precipitate in the solution. After incubation the procedure described under I was followed.

Preparation Procedure IV

Tissue blocks for histochemical study with light microscopy were frozen to about −20°C, within 15 minutes after death, and sectioned in a cryostat. ATPase activity was demonstrated in the sections according to a technique described by Wachstein and Meisel (4, 5) and modified by Willighagen (6, 7) in such a way that tissue sections were prefixed for 10

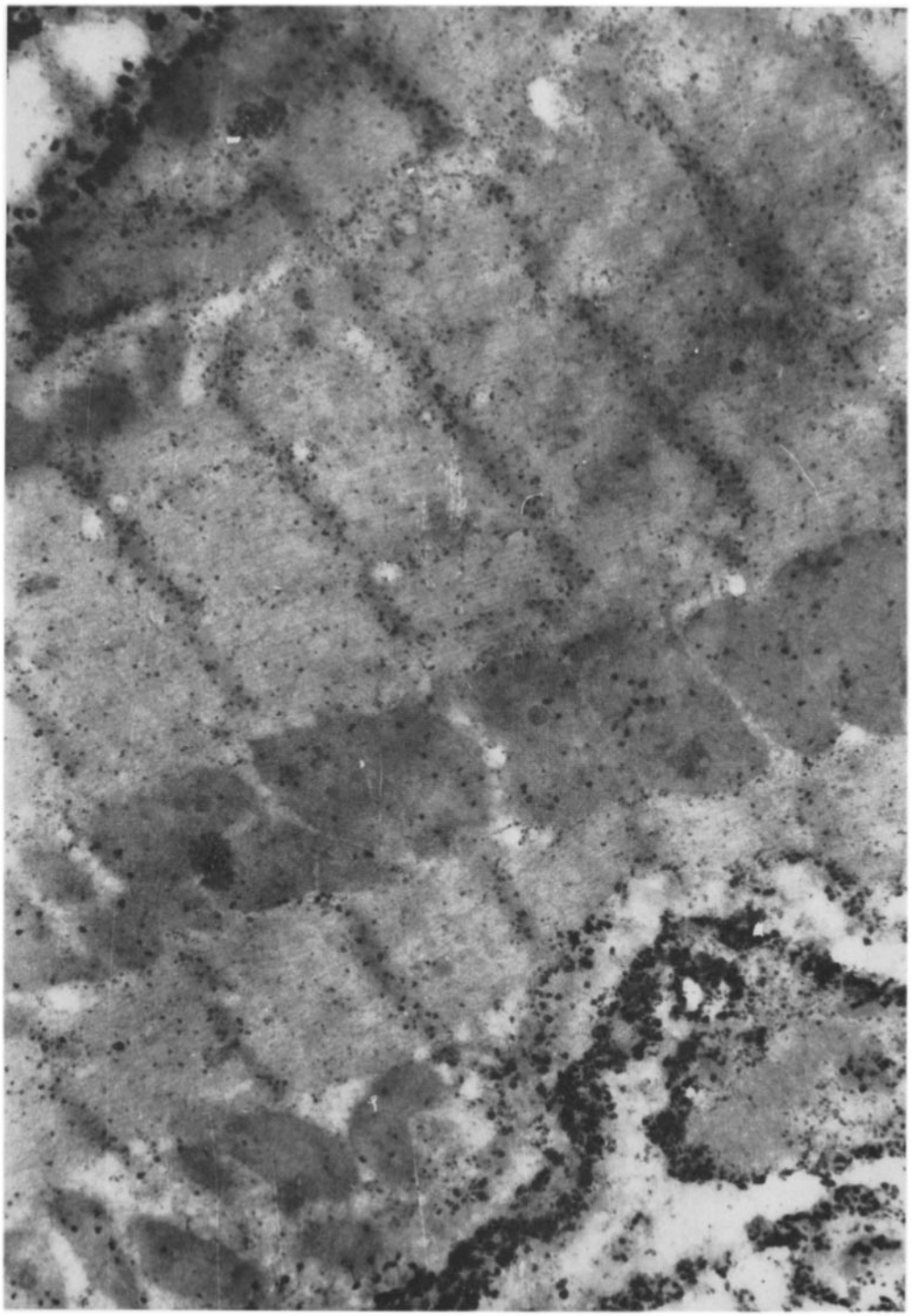


FIGURE 1

Electron micrograph of mouse heart muscle, showing a precipitate indicating ATPase activity on the C_z bands and on the cell membrane. At the lower right, part of an endothelial cell with a heavy precipitate is visible. Preparation procedure II. Magnification, 23,000.

minutes in a solution containing 4 per cent neutral formaldehyde, 6 per cent Dextran (mol wt 18,000), and 2 per cent calcium chloride. Before incubation, tissue sections were washed in distilled water. Incubation was continued for 45 minutes at 37°C. Also, similarly prepared tissue sections which were not prefixed in formaldehyde were studied.

Preparation Procedure V

Lastly, tissue blocks were fixed in formaldehyde for routine light microscopy.

RESULTS

Hematoxylin-eosin-stained sections of formaldehyde-fixed tissue (preparation procedure V) showed normal heart muscle. The fine structure as revealed by the electron microscopical examination of thin sections (preparation procedure I) corresponded to that which is extensively described in the literature (8–14). The muscle fibers in our material appeared to be contracted.

Electron microscopical examination of several tissue blocks incubated in the ATP-containing medium (preparation procedure II) showed a precipitate, in the majority of the blocks, in an outer zone consisting of several fibers. The amount of the precipitate decreased gradually towards the center of the block where there was no precipitate at all. A zonal distribution of the precipitate, after prefixation with OsO_4, has been found for various enzyme tests (15–17). The majority of our preparations, however, did not show an outermost peripheral zone in which precipitate was lacking. It is further to be noted that in spite of a decrease in the total amount of precipitate towards the center of the block, the pattern of distribution of the precipitate relative to the fibrils and other components remained unaltered. Only in the deepest parts of the tissue blocks, where some formation of precipitate had occurred, did we find a change in the distribution pattern, for here the precipitate was mostly confined to the cell membrane and the endothelial cells. It thus appears that the penetration of the incubation medium is sufficient only in the outer zones.

Precipitate consisting of very fine to somewhat coarser granules, presumably indicating ATPase activity, was found at the following sites:

(*a*) C_z bands (Fig. 1),

(*b*) Intercalated discs (Fig. 2), as well as on the related

(*c*) Cell membrane (Fig. 1), and

(*d*) Endothelial cells of the blood vessels (Fig. 1).

In addition, a much less pronounced precipitate was found to be scattered through the A zone of the sarcomere.

The implications of the occurrence of precipitate in some of the mitochondria is discussed by Persijn *et al.* (3).

Electron microscopical examination of several blocks of the control tissue, incubated in media in which either monophenyl phosphate or AMP was substituted for ATP (preparation procedure IIIA), revealed in these cases only very few precipitate granules with no constant or specific localization. Electron microscopical examination of tissue incubated in the ATPase medium with PCMB added (preparation procedure IIIB) revealed the precipitate in identical regions of the tissue blocks just as when no PCMB was added. However, a decrease was evident in the precipitate

FIGURE 2

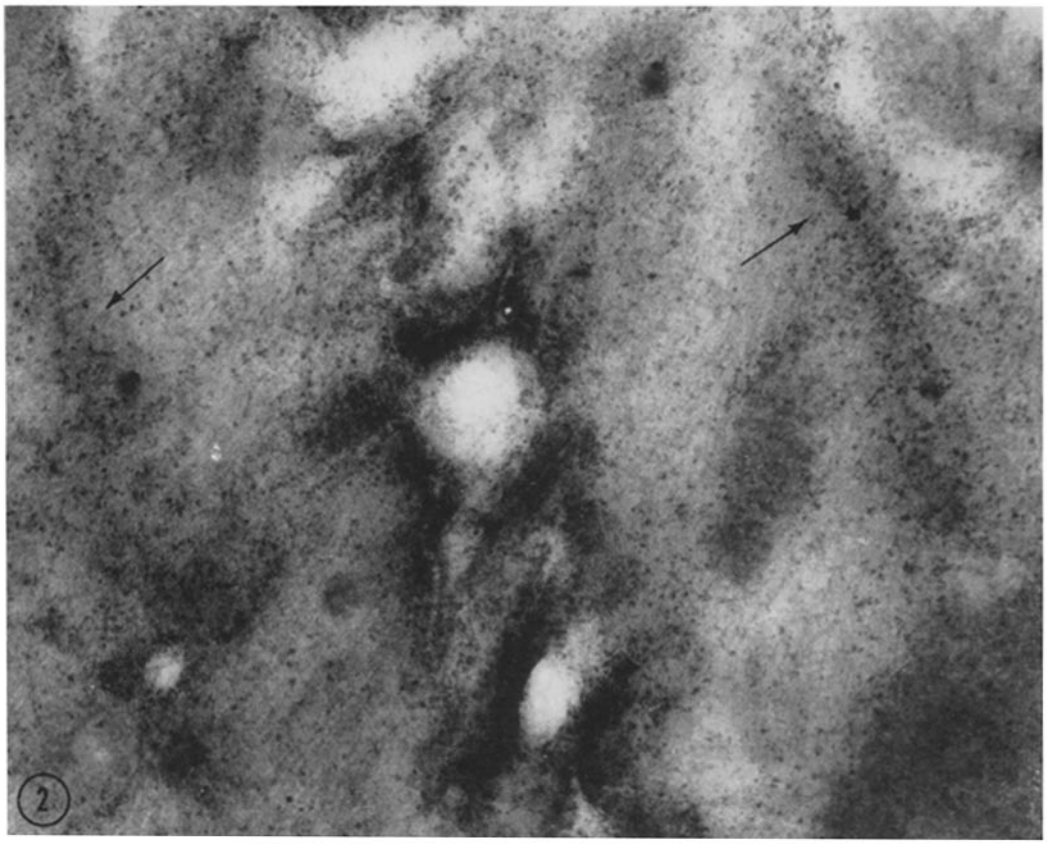
Electron micrograph of mouse heart muscle, showing a precipitate indicating ATPase activity on the intercalated discs. On both sides of the disc a C_z band (arrow) with a precipitate is seen. Preparation procedure II. Magnification, 35,000.

FIGURE 3

Light micrograph of mouse heart muscle, showing a precipitate indicating ATPase activity on the intercalated disc, cell membrane, and cross-striations. Preparation procedure IV. Magnification, 1880.

FIGURE 4

Light micrograph of mouse heart muscle with precipitate indicating ATPase activity on the cross-striations. A heavy precipitate is also visible on the blood vessel wall. Preparation procedure IV. Magnification, 1880.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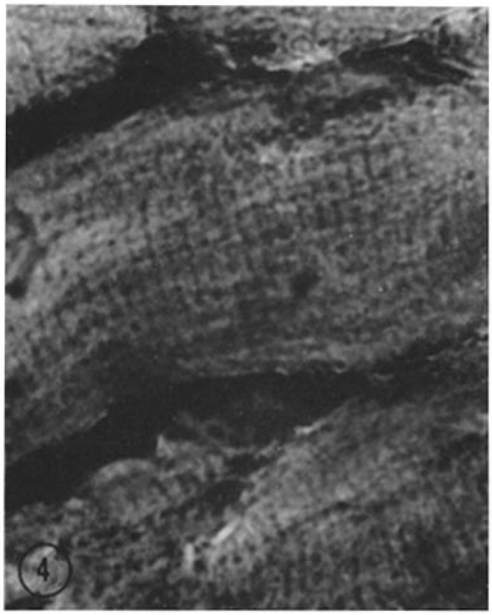
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on the C_z bands relative to other structures in the same cells, such as the cell membrane and intercalated discs. In many cases, precipitate on the C_z bands was even absent, while precipitate on the other components of the same cell was present.

In the light microscopical examination of the ATPase activity (preparation procedure IV), we found some precipitate in the intercalated disc (Fig. 3), on the cell membrane (Figs. 3 and 4), and on the cross-striations (Fig. 4), the latter probably representing the electron-microscopically visible C_z bands. Also, a very heavy precipitate was found in the wall of the blood vessels. The localization of the precipitate occurring in both formaldehyde-prefixed and unfixed sections was essentially identical. In the unfixed sections, however, there occurred an additional diffuse precipitate which made the study of the above-mentioned precipitate on the cross-striations and intercalated disc somewhat difficult.

CONCLUSIONS

(*a*) With the method used for light microscopy and electron microscopy, it became apparent that Mg-activated ATPase activity can be demonstrated in heart muscle of the mouse.

(*b*) A fairly constant localization of the precipitate was found electron microscopically on the C_z bands. Furthermore, the intercalated discs, the cell membrane, and the endothelial cells were also found to be positive.

(*c*) The localization of the activity of an Mg-activated ATPase as indicated by the finding of a heavy precipitate on the C_z bands of this heart muscle differs from the localization of the electron microscopically visible Ca-activated ATPase activity reported in the A band region of the skeletal muscle by Tice and Barrnett (2), which probably represents myosin ATPase.

(*d*) Since examination showed practically no precipitate in several tissue blocks when monophenyl phosphate or AMP was used as a substrate, it may be assumed that the activity of monophosphatases will probably not be a source of error in the interpretation of our results.

(*e*) In order to attempt to distinguish the different ATP-splitting enzymes at the electron microscopical level, we used PCMB as a sulfhydryl inhibitor. When this procedure was followed, we found that the formation of precipitate on the C_z bands had decreased. Since nothing is known, however, about the rate of penetration of PCMB in relation to other constituents of the medium or about its activity at the pH existing locally in the cytoplasm, we have to be cautious in the interpretation of the results of this experiment on inhibition.

(*f*) The precipitate which was seen light microscopically on the cross-striations corresponds with the electron-microscopically visible precipitate on the C_z bands. Both electron microscopy and light microscopy showed a precipitate located on the intercalated disc.

Received for publication, October 17, 1961.

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