Biochimica et Biophysica Acta, 468 (1977) 495—501

BBA Report

BBA 71300

AN ELECTRON MICROSCOPIC HISTOCHEMICAL INVESTIGATION OF THE LOCALIZATION OF CREATINE PHOSPHOKINASE IN HEART CELLS

VICTOR G. SHAROV, VALDUR A. SAKS*, VLADIMIR N. SMIRNOV and EVGENII I. CHAZOV

Laboratory of Cardiac Metabolism, U.S.S.R. Research Center for Cardiology, Moscow (U.S.S.R.)

(Received April 25th, 1977)

Summary

The results of an electron microscopic histochemical investigation performed in the current study indicate that in heart cells creatine phosphokinase is localized:(1) inside mitochondria mainly on the cristae membranes, (2) on the membrane of the sarcoplasmic reticulum, (3) on myofibrils (and in cytoplasm), (4) on the plasma membrane of the cells, (5) on the membrane of the cell nuclei.

The important role of the creatine phosphokinase reactions in energy metabolism of heart and skeletal muscle cells has been established by investigations from several laboratories [1—7]. In heart cells about 30 per cent of the creatine phosphokinase activity is represented by that of the mitochondrial isoenzyme localized on the outer side of the inner mitochondrial membrane [3—5]. Due to its tight functional coupling with ATP-ADP translocase mitochondrial creatine phosphokinase ensures effective creatine phosphate production in heart mitochondria in the presence of creatine [5—7]. This creatine phosphate can be used for ATP synthesis in the creatine phosphokinase reactions in myofibrils and on the sarcoplasmic reticulum membrane where a significant amount of the MM isoenzyme of creatine phosphokinase is located [8—10]. Recently it has been shown that a part of the MM isoenzyme is bound also to the plasma membrane of heart cells and is functionally coupled to ($Na^+ + K^+$)-ATPase [11]. However, the histochemical and electron microscopic study of the heart muscle recently

*To whom correspondence should be addressed.

reported has failed to show the localization of creatine phosphokinase on cellular structures others than mitochondria, sarcoplasmic reticulum and myofibrils [12]. Therefore, the purpose of this investigation was to re-examine the localization of creatine phosphokinase in heart cells by an electron microscopic technique with special attention to the plasma membrane.

The rat hearts were obtained from two healthy animals and were immediately frozen in liquid nitrogen. 20—40 μm thick cryostat sections were cut. The sections were fixed in 2.5 % glutaraldehyde solution buffered by 0.1 M sodium cacodylate, pH 7.4, for 30 min at 4° C. After thawing the sections in 0.1 M sodium cacodylate buffer for 30 min they were incubated for 45 min at room temperature in a dark place with a mixture of the following composition to activate the creatine phosphokinase reaction: 50 mM Tris·HCl, pH 7.2, 1 mM NADP, 1 mM ADP, 10 mM AMP, 0.33 M glucose, 10 mM magnesium acetate, 6 mM creatine phosphate, 2 I.U. per ml hexokinase, 2 I.U. per ml glucose-6-phosphate dehydrogenase, 0.05 mg per ml phenazine metasulfate and 0.2 mg per ml tetranitro blue tetrazolium. After incubation, the sections were rinsed in 0.1 M sodium cacodylate buffer for 30 min and then postfixed in 1 % osmium tetroxide buffered by 0.1 M sodium cacodylate for 1 at 4° C. After that the sections were dehydrated in an ascending series of ethanol solutions and embedded in a mixture of Epon/Araldite. Thin sections were cut on Reichert OM U-3 microtome, stained with citrate and studied with electron microscope JEM 100B (Japan).

Control sections were incubated with the creatine phosphokinase reaction mixture with the following exceptions: (1) 1-fluoro-2,4-di-nitrobenzene (0.1 mM) was used to inhibit creatine phosphokinase, (2) creatine phosphate was omitted from the complete mixture.

Isolation of a plasma membrane preparation from rat heart was carried out by a method of Kidwai et al. [13]. A preparation of sarcoplasmic reticulum was obtained by a method described recently [14]. A heart nuclei preparation was isolated by a method already described [15].

In the experiments described below more than 200 thin sections of heart tissue have been studied. The results presented were typical for more than 60 % of sections investigated.

In the presence of the hexokinase-glucose-6-phosphate dehydrogenase system and tetranitro blue tetrazolim the reverse creatine phosphokinase reaction results in formation of electron opaque deposits of spherical or triangular shape with sizes in the range of 2.0—10 nm (Plates 1—6). Histochemical control of the method used has been carried out by several authors [12,16]. It has been shown that in heart tissue sections incubated without each one of the histochemical ingredients including creatine phosphate or enzymes no formation of formazan deposits occurs [12,16]. The liver cells which do not contain creatine phosphokinase also showed no staining in the complete medium [2,12]. Thus, formation of formazan deposits can be considered to be a result of the creatine phosphokinase reaction under conditions described. In heart cells the deposit is seen inside mitochondria where its main part seems to be localized on the cristae membranes, on the membrane of sarcoplasmic reticulum and is also almost homogenously dis-

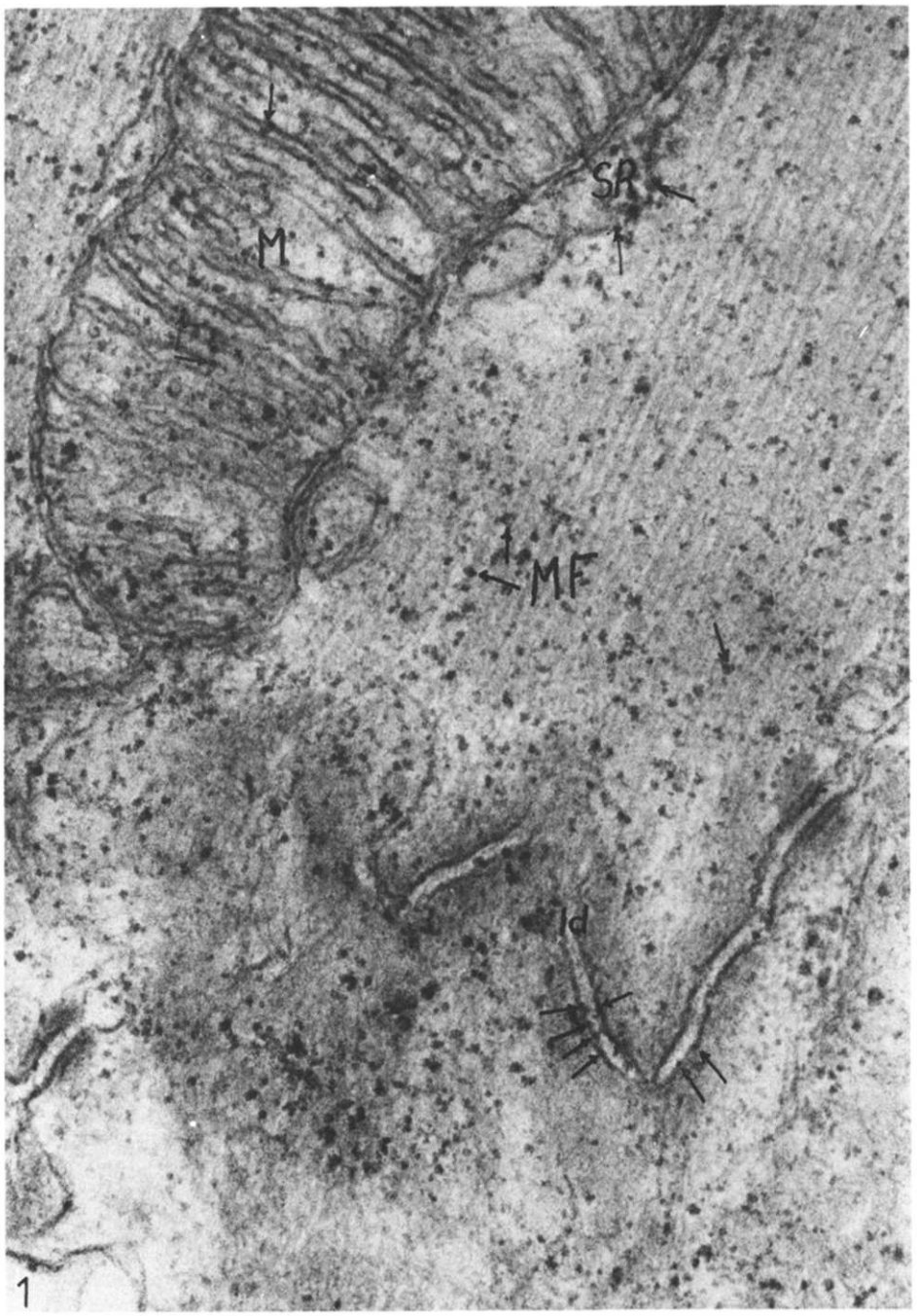

Plate 1. Electron micrograph of rat cardiac muscle showing creatine phosphokinase activity. The myocardium was fixed in glutaraldehyde and incubated in the medium with tetranitro blue tetrazolium as described in the text. M, mitochondria; SR, sarcoplasmic reticulum; MF, myofibrils; Id, intercalated disc. Arrows indicate typical places of the deposit localization due to the creatine phosphokinase reaction. 160 000 X.

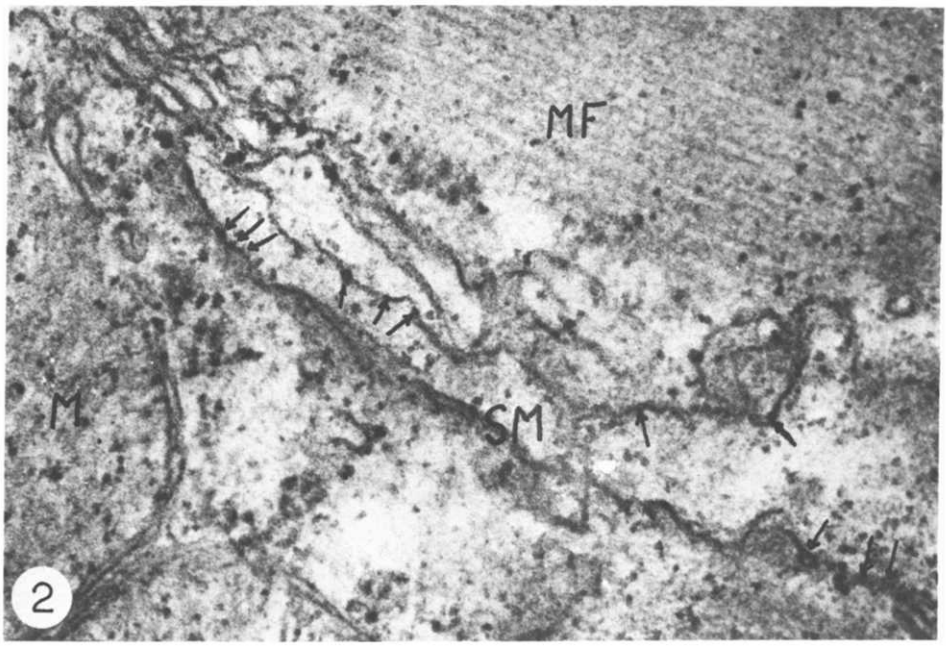

Plate 2. Electron micrograph of rat cardiac muscle showing sarcolemmal membrane area of the cell. SM, sarcolemmal membrane; other symbols as in the Legend to Plate 1. 160 000 X.

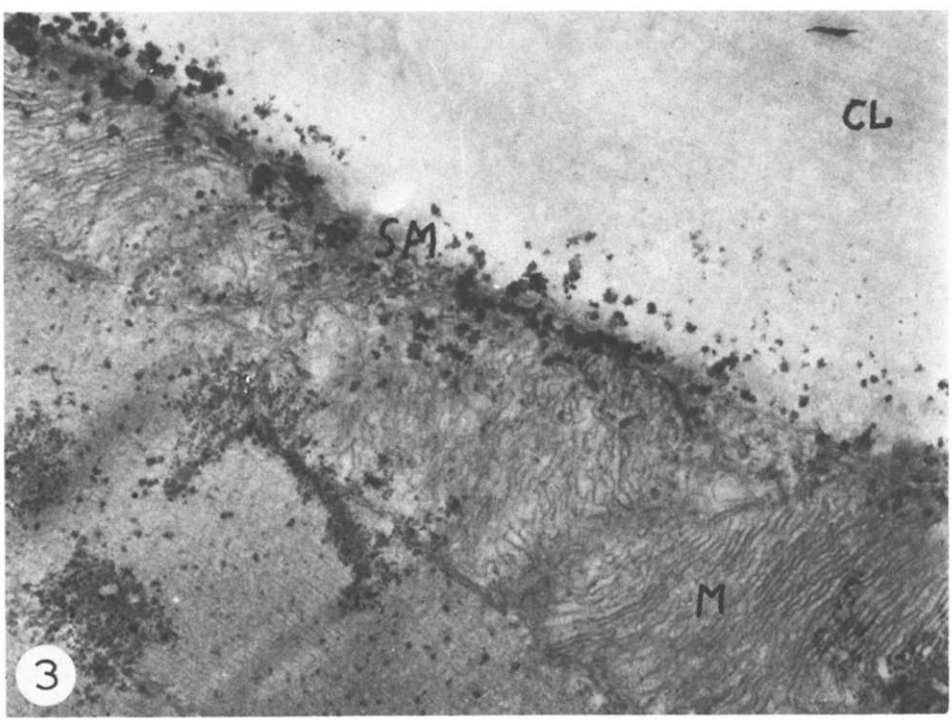

Plate 3. Electron micrograph of the cardiac cell showing intensive deposit formation at the sarcolemmal membrane due to the creatine phosphokinase reaction. CL, collagen; other symbols as in the Legend to Plate 1. 60 000 X.

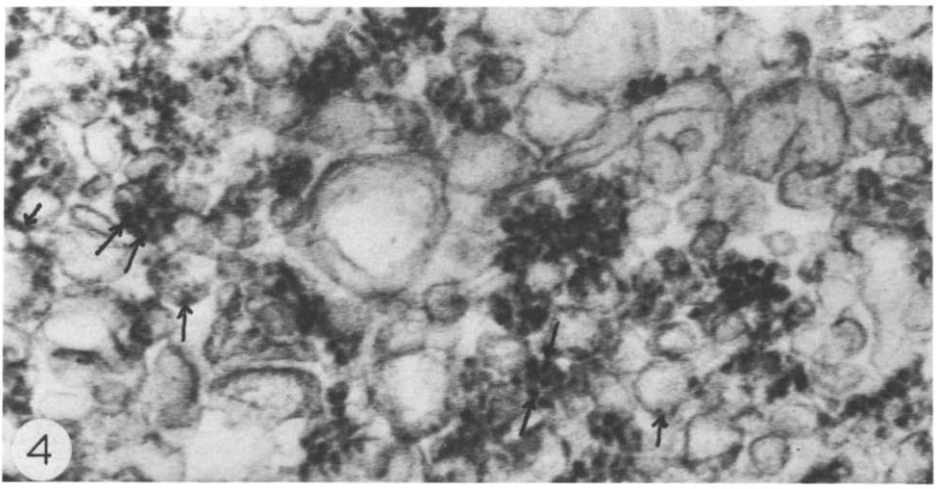

Plate 4. Electron micrograph of a rat heart plasma membrane preparation. A preparation was fixed in glutaraldehyde and incubated in the medium with tetranitro blue tetrazolium. Arrows indicate typical places of creatine phosphokinase localization. 120 000 X.

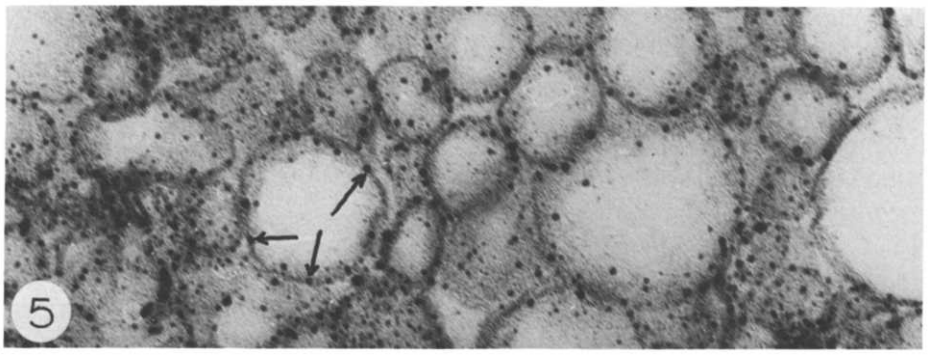

Plate 5. Electron micrograph of an isolated sarcoplasmic reticulum preparation from heart. A preparation was treated as described in the Legend to Plate 4. 120 000 X.

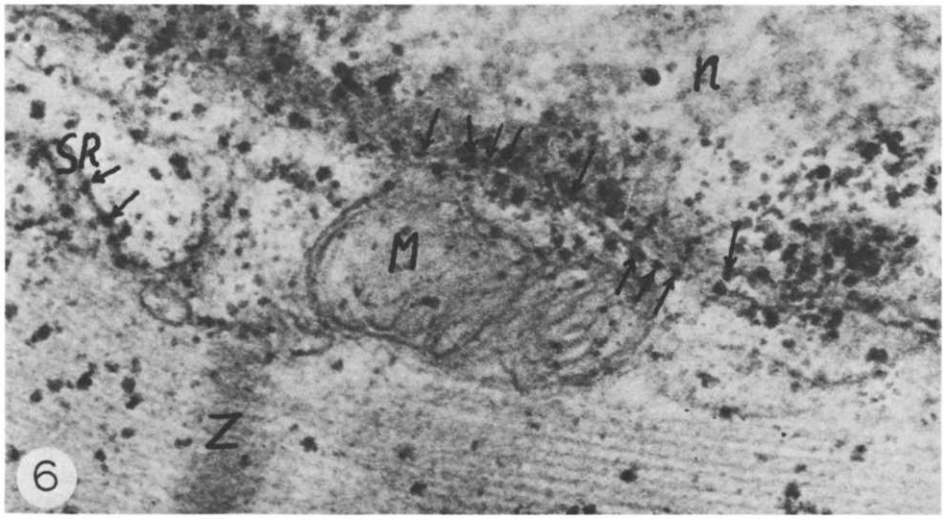

Plate 6. Electron micrograph of rat cardiac muscle cell showing the localization of creatine kinase at the membrane of the nucleus. N, nucleus; Z, Z-line; other symbols as described in the Legend to Plate 1. 160 000 X.

tributed on the myofibrils (Plate 1). Obviously, a part of the deposits seen on the myofibrils may be formed due to cytoplasmic creatine phosphokinase. Such a localization of creatine phosphokinase is consistent with all earlier data [3—10, 12]. Additionally, it can be seen that the deposit is attached also to the plasma membrane at intercalated discs (Plate 1) as well as at the remainder of the sarcolemma (Plate 2). Very intensive deposit formation at the surface membrane of heart cells is demonstrated in Plate 3. These data clearly indicate the localization of creatine phosphokinase on the heart cell plasma membrane. That conclusion is confirmed also by the results of the investigation of an isolated plasma membrane preparation under similar conditions. As can be seen from Plate 4, in this case the intensive deposit formation also occurs; a similar picture can be seen when isolated sarcoplasmic reticulum is investigated (Plate 5). Finally, the intensive deposit formation due to the creatine phosphokinase reaction can be seen in the heart cell nucleus, the main amount of the deposit being localized in the area of the nucleus membrane (Plate 6).

No deposit formation has been observed in control experiments when heart tissue had been pretreated before the creatine phosphokinase reaction with a 0.1 mM solution of 1-fluoro-2,4-dinitrobenzene which inhibits the creatine phosphokinase reaction [1]. Without 1-fluoro-2,4-dinitrobenzene, when only creatine phosphate was omitted from the complete mixture the deposit formation was much less intensive (occurring due to some amounts of intracellular creatine phosphate remaining) and often not observed. These data are consistent with the results of control experiments performed earlier [12,16].

The localization of creatine phosphokinase on the plasma membrane, sarcoplasmic reticulum, nuclei and in mitochondria can be revealed independently by measurements of creatine phosphokinase activity of isolated and purified preparations of those cellular structures (Table I).

The results obtained in this study indicate that all cellular structures of the heart where the reactions of energy utilization occur contain significant activity of creatine phosphokinase, the latter being present also in mitochondria. These data may be taken to indicate that creatine phosphate is a source of energy for all ATPase reactions maintaining both contraction and ion transport across cellular membranes.

Obviously, the significant deposit formation occurs only at sites of enzyme localization most available to the reaction mixture. Since there are

TABLE I

CREATINE PHOSPHOKINASE ACTIVITIES OF ISOLATED PREPARATIONS OF RAT HEART

The values of creatine phosphokinase activities were determined by the reverse reaction by a method described earlier [6,11] at 30° C, pH 7.4.

Preparation	Specific activity (μmol per min per mg of protein)
1. Mitochondria	2.6
2. Plasma membrane	0.6
3. Sarcoplasmic reticulum	0.4
4. Nuclei	0.9

obvious difficulties for diffusion of the reaction mixture components in heart cells and since some inactivation of the enzyme occurs during the fixation procedures [17], it was neccessary to carry out a large number of investigations to obtain reliable results. It may be that the difficulties mentioned contribute in some way to the discrepances between a part of the results of the current study and those of a study reported earlier [12].

The authors thank N.V. Lipina, T.S. Levchenko and N. Volkova for their skillful assistance in carring out the isolation procedures.

References

1 Mommaerts, W.F. (1969) Physiol. Rev. 49, 427—508
2 Seraydarian, M.W. and Abbott, B.C. (1976) J. Mol. Cell. Cardiol. 8, 741—746
3 Scholte, H.R. (1973) Biochim. Biophys. Acta 305, 413—427
4 Jacobus, W.E. and Lehninger, A.L. (1973) J. Biol. Chem. 248, 4803—4810
5 Saks, V.A., Chernousova, G.B., Voronkov, Y.I., Smirnov, V.N. and Chazov, E.I. (1974) Circ. Res. Suppl. III, 34, 138—149
6 Saks, V.A., Chernousova, G.B., Gukovsky, D.E., Smirnov, V.N. and Chazov, E.I. (1975) Eur. J. Biochem. 57, 273—290
7 Saks, V.A., Lipina, N.V., Smirnov, V.N. and Chazov, E.I. (1976) Arch. Biochem. Biophys. 173, 34—41.
8 Jacobus, W.E. and Baskin, R.J. (1974) Circulation 50, 111—118
9 Ottoway, H.R. (1967) Nature 215, 521—522
10 Saks, V.A., Chernousova, G.B., Vetter, R., Smirnov, V.N. and Chazov, E.I. (1976) FEBS Lett. 62, 293—296
11 Saks, V.A., Lipina, N.V., Sharov, G.V., Smirnov, V.N., Chazov, E.I. and Crosse, R. (1977) Biochim. Biophys. Acta 465, 550—558
12 Baba, N., Kim, S. and Farrell, E.C. (1976) J. Mol. Cell. Cardiol. 8, 599—617
13 Kidwai, A.M., Radcliff, M.A., Duchon, G. and Daniel, E.E. (1971) Biochem. Biophys. Res. Commun 45, 901—905
14 Levitsky, D.O., Kuzmin, A.V., Levchenko, T.S., Smirnov, V.N. and Chazov, E.I. (1976) Biochim. Biophys. Acta 443, 468—484
15 Widnell, C.L., Hamilton, T.H. and Tata, J.R. (1967) J. Cell. Biol. 32, 766—772
16 Sjovall, K.A. (1970) Histochemie 10, 336—340
17 Geyer, G. (1973) Ultrahistochemie, pp. 100—190, Veb Gustav Fischer Verlag, Jena