PRELIMINARY NOTES

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A myocardial sarcolemma preparation and the ouabain-sensitive (Na^+-K^+)-ATPase

It is generally agreed that the (Na^+-K^+)-stimulated, ouabain-inhibited ATPase of cell membranes serves as a "Na^+ pump" for the active transport of Na^+ out of mammalian cells. As noted by SKOU[1], it is essential to demonstrate the ATPase location in the outer membrane of the cell, before this Na^+ pump function can be attributed to the (Na^+-K^+)-stimulated ATPase activity. The existence of a (Na^+-K^+)-stimulated, ouabain-inhibited ATPase in heart muscle ((Na^+-K^+)-ATPase) was initially reported in 1962 (refs. 2 and 3), but the subcellular localization of this enzyme within the myocardium remains a subject of controversy. Thus, the (Na^+-K^+)-ATPase activity has been localized to both a sarcoplasmic reticular[4-9] and sarcolemmal[10-12] sites in the ventricular myocardium. However, these variable conclusions might result from not having a clearly defined preparation of the myocardial sarcolemma. Accordingly, this study was aimed at developing such a preparation of myocardial sarcolemma and applying it to the subcellular localization of the myocardial (Na^+-K^+)-ATPase.

Canine cardiac myofibers were prepared from trimmed left ventricle of the dog homogenized for 15 sec in a 1:6 (w/v) dilution contained in 0.25 M sucrose, 0.001 M ethyleneglycol-bis-(aminoethyl)tetraacetic acid (EGTA), and 0.020 M Tris maleate (pH 7.4). The initial sediment, which was obtained after 30 min at 2200 $\times$ g, was washed 6 times in the same media, and finally resedimented at 300 $\times$ g, for 4 min. The presence of sarcolemmal membranes at this stage was assured by the preponderance of striated cellular fragments measuring 10 μ $\times$ 100 μ. Some of these fibers retained intercalated discs when viewed with the phase interference microscope. This fiber preparation was then extracted with 1.0 M KI or NaI for 16 h at 4° and washed in the initial isolation media. Mitochondria were separated by centrifuging the initial 2200 $\times$ g supernatant at 16000 $\times$ g for 12 min and washed once. The 16000 $\times$ g supernatant was brought to 0.6 M with KCl and microsomes were collected by spinning at 211000 $\times$ g for 20 min and washed. ATPase activity was assayed by the method of STAM AND HONIG[13]. The assay media contained 0.25 M sucrose, 0.005 M ATP, 0.005 M $MgCl_2$, 0.020 M Tris maleate (pH 7.4) with or without 0.120 M NaCl and 0.012 M KCl. Cytochrome oxidase and glucose-6-phosphatase activities were used as enzymatic markers for mitochondria and microsomes, respectively[14,15].

Initial experiments showed that the methods used to prepare skeletal muscle sarcolemma gave incomplete extraction of myofibrillar contents from cardiac fibers. In contrast, the extraction of cardiac fibers with 1.0 M KI or NaI led to complete loss of cross striations leaving sarcolemma sacs which approximated the size of the original striated fiber. Examples of such preparations are shown in Figs. 1A and 1B.

The absence of contractile filaments within these membrane structures was also confirmed by ultrastructural examination. Such preparations were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated, and imbedded in Araldite or Epon. Following appropriate sectioning, material was viewed under an RCA III MU electron microscope. The debris within the sarcolemma included occasional swollen mitochondria and many small vesicles. However, myofilaments were not observed.

In Table I are shown the specific activities of Mg^{2+}- and (Mg^{2+}-Na^{+}-K^{+})-

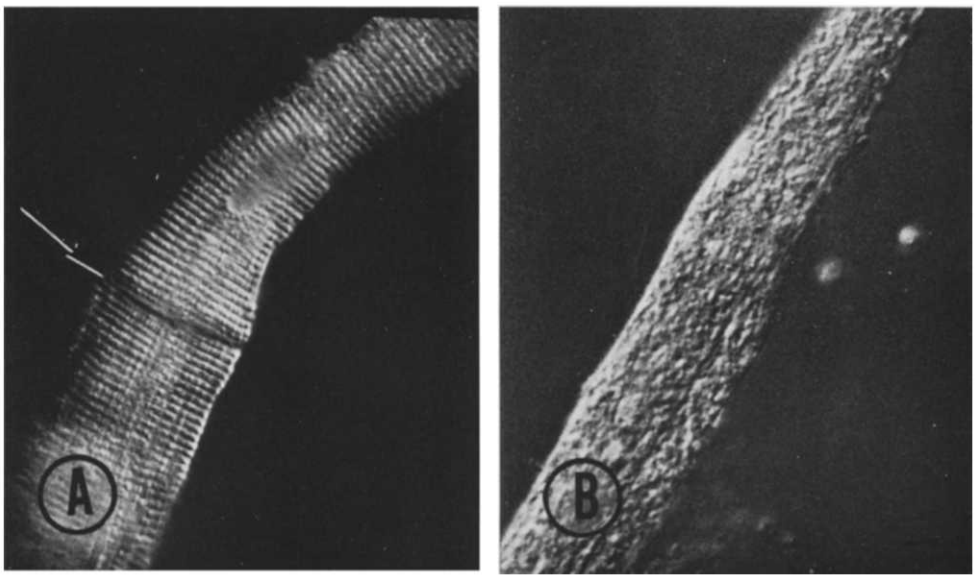

Fig. 1. Dog cardiac fiber and sarcolemma preparations. (A) Adjacent fragments of two myocardial cells still attached by an intercalated disk (arrow). The cells are striated, retain sharp margins at the sarcolemma. (B) A cardiac fiber further treated with 1.0 M NaI and washed in 0.25 M sucrose. The cellular outline is retained with sharp margins but cross-striations are no longer present. Invaginations of the surface highlight adjacent ridges. (Phase interference optics, × 650.)

TABLE I

COMPARISON OF THE SPECIFIC ACTIVITIES OF ATPases CYTOCHROME OXIDASE AND GLUCOSE-6-PHOSPHATASE IN THE VARIOUS MYOCARDIAL PREPARATIONS

The units are μmoles P_i per min per mg protein for ATPase, nmoles cytochrome *c* oxidized per min per mg protein for cytochrome *c* oxidase, and μmoles P_i per h per mg protein for glucose-6-phosphatase. All values represent the mean of from 4 to 7 replicate assays on paired preparations. In all instances the S.E. of the mean was less than 6 % of the mean.

Preparation	*ATPase*			*Cytochrome oxidase*	*Glucose-6-phosphatase*
	Mg^{2+} (Q_{P_i})	Na^{+}-K^{+}-Mg^{2+} (Q_{P_i})	$\frac{Na^{+}\text{-}K^{+}\text{-}Mg^{2+}}{(Mg^{2+})}$		
Whole homogenate	0.172	0.140	0.81	241	0.205
Myofibers	0.138	0.124	0.89	46	0.160
Mitochondria	0.612	0.442	0.71	1318	0.064
Microsomes	0.396	0.295	0.75	94	0.952
Sarcolemma	0.060	0.148	2.48	48	0.202

ATPase, cytochrome oxidase, and glucose-6-phosphatase of the whole homogenate, washed fibers, mitochondria, microsomes and sarcolemma. ATPase activity that was (Na^+-K^+)-stimulated was only found in the sarcolemmal preparation. Moreover, glucose-6-phosphatase activity in the sarcolemma was less than 21 % that of the microsome fraction, while the cytochrome oxidase of the sarcolemma was less than 4 % that of the mitochondria. In 6 preparations, prepared with NaI, Q_{P_i} (μmoles P_i appearing per min per mg protein) stimulated with Mg^{2+} alone averaged 0.061 $\pm$ 0.002 (S.E.), in the presence of Na^+-K^+-Mg^{2+} as well Q_{P_i}, averaged 0.158 $\pm$ 0.007, representing a 2.61 $\pm$ 0.11-fold stimulation by Na^+-K^+.

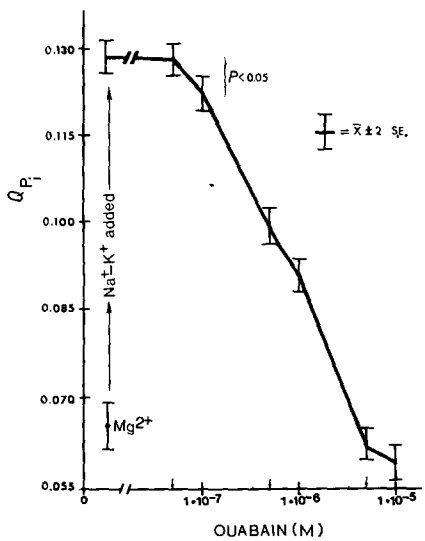

Fig. 2. Ouabain and the sarcolemmal (Na^+-K^+)-ATPase. The Q_{P_i} of the sarcolemma in the presence of Na^+-K^+ stimulation is plotted as a function of the molar ouabain concentration. Data is presented as the mean $\pm$ 2 S.E. The threshold for ouabain was between $5 \cdot 10^{-8}$ and $1 \cdot 10^{-7}$ M. Maximum ouabain effect represented a 54 % depression of the total ATPase activity and a 100 % depression of the Na^+-K^+ stimulation. Half-maximal depression (I_{50}) was seen at $5 \cdot 10^{-7}$ M.

As shown in Fig. 2, the stimulation of the ATPase in sarcolemma preparation by Na^+ and K^+ is inhibited by ouabain. The threshold for this ouabain inhibition was between $5 \cdot 10^{-8}$ and $1 \cdot 10^{-7}$ M. In the 6 sarcolemma preparations extracted with NaI and 8 extracted with KI, $5 \cdot 10^{-6}$ M ouabain inhibited the Na^+-K^+ stimulation by 91 $\pm$ 3 % and 92 $\pm$ 4 %, respectively. Paired studies with preparations of mitochondria and microsomes ATPase activity was not Na^+ stimulated and was only inhibited by 8 $\pm$ 4 % following ouabain ($5 \cdot 10^{-6}$ M).

In further studies it was shown that the ATPase of the sarcolemma could be activated by $1.0 \cdot 10^{-3}$ M Ca^{2+} ($CaCl_2$) in the absence of Mg^{2+} ($MgCl_2$), while Mg^{2+} was required for Na^+-K^+ stimulation. Moreover, in the presence of Mg^{2+}, $1.0 \cdot 10^{-3}$ M Ca^{2+} completely inhibited the stimulation of ATPase by Na^+-K^+. Sodium azide ($2.5 \cdot 10^{-4}$ M) and dinitrophenol ($2.5 \cdot 10^{-4}$ M) were without effect on the ATPase of the sarcolemma. However, F^- ($10 \cdot 10^{-2}$ M NaF) completely inhibited the stimulation by Na^+-K^+ but did not affect the Mg^{2+}-activated ATPase activity.

It is concluded that sarcolemmal preparations of heart muscle can be obtained

which are free of filament contamination by extraction of washed fibers with 1.0 M NaI or KI. Enzymatic markers revealed minimal contamination by sarcoplasmic reticulum and mitochondria. This preparation of sarcolemmal membranes contains the ATPase which is stimulated by Na^+ and K^+ and inhibited by ouabain. These responses are similar to those observed in the red cell ghost[16]. This study does not exclude the existence of an additional (Na^+-K^+)-stimulated ATPase of the sarcoplasmic reticulum but no evidence has been obtained to support such a possibility. In contrast, the above results support the view that a (Na^+-K^+)-stimulated ATPase in the sarcolemma is a major site of action for the inotropic effect of ouabain. Furthermore, a physiologic role for calcium may be postulated as a modifier of the (Na^+-K^+)-stimulated ATPase of the sarcolemma.

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