A SODIUM AND POTASSIUM-STIMULATED ADENOSINE TRIPHOSPHATASE FROM CARDIAC TISSUES. I. PREPARATION AND PROPERTIES

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In recent years attention has been given to an adenosine triphosphatase (ATPase), which has been implicated in active cation transport across membranes. This enzyme system has been extensively studied in crab nerve (Skou, 1957,1960), red blood cell membranes (Post, 1959; Dunham and Glynn, 1961) and cerebral tissues (Jarnefelt, 1961; Aldridge, 1962; Schwartz et al, 1962; Skou, 1962).

The present report is concerned with the preparation and some properties of an active sodium and potassium-dependent ATPase from heart muscle.

METHODS

The procedure of homogenization and differential centrifugation of the tissue was similar to the one previously described for brain (Schwartz et al, 1962) with the exception of the isolation medium. This consisted of 0.25M sucrose, 5mM ethylenediamine tetraacetic acid (EDTA), 30mM histidine HCl and sufficient 2-amino-2-methyl-1,3-propanediol buffer (AMPD) to bring the pH to 6.8 (this required about 50mM/liter). Prior to using, 0.2% sodium deoxycholate was added. Adult rats, weighing between 300-400 gm, were decapitated and bled. The hearts were removed, wiped free of blood, weighed, minced in cold medium and homogenized. Fractions were collected at 600 x g (average) for 15 minutes

(P_6), 10,000 x g for 30 minutes (P_{10}), 20,000 x g for 30 minutes (P_{20}), 80,000 x g for 30 minutes (P_{80}) and 100,000 x g for 70 minutes (P_{100}). The pellets were then homogenized lightly and suspended in 0.25M sucrose containing 1mM EDTA, 30mM histidine HCl, 50mM AMPD, pH 7.00 and stored at -5°C.

RNA and esterase activity were estimated as indicated in Table 1; 5-nucleotidase activity was determined by the procedure of Heppel and Hilmoe (1955); glucose-6 phosphatase activity by the method of Swanson (1955) and protein by the biuret procedure.

TABLE 1

RNA CONTENT AND ESTERASE ACTIVITY

Fraction	ESTERASE ACTIVITY µl CO_2/mg Protein/Hour	RNA µg RNA/mg Protein
P_6	225	11.0
P_{10}	490	7.6
P_{20}	980	4.3
P_{80}	1190	4.5
P_{100}	1170	5.4
SPN	485	5.5

Esterase activity was measured by the procedure of Hulsman (1961). RNA was determined by the method of Hutchinson et al (1962).

ATPase activity was measured at 37°C in a shaking bath, in Tris(Sigma 121) buffer, at pH 7.00. Phosphate was estimated by the method of Fiske and Subbarow (1929), using amidol as the reducing agent (20 min. color development) at 660 mµ. The ATP was purchased from Sigma either in the Tris form or was prepared from the disodium salt (Schwartz et al, 1962).

RESULTS AND DISCUSSION

The present procedure of isolation affords a convenient method of obtaining membrane preparations low in RNA and high in esterase activity, the latter indicating the presence of a high

concentration of microsomal material (Table 1) in fractions P_{20}, P_{80} and P_{100}.

In agreement with Hulsman (1961), almost no glucose-6 phosphatase activity was found in heart muscle suspensions. In addition, no significant 5'-nucleotidase activity could be detected in the P_{20}, P_{80} or P_{200} fractions of four preparations. It is concluded, therefore, that most, or all, of the inorganic phosphate which appeared in the reaction mixture after incubation arose from the action of ATPases specifically on ATP. The rate of enzymic hydrolysis is linear over the first 5-8 minutes only.

The presence of a specific Na^++K^+ dependent ATPase may be demonstrated in fresh rat preparations by the use of ouabain, a cardiac glycoside which specifically inhibits this enzyme and active transport of Na^++K^+ (Skou, 1957, 1960). It may be seen both from Table 2 and Table 3, that the addition of Na^++K^+ appears not to induce any stimulation of the ATPase in fresh preparations. In fact, in some cases a slight inhibitory effect is noted. Nevertheless, ouabain is able to exert some inhibition on the activity resulting from Na^++K^+ addition. This would suggest that the high "basic" Mg^{++} activity "masks" the activity or stimulation induced by Na^++K^+. Storage of the enzymes at -5°C, however, effects a rapid loss of the Mg^{++} activity with a much less rapid loss of the Mg^{++}+Na^++K^+ enzyme activity, thus revealing a marked 'activity ratio' about the twelfth day after preparation. (Preincubation of the enzyme suspension in the assay medium in ice without ATP for about 15 minutes will also cause a drop in the Mg^{++}-ATP activity but no change in the Mg^{++}+Na^++K^+ activity compared to control values.) The activity due to Na^++K^+ may be almost completely abolished by the addition of ouabain in high concentration.

The storage properties of the heart enzyme system appear to be different from those of the brain enzyme. It was found that

TABLE 2

ATPase activity of rat cardiac muscle: the effect of storage at -5° C

Day	μg Pi/mg Protein/Hour (A) Mg	(B) Mg+Na+K	(B) ÷ (A) Activity Ratio
PREP. 1, 2 and 3, P_{20}			
0	205	190	0.93
2	175	180	1.03
9	76	128	1.68
12	65	142	2.20
20	28	112	4.40
25	20	108	5.40
PREP. 1 and 2, P_{80}			
2	350	380	1.08
5	240	330	1.40
6	150	250	1.68
8	130	245	1.90

The standard incubation mixture consisted of 3 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl, 3 mM Tris-ATP, 30 mM Tris, pH 7.0; generally 0.1 ml of enzyme suspension was added after 5 minutes temperature equilibrium at 37°C. Incubation was continued for 15-30 minutes in a Dubnoff shaker. The reaction was stopped by the addition of 0.1 ml cold 50% TCA and the tubes immersed in an ice bath for 15 minutes. Column (A) represents the activity in the presence of Mg alone; (B) in the presence of $Mg^{++}+Na^{+}+K^{+}$.

TABLE 3

The effect of ouabain on the $Na^{+}+K^{+}$-stimulated ATPase of rat cardiac muscle

PREP	Day	Mg	Mg+K	Mg+Na	Mg+Na+K	Ouabain+Mg+Na+K	
P_{20}	0	205	--	--	190	(10^{-3}M)	160
P_{20}	9	69	71	71	125	(10^{-4}M)	93
						(10^{-3}M)	76
P_{20}	30	19	--	--	86	(10^{-4}M)	20
P_{20}	5	104	--	--	115	(10^{-3}M)	88

Ouabain in the concentrations indicated was added directly to the incubation tubes before the addition of the enzyme. In no case did the drug affect the enzyme in the presence of Mg alone or Mg+Na or Mg+K. The incubation conditions and assay were identical to that of Table 2.

storage at -5 to 0°C caused a parallel and very slow diminution of the ATPase activities of the brain enzyme in the presence or absence of Na^+ (Schwartz et al, 1962).

The differential effect of freezing and storage on the heart preparations suggest the presence of at least 2 components or enzymes, one Mg^{++} dependent, and the other $Mg^{++}+Na^+ +K^+$ dependent. Further evidence of this may be derived from a reciprocal plot of substrate vs. enzyme activity. These have revealed, in three preparations, an average K_m for the Mg^{++} enzyme of 3.1×10^{-3} M, and for the $Mg^{++}+Na^+ +K^+$ enzyme of 1.7×10^{-3}M. The maximal velocities of the Mg^{++} and the $Mg^{++}+Na^+ +K^+$ ATPases respectively were 122 and 170 µg Pi/mg protein/hour. In contrast, Lineweaver-Burk plots for the brain enzyme revealed the same maximal velocities for both the $Mg^{++}+Na^+ +K^+$ ATPases (Schwartz et al, 1962). Although the preparations are crude, it may be tentatively assumed that the heart ATPase "system" consists of either 2 ATPases with different metal requirements, or one ATPase with 2 different sites of activity.

The sediments collected at 600 x g, 10,000 x g, 100,000 x g and the SPN revealed a Mg^{++} ATPase but no $Mg^{++}+Na^+ +K^+$ activity, even after storage. In addition, ouabain produced no effect. Of particular interest is the P_{100} fraction, which had high esterase-active membrane fragments and still exhibited no $Na^+ +K^+$ ATPase activity. Similar results were obtained with brain (Schwartz, et al, 1962) and would suggest a localization of the enzyme(s) in or on specific aspects of the endoplasmic reticulum.

Note:

After completion of the manuscript two recent papers, one by Auditore (1962) and an abstract by Yu and Lee (1962) describing a $Na^+ +K^+$-dependent ATPase system in rabbit ventricle and in guinea pig heart, respectively, came to the attention of this

author. These enzyme systems are apparently similar to the one described in the present study.

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