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Sodium - Potassium Activated G-Strophanthin Sensitive ATPase in Cardiac Muscle.* (27589)

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Skou(1,2) demonstrated the existence of an enzymatic activity in peripheral crab nerves which possessed such properties that he considered whether the enzyme might be involved in active transport of Na and K in the nerve fiber. The substrate for the enzyme is ATP, appears to have an absolute requirement for Mg and can be activated further by Na and K. The Na + K active component of the enzyme exhibits a unique susceptibility to inhibition by very low concentrations of G-Strophanthin, a supposedly specific inhibitor of the coupled Na + K transport system(3).

If such an enzymatic activity is a participant of the Na + K active transport system it may be assumed that the enzyme should be present in other tissues where active Na and K transport occurs. The purpose of this investigation was to search cardiac muscle for an enzymatic activity similar to that first described by Skou(1).

Materials and methods. Preparation of enzyme: Two different procedures were employed to prepare the enzyme. In the first procedure rabbit ventricles were homogenized in 9 volumes of ice cold distilled water using an all-glass Potter Elvehjem homogenizer. The water homogenate was centrifuged for 10 minutes at 2000 × *g* and sediment discarded. The supernate was dialyzed against a large volume of ice cold water at 5°C and was changed once within a 24-hour period. This dialyzed preparation represented the source of enzyme and is referred to as the water extract. A small amount of sediment formed during dialysis and was included in the preparation.

The second procedure was that described to me by Skou with some modification; rabbit ventricles were homogenized in 9 volumes of ice cold sucrose containing 0.1% deoxycholate (DOC), .030 mM L-histidine, and .005 M ethylenediamine tetraacetic acid (EDTA), and the homogenate subjected to the differential centrifugation technic(4). The debris-nuclear (500 × *g*, 10 min), mitochondrial (10,000 × *g*, 10 min), and microsomal (72,000 × *g*, 45 min) fractions were isolated using a Lourdes refrigerated Vacu-Fuge. The microsomes were washed once in a medium consisting of .25 M sucrose, .05% DOC, .030 mM L-histidine and .001 M EDTA. All fractions were resuspended in a suitable amount of cold .25 M sucrose containing .030 mM L-histidine, rehomogenized to ensure a fine suspension of enzyme and immediately assayed for ATPase activity.

Reaction mixture: The activity of these preparations was determined at 37°C in a total volume of 2.5 ml containing 0.2 ml of enzyme preparation buffered with imidazole (100 μmoles), histidine (100 μmoles) and brought to pH 7.1 with approximately 50 μmoles of HCl. Incubation time was 60 minutes. The reaction mixture for routine assays was charged with 6 μmoles of Mg and 5 μmoles of ATP which was rendered free of Na and K by passing it through a Dowex 50 cation-exchange column in the tris form and finally buffered to pH 7.0 with trishydroxymethyl aminomethane (tris). The Dowex

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TABLE I. Influence of Na, K, and G-Strophanthin of Rabbit Ventricle ATPase.

No.	Preparation	ATPase activity (μmoles Pi per mg protein per hr)		
		—	Na + K	Na + K + S
5	Water extract	1.44 ± .33*	3.60 ± 1.30	1.65 ± .21
3	Microsome	4.72 ± .71	10.44 ± 1.81	5.52 ± .47

* Avg and S.D.

50 column in the tris form was prepared as follows: a small amount of Dowex 50 resin in the hydrogen form was suspended in distilled water and to this was added sufficient saturated tris solution slowly to raise the internal pH of the resin to pH 7.0. pH measurements were made using a Beckman Zeromatic pH meter. The wet resin was packed in a pyrex column 15 cm long and .5 cm in diameter. A known solution of ATP (Sigma Biochem.) was passed through this column at 5°C. When tested individually or together the final concentration of Na and K in the reaction mixture was .080 M and .026 M respectively. All cations were added as their chlorides. The rate of hydrolysis of ATP was linear with time and enzyme concentration under these experimental conditions. The reaction was stopped by addition of 1.5 ml of 8% perchloric acid. The solution was filtered and analyzed for presence of inorganic phosphorus on a Model B Spectrophotometer at 970 mμ. The method employed was that of Fiske and Subbarow(5) using amidol as the reducing agent.

Protein determinations were carried out employing the procedure described by Lowry (6).

Results. The influence of Mg, K, Na, and G-Strophanthin in various combinations on ATPase activity in dialyzed water extracts and microsomal suspensions has been summarized in Table I.

Although both preparations show some activity in absence of added cations, Mg appears an obligatory requirement for enzyme activity. Addition of Mg increased enzyme activity at least 4-fold. With 5 μmoles of ATP in the medium, maximum enzyme stimulation occurred when concentration of Mg exceeded 5 μmoles. Higher Mg concentrations did not inhibit enzyme activity.

With Mg in the medium, small amounts of Na or K added separately to both preparations increased dephosphorylation of ATP slightly. Together, however, Na and K greatly stimulated enzyme activity. The average per cent Na + K activation was 157 for the water extract and 121 for the microsomal preparation. This increment in activity was almost completely abolished by G-Strophanthin at a concentration of 2.7×10^{-6} M. G-Strophanthin did not affect the increase in enzyme activity produced by Mg.

Water extracts prepared from fresh ventricles showed little or no Na + K dependent ATPase activity. On the other hand, water extracts made from fresh frozen (−10°C for several hours) ventricles always possessed more Na + K dependent ATPase activity. This observation prompted a study of the influence of ageing on ATPase activity of the intact ventricle. Rabbit ventricles divided into 4 equal parts were placed in a deep freeze (−10°C). Water extracts of these samples were prepared daily for 4 consecutive days and assayed for Na + K dependent and independent ATPases. The results summarized in Table II revealed a loss of the Na + K independent ATPase and an increase in Na + K dependent ATPase activity.

Activity-pH curves have been constructed for both enzyme preparations and are re-

TABLE II. Influence of Ageing on Na + K-Independent and Dependent ATPases.

System	ATPase (μmoles Pi per mg protein per hr)			
	Day 1	Day 2	Day 3	Day 4
E	2.99	2.33	2.27	2.33
E + Na + K	3.43	3.23	3.52	4.06
E + Na + K + S	2.96	2.71	2.70	2.70
% Na + K-activation	14	38	55	74
% S-inhibition	100	58	66	79

E = Enzyme + Mg + ATP.
% S = Inhibition of Na + K activation by G-Strophanthin (10^{-6}M).

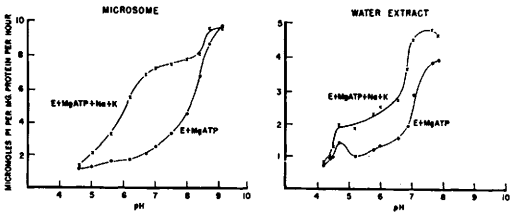

FIG. 1. Relation between water extract ATPase and microsomal ATPase activities and pH in presence and absence of sodium and potassium.

ported in Fig. 1. The buffer system used to carry out these experiments consisted of imidazole, HCl and tris. The forms of the water extract and microsomal curves obtained for the Na + K independent ATPases are very similar. Optimum enzyme activity was recorded at pH levels between 8.5-9.2. The Na + K dependent activity, on the other hand, was active over a wide range of pH. This activity was more pronounced with the microsomal enzyme preparation. Moreover, it should be noted that the specific activity of the microsomal preparation was at least twice as great as that of the water extract.

Discussion. An adenosinetriphosphatase has been found in water extracts of aged rabbit ventricles and freshly prepared microsomes that appears to depend on the relative concentrations of Na, K and Mg for its activity. The enzyme appears to possess 2 active components. One is stimulated by Mg and the other by Na and K added separately or together. In the absence of added cation there is always a little residual activity which is probably due to unremovable Mg tightly bound by the enzyme molecule. The Na + K component but not the Mg component is susceptible to inhibition by 10^{-6} M G-Strophanthin, a concentration level, which also promotes K loss from rabbit ventricles(7).

Although the enzyme in the present study appears to share some properties with the first described by Skou(1) in crab nerves and more recently by others(8,9,10) in other tissues, certain dissimilar properties are apparent. With the crab nerve enzyme Na alone but not K alone produced a slight stimulation of enzyme activity. Addition of Na and K together in presence of Mg and ATP appears to be a definite requirement for the erythrocyte enzyme activation. Na or K added separately to the cardiac enzyme preparation, however, consistently stimulated enzyme activity.

All cellular fractions contained Mg activated Na + K independent ATPase activity. Most of the Mg + Na + K dependent ATPase activity, however, was found in the microsomes. These data suggest that a family of Mg activated ATPases exist in cardiac tissue.

It has been established that myosin ATPase shows pH optima around 6.4 and 9.5. A similar pH optimum was obtained for the Na + K independent ATPase activity found in the water extract and microsomal fraction. Of interest also is the broad pH optimum shown by the Na + K dependent ATPase in both preparations. Preliminary experiments with myosin revealed the presence of a Mg activated ATPase but lack of a Na + K dependent, G-Strophanthin sensitive ATPase.

Similar Na + K dependent and independent ATPases have been demonstrated in rabbit atria, brain and human ventricle.

Summary. An ATPase has been demonstrated in water extracts and microsomal suspensions prepared from rabbit ventricles. It has an absolute requirement for Mg and it can be further stimulated by Na and K. The Na + K active component is susceptible to inhibition by G-Strophanthin. Ageing increases the Mg + Na + K/Mg ATPase ratio.

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