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A STUDY OF THE SODIUM- AND POTASSIUM-ACTIVATED ADENOSINETRIPHOSPHATASE ACTIVITY OF HEART MICROSOMAL FRACTION*

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Abstract—The microsomal fraction is isolated from guinea pig heart. This fraction is found to contain the Na^+- and K^+-activated ATPase in the presence of Mg^{2+}. The maximal ATPase activity is obtained at Na^+ and K^+ concentrations of 100 mM and 10 mM respectively. Calcium inhibits totally the Na^+- and K^+-activated portion of ATPase in the presence of Mg^{2+}; however Ca^{2+} itself stimulates ATPase in the absence of Mg^{2+}. Na^+ increases further the Ca^{2+}-activated ATPase but not K^+. Strophanthin-K initially stimulates then depresses the Na^+–K^+-activated ATPase in the presence of Mg^{2+}. Both catecholamines and sympathetic blocking agents depress the Na^+–K^+-activated ATPase. However, when these agents are present together in the reaction mixture, no depression of Na^+–K^+-activated ATPase activity is observed. Similarly, both acetylcholine and atropine inhibit the Na^+–K^+-activated ATPase, but no inhibition of Na^+–K^+-activated ATPase is observed when acetylcholine and atropine are added together.

SINCE the work of Skou on crab nerve, the presence of sodium (Na^+)- and potassium (K^+)-activated ATPase† of cellular membrane or microsomal fraction has been well documented.[1–10] The influence of Na^+ and K^+ on the ATPase activity is such as to suggest strongly the involvement of this enzymactic activity with the active transport of Na^+ and K^+ at cell membranes. Furthermore, this Na^+–K^+-activated enzyme from various tissues is markedly influenced by cardiac glycosides in concentrations comparable to their therapeutic concentrations. In view of the unique response of the heart to cardiac glycosides, it is of interest to study the presence of Na^+–K^+-activated ATPase in heart muscle and to investigate the influence of cardiac glycosides on this system. In addition to the cardiac glycosides, physiologically important agents having a profound effect on heart, such as catecholamines, acetylcholine, and others, were studied with regard to their effects on the Na^+–K^+-activated ATPase from heart tissue.

METHODS

Preparation of enzyme

Adult guinea pigs of either sex were struck on the head and hearts were quickly excised and weighed. The tissue was minced with scissors and ground in 10 volumes

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† Throughout this paper ATPase (adenosinetriphosphatase) will be used to denote a dephosphorylating system which leads to the liberation of inorganic phosphate (IP) from adenosinetriphosphate (ATP).

of 0·32 M sucrose in a glass homogenizer with a Teflon plunger. The suspension was transferred to the chamber of a piston press homogenizer (Emanuel and Chaikoff[11,12]) fitted with a plunger of 30-μ clearance. The press was immediately operated and the suspension collected in an ice-cold plastic container. The homogenates were centrifuged for 10 min at 800 × *g* and the sediment discarded. The supernatant was taken to 11,000 × *g* for 35 min in a Lourdes refrigerated centrifuge, and the resulting supernatant was placed in a no. 40 rotor of a Spinco preparative ultracentrifuge which was taken to 130,000 × *g* for 95 min. The final precipitate was washed with the sucrose solution and recentrifuged in the Spinco, and the final precipitate was suspended in Tris buffer (0·125 M, pH 6·8), whose volume ratio to original tissue was 1:1. This will be called the microsomal suspension.

Adenosinetriphosphatase assay

Triplicate reaction mixtures of 1 ml were prepared in 5-ml tubes. Each incubation medium contained Tris buffer (0·125 mM), ATP (3 μM), and 0·02 ml of microsomal suspension in addition to activating metals or agents as indicated in the figure legends. The tube lacking only the microsomal suspension served as control. All incubations were carried out at 37°.

Other methods

Protein was determined by the biuret method. Tris–ATP with a little Na^+ was prepared from the disodium salt (Sigma) with Dowex-50 resin in the H^+ form according to the procedure of Järnfelt[13] or purchased from Sigma as Tris–ATP. Inorganic phosphate was determined by the method of Fiske and Subbarrow.[14] The substances used in these experiments were L-norepinephrine bitartrate monohydrate, L-epinephrine bitartrate, acetylcholine, and dichloroisoprotenerol (DCI). Triple-distilled water was used throughout.

RESULTS

ATPase activity of different fractions

Table 1 shows the ATPase activity of the various fractions employed. In studying their ability to liberate inorganic phosphate from ATP, conditions indicated in Table 1

TABLE 1. ATPASE ACTIVITY OF THE VARIOUS FRACTIONS EMPLOYED

Description of centrifuged preparation	Centrifugal force, *g*	Time, min	ATPase activity, μmoles IP/mg protein/hr	
			Mg^{2+} alone	$Mg^{2+} + Na^+ + K^+$
Nuclear	600	10	16·3	17·8
Mitochondrial	11,000	30	12·3	11·6
Microsomal	130,000	95	6·2	15·5
Supernatant			2·2	2·4

The incubation medium (total volume of 1 ml); Tris buffer (pH 6·7) 0·125 M; $MgCl_2$ 3 mM; ATP 3 mM; and microsomal or other suspension 0·2 ml. Incubation time 15 min. ATP used is Tris-ATP in all experiments.

were chosen. Magnesium alone activates the ATPase initially in all fractions. The addition of Na^+ (100 mM) and K^+ (10 mM) stimulates the Mg^{2+}-activated ATPase further only in the microsomal fraction. Originally, the simple homogenization by a Teflon-glass homogenizer of heart tissue was employed. In this method the similar

procedure of fractionation yielded rather variable results in that the Na^+–K^+-activated ATPase was localized in different fractions depending on the degree of homogenization. This inconsistent localization of Na^+–K^+-activated ATPase was overcome by standardizing the initial homogenization of tissue in the Teflon-glass homogenizer followed by the careful and constant timing of homogenization in the Emanuel and Chaikoff homogenizer. The electron microscopic examination of this fraction* showed that it is composed mainly of membrane fragments and sarcotubular system of smooth type similar to that from the skeletal muscle described by Muscatello *et al.*[15] The ATPase activity of this fraction will be called the microsomal ATPase.

The effects of Na^+ and K^+ on the microsomal ATPase

The addition of sodium alone had a marked stimulatory effect on Mg^{2+}-activated ATPase, the degree of stimulation being dependent upon the Na^+ concentration. The results are shown in Fig. 1. The microsomal suspension liberates IP from ATP at

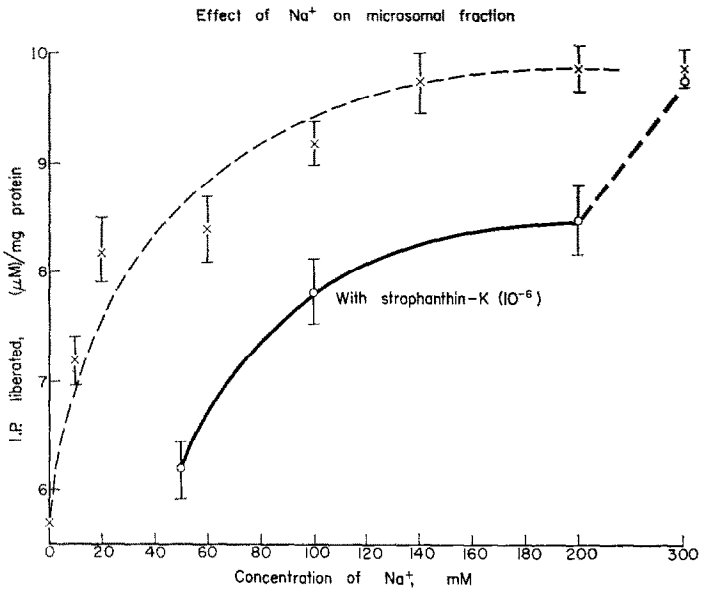

FIG. 1. The effect of Na^+ on microsomal ATPase. In this and all following figures, IP liberated is expressed in terms of μmoles of IP liberated per mg protein of microsomal suspension per hr, and each point indicates a mean $\pm$ standard error (S.E. indicated by bar). Each point is mean of 8 experiments. Incubation medium total volume 1 ml; Tris buffer (pH 6·7) 0·125 M; $MgCl_2$ 3 mM; ATP 3 mM. Effect of strophanthin-K (10^{-6}) is also included.

the rate of 5·7 μmoles/mg protein per hr in the presence of Mg^{2+} alone. The addition of Na^+ (20 mM) already has a significant stimulatory effect on the Mg^{2+}-activated ATPase which reaches approximately the maximum at Na^+ concentrations around 140 mM.

Potassium alone does not stimulate Mg^{2+}-activated ATPase. The degree of stimulation by K^+ is dependent upon both the concentration of Na^+ present initially and that

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of K^+ added. This is shown in Fig. 2 in which, with the higher Na^+ concentration of 100 mM, the maximal activation is reached with K^+ concentration of 10 mM, whereas with 20 Mm Na^+, the same concentration of K^+ (10 mM) is inhibitory. The activation of Mg^{2+} ATPase by Na^+ and K^+ is dependent on the pH of incubation medium, as shown in Fig. 3. The solution is buffered with Tris-maleate (0·125 M). The range of pH

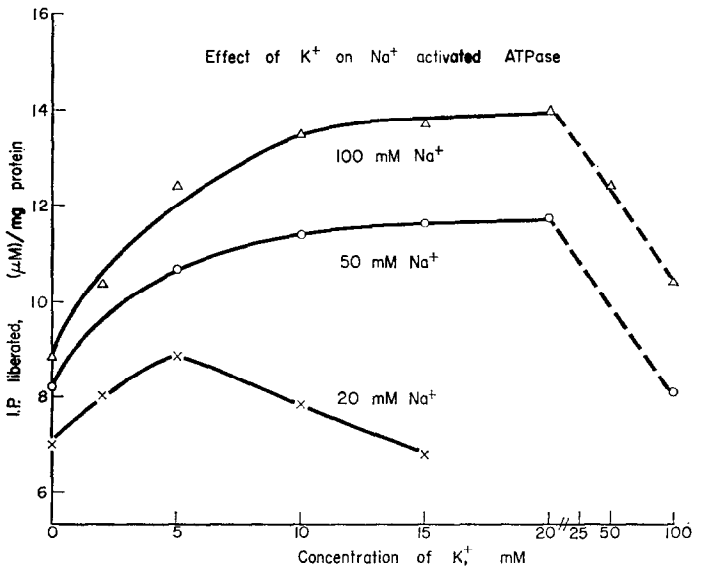

FIG. 2. The effect of K^+ on Mg^{2+}–Na^+-activated ATPase. Conditions of incubation same as Fig. 1. Average of 6 experiments.

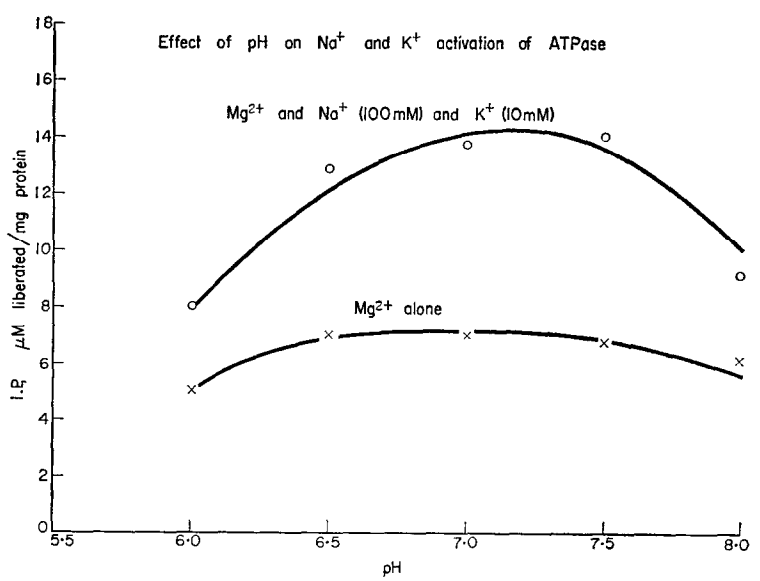

FIG. 3. The effect of pH on Mg^{2+}–Na^+–K^+-activated ATPase. Incubation medium: Tris-maleate buffer 0·125 M; $MgCl_2$ 3 mM; ATP 3 mM; NaCl 100 mM; and KCl 10 mM. Average of 5 experiments.

6·5 to 7·5 appears to be optimum for Na^+–K^+-activated ATPase. The pH of 6·7 was chosen in all following experiments.

The effect of Ca^{2+} on the microsomal ATPase

The Na^+–K^+-activated ATPase of this microsomal fraction is very sensitive to Ca^{2+}, and Ca^{2+} in a concentration of 1 mM completely inhibits the Na^+–K^+-activated ATPase without influencing Mg^{2+}-activated ATPase. However, in the absence of Mg^{2+}, the addition of Ca^{2+} alone increases ATPase activity. The degree of activation is dependent on the amount of Ca^{2+} added and this is shown in Fig. 4 and 5. The effect

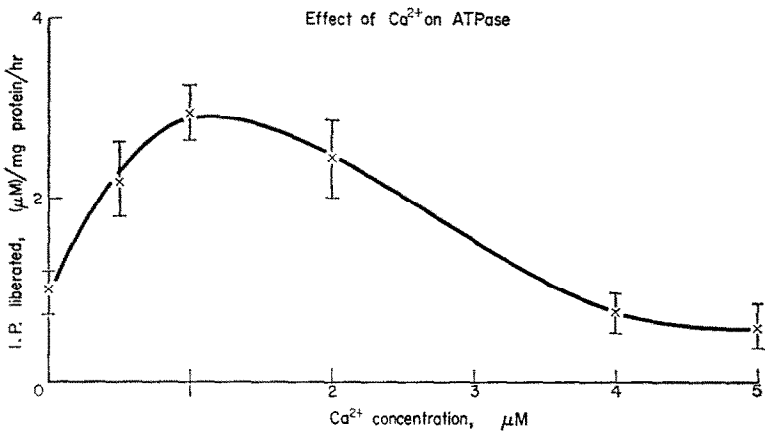

FIG. 4. The effect of low concentrations of Ca^{2+} on microsomal ATPase. Tris buffer 0·125 M; ATP 3 mM. Mean ± S.E. of 9 experiments. Ca^{2+} concentration indicates the amount of Ca^{2+} added.

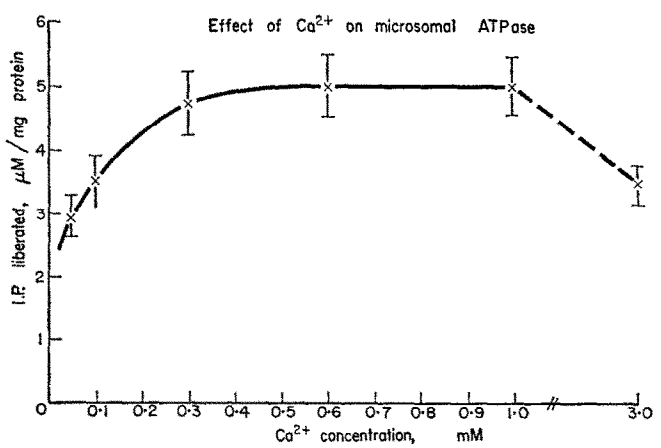

FIG. 5. The effect of high concentrations of Ca^{2+} on microsomal ATPase. Incubation medium same as Fig. 4. Mean ± S.E. of 9 experiments.

of Ca^{2+} on ATPase is diphasic. At the lower range of 1 to 2 μM (in Fig. 4), Ca^{2+} stimulates ATPase significantly, and then the further increase in Ca^{2+} concentration to 5 μM causes the disappearance of this Ca^{2+}-activation. Increasing the concentration of Ca^{2+} further does not influence ATPase until approximately 100 μM is reached. At this concentration of Ca^{2+}, the ATPase is beginning to be stimulated again, reaching maximum at around 0·5 mM (Fig. 5).

The ATPase activated at the higher concentrations (0·5 mM) of Ca^{2+} (high-Ca^{2+}-activated ATPase) is further stimulated by the addition of Na^+ but not by K^+. The effect of Na^+ on this high-Ca^{2+}-activated ATPase is shown in Fig. 6. The threshold

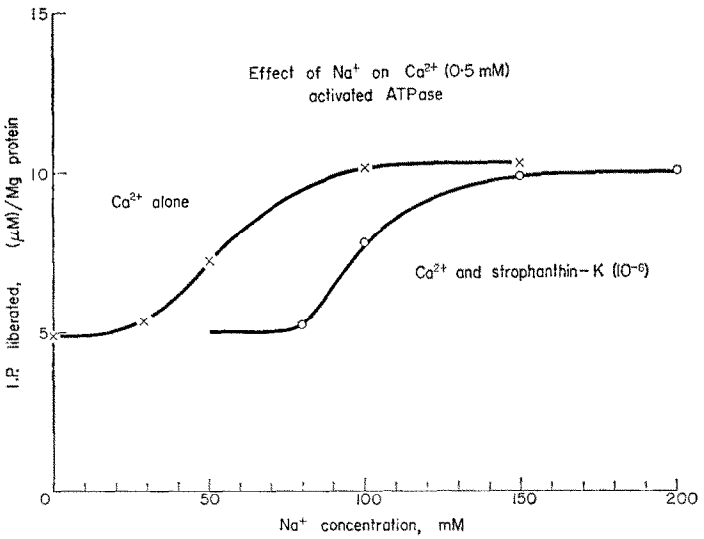

FIG. 6. The effect of Na^+ on Ca^{2+} (high concentration)-activated ATPase. Effect of strophanthin-K (10^{-6}) is also shown. Average of 8 experiments.

concentration of stimulating effect of Na^+ is approximately 50 mM, and at about 100 mM the effect of Na^+ reaches maximum. This Na^+-activated Ca^{2+} ATPase is not influenced by the addition of K^+ up to 100 mM.

Effect of cardiac glycosides

Ouabain and strophanthin-K were used to investigate the effect of cardiac glycosides. The effects of these agents were very similar; therefore, only that of strophanthin-K will be presented.

The cardiac glycosides are found to influence the Na^+–K^+-activated ATPase profoundly without having a significant effect on Mg^{2+}- or Ca^{2+}-activated ATPase. The effect of strophanthin-K on the Na^+-activated Mg^{2+} ATPase is shown in Fig. 1. As can be seen here, strophanthin-K in a concentration of 10^{-6} almost completely depresses the Na^+-activated ATPase when the Na^+ concentration is around 40 to 50 mM. However, upon increasing the Na^+ concentration, the inhibitory effect of strophanthin-K diminishes, and when the Na^+ concentration is increased to 300 mM, the inhibitory effect of strophanthin-K is almost completely reversed. The effect of

strophanthin-K on the Na^+–K^+-activated ATPase is shown in Fig. 7. Strophanthin-K at very low concentrations stimulates the Na^+–K^+-activated ATPase; however, at higher concentrations it inhibits the ATPase. Even at low concentrations of strophanthin-K, such as 10^{-11}, when the incubation time is prolonged more than 15 min, this initial stimulating effect disappears. Thus the initial stimulatory effect of strophanthin-K

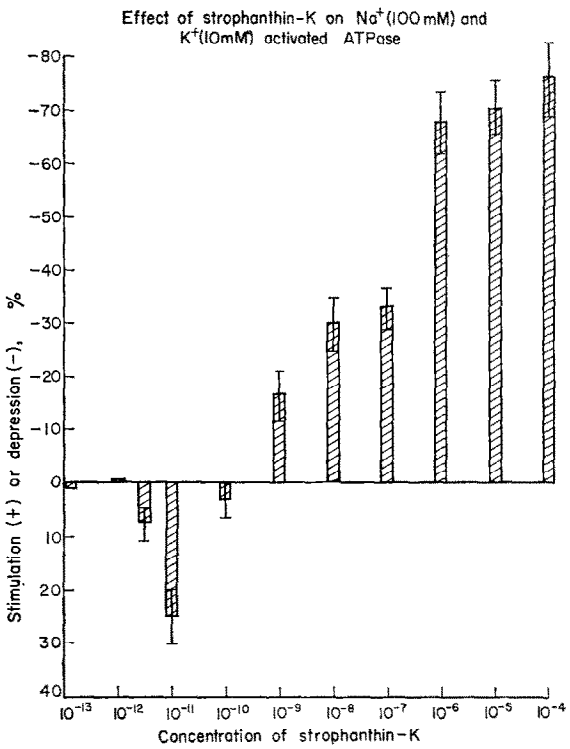

FIG. 7. The effect of strophanthin-K on Mg^{2+}–Na^+–K^+-activated ATPase. Mean ± S.E. of 9 experiments. Incubation medium same as in Fig. 3; pH 6·7.

appears to be transitory. At a concentration of 10^{-8}, strophanthin-K starts inhibiting the Na^+–K^+-activated ATPase considerably.

The inhibitory effect of strophanthin-K (10^{-6}) on the Na^+–K^+-activated ATPase was further investigated. As shown previously, strophanthin-K inhibits the Na^+-activated portion of ATPase (Fig. 1). However, when the Na^+ concentration is increased to 300 mM to overcome the inhibitory effect of strophanthin-K on Mg^{2+}–Na^+-activated ATPase, the cardiac glycoside inhibits completely the stimulating effect of K^+ (10 mM) on the Mg^{2+}–Na^+-activated ATPase.

The effect of strophanthin-K on the ATPase which is activated by the lower concentrations (such as 2 μM) of Ca^{2+} (low-Ca^{2+}-activated ATPase) is shown in Fig. 8. Here again a very low concentration of strophanthin-K (10^{-11}) appears to stimulate the low-Ca^{2+}-activated ATPase slightly (statistically not significant). As the concentration of strophanthin-K is increased, however, the marked depression of the low-Ca^{2+}-activated ATPase by strophanthin-K is observed. On the other hand, strophanthin-K

(up to 10^{-6}) has no effect on the high-Ca^{2+}-activated ATPase. However, the Na^+-activated portion of high-Ca^{2+}-activated ATPase is inhibited by strophanthin-K, as shown in Fig. 6. This inhibition of Na^+ activation by strophanthin-K in high-Ca^{2+}-activated ATPase is reversed by increasing the Na^+ concentration to 150 mM or more, as can be seen in Fig. 6.

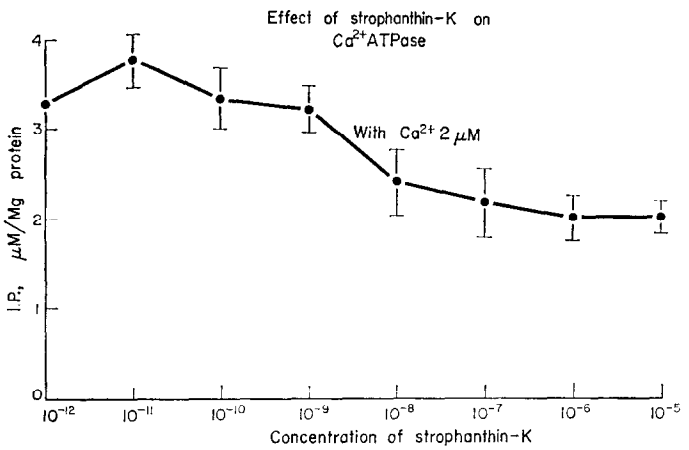

FIG. 8. The effect of strophanthin-K on low-Ca^{2+} (2 μM)-activated ATPase. Mean $\pm$ S.E. of 7 experiments.

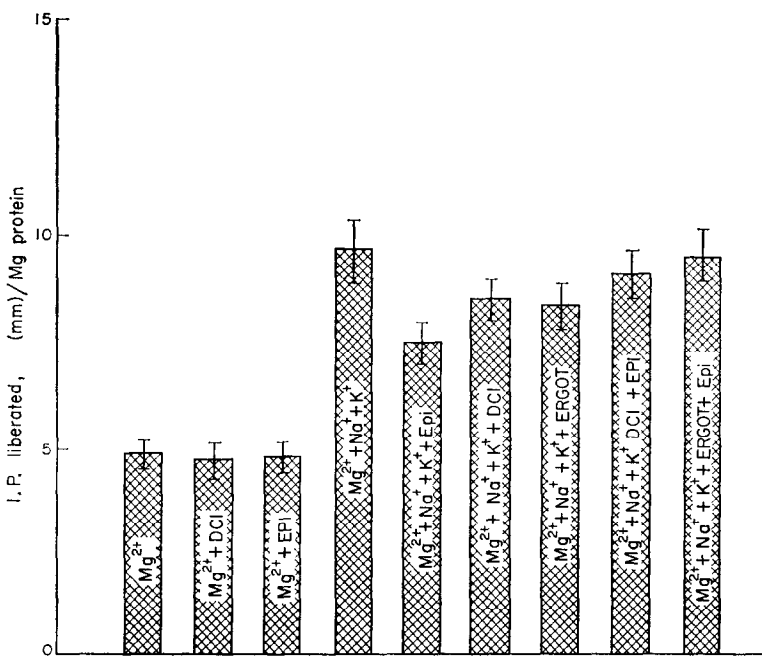

FIG. 9. The effect of epinephrine and its antagonists on Mg^{2+}–Na^+–K^+-activated ATPase. Concentrations: epinephrine 10^{-6}, ergotamine 10^{-5}, DCI 10^{-5}; Na^+ 100 mM, K^+ 10 mM. Mean $\pm$ S.E. of 6 experiments.

Effect of catecholamines and their pharmacological antagonists on ATPase

In view of the profound physiological action of catecholamines on heart muscle, effects of epinephrine and norepinephrine on the Na^+- and K^+-activated Mg^{2+} ATPase and the influence of pharmacological antagonists such as dichloroisoprotenerol and ergotamine were studied. The effects of epinephrine and its antagonists (DCI or ergotamine) are shown in Fig. 9. As can be seen here, epinephrine in a concentration of 10^{-6} depresses the Na^+–K^+-activated ATPase. Similarly, DCI and ergotamine depress the Na^+–K^+-activated ATPase. When both epinephrine and DCI are present, however, there is no significant depression of Na^+–K^+-activated ATPase (see Fig. 9). Similarly with ergotamine (10^{-5}) and epinephrine, each antagonizes the inhibitory effect of the other. Similar results are obtained with norepinephrine on one hand and DCI and ergotamine on the other.

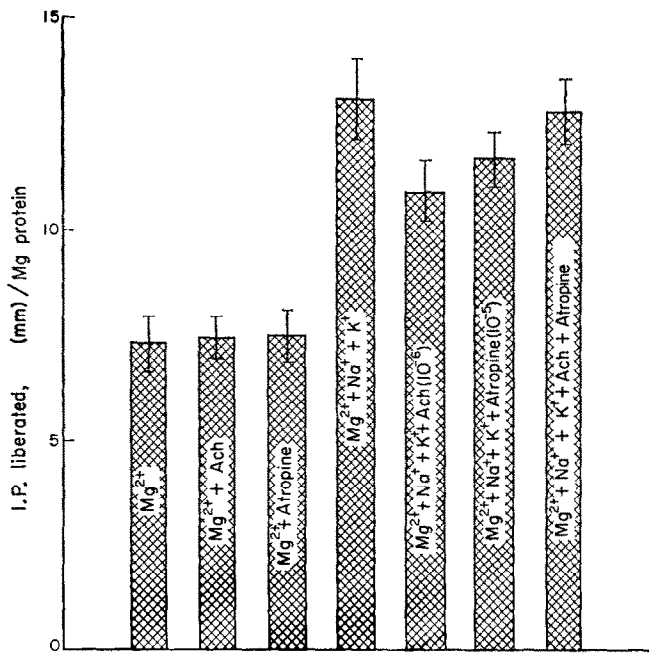

FIG. 10. The effect of acetylcholine and atropine on Mg^{2+}–Na^+–K^+-activated ATPase. Concentrations: acetylcholine 10^{-6}, atropine 10^{-5}; Na^+ 100 mM, K^+ 10 mM. Mean ± S.E. of 6 experiments.

Effect of acetylcholine and its pharmacological antagonist atropine

As can be seen in Fig. 10, when either acetylcholine (10^{-6}) or atropine (10^{-5}) alone is added to the reaction mixture, the Na^+–K^+-activated ATPase is depressed, but if acetylcholine and atropine are added together, no inhibition of the Na^+–K^+-activated ATPase is observed.

DISCUSSION

The localization and activity of the Na^+–K^+-activated Mg^{2+} ATPase

Previously the Na^+–K^+-activated ATPase was demonstrated in the microsomal fraction of brain, nerve, heart, and other tissues.[1–7,10] The present study is concerned with some aspects of characteristics of the Na^+–K^+-activated ATPase of the heart microsomal fraction. The electron microscopic examination of the present preparation shows that this fraction is composed mainly of fragments of cell membrane and the endoplasmic reticular system of smooth type which is similar to the sarcotubular system described by Muscatello *et al.*[15] In the present preparation, Na^+ alone increases ATPase activity in the presence of Mg^{2+}, and the degree of activation amounted to 70 to 80 per cent of Mg^{2+}-activated ATPase at Na^+ concentration of 120 mM. The total ATPase activity is approximately 10 μmoles IP liberated/mg protein per hr under this condition. The addition of K^+ further stimulates the Na^+-activated Mg^{2+} ATPase and brings the total ATPase activity to the level of 13 to 16 μmoles IP liberated/mg protein per hr at a concentration of 10 mM of K^+. The activation by K^+ is the function of concentration of Na^+ already present, and this suggests that the competition between K^+ and Na^+ exists for the site of enzyme.

The effect of Ca^{2+} on the microsomal ATPase

In the presence of Mg^{2+}, Ca^{2+} has been found to be a potent inhibitor of the Na^+–K^+-activated ATPase of the microsomal fraction. However, in the absence of Mg^{2+}, the addition of Ca^{2+} in the reaction mixture further increases the ATPase activity of this microsomal fraction. This activation by Ca^{2+} is diphasic. At low concentrations ranging from 1 to 2 μM, Ca^{2+} activates ATPase to the extent of one-third of Mg^{2+} activation. However, the increase of Ca^{2+} concentration to 5 μM results in the disappearance of this activating effect of Ca^{2+}. It should be noted, however, that the true concentration of Ca^{2+} in the medium at this range is unknown because of the contamination of the medium by Ca^{2+} which is present in the triple-distilled water and reagents used here. Upon further increasing Ca^{2+} concentration, the ATPase activity again begins to be activated at approximately 0·1 mM of Ca^{2+} and reaches maximum at 0·5 mM. Both microsomal ATPases activated by the higher and the lower concentrations of Ca^{2+} are stimulated by the presence of Na^+, but it requires rather high concentrations (50 mM or more) of Na^+ to have a significant stimulant effect. On the other hand, K^+ has no effect on these Ca^{2+} ATPases regardless of the presence or absence of Na^+ ion.

The effect of cardiac glycosides

It has been known that the cardiac glycosides are specific inhibitors of the Na^+–K^+-activated ATPase of membrane or microsomal fraction.[4,5,9] In view of the profound effect of cardiac glycosides on the ion transport and the subsequent loss of K^+ from the cell after the administration of these agents,[16–18] a considerable effort has been directed into the investigation of the relationship between this inhibitory effect on the Na^+–K^+-activated ATPase and the well-known positive inotropic effect of the cardiac glycosides on heart.[9,19–22] The present data further elucidate this problem. It is found that the effect of cardiac glycosides on the alkali metal-activated ATPase is of rather complicated nature. The effect of strophanthin-K on Mg^{2+}–Na^+–K^+-activated ATPase is diphasic, initial stimulation being by remarkably low concentrations (the

range of 10^{-11}) and the subsequent depression beginning with a higher concentration of 10^{-9}. This confirms the results obtained by Repke.[9] The initial stimulatory effect is very transient. Thus, if the incubation period is prolonged more than 15 min, even at a very low concentration of 10^{-11}, the stimulant effect is not observed. This is apparently due to the onset of depression at the later stage which masks the initial stimulation. It appears that the inhibitory effect of strophanthin-K on alkali metal-activated ATPase is due to the inhibition of both the Na^+-activated and K^+-activated portions of Mg^{2+} ATPase. As can be seen in Fig. 1, Na^+ activation is inhibited by the glycoside, and it appears to be of competitive nature since the inhibitory effect of the cardiac glycoside is reversed by increasing the Na^+ concentration to 300 mM. When the excess amount of Na^+ (300 mM) is present, strophanthin-K blocks the further stimulation of Mg^{2+}–Na^+-activated ATPase by K^+. Thus, both Na^+- and K^+-activated portions of Mg^{2+}-ATPase appear to be inhibited by the cardiac glycosides.

When Ca^{2+} is present alone, Na^+ also activates Ca^{2+}-ATPase but not K^+. This Na^+-activated ATPase in the presence of Ca^{2+} is also inhibited by the cardiac glycosides. Here again the inhibition is of competitive nature, and the increase of Na^+ concentration to 200 mM reverses the inhibitory effect of cardiac glycosides in both the low-Ca^{2+}-activated and the high-Ca^{2+}-activated ATPase. The more interesting effect of the cardiac glycosides is their effect on ATPase activity which is activated by lower concentrations of Ca^{2+}. Initially, the very small concentrations of the glycoside appear to have a slightly stimulating effect (see Fig. 8), but it is not statistically significant. The main action of strophanthin-K as well as ouabain on this low-Ca^{2+}-activated ATPase is the inhibition. In concentrations of 10^{-7} to 10^{-8}, strophanthin-K inhibits markedly the activating effect of the lower concentrations of Ca^{2+}.

The effect of catecholamines

It is noted that catecholamines, both epinephrine and norepinephrine, inhibit the Na^+–K^+-activated ATPase without influencing Mg^{2+}-activated ATPase. If the claim that this Na^+–K^+-activated ATPase is associated with the sodium pump mechanism in cellular membrane is correct, then this may mean that those catecholamines would facilitate the depolarization through the inhibitory means of the sodium pump, and this may be the basis for the potassium loss observed in muscle after the application of those amines.[23,24] It is to be noted that the sympathetic blocking agents such as DCI and ergotamine also have the depressant effect on the Na^+–K^+-activated ATPase. It is interesting, however, that no inhibition of Na^+–K^+-activated ATPase is observed when these pharmacological antagonists (sympathetic amines and blocking agents) are present together in the reaction medium.

Effect of acetylcholine and atropine

Since acetylcholine is shown to induce K^+ loss in heart muscle and the pharmacological antagonist atropine blocks this K^+ loss,[25–27] the effect of acetylcholine and atropine on the Na^+–K^+-activated ATPase of heart microsomal fraction is studied. The results in Fig. 10 show that both acetylcholine and atropine inhibit the Na^+–K^+-activated ATPase but that this inhibitory effect disappears when both acetylcholine and atropine are added together to the reaction mixture. This may suggest that the effect of these drugs on the microsomal fraction *in vitro* as presented here may have some bearing on the physiological action of these agents *in vivo*.

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