BBA 75134

STUDIES ON (Na^+-K^+)-ACTIVATED ATPase

XX. PROPERTIES OF (Na^+-K^+)-ACTIVATED ATPase IN RAT LIVER

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(Received November 27th, 1967)

SUMMARY

1. The ouabain-sensitive (Na^+-K^+)-activated ATPase enzyme system was present in rather low activity (0.37 mole/kg dry weight per h) in rat liver.

2. Pretreatment with 1.5 M urea decreased the Mg^{2+}-activated ATPase activity without significantly affecting the (Na^+-K^+)-ATPase activity, thus causing the relative activity of the latter to rise from 13 % to 37 %. This permitted to determine the properties of the (Na^+-K^+)-ATPase system with greater accuracy.

3. The (Na^+-K^+)-ATPase required for activation both Na^+ (K_m = 6 mM) and K^+ (K_m = 0.9 mM). Rb^+ could replace K^+ in activating the enzyme (K_m = 0.8 mM). Maximal activation of the (Na^+-K^+)-ATPase system required 2 mM Mg^{2+} at an ATP concentration of 2 mM.

4. The pH optimum for (Na^+-K^+)-ATPase was 7.3, while the Mg^{2+}-activated ATPase activity had a pH optimum of 8.7.

5. The optimal temperature for (Na^+-K^+)-ATPase and for Mg^{2+}-ATPase activity was 45°.

6. The (Na^+-K^+)-ATPase was inhibited by the digitalis glycoside ouabain (pI_{50} = 3.9) and the Erythrophleum alkaloid erythrophleine (pI_{50} = 5.1).

INTRODUCTION

In connection with a study of active cation transport in regenerating rat liver[1], it seemed to us necessary to examine the properties of the (Na^+-K^+)-activated ATPase ((Na^+-K^+)-ATPase) system of this organ. After SKOU[2] had first described this enzyme system in crab nerve, many other workers have shown it to be closely related to the cardiac glycoside-sensitive active cation transport system in a great variety of animal tissues. POST *et al.*[3] and DUNHAM AND GLYNN[4] demonstrated this for human erythrocyte membranes. In a systematic study of the presence of the (Na^+-K^+)-ATPase in all tissues known to have a cardiac glycoside-sensitive cation transport BONTING, CARAVAGGIO AND HAWKINS[5] found the enzyme system to be present in all 21 tissues from 10 species. In addition a quantitative correlation between active cation transport and (Na^+-K^+)-ATPase activity could be demonstrated in a number of tissues[6].

The occurrence of this enzyme system in rat liver has previously been reported[5,7,8], but the present paper gives for the first time a detailed account of its properties.

METHODS

Livers were removed from adult male Wistar rats of about 250 g, 3–3.5 months of age, immediately after sacrificing the animal by stunning and decapitation. Tissue preparation and assay of (Na^+-K^+)-ATPase activity were carried out as previously described (BONTING, CARAVAGGIO AND HAWKINS[9]) with the minor modification that all volumes used were 50-fold magnified. Aqueous homogenates (10 %, w/v) were prepared in Potter–Elvehjem tissue grinders. Aliquots of each homogenate were lyophilized at $-20°$ and stored at $-25°$ until use. For assay, the frozen-dried homogenates were reconstituted with distilled water to contain 8 mg original wet weight of tissue/ml. Medium A (complete) gave total ATPase activity, Medium B (no K^+), C (no Na^+), D (Medium A *plus* 10^{-4} M ouabain) and Medium E (no K^+, 10^{-4} M ouabain) gave Mg^{2+}-activated ATPase activity. Activity in Medium A *minus* the average activity in inhibitory Media B, C, D and E gave the (Na^+-K^+)-activated ATPase activity.

Treatment of the enzyme preparation with concentrated urea solution was performed as follows: after incubation for 15 min at 0° in the presence of 1.5 M urea, the homogenate was centrifuged for 1 h at 100000 $\times g$ to remove practically all of the urea. The precipitate was taken up in the required amount of water and after another period of 30 min at 0° was used for the (Na^+-K^+)-ATPase assay.

The Mg^{2+}-activation curve was obtained by varying the Mg^{2+} concentration in Media A and E from 0 to 6 mM, while maintaining the ATP concentration at 2 mM. The Na^+-activation curve was obtained by adding graded amounts of NaCl (0–150 mM) to Medium C. The K^+-activation curve was obtained by adding KCl (0–36 mM) to Medium B. In a similar way the Rb^+-activation curve was obtained by adding RbCl (0–36 mM) to Medium B. The pH–activity curves were obtained by preparing Media A and E with Tris–histidine buffers (final concentration of each compound: 50 mM) in the pH range from 6.0 to 7.4, and with Tris–HCl buffers (100 mM) in the pH range from 7.4 to 9.0. The pH of each resulting medium was measured and used in plotting the assay results. Inhibition curves for ouabain and erythrophleine were determined by adding the inhibitor (10^{-8}–10^{-2} M) to Medium A (5 mM K^+). The effect of temperature on activity was determined by measuring the ATPase activities in Media A and E at the given temperatures.

RESULTS AND DISCUSSION

The relative ATPase activities in the various inhibitory media are shown in Table I. There was an average 13 % inhibition of ATPase activity upon omission of Na^+ or K^+ or addition of 10^{-4} M ouabain, indicating that the inhibited ATPase activity represents the ouabain-sensitive, (Na^+-K^+)-activated ATPase. The inhibition in the Na^+-free Medium C was somewhat higher than in the other media. This is presumably due to a slight Na^+-sensitivity of the Mg^{2+}-activated ATPase, as previously observed in rabbit brain[12], rabbit kidney[12], rabbit ciliary body[12], cat choroid plexus[12,13], calf retinal rods[14], the salt gland of the herring gull[15], human leukocytes[16] and the rectal gland of the elasmobranchs[17]. Inhibition in the K^+-free Medium B was incomplete due to partial activation of the (Na^+-K^+)-activated ATPase by the small amount of tissue potassium present in the incubation mixture, since the half-maximal

TABLE I

RELATIVE ATPase ACTIVITY IN VARIOUS SUBSTRATE MEDIA

Composition of substrate media in mmoles per l (final concentration): Medium A: ATP, Na salt, 2; Mg^{2+}, 1; K^+, 5; Na^+, 58; CN^-, 10; EDTA, 0.1; Tris buffer, 92. Medium B: same as A, except K^+ replaced by Na^+. Medium C: ATP, Tris salt, 2; Mg^{2+}, 1; K^+, 9; CN^-, 5; EDTA, 0.1; Tris buffer, 147. Medium D: same as A, except 10^{-4} M ouabain present. Medium E: ATP, Na salt, 2; Mg^{2+}, 1; Na^+, 62; CN^-, 10; EDTA, 0.1; ouabain, 0.1; Tris buffer, 91. ATPase activity in Medium A (total ATPase activity) set at 100; data for Media B, D and E (means with standard errors from 18 determinations) and for Medium C (means with standard errors from 4 determinations) indicate the activity remaining upon inhibition of the (Na^+-K^+)-ATPase activity.

Medium	%
A (complete)	100
B (no K^+)	91.4 ± 0.5
C (no Na^+)	76.6 ± 0.9
D (10^{-4} M ouabain)	92.3 ± 0.4
E (no K^+, 10^{-4} M ouabain)	88.2 ± 0.4
Average of Media B, C, D, E	87.1 ± 3.6

activation concentration for K^+ was only 0.9 mM (Fig. 2). The relative insensitivity of the rat to ouabain (Fig. 7) would lead to incomplete inhibition by Medium D (10^{-4} M ouabain).

In normal rat liver the mean Mg^{2+}-ATPase activity in Media B, D and E was 3.63 ± 0.09 ($n = 18$) and the mean (Na^+-K^+)-ATPase activity 0.374 ± 0.017 ($n = 18$), both expressed in moles ATP hydrolyzed per kg dry weight per h at 37°. Since the relative (Na^+-K^+)-ATPase activity was rather low, and the (Na^+-K^+)-activated ATPase was determined by a differential assay, it is understandable that difficulties were encountered in studying the properties of the enzyme, *e.g.* Na^+- and K^+-activation, ouabain inhibition and pH-dependence. Therefore a pretreatment of the enzyme preparation with concentrated urea solution was performed. By this method, as described by GLYNN and collaborators[10] and by SKOU[11], the Mg^{2+}-ATPase activity decreased considerably, while the (Na^+-K^+)-ATPase activity did not change significantly. After treatment with 1.5 M urea the (Na^+-K^+)-ATPase activity in rat liver

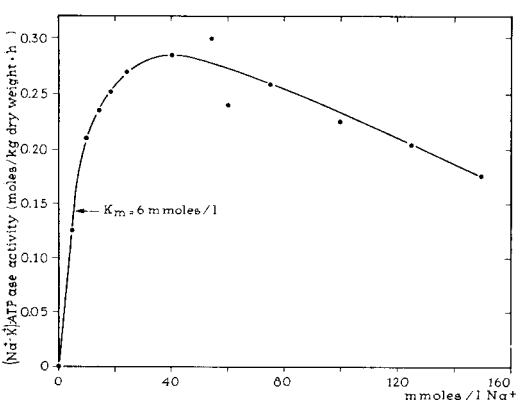

Fig. 1. Effect of Na^+ concentration on (Na^+-K^+)-ATPase activity in rat liver homogenate after urea treatment.

was 0.330 ($\pm$0.021) mole/kg dry wt. per h (n = 11) and the Mg^{2+}-ATPase activity was 0.451 ($\pm$0.100) mole/kg dry wt. per h (n = 11). The percentage $(Na^+\text{-}K^+)$-ATPase, related to total ATPase, was now 36.5 % ($\pm$3.7 %).

After treatment of the enzyme preparation with 0.1 % deoxycholate, according to AHMED AND JUDAH[8], for 30 min at 0°, the $(Na^+\text{-}K^+)$-ATPase activity did not significantly change, but the decrease in Mg^{2+}-ATPase activity was less than after pretreatment with urea. Also in view of the reported[8] decline in $(Na^+\text{-}K^+)$-ATPase activity by use of deoxycholate, pretreatment with urea was considered to be more convenient for our purposes.

Fig. 1 represents the Na^+-activation curve for the $(Na^+\text{-}K^+)$-ATPase system of rat liver. The K^+ concentration was kept constant at 5 mM. The activity reached a maximum at 40 mM Na^+. Half-maximal activation by Na^+ occurred at 6 mM Na^+, somewhat lower than in some other tissues[15,17].

The K^+-activation curve is given in Fig. 2. In this case, the Na^+ concentration was kept constant at 60 mM. Maximum $(Na^+\text{-}K^+)$-ATPase activity was reached at

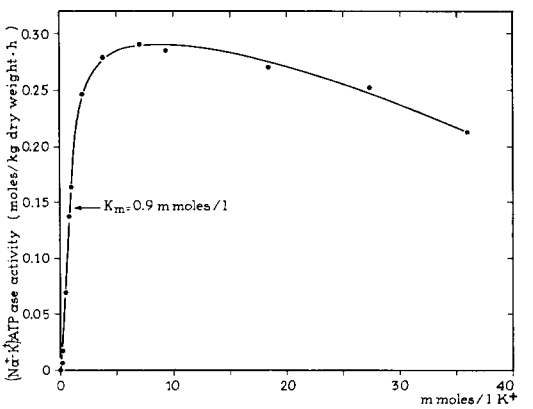

Fig. 2. Effect of K^+ concentration on $(Na^+\text{-}K^+)$-ATPase activity in rat liver homogenate after urea treatment.

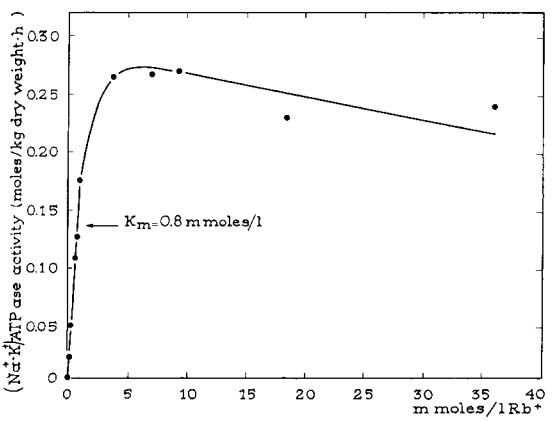

Fig. 3. Effect of Rb^+ concentration on $(Na^+\text{-}K^+)$-ATPase activity in rat liver homogenate after urea treatment.

5 mM K^+. Half-maximal activation by K^+ occurred at 0.9 mM K^+, as in most other tissues[9,15,17].

In studies of active transport Rb^+ is usually taken to behave identically with K^+, but this assumption may not be valid for all tissues, as pointed out in a recent report[18] on ion uptake in marine algae. Therefore it was necessary to compare the effect of Rb^+ on the (Na^+-K^+)-ATPase system with that of K^+. The Rb^+ concentration

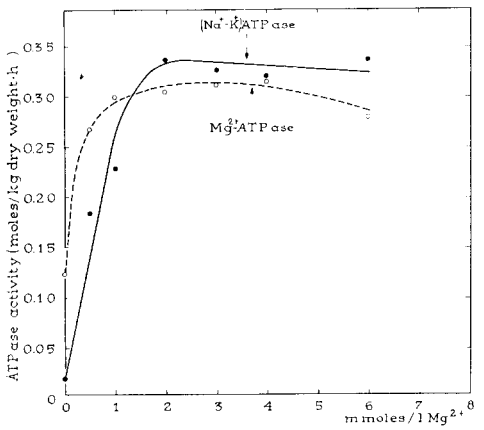

Fig. 4. Effect of Mg^{2+} concentration on Mg^{2+}-ATPase (○---○) and (Na^+-K^+)-ATPase (●—●) activities in rat liver homogenate after urea treatment.

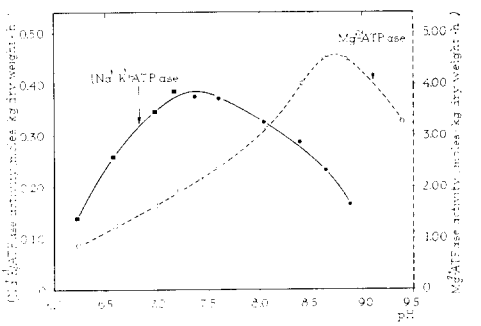

Fig. 5. Effect of pH on Mg^{2+}-ATPase (□ · · · □, Tris–histidine buffers; ○ · · · ○, Tris–HCl buffers) and (Na^+-K^+)-ATPase (■—■, Tris–histidine buffers; ●—●, Tris–HCl buffers) activities in rat liver homogenate. (Na^+-K^+)-ATPase activity was determined after urea treatment.

was varied from 0 to 35 mM in the absence of K^+, while the Na^+ concentration was kept constant again at 60 mM. Maximum activity was reached at 6 mM Rb^+ and half-maximal activation by Rb^+ occurred at 0.8 mM Rb^+ (Fig. 3). In rabbit lens[9] about the same K_m value for Rb^+ was observed. The activation by Rb^+ appears indeed to be very similar to that by K^+, and thus it seems to be justified in a study of active transport in rat liver to substitute Rb^+ for K^+.

Fig. 4 shows the effect of increasing the Mg^{2+} concentration from 0 to 6 mM, while keeping the ATP level constant at 2 mM. In the absence of added Mg^{2+} there was virtually no (Na^+-K^+)-ATPase activity, while the Mg^{2+}-ATPase activity was about 40 % of maximum. Maximal (Na^+-K^+)-ATPase activity was reached at 2 mM Mg^{2+}, *i.e.* at a Mg^{2+}/ATP ratio of about 1. The level of 1 mM Mg^{2+}, used in our standard incubation medium, gave 19 % suboptimal activity. Since our studies were essentially of a comparative nature, this was considered to be acceptable for our purposes.

The pH–activity curves for (Na^+-K^+)-ATPase and Mg^{2+}-ATPase are shown in Fig. 5. Since the determination of Mg^{2+}-ATPase activity at different pH values after treatment with 1.5 M urea gave variable results, the pH–activity curve for this enzyme system was obtained without urea pretreatment. The optimum for (Na^+-K^+)-ATPase was at pH 7.3, for Mg^{2+}-ATPase the optimum pH was at 8.7. These values agree very well with the optima for both enzyme systems, found in other tissues[9,15].

The effect of temperature on (Na^+-K^+)-ATPase and Mg^{2+}-ATPase activities is represented in Fig. 6. For both enzyme systems maximum activity was reached at

45°. This is in good agreement with the findings of SCHONER *et al.* in ox brain[19].

Fig. 7 shows the ouabain inhibition curve for (Na+-K+)-ATPase in the presence of 5 mM K+. Complete inhibition did not occur until at 10^{-2} M ouabain concentration. The negative logarithm of the half-maximal inhibition concentration was $pI_{50} = 3.9$. This curve demonstrates again the low sensitivity of the rat to ouabain, as previously established by REPKE, EST AND PORTIUS[20] in a study of digitalis action on rat heart muscle.

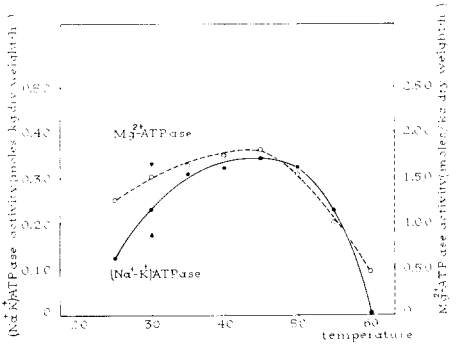

Fig. 6. Effect of temperature on Mg^{2+}-ATPase (○···○) and (Na+-K+)-ATPase (●—●) activities in rat liver homogenate. (Na+-K+)-ATPase activity was determined after urea treatment.

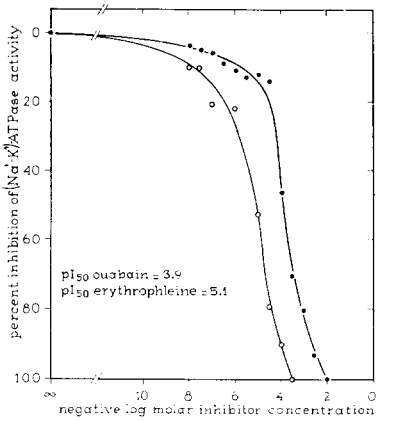

Fig. 7. Effect of ouabain (●—●) and erythrophleine (○—○) on (Na+-K+)-ATPase activity in rat liver homogenate after urea treatment. pI_{50} is the negative logarithm of the molar inhibitor concentration, causing half-maximal inhibition.

In view of the demonstrated similarity between the cardiac glycosides and the Erythrophleum alkaloids in their behavior towards (Na+-K+)-ATPase[12], the effect of erythrophleine was compared with that of ouabain (Fig. 7). Neither substance inhibited Mg^{2+}-activated ATPase, while (Na+-K+)-ATPase was inhibited for 48% by ouabain ($pI_{50} = 3.9$) and for 90% by erythrophleine ($pI_{50} = 5.1$) at 10^{-4} M concentrations. Thus erythrophleine is a more effective inhibitor than ouabain for rat liver, as was also found previously in other tissues. The difference in the pI_{50} for these two inhibitors was of the same order in all tissues: rabbit brain[12]: 0.6, cat choroid plexus[13]: 1.1, toad bladder[21]: 1.3, rat liver: 1.2.

The question arises whether the pretreatment with urea might change the enzymic properties of the (Na+-K+)-ATPase. In preliminary experiments, in which no urea treatment was applied, the same half-maximal inhibition concentration for ouabain was obtained as after urea treatment ($pI_{50} = 3.9$). The optimum pH for (Na+-K+)-ATPase without urea treatment was 7.3–7.4, very close to the value of 7.3 found after urea treatment. We may, therefore, conclude that the kinetic properties of the liver (Na+-K+)-ATPase were not significantly changed by the urea treatment.

ACKNOWLEDGEMENTS

The technical assistance of Miss E. D. KUHLMANN is gratefully acknowledged. Erythrophleine sulfate was a gift of E. Merck, A.G., Darmstadt, Germany.

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