Vol. 82, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 15, 1978 Pages 13-18

ACTIVATION OF Na^+-K^+-ADENOSINE TRIPHOSPHATASE BY SPERMINE

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Received March 13,1978

SUMMARY Spermine activated Na^+-K^+-ATPase when the concentrations of K^+ and ATP were low, whereas it inhibited K^+-dependent and ouabain-inhibitable monophosphatase. The activating effect of spermine was not due to the substitution for K^+ or Na^+. Excess K^+ inhibited Na^+-K^+-ATPase partially, and reduced the spermine activation. When 1 mM ATP was used, spermine at higher concentrations inhibited Na^+-K^+-ATPase, and did not activate at all. It is suggested that the K^+-sites essential to Na^+-K^+-ATPase and the K^+-phosphatase co-exist at different places of the enzyme.

It is believed that ouabain-inhibitable and K^+-dependent monophoaphatase and Na^+-K^+-ATPase(ATPphosphohydrolase EC.3.6.1.3) activities are derived from the same enzyme molecule (1,2). However, their relationship is still not well understood. Both enzyme activities in resealed red cell membranes require K^+ outside the cells (3). The enzyme preparation is phosphorylated by forming an acylphosphate bond from [^{32}P]acetylphosphate as well as from [^{32}P]-ATP (4). Ouabain inhibits the two enzyme activities. There is a similarity between the cation sensitivity of the K^+-phosphatase and the final dephosphorylation step in the reaction sequence of Na^+-K^+-ATPase. It is suggested that K^+-binding sites are common to the two enzyme activities.

Spermine inhibits specifically the K^+-phosphatase (5,6). We expected that spermine would also inhibit Na^+-K^+-ATPase. The inhibition of K^+-phosphatase by spermine is competitive with K^+. Mg^{2+} and Ca^{2+} as well as spermine seem to bind to the K^+-sites for the K^+-phosphatase (6,7). The effects of spermine on Na^+-K^+-ATPase activity should be observed with various concentrations of K^+ and

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Mg^{2+}. Recently, Heinrich-Hirsch et al. reported that spermine inhibited Na^+-K^+-ATPase in the presence of high concentrations of K^+, Na^+, Mg^{2+} and ATP (8). We observed an activation of Na^+-K^+-ATPase by spermine, when the concentrations of K^+ and ATP were low.

EXPERIMENTAL Plasma membranes were purified by the pH 9.0 treatment of microsomes from frozen rabbit kidneys as reported previously (7). Na^+-K^+-ATPase activity was measured in the presence of 1 mM $MgCl_2$, 0.5 mM KCl, 10 mM NaCl, 5 μM ATP, 20 μM EDTA, 0.05% mercaptoethanol and 25 mM Tris-HCl buffer, pH 7.5, with or without 0.2 mM ouabain in a final volume of 1.0 ml, unless otherwise specified. After incubation at 37° for 30 min, the released inorganic phosphate was determined by using the color reagent as described previously (9). The reaction was linear with time up to 30 min under these conditions. When the enzyme reaction medium contained 5 mM p-nitrophenylphosphate, the inorganic phosphate was determined by Fiske-SubbaRow method (10) in the presence of sodium dodecyl sulfate (11). K^+-phosphatase activity was assayed with 5 mM p-nitrophenylphosphate as the substrate. The released p-nitrophenol was determined by the procedure as described previously (7). Both Na^+-K^+-ATPase and K^+-phosphatase activities were abolished completely by the addition of 0.2 mM ouabain under the present experimental conditions. The two activities were calculated by subtraction of the activity in the presence of ouabain from the total activity. The activities were expressed as μmoles Pi or p-nitrophenol per mg protein per min. Protein concentrations were measured by the method of Lowry et al.(12) using albumin as standard.

RESULTS

When Na^+-K^+-ATPase activity was assayed under approximately optimal conditions with 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$ and 3 mM ATP at pH 7.5, spermine below 1.0 mM showed no effect on the activity (Data are not shown). One possibility is that high cation concentrations protect the activity from the effect of spermine. Another possibility is that ATP or Na^+ protects the activity from the spermine inhibition, since in the previous experiments (6) the assay mixture of K^+-phosphatase contained neither ATP nor Na^+.

Simultaneous Observation of Na^+-K^+-ATPase and K^+-Phosphatase. Na^+-K^+-ATPase and K^+-phosphatase were observed simultaneously in the presence of 0.5 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 0.1 mM ATP and 5 mM p-nitrophenylphosphate (Fig. 1-A). The addition of Na^+ and ATP

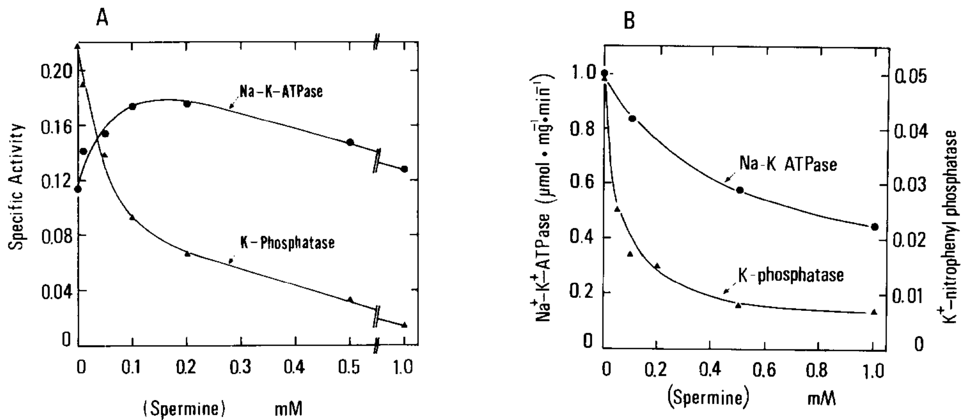

Fig. 1. Effect of spermine on Na^+-K^+-ATPase and K^+-phosphatase under the same conditions. The complete reaction mixture contained 8 μg of membrane proteins, 0.1 mM ATP (A) or 1.0 mM ATP (B), 5 mM p-nitrophenylphosphate and spermine at the indicated concentrations in 1 ml of the routine assay mixture described in EXPERIMENTAL. The K^+-phosphatase was measured following the appearance of p-nitrophenol. For estimation of Na^+-K^+-ATPase, Pi was determined at the end of the incubation, and Pi from ATP was calculated by subtracting the amount of the p-nitrophenol expressed as μmoles from the total amount of Pi. It was confirmed that the amount of liberated p-nitrophenol expressed as μmoles was the same as that of liberated Pi expressed as μmoles when ATP was absent from the incubation medium.

stimulated markedly the K^+-phosphatase as compared with the activity in the absence of them as reported previously (13). Spermine inhibited K^+-phosphatase, while it activated Na^+-K^+-ATPase. The spermine concentration required for 50% inhibition of K^+-phosphatase was approximately 70 μM which was similar to that obtained without Na^+ and ATP. The activation of Na^+-K^+-ATPase was maximum, 53%, at 0.1 mM spermine. In the presence of 1.0 mM spermine the ATPase activity was still stimulated by 12%, while the K^+-phosphatase was inhibited by 93%.

The activation of Na^+-K^+-ATPase by spermine was also observed in the absence of p-nitrophenylphosphate at low ATP concentrations (Fig. 2). In the concentration range of spermine which activates

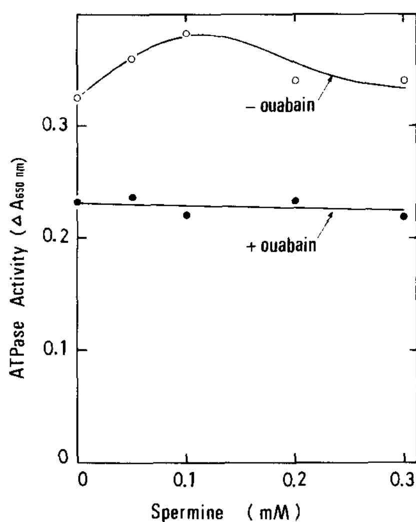

Fig. 2. Effect of spermine on the ATPase in the presence and absence of ouabain. The concentrations of ATP and spermine were 20 μM and 0.1 mM, respectively. The concentrations of other assay reagents were the same as those described in EXPERIMENTAL.

Na^+-K^+-ATPase, ouabain-insensitive ATPase activity was not affected by spermine.

K^+-Activation Curve in the Presence of Spermine. Effects of K^+ concentrations on Na^+-K^+-ATPase were investigated in the presence and absence of 0.1 mM spermine (Fig. 3-A). No activation by spermine was observed without K^+. This indicates that spermine did not substitute for K^+. The maximum Na^+-K^+-ATPase activity was obtained at approximately 1 mM K^+ both in the presence and absence of spermine. These suggest that the spermine-binding sites are different from the K^+-sites for the ATPase activity.

Without Na^+ no ouabain-inhibitable ATPase activity was observed. Increasing Na^+ up to 100 mM did not reduce the spermine activation (Fig. 3-B).

Effect of Spermine at an High Concentration of ATP. The properties of Na^+-K^+-ATPase with low ATP concentrations are different

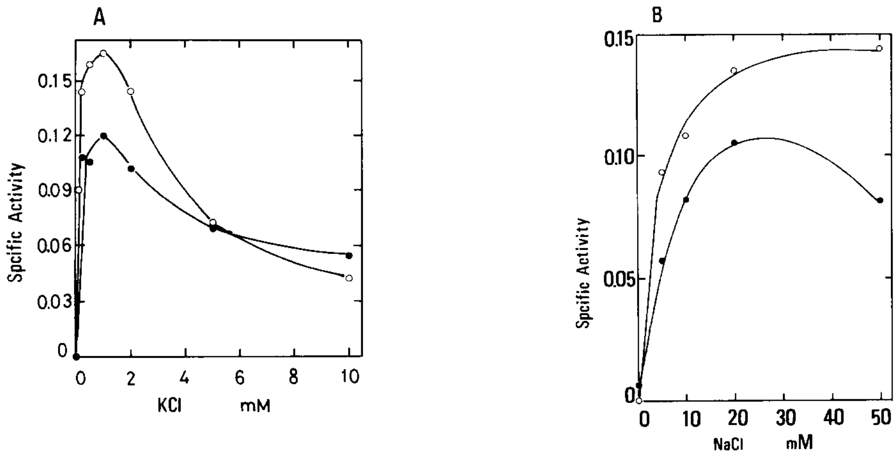

Fig. 3. Effect of K^+ or Na^+ on Na^+-K^+-ATPase in the presence and absence of spermine. Na^+-K^+-ATPase was assayed in the presence of various concentrations of KCl (A) or NaCl (B) with or without 0.1 mM spermine. The concentrations of other assay reagents were the same as those described in EXPERIMENTAL. (●)without spermine, (○)with spermine.

from those with its high concentrations. With μM concentrations of ATP there is a low level of Na^+-dependent and ouabain-inhibitable ATPase activity which is partially inhibited by K^+ (14), whereas at high ATP concentrations K^+ is essential to the ATPase activity. When 1 mM ATP was used without changing the concentrations of cations, spermine inhibited Na^+-K^+-ATPase (Fig. 1-B).

DISCUSSION

The results presented in this study demonstrate that the properties of K^+-sites required for Na^+-K^+-ATPase activity at low ATP concentrations was different from those required for K^+-phosphatase activity. Spermine activated Na^+-K^+-ATPase only in the simultaneous presence of K^+ and Na^+, while it inhibited K^+-phosphatase by competing with K^+. The spermine activation was reduced by excess K^+, and not by excess Na^+. Excess K^+ inhibited Na^+-K^+-ATPase partially. Spermine seems to block the sites for

the inhibitory K^+. The effective concentration ranges of spermine on the two enzyme activities were the same. The sites of spermine on Na^+-K^+-ATPase and K^+-phosphatase are probably common. Therefore, the K^+-sites essential to K^+-phosphatase activity may exist at a place different from that essential to Na^+-K^+-ATPase activity.

In the presence of low ATP concentrations where the spermine activation is observed, the ATP-sites for its regulatory action (Site I) are not saturated (Apparent Km=181 μM) (15). Then, the stable complex, K-E, is accumulated. The K-E complex is formed from the phosphorylated enzyme, E_2-P, which is hydrolyzed by K^+. If the K^+-sites of K-E differ from the K^+-sites of E_2-P, it is possible to explain the spermine activation by the stability of K-E, that is, spermine dissociates K^+ from K-E competitively and the enzyme is changed to the form reactive with ATP at low concentrations.

At high ATP concentrations K-E is not accumulated. The inhibition of Na^+-K^+-ATPase by high spermine concentrations can be explained by the competition of spermine with K^+ which hydrolyzes E_2-P.

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