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Regulation of Intermediary Phosphorylation of K^+-ATPase from Pig Gastric Mucosa by Sodium Ions *, **

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Vesicles from the microsomal fraction of gastric mucosa hydrolyze ATP with a concomittant K^+-dependent uptake of H^+.[1] Broken membranes derived from these vesicles contain a K^+-stimulated ATPase which is believed to constitute an integral part of the proton pump. In the presence of Mg^{2+} and ATP a phosphorylated form of the ATPase appears.[2] The extent of phosphorylation is reduced by K^+. In a recent report evidence was presented that the phosphoenzyme is an intermediate in the hydrolysis of ATP.[3] It was found also that Na^+ inhibited the K^+-stimulated hydrolysis of ATP. This study shows that already low concentrations of Na^+ effectively reduce the rate of formation of the phosphoenzyme intermediate.

Experimental. The Tris-salt of ATP was prepared as described previously.[4] [γ-^{32}P]ATP was a product of New England Nuclear. K^+-ATPase was prepared from the gastric mucosa of pig stomachs (fraction GII, Ref. 5). The ATPase activity was about 6 μmol (mg protein)$^{-1}$ h^{-1} at 21 °C in the presence of 5 μM ATP, 2 mM $MgCl_2$ and 10 mM KCl in 40 mM Tris-HCl buffer, pH 7.4. Phosphorylation experiments were carried out at 20 – 22 °C at 5 μM ATP by means of a rapid-mixing apparatus.[4] Maximal amount of phosphoenzyme was obtained by phosphorylation of the enzyme in this apparatus, or by calculation of the upper limit to which the experimental values extrapolated in a time-dependent study. Both methods gave maximally about 1.5 nmol per mg protein. Curve fitting of experimental data points was performed by the method of least squares on a Wang 600 calculator assuming first-order or pseudo first-order kinetics. The correlation coefficient was 0.997 or better in all experiments.

Results and discussion. In order to investigate further interactions of Na^+ with the K^+-ATPase, the rate of formation of the phosphoenzyme intermediate was studied at various

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** The abbreviations used are: K^+-ATPase, potassium-stimulated ATP phosphohydrolase; (Na^+,K^+)-ATPase, sodium plus potassium ion transport ATP phosphohydrolase.

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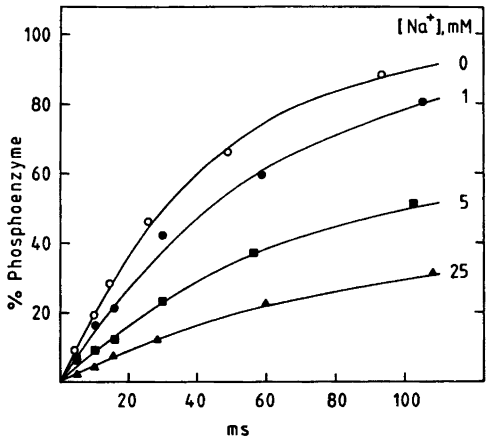

Fig. 1. Phosphorylation of K^+-ATPase in the presence of 5 μM ATP, 2 mM $MgCl_2$ in 40 mM Tris-HCl buffer, pH 7.4, and at various concentrations of NaCl.

concentrations of Na^+. K^+-ATPase was incubated with 5 μM [γ-^{32}P]ATP and 2 mM $MgCl_2$ in 40 mM Tris-HCl buffer, pH 7.4 (Fig. 1). Inclusion of 1 mM NaCl in the incubation medium reduced the rate as well as the extent of phosphorylation. A further increase of the concentration of Na^+ resulted in a progressive reduction of the rate and extent of phosphorylation. The pseudo first-order rate constant of the phosphorylation was reduced to 72 % at 1 mM Na^+, and to about 59 and 46 % at 5 and 25 mM Na^+, respectively (Fig. 2).

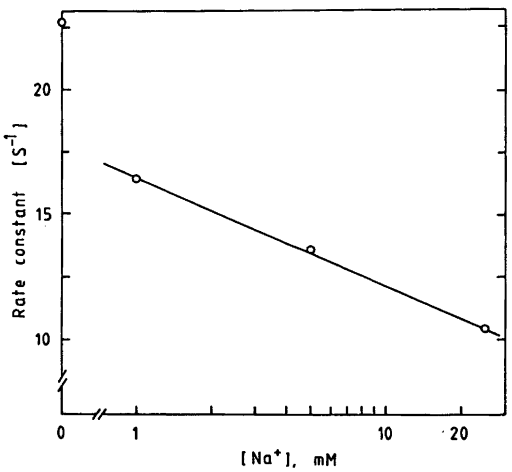

Fig. 2. Dependence of the pseudo first-order rate constant of the phosphorylation of K^+-ATPase on the concentration of Na^+.

These results indicate that Na^+ has a regulatory role in the K^+-stimulated ATPase reaction by an inhibitory effect on the intermediary phosphorylation. Since the function of the K^+-ATPase is essential for the acid secretion in the stomach,[1] Na^+ may also have a regulatory role in the production of acid. In unstimulated normal cells intracellular concentrations of 35 ans 23 meq Na^+/kg have been reported.[6,7] Depletion of the ATP in the gastric mucosa by treatment with 2,4-dinitrophenol increases the Na^+ content of the tissue.[7] From the present investigation it is not possible to determine from which side of the membrane Na^+ inhibits the K^+-stimulated ATPase. The results, however, infer an important regulatory function of the Na^+-pump (Na^+,K^+-ATPase) in the regulation of gastric ion secretion. In addition, the results show the importance of having a strict control of the Na^+ concentration in kinetic studies on the K^+-ATPase.

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