The effects of Na⁺ on gastric H,K-ATPase were investigated using leaky and ion-tight H,K-ATPase vesicles. Na⁺ activated the total ATPase activity in the absence of K⁺, reaching levels of 15% relative to those in the presence of K⁺. The Na⁺ activation, which takes place at the luminal side of the membrane, depended on the ATP concentration and the type of buffer used. The steady-state ATP phosphorylation level, studied with leaky vesicles, was reduced by Na⁺ due to both activation of the dephosphorylation reaction and a shift to E₂ in the E₁↔E₂ equilibrium. By studying this equilibrium in ion-tight H,K-ATPase vesicles, it was found that Na⁺ drives the enzyme via a cytosolic site to the nonphosphorylating E₂ conformation. No H⁺-like properties of cytosolic Na⁺ could be detected. We therefore conclude that Na⁺ behaves like K⁺ rather than like H⁺ in the H,K-ATPase reaction.

H,K-ATPase is an intrinsic membrane protein complex, which is located in the secretory vesicles of the gastric parietal cell and is able to generate a proton gradient of 10⁶ across the membrane in exchange for potassium. Na,K-ATPase, present in plasma membranes of all mammalian cells, is responsible for the maintenance of the intracellular levels of potassium and sodium and can generate a Na⁺ gradient of about 10¹-². The catalytic α-subunits of both ATPases have been cloned from several species and have a molecular mass of 112–114 kDa (1, 2). The homology between these two ATPases is higher than with other transport ATPases (3, 4). Na,K-ATPase and H,K-ATPase have additionally in common that they both contain a phosphorylated at an aspartyl residue, (ii) they are inhibited by sub-micromolar vanadate concentrations, and (iii) two different enzyme conformations (E₁ and E₂) can be distinguished. The E₁ form has high-affinity cation binding sites at the cytosolic side, whereas the E₂ form has high affinity cation binding sites at the luminal side of the membrane. The Post-Albers scheme, which is based on the alternate formation of these two conformations, is often used to explain the reaction mechanisms of both Na,K-ATPase and H,K-ATPase. In the overall H,K-ATPase reaction cycle (7, 8), where H⁺ is transported from the cytosol to the lumen in exchange for K⁺ (steps 1–7, Fig. 1), different partial reactions can be distinguished such as (i) the steady-state ATP phosphorylation reaction (steps 2, 3, and 4), (ii) the dephosphorylation reaction (steps 5 and 6), and (iii) the E₁↔E₂ transition (steps 7 and 1).

Due to the common characteristics the ion specificities of the two ion transporting enzymes have been studied intensively. Proton-like effects of sodium on H,K-ATPase (a "Na,K"-ATPase activity) (9) and sodium-like effects of protons on Na,K-ATPase (a "H,K"-ATPase activity) have been claimed (10), although the latter effects were not found when ATP phosphorylation was studied (11). In Na,K-ATPase, Na⁺ shows, besides effects of its own, K⁺-like properties in the absence of K⁺. This Na₉Na⁺ATPase or Na₉ATPase activity is the result of activation of both the ATP phosphorylation, and the dephosphorylation reaction by Na⁺ (12). The data regarding the effects of Na⁺ on H,K-ATPase is somewhat confusing. In some H,K-ATPase studies an identical activation by Na⁺ of the ATP hydrolysis reaction, a H₉Na₉ATPase activity, has been observed (13). Similar K⁺-like effects of Na⁺ have been found on the rate of ATP phosphorylation (13–15), but the dephosphorylation reaction has been claimed to be either activated (16), or insensitive toward Na⁺ (15). Furthermore, two studies (9, 17) indicate that Na⁺ behaves more like H⁺ and drives the enzyme to an E₁ conformation.

In preliminary experiments De Jong (18) observed that the K₀₉ for ATP in the phosphorylation reaction was considerably increased by Na⁺. Such an effect of Na⁺ cannot easily be explained when Na⁺ behaves like H⁺. It could be explained, however, when Na⁺ behaves as a K⁺ analog. In that case the ion activates the dephosphorylation reaction and drives the enzyme into the E₂ conformation.

With the use of ion-tight H,K-ATPase vesicles, where no activation of the dephosphorylation process by extravascular cations can occur (19), and by comparing their properties with those of leaky vesicles, where such activation does occur, the effects of Na⁺ on the total ATPase reaction, the steady-state ATP phosphorylation level, the dephosphorylation reaction, and the E₁↔E₂ transition were investigated. The results show that Na⁺ displays K⁺-like actions under these reaction conditions, thus activating the dephosphorylation process at the luminal side of the membrane and driving the enzyme into an E₂ conformation by interacting at the cytosolic side.

MATERIALS AND METHODS

H,K-ATPase Preparations—Gastric H,K-ATPase was purified from pig gastric mucosa as reported previously (20). Fresh (ion-tight) H,K-ATPase vesicles were collected at the 0.25 M sucrose interface and stored at 4 °C. Leaky vesicles were prepared by diluting these ion-tight vesicles in 20 mM Tris acetate (pH 7.0), followed by centrifugation (100,000 x g), resuspension in water and freeze drying. This preparation was stored at -20 °C for up to 1 year. The ion-tight H,K-ATPase vesicles were collected at the 0.25 M sucrose, 7% Ficoll (w/v) interface and stored at 4 °C. Leaky vesicles were prepared by diluting these ion-tight vesicles in 20 mM Tris acetate (pH 7.0), followed by centrifugation (100,000 x g), resuspension in water and freeze drying. This preparation was stored at -20 °C for up to 1 year.

Protein Determination—Protein was determined with the Bio-Rad protein assay (21) using bovine serum albumin as a standard. All data was expressed in Lowry protein values which are 1.5 times higher than

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* This work was sponsored by Netherlands Foundation for Scientific Research (NWO) Grant 900-522-068. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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ATPase activities were determined with a radiochemical method. For incubation for 1-30 min at 37 °C the reaction was stopped by adding

to this buffer, contained 6

of H,K-ATPase was added to 100

and 20 mM Tris acetate (pH 7.0), was totally insensitive to NaCl. All other chemicals were of analytical grade.

**RESULTS**

The Effect of NaCl on the Steady-state ATP Phosphorylation Level—Fig. 2A shows the combined effects of ATP and NaCl on the steady-state ATP phosphorylation level of gastric H,K-ATPase. At 22 °C and pH 7.0, in the absence of NaCl the ATP affinity was very high, the K_{0.5} being about 0.01 μM (19). Upon increasing the [Na⁺], the ATP affinity decreased. The maximal phosphorylation level tended to be slightly reduced at higher [Na⁺], indicating that the inhibition is not simply a competition between Na⁺ and ATP. If the effect of Na⁺ on the steady-state ATP phosphorylation was compared with its effect on the overall ATP hydrolysis rate, at 37 °C, and in the presence of 20 μM ATP (Fig. 2B), it was observed that, whereas the ATP phosphorylation decreased (I_{50} = 150 μM) the ATPase activity was activated at low K_{0.5} (14 μM), and inhibited at high NaCl concentrations. These observations suggest that the rate-limiting step in the reaction cycle changes at the different [Na⁺] tested. From the data of Fig. 2B the turnover number of the enzyme can be calculated using the equation ν = k ([E-P]), where ν represents the rate of ATP hydrolysis and k the dephosphorylation rate constant. NaCl increased this turnover number up to a value of 350 min⁻¹ in the presence of 60 mM NaCl. This value is about 15% of the maximal activity obtained in the presence of 1 mM ATP and 10 mM K⁺ (19). At high concentrations of NaCl (>100 mM) the turnover number decreased again. The stimulatory effects of Na⁺ suggest that this ion, like K⁺, increases the dephosphorylation rate, which is the rate-limiting step in the H,K-ATPase reaction cycle under normal conditions.

The Effect of Ouabain and SCH 28080 on the Na⁺ Activation—The Na⁺-activated ATP hydrolysis rate, measured at 37 °C in the presence of 50 mM NaCl, 20 μM ATP, 0.12 mM MgCl₂, and 20 mM Tris acetate (pH 7.0), was totally insensitive toward the specific Na⁺,K⁺-ATPase inhibitor ouabain, indicating that the activation of the ATP hydrolysis is not due to contamination with Na⁺,K⁺-ATPase. Moreover, the specific H,K-ATPase inhibitor SCH 28080 inhibited under these conditions the ATP hydrolysis by nearly 95% (I_{50} = 0.08 μM). Either in the presence of 1 mM KCl instead of 50 mM NaCl, or in the presence of both NaCl and KCl the I_{50} value for SCH 28080 increased to 0.2 μM, probably due the antagonism between SCH 28080 and K⁺. Although ouabain (1 mM) did not change the inhibition profile of SCH 28080, it was included in most experiments to ensure that any contaminating Na⁺,K⁺-ATPase activity was blocked. Corrections for the basal Mg-ATPase activity, which is the activity in the absence of K⁺ or Na⁺ and in the presence of 0.1 mM SCH 28080, were also made.

**Comparison of the Na⁺- and K⁺-activated ATPase Activity of H,K-ATPase—**The properties of the overall H,K-ATPase activity depend on the conditions in which the assay is performed (19). Fig. 3A shows that the maximal H,K-ATPase activity and the degree of K⁺ activation of the ATP hydrolysis depend on the ATP concentration. At low ATP concentrations (5 μM), K⁺ activation occurred with a K_{0.5} value of 0.04 μM. The activity obtained with optimal K⁺ concentrations (0.4 mM) was about 20% of the maximal activity obtained with 0.5 mM ATP and 5 mM K⁺. At an ATP concentration of 5 mM the K_{0.5} value for K⁺...
Fig. 2. The effect of Na+ on the steady-state ATP phosphorylation level and on the ATPase activity. A, the combined effect of Na+ and ATP on the steady-state ATP phosphorylation level. A leaky H,K-ATPase preparation (0.012–200 μg/80 μl) was preincubated at 22 °C in the presence of 50 mM Tris acetate (pH 7.0), 0.1 mM MgCl₂, 0.2 mM ouabain, and 12.5, 25, 62.5, 125, and 250 mM NaCl. After 20 min the steady-state ATP phosphorylation level was determined by incubating for 5 s with 0.006–80 μM [γ-32P]ATP (20 μl). The ATP phosphorylation level (nmol of E-P per mg of protein) at the different [NaCl] and [ATP] present during the phosphorylation period is plotted. B, comparison between the effects of Na+ on the steady-state ATP phosphorylation level and the ATP hydrolysis rate. H,K-ATPase (0.01 mg/ml (□) and 0.125 mg/ml (△)) was incubated at 37 °C in the presence of 20 μM [γ-32P]ATP, 0.12 mM MgCl₂, 50 mM Tris acetate (pH 7.0) and NaCl as indicated. After 3 (□) or 120 (△) s the reactions were terminated and the steady-state ATP phosphorylation level (O, nmol E-P per mg protein) or the ATPase activity (®, μmol of ATP hydrolyzed per mg of protein/h) were determined as described under "Materials and Methods."

Fig. 3. The effect of KCl and NaCl on the H,K-ATPase activity at varying concentrations of ATP. A leaky H,K-ATPase vesicle preparation (1–400 μg/ml) was incubated for 1–10 min at 37 °C in the presence of 30 mM Tris-HCl (pH 7.0), 5–5000 μM [γ-32P]MgATP, 0.1 mM MgCl₂, 0.1 mM ouabain, and the KCl (A) or NaCl (B) concentrations as indicated. Maximally 30% of the ATP was converted. Activity is given as μmol of ATP hydrolyzed per mg of protein/h.

was 1.0 mM, which is 25 times the value at 5 μM ATP. High concentrations of K+ inhibited the ATP hydrolysis rate, the inhibition occurring at lower [K+] when less ATP was used.

The activation of the ATPase activity by Na+ depended, like the K⁺-activation, on the ATP concentration (Fig. 3B). In the presence of 5 μM ATP we observed a Kₐ for Na⁺ of 10–20 mM while in the presence of 5 mM ATP this value increased to about 100 mM. The maximal Na⁺-ATPase activity reached levels of about 15–25 μmol of ATP hydrolyzed/mg of protein per h, which is about 15% of the activity obtained in the presence of K⁺.

The Affinity for ATP in the Na⁺-activated ATPase Reaction—The data of Fig. 3B show that the maximal Na⁺-ATPase activity hardly changed with the concentrations of ATP used. This indicates that the affinity for ATP is far below 5 μM. In the presence of 20 mM NaCl only one (high) affinity ATP site could be detected, Kᵣ for ATP = 0.25 μM. High concentrations of ATP did not increase the Na⁺-ATPase activity further as they did in the presence of KCl. The inhibitory action at high cation concentrations apparently overruled the activation process.

Effect of the K⁺-antagonist Imidazole on the Na⁺-ATPase Activity—Imidazole, a tertiary amine, is an antagonist of the activation by K⁺ of the H,K-ATPase activity (19). In the presence of 20 μM ATP, 0.12 mM Mg²⁺ and 50 mM imidazole/acetate (pH 7.0), a Kᵣ for Na⁺ of 80 mM for Na⁺ was determined. This value is about five times higher than in the presence of 50 mM Tris acetate (see Fig. 3B). So, there is an antagonism between tertiary amines and Na⁺, which is similar to the antagonism between tertiary amines and K⁺.

The Specificity of the Na⁺ Activation—In order to test whether the activating effect of Na⁺ is due to Na⁺ itself or to a contamination by K⁺, two different types of experiments were carried out. First, the amount of K⁺ in the different media was determined with a flame photometer. The 125 mM NaCl stock solution used for most experiments contained only 4.8 μM K⁺.
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The extra addition of K⁺ to NaCl media was completely recovered, indicating that high [Na⁺] did not disturb the K⁺ determination. Since K⁺ at these concentrations had hardly any effect, this finding already suggests that the Na⁺ effect is not due to contaminating K⁺.

Second, with the use of the K⁺ ionophores, valinomycin and nigericin, the K⁺ activation of ATPase activity was studied in ion-tight vesicles. In this type of H,K-ATPase vesicles the K⁺ activation site is located intravesicularly (20). Fig. 4A shows that in these vesicles the basal (Mg-ATPase) activity was very low and that activation by extravesicular (cytosolic) K⁺ was not possible. In the presence of nigericin, a K⁺ for H⁺ exchanger, the K⁺ activation profile was nearly identical to that of a leaky H,K-ATPase preparation, in which the K⁺ activation site is freely accessible (Fig. 3A). In the presence of the specific K⁺ ionophore valinomycin, however, there was only a slight activation, probably due to the ionophore-induced voltage difference across the vesicle membrane (23). The lack of activation could partially be overcome by the extra addition of the protopore valve.

The Effects of Na⁺ and K⁺ on the Dephosphorylation Reaction—The dephosphorylation reaction was studied in leaky H,K-ATPase preparations. Fig. 5A shows that both Na⁺ and K⁺, but not choline chloride, reduce the phosphorylation level at suboptimal ATP concentrations. In the presence of either 0.4 mM K⁺ or 35 mM Na⁺ (ratio Na⁺/K⁺ = 88) the amount of phosphoenzyme obtained was reduced by 50%. Other related monovalent cations such as Tl⁺, Rb⁺, NH₄⁺, Cs⁺, and Li⁺ had I₅₀ values of 0.005, 0.4, 0.5, 20, and 65 mM, respectively. This result indicates that Na⁺, like K⁺ and the other monovalent cations, drives the enzyme to the E₂ configuration.

In the absence of any H⁺-like properties of Na⁺ under these conditions. Na⁺ only led to a decrease in the steady-state ATP phosphorylation level (19), as only the Na⁺ enzyme level at suboptimal ATP concentrations, demonstrating the absence of any H⁺-like properties of Na⁺ under these conditions. Na⁺ only led to a decrease in the steady-state phosphorylation level in a preparation in which both cytosolic and luminal ion binding sites are accessible. In intact H,K-ATPase vesicles no enhancement of this process was observed, showing that the Na⁺-activated dephosphorylation rate was also reduced in the presence of ATP (data not shown).

E₁-E₂ Transition—In closed vesicles no activation of the luminal K⁺ (or Na⁺)-site by extravesicular ligands can occur, see above and Ref. 20. At low ATP concentrations it is feasible to study the effects of these ligands on the E₁-E₂ transition by determining the steady-state ATP phosphorylation level (19), as only the E₁ enzyme can be phosphorylated by ATP. Fig. 5B shows that both Na⁺ and K⁺, but not choline chloride, reduce the phosphorylation level at suboptimal ATP concentrations. In the presence of both 0.4 mM K⁺ or 35 mM Na⁺ (ratio Na⁺/K⁺ = 88) the amount of phosphoenzyme obtained was reduced by 50%. Other related monovalent cations such as Tl⁺, Rb⁺, NH₄⁺, Cs⁺, and Li⁺ had I₅₀ values of 0.005, 0.4, 0.5, 20, and 65 mM, respectively. This result indicates that Na⁺, like K⁺ and the other monovalent cations, drives the enzyme to the E₂ configuration.

E₁-E₂ Studies at pH 8.0—When the extravesicular proton concentration in these experiments was reduced 10 times, by changing the pH to 8.0, the affinity for ATP in the steady-state ATP phosphorylation reaction decreased, Kₐ₅₀ = 0.2 μM (Fig. 6) compared to 0.01 μM at pH 7.0 (see Fig. 2A, Ref. 19). Fig. 6 shows, in addition, that Na⁺ did not increase the phosphoenzyme level at suboptimal ATP concentrations, demonstrating the absence of any H⁺-like properties of Na⁺ under these conditions. Na⁺ only led to a decrease in the steady-state phosphorylation level (I₅₀ values for Na⁺ were 6, 18, and 40 mM in the presence of 0.2, 2, and 20 μM ATP, respectively), underlining once more the ATP/Na⁺ antagonism.

**FIG. 4.** The effect of ionophores on the ATPase activity in ion-tight H,K-ATPase vesicles in the presence of varying concentrations of either K⁺ or Na⁺. Ion-tight H,K-ATPase vesicles (60 μg/ml) were preincubated at room temperature in 20 mM Tris acetate (pH 7.0) and 250 μM sucrose with 100 μM of the ionophores: nigericin (☐), valinomycin (□), valinomycin plus CCCP (●), or with 2% ethanol (■) as a control. After 8 min 10 μl of the enzyme suspension was mixed with 80 μl of different KCl or NaCl solutions and incubated for 2 min at 37 °C. The K⁺- or Na⁺-dependent ATPase activity was determined by incubating another 2 min with 10 μl of 50 μM [γ-32P]ATP and 0.15 mM MgCl₂ (final concentrations) in 250 mM sucrose, 20 mM Tris acetate. The total ATPase activity, including the basal Mg-ATPase activity is plotted as function of the KCl (A) or NaCl (B) concentrations present during the ATPase assay. Activity is given as μmol of ATP hydrolyzed per mg of protein/h.

**FIG. 5.** The effects of Na⁺ (Fig. 5A) and K⁺ (Fig. 5B) on the ATPase activity in ion-tight H,K-ATPase vesicles in the presence of Na⁺ or K⁺. Ion-tight H,K-ATPase vesicles (60 μg/ml) were preincubated at room temperature in 20 mM Tris acetate (pH 7.0) and 250 μM sucrose with 100 μM of the ionophores: nigericin (☐), valinomycin (□), valinomycin plus CCCP (●), or with 2% ethanol (■) as a control. After 8 min 10 μl of the enzyme suspension was mixed with 80 μl of different KCl or NaCl solutions and incubated for 2 min at 37 °C. The K⁺- or Na⁺-dependent ATPase activity was determined by incubating another 2 min with 10 μl of 50 μM [γ-32P]ATP and 0.15 mM MgCl₂ (final concentrations) in 250 mM sucrose, 20 mM Tris acetate. The total ATPase activity, including the basal Mg-ATPase activity is plotted as function of the KCl (A) or NaCl (B) concentrations present during the ATPase assay. Activity is given as μmol of ATP hydrolyzed per mg of protein/h.

**FIG. 6.** The effect of ionophores on the ATPase activity in ion-tight H,K-ATPase vesicles in the presence of varying concentrations of either K⁺ or Na⁺. Ion-tight H,K-ATPase vesicles (60 μg/ml) were preincubated at room temperature in 20 mM Tris acetate (pH 7.0) and 250 μM sucrose with 100 μM of the ionophores: nigericin (☐), valinomycin (□), valinomycin plus CCCP (●), or with 2% ethanol (■) as a control. After 8 min 10 μl of the enzyme suspension was mixed with 80 μl of different KCl or NaCl solutions and incubated for 2 min at 37 °C. The K⁺- or Na⁺-dependent ATPase activity was determined by incubating another 2 min with 10 μl of 50 μM [γ-32P]ATP and 0.15 mM MgCl₂ (final concentrations) in 250 mM sucrose, 20 mM Tris acetate. The total ATPase activity, including the basal Mg-ATPase activity is plotted as function of the KCl (A) or NaCl (B) concentrations present during the ATPase assay. Activity is given as μmol of ATP hydrolyzed per mg of protein/h.

These observations indicate that the activation of the ATP hydrolysis by Na⁺ is not due to a contamination by K⁺, but that Na⁺ itself activates the dephosphorylation process at the luminal (intravesicular) side of the membrane.

The Effects of Na⁺ and K⁺ on the Dephosphorylation Reaction—The dephosphorylation reaction was studied in leaky H,K-ATPase preparations. Fig. 5A shows that both Na⁺ and K⁺ enhance its rate, with an apparent half-time (K₅₀) of 10 and 0.92 mm, respectively (ratio Na⁺/K⁺ = 500). Choline chloride had no effect on the dephosphorylation process, excluding effects of ionic strength.

In the absence of any H⁺-like properties of Na⁺ under these conditions. Na⁺ only led to a decrease in the steady-state phosphorylation level (I₅₀ values for Na⁺ were 6, 18, and 40 mM in the presence of 0.2, 2, and 20 μM ATP, respectively), underlining once more the ATP/Na⁺ antagonism.

**DISCUSSION**

In this study data is presented which clearly shows that Na⁺ ions behave like K⁺ ions in the H,K-ATPase reaction cycle. Na⁺ activates the dephosphorylation reaction (steps 5 and 6, Fig. 1) in a preparation in which both cytosolic and luminal ion binding sites are accessible. In intact H,K-ATPase vesicles no enhancement of this process was observed, showing that the Na⁺-activation site, like the K⁺-site (19, 25), is located intravesicularly (the luminal side). In ion-tight vesicles the phosphorylation capacity was used as a measure for the relative amount of the E₁ form of H,K-ATPase, as only the E₁ form can
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The observation that cytosolic Na⁺ has K⁺-like properties, driving the enzyme to the E₂ form, is in line with the inhibition of the ATP phosphorylation rate at relative low concentrations activated the hydrolysis of ATP, via the dephosphorylation reaction (steps 5 and 6), and inhibited ATPase reaction at high concentrations by driving the E₁→E₂ equilibrium to the right (steps -1 and -7). The combination of both effects explains the increasing effect of Na⁺ on the K₀.₅ for ATP in the phosphorylation reaction (Fig. 1A).

In both Na⁺,K-ATPase and H,K-ATPase, K⁺ activates the dephosphorylation reaction. The role of K⁺ can be performed in both enzymes by Na⁺ (Refs. 27 and 28 and this study), although the affinity for Na⁺ is much lower than that of K⁺. In both enzymes K⁺ also drives the equilibrium E₁→E₂ to the E₂ form, whereas Na⁺ (for Na,K-ATPase) and H⁺ (for H,K-ATPase) shifts the equilibrium to the E₁ form. The present study shows that with H,K-ATPase Na⁺ can perform the latter role of K⁺, but not that of H⁺. With Na,K-ATPase there is no indication for an E₂ promoting effect of Na⁺ in the absence of K⁺. The ion specificity of Na⁺ and H⁺ as E₁ promoters in Na,K-ATPase and H,K-ATPase, respectively, is much more prominent. Neither an effect of H⁺ on the steady-state phosphorylation level of Na,K-ATPase (11) nor of Na⁺ on this parameter of H,K-ATPase (this study) was observed.

The data seems to conflict with studies by Rabon et al. (17), who used a fluorescein isothiocyanate-labeled H,K-ATPase preparation to test the effects of Na⁺. The fluorescence of this modified enzyme, incapable of being phosphorylated by ATP, increased in the presence of Na⁺. Although an antagonism between H⁺ and Na⁺ was observed, the increase in fluorescence was interpreted as an increase in the E₁ form of the enzyme, analogous to that with Na,K-ATPase. It has not been proven, however, that an increase in fluorescence under these conditions is due to Na⁺ activation of the dephosphorylation reaction (steps 5 and 6), and inhibited ATPase reaction at high concentrations by driving the E₁→E₂ equilibrium to the right (steps -1 and -7). The combination of both effects explains the increasing effect of Na⁺ on the K₀.₅ for ATP in the phosphorylation reaction (Fig. 1A).

Variable effects of Na⁺, in the absence of K⁺, on the overall H,K-ATPase activity (steps 1–7) have been reported (13, 15). These variations might be due to differences in assay conditions, since we demonstrate that high concentrations of ATP (Fig. 3B), Mg²⁺, and imidazole have marked effects on the Na⁺ (and the K⁺ (19)) affinity in the overall ATPase reaction. Moreover, high [Na⁺] inhibits the latter activity. In the overall ATPase experiments we were able to show that Na⁺, like K⁺, at relative low concentrations activated the hydrolysis of ATP, via the dephosphorylation reaction (steps 5 and 6), and inhibited the ATPase reaction at high concentrations by driving the E₁→E₂ equilibrium to the right (steps -1 and -7). The combination of both effects explains the increasing effect of Na⁺ on the K₀.₅ for ATP in the phosphorylation reaction (Fig. 1A).

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Na⁺ inactivates the enzyme, consistent with its localization in the luminal site of the membrane. Nonetheless, Mg²⁺ is able to reverse the Na⁺ effect, suggesting that the second step of the ATPase reaction is affected. The presence of Na⁺ has an H⁺-like effect on the enzyme, as Na⁺ is able to mimic the action of K⁺. This effect is similar to that observed for Na⁺ in the presence of ATP, where Na⁺ can substitute for K⁺ in the activation of the enzyme.

When we also used 2 mM Mg²⁺ we found that the maximal activity of the enzyme was doubled from 0.16 mM K₀ to 0.33 mM Mg²⁺ (conditions of Wallmark et al. [16]). The maximal H,K-ATPase activity was not affected. Hence, Mg²⁺ decreases the affinity of the enzyme for K⁺ and consequently the K⁺/Na⁺ antagonism.

Another fundamental question is: if Na⁺ can substitute for K⁺ as activating cation for H,K-ATPase, why then is the maximal activity only 15% of that with K⁺? The most likely explanation is the difference between the affinities at the luminal and the cytosolic K⁺ sites. Since Na⁺ has a higher affinity for the cytosolic site, the presence of Na⁺ is due to a decreased maximal rate of dephosphorylation in the presence of the latter ion.

In the Post-Albers model (Fig. 1) the activating K⁺ site (and/or Na⁺ site) is located at the luminal side of the membrane. Upon K⁺ binding to the E₂P complex the enzyme changes to a conformation with "low" affinity for K⁺ at the cytosolic side. Our findings indicate that the ligands (K⁺ or Na⁺) which are promoters of this "on reaction" (steps 5 and 6) are, at the same time inhibitors of the "off reaction" (steps 7 and 8). Inhibitors of the on reaction, like H⁺ (25), ATP (24), and tertiary amines (19), are in parallel, promoters of the off reaction.

Acknowledgment—We thank K. M. Garner for preparation of the manuscript.

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