Sodium Acts as a Potassium Analog on Gastric H,K-ATPase*

(Received for publication, November 23, 1994, and in revised form, January 12, 1995)

Herman G. P. Swarts, Corné H. W. Klaassen, Feico M. A. H. Schuurmans Stekhoven, and Jan Joep H. H. M. De Pont†

From the Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands

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 $E_2$ in the $E_1 <\leftrightarrow E_2$ 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 $E_2$ 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 10¹² (1, 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 50%. The β subunit of Na,K-ATPase is absent in other related ATPases like Ca-ATPases or Na-ATPase activity is the result of activation of both partial reactions. 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 $E_1 <\leftrightarrow E_2$ 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 Na,Na⁺-ATPase or Na-ATPase 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 (9,17) a sodium-like effect of Na⁺ 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 $E_1$ conformation.

In preliminary experiments De Jong (18) observed that the $K_{0.5}$ 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 $E_2$ conformation.

With the use of ion-tight H,K-ATPase vesicles, where no activation of the dephosphorylation process by extravesicular 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 $E_1 <\leftrightarrow E_2$ transition were investigated. The results show that Na⁺ displays K⁺-like actions under those reaction conditions, thus activating the dephosphorylation process at the luminal side of the membrane and driving the enzyme into an $E_2$ 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 0.35 M sucrose and 7% Ficoll (w/v) in 0.25 M sucrose and 7% Ficoll (w/v) in 0.25 M sucrose and 7% Ficoll (w/v) and freeze drying. This preparation was stored at -20°C. In the experiments in which ion-tight H,K-ATPase vesicles were used the osmolality was kept constant with 0.25 M sucrose and 50 mM Tris acetate (pH 7.0).

**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

---

*This work was sponsored by Netherlands Foundation for Scientific Research (NWO) Grant 900-522-086. 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.

† To whom correspondence should be addressed: Dept. of Biochemistry, University of Nijmegen, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: 31-80-614260; Fax: 31-80-540925.
The normal reaction cycle turns clockwise. In the text, opposite reactions are notated by negative signs.

The Bio-Rad values (20).

**K**⁺ or Na⁺-activated Hydrolysis of ATP—The K⁺- and Na⁺-activated ATPase activities were determined with a radiochemical method. For this purpose 0.1–2 mM of H,K-ATPase was added to 100 µl of medium, which contained 0.01 µCi to 5 mCi [γ-32P]MgATP (specific activity 0.15–200 mCi mmol⁻¹) 0.1–5 mM MgCl₂, 0.1 mM ouabain, 20–50 mM Tris-HCl (pH 7.0), and varying concentrations of either KCl or NaCl. After incubation for 1–30 min at 37 °C the reaction was stopped by adding 500 µl of ice-cold 10% (w/v) charcoal in 6% (w/v) trichloroacetic acid and after 10 min at 0 °C the mixture was centrifuged for 10 s (10,000 **g**). To 0.15 ml of the clear supernatant containing the liberated inorganic phosphate ([γ-32P]), 3 ml of OptiFluor (Canberra Packard, Tilburg, The Netherlands) was added and the mixture was analyzed by liquid scintillation analysis. In general, blanks were prepared by incubating the enzyme in the absence of KCl but in the presence of 0.1 mM SCH 28080.1

**Steady-state ATP Phosphorylation Level**—H,K-ATPase vesicles were incubated at room temperature in 0.25 M sucrose, 0.12 mM MgCl₂, and 50 mM Tris-acetate (pH 7.0). The reaction was stopped by adding 5 ml of 5% (w/v) trichloroacetic acid in 0.1 M phosphoric acid. After filtration over a Schleicher & Schuell filter (type ME28, 1.2-µm pore width, Dassel, Germany) and washing with stopping solution, the ATP-protein content was determined. Blanks were prepared by denaturing the enzyme prior to incubation with the phosphorylation medium (20).

**The Eᵢ→E₂ Transition**—Under iso-osmotic conditions ion-tight H,K-ATPase vesicles were incubated at room temperature in 0.25 M sucrose, 20 mM Tris acetate (pH 7.0), 0.1 mM MgCl₂, and NaCl, KCl, or cholinel in the concentrations indicated. After 10 s the ATP hydrolysis capacity was determined by incubating with another 3 s in the presence of 50 mM [γ-32P]ATP. The phosphoenzyme complex was collected as described above.

**Dephosphorylation Studies**—After 10 s phosphorylation with 1 µM [γ-32P]ATP (see above), 10 volumes of 20 mM non-radioactive ATP and the ligand to be tested were added and incubated for 3–10 s at room temperature. The reaction was stopped by adding 5 ml of stopping solution. The amount of [γ-32P]phosphoenzyme was determined as described above. Dephosphorylation is expressed as the decrease in acid-stable phosphoenzyme during the incubation period (% hydrolysis).

**Sodium and Potassium Determinations**—The Na⁺ and K⁺ contents of the enzyme preparations and media were determined flame photometrically (PJM 6342, Eppendorf, Hamburg, Germany). When the K⁺ levels were determined in 125 mM NaCl solutions, the recovery of KCl was checked by the addition of 10 µM KCl as an internal standard. The recovery of K⁺ was 10.6 ± 1.6 µM (mean ± S.D., n = 6). The K⁺ concentrations of 125 mM CsCl, LiCl, NaCl, NH₄Cl, RbCl, and choline chloride in 20 mM Tris acetate (pH 7.0) were 6.8, 2.5, 4.8, 5.2, 320, and 3.8 µM, respectively. The homogenization buffer (0.25 M sucrose (BDH, Poole, UK), 20 mM Tris acetate, pH 7.0) contained 4.4 mM K⁺. TIC (10 ml), diluted in this buffer, contained 6 µM K⁺.

**Calculations**—The Kᵢ₀ value is defined as the concentration of effector giving the half-maximal activation or phosphorylation level and the Iₜₜ as the value giving 50% inhibition of the activity or level. From data on the phosphorylation level (E-P) and ATP hydrolysis rate, v, the apparent dephosphorylation rate constant, k (turnover number), was calculated using the equation: v = k(E-P) (22).

**Chemicals**—[γ-32P]ATP (3.0 mCi mmol⁻¹, Radiochemical Centre) was diluted with nonradioactive Tris-ATP (pH 7.0). Nigericin, valinomycin (Sigma), and CCCP (Aldrich) were dissolved in ethanol and diluted to their final concentrations of maximally 100 µM in 1% ethanol. SCH 28080 (2-methyl-8-phenylmethoxyimidazo-[1-2-a]pyridine-3-acetonitrile) was kindly provided by Dr. B. Wallmark, Hässle, Mölndal, Sweden. 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 Kᵢ₀ 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 (Iₜₜ = 150 µm⁻¹), the ATPase activity was activated at low Kᵢ₀ (Iₜₜ = 14 µm⁻¹), and inhibited at high NaCl concentrations. These observations suggest that the rate-limiting step in the reaction cycle changes at the different Na⁺ and K⁺ levels.

**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% (Iₜₜ = 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 Iₜₜ 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 Na⁺ 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 concentration (5 µM), K⁺ activation occurred with a Kᵢ₀ 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 Kᵢ₀ 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 (0) and 0.125 mg/ml (O) 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 (O) or 120 ((O) 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. Maximal 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 \( K_{0.5} \) 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, \( K_{0.5} \) 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 \( K_{0.5} \) 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⁺.
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 protophore CCCP. When similar experiments (Fig. 4B) were carried out in the presence of Na⁺, activation of the ATP hydrolysis was only observed in the presence of nigericin, which ionophore can also, but to a lesser extent, exchange Na⁺ for H⁺ (23). Valinomycin either alone or in combination with CCCP could not induce a Na⁺-activated Pᵢ production due to the absolute selectivity of this ionophore for K⁺.

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 Kᵣ₅₀ of 10 and 0.02 mM, respectively (ratio Na⁺/K⁺ = 500). Choline chloride had no effect on the dephosphorylation process, excluding effects of ionic strength.

In ion-tight vesicles Na⁺ was unable to activate the dephosphorylation reaction. In the presence of nigericin the dephosphorylation reaction was again activated. This shows directly that the activating Na⁺ site is located intravesicularly. Extravesicular (cytosolic) Na⁺ did not change the kinetics of the K⁺-activated dephosphorylation reaction (studied with valinomycin and CCCP, data not shown).

Helmach-De Jong et al. (24), showed that ATP inhibits the basal and the K⁺-activated dephosphorylation reaction. With the use of ion-tight vesicles, in combination with valinomycin and CCCP it was possible to show that the site of inhibition is located at the extravesicular (cytosolic) side of the membrane. Parallel to K⁺, the Na⁺-activated dephosphorylation rate was also reduced in the presence of ATP (data not shown).

The Effect of Na⁺ and K⁺ on the 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 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 conformation.

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, I₅₀ = 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.

**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...
**Na**⁺ Acts as K⁺ Analog on Gastric H,K-ATPase

...and the reduction of the H⁺-transport rate excluding H⁺-like properties of Na⁺. 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 (13–15) and the reduction of the H⁺-transport rate (15, 25, 28) by this ion.

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 1–7). The combination of both effects explains the increasing effect of Na⁺ on the K₉.₅ 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 increased E₁ formation.
circumstances actually means a shift of the $E_2\rightarrow E_1$ equilibrium to the right. Another interpretation is that the $E_2$Na form has a higher fluorescence than the $E_1$K form.

A comparable "H"-like effect of Na has also been observed by Polvani et al. (8), who measured an increased $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Our observation of an antagonism between ATP and either K+ or Na+ was not made by Wallmark et al. (16). The main difference between their and our approach is that they used constant (2 mm) Mg2+ and we always used 0.1 mm Mg2+ in excess of [ATP]. In our experience the latter combination gives maximal activities. When we also used 2 mm Mg2+ we found the effect of ATP on the $K_{0.5}$ for K* activation to be reduced, e.g. at 20 µM ATP the $K_{0.5}$ for K* doubled from 0.16 mm at 12 mm Mg2+ (our conditions) to 0.33 mm at 2 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*/ATP antagonism.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Our observation of an antagonism between ATP and either K+ or Na+ was not made by Wallmark et al. (16). The main difference between their and our approach is that they used constant (2 mm) Mg2+ and we always used 0.1 mm Mg2+ in excess of [ATP]. In our experience the latter combination gives maximal activities. When we also used 2 mm Mg2+ we found the effect of ATP on the $K_{0.5}$ for K* activation to be reduced, e.g. at 20 µM ATP the $K_{0.5}$ for K* doubled from 0.16 mm at 12 mm Mg2+ (our conditions) to 0.33 mm at 2 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*/ATP antagonism.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Our observation of an antagonism between ATP and either K+ or Na+ was not made by Wallmark et al. (16). The main difference between their and our approach is that they used constant (2 mm) Mg2+ and we always used 0.1 mm Mg2+ in excess of [ATP]. In our experience the latter combination gives maximal activities. When we also used 2 mm Mg2+ we found the effect of ATP on the $K_{0.5}$ for K* activation to be reduced, e.g. at 20 µM ATP the $K_{0.5}$ for K* doubled from 0.16 mm at 12 mm Mg2+ (our conditions) to 0.33 mm at 2 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*/ATP antagonism.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Our observation of an antagonism between ATP and either K+ or Na+ was not made by Wallmark et al. (16). The main difference between their and our approach is that they used constant (2 mm) Mg2+ and we always used 0.1 mm Mg2+ in excess of [ATP]. In our experience the latter combination gives maximal activities. When we also used 2 mm Mg2+ we found the effect of ATP on the $K_{0.5}$ for K* activation to be reduced, e.g. at 20 µM ATP the $K_{0.5}$ for K* doubled from 0.16 mm at 12 mm Mg2+ (our conditions) to 0.33 mm at 2 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*/ATP antagonism.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.

Another fundamental question is: if Na+ can substitute for K+ as activating cation for H,K-ATPase, why then is the maximal activities. When we also used 2 mM Mg2+ we found $^{22}$Na uptake in H,K-ATPase vesicles, under special conditions of low [H+] (pH > 8.0), [Na+] between 2 and 5 mm, and the presence of intravesicular K+. The $^{22}$Na uptake and the ATP hydrolysis rates were in a 1:1 ratio activated by luminal K+. Cytosolic Na+ failed to increase the hydrolytic rate of ATP, what should have been the case if Na+ has H*-like properties. Therefore, it is most likely that they measured H+- and K+-activated ATP hydrolysis together with an exchange of cytosolic $^{22}$Na+ for luminal K+ (normally the K+-K* exchange) and not a Na,K-ATPase activity.