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Half of the ($Na^+ + K^+$)-transporting-ATPase-associated K^+-stimulated *p*-nitrophenyl phosphatase activity of gastric epithelial cells is exposed to the surface exterior

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Ouabain inhibited $^{86}RbCl$ uptake by 80% in rabbit gastric superficial epithelial cells (SEC), revealing the presence of a functional Na^+,K^+-ATPase [($Na^+ + K^+$)-transporting ATPase] pump. Intact SEC were used to study the ouabain-sensitive Na^+,K^+-ATPase and K^+-pNPPase (K^+-stimulated *p*-nitrophenyl phosphatase) activities before and after lysis. Intact SEC showed no Na^+,K^+-ATPase and insignificant Mg^{2+}-ATPase activity. However, appreciable K^+-pNPPase activity sensitive to ouabain inhibition was demonstrated by localizing its activity to the cell-surface exterior. The lysed SEC, on the other hand, demonstrated both ouabain-sensitive Na^+,K^+-ATPase and K^+-pNPPase activities. Thus the ATP-hydrolytic site of Na^+,K^+-ATPase faces exclusively the cytosol, whereas the associated K^+-pNPPase is distributed equally across the plasma membrane. The study suggests that the cell-exterior-located K^+-pNPPase can be used as a convenient and reliable '*in situ*' marker for the functional Na^+,K^+-ATPase system of various isolated cells under non-invasive conditions.

INTRODUCTION

The reaction mechanism of the univalent-cation-transporting ATPase systems such as Na^+,K^+-ATPase and gastric H^+,K^+-ATPase can be broadly divided into two principal steps: a kinase step involving ATP for the generation of enzyme–phosphate ($E \sim P$) complex, and a K^+-requiring phosphatase step responsible for the hydrolysis of $E \sim P$ into E and P_i. The latter step has been thought to be intimately related to K^+-stimulated *p*-nitrophenyl phosphatase (K^+-pNPPase), which is always co-purified with the ATPase systems (Skou, 1965; Schrijen *et al.*, 1983; Forte *et al.*, 1976; Nandi & Ray, 1984). Since K^+-pNPPase is associated with activity of the Na^+,K^+-ATPase complex (Judah *et al.*, 1962; Garrahan *et al.*, 1970; Robinson, 1970), K^+-pNPPase has generally been accepted as a manifestation of the phosphatase step, although the evidence is conflicting (Fujita *et al.*, 1966; Kepner & Macey, 1968; Ottolenghi, 1979; Fleary *et al.*, 1984; Ball, 1984).

Recent studies from our laboratory (Ray & Nandi, 1985*a*, 1986; Ray *et al.*, 1987) demonstrated that the associated K^+-pNPPase does not represent a partial step in the reaction sequence of gastric H^+,K^+-ATPase activity. This conclusion was based on distinctive differences in the orientation of the catalytic and K^+ effector sites of H^+,K^+-ATPase and K^+-pNPPase reactions across the membrane bilayer, and differential effects of several inhibitors on the two enzymic activities. Notable aspects of the study (Ray & Nandi, 1986) include the observation that, whereas the ATP-hydrolytic site is located exclusively facing the cytosol, the pNPP-hydrolytic site is distributed equally across the bilayer. Also, in contrast with the ATP-hydrolytic activity, the K^+ site regulating the pNPP hydrolysis is present on the same side of the membrane as the pNPP-hydrolytic site. Furthermore, the two K^+ sites (one on each side of the bilayer) regulating the pNPPase activity have identical characteristics but different from the one regulating ATPase.

Since gastric H^+,K^+-ATPase and Na^+,K^+-ATPase share numerous features (Ray & Forte, 1976; Kirley *et al.*, 1985; Ray & Nandi, 1985*a*, 1986), it was of interest to investigate whether the latter enzyme, like the former, also shows similar differential orientation of the catalytic and K^+ effector sites (Ray & Nandi, 1986). The effects of inhibitors like ATP and spermine on the Na^+,K^+-ATPase-associated K^+-pNPPase activity were also examined in superficial epithelial cells (SEC) as previously studied with H^+,K^+-ATPase (Ray & Nandi, 1986) in gastric microsomes, in order to evaluate the unique features of the two univalent-cation transporters.

A preliminary account of some of this work has already been published (Nandi *et al.*, 1986).

METHODS AND MATERIALS

Isolation of surface cells

The SEC were isolated as described by Tanaka *et al.* (1982) and as reported recently (Bailey *et al.*, 1987). The cells meet several criteria of viability (Tanaka *et al.*, 1982): (1) more than 95% of the cells exclude the vital dyes Trypan Blue and erythrosine B; (2) they maintain expected intracellular Na^+ and K^+ concentration gradients; (3) their O_2 consumption responds in expected

Abbreviations used: Na^+,K^+-ATPase, ($Na^+ + K^+$)-transporting ATPase; K^+-pNPPase, K^+-stimulated *p*-nitrophenylphosphatase; SEC, superficial epithelial cells; Mg^{2+}-ATPase, Mg^{2+}-stimulated ATPase; H^+,K^+-ATPase, ($H^+ + K^+$)-transporting ATPase; pNPP, *p*-nitrophenyl phosphate.

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fashions to glucose and agents affecting oxidative phosphorylation; (4) there is no response of O_2 consumption to a normally impermeant glucose 1-phosphate; (5) measurement of phospholipid release suggests negligible cellular deterioration after a 1 h period; (6) ultrastructurally the cells show normal nuclear and cytoplasmic elements after suspension for 1 h. Differential staining of the isolated cells indicates that less than 1 % of the cells are parietal ones, hence consistent with the absence of H^+,K^+-ATPase activity in the SEC.

Studies on ^{86}Rb uptake

The SEC were incubated for 5 min at 37 °C in the presence or absence of ouabain (1.0 mM) and/or furosemide (2.0 mM) before the addition of ^{86}RbCl (4.8 mM, sp. radioactivity 0.5 mCi/mmol; New England Nuclear). The uptake of $^{86}Rb^+$ was monitored at different time intervals, and the incubation was terminated by dilution of the cell suspension in ice-chilled respiratory medium containing 5.0 mM-RbCl. After termination the cells were harvested by centrifugation (500 **g**, 5 min) at 10 °C, dissolved in Aquasol and counted for radioactivity. A system prepared using the above conditions in the presence of ouabain (1.0 mM) but without further incubation ('zero time') served as a blank.

Assay of Na^+,K^+-ATPase and K^+-pNPPase

Conditions for assay (Ray, 1978, 1980) in intact and lysed cell preparations were as follows: The incubation mixture for Na^+,K^+-ATPase (when not specified) contained 50 μmol of Pipes, pH 7.0, 2 μmol of $MgCl_2$, an appropriate amount of either lysed or intact cell suspension, in the presence or absence of 100 μmol of NaCl and 20 μmol of KCl, in a final volume of 1.0 ml. After a preincubation period of 5 min at 37 °C, the reactions were initiated by adding 2 μmol of Tris/ATP. After 5 min the reactions were terminated with 1 ml of ice-cold 10 % (w/v) trichloroacetic acid and assayed for P_i by the method of Sanui (1974). For pNPPase the reactions were initiated by pNPP under conditions similar to those described above, using the following incubation system. The incubation mixture contained, in a total volume of 1.0 ml, 50 μmol of Tris buffer, pH 7.5, 5 μmol of $MgCl_2$, 5 μmol of pNPP, an appropriate amount of either lysed or intact cell suspension, in the presence or absence of 20 μmol of KCl. For assay of intact cells, iso-osmoticity of the medium was maintained at 250 mM by using sucrose. For the preparation of lysed cells, the intact cells were quickly frozen (ethanol/solid CO_2) and thawed (at 37 °C with shaking) four times within a period of 15–20 min and then homogenized briefly by using a Dounce homogenizer with a loose-fitting pestle.

Measurement of permeability of the SEC

Permeability of the SEC to pNPP was determined as follows. The SEC were incubated at 37 °C without and with digitonin (20 μg/ml) (Norris & Hersey, 1985) in a total volume of 7.0 ml of the respiratory medium containing 10^6 cells/ml with either 5 mM-[^{3}H]pNPP (0.5 μCi/ml; sp. radioactivity 0.1 mCi/mmol; New England Nuclear) or [^{14}C]polydextran (0.5 μCi/ml; sp. radioactivity 0.99 mCi/g). Aliquots (1 ml) were transferred at 0, 5, 10, 20 and 30 min intervals to 4 ml of prechilled (0–4 °C) respiratory medium and immediately centrifuged (200 **g**, 5 min) at 4 °C. The pellet was dissolved in Aquasol and counted for radioactivity. The extent of pNPP adherence to the cells or trapped in the intercellular space of the SEC was calculated from the [^{14}C]polydextran association with the control (untreated) cell pellet.

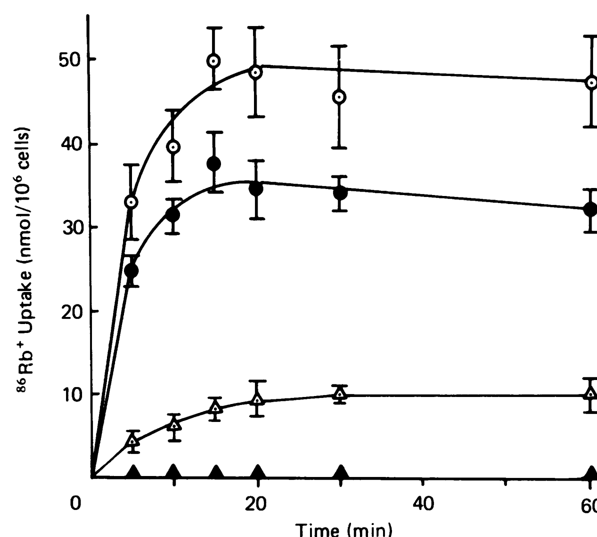


Fig. 1. Time course of $^{86}Rb^+$ uptake in the presence or absence of ouabain or furosemide

The SEC cells were incubated for 5 min at 37 °C in the presence or absence of ouabain (1.0 mM) and/or furosemide (2.0 mM) before the addition of ^{86}RbCl (4.8 mM, sp. radioactivity 0.5 mCi/mmol). The uptake of $^{86}Rb^+$ was monitored at the indicated time. Symbols: ⊙, control; △, with ouabain; ●, with furosemide; ▲, with ouabain and furosemide. It should be pointed out that no detectable uptake of $^{86}Rb^+$ was observed in the lysed SEC (results not shown). The details are given in the Methods and materials section. The data are means ± S.D. for four different SEC preparations.

RESULTS AND DISCUSSION

Fig. 1 demonstrates that SEC can take up ^{86}RbCl ($^{86}Rb^+,K^+$) by the mediation of an ouabain-inhibitable Na^+,K^+-ATPase pump. It is noteworthy that, whereas about 80 % of the $^{86}Rb^+$ uptake is blocked by ouabain, the remaining 20 % is inhibited by furosemide (Fig. 1). Such ouabain-resistant 'loop diuretics' (furosemide or bumetanide)-sensitive $^{86}Rb^+(K^+)$ influx is due presumably to the $Na^+/K^+/Cl^-$ co-transport system demonstrated recently in a variety of different cell types (Dunham *et al.*, 1980; Herbert & Andreoli, 1984; Bourrit *et al.*, 1985; Burnham *et al.*, 1985). It is interesting that the ouabain- and furosemide-sensitive components of the ^{86}Rb uptake in SEC (Fig. 1) are nearly identical with those reported by Bourrit *et al.* (1985) in macrophages and by Burnham *et al.* (1985) in the luminal membranes of the kidney. The data strongly suggest that the plasmalemmal integrity of the SEC towards various univalent-cation-transporting functions remains largely unaffected by the present isolation procedure. Hence these cells appeared quite suited for locating any ATP- or pNPP-hydrolytic activity exposed to the exterior.

In order to localize any enzymic activity on the cell exterior, it is important that the substrate or product does not penetrate the cells appreciably under the

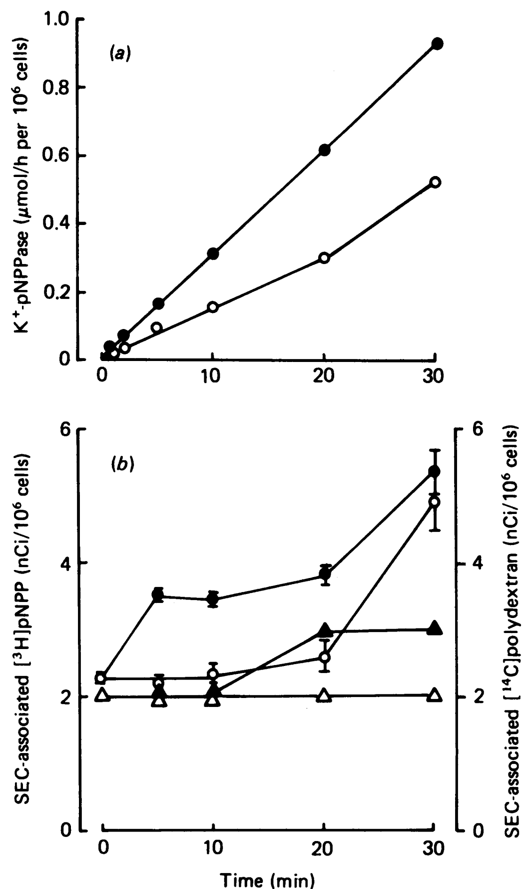


Fig. 2. Time course of pNPP hydrolysis and permeability of SEC to pNPP and polydextran

(*a*) Time course of K^+-dependent hydrolysis of pNPP in the intact and lysed SEC. Symbols: ○, intact cells; ●, digitonin-permeabilized cells. The SEC (3.5×10^6) were treated without (intact) and with digitonin (20 μg/ml) in 1 ml of the respiratory medium for 20 min at 37 °C as detailed in the Methods and materials section. After digitonin treatment, the cells were centrifuged at 200 *g* for 5 min. The pellet was resuspended in 50 mM-Tris buffer, pH 7.5, containing 0.2 M-sucrose, and immediately assayed for K^+-pNPPase. The details of the K^+-pNPPase assay are given in the Methods and materials section. (*b*) Permeability of the SEC to pNPP and polydextran in the absence and presence of digitonin. SEC-associated [^{3}H]pNPP: ○, control; ●, in the presence of digitonin (20 μg/ml); SEC-associated [^{14}C]polydextran: △, control; ▲, in the presence of digitonin (20 μg/ml). The details of the experimental conditions, including the assay procedures, are given in the Methods and materials section. For the [^{3}H]pNPP uptake study, the values are means ± S.E.M. ($n = 4$), and for the [^{14}C]polydextran study the values are an average for duplicate experiments. The duplicate values were within 10% of each other.

conditions of the assay. Fig. 2 demonstrates that the extent of association of both [^{3}H]pNPP and [^{14}C]polydextran with the control (in the absence of digitonin) cells are nearly the same within 10 min of incubation, suggesting no appreciable permeation of pNPP under such conditions. Whereas the control cells do not show any [^{14}C]polydextran uptake beyond even the 10 min period, pNPP shows a slow uptake between 10 and 20 min and a subsequent rapid uptake beyond the 20 min period. In the presence of digitonin, on the other hand, [^{14}C]polydextran uptake becomes appreciable beyond 10 min, but [^{3}H]pNPP uptake occurs as early as 5 min. Measurement of the kinetics of the K^+-stimulated pNPP hydrolysis demonstrates a linear rate of hydrolysis by the control cells up to 20 min, whereas a linear rate showing a doubling of the control rate is observed with the digitonin-treated SEC throughout the entire 30 min incubation period (Fig. 2*a*). It is noteworthy that the former observation of enhanced rate of pNPP hydrolysis by the control cells between 20 and 30 min concurs with the rapid permeation of pNPP observed during such a period (Fig. 2*b*). These data clearly demonstrate that the extent of pNPP permeation in the intact SEC and consequent hydrolysis at an intracellular site are negligible within the 10 min incubation period at 37 °C. Fig. 2(*a*) also demonstrates that digitonin treatment (30 min at 37 °C) totally eliminates the permeability barrier, allowing pNPP to be hydrolysed at both the exterior and the interior of the cells.

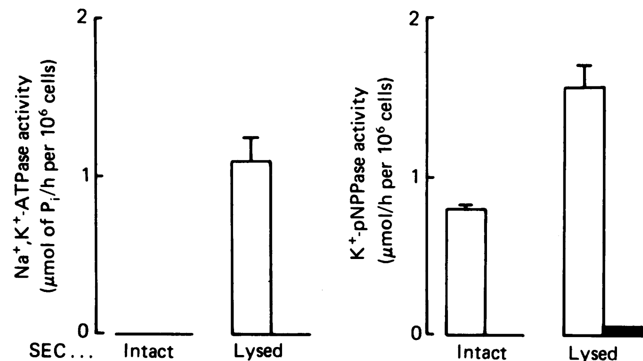


Fig. 3. Na^+,K^+-ATPase and K^+-pNPPase activities of intact and lysed SEC cells

Basal activities (μmol/h per 10^6 cells) represent values (means ± S.D.) in the presence of Mg^{2+} alone and were 4.1 ± 0.3 for ATPase (lysed cells) and 0.25 ± 0.03 for pNPPase in the intact and 0.5 ± 0.03 for lysed SEC respectively. No basal or Na^+,K^+-ATPase was detected in intact SEC. The activity values (μmol/h per 10^6 cells) were obtained by subtracting the basal activity from the total and are 0 and 1.14 ± 0.14 for the Na^+,K^+-ATPase, and 0.77 ± 0.04 and 1.53 ± 0.16 for the K^+-pNPPase, in the intact and lysed cells respectively. The open and closed bars represent the absence and the presence of 1 mM-ouabain respectively. Results are means ± S.D. for four different cell preparations, each assay being done in triplicate. Details are given in the Methods and materials section.

The data on the orientation of the ATP and pNPP hydrolytic sites associated with the Na^+,K^+-ATPase complex of SEC are shown in Fig. 3. The information about the sidedness of the hydrolytic sites across the plasma membrane was gathered by assaying the ouabain-sensitive ATP and pNPP hydrolysis in intact and lysed SEC. Thus, whereas the activities (μmol/h per 10^6 cells) of the Na^+,K^+-ATPase associated with the intact and lysed cells were 0 and 1.14 ± 0.14 respectively, the activities of the K^+-pNPPase were 0.77 ± 0.04 and 1.53 ± 0.16 respectively. The data clearly demonstrate that, whereas 100% of the ATP-hydrolytic sites are facing the cytosol, the pNPP-hydrolytic activities are distributed equally across the bilayer.

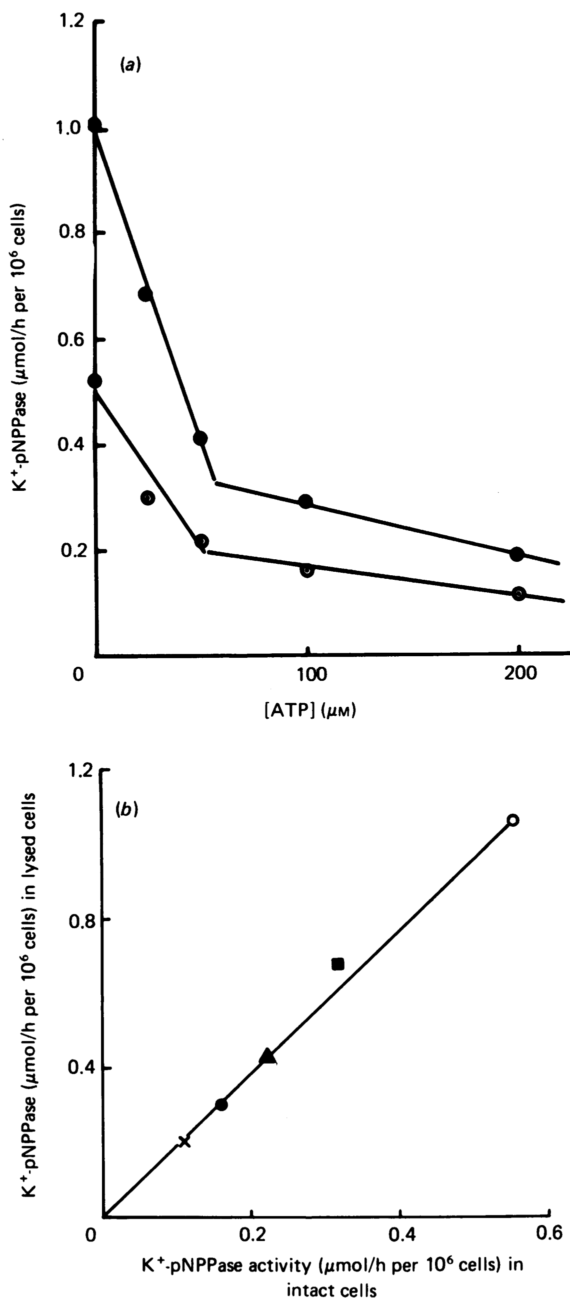


Fig. 4. Inhibition of K$^+$-pNPPase of SEC by ATP

(*a*) Effects of increasing concentrations of ATP on K$^+$-pNPPase activities associated with the intact (⊙) and lysed (●) SEC preparations. The details of the assay are given in the Methods and materials section. The data are typical results from four separate experiments using four different SEC preparations. (*b*) Relationship between the K$^+$-pNPPase activities associated with the intact and lysed SEC in the presence of various concentrations of ATP [○, none (control); ■, 25 μM; ▲, 50 μM; ●, 100 μM; ×, 200 μM]. The data are taken from (*a*) and replotted. A linear relationship is clearly evident from the plot.

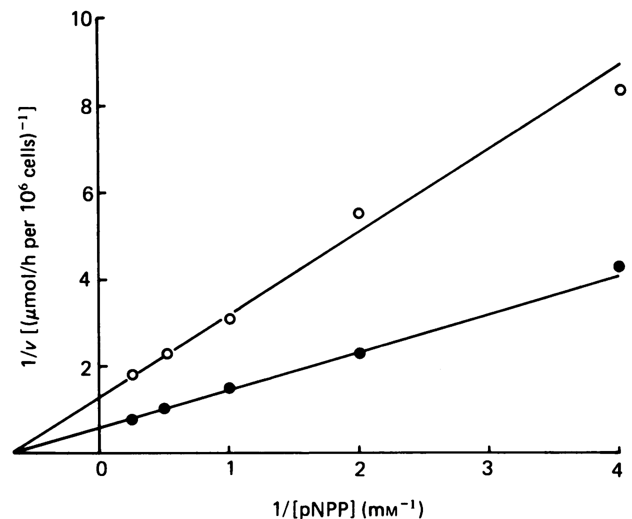


Fig. 5. Effects of increasing concentrations of pNPP on the K$^+$-pNPPase activities of the intact (○) and lysed (●) SEC

The concentration of K$^+$ was 20 mM in each case. A Lineweaver–Burk plot of the data is shown. Studies were made with three different freshly prepared SEC populations. The mean [$\pm$S.E.M. ($n = 3$)] $1/v$ (reciprocal of reaction velocity) values were 8.3 ± 0.1, 5.5 ± 0.08, 3.02 ± 0.09, 2.28 ± 0.03 and 1.70 ± 0.06 for the intact SEC, and 4.31 ± 0.1, 2.31 ± 0.1, 1.48 ± 0.07, 1.03 ± 0.06 and 0.83 ± 0.03 for the lysed SEC at the corresponding reciprocal of pNPP concentration of 4, 2, 1, 0.5 and 0.25 respectively (for units see the axis legends). The lines were drawn by eye. Statistical (non-linear regression) analysis of the data revealed that the K_m (mM) and $V_{max.}$ (μmol/h per 10^6 cells) values for the intact and lysed cell preparations were 1.54 ± 0.37, 0.86 ± 0.16 and 1.67 ± 0.21, 1.81 ± 0.19 respectively. The details of the pNPPase assay are given in the Methods and materials section.

It is acknowledged that K$^+$-pNPPase activity associated with both gastric H$^+$,K$^+$- and Na$^+$,K$^+$-ATPases is inhibited by ATP (Fujita *et al.*, 1966; Ray & Nandi, 1986). Since our recent studies (Ray & Nandi, 1986) with gastric microsomal vesicles demonstrated that the cytosolic K$^+$-pNPPase has a higher affinity towards ATP than the one exposed to the lumen, we tested the ATP-sensitivity of two K$^+$-pNPPase activities in SEC. The results (Figs. 4*a* and 4*b*) demonstrated that, unlike gastric H$^+$,K$^+$-ATPase, K$^+$-stimulated pNPP-hydrolytic sites associated with Na$^+$,K$^+$-ATPase located on the cell exterior and interior have nearly equal affinities for ATP.

The K_m for the cell-exterior- and cell-interior-located K$^+$-stimulated pNPP-hydrolytic sites were also evaluated. Fig. 5 shows that, whereas the $V_{max.}$ is nearly doubled after lysis, the K_m values for the K$^+$-pNPPase activity associated with the intact and lysed cells are closely similar. Thus the K_m for pNPP and $V_{max.}$ values for the intact and lysed SEC were 1.54 ± 0.37 and 0.86 ± 0.16, and 1.67 ± 0.21 mM and 1.81 ± 0.19 μmol/h per 10^6 cells respectively. The data suggest that the exterior and interior sites have equal substrate affinities. Besides the K_m, our study (see below) also revealed that both the exterior- and interior-located pNPPase of the SEC have equal affinities towards K$^+$ and display an identical spermine/K$^+$ antagonism.

The polyamine spermine, which was previously demonstrated (Ray & Nandi, 1986) to compete with K$^+$ for the K$^+$-pNPPase activity associated with gastric microsomal H$^+$,K$^+$-ATPase, also produced a similar inhibition of the Na$^+$,K$^+$-ATPase-associated K$^+$-pNPPase of SEC (Figs.

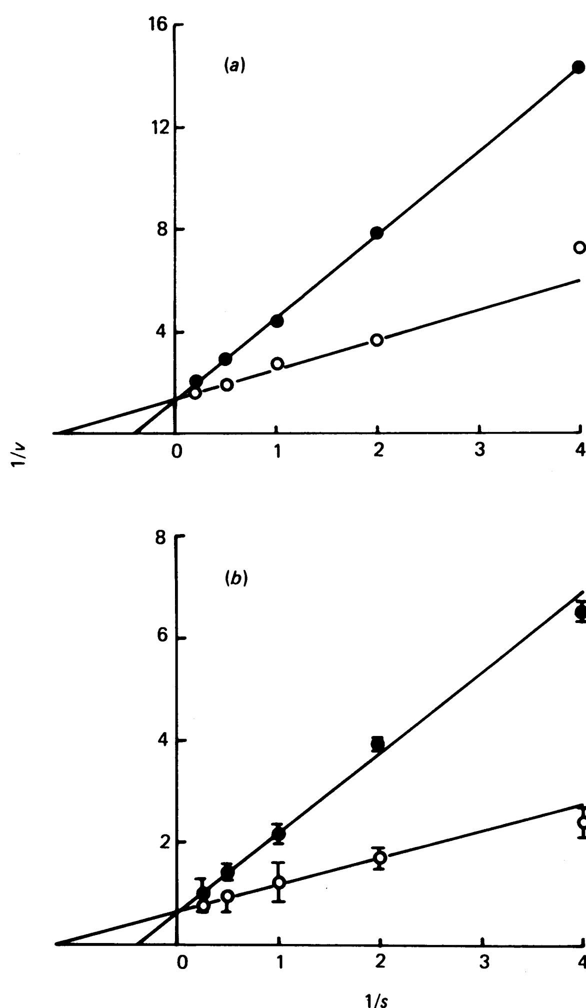


Fig. 6. Kinetics of inhibition of K^+-pNPPase activity associated with intact (*a*) and lysed (*b*) SEC

A Lineweaver–Burk plot of the data is shown. The concentrations of K^+ (denoted by *s*) were varied (0–5 mM) at 5 mM-pNPP. Symbols: ○, without spermine; ●, with spermine (0.5 mM). The data in (*a*) represent values from one study typical of four separate studies using four different SEC preparations (each showing nearly identical values) and in (*b*) represent means (±S.E.M.) for all four studies. The lines were drawn by eye. Statistical (non-linear regression) analysis of the data showed significant differences only in the K_m values with and without spermine in the two groups. The K_m (mM) and $V_{max.}$ (µmol/h per 10^6 cells) values (±S.E.M.) were as follows. For intact SEC (*a*): (1) control, 1.75±0.35 and 0.97±0.2; (2) 0.5 mM-spermine, 2.32±0.16 and 0.73±0.045 respectively; for the lysed SEC (*b*): (1) control, 1.63±0.15 and 1.80±0.18; (2) 0.5 mM-spermine, 1.9±0.5 and 1.33±0.3 respectively. The x^2 values showed no significant difference from the fit. The details of the assay are given in the Methods and materials section.

6*a* and 6*b*). The Na^+,K^+-ATPase activity, however, was found to be somewhat (about 20%) stimulated at pH 7.5 (P. K. Das, J. Nandi & T. K. Ray, unpublished work) by 0.5 mM-spermine. The data strongly suggest that, analogously to H^+,K^+-ATPase system (Ray & Nandi, 1986), the K^+ effector site responsible for the hydrolysis of ATP is different from those responsible for the hydrolysis of pNPP.

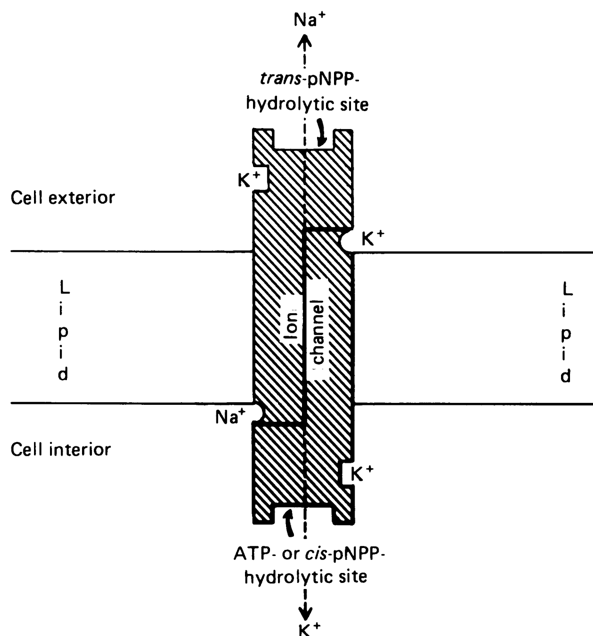
It is clear from the data in Fig. 6 that the affinities of the K^+ sites regulating the K^+-pNPPase activity located at the exterior and interior of the cells are nearly identical. Thus the K_a values for K^+ in the intact and lysed SEC preparation were 1.75±0.35 and 1.63±0.5 mM respectively. During spermine (0.5 mM)/K^+ antagonism, the affinity of the K^+-pNPPase for K^+ in the intact and lysed SEC decreased to nearly the same extent and were 2.32±0.16 and 1.9±0.5 mM. This information provides strong support for the closely similar nature of the exterior- and interior-located K^+ effector sites of the SEC-associated K^+-pNPPase activities. Recent studies with gastric microsomal H^+,K^+-ATPase also revealed a similarly identical nature of the exterior- and interior-associated K^+ effector site of the accompanying K^+-pNPPase activity (Ray & Nandi, 1986).

The present data demonstrate that the univalent-cation-transporting ATPase systems Na^+,K^+-ATPase and gastric H^+,K^+-ATPase are identical with respect to the orientation of the ATP- and pNPP-hydrolytic sites and the K^+ sites responsible for the regulation of the corresponding hydrolytic processes. Similarities also exist in the nature of the spermine and ATP inhibition of the associated K^+-pNPPase reaction.

The only noteworthy difference between the SEC Na^+,K^+-ATPase- and the gastric H^+,K^+-ATPase-associated K^+-pNPPase activities appears to be in the affinities for ATP during inhibition of the K^+-pNPPase by the nucleotide. Thus, whereas the K_i values for ATP during ATP inhibition of both the exterior and interior K^+-pNPPase of the SEC are identical (about 0.04 mM), they were found to be appreciably different in the gastric microsomal H^+,K^+-ATPase system, the K_i values for the exterior and interior of the parietal cells appearing to be about 0.27 and 1.0 mM respectively (Ray & Nandi, 1986).

The above observations point towards a common mechanistic principle (Scheme 1) upon which the operation of the two different ATPase systems having distinct physiological functions are based (Ray & Nandi, 1986). This concept has been further supported by a recent observation (Ray & Nandi, 1985*a*) that, at alkaline pH (8.5), the H^+,K^+-ATPase loses its responsiveness to K^+ alone, but becomes dependent on a combination of both Na^+ and K^+, thus behaving essentially like Na^+,K^+-ATPase. An analogous effect was demonstrated (Skou & Esmann, 1980; Hara *et al.*, 1986) for Na^+,K^+-ATPase, which, under acidic conditions (pH 6.0), loses its sensitivity to Na^+ and acts essentially like H^+,K^+-ATPase.

Although cell-surface K^+-pNPPase activity does not represent the phosphatase step of the univalent-cation-transporting ATPase systems, it is an integral part of the system, as shown by the high activity in pure and homogeneous preparations of H^+,K^+-ATPase (Nandi & Ray, 1984; Nandi *et al.*, 1987) and Na^+,K^+-ATPase (Jorgensen & Peterson, 1979). There is evidence (Ray *et al.*, 1982; Ray & Nandi, 1985*b*, 1986; Nandi & Ray, 1986) to demonstrate a good correlation between the ion-transporting ability of the gastric microsomal H^+,K^+-ATPase and enrichment of the associated K^+-pNPPase. Since both ATPase and associated pNPPase activities also respond to numerous inhibitors (Jorgensen & Peterson, 1979; Ray & Fromm, 1981) in a generally similar fashion, the K^+-pNPPase exposed to the surface of intact viable cells may be highly useful as a non-invasive '*in situ*' marker for univalent-cation-



Scheme 1. Model of the SEC Na^+,K^+-ATPase system

A similar model was recently proposed for gastric H^+,K^+-ATPase (Ray & Nandi, 1986). Two identical catalytic subunits (about 100 kDa each) are arranged in close apposition and in an opposite direction across the bilayer. The high-affinity K^+ and Na^+ sites (designated by open half circles) regulating the catalysis of ATP alone and two other low-affinity K^+ sites (shown by the open half rectangle) regulating the hydrolysis of pNPP are shown. The latter K^+ sites have also been suggested to be involved in the vectorial translocation of ions in each direction (Ray & Nandi, 1986). The Na^+ and K^+ ions bound to the cytosolic Na^+ site and high-affinity K^+ site respectively are translocated during the ATPase reaction through the respective ion channels. The low-affinity K^+ sites may regulate the ion channel, as suggested from recent data (Ray *et al.*, 1982; Nandi & Ray, 1984, 1986; Bailey *et al.*, 1987). (Adapted from Ray & Nandi, 1986.)

transporting ATPase systems associated with various cell types, either freshly isolated or maintained in culture. The feasibility of the outer K^+-pNPPase as such a marker has recently been documented by Bailey *et al.* (1987) in freshly isolated viable SEC. Many useful applications of this cell-surface marker can be visualized. Thus the well-known phenomenon of concurrent bursts of Na^+,K^+-ATPase activity during proliferation of B-cells (membrane form) after mitogenic or antigenic stimulation could conveniently be monitored by the surface K^+-pNPPase activity. Hence the two primary states of the immunogenic B-cells such as the membrane form and the secretory form (fully differentiated) may be identified by using this non-invasive approach in conjunction with other existing techniques. Since most transport events across the plasma membranes are linked, directly or indirectly, with the Na^+,K^+-ATPase pump, the cell-surface K^+-pNPPase could have numerous other uses in correlating various physiological functions.

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