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J Clin Invest. 1991;87(5):1547-1552. https://doi.org/10.1172/JCI115167.

Research Article

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A Potential Role for Guanine Nucleotide-binding Protein in the Regulation of Endosomal Proton Transport

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Abstract

The effects of guanosine 5'-triphosphate (GTP) and GTP-γ-S, known activators of GTP binding proteins, on proton transport were investigated in endosome-enriched vesicles (endosomes). Endosomes were prepared from rabbit renal cortex following the intravenous injection of FITC-dextran. The rate of intravesicular acidification was determined by measuring changes in fluorescence of FITC-dextran. Both GTP and GTP-γ-S stimulated significantly the initial rate of proton transport. In contrast, GDP-β-S, which does not activate GTP binding proteins, inhibited proton transport. The rank order of stimulation was GTP-γ-S > GTP > control > GDP-β-S. GTP-γ-S stimulation of proton transport was also observed under conditions in which chloride entry was eliminated, i.e., 0 mM external chloride concentration in the presence of potassium/valinomycin voltage clamping. GTP-γ-S did not affect proton leak in endosomes as determined by collapse of H^+ ATPase-generated pH gradients. ADP ribosylation by treatment of endosomal membranes with pertussis toxin revealed two substrates corresponding to the 39–41 kD region and comigrating with α_i subunits. Pretreatment of the membranes with pertussis toxin had no effect on proton transport in the absence of GTP or GTP-γ-S. However, pretreatment with pertussis toxin blocked the stimulation of proton transport by GTP. In contrast, as reported in other membranes by others previously, pertussis toxin did not prevent the stimulation of proton transport by GTP-γ-S. These findings, taken together, indicate that GTP binding proteins are present in endosomal membranes derived from renal cortex and that activation of G protein by GTP and GTP-γ-S stimulates proton transport in a rank order identical to that reported for other transport pathways modulated by G_i proteins. Therefore, these studies suggest that G proteins are capable of stimulating the vacuolar H ATPase of endosomes directly. (*J. Clin. Invest.* 1991. 87:1547–1552.) Key words: endosome • H^+ATPase • G protein

Introduction

Previously we have characterized a population of endosome-enriched membranes derived from rabbit renal cortex that contains a proton translocating ATPase (H^+ATPase) and an electoneutral Na^+/H^+ exchanger (1). Recent studies in our laboratory have demonstrated that 8-bromoadenosine cyclic monophosphate has a direct inhibitory effect on the endosomal H^+ ATPase in all populations (fractions) and a chloride-dependent stimulatory effect at lower concentrations in the lighter populations (2). Since endosomal acidification is a requirement for maintaining the integrity of endosomal functional (3), these results suggest that cAMP could play a role in regulating endosomal function in the renal proximal tubule. Moreover, since an ATP-dependent proton pump contributes to proximal tubule acidification (4), it is conceivable that cAMP could be involved in the regulation of transepithelial proton secretion by modulation of the H^+ ATPase in apical membranes of the proximal convoluted tubule.

Cyclic AMP, through second messenger pathways, participates in the regulation of numerous intracellular functions. Furthermore, cAMP synthesis is regulated, in part, by activation of guanine nucleotide-binding proteins (G proteins)[1] (5). The G protein family of membrane-bound regulatory proteins may serve as intermediaries in a variety of transmembrane signaling processes (6). In the intracellular pathway, G proteins couple ligand-bound receptors to effector proteins such as adenylyl cyclase, which, in turn, increase or decrease cAMP production, activate protein kinase, and produce changes in intracellular metabolism. In addition to this intracellular pathway of second messenger activation, certain G proteins have been demonstrated recently to regulate directly ion channel activity (7). Examples include the cardiac dihydropyridine-sensitive Ca^{++} channel (G_s) (8), the cardiac K^+ channel (G_K) (9), and certain renal chloride (G_i) (10) and sodium channels (G_i) (11). Since there are multiple transport pathways that participate either directly or indirectly in endosomal acidification, such as the H^+ ATPase, a proton leak pathway, and a chloride entry pathway, it is possible that G proteins could act to regulate either or all of these transport functions.

The purposes of this study, therefore, were to determine if G proteins can modulate endosomal acidification and attempt to define which proton transport pathways are involved. Prior to this study, a possible role for G-proteins in the regulation of H^+ ATPase has not been reported in any epithelia.

Methods

Preparation of membrane vesicles. The preparation of endosomal vesicles from rabbit renal cortex has been described in detail previously (1). Briefly, female New Zealand White rabbits were killed 5 min after the intravenous injection of fluorescein-isothiocyanate dextran (FITC-dextran) (75 mg). The kidneys were perfused in situ with 30 ml of 250 mM sucrose, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic

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Received for publication 5 February 1990 and in revised form 21 December 1990.

J. Clin. Invest.

0021-9738/91/05/1547/06 $2.00
Volume 87, May 1991, 1547–1552

1. *Abbreviations used in this paper:* G protein, guanine nucleotide-binding protein; GDP, guanosine 5'-diphosphate; GTP, guanosine 5'-triphosphate; HRP, horseradish peroxidase; NAGS, *N*-acetyl glucosaminidase; NEMS, *N*-ethylmaleimide; PTX, pertussis toxin.

acid-tris (hydroxymethyl) amino-methane (Hepes/Tris), pH 7.0, and 5 mM EGTA, removed, and the cortices stripped. The cortices were homogenized in an Omni Mixer (OCI Instruments, Omni International, Waterbury, CT). The homogenate then underwent differential centrifugation. The pellet from the last centrifugation (100,000 *g*) was bottom loaded under a linear sucrose density gradient (35–48% wt/vol; total vol = 36 ml) and then underwent equilibrium density centrifugation at 100,000 *g* overnight. The gradients were fractionated on ice in 3-ml aliquots to yield 12 fractions, diluted 1:10 in 10 mM Hepes/Tris buffer, and pelleted at 100,000 *g* for 1 h.

Protein and enzyme determinations. Protein was determined using a BCA kit (Pierce Chemical Co., Rockford, IL). Maltase was measured with glucose assay kit 15-10 from Sigma Chemical Co., St. Louis, MO, using maltose as a substrate. Na^+-K^+-ATPase was measured by the method of Schoner et al. (12). Succinate dehydrogenase (SDH) was measured by the method of Ackrell et al. (13). *N*-acetyl glucosaminidase (NAGS) was measured by the method of Vaes (14) with the following two modifications: 8 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide was used and the reaction was stopped by the addition of 2.5 ml of 400 mM glycine-sodium hydroxide buffer, pH 10.8.

Measurement of proton transport. Proton transport activity was measured monitoring changes in fluorescence of FITC-dextran (excitation 470 nm, emission 525 nm) using a spectrofluorometer (Alphascan; Photon Technology International Inc., South Brunswick, NJ). 10–20 μl of vesicles (25–40 μg protein) was placed in 1 ml of buffer containing 150 mM sucrose, 100 mM KCl or other salt as specified, 10 mM Hepes-Tris pH 7.0, 3 mM $MgCl_2$, 1 mM ouabain, 1 mM DTT, and, where specified, 15 μM guanosine 5′ triphosphate (GTP), GTP-γ-S, or GDP-β-S. For experiments employing pertussis toxin, vesicles were incubated for 30 min at room temperature in the presence of activated pertussis toxin (500 ng/ml) and NAD (100 μM). Where indicated, GTP or GTP-γ-S (15 μM) was added to the pretreated vesicles 5 min before initiating proton transport. The reaction was initiated by the addition of Tris-ATP, pH 7.0 (1 mM final concentration). The change in fluorescence over time was followed. The initial rate of proton transport was determined from the slope of the tangent line (refer to Fig. 1, reference 2) and expressed as fluorescence units per second per microgram protein (FU s^{-1} μg $protein^{-1}$).

Proton leak rates were determined by generating equivalent pH gradients in the presence or absence of 15 μM GTP-γ-S. Either *N*-ethylmaleimide (NEM, 5 mM) or glucose (10 mM) and hexokinase (10 U/ml) was then added to inhibit the H^+-ATPase. Addition of NEM or glucose and hexokinase induces an efflux (i.e., leak) of protons from the vesicle and is indicated by an increase in fluorescence. Rates of efflux under control conditions were compared with rates of efflux under conditions in which 15 μM GTP-γ-S was present. Results are expressed as FU s^{-1} μg $protein^{-1}$.

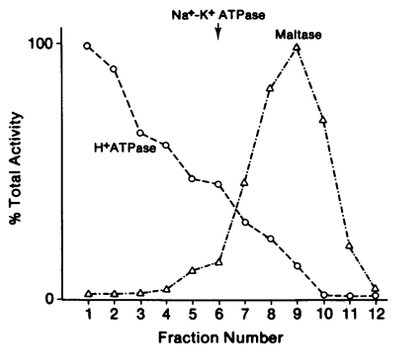

Figure 1. Distribution of marker enzyme activity (H^+ ATPase, Na^+-K^+ ATPase, and maltase). The ordinate is relative enzyme activity. The fraction with highest activity was assigned 100% and the remaining fractions are expressed relative to that value for each enzyme. The abscissa is fraction number. The lightest fractions (least dense vesicles) are represented by the lowest numbers, and were found at the top of the gradient. As indicated by the arrow, peak Na^+-K^+ ATPase activity (basolateral membranes) was in fraction 6. Distribution of other markers: H-ATPase, *open circles;* maltase, *open triangles.*

Demonstration of G proteins. Identification of GTP-binding proteins was accomplished according to the method of Ribeiro-Neto et al. (16) as modified by Codina et al. (17). For [^{32}P]ADP ribosylation with pertussis toxin (PTX), the toxin (240 ng/μl) was incubated with 50 mM DTT and 1 mM AMP-P-(NH)-P at 32°C for 30 min and then diluted with 10 mM Tris-HCl, pH 7.8, containing 0.05% BSA to 60 ng/μl. 150 ng of the activated toxin was incubated in 15 μl containing 10 mM Tris-HCl, pH 8.0, 1 mM EDTA 10 mM thymidine, 1 mM GTP, 100 μM GDP-β-S, 1 mM ATP, 2 mM DTT, 0.3% Lubrol PX, 10×10^6 cpm ^{32}P-NAD, and 20 μg membrane at 32°C for 30 min. After incubation the sample was diluted in 40 μl of Laemmli buffer (18) containing 4 mM NAD and then applied to an SDS-urea gel as described previously (17). The gel was stained with Coomassie blue, destained, and dried. The dried gel was exposed for 4 h at room temperature using two amplifier screens and X5R-5 film (Eastman Kodak Co., Rochester, NY).

Materials. GTP, ATP, *N*-ethylmaleimide, hexokinase, glucose, and valinomycin were obtained from Sigma Chemical Co. [^{32}P]ATP was obtained from Dupont New England Nuclear Research Products, Boston, MA. GTP-γ-S and GDP-β-S were obtained from Boehringer Mannheim Biochemicals, Indianapolis, IN. BCA was obtained from Pierce Chemical Co., and pertussis toxin from List Biological Laboratories, Inc., Campbell, CA.

Statistical methods. Where applicable, results are expressed as means±SEM. Differences between means were evaluated by the paired *t* test where appropriate. $P < 0.05$ indicates statistical significance.

Results

Our previous studies (1, 2) employed horseradish peroxidase (HRP) as a marker for endocytosis and acridine orange as a measure of proton transport. The disadvantage of this technique is that there is a possibility that acridine orange could detect proton transport in vesicles that do not contain HRP, i.e., vesicles that are not of endosomal origin. To circumvent this problem, we employed in these studies FITC-dextran, a compound that is both a marker of endocytosis and pH sensitive. Thus, proton transport is measured only in those vesicles formed by the fluid phase endocytosis of FITC-dextran. (ATP-dependent proton transport was not observed in membranes prepared from sham injected rabbits in which FITC-dextran was added immediately in vitro before homogenization. Thus, random, nonspecific entrapment of FITC-dextran within vesicles does not occur in this preparation.) The distribution of marker enzyme activity is displayed in Fig. 1. As was found in previous studies with HRP (1, 2), the least dense endosomes (i.e., fraction 1) were most enriched in H^+ ATPase activity compared with the other fractions. Moreover, fraction 1 was distinct from enzyme markers of basolateral membranes (Na^+/K^+ ATPase), and brush border membranes (maltase), as well as lysosomal (NAGS) and mitochondrial (SDH) markers (not shown). Therefore, fraction 1 endosomes were employed in these studies. However, similar results were obtained using the heavier endosomal fractions 2–6 (data not shown).

As displayed in Fig. 2, the rate of proton transport was increased in the presence of 15 μM GTP-γ-S, a nonhydrolyzable analogue of GTP that irreversibly activates G proteins by preventing hydrolysis to guanosine 5′-diphosphate (GDP) (deactivation). When multiple experiments were compared, the initial rate of proton transport was significantly faster in the presence of GTP-γ-S (8.50±1.43 vs. 13.48±1.43 FU s^{-1} μg $protein^{-1}$, control vs. GTP-γ-S, $P < 0.002$, $n = 9$ different membrane preparations). The rate of transport in the presence of GTP was also significantly faster than control (8.50±1.43 vs.

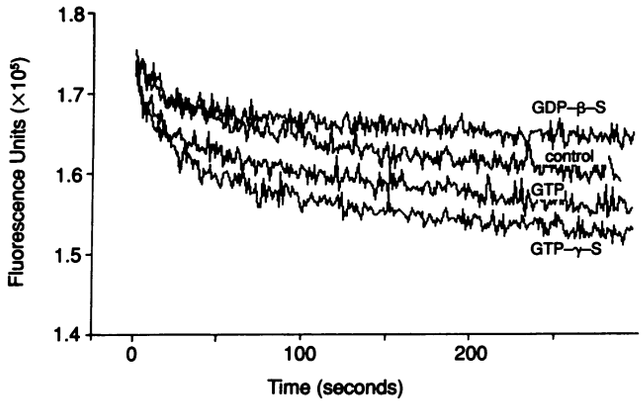

Figure 2. Effects of GTP, GTP-γ-S, and GDP-β-S on the initial rate of endosomal proton transport. The abscissa is time in seconds. The ordinate is fluorescence units. 10 μl (20–25 μg protein) of vesicles was employed in these experiments.

10.97±1.58 control vs. GTP, $P < 0.001$). GTP-γ-S stimulated proton transport to a slightly greater degree than GTP (13.48±1.43 vs. 10.97±1.58 GTP-γ-S vs. GTP $P < 0.05$). To determine the specificity of the GTP and GTP-γ-S effects, experiments were also performed using GDP-β-S, a GDP analogue that does not activate G proteins. As shown in Fig. 2, GDP-β-S exhibited a small, inhibitory effect on proton transport (8.50±1.43 vs. 6.04±1.15, control vs. GDP-β-S, $P < 0.05$). The inability of GDP-β-S to reproduce the GTP or GTP-γ-S effects suggests that the effects of GTP and GTP-γ-S represent activation of a G protein.

It is known that GTP can support proton pumping by H^+ ATPase in other systems. Thus, this effect of GTP could explain the stimulation of proton transport observed in Fig. 2. However, neither 15 μM GTP-γ-S nor GTP were capable of supporting proton transport in the absence of ATP (not shown). Additional studies demonstrated that concentrations of GTP and GTP-γ-S as high as 1 mM were also not capable of supporting proton transport in the absence of ATP (not shown). Thus, the stimulatory effects of GTP on proton transport are not by hydrolysis of GTP in a manner similar to that of ATP.

Since there are several transport pathways which can contribute directly or indirectly to endosomal acidification, i.e., H^+ ATPase, Cl^- transport, and proton leak pathways, the effects of GTP-γ-S on the individual pathways were then investigated. First proton leak pathways were assessed. pH gradients were generated in the presence or absence of 15 μM GTP-γ-S. After equivalent pH gradients were generated, *N*-ethyl maleimide (NEM) (5 mM), an inhibitor of the H^+ ATPase, was added. This maneuver produced an upward deflection of the tracing that indicates proton efflux through leak pathways. GTP-γ-S had no effect on proton leak rates (15.81±2.99 vs. 15.24±3.66, control vs. GTP-γ-S, NS, $n = 4$ different membrane preparations). Since NEM may not inhibit the H^+ ATPase instantaneously, additional leak rate experiments were performed using glucose and hexokinase. The experimental protocol was similar to the NEM experiments except that the external buffer contained glucose (10 mM). After equal pH gradients were generated, hexokinase (10 U/ml), was added. This maneuver induces leak rates by rapidly removing ATP from the external buffer. Consequently, the H^+ ATPase is inhibited and proton leak ensues. GTP-γ-S had no effect on proton leak rates (17.39±3.62 vs. 17.44±4.36, NS, $n = 4$ different membrane preparations). Thus, these experiments indicate that GTP-γ-S does not affect proton leak in these vesicles.

GTP-γ-S could conceivably alter chloride entry into the endosomal vesicles, which would in turn alter charge compensation for the electrogenic H^+ ATPase and thereby modify proton transport. Thus, experiments were performed under conditions in which the chloride contribution to proton transport was eliminated by removing extravesicular Cl^- and by "voltage clamping" the membranes. These studies were based on the assumption that, if the effects of GTP-γ-S on proton transport were still observed in the absence of chloride in association with voltage clamping, then such effects could be attributed to a direct action of GTP-γ-S on the H^+ ATPase rather than an effect on the chloride entry pathway. These experiments were performed by placing vesicles loaded with 100 mM potassium gluconate into buffer containing 100 mM potassium gluconate, 150 mM sucrose, 10 mM Hepes-Tris pH 7.0, 3 mM $MgSO_4$, and 1 mM ouabain. Valinomycin (5 μM) was then added to provide a maximal potassium conductance (1) and, thus, voltage clamp the membranes. The reaction was started by the addition of ATP and proton transport was measured as described in Methods. A representative experiment is depicted in Fig. 3. The control rate of proton transport was extremely low under these conditions (see below). Addition of valinomycin produced a measurable rate of proton transport indicating that these experimental conditions provided adequate charge compensation for the H^+ ATPase. In spite of the adequate voltage clamp the rates of proton transport were significantly lower than those observed in the presence of Cl^- (11.83±1.34 vs. 4.30±1.21, 106 mM Cl^- vs. 0 mM Cl^- $P < 0.001$, $n = 4$ membrane preparations). This difference was due, in part, to the absence of Cl^- which has been shown to stimulate the H^+ ATPase (2, 19). In addition we have found it necessary to incubate these vesicles for 12–16 h at 4°C to ensure equilibration of potassium gluconate across the membrane. Approximately 15% of the H^+ ATPase activity is lost during the incubation process. Experiments were then performed in the presence of valinomycin and GTP-γ-S (15 μM). Under these conditions, proton transport was faster than that observed in the presence

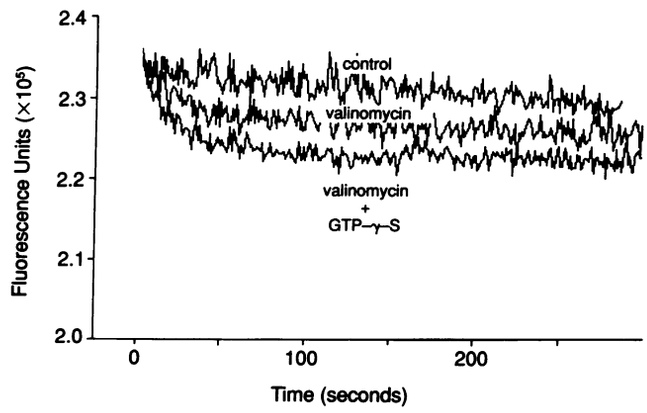

Figure 3. Effects of GTP-γ-S on proton transport under O $[Cl^-]$, voltage-clamped conditions. Axes are the same as in Fig. 2. 20 μl (40–50 μg protein) was employed in these experiments.

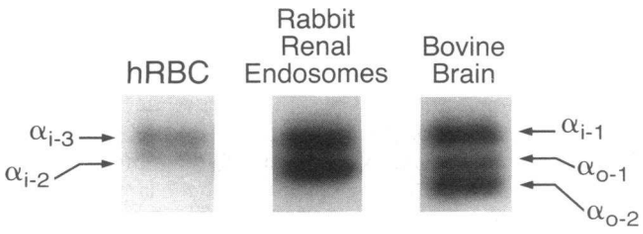

Figure 4. Autoradiograph of PTX-treated membranes. The left lane (control) depicts α subunits ADP-ribosylated by PTX in human red blood cells (*hRBC*). The middle lane depicts proteins ADP ribosylated in renal endosomal membranes derived from fraction 1 (see text). The right lane (control) depicts α subunits ADP ribosylated in membranes prepared from bovine brain.

of valinomycin alone (4.30±0.61 vs. 6.14±0.55, valinomycin vs. valinomycin plus GTP-γ-S, $P < 0.002$, $n = 5$ different membrane preparations). Thus, these findings suggest that the increase in acidification rates observed during application of GTP-γ-S was most likely a result of direct stimulation of the H^+ ATPase.

Since G proteins are commonly membrane bound and are substrates for ADP-ribosylation, experiments were then performed to determine if G proteins, specifically G_i, are present in these same membrane fractions. A representative autoradiograph of endosomal fraction 1, after ADP-ribosylation by PTX, is depicted in the middle panel of Fig. 4. Following PTX treatment, two substrates were detected corresponding to the 39–41 kD region. These bands colocalize with α_{i2} and α_{i3} subunits (Fig. 4, left panel). However, the endosomal bands also migrate closely with α_{i1}, α_{o1}, and α_{o2} (Fig. 4, right panel). Thus, it cannot be stated conclusively that the endosomal bands represent specifically α_{i2} and α_{i3}. It should be noted that no substrates other than those displayed in Fig. 4 were detected on the remainder of the gel.

Since pertussis toxin substrates were detected in these membranes, the effects of PTX on endosomal proton transport were then investigated. Fig. 5 demonstrates that in the absence of GTP or GTP-γ-S, PTX alone had no significant effect on proton transport (11.48±1.09 vs. 11.48±2.07, $n = 5$ different membrane preparations). It was next determined if PTX could block the stimulatory effects of GTP on proton transport. As depicted in Fig. 6, the stimulatory effects of GTP noted previously were inhibited significantly by PTX (15.15±1.28 vs. 11.55±1.17; GTP vs. GTP/PTX, $P = 0.005$, $n = 5$ different membrane preparations). The inhibition by PTX reproduced a rate of transport similar to that observed in the absence of GTP (compare to control tracing, Fig. 6). Next, the effects of PTX on the stimulation of proton transport by GTP-γ-S were investigated. In contrast to the GTP experiments, PTX did not block the stimulation of proton transport produced by GTP-γ-S (Fig. 7) (17.79±2.88 vs. 17.68±3.06; GTP-γ-S vs. PTX/GTP-γ-S; P = NS, $n = 5$ different membrane preparations).

Discussion

The purpose of these studies was to determine if G proteins participate in the regulation of proton transport by endosomal fractions from rabbit renal cortex. The results demonstrate that G proteins reside in endosomal membranes and that known activators of G proteins, GTP-γ-S and GTP, stimulate endosomal proton transport. That this effect is specific is evidenced by the observation that GDP-β-S, a GTP analogue that does not activate G proteins, does not stimulate proton transport. Moreover, PTX blocked the stimulation of proton transport by GTP, but not the stimulation by GTP-γ-S. Such a pattern is similar conceptually to results reported previously for ion channels (7, 10, 11) where it was observed that PTX blocked the activation of channel activity by GTP but did not block the activation of channel activity by GTP-γ-S.

The inhibition of proton transport by GDP-β-S provides further support for the view that G proteins can affect directly endosomal proton transport. It is likely that this membrane preparation contains residual quantities of GTP. Such residual GTP could provide "baseline" stimulation and could serve, as well, to displace a portion of the added GTP-γ-S. Moreover, GDP-β-S could interrupt the GTP-G protein cycle and thereby inhibit proton transport.

The proton leak studies indicate that the effect of GTP-γ-S is not on the proton leak pathway per se. Furthermore, the studies performed under O [Cl^-], voltage-clamped conditions suggest that the G protein effect is not on the chloride entry pathway (Fig. 3). However, since membrane potential was not measured directly, the possibility exists that GTP-γ-S could reduce membrane potential, even in the absence of Cl^-, and, thereby, stimulate proton transport. Further support for our view that the G protein stimulation of acidification is independent of electrogenic Cl^- entry has been suggested by the studies of Bae and Verkman (20). These investigators observed in preliminary studies that GTP-γ-S had no effect on Cl^- influx into rabbit renal endosomes prepared similarly. Thus, the proton leak and O [Cl^-] voltage clamp experiments suggest, but do not prove, that the G protein–mediated increase in acidification could be through direct stimulation of the H^+ ATPase.

Although the G protein effect appears to be through the H^+ ATPase, it is not known whether the G protein is coupled directly to the H^+ ATPase, as is the case with ion channels (7), or acts through a second messenger such as cAMP (6). In regard to the latter possibility, it is interesting to note that we have demonstrated previously that cAMP modulates proton transport in these vesicles (2). Specifically, cAMP inhibits directly the H^+ ATPase, a finding that is in opposition, directionally, to the

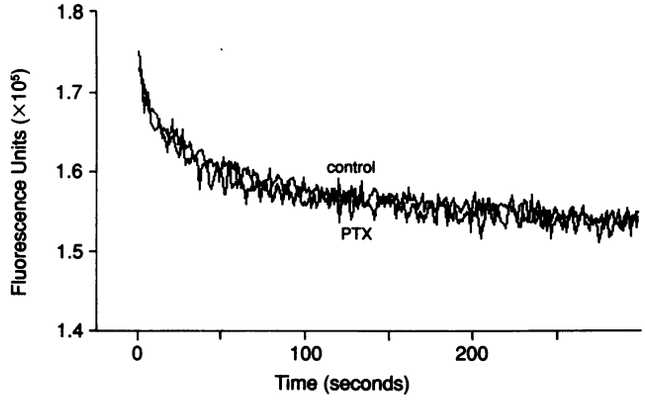

Figure 5. Effects of PTX on endosomal proton transport. Axes are the same as in Fig. 2. Membranes were incubated in the presence of PTX for 30 min before the addition of ATP.

stimulation observed in the presence of GTP and GTP-γ-S. While our studies were performed in the absence of exogenous cAMP, this does not preclude the mediation of the G protein effect by cAMP. It is possible that endosomal membranes contain adenylyl cyclase and, thus, could produce cAMP from the ATP or GTP added as substrate for the H^+ ATPase. If correct, the seemingly opposite effects of GTP and cAMP on the H^+ ATPase could be explained. One possibility would be that the G protein involved is G_i, a G protein known to inhibit adenylyl cyclase. Inhibition of adenylyl cyclase would decrease cAMP levels which could then increase H^+ ATPase activity, thereby relaxing a tonic inhibition and stimulating proton transport.

These studies demonstrate that G proteins, most likely α_i subunits, are present in these membranes and are ADP-ribosylated by pertussis toxin. Specific G protein subunits were demonstrated to be present in the endosomal membrane fractions after incubation with pertussis toxin in the presence of NAD. Two specific bands in the range of 39–41 kDa were observed on SDS urea gels which could correspond to α_i subunits α_{i3} and α_{i2}, respectively (Fig. 4). The functional studies performed, i.e., response to GTP-γ-S but not GDP-β-S, and the rank order of stimulation (GTP-γ-S > GTP > control > GDB-β-S), are all entirely consistent with a G_i protein effect in these membranes. More importantly, the effect of PTX to block the stimulation of proton transport by GTP but not by GTP-γ-S is also consistent with a G_i protein effect. Precedent for this pattern of response has been established by Sunyer and colleagues (21) who reported similar effects of PTX and GTP analogues on cAMP production in cell membranes containing only G_i-regulated adenylyl cyclase. Precise delineation of the specific subunits (i.e., α_{i3}, α_{i2}, α_o, etc.) will require studies employing subunit-specific antibodies.

It is known that endosomal acidification is necessary for endosomal function (3). Furthermore, in the proximal tubule, it has been shown that alkalinization of acidic compartments inhibits protein degradation by the cell (22). Thus, G proteins could play a role in modulating proximal tubule endosomal function by regulating endosomal acidification. Consistent with this view is the recent demonstration that GTP-γ-S inhibits endocytosis in macrophages (23). Further evidence of a regulatory function for GTP-binding proteins in the vesicular pathway is based on the observation that protein transport among Golgi stacks (24) and recycling of phosphomannosyl receptors (25) is inhibited by GTP-γ-S in cell free systems.

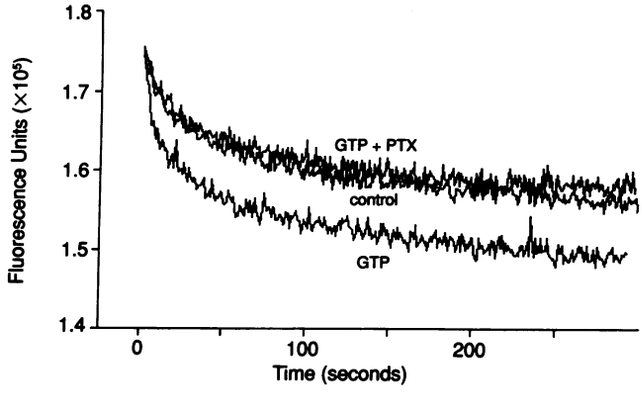

Figure 6. Effects of PTX on endosomal proton transport in the presence of GTP. Axes are the same as in the previous figure. A control tracing (no GTP or PTX present) is displayed for comparison.

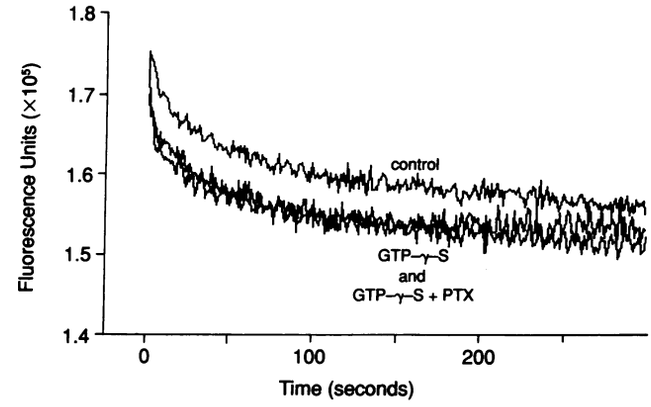

Figure 7. Effects of PTX on endosomal proton transport in the presence of GTP-γ-S. Axes are the same as in the previous figure. A control tracing (no GTP-γ-S or PTX present) is displayed for comparison.

The precise function of the endosomes studied in these experiments has not been described fully. However, since a H^+ ATPase on the apical membrane is felt to contribute to proximal tubule acidification (4), it is conceivable that endosomes may function to modulate apical proton transport by inserting or removing proton pumps from the apical membrane. If the H^+ ATPase on the apical membrane is identical to the endosomal H^+ ATPase (26), then G proteins could modulate luminal acidification in a manner similar to that postulated by Liu and Cogan (27). The type of experimental approach employed by these investigators would be unable to distinguish between a primary effect of angiotensin II or parathyroid hormone through G proteins on the Na^+/H^+ antiporter as opposed to the H^+ ATPase per se.

In summary, these studies demonstrate that G proteins are present in endosomes derived from rabbit renal cortex. Furthermore, it is suggested that G proteins can modulate endosomal proton transport by stimulating the H^+ ATPase. Further studies are required to determine the physiological significance of the G protein effect and to define with greater precision the specific G protein subunits responsible for this regulatory mediation.

Acknowledgments

The authors gratefully acknowledge the advice and assistance of Lutz Birnbaumer, Ph.D. The authors also wish to acknowledge the secretarial assistance of Ofilia Chlamon and Connie Clack.

Support for these studies was obtained from National Institutes of Health grant DK-39331 (Dr. Gurich) and DK-30603 (Dr. DuBose). Dr. Codina was supported in part by grant DK-19318 to Dr. Birnbaumer.

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