A population of gastric membrane vesicles of high K+ permeability and of low density than endoplasmic tubulovesicles containing (H+-K+)-ATPase was detected in gastric mucosal microsomes from the rat fasted overnight. The K+-transport activity as measured with 86RbCl uptake had a $K_m$ for Rb+ of 0.58 ± 0.11 mM and a $v_{max}$ of 13.7 ± 1.9 nmol/min/mg of protein. The 86Rb uptake was reduced by 40% upon substituting Cl- with SO$_4^{2-}$ and inhibited noncompetitively by ATP and vanadate with a $K_i$ of 3 and 30 μM, respectively; vanadate also inhibited rat gastric (H+-K+)-ATPase but with a $K_i$ of 0.09 μM. Carbachol or histamine stimulation decreased the population of the K+-permeable light membrane vesicles, at the same time increased K+-transport activity in the heavy, presumably apical membranes of gastric parietal cells, and enabled the heavy microsomes to accumulate H+ ions in the presence of ATP and KCl without valinomycin. The secretagogue-induced shift of K+ permeability was blocked by cimetidine, a H$_2$-receptor antagonist. Four characteristics of the K+ permeability as measured with 86RbCl were common in the resting light and the heavy microsomes: (a) $K_m$ for Rb+, (b) anion sensitivity (Cl- > SO$_4^{2-}$), (c) potentiation of various divalent cations (Hg$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$) to inhibit Rb+ uptake, and (d) inhibitory effect of ATP, although the nucleotide sensitivity was latent in the stimulated heavy microsomes. The $v_{max}$ for 86RbCl uptake was about 10 times greater in the resting light than the stimulated heavy microsomes. These observations led us to propose that secretagogue stimulation induces the insertion of not only the tubulovesicles containing (H+-K+)-ATPase, but also the light membrane vesicles containing KCl transporter into the heavy apical membranes of gastric parietal cells.

During stimulation of gastric acid secretion, two major molecular changes have been proposed to occur at the apical membranes of gastric parietal cells; insertion of intracellular vesicles containing (H+-K+)-ATPase and induction of a KCl transporter. The secretagogue-induced shift of K+ permeability in the parietal cells and that insertion of these vesicles into the apical membranes may account for induction of the KCl pathway.

**Materials and Methods**

Male Sprague-Dawley rats weighing about 230 g were fasted overnight. Histamine (30 mg/kg) or carbachol (600 μg/kg) was given subcutaneously. Cimetidine (100 mg/kg) was injected intraperitoneally. In some experiments, cimetidine was given 1 h before the secretagogue treatments. The animals were killed by cervical dislocation 60 min after carbachol and cimetidine or 30 min after histamine. To prepare gastric mucosal microsomes, the fundic region of the stomach was scraped with a glass slide. The tissue scrapings from 12 stomachs were suspended in 40 ml of homogenizing buffer containing 250 mM sucrose, 2 mM MgCl$_2$, 1 mM EGTA, 1 and 2 mM Heps/Tris, pH 7.4. Tissue homogenates were prepared and fractionated by differential centrifugation as described previously (4). Resolution of the gastric microsomes into the light and the heavy fractions was also described previously (4). Briefly, the supernatant after removal of the nuclei-mitochondria-enriched fraction (20,000 × g for 15 min), about 15 ml, was layered over a gradient of the homogenizing buffers of different density; one (10 ml) was prepared in 40% H$_2$O and contained 270 mM sucrose with the rest of the buffer components, and the other (5 ml) in 99.8% D$_2$O and 300 mM sucrose. The centrifugation was carried out in a SW 28 rotor at 24,000 rpm for 30 min.

No distinctive interfacial bands were observed, most likely due to formation of a continuous gradient as a result of H$_2$O diffusion. The heavy microsomes refer to the membranous materials sedimented to form a pellet. The light microsomes were recovered from the gradient media by a second centrifugation at 170,000 × g for 35 min. In some experiments, the light microsomes were fractionated by piecing the bottom of the centrifuge tube. Fractions were numbered from the top of the tube; thus F-1, -2, and -3 (5 ml each) were obtained from the region where the sample layer was placed initially, and F-4 to -8 (3 ml each) from the H$_2$O layers. Occasionally, the subfractions, F-3, -4, and -5, were combined and designated as L-1, and the pooled fraction of F-6, -7, and -8 as L-2. All the fractions were centrifuged at 170,000 × g for 35 min and the membranous materials were resuspended in a buffer containing 240 μM sucrose and 10 mM Pipes/Tris, pH 7.0, to a final concentration of about 10 mg/ml. The heavy gastric microsomes were further purified using Percoll gradient centrifugation: a mixture of 4 parts of the heavy microsomal suspensions (in the homogenizing buffer) and 1 part of Percoll in 240 mM sucrose.

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1 The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; Pipes, 1,4-piperazinetetraacetic acid; Heps, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
were centrifuged at 18,000 rpm in a Beckman rotor, 60 Ti, for 30 min. The distinctive membrane band near the top of the gradient was collected and applied to a column (15 x 20 cm) of Sephacryl S-1000, fine (9), equilibrated with the homogenizing buffer containing 10 mM Tris/HCl additionally. The membranes were eluted ahead of Percoll and concentrated by centrifugation as described above.

Uptake of "RbCl by various membrane fractions was measured by the membrane filtration technique. Typically the incubation medium contained 1 mM RbCl, unless indicated otherwise, 240 mM sucrose, 0.1 mM EDTA, 10 mM Pipes/Tris, pH 7.0, and radioactive "RbCl (3.6 x 10^6 cpm/100μl). MgCl₂ when indicated, was added to a final concentration of 1 mM and EDTA was deleted. Additions of other solutes when indicated were made by replacing sucrose iso-osmotically. The transport reaction was initiated by mixing 10 μl of membrane suspensions (~100 μg of membrane protein) with 100 μl of the incubation media. After a designated period of incubation at 24 °C, the transport activity was stopped by diluting with 2 ml of ice-cold washing solution containing 240 mM sucrose and 1 mM Tris/HCl, pH 7.0. The suspension was immediately filtered over a Millipore filter (HAWP 0.45-μm pore size). The filters were washed five times with 2 ml of washing solution. Nonspecific binding of "Rb to membranes was estimated by measuring the amount of "Rb associated with the membranes in the presence of 120 mM KCl. The nonspecific component thus obtained was largely time-independent and amounted to 5-10% of the radioactivity found at equilibrium in these gastric microsomes. The rest of the "Rb associated with the membranes was sensitive to osmolality changes with sucrose or mannitol. For determination of kinetic parameters, the initial rate of "RbCl uptake was obtained from the rate observed during the first 15 s of incubation period for the resting light microsomes and from the linear slope of the uptake observed during the first 3 min for the stimulated heavy microsomes at 24 °C. The effect of ATP, other nucleotides and vanadate on the uptake was examined under the same conditions. In the case of vanadate, the membranes were preincubated with the inorganic ion in the presence or absence of Mg²⁺ (1 mM) for 2 min. Various divalent cations, sulfhydryl agents, and inhibitors of anion transport were also tested for their effect on RbCl transport in the gastric microsomes. The inhibitory potency of these agents was compared on the basis of their concentration to inhibit 50% of RbCl uptake at equilibrium, which was reached in 2 min for the resting light microsomes or 8 min for the stimulated heavy microsomes at 24 °C. A typical dose-response curve consisted of data obtained with at least five different concentrations of inhibitors.

The degree of H⁺ ion accumulation in gastric microsomal membrane vesicles was estimated by measuring the uptake of [³²P]amino- pyrine as described previously (4). Also, (H⁺-K⁺)-ATPase in mem- brane fractions was determined by measuring K⁺-dependent release of inorganic phosphate from ATP as described (4). The gastric membrane fractions were analyzed for the following markers: succi- nate-cytochrome c reductase as a marker for mitochondria (10) and NADPH-cytochrome c reductase for endoplasmic reticulum (11). Polyacrylamide slab gel electrophoresis (10% gel) of membrane samples was carried out using Tris/glycine/sodium dodecyl sulfate buffer (12). Protein was determined by the method of Lowry et al. (13) using bovine serum albumin as standard.

"RbCl and [³²P]amino-pyrine were purchased from New England Nuclear. Furosemide, 4,4'-disothiocyano-2,2'-disulfonic acid stilbene, 5,5'-dithiobis-(2-nitrobenzoic acid), and N-ethylmaleimide were obtained from Sigma. A sample of omeprazole was prepared by Dr. J. C. Sih in the Upjohn Company. All other materials were of reagent grade quality and obtained from standard sources.

**RESULTS**

Fig. 1 shows "RbCl uptake at 24 °C in gastric mucosal microsomes prepared from the fasted or the carbachol-stimulated or the cimetidine-pretreated and carbachol-stimulated rats. In the resting animals, the light microsomes showed a rapid and high RbCl uptake; the half-time to reach equilibrium was 10 s at 24 °C and 40 s at 4 °C (Fig. 2) and its equilibrium level was almost twice as high as that observed with the heavy microsomes. By contrast, in the stimulated rats the heavy microsomes were associated with a higher level of RbCl transport activity than the light ones. The maximum level of "RbCl uptake was 8 to 10 nmol/mg protein in both the resting light and the stimulated heavy microsomes and was 4 to 5 nmol/mg protein in the resting heavy and the stimulated light microsomes. Pretreatment with cimetidine partially blocked the carbachol-induced increase of "RbCl uptake in the heavy microsomes. Histamine also brought...
FIG. 2. Temperature dependence of $^{86}$RbCl uptake in the resting light microsomes. $^{86}$RbCl uptake was measured at 24°C (○) or 4°C (●) in the light microsomes from the rats fasted overnight. Other experimental conditions were the same as described in the legend of Fig. 1.

FIG. 3. Comparison of H$^+$ transport activity as measured with $[^{14}C]$aminopyrine uptake in rat gastric heavy microsomes. The heavy microsomes were prepared from the rats fasted overnight (○), or treated with carbachol (●), or pretreated with cimetidine and then with carbachol (△). The transport activity was measured in the medium containing 40 mM KCl, 160 mM sucrose, 0.5 mM Mg$^{2+}$-ATP, 10 mM Pipes/Tris, pH 7.0, without valinomycin at 24°C. Each point with a bar represents the mean and standard deviation of measurements from three separate experiments, each consisting of duplicate determinations.

about a similar shift of $^{86}$RbCl transport activity from the light to the heavy microsomes which was completely blocked by cimetidine (data not shown).

Fig. 3 shows time course profiles for H$^+$ accumulation as measured with $[^{14}C]$aminopyrine uptake in the heavy microsomes in the presence of 40 mM KCl and 0.5 mM Mg$^{2+}$-ATP (without valinomycin). The degree of H$^+$ ion accumulation in the heavy microsomes was largely proportional to their ability of RbCl uptake; thus the carbachol-stimulated heavy microsomes showed the highest level of H$^+$ accumulation followed by the cimetidine-carbachol treated and the resting heavy microsomes. It should be noted that the specific activity of (H$^+$-K$^+$)-ATPase in these three types of the heavy microsomes was not considerably variable ranging from 18 to 24 μmol of Pi/h·mg of protein, and that the light microsomes accumulated H$^+$ ion only in the presence of valinomycin and a high concentration (150 mM) of KCl (4). These observations suggest that the transport activity of RbCl is coupled to (H$^+$-K$^+$)-ATPase in the same population of the heavy microsomal
Wolosin and Forte (14) have noted the inhibitory effect of divalent cations on RbCl transport in the heavy gastric membranes from histamine-stimulated rabbits. In this study we have found that various divalent cations inhibited RbCl uptake not only in the stimulated heavy but also in the resting light microsomes with almost indistinguishable potency. For instance, in both of the microsomes HgCl₂, CuCl₂, CdCl₂, and ZnCl₂ inhibited 50% of RbCl uptake at equilibrium at the concentration of 0.25 ± 0.05, 0.55 ± 0.1, 0.85 ± 0.15, and 0.9 ± 0.1 mM, respectively. BaCl₂ was not effective at 1 mM. KCl at 1 mM inhibited 50% RbCl uptake at equilibrium in both of the microsomes. Since the concentration of RbCl in these uptake studies was 1 mM, Rb⁺ and K⁺ are apparently handled indistinguishably by the rat gastric transport system. Choline chloride even up to 50 mM had no effect on the RbCl transport. Also ineffective agents are 5,5'-dithiobis-(2-nitrobenzoic acid), N-ethylmaleimide, pyridoxal phosphate, anthracene 9-carboxylic acid, 4,4'-diisothiocyanato-2,2'-disulfonic acid stilbene, and furosemide at the concentration of 1 mM. Omeprazole, a specific inhibitor of (H⁺-K⁺)-ATPase (15), showed no effect at 0.1 mM on the RbCl transport activity.

We have examined the distribution pattern of (H⁺-K⁺)-ATPase and the transport activity of RbCl in the heavy and the light microsomes as a function of time after subcutaneous administration of a single dose (600 µg/kg) of carbachol (Fig. 4). (H⁺-K⁺)-ATPase activity in the light microsomes decreased to less than 50% of the resting level within 30 min and remained low for 2 h. The specific activity of the ATPase in the heavy microsomes, on the other hand, showed no significant changes during stimulation. In earlier studies (4), the evidence has been presented that carbachol stimulation increased the overall content of (H⁺-K⁺)-ATPase in the heavy microsomes by increasing their population, not their specific activity of the ATPase. The transport level of RbCl in the light microsomes at equilibrium (2 min) also decreased rapidly with carbachol treatment and its time course profile mimicked that of (H⁺-K⁺)-ATPase. Concomitantly, we have observed a sharp increase in the transport level of RbCl in the heavy microsomes. From these data, we may suggest a reciprocal
relationship of RbCl transport activity in the resting light and the stimulated heavy microsomes.

An unequal distribution of (H⁺-K⁺)-ATPase and RbCl transport activity was observed among the eight subfractions of the resting light microsomes from the 3H₂O gradient media (Fig. 5). Most of RbCl transport activity was associated with F-3, -4, and -5, whereas (H⁺-K⁺)-ATPase activity was about equally concentrated in F-4, -3, -6, and -7 (Panel A), in the L-2, the other combined light microsomal portion including F-6, -7, and -8 (Panel B), and in the heavy microsomes (Panel C) prepared from the rats fasted overnight. Similar studies with the L-1, the L-2, and the heavy microsomes prepared from the carbachol-stimulated rats are shown in Panels D, E, and F, respectively. The transport activity was measured at the concentration of 1 mM RbCl without Mg⁺⁺ at 24 °C and was corrected for nonspecific component (see "Methods and Materials"). The specific activity of (H⁺-K⁺)-ATPase was 43, 35, 18, 21, 15, and 21 μmol of Pi/h·mg of protein for the membrane preparations shown from Panel A to F, respectively.

Edwards and others (16) have proposed ATP-inhibitable passive transport of Rb⁺ by hog gastric (H⁺-K⁺)-ATPase. We have examined the effect of ATP on transport of ³⁵RbCl at 1 mM in the L-1 (a combined light microsomal fraction containing F-3, -4, and -5), the L-2 (the other light microsomal portion including F-6, -7, and -8), and the heavy microsomes (Fig. 7). ATP at 1 mM in the absence of Mg⁺⁺ blocked more than 90% of RbCl transport in the L-1 of the resting light microsomes (Panel A) but had no considerable effect in the L-2 or in the resting heavy microsomes (Panels B and C). The specific activity of (H⁺-K⁺)-ATPase was 43, 35, and 21 μmol of Pi/h·mg of protein for the L-1, the L-2, and the heavy microsomes, respectively. These observations apparently point out that the ATP-sensitive transport activity of RbCl (at 1 mM) in the L-1 fraction is not likely related to (H⁺-K⁺)-ATPase, since such a nucleotide-inhibitable transport activity was absent in the L-2 which contained nearly as much ATPase activity as L-1. Fig. 7 further shows that the L-1 from the carbachol-stimulated light microsomes lost a majority of ATP-sensitive transport activity of RbCl (Panel D) and that the L-2 and the heavy microsomes gained higher levels of ATP-insensitive RbCl transport activity as compared to the corresponding fractions from the resting animals.

Fig. 8 shows the effect of valinomycin, ATP, or in combination on ³⁵RbCl transport activity and H⁺ accumulation as measured with [¹⁴C]aminopyrine uptake in the resting L-1 (Panels A and C) and L-2 (Panels B and D). In these particular preparations, the specific activity of (H⁺-K⁺)-ATPase was 35 and 31 μmol of Pi/h·mg of protein for the L-1 and the L-2, respectively. With the L-1 fraction, valinomycin increased the uptake of RbCl by about 25%, but addition of ATP (1 mM) in the presence of valinomycin (without Mg⁺⁺) decreased the transport level by almost 70%. These observations indicate that the L-1 fraction consists of at least two populations of membrane vesicles differing in Rb⁺ and Cl⁻ permeability; in one vesicle population which shows ATP-sensitive RbCl transport, the nucleotide shut off both K⁺ and Cl⁻ permeability and therefore even valinomycin could not catalyze a bulk transfer of Rb⁺ due to the lack of the anion permeability. The other population of the membrane vesicles appeared to be only devoid of Rb⁺ permeability so that valinomycin, in the presence of Cl⁻, was able to transport a bulk of the cation. Since H⁺ accumulation in the L-1 was observed only in the presence of valinomycin (with KCl) and ATP (Panel C, Fig. 8), (H⁺-K⁺)-ATPase seemed to be located in the membrane vesicles where ATP-insensitive and valinomycin-dependent
uptake. In these membrane vesicles intravesicular acidification by ATP-sensitive portion amounted to less than 10% of the total measured with the L-1 valinomycin and ATP. resting light microsomes, L-1 and L-2, in the presence of protein for the L-1 and L-2, respectively.

Cumulation was measured in the absence of Mg2+ at medium containing 150 mM KCl, 0.5 mM Me-ATP, 10 mM Pipes/Tris, pH 7.0, with or without valinomycin (1 pg/ml), or with both ATP and valinomycin (A). The RbCl transport was measured in the absence of Mg2+ at 24 °C and was corrected for nonspecific components. Accumulation of [14C]aminopyrine in the resting L-1 (Panel C) and the L-2 (Panel D) was measured in a medium containing 150 mM RbCl, 0.5 mM Mg2+-ATP, 10 mM Pipes/Tris, pH 7.0, with or without valinomycin (1 µg/ml). The specific activity of (H+-K')-ATPase was 35 and 31 µmol of P/h·mg of protein for the L-1 and L-2, respectively. The time course uptake of Rb+ in the resting L-1 (Panel A) or the L-2 (Panel B) in the presence of 1 mM ATP (1 mM) or with both ATP and valinomycin (A). The RbCl transport was measured with [14C]aminopyrine uptake in the presence of 1 mM RbCl at 24 °C. Each point represents the mean and experimental variations of triplicate measurements. We have compared the effect of sulfate and chloride anion on uptake of Rb+ at 1 meq in the resting L-1 and the stimulated heavy microsomes (Fig. 9). In both of the membrane fractions, sulfate anion reduced the transport activity of Rb+ by 40% as compared to that observed with chloride anion. It should be noted that the transport activity in the stimulated heavy microsomes was measured in the presence of 1 mM ATP to eliminate ATP-sensitive RbCl uptake, although it was a minor component. Interestingly, we have observed the latency of ATP-sensitive RbCl transport activity in the stimulated heavy microsomes. For instance, as the heavy microsomes were incubated at 24 °C for 30 min, nearly 40% of the total RbCl uptake became inhibited by ATP (1 mM) (Fig. 10). It should be emphasized that the stimulated heavy microsomes, when kept at 0 °C, showed largely ATP-insensitive RbCl uptake; less than 10% of the total uptake being inhibited by the nucleotide (Panel A, Fig. 10). The appearance of the ATP sensitivity is not due to pumping of Rb+ by (H+-K')-ATPase, since ATP was added in the absence of Mg2+ and addition of EDTA up to 1 mM had no effects on the above results. The temperature-dependent appearance of the ATP sensitivity suggests certain enzymatic modification of the putative RbCl transporter in the stimulated heavy microsomes.

The stimulated heavy microsomes from the D2O gradient were further purified through gradient centrifugation using Percoll. The membrane band near the top of the self-formed gradient was enriched 2-fold with (H+-K')-ATPase as compared to the starting membrane fraction; the specific activity of (H+-K')-ATPase was 45 µmol of P/h·mg of protein. The ATPase-enriched fraction was freed of Percoll through a column chromatography with Sephacryl S-1000 fine. Sodium dodecyl sulfate-gel electrophoresis pattern of the Percoll-purified stimulated heavy microsomes is shown in Fig. 11 (Lane 3) in comparison with those of the resting L-1 and L-2 (Lanes 1 and 2). The purified heavy microsomes were made of three main polypeptides at the molecular weight of 93,000 representing (H+-K')-ATPase, 81,000 and 45,000. The other two polypeptides appeared to be rather minor components in the resting light microsomes.

Table I shows v,, and K,, for RbCl uptake in the resting L-1, the stimulated heavy microsomes and the Percoll purified
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FIG. 11. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns. From the left, molecular weight standards (the same as described in the legend of Fig. 6), the resting L-1, the resting L-2, and the Percoll-purified stimulated heavy microsomes. 15 μg of membrane proteins were applied. The slab gel (10%) was stained with Coomassie Blue.

TABLE I
Kinetic parameters for RbCl uptake in various gastric microsomal fractions

The rate of 86RbCl uptake was measured in the Mg2+-free sucrose/Pips buffer, pH 7.0, varying concentration of RbCl from 0.1 to 4 mM. We have used the rates measured during the first 15-s incubation at 24 °C for the resting light and the rates during the first 3 min for the heavy microsomal fractions. For the stimulated heavy microsomes, 1 mM ATP was included in the above media.

| Fraction                  | $K_v$ (nmol/min-mg protein) | $K_m$ (mM) |
|---------------------------|-----------------------------|-----------|
| Resting light microsomes  | 13.7 ± 1.9                  | 0.58 ± 0.11|
| Stimulated heavy microsomes | 0.91 ± 0.10               | 0.54 ± 0.06|
| Percoll purified heavy microsomes | 1.7                      | 0.56      |

fraction, $K_v$ was around 0.55 mM for all the membrane fractions and the $K_v$ for the resting L-1 was over 10 times greater than that for the stimulated heavy microsomes. The Percoll purified heavy microsomes showed a $r_{max}$ two times faster than the starting heavy fractions. These data suggest that RbCl-transport activity was co-purified with (H+-K+)-ATPase in the heavy microsomes.

We have examined $K_v$ values of several nucleotides including ATP and Mg2+-vanadate for RbCl uptake in the resting L-1 fraction. $K_v$ of ATP was 3 μM and was not affected in the presence of 1 mM Mg2+. ADP and ITP also inhibited the RbCl uptake with a $K_v$ of 30 and 50 μM, respectively, and AMP was not effective even at 1 mM. Vanadate only in the presence of Mg2+ (1 mM) showed $K_v$ of 30 μM. Similar values of $K_v$ for ATP and vanadate were observed for RbCl uptake in the carbachol-stimulated heavy microsomes after aging at 24 °C.

FIG. 12. Lineweaver-Burk plots of the rate of 86RbCl uptake by the resting light microsomes, L-1, versus the concentration of RbCl varying from 0.2 to 4 mM in the absence (○) or presence of ATP 2 μM (△) or 5 μM (●). The rate of uptake was measured during the first 15 s of incubation time at 24 °C.

TABLE II
Specific activities of the marker enzymes for mitochondria and endoplasmic reticulum in the various gastric membranes

Succinate-cytochrome c reductase, and NADPH-cytochrome c reductase in the presence of KCN were measured spectrophotometrically as described (10, 11). The enzyme activities in the homogenates of the carbachol-stimulated or the resting gastric mucosa were not significantly different. The data represent the average of duplicate measurements.

| Fractions               | Succinate-cytochrome c reductase (nmol/min-mg protein) | NADPH-cytochrome c reductase (nmol/min-mg protein) |
|-------------------------|--------------------------------------------------------|-----------------------------------------------------|
| Carbachol-stimulated    | 57.1                                                   | 4.0                                                 |
| L-1                     | 5.5                                                    | 9.2                                                 |
| L-2                     | 18.7                                                   | 17.3                                                |
| Heavy                   | 30.3                                                   | 5.8                                                 |
| Percoll purified, heavy | 6.5                                                    | 11.4                                                |

We have examined $K_v$ values of several nucleotides including ATP and Mg2+-vanadate for RbCl uptake in the resting L-1 fraction. $K_v$ of ATP was 3 μM and was not affected in the presence of 1 mM Mg2+. ADP and ITP also inhibited the RbCl uptake with a $K_v$ of 30 and 50 μM, respectively, and AMP was not effective even at 1 mM. Vanadate only in the presence of Mg2+ (1 mM) showed $K_v$ of 30 μM. Similar values of $K_v$ for ATP and vanadate were observed for RbCl uptake in the carbachol-stimulated heavy microsomes after aging at 24 °C.
As shown in Fig. 12, ATP decreased \( v_{\text{max}} \) and increased \( K_m \) for RbCl uptake in the resting L-1. These data suggest allosteric effects of ATP on RbCl transport in the resting light microsomes. We have found that rat (H\(^+-K^+\))-ATPase has a \( K_m \) for Rb\(^+\) of 1.2 mM while its \( K_m \) for Mg\(^{2+}\)-vanadate was 0.03 \( \mu \)M. These parameters were obtained with 20 mM RbCl, 2 mM Mg\(^{2+}\)-ATP, when they are not limiting substrates, and in the presence of nigericin.

The various gastric microsomal fractions were examined for their contents of mitochondrial and endoplasmic reticulum membranes (Table II). The activity of succinate-cytochrome c reductase, a marker for mitochondria, was not enriched in the rat gastric microsomes as compared to the homogenates and also was not noticeably different between the membranes from the resting or the carbachol-stimulated animals. The activity of NADPH-cytochrome c reductase, a marker for endoplasmic reticulum, was 1.5- to 4-fold enriched in the various gastric microsomal membranes in comparison with that of the homogenates, but the enzyme level of each fraction remained unchanged whether the membranes were prepared from the resting or the carbachol-stimulated animals. These data dissociated the RbCl transport activity from mitochondrial or endoplasmic reticulum membranes.

DISCUSSION

Earlier we have shown that (a) both the light and the heavy microsomes from rat gastric mucosa were enriched with (H\(^+-K^+\))-ATPase, and (b) intravesicular H\(^+\) accumulation is valinomycin-independent in the carbachol-stimulated heavy microsomes, but ionomycin-dependent in the light or the resting heavy microsomes (4). Recently, Wolosin and Forte (17) and Couppaletti and Sachs (18) have reported the appearance of large KCl or Cl\(^-\) conductance in histamine-stimulated rabbit gastric membranes. We have also observed in this study that transport activity of RbCl in rat gastric heavy microsomes increased with carbachol stimulation and was apparently responsible for valinomycin-independent intravesicular H\(^+\) accumulation (Fig. 3). What had been unexpected, however, was the rapid and high level of RbCl uptake in the resting light microsomes (Fig. 1). Three aspects of this transport activity are of interest: its functional and physical identity in relation to gastric (H\(^+-K^+\))-ATPase, its connection with the similar transport activity in the heavy microsomes, and its role in the acid secretory process.

To begin with its functional identity, the RbCl transport activity in the resting light microsomes is not likely to involve a Rb\(^+\)-H\(^+\) or Rb\(^+-\)K\(^+\) exchange by gastric (H\(^+-K^+\))-ATPase (16) because of the following observations: (a) substitution of Cl\(^-\) with SO\(_4^2-\) decreased the Rb uptake by 40\%, (b) vanadate inhibited the activity of (H\(^+-K^+\))-ATPase with a \( K_{\text{inhibitor}} \) of 0.03 \( \mu \)M, but was 1000 times less sensitive to the RbCl transport activity (its \( K_{\text{inhibitor}} \) being 30 \( \mu \)M). (c) ATP noncompetitively inhibited the RbCl uptake with a \( K_{\text{inhibitor}} \) of 3 \( \mu \)M while the cation exchange by hog gastric (H\(^+-K^+\))-ATPase has been reported to have a \( K_{\text{inhibitor}} \) of 30 \( \mu \)M for ATP and to be anion-insensitive (16). Furthermore, the Rb\(^+\)-Rb\(^+\) exchange by (Na\(^+-\)K\(^+\))-ATPase, a membrane enzyme analogous to (H\(^+-\)K\(^+\))-ATPase, was rather stimulated at 3 \( \mu \)M of ATP (19). In fact, observations made in this study suggest that (H\(^+-\)K\(^+\))-ATPase and the putative RbCl transporter were located in different populations of membrane vesicles in the resting light microsomes. For instance, the RbCl transport activity was concentrated in the L-1, the light microsomal subfraction of lower density as resolved with H\(_2\)O gradient centrifugation (Fig. 5), but not in the L-2, although both the fractions were equally enriched with (H\(^+-\)K\(^+\))-ATPase and require valinomycin for intravesicular acidification (Fig. 8). Furthermore, the RbCl uptake in the L-1 was inhibited by ATP (Fig. 7) and could not be restored even with valinomycin, therefore is likely to be dissociated from those membrane vesicles containing (H\(^+-\)K\(^+\))-ATPase which should have become permeable to RbCl with valinomycin in the presence of ATP (Fig. 8).

Despite their apparent spatial individuality, (H\(^+-\)K\(^+\))-ATPase and the RbCl transport activities in the light microsomes decreased in unison with carbachol stimulation (Fig. 4). At the same time, the increased population of the heavy microsomal vesicles contained (H\(^+-\)K\(^+\))-ATPase (4) and became permeable to RbCl (Figs. 3 and 4). Several lines of evidence suggest that the same transporter is involved in the RbCl uptake in the resting light and the stimulated heavy gastric microsomes; (a) the same \( K_m \) for Rb\(^+\), (b) the similar anion effect (Cl\(^-\) > SO\(_4^2-\)), (c) the equal sensitivity to various divalent cations, and (d) inhibition by ATP, although the nucleotide sensitivity was latent in the stimulated heavy microsomes (Fig. 10). The appearance of the ATP sensitivity was temperature-dependent and conceivably reflects some type of enzymatic modification of the RbCl transporter, perhaps involving its dephosphorylation. Whatever the modifications are, it appears that \textit{in vivo} the KCl transporter remains inactive during the resting state due to its ATP sensitivity and may become active upon its seemingly reversible conversion to the ATP-insensitive form with secretagogue stimulation. Thus these observations support our proposition that the same KCl transporter is engaged in the RbCl uptake in the resting light or the stimulated heavy microsomes. Even the only observed difference in the \( v_{\text{max}} \) of RbCl uptake, being 10 times greater for the resting light than the stimulated heavy microsomes, would be expected, if the light membrane vesicles concentrated with the transporter were fused into the heavy apical membranes of greater surface area.

The involvement of the RbCl transport activity in the acid secretory process of gastric parietal cells was alluded to in the preceding discussion. This proposal has been supported by three observations. First, cimetidine, which blocked the histamine- or carbachol-induced shift of the RbCl transport activity from the light to the heavy gastric microsomes, has been known to limit its effect in the gastric mucosa primarily to the parietal cells (20-22). Second, the RbCl transport activity in the stimulated heavy microsomes which seems to be originated from the resting light microsomes is not only related to valinomycin-independent H\(^+\) accumulation by (H\(^+-\)K\(^+\))-ATPase, but has been co-purified with the ATPase through Percoll gradient centrifugation. Finally, the mitochondrial or endoplasmic reticulum of the gastric cells could not be responsible for the RbCl transport activity, since the specific activities of their marker enzymes in the various microsomal membranes was not affected at all with carbachol stimulation. In conclusion, gastric parietal cells appear to possess a population of intracellular membrane vesicles containing KCl transporter in addition to those containing (H\(^+-\)K\(^+\))-ATPase, both of which may converge at the apical membranes upon secretagogue stimulation.

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