Biochimica et Biophysica Acta, 772 (1984) 167–175
Elsevier

BBA 72080

EFFECT OF CARBACHOL OR HISTAMINE STIMULATION ON RAT GASTRIC MEMBRANES ENRICHED IN (H^+-K^+)-ATPase

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(Received October 4th, 1983)
(Revised manuscript received January 4th, 1984)

Key words: (H^+-K^+)-ATPase; Carbachol; Histamine; Electron microscopy; (Rat gastric membrane)

We have examined histamine- or carbachol-induced changes in rat gastric membranes enriched in K^+-stimulated ATPase. Stimulation of secretion by both secretagogues in vivo produced a class of microsomal membranes which exhibited valinomycin-independent, KCl-dependent H^+ transport. In contrast, membrane vesicles isolated from cimetidine inhibited resting mucosa exhibited largely the ionophore-dependent H^+ transport. In addition, only in the carbachol-stimulated membranes a portion of the ionophore-independent H^+ transport was refractory to cimetidine pretreatment. The gastric microsomal membranes were resolved into light and heavy fractions by centrifugation over isotonic ^2H_2O media. The ionophore-independent H^+ transport was almost exclusively associated with the heavy microsomal fraction while the ionophore-dependent H^+ transport was detected in the light fraction. Also, these fractions were considerably different from each other in their appearance in electron micrographs and SDS gel electrophoresis patterns. Secretagogue stimulation increased the population of the heavy microsomal membrane vesicles exhibiting the valinomycin-independent, K^+-dependent H^+ transport and their overall content of K^+-stimulated ATPase. Cimetidine treatment, on the other hand, increased the ATPase activity associated with the light microsomes, and produced the heavy microsomal membranes showing only a marginal degree of the ionophore independent H^+ accumulation, even though they were very similar to the carbachol-stimulated heavy membranes in the specific activity of K^+-stimulated ATPase, SDS gel patterns and appearance in electron micrograph. These observations suggest that activation of secretion involves at least two distinctive events; transformation of the light to the heavy gastric membranes containing a K^+-dependent H^+ pump and an increased KCl permeability in the latter.

Introduction

(H^+-K^+)-ATPase is an electroneutral H^+-K^+ exchange system located primarily in gastric parietal cells [1–5]. This enzyme has been recognized as the acid pump involved in the terminal steps of the acid secretory process [6]. Physiological secretagogues such as histamine, acetylcholine and gastrin are known to activate the acid pump through mediation by second messengers, cAMP and/or Ca^{2+} [7–13]. Although these messengers are known to activate various cellular protein kinases [14,15], the molecular changes directly related to activation of (H^+-K^+)-ATPase are not fully understood at present.

Several recent studies have related KCl transport to gastric acid secretion. For instance, K^+-dependence of acid secretion has been demonstrated

Abbreviation: Pipes, 1,4-piperazinediethanesulphonic acid.

in frog stomach [16], isolated gastric glands [17–19] and gastric membrane vesicles enriched with (H^+-K^+)-ATPase [20]. Chloride conductance has been detected upon aging or limited trypsin digestion of hog gastric membranes [21]. More recently, Wolosin and Forte [22,23] have reported that histamine stimulation in the rabbit produced a population of vesicles containing an ionophore-independent, K^+-dependent H^+ pump. They concluded that changes in the sedimentation properties of these gastric membranes were due to increase in density and size of the isolated vesicles [24].

In this study, we explored these observations further in the rat using histamine and carbachol. Both secretagogues induced an ionophore-independent, K^+-dependent H^+ transport activity in the gastric microsomal membrane vesicles, although the magnitude and duration of their effects, and their sensitivity to cimetidine were variable. Furthermore, we have been able to resolve the rat microsomes into light and heavy fractions by centrifugation over isotonic sucrose-^2H_2O media. Both fractions were enriched with K^+-stimulated ATPase, but greatly differed in their polypeptide compositions, appearances in electronmicrographs and H^+ transport requirements.

Methods and Materials

Male Sprague-Dawley rats weighing about 210 g were fasted for 16 h. Histamine (30 mg/kg) or carbachol (350 μg/kg) was given subcutaneously. Cimetidine (100 mg/kg) was injected intraperitoneally. The dose of the secretagogues or cimetidine was chosen to give the maximal response on the dose-response curve. In some experiments, cimetidine was given 45 min before the treatment with the secretagogues in order to study possible interaction between the H_2 blocker and the secretagogues. Typically, the rats were killed by cervical dislocation 30 min after a final treatment, unless stated otherwise.

The gastric microsomes enriched in K^+-stimulated ATPase were prepared as described before [24]. Briefly, the mucosal tissues from gastric fundic region were obtained by scraping with a glass slide. The scrapings from 10 rat stomachs were suspended in 40 ml of homogenizing buffer containing 250 mM sucrose/2 mM $MgCl_2$/1 mM EGTA/2 mM Hepes/Tris (pH 7.4). The tissues were homogenized with 20 strokes of a motor-driven (1500 rpm) Teflon pestle in a Potter-Elvehjem homogenizer. The homogenates were fractionated by differential centrifugation; the fraction enriched in nuclei and mitochondria was obtained by centrifuging at 20000 × g for 15 min and the microsomes at 170000 × g for 35 min.

Resolution of the gastric microsomes was attempted by centrifugation over homogenizing buffers prepared in deuterium oxide. Typically, the supernatant after the nuclei-mitochondria-enriched fraction was layered over a gradient of 10 ml and 4 ml of homogenizing buffers prepared in 40 and 99.8% ^2H_2O, respectively. The actual density of the sucrose-^2H_2O buffers was 1.07 and 1.13 in the order given above. The centrifugation was carried out in a SW 24.1 rotor at 24000 rpm for 30 min. The microsomes were separated into two fractions; the heavy microsomes being sedimented to form a pellet and the light ones remaining in the ^2H_2O media. The latter were recovered after dilution by a second centrifugation at 170000 × g for 35 min.

Assay procedures

(H^+-K^+)-ATPase activity in various membrane fractions was determined by measuring K^+-dependent release of inorganic phosphate from ATP. About 50 μg membrane proteins were added to 1 ml incubation buffers containing 40 mM Tris-acetate (pH 7.4), 180 mM sucrose, 2 mM $MgCl_2$, 2 mM ATP and 0.56 μM dicyclohexylcarbodiimide with or without 7 mM KCl and 7 mM NH_4Cl. The mixtures were incubated for 8 min at 37°C. The reaction was terminated by adding 1 ml ice-cold 10% trichloroacetic acid containing 0.1 g of HCl-washed charcoal. The amount of inorganic phosphate released was determined by the method of Tsai et al. [25]. Addition of nigericin (5 μg/ml) instead of NH_4Cl in the incubation buffers led to the same rate of K^+-dependent ATP hydrolysis.

The various membrane fractoins were analyzed for the following markers; succinate-cytochrome c reductase as a marker for mitochondria [26], glucose-6-phosphatase for endoplasmic reticulum [27] acid phosphatase using glycerophosphate for

lysosomes [28], and RNA for rough endoplasmic reticulum [29].

The degree of H^+ ion accumulation in gastric microsomal membrane vesicles was estimated by measuring the uptake of amino[^{14}C]pyrine or absorbance changes in acridine orange [30,31]. For aminopyrine uptake, 5–10 μl of membrane suspensions (100–150 μg protein) were mixed with 500 μl of a buffer containing 150 mM KCl/10 mM Pipes/Tris (pH 7.0)/0.5 mM Mg^{2+}-ATP/2 μg oligomycin/3 μM amino[^{14}C]pyrine (99.5 mCi/mM). In some experiments, the KCl concentration was lowered to 35 mM while maintaining isoosmolarity with sucrose. The mixtures were incubated at 22°C for an indicated time. The reaction was terminated by filtering the mixture over a Millipore filter (HAWP, 0.45 μm). The filters were washed with ice-cold 150 mM KCl solution and counted in 10 ml of Instagel scintillation cocktail (Packard). The data were corrected for non-specific adsorption of amino[^{14}C]pyrine in the filter.

For uptake of acridine orange, similar media described above were employed except that acridine orange at 15 μM was present in place of aminopyrine. Changes in the absorbance of acridine orange were followed spectrophotometrically at 492 nm, as described elsewhere [30]. Also, fluorescence quenching of acridine orange was measured in a Perkin-Elmer MPF 44 scanning fluorimeter with 492 nm excitation and 546-nm emission wavelengths. The concentration of acridine orange was 1 μM. In some experiments, the concentrations of K^+ and Cl^- in the reaction mixture were varied by mixing stock solutions of K_2SO_4 and choline chloride while maintaining isoosmolarity with sucrose.

For electronmicrographs, the membrane fractions were resuspended in a solution of 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.0). After fixation for 24 h at 4°C, the fractions were sedimented, postfixed in 1% OsO_4 in phosphate buffer, dehydrated in graded ethanol solution, and infiltrated with epoxy resin. Thin sections were stained by flotation on uranyl acetate and lead citrate solutions and were examined with a Philips 301 Transmission Electron Microscope.

Polyacrylamide slab gel electrophoresis (12% gel) of membrane samples was carried out using Tris-glycine-sodium dodecyl sulfate buffer [32]. Protein was determined by the method of Lowry et al. [33] using bovine serum albumin as a standard.

Materials

Amino[^{14}C]pyrine was purchased from New England Nuclear. Nigericin was obtained from Calbiochem. Valinomycin, histamine and carbachol were purchased from Sigma. Pentagastrin was obtained from Fox Chemical. All other materials were of reagent grade quality and obtained from standard sources.

Results

Table I shows the specific activity and the distribution of K^+-stimulated ATPase in subcellular fractions obtained from homogenates of the gastric mucosa of the rats treated with cimetidine, histamine or carbachol. The secretagogue treatments markedly reduced both the specific activity and total recovery of the ATPase in the microsomal fraction (170000 × *g* for 35 min) but increased recovery of the enzyme activity in the fraction enriched with nuclei and mitochondria (20000 × *g* for 15 min) in comparison with the cimetidine treatment. For example, the K^+-dependent ATPase activity was 43.8 μmol/h per mg protein in the gastric microsomes from the cimetidine-treated rats, but only 29 and 20 μmol/h per mg protein in those from the histamine- or carbachol-treated rats, respectively. The amount of the ATPase activity in the microsomes relative to that in the homogenates was 42, 29 and 22% for the cimetidine, histamine and carbachol group, respectively. The nuclei and mitochondria-enriched fraction from the secretagogue-treated rats contained almost 60% of the ATPase activity in the homogenates, while the same fraction from the cimetidine-treated rats contained only 30%.

Transport of H^+ ions was examined in the various microsomes using amino[^{14}C]pyrine as an internal pH probe (Fig. 1). In the medium containing valinomycin (5 μg/ml), 0.5 mM Mg^{2+}-ATP and 150 mM KCl, the microsomes from the cimetidine-treated rats showed the highest level of H^+ accumulation at equilibrium followed by those from the histamine- and the carbachol-treated rats.

TABLE I

SPECIFIC ACTIVITY AND DISTRIBUTION OF K^+-STIMULATED ATPase IN THE SUBCELLULAR FRACTIONS OF THE GASTRIC MUCOSA FROM THE RATS TREATED WITH CIMETIDINE, HISTAMINE OR CARBACHOL

K^+-dependent ATP hydrolysis was measured under K^+ permeable conditions using 7 mM NH_4Cl or nigericin with KCl (7 mM). Dicyclohexylcarbodiimide at the concentration of 0.56 μM was present in the incubation media to inhibit mitochondrial ATPase activity. The values in the parenthesis represent the percentage in each fraction of the total ATPase activity in the homogenates.

Treatments	K^+-stimulated ATPase activity (μmol/h per mg protein)		
	Homogenate	Nuclei- and mitochondria-enriched	Microsomal
Cimetidine	11.2 ± 0.9	7.1 ± 0.8 (30 ± 4)	43.8 ± 3 (42 ± 5)
Histamine	9.5 ± 1.5	11.0 ± 1.8 (54 ± 3)	29.0 ± 3.8 (29 ± 3)
Carbachol	10.1 ± 1.3	13.1 ± 1.1 (69 ± 2)	19.7 ± 3.0 (22 ± 4)

Under these conditions, the degree of valinomycin-dependent intravesicular acidification reflected the specific activity of K^+ stimulated ATPase activity in the microsomes, which in turn represent the population of the membrane vesicles bearing the ATPase. It should be noted that more than 95% of aminopyrine accumulation was abolished with nigericin (5 μg/ml).

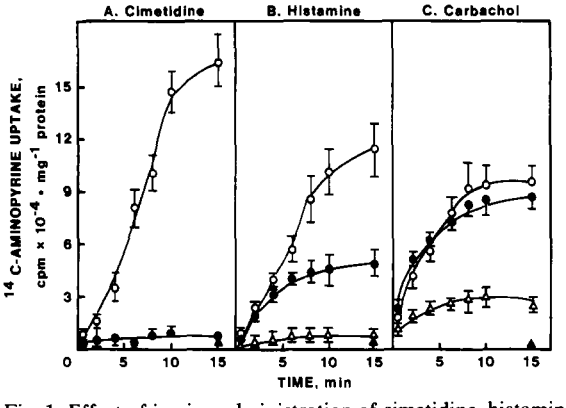

Fig. 1. Effect of in vivo administration of cimetidine, histamine or carbachol on H^+ transport in isolated rat gastric microsomes enriched in (H^+-K^+)-ATPase. The rats were fasted for 16 h and treated with the secretagogues (circles) or treated with a saturating dose of cimetidine 45 min prior to the administration of the secretagogues (open triangles). Amino[^{14}C]pyrine uptake was used to estimate the extent of H^+ accumulation in the membrane vesicle. The microsomes were suspended in the medium containing 150 mM KCl/0.5 mM Mg^{2+}-ATP/10 mM Pipes-Tris (pH 7.0) with (○) or without (●, △) valinomycin (5 μg/ml). The level of amino[^{14}C]pyrine in the medium was 3 μM, 400 cpm/μl of the medium, at the beginning of the reaction. The suspension was incubated at 22°C for an indicated time. The amount of amino[^{14}C]pyrine trapped within the vesicles was measured using rapid membrane filtration techniques and corrected for nonspecific adsorption of the filters. Addition of nigericin (5 μg/ml) released almost all the trapped aminopyrene from the vesicles as shown (▲).

In the absence of valinomycin, the microsomes from the cimetidine-treated rats showed the lowest level of intravesicular acidification, being just barely above the level observed upon addition of nigericin. In contrast, microsomes from the histamine-treated rats showed H^+ accumulation, though less than that observed with the microsomes from the carbachol-treated rats. Valinomycin had an almost negligible effect on the microsomes from the carbachol-treated animals. The microsomes from the rats fasted for 16 h were found to be similar to those from the cimetidine-treated ones with respect to their inability to accumulate H^+ ions in the absence of valinomycin (data not shown). H^+ movements in the microsomes demonstrated an absolute requirement for KCl and were measured in the presence of oligomycin (2 μg/ml).

Duration of the histamine or carbachol-induction of the activated H^+ transport was examined by comparing valinomycin-independent H^+ uptake by the microsomes prepared 30 min (as shown in Fig. 1B and C) and 80 min after administration of the single dose of the secretagogues. The carbachol effect was maintained at least up to 80 min without decay, but the histamine effect 80 min after the injection was reduced to less than one

half of the level observed 30 min after the drug administration. We have also examined the effect of cimetidine on the action of the secretagogues. Treatment of the rats with cimetidine 45 min prior to histamine administration completely abolished the secretagogue induction of the H^+ transport reaction; that is, the membrane vesicles were unable to accumulate H^+ ions without valinomycin (Fig. 1, panel B). The H_2 blocker, however, only partially inhibited the similar effect by carbachol. In this case, the microsomes from the rats treated successively with cimetidine and carbachol demonstrated a level of intravesicular acidification in the absence of valinomycin equal to one-third of that observed with the microsomes from the rats treated with carbachol alone (Fig. 1, Panel C).

The gastric microsomes were resolved into a heavy and a light fraction by limited centrifugation over homogenizing buffers prepared in 40 and 99.8% ^2H_2O. The heavy fraction sedimented at the bottom contained about 40–45% of the microsomal proteins regardless of the treatments. The rest of the microsomal membranes was recovered as the light fraction from the ^2H_2O media.

The heavy and the light microsomes were compared for their ability to accumulate H^+ ions in the absence of valinomycin (Fig. 2). The concentration of KCl in the transport medium was 35 mM. Only the heavy microsomes were able to acidify considerably their intravesicular space without valinomycin, although the extents of H^+ uptake varied among them according to the treatments given to the rats. For example, the carbachol treatment induced the highest level of intravesicular acidification in the heavy microsomes followed by the histamine treatment, reaching about 50% of the carbachol level. The cimetidine treatment led to the lowest level of H^+ uptake in the heavy microsomes, but still significantly higher than that observed on adding nigericin.

The concentration of KCl in the transport medium was raised to 150 mM since the valinomycin effect was not apparent with the low concentration of KCl [34]. The extent of H^+ accumulation by the heavy microsomes was reduced to almost one-fourth that observed at the low KCl (35 mM), while their relative magnitude depending on the various treatments was unchanged. Valinomycin showed no stimulating effect on H^+-accumulation by the heavy microsomes from the carbachol- or histamine-treated rats, but stimulated H^+ uptake by those from the cimetidine-treated rats (data not shown). It should be noted

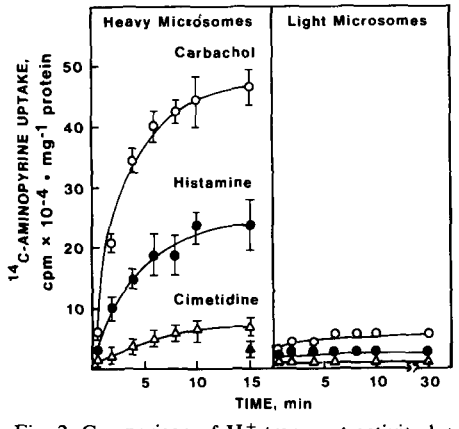

Fig. 2. Comparison of H^+ transport activity by the heavy and the light microsomes in the absence of valinomycin. The microsomes of different density were separated by centrifuging over isotonic sucrose-^2H_2O media. The microsomes were prepared from the rats treated with carbachol (○), histamine (●) or cimetidine (△). The transport activity was measured in the medium containing 35 mM KCl/0.5 mM Mg^{2+}-ATP/10 mM Pipes-Tris (pH 7.0). Addition of valinomycin (5 μg/ml) showed no effect but nigericin (5 μg/ml) reduced aminopyrine accumulation (▲).

TABLE II

SPECIFIC ACTIVITY OF K^+-STIMULATED ATPase IN THE LIGHT AND THE HEAVY GASTRIC MICROSOMES FROM THE RATS TREATED WITH CIMETIDINE, HISTAMINE AND CARBACHOL

The conditions for measurements of K^+-dependent ATP hydrolysis were the same as described in Table I. The light and the heavy microsomes were obtained by centrifugation over isotonic sucrose-^2H_2O media. The relative protein contents were 60 and 40% for the light and the heavy microsomes, respectively. The total amounts of the microsomal proteins were not affected noticeably by the treatments.

Treatments	K^+-stimulated ATPase activity (μmol/h per mg protein)	
	Light microsomes	Heavy microsomes
Cimetidine	52.5 ± 1.4	23.1 ± 1.9
Histamine	32.8 ± 3.6	18.1 ± 1.9
Carbachol	19.5 ± 1.6	24.6 ± 2.3

that the specific activity of K^+-stimulated ATPase was similar among the heavy microsomes from the cimetidine- and carbachol-treated rats, the value being 23.1 and 24.6 μmol/h per mg protein, respectively (Table II). The ATPase activity in the heavy microsomes from the histamine-treated rats was 18.1 μmol/h per mg protein.

The light microsomes which showed little H^+ uptake at either 35 or 150 mM KCl were stimulated to acidify their intravesicular space by valinomycin in the high KCl medium (Fig. 3). Again, the extent of intravesicular acidification was variable among the light microsomes depending on the treatment the animals received. The highest level was observed with the cimetidine treatment followed by the histamine and the carbachol treatments. The specific activity of K^+-stimulated ATPase in the light microsomes from the cimetidine-, histamine- or carbachol-treated rats was 52.5, 32.8 and 19.5 μmol/h per mg protein, respectively. It appeared that the degree of intravesicular acidification per unit of the ATPase activity in the light microsomes was constant, regardless of the treatments.

Our preliminary studies showed that pentagastrin was very similar to histamine in the rat with respect to its ability to induce ionophore-independent, K^+-dependent H^+ transport in the heavy microsomal membranes and its complete sensitivity to cimetidine.

Since in the case of carbachol treatment about 70% of (H^+-K^+)-ATPase activity were found in the fraction enriched with nuclei and mitochondria (20 000 × g), the fraction was rehomogenized and subjected to differential centrifugation. The microsomes thus obtained possessed again 12% of K^+-stimulated ATPase activity of the original homogenates. When the microsomes were resolved using the sucrose-^2H_2O media, about 70% of the microsomal ATPase activity were located in the heavy microsomes, which were found indistinguishable from those prepared originally with respect to their specific activity of K^+-stimulated ATPase and H^+ ion transport characteristics.

Fig. 4 shows SDS gel electrophoresis patterns of the light and the heavy microsomes from the rats treated with carbachol, pentagastrin, histamine or cimetidine. It is noteworthy that the most prominent band in the gel patterns of either the light or the heavy microsomes is the one at the region of 100 kDa where (H^+-K^+)-ATPase is expected [35]. The polypeptide patterns of the heavy microsomes were not noticeably variable whether the animals were treated with the secretagogues or cimetidine.

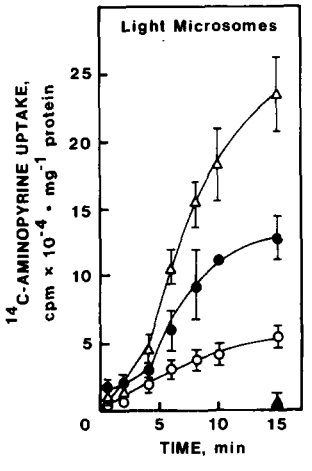

Fig. 3. H^+ transport activity by the light microsomes in the presence of valinomycin. The same symbols as in Fig. 2. The transport medium consists of 150 mM KCl, 0.5 mM Mg^{2+}-ATP and 10 mM Pipes-Tris (pH 7.0) with valinomycin (5 μg/ml).

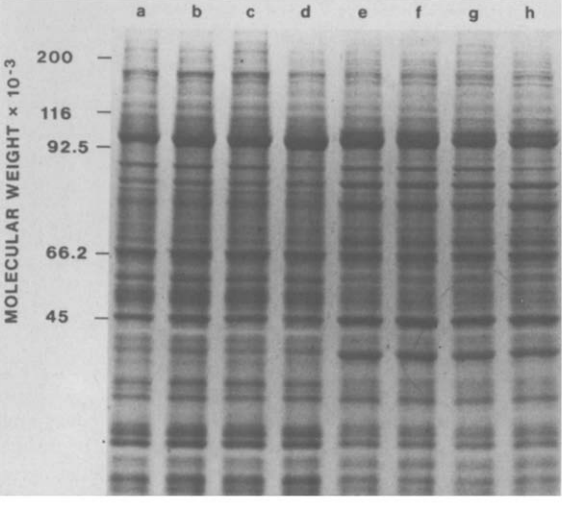

Fig. 4. SDS-polyacrylamide gel electrophoretic patterns. The light microsomes (a, b, c and d) and the heavy microsomes (e, f, g and h) from the rats treated with carbachol, histamine, pentagastrin or cimetidine, respectively. 15 μg of membrane proteins were applied. The slab gel (12%) was stained with Coomassie blue.

On the other hand, in the gel patterns of the light microsomes the intensity of the band of 100 kDa was considerably reduced with the carbachol treatment as compared to the cimetidine. This observation is consistent with the difference observed in their (H^+-K^+)-ATPase activity; 19.5 and 52.5 μmol/h per mg protein for the light microsomes from the carbachol and the cimetidine-treated rats, respectively (Table II). The other regions of the gel patterns of the light microsomes were not variable under the treatments with the secretagogues or cimetidine. It should be noted, however, that considerable differences were observed between the gel patterns of the light and the heavy microsomes. For instance, the mjaor bands in the gel patterns of the heavy microsomes besides the one at 100 kDa were located at the regions of 105, 82, 76, 45 and 42 kDa. These bands were either of less intensity or absent in the patterns of the light microsomes.

Fig. 5 shows the electron micrographs of the light microsomes from the cimetidine-treated rats (A) and of the heavy microsomes from the carbachol-treated animals (B). The average size of the membrane vesicles present in the light or the heavy microsomes was not significantly different. However, numerous compact nubs are present at the outer surface of almost all the heavy microsomal membrane vesicles, but absent in a majority of the light microsomal membrane vesicles. It should be noted that the heavy microsomes from the cimetidine-treated rats showed the same mor-

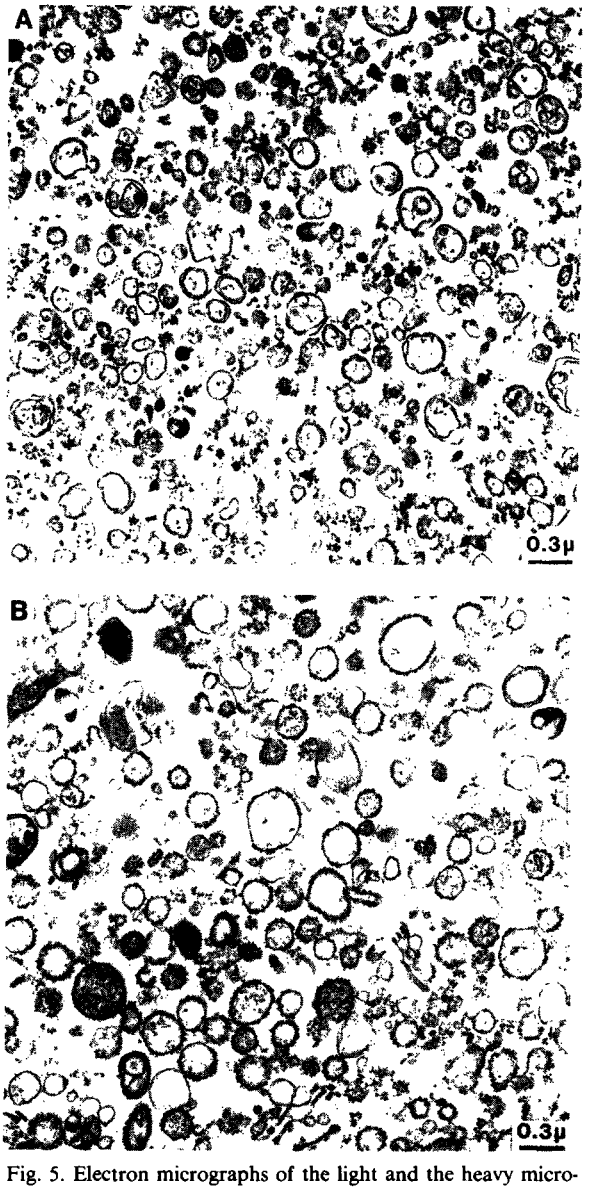

Fig. 5. Electron micrographs of the light and the heavy microsomes from the cimetidine- or carbachol-treated rats, respectively.

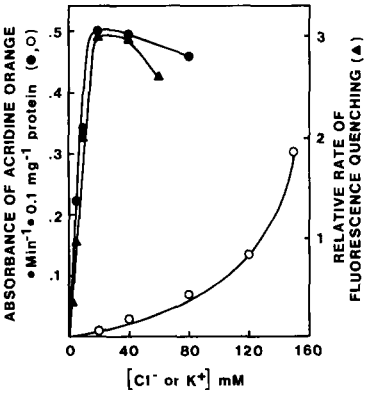

Fig. 6. Dependency of H^+-transport in rat gastric microsomes on chloride or K^+; the heavy (●, ▲) and the light (○) microsomes from the carbachol- and the cimetidine-treated rats, respectively. The incubation medium contained 18 mM K_2SO_4 with indicated amounts of choline chloride (●, ○) or 140 mM choline chloride with indicated amount of K^+ as K_2SO_4. Isoosmorality was maintained by adding sucrose. Changes in the absorbance of acridine orange were measured at 492 nm as described [30]. Fluorescence quenching of acridine orange was measured with 492 nm excitation and 546 nm emission wavelengths. Relative initial rates were plotted.

phological characteristics as those from the carbachol-treated rats.

We have compared chloride ion dependency of H^+ uptake in the heavy microsomes from the carbachol-treated or the light microsomes from the cimetidine-treated rats (Fig. 6). In the presence of sulfate as the only counteranion, as expected K^+ (36 mM) could not stimulate H^+ accumulation in the heavy or the light microsomes with or without valinomycin. Addition of chloride ions dose-dependently stimulated H^+ uptake at a fixed K^+ concentration, 36 mM. The chloride concentration required to obtain the half maximal initial rate of stimulation of H^+ uptake was 7 mM in the heavy microsomes and more than 100 mM in the light microsomes with valinomycin. The K^+ concentration required to give the half maximal initial rate of H^+ uptake at a fixed chloride concentration (140 mM) was again 7 mM in the heavy microsomes without valinomycin.

Finally, these two fractions were compared for the extent of contamination by intracellular organelles. The level of succinate-cytochrome *c* reductase, a marker for mitochondria, in the light and the heavy microsomes was 1/4 and 1/2 of that in the homogenates, respectively. The levels of glucose-6-phosphatase or acid phosphatase, a marker for endoplasmic reticulum or lysosomes, respectively, were not significantly different between the light and the heavy microsomes, being about equal to those of the homogenates. Furthermore, the content of RNA was 40 and 30 $\mu g/mg$ protein for the light and the heavy fraction, respectively.

Discussion

In this study we have shown that a K^+-stimulated ATPase was located in two types of gastric microsomal membranes, the light and the heavy microsomes as resolved by a limited centrifugation over isotonic sucrose-^2H_2O media. Electron micrographs showed considerable morphological variations between the two fractions. Most apparent, dense nubs were found at the outer surface of almost all the heavy microsomal membranes, but were absent in a majority of the light microsomal membranes. Since the contents of RNA and glucose-6-phosphatase activity were not considerably different between the light and heavy microsomes, the membranes with the nubs do not represent rough endoplasmic reticulum. Also, both fractions showed no significant enrichment of mitochondrial or lysosomal marker enzymes. However, the identity of the 'nubs' and their possible role in acid secretory process are not clear and further studies are needed.

We have also observed considerable differences between the SDS gel patterns of the light and the heavy microsomes. Both types of the microsomes, although enriched with gastric parietal cell membranes containing K^+-stimulated ATPase, are likely to be contaminated somewhat with other cellular or intracellular membranes. The differences in their gel patterns, therefore, may reflect not only the possible variations in the protein compositions between the light and the heavy membranes of gastric parietal cells, but also the possible diversity in other contaminating membranes. In this connection, it was noted that treatments with the secretagogues or cimetidine caused no apparent changes among the gel patterns of the heavy or the light microsomes except the intensity of the band at the region of 100 kDa. These observations can be interpreted to mean that at least the degree of contamination by other membranes was not variable within the heavy or the light microsomes following the various treatments.

Of particular importance are the functional changes in the light and the heavy microsomes brought about with the secretagogues in comparison with cimetidine. Several changes are noteworthy: (1) reduction of K^+-stimulated ATPase activity in the light microsomes; (2) an increased distribution of the enzyme activity in the nuclei and mitochondria-enriched fraction which can in turn be isolated as predominantly heavy microsomes; (3) detection of an ionophore-independent, K^+-dependent H^+ pump in the heavy microsomal membranes. It is reasonable to assume a functional relationship between secretagogue-induced redistribution of K^+-stimulated ATPase and activation of the ionophore-independent H^+ transport pathway. This assumption underlies the studies of the 'activated' state in rabbit where H^+ transport is typically thought to be H^+-K^+ exchange with pump activity increased by enhanced permeability to K^+ and Cl^-.

In this study we have shown that carbachol and histamine differed in the duration of their effects on the apical membranes of gastric parietal cells and the sensitivity to the H_2 blocker' cimetidine. These differences may arise from the diversity in the mode of interactions between the secretagogues and gastric parietal cells. Currently, two leading hypotheses are available; one proposes histamine as the final messenger for all the secretagogues and the other holds that each secretagogue interacts with its own receptor at the surface of gastric parietal cells [35–37]. The observed reduction of the carbachol effect on the parietal cell membranes by cimetidine pointed to the important role of histamine in the action of carbachol. However, since a significant portion of the carbachol effect survived the cimetidine pretreatment, a histamine-independent interaction of carbachol is suggested.

Regardless of their modes of interaction with the parietal cells, carbachol and histamine bring about qualitatively similar changes; activation of an ionophore independent H^+-transport pathway and an apparent increase in heavier membranes containing this mechanism and a K^+-stimulated ATPase.

Acknowledgement

We thank Drs. George Sachs and John Cuppoletti for their comments and helpful discussions.

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