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EFFECT OF OMEPRAZOLE ON GASTRIC SECRETION IN H^+,K^+-ATPase AND IN PEPSINOGEN-RICH CELL FRACTIONS FROM RABBIT GASTRIC MUCOSA*

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Abstract—In order to study the effects of the substituted benzimidazole omeprazole on gastric secretory functions, parietal cells and chief cells from rabbit gastric mucosa were separated and enriched by density gradient centrifugation in Percoll. H^+,K^+-ATPase activity, as well as a 100,000 dalton protein, was found to copurify with a cell fraction morphologically characterized as mainly parietal cells (purity ~ 65%), while pepsinogen copurified with a cell fraction morphologically characterized as chief cells (purity ~ 90%). A spontaneous pepsinogen release (9.9 μg/mg cell dry wt × 2 hr), unaffected by both atropine and omeprazole, was found in the chief cell fraction. The release was approximately doubled by both carbacholine (4×10^{-5} M) and dibutyryl cAMP (db-cAMP, 10^{-3} M). The cholinergic stimulation was selectively blocked by atropine, while omeprazole had no effect on pepsinogen release induced by either of the secretagogues. On the other hand, omeprazole inhibited both db-cAMP- and histamine-stimulated acid secretion quantified as [^{14}C]aminopyrine (AP) accumulation in the parietal cell fraction. Cimetidine counteracted only acid secretion induced by histamine. These findings indicate that omeprazole has a specific effect on acid secretion, and are consonant with the hypothesis that the effect is due to H^+,K^+-ATPase inhibition.

Substituted benzimidazoles are potent inhibitors of gastric hydrochloric acid secretion *in vivo* in rat, dog and man [1–3]. In *in vitro* test systems, such as the isolated guinea-pig mucosa, this class of compounds inhibits the acid secretory response to both histamine and dibutyryl cAMP(db-cAMP) [4] (H. Larsson, E. Carlsson and G. Sundell, personal communication). In isolated gastric glands or enriched parietal cell preparations, the benzimidazoles inhibit basal acid formation, and that induced by histamine, db-cAMP and K^+, and in permeable gastric glands omeprazole prevents ATP-induced acid secretion [5–8]. When tested on purified H^+,K^+-ATPase, the benzimidazoles inhibit both ATPase activity and the hydrogen-ion transport function of this enzyme [9, 10]. These data indicate that the benzimidazoles inhibit acid formation by an action peripheral to cAMP at the level of the gastric proton pump, the H^+,K^+-ATPase. In the present study, special attention has been paid to the effect of omeprazole [5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1H-benzimidazole] on secretory functions in different cell fractions from gastric mucosa in order to make further evaluations of its action on the gastric secretory processes. For this purpose, a method of separating and enriching parietal and chief cells from rabbit gastric mucosa by the use of density gradient centrifugation in Percoll has been developed. The different cell fractions were characterized morphologically and by analysis of the content of pepsinogen and H^+,K^+-ATPase. Furthermore, the effects of different secretagogues and inhibitors of [^{14}C]aminopyrine (AP) accumulation and pepsinogen release were studied.

* Preliminary data have been published in abstract form [*Scand. J. Gastroent.* Suppl 78, Abstr. No. 442 (1982)].

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MATERIALS AND METHODS

Cell preparation. Cell dispersion: Male albino rabbits of New Zealand strain, weighing ~2.5 kg, were anaesthetized with Mebumal® (30 mg/kg, i.v.) and the stomachs were perfused *in situ* through the vascular system with phosphate-buffered saline [PBS (in mM): NaCl, 149.6; K_2HPO_4, 3.0; NaH_2PO_4, 0.64], pH 7.3, according to Berglindh and Öbrink [11]. After the stomachs had been excised, the antral part was cut off and discarded. Mucus was wiped off from the gastric mucosa, which was then dissected free from underlying muscular layers. The tissue was minced with a pair of scissors before incubation with pronase, 9 U/ml (Boehringer, Mannheim, F.R.G.). After 15 min of pronase treatment, the mucosa fragments were allowed to settle and the enzyme solution was sucked off and discarded. The settled material was rinsed three times and then treated for 45 min with collagenase, 90 U/ml (Sigma Type I). The enzyme incubations were performed in 50 ml of a buffered solution containing (in mM): NaCl, 130.0; $NaHCO_3$, 12.0; NaH_2PO_4, 3.0; Na_2HPO_4, 3.0; K_2HPO_4, 3.0; $MgSO_4$, 2.0; $CaCl_2$, 1.0 (omitted in the pronase incubation); rabbit albumin, 1.0 mg/ml (fraction V, Sigma, St Louis, MO); and glucose, 2.0 mg/ml, pH 7.4, at 37° in 250 ml flasks which were gassed with 100% oxygen, sealed and gently stirred with a magnetic bar.

After the collagenase treatment, the flask content was filtered through a 70 μm nylon mesh and the cells were spun down at 50 *g* for 5 min. The supernatant containing small cells and digested material was discarded and the pelleted cells were gently resuspended and rinsed three times. All rinsings were performed in the incubation medium containing (in mM): NaCl, 132.4; KCl, 5.4; Na_2HPO_4, 5.0; NaH_2PO_4, 1.0; $MgSO_4$, 1.2; $CaCl_2$, 1.0; albumin, 1.0 mg/ml; and glucose, 2.0 mg/ml. Finally, the cell pellet was resuspended in 37% (v/v) Percoll (Pharmacia Fine Chemicals) and passed through a 30 μm nylon mesh giving a crude cell suspension free from clumps. All Percoll solutions were made up in PBS and contained 2 mM EDTA to prevent aggregation of cells.

Gradient formation: A 49% (v/v) Percoll solution in PBS was centrifuged in 50 ml polycarbonate (Nalge) tubes for 30 min at 20,000 *g* in a Beckman J2-21 centrifuge equipped with a JA-17 fixed angle rotor. The continuous gradient formed was checked with density marker beads (Pharmacia Fine Chemicals) and was found to be S-shaped with densities ranging from 1.02 to 1.14 g/cm^3.

Cell separations: 5 ml of the crude suspension in 37% Percoll was layered at the top of each pre-formed 49% Percoll gradient and centrifuged for 30 min at 400 *g* in a Rotixa centrifuge equipped with a swing-out rotor. The cells separated into four more or less distinct bands in the gradient. Four fractions at densities of 1.02–1.04 (fraction 1), 1.05–1.06 (2), 1.07–1.08 (3) and 1.09–1.10 g/cm^3 (4), respectively, were collected and washed free from Percoll for use in further studies.

Identification and viability of cells. Using phase-contrast microscopy, parietal cells were identified either by their large size and dark appearance in relation to other cells, or by their staining with nitrotetrazolium blue (NTB) for succinic dehydrogenase [12]. Cell viability was tested by the trypan blue exclusion technique.

In fractions 2 and 4, electron microscopy was used to identify both parietal and chief cells. For this purpose the cells were fixed for 1 hr at room temperature in 100 mM phosphate buffer (Na_2HPO_4, 75 mM; $NaH_2PO_4 \cdot H_2O$, 25 mM) containing 2% glutaraldehyde (final pH 7.2–7.4), post-fixed with OsO_4, dehydrated and embedded in epon. Sections (80 nm) were contrasted with uranyl acetate and lead hydroxide, and examined at 60 kV in a Philips EM 300 electron microscope.

AP accumulation. Acid secretion was measured as the accumulation of the weak base [^{14}C]-aminopyrine (pK_a 5) [13]. AP, which in its neutral form rapidly passes membranes, is charged and therefore trapped in acid compartments, and can thus serve as an indirect measure of the pH difference between the acid compartments and surrounding areas.

Cells (~1 mg dry wt) were incubated at 37° in 0.5 ml incubation medium containing 3.7 kBq [^{14}C]AP (The Radiochemical Centre, Amersham, Bucks., U.K.) in an orbital shaking water bath (150 rpm). After 90 min the cells and medium were separated by centrifugation at 1000 *g* for 2 min. An aliquot of the medium was added to 10 ml Instagel (Packard) for radioactivity counting in a Searle Mark III 6880 liquid scintillation counter. The remaining medium was discarded and the cell pellet dried overnight at 100°, weighed on a Mettler ME 30 microbalance and dissolved in 1 ml Soluene (Packard); its radioactivity was determined after addition of 10 ml Dimilume (Packard). All experiments were carried out in duplicate or triplicate. The AP ratios were calculated according to Berglindh *et al.* [13].

Pepsinogen content and release. Prior to the determination of cell pepsinogen content in the crude suspension, fractions 2 and 4, the cells were lysed with distilled water. For pepsinogen release studies a quantity of chief cells (fraction 4) corresponding to *ca* 1 mg dry wt was incubated in a 6 ml scintillation vial containing 0.5 ml incubation medium at pH 7.4. The vials were agitated (150 rpm) in an orbital shaking water bath. After 2 hr at 37° the cells were spun down at 1000 *g* for 2 min, and the pepsinogen content of the medium was assayed. The cell pellet for each sample was dried overnight at 100° and weighed. Pepsin analyses were performed according to Berstad [14] with human haemoglobin as substrate, and expressed as μg pepsinogen/mg dry wt × 2 hr. The pH curve for rabbit pepsin activity was found to have an optimum at pH 1.8 and, consequently, the activity of the enzyme was assessed at this pH. Crystalline porcine pepsin (Koch Light Laboratories, 3× crystallized) was used as a standard.

Determination of microsomal ATPase activity. To determine microsomal ATPase activity in the crude suspension, fractions 2 and 4, the cell fractions were homogenized with a glass–Teflon homogenizer in a 0.25 M sucrose solution containing 5 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], buffered with Tris to pH 7.4. A microsomal fraction was prepared by differential centrifugation of the homogenate (15,000 *g* for 20 min followed by centrifugation of the supernatant at 100,000 *g* for 60 min) in a Kontron TGA-65 ultracentrifuge with a TST 60 swing-out rotor. The enzyme activity of the resulting pellet was determined at 37° in a medium consisting of 2 mM Na_2ATP, 2 mM $MgCl_2$, 5 mM PIPES buffered with Tris to pH 7.4, and 10 μg of microsomal protein in a total volume of 1 ml. K^+ stimulation was measured after addition of 10 mM KCl, 0.5 μg gramicidine or nigericin, and 0.1 mM ouabain. The reaction was stopped after 10 min by the addition of 1 ml 10% perchloric acid. Reaction rates were followed by the release of inorganic phosphate from ATP and the enzyme activity was expressed as μmole P_i/mg protein × hr. Inorganic phosphate was analysed according to Yoda and Hokin [15]. All analyses were performed on the same day as the preparation.

Gel electrophoresis of the microsomal fractions. Samples (*ca* 40 μg membrane protein) of the microsomal fractions were denatured at 100° for 10 min in a buffer containing 20 mM Tris–HCl, 2 mM EDTA and 2% SDS, pH 8.0. The samples were applied on top of a density gradient polyacrylamide slab gel with a density ranging from 2 to 16%. The gels were pre-equilibrated for 1 hr at 40 V in a buffer containing 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA and 0.2% SDS, pH 7.4. After application, the samples were electrophoresed at 150 V for 30–60 min after the tracking dye had migrated off the

Table 1. Distribution and viability of parietal and chief cells before and after separation

Preparation	Light microscopy*		Electron microscopy	
	Parietal cells %	Cell viability %	Parietal cells %	Chief cells %
Crude susp	29 ± 1 (10)	87 ± 2 (13)		
Fraction 1	73 ± 3 (3)	63 ± 3 (3)		
2	66 ± 3 (9)	75 ± 2 (6)	71	25
3	33 ± 3 (3)	86 ± 5 (3)		
4	7 ± 1 (9)	93 ± 3 (6)	5	94

Mean ± S.E.M. Numbers in parentheses indicate number of experiments.
The viability tests were performed using the trypan blue exclusion technique.
* The parietal cells were identified either by using the phase-contrast technique or after staining with nitrotetrazolium blue (NTB) for succinic dehydrogenase.

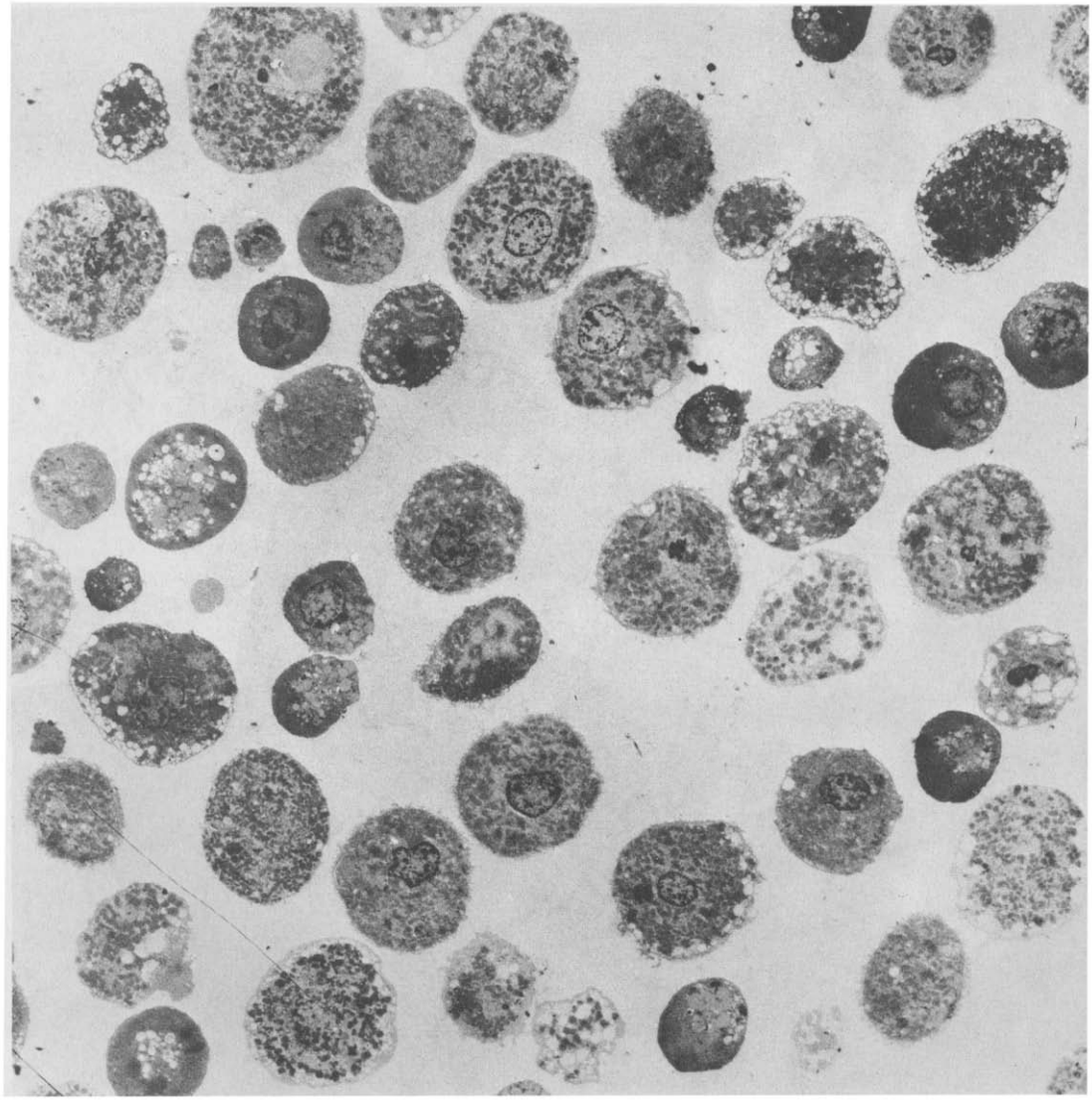

Fig. 1. Electron microscopic survey of cell fraction 2, rich in parietal cells. ×600.

bottom of the gel. The gels were stained overnight in 0.2% Coomassie Blue R 250 in 50% methanol–10% acetic acid in water. Destaining was performed at 12 V for 75 min using 30% methanol–10% acetic acid in water. Finally, the gels were scanned on a LKB 2202 ultroscan laser densitometer (LKB Instruments, Sweden).

RESULTS

Characterization of the cell fractions

Light and electron microscopy and test of viability. As can be seen from Table 1, the parietal cell content in the crude suspension was relatively high, 29 ± 1%, and the cell viability 87 ± 2%. After gradient centrifugation, the parietal cells were most abundant in fractions 1 (73 ± 3%) and 2 (66 ± 3%) (i.e. in the upper part of the gradient at densities <1.07 g/cm^3), less frequent in fraction 3 (33 ± 3%), and almost lacking in fraction 4 (7 ± 1%), with a density ≥ 1.09 g/cm^3. In this latter fraction chief cells were highly enriched (94%). Table 1 also shows that the cell viability, as judged by their ability to exclude trypan blue, was lower in fractions 1 and 2 than in fractions 3 and 4. The light microscopic findings were confirmed by electron microscopy. Parietal cells and chief cells accounted for nearly all of the cells (Table 1) in fractions 2 and 4, respectively (Figs. 1 and 2).

AP accumulation. Both unstimulated and stimulated (i.e. 5×10^{-5} M histamine or 10^{-3} M db-cAMP) AP accumulation were highest in fractions 1 and 2 (Fig. 3). In fractions 3 and 4, histamine and db-cAMP were without, or had a minor, stimulatory effect. Fraction 2 was chosen for further studies of parietal cells and fraction 4 for chief cells. Fraction 2 was selected in preference to fraction 1 because of a higher cell viability and a somewhat better response to histamine stimulation.

Microsomal ATPase activity and pepsinogen content in fractions 2 and 4. In order to characterize fractions 2 and 4 further, microsomal Mg^{2+}-ATPase and H^+,K^+-ATPase activities were measured and

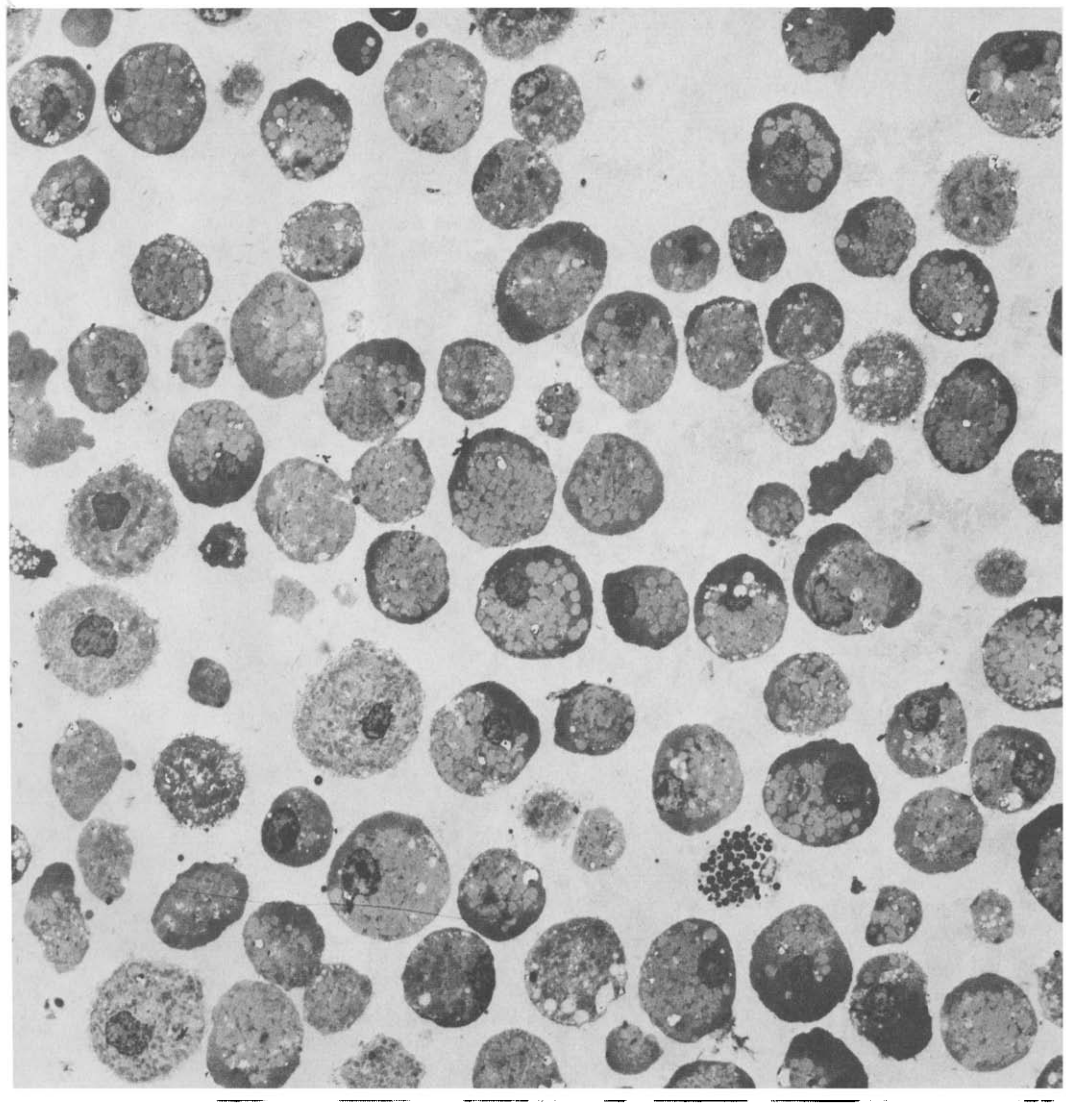

Fig. 2. Electron microscopic survey of cell fraction 4, rich in chief cells. ×600.

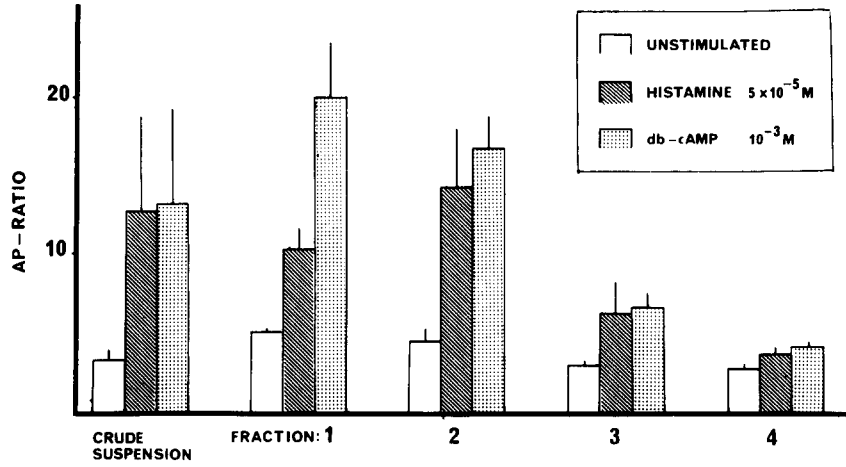

Fig. 3. Basal and stimulated AP accumulation in the crude cell suspension and in cell fractions after separation in a 49% Percoll density gradient. After 90 min incubation at 37°, cells and incubation medium were separated, and the AP ratio was calculated on the basis of the distribution of AP between cells and incubation medium. Mean ± S.E.M., $n = 3$.

the pepsinogen content analysed (Fig. 4). The Mg^{2+}-ATPase activity was higher in the crude suspension, and higher in fraction 2 than in fraction 4. The K^+-stimulated ATPase activity, measured as the difference between the basal Mg^{2+}-ATPase and the Mg^{2+} plus K^+-stimulated activities in the presence of gramicidin and ouabain, was highest in fraction 2 and was very low in fraction 4 (Fig. 4). SDS-Polyacrylamide gel electrophoresis showed a characteristic protein band in the 100,000 dalton region in the crude suspension and fraction 2. In fraction 4, however, this band consisted of only 30% of the amount found in fraction 2 (Fig. 5). In contrast to the distribution of the H^+,K^+-ATPase, pepsinogen was found mainly in fraction 4 and in only small amounts in fraction 2 (Fig. 4). Therefore, the pepsinogen content seems to parallel the morphologically assessed occurrence of chief cells in these fractions.

Effect of omeprazole and cimetidine on basal and stimulated AP accumulation in the parietal cell fraction

In the unstimulated state the cells in the parietal cell fraction (fraction 2) accumulated AP to an AP ratio of 6 ± 0.8 ($n = 12$). This basal AP uptake was not affected by the H_2-antagonist cimetidine at 10^{-4} M, but was totally inhibited by omeprazole at 3×10^{-6} M. Both histamine and db-cAMP stimulated AP accumulation in a concentration-dependent manner, with EC_{50} values of 3×10^{-6} and 6×10^{-4} M, respectively. Maximal AP uptake was recorded for

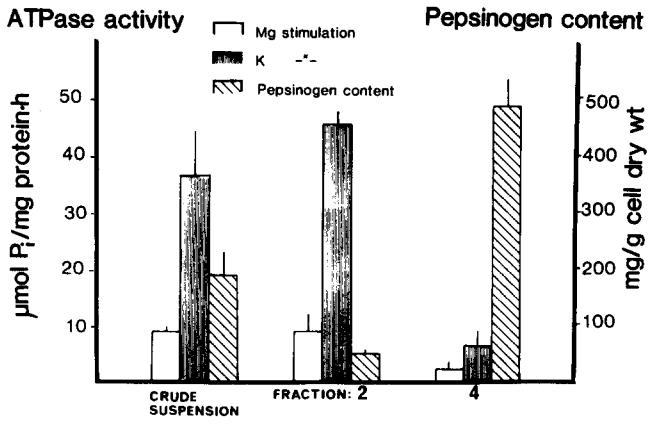

Fig. 4. Microsomal ATPase activities and pepsinogen contents in the crude suspension, fractions 2 and 4. Microsomes were collected after homogenization and differential centrifugation. The K^+ stimulation was measured after the addition of 10 mM KCl and 0.5 µg gramicidin and 0.1 mM ouabain to a solution containing 2 mM Na_2ATP, 2 mM $MgCl_2$ and 5 mM PIPES (pH 7.4) in a total volume of 1 ml. Reaction rates were followed by the release of inorganic phosphate. The pepsinogen content was measured with haemoglobin as substrate after breaking the cells with distilled water. Mean ± S.E.M., $n = 3$–5.

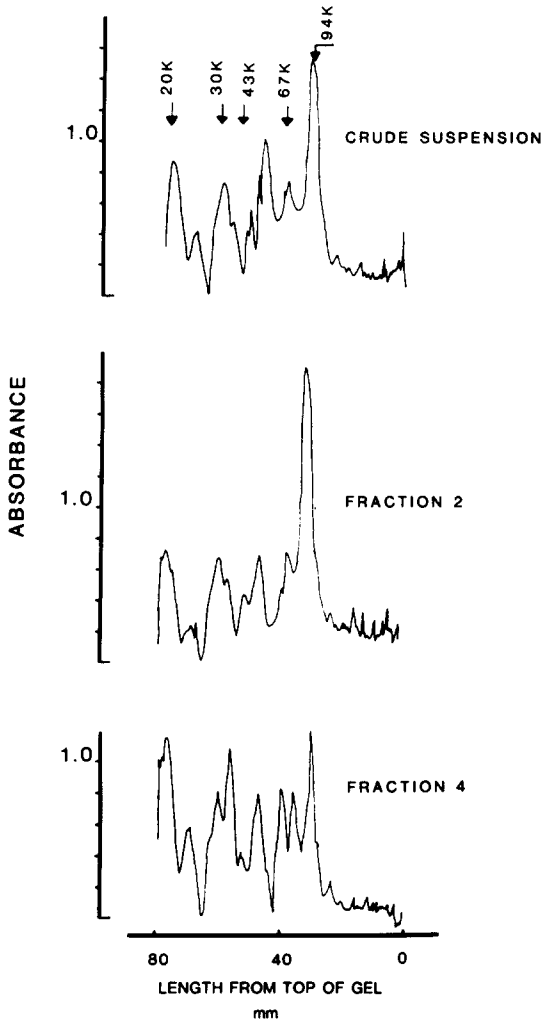

Fig. 5. Protein profiles of density gradient polyacrylamide slab gel electrophoresis of crude suspension, fraction 2 containing enriched parietal cells and fraction 4 with enriched chief cells.

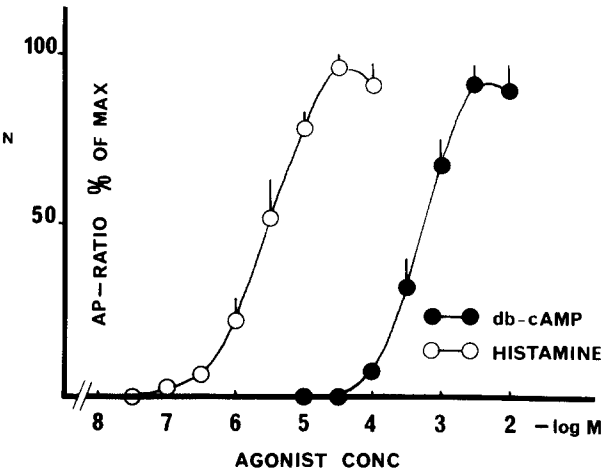

Fig. 6. Concentration response curves for histamine and db-cAMP on AP accumulation in the parietal cell fraction (fraction 2). Experiments were performed as described in Fig. 3. Absolute AP ratios: unstimulated, 6 ± 1; maximum histamine stimulation, 47 ± 11; maximum db-cAMP stimulation, 74 ± 16. Mean ± S.E.M., $n = 5$.

histamine at 3×10^{-5} M (47 ± 1) and for db-cAMP at 3×10^{-3} M (74 ± 16) (Fig. 6). Figure 7 shows the effect of cimetidine and omeprazole on maximal AP accumulation stimulated by either histamine or db-cAMP. Cimetidine at a concentration of 10^{-4} M inhibited the histamine-stimulated AP uptake by 90%, but was without effect on db-cAMP stimulation. In contrast, omeprazole equally well inhibited the responses to both agonists with an EC_{50} value of 3×10^{-7} M.

Effects of omeprazole and atropine on pepsinogen release from the chief cell fraction

In the absence of added secretagogues, a basal release of pepsinogen from the chief cell fraction (fraction 4) was found which was linear with time for at least 2 hr (data not shown). Neither atropine

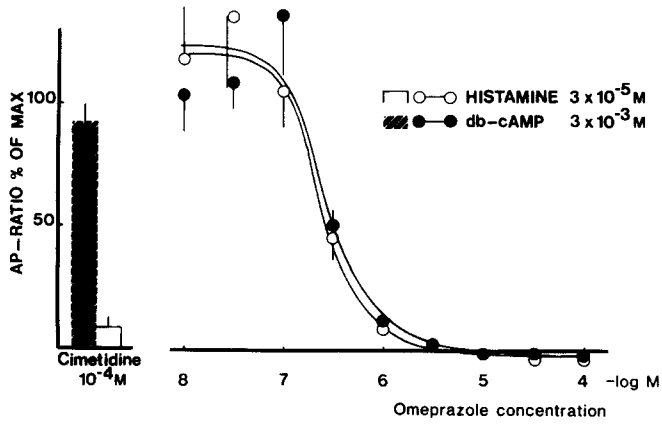

Fig. 7. Effect of cimetidine (10^{-4} M) and omeprazole (10^{-8}–10^{-4} M) on maximal histamine- (3×10^{-5} M) and db-cAMP-(3×10^{-3} M) stimulated AP accumulation in the parietal cell fraction (fraction 2). Experiments were performed as described in Fig. 3. Means ± S.E.M., $n = 3$–5.

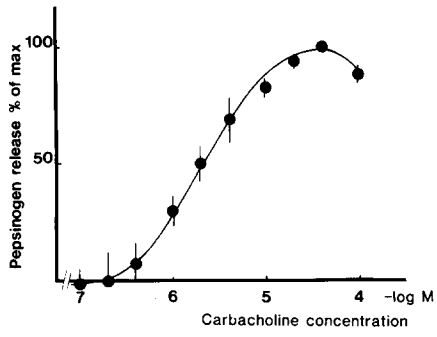

Fig. 8. Concentration response curve for carbacholine stimulation of pepsinogen release in the chief cell fraction (fraction 4). Cells were incubated for 2 hr at 37° at pH 7.4. The pepsinogen released into the incubation medium was converted to pepsin and estimated according to the method of Berstad [14] at pH 1.8. The cells in each sample were dried and weighed. The data are expressed as the percentage of the maximal response within each experiment. Absolute values of μg pepsinogen released per mg cell dry wt × 2 hr: unstimulated, 9.9 ± 4.2; stimulated, 22.1 ± 6.6; $n = 4$–5.

nor omeprazole affected basal release (9.9 ± 4.2 μg/mg cell dry wt × 2 hr) in any of the concentrations tested (Fig. 9). Carbacholine concentration dependently stimulated pepsinogen release, with an EC_{50} of 2×10^{-6} M (Fig. 8) and with the maximal secretion rate attained at 4×10^{-5} M (22.1 ± 6.6 μg/mg cell dry wt × 2 hr). Db-cAMP (10^{-3} M) stimulated pepsinogen release to nearly the same extent as carbacholine (Fig. 9). Since db-cAMP has light absorption at 280 nm, and thus interferes with the pepsin assay, care was taken to use correct blanks in these studies. Atropine totally antagonized the carbacholine-stimulated release at a concentration of 5×10^{-6} M (Fig. 9), but was without effect on db-cAMP-stimulated release even at a concentration of 5×10^{-5} M (Fig. 9). Omeprazole at concentrations of up to 10^{-5} M had no effect on pepsinogen release stimulated by either of the two agonists (Fig. 9).

DISCUSSION

Isolation procedures for gastric mucosal cells have so far mainly focused on parietal cell enrichment [16–18]. Little attention has been directed to chief cells and the possibility of isolating and using these cells in the study of pepsinogen production and release [19].

The present preparation method yielded enriched fractions of both morphologically and functionally well-defined parietal cells and chief cells. Thus, the parietal cells (purity ~65%) were stimulated with both histamine and db-cAMP to uptake of AP, indicating a high viability with regard to the acid secretory function. Furthermore, the H^+,K^+-ATPase activity was found to copurify with this fraction. In analogy, a high pepsinogen content was found in the chief cell fraction, and the cells in this fraction (purity ~ 90%) released pepsinogen in response to stimuli such as carbacholine and db-cAMP.

Immunohistochemical studies have demonstrated that the gastric H^+,K^+-ATPase is located in the parietal cells [20, 21]. These findings are in agreement with our results: the H^+,K^+-ATPase activity was enriched in the PC fraction. Furthermore, this fraction contained a microsomal 100,000 dalton protein band, virtually absent in the chief cell fraction. There is considerable evidence supporting the hypothesis that this protein is the gastric H^+,K^+-ATPase [22]. Therefore, the results suggest that the gastric H^+,K^+-ATPase activity can be used as a marker for the parietal cell.

Pepsinogen was released spontaneously from the chief cell fraction. Such a basal release has previously been reported in other *in vitro* systems as well

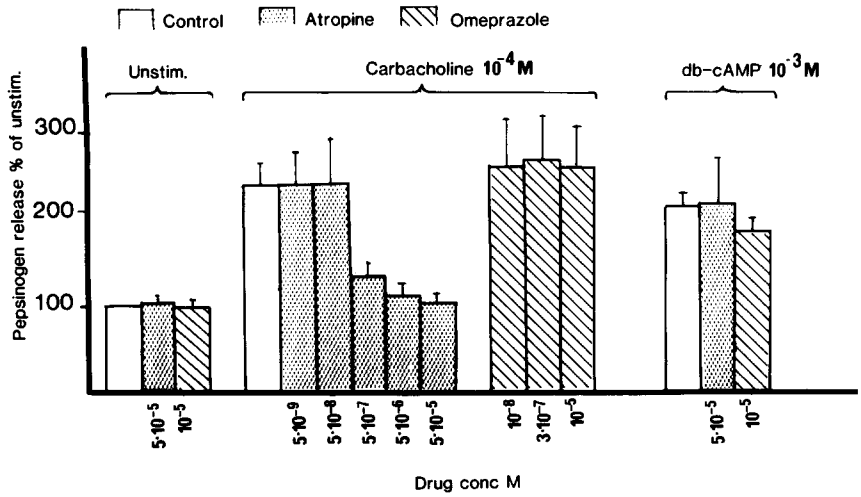

Fig. 9. Effects of atropine and omeprazole on unstimulated and on carbacholine- and db-cAMP-stimulated pepsinogen release in the chief cell fraction (fraction 4). Values are expressed as the percentage of the unstimulated release in each experiment. 100% = 9.9 μg/mg cell dry wt × 2 hr. Mean ± S.E.M., $n = 4$–10.

[23–25]. However, the mechanism and the physiological importance of this basal release are unknown and remain to be clarified. The basal release was unaffected by both omeprazole and atropine. This is contrary to the findings in the *in vitro* perfused mouse stomach, where the benzimidazoles in very high concentrations (10^{-4} M) were found to stimulate basal pepsinogen release [26].

Pepsinogen release was stimulated by carbacholine and by db-cAMP. Atropine totally antagonized carbacholine- but not db-cAMP-stimulated release, a finding supporting the involvement of muscarine receptors in stimulation of the chief cell secretion. Omeprazole had no effect on the stimulated release of pepsinogen in the chief cell fraction, regardless of the stimulus used. This contrasted with the profound inhibitory effects of omeprazole on basal as well as on stimulated AP accumulation in the parietal cell fraction. Thus, despite the fact that db-cAMP was an effective agonist for both pepsinogen and acid secretion, omeprazole only inhibited the acid secretory function, obviously at a site of action inside the parietal cell.

Our findings demonstrate that the substituted benzimidazole omeprazole has a specific inhibitory effect on the acid secretory function of the parietal cells, and support the previously proposed hypothesis of an action mediated through inhibition of the gastric H^+,K^+-ATPase [9, 10].

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