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STIMULATED CHLORIDE TRANSPORT BY ISOLATED PARIETAL CELLS

DAVID M. GLICK

The Medical College of Wisconsin, Milwaukee, Wis. 53233, U.S.A.

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Abstract—A preparation of rabbit parietal cells has been shown to respond to effectors of gastric acid secretion. The cells were isolated, aligned at the interface of two aqueous phases, and the transport of Cl^- from one phase to the other effected by the cells under various pharmacological stimuli, was measured. Ca^{2+}, in contrast to Mg^{2+}, was shown to be necessary to elicit a response with histamine. This response was inhibited by prostaglandin E_1. Dibutyryl cyclic AMP was without effect. Carbachol and catecholamines also stimulated secretion, but this was not inhibited by prostaglandin E_1. Because basal Cl^- transport in the presence of Ca^{2+} is in a direction opposite to that of the stimulated transport (and to the low basal transport in the presence of Mg^{2+}), at least two secretion mechanisms appear operative.

THE DEVELOPMENT of methods for isolating parietal cells[1] and for observing their function *in vitro*[2] has made possible the determination of the response of parietal cells to various effectors of gastric acid secretion, uncomplicated by extraneous considerations. The cells, freed from the mucosa by collagenase digestion, are aligned according to their electric polarity at the interface of two aqueous phases. This electric polarity corresponds to a functional polarity, so the cells transport ions from one aqueous phase to the other.[2] This communication reports the practicality of using a chloride-specific electrode to monitor cell function, the responsiveness of cells to histamine, dibutyryl cyclic AMP, prostaglandin E_1, carbachol, catecholamines and pentagastrin, and the involvement of calcium and magnesium, these characteristics indicating that the system is potentially a good model for gastric function *in vivo*.

EXPERIMENTAL

The cell preparation was made essentially as described.[1] The mucosa of a young (3 lb) female Swiss white rabbit was separated from the muscularis by dissection and then minced and shaken at 30° for 1 hr in 50 ml of a solution that was 122 mM in Na^+, 4 mM in K^+, 116 mM in Cl^-, and 10 mM in HCO_3^-, and that contained 3·4% glucose and 40 mg crude collagenase (P-L Biochemicals, Milwaukee, Wis.) at pH 7·5. As noted, in a few experiments, a purified collagenase (Sigma Chemical Co., St. Louis, Mo.) was employed. The digested preparation was strained, chilled and centrifuged in the cold at 800 *g* to collect the cells. The sedimented material was repeatedly resuspended in the above solution (without collagenase) and resedimented to wash out mucins. Finally, the collected cells were suspended in a solution made 6% (w/w) in Ficoll (Pharmacia, Piscataway, N.J.) and layered over a 14% and a 19% Ficoll solution in the electrolyte–glucose medium described above. This was centrifuged at

800 *g* for 20 min, and those cells that had penetrated the bottom layer were collected, washed, and suspended in a solution that was 125 mM in Na^+, 115 mM in Cl^-, 10 mM in HCO_3^-, 50 mM in Na-TES* buffer (pH 7·5) and 3·4% glucose. The cells were typically about 60 per cent parietal cells, as judged by their staining with hematoxylin and eosin, with most of the remaining cells being chief cells and some mucus neck cells. The preparation was used for measurement within 1 hr.

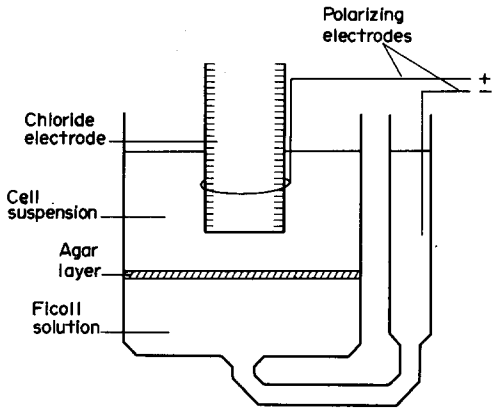

FIG. 1. Diagrammatic representation of apparatus for measurement of chloride transport. The vessel has a 2·22-cm inside diameter, and the body of it has a water jacket (not represented) for temperature control.

Transport of chloride by the preparation was measured in a thermostatted glass vessel shown diagrammatically in Fig. 1. A lower phase consisting of 4 ml of the same solution in which the cells were finally suspended, but made denser by the inclusion of 22% Ficoll, supported the upper phase, which was a cell suspension of the composition indicated. A thin layer of agar protected the boundary between the two phases. Into the 1-ml upper phase was inserted a chloride-specific combination electrode (model 96-17, Orion Research, Cambridge, Mass.). In the upper phase was a platinum-iridium wire connected to the positive pole of a variable power source, and a similar wire in the side arm connected the lower phase to the negative pole. As the cells sedimented onto the agar layer, a 6-V potential aligned the cells in its field. After 20 min, the power supply was disconnected and readings from the chloride-specific electrode were taken on a model EU-200-30 electrometer and model EU-205-11 recorder (Heath Co., Benton Harbor, Mich.). The response of the electrode, which is proportional to the logarithm of the chloride concentration, was calculated to yield the ratio of the chloride concentration at any given time to that at the beginning of the recording period. The best idealized linear slope during the first 15 min was then calculated and reported.

RESULTS AND DISCUSSION

Although parietal cells are obviously of interest because of their hydrogen ion secretion, the change in pH that would have to be accepted during measurement of hydrogen ion transport is intolerable. Therefore, transport of chloride, the counter ion, was studied. An increase of, for example, 1 mM means a change in less than 1 per

* Na-TES, *N*-Tris-(hydroxymethyl)-2-aminoethane sulfonic acid, sodium salt.

cent in chloride concentration, but in an unbuffered solution it means a drop below pH 1. Indeed, in one case, the upper and lower phases were sampled after an experiment performed without buffer, and the upper phase showed a pH near 4, and the lower phase, a pH near 10. This extreme change in conditions during an experiment was judged too severe to allow interpretation of any results. Therefore, chloride transport has been observed in hydrogen ion-buffered solutions, and the results must be considered only potentially informative of the cells' handling of hydrogen ions.

The cells respond to histamine stimulation, in the presence of 10^{-3} M calcium, by secreting chloride ions across their more negatively charged surface (Fig. 2). Dibutyryl cyclic AMP does not reproduce this histamine effect. No stimulation is seen when magnesium replaces calcium, but a basal chloride transport is seen, even in the absence of histamine or the nucleotide. The histamine-stimulated chloride transport in the presence of calcium is abolished when prostaglandin E_1 is included in the medium (Fig. 3).

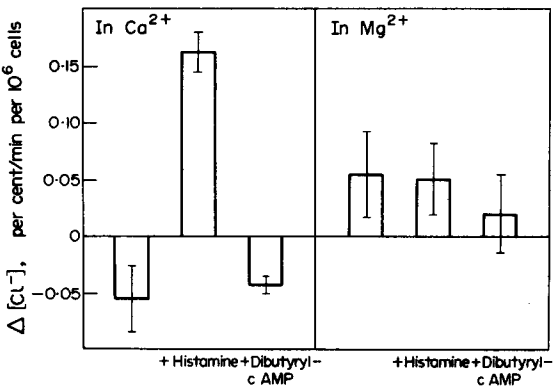

FIG. 2. Transport of chloride to the side of parietal cells that orients toward the anode in an electric field. Measurements were made in the presence of 10^{-3} M $MgCl_2$ or $CaCl_2$ and in the presence or absence of 10^{-3} M histamine diphosphate or 10^{-3} M dibutyryl cyclic AMP (P-L Biochemicals). All cell suspensions were, in addition, 75 mM in Na^+, 50 mM in K^+, 115 mM in Cl^-, 10 mM in HCO_3^-, 50 mM in Na-TES (pH 7·5), and 3·4% in glucose, 30°. The vessel contained 1 to 2 × 10^6 cells, as determined in a hemacytometer.

Chloride transport may also be stimulated by carbachol (calcium being present), but in contrast to histamine-stimulated transport, the carbachol effect is not inhibited by prostaglandin E_1 (Fig. 4). Catecholamines also permit chloride transport, but at an apparently lower level than do histamine or carbachol, and this stimulation, too, is not inhibited by prostaglandin E_1 (Fig. 5).

Many attempts were made to demonstrate stimulation of the cells by pentagastrin, but the results were quite variable (Fig. 6). Because of the possibility that hydrolytic enzymes present in the crude collagenase preparation used to disrupt the mucosa were destroying gastrin receptor sites on the cells, or were persisting through the washes and were destroying the pentagastrin, some experiments were attempted with a purified collagenase preparation. These showed a stronger transport of chloride and were more consistent.

The transport of chloride in this system is potentially a good model for gastric acid secretion: it responds to histamine and prostaglandin E_1, and to pentagastrin and

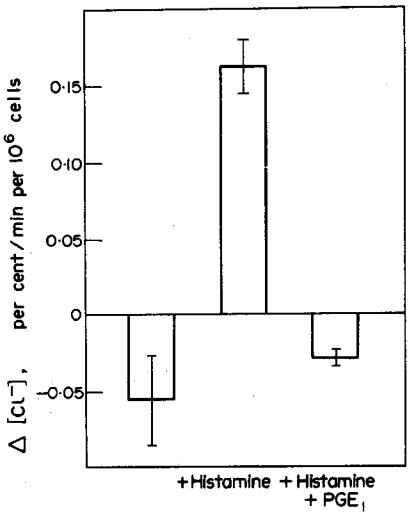

FIG. 3. Inhibition of histamine-stimulated transport by prostaglandin E_1. Conditions as for Fig. 2; prostaglandin E_1 (courtesy of Upjohn Co., Kalamazoo, Mich.) was 10 μg/ml, when present.

carbachol (surrogates for gastrin and acetyl choline), as does the gastric mucosa *in vivo*. That at least two chemical sequences may signal chloride transport is indicated by the two different directions of transport, and by the transport in the presence of magnesium being distinct from the basal or the histamine- or carbachol-stimulated transport in the presence of calcium (Fig. 2). That prostaglandin inhibits histamine- but not carbachol- or catecholamine-stimulated transport may or may not indicate that these effectors use different mechanisms. As cells are normally exposed to a relatively high magnesium concentration, this cation is unlikely to be a modulator of parietal cell function *in vivo*. Calcium (10^{-3} M), on the other hand, permits consider-

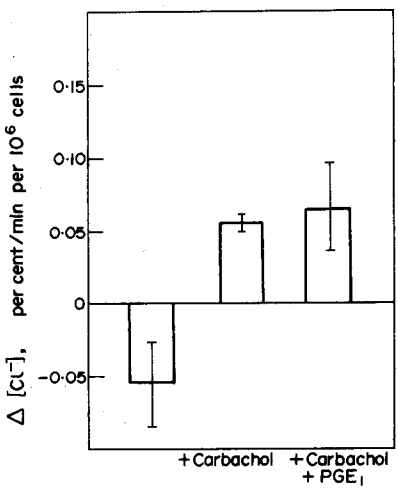

FIG. 4. Stimulation of transport by carbachol. Conditions as for Fig. 2; carbachol was 10^{-4} M, and prostaglandin E_1 was 10 μg/ml, when present.

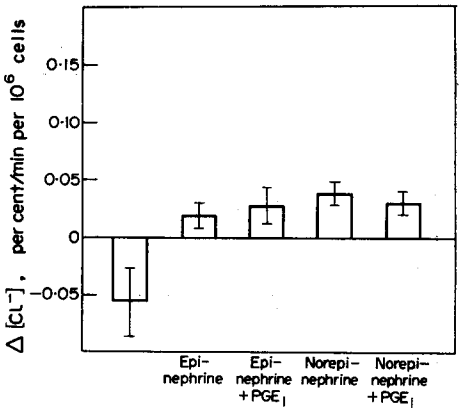

FIG. 5. Stimulation of transport by catecholamines. Conditions as for Fig. 2; epinephrine and norepinephrine were 10^{-3} M, and prostaglandin E_1 was 10 μg/ml, when present.

able changes, and may well be a physiological effector of the cells' function in conjunction with histamine. Calcium, of course, has been proposed for a major role in the regulation of secretions.[3]

The negative results with the cyclic AMP analogue were surprising in light of reports of the involvement of cyclic AMP in gastric acid secretion in some mammals.[4–7] These data may indicate only that the dibutyryl derivative is a poor model for cyclic AMP, or that the conditions of the experiment were inappropriate. However, an assay for adenylate cyclase activity (courtesy of Dr. R. G. Kemp) in a broken cell preparation showed only a 1·5-fold stimulation of the enzyme in the presence of 10^{-3} M histamine, as compared with the unstimulated level of 0·96 nmole cyclic AMP formed 10 min/mg of protein. By contrast, 10^{-2} M NaF stimulated 3·6-fold,

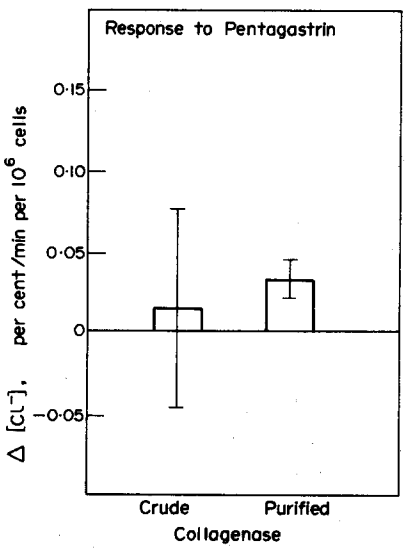

FIG. 6. Pentagastrin (Ayerst, New York)-stimulated transport by cells prepared with crude or with purified collagenase.

and the weak stimulation by histamine may even be due to contaminating cells in the preparation. The inhibition of stimulated chloride transport by prostaglandin E_1 corresponds to its action *in vivo*.[7]

A detailed physical description of the barrier between the upper and lower phases is difficult. The cells, having a diameter near 20 μm, can just cover the agar membrane as a monolayer, but undoubtedly there must be small uncovered areas. These could allow leaks in the barrier, leading to an underestimate of chloride transport, but as the chloride gradient is small, this probably is not a serious problem. The inclusion of a buffer in the medium prevents any significant change in pH. There is no easy way to confirm that the cells are oriented so that their mucosal sides are toward the anode of the polarizing field (as inferred from the direction of stimulated secretion), because isolated parietal cells are not obviously polar in appearance under the light microscope.

The stimulation of chloride transport by catecholamines, unmediated by gastrin as this apparently is, is unexpected. The low level of transport is similar to the basal secretion seen where magnesium replaces calcium, and may reflect the same mechanism.

Card and Marks[8] reported the maximal output from the human stomach to be 23 m-equiv $H^+/hr/10^9$ parietal cells, for histamine stimulation. That, despite the difference in species and the difference between a living individual and an isolated cell preparation, the values fall so close together, as they do, is quite encouraging.

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