ATP DEPENDENCE OF H^+ SECRETION

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ABSTRACT

Cells in isolated rabbit gastric gland were made permeable to ATP by high voltage discharge across a gland suspension. In both normal (5.4 mM K^+) and high K^+ (108 mM) medium, this electrical shock resulted in a marked reduction in the ability of the parietal cell to produce and accumulate acid. Acid production was monitored both microscopically by acridine orange accumulation in the secretory canaliculus and by accumulation of the weak base [^{14}C]aminopyrine. In 108 mM K^+ solutions but not in 5.4 mM K^+ solutions, 5 mM ATP was able to restore the accumulation of these probes to control (unshocked) levels. When shocked glands had been previously stimulated by secretagogues, the aminopyrine accumulation ratio was only partly restored by ATP. Inhibition of mitochondrial respiration by cyanide, azide, or Amytal abolished acid secretion; the subsequent addition of ATP to shocked glands increased the aminopyrine accumulation ratio to 47 and resulted in an acridine orange fluorescence indistinguishable from that of histamine-stimulated, unshocked glands. We conclude that ATP can act as a substrate for H^+ secretion in the parietal cell, and that perhaps no additional energy source is necessary.

The gastric proton pump generates a pH of 0.8 in the gastric lumen, creating one of the largest ion gradients known to biology. The site of acid secretion in mammals is the luminally facing surface of the secretory canaliculus of the parietal cell (1). The energy source for this proton pump has not been conclusively established.

Before the discovery of ATP, a redox pump was postulated in which an appropriately oriented oxidation-reduction reaction would result in the separation of H^+ and electrons (2). Data showing the absolute O_2 dependence of acid secretion in amphibian mucosa (3) and a maximum H^+:O ratio of 2 in dogs (4) provides evidence for this mechanism.

ATP was implicated in H^+ secretion by studies of the action of uncouplers (dinitrophenol, *m*-chlorocyano carbonyl phenylhydrazone), phosphorylation inhibitors (aurovertin, atractylate), and respiratory inhibitors (Amytal) on mitochondria (5, 6). A K^+-activated ATPase was suggested on the basis of the discovery of a K^+-dependent acetyl phosphatase (7) and later was shown to be present in frog (8), dog (9), and hog (10) gastric membrane. The H^+ translocation properties of gastric membrane vesicles from these species (11, 12, 13) provide the most direct evidence to date for an ATP-driven proton pump. This hypothesis has been further strengthened by the demonstration that gastric ATPase is located on the microvilli of secretory canaliculi (14).

On the other hand, whereas measurements of phosphorylation potential (ATP/[ADP + P_i]) in dog gastric biopsies (15) showed the expected decrease with the onset of acid secretion, no change in ATP:ADP or phosphocreatine:creatine (PCr:Cr) ratios were observed in either the dog or the

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frog (15, 16). Moreover, in the frog, when acid secretion and short circuit current are inhibited by Amytal and ATP levels are reduced to 10% of control, the addition of, for example, ascorbate, which would bypass the Amytal blockade of respiration, results in restoration of short-circuit current, partial restoration of ATP levels, and yet no resumption of HCl transport (17). This was interpreted as being the result of the dependence of HCl secretion on an oxidation step requiring complex I activity. In support of this interpretation, it was found that spectroscopic data (from frogs) and metabolite level data (from dogs) implied a crossover between NAD^+ and FAD (18). More recently, however, spectroscopy of rabbit gland suspensions showed no steady-state redox differences between rest and secretion, and, in contrast to the frog, cytochrome oxidase was at least 75% oxidized during secretory activity.[1]

Judging from the foregoing, it seems to us that there is considerable room for dispute as to the primary source of energy for acid secretion. The issues can be summarized by the questions: can ATP supply some of the energy for H^+ secretion in intact parietal cells; and can ATP act as the sole energy source for acid secretion? The latter question is also raised by the finding that the initial stoichiometry of H^+ removed from the medium to ATP hydrolyzed by gastric vesicles equilibrated with KCl is 4, a value greatly in excess of the value of 1 anticipated for an ATPase generating a ΔpH of 6.6 to 7 (13).

In designing experiments to assess this question, we utilized two recent advances in isolated cell technology. Firstly, it has been shown that high-voltage pulses applied to suspensions of erythrocytes (19) or adrenal medullary cells (20) result in the formation of pores in the cell membranes capable of admitting dyes such as trypan blue and solutes such as Ca^{++} or ATP (20). This allows the penetration of solutes otherwise excluded. Secondly, isolated rabbit gland preparation has been characterized in some detail as providing an adequate model for mammalian acid secretion (21, 22). In particular, one can use the accumulation of the weak base [^{14}C]aminopyrine (22) and the metachromatic fluorescence of acridine orange (1) as indices of the site and level of acid production by the parietal cell. It appears that, in the parietal cells of the gastric glands, pores can be formed in the bounding membrane of the cell without pores necessarily being formed in the membrane bounding the acidic compartment.

In this paper, we describe some of the properties of shocked rabbit gland suspensions, with particular reference to the presence of medium K^+ and Ca^{++} and to the responses of these suspensions to the addition of ATP, with or without mitochondrial respiratory blockade.

MATERIALS AND METHODS

Gastric Glands

Gastric glands were prepared from rabbit gastric mucosa by the collagenase digestion procedure detailed previously (21). For shocking, the glands were suspended in a medium containing (in mM): Na^+, 143.4; K^+, 5.4; Ca^{++}, 1.0; Mg^{++}, 1.2; Cl^-, 139.8; SO_4^{--}, 1.2; HPO_4^{--}, 5.0; $H_2PO_4^-$, 1.0; glucose, 2 mg/ml, pH 7.4 (defined as normal K^+ medium) or in high K^+ medium containing 108 mM K^+ and 40.8 mM Na^+, with the other ions held constant. After shock (see below), the glands were transferred to a medium devoid of Ca^{++} and containing EGTA to give a final concentration of 2 mM for studies of aminopyrine or acridine orange uptake under a variety of conditions. In some instances, glands were kept in Ca^{++}-containing medium or were kept free of Ca^{++} throughout the procedure. All media also contained 2 mg/ml of rabbit albumin (Sigma Chemical Co., St. Louis, Mo.).

Shock Procedure

Suspensions of isolated glands were subjected to a capacitative discharge of 3.0 kV for 200 μs across 1-cm^2 stainless steel plates separated by 1 cm. In initial microscope examination of the shocked glands, the fraction of cells taking up trypan blue (mol wt 961) was measured as a function of the number of shocks. Fig. 1 is a plot of these results. From these findings, and with consideration for the remaining structural integrity of the glands, the routine procedure chosen was four 3-kV shocks to yield a preparation highly permeable to ATP.

It was noted that when shocking was carried out in the presence of 1 mM Ca^{++}, the glands not only remained intact but gave an "all-or-nothing" response with regard to trypan blue uptake. That is, either none of the cells or all of the cells in a given gland took up the dye. When shocking was carried out in the absence of Ca^{++}, this was not observed—uptake of trypan blue in a given gland was patchy, and many more isolated cells were seen. Accordingly, shocking was routinely carried out in normal Ca^{++} medium. Reversal of this effect was tested by incubating shocked glands at room temperature or 37°C for up to 30 min, with or without ATP, and then adding trypan blue. No reduction in the number of glands taking up trypan blue was found; hence, the leakiness to trypan blue was not reversed under our experimental conditions.

Quantitation of Glandular Volume

Because the [^{14}C]aminopyrine ratio calculations depend on the volume of cell water, and because the induction of holes in the cell membrane almost certainly results in complete or partial loss of volume regulation, glandular volume was measured under the various experimental conditions chosen. Methods of measurement previously described in detail were used (21), with measurement of total water and the [^{14}C]inulin space.

[1] Berglindh, T., and G. Sachs. Unpublished observations.

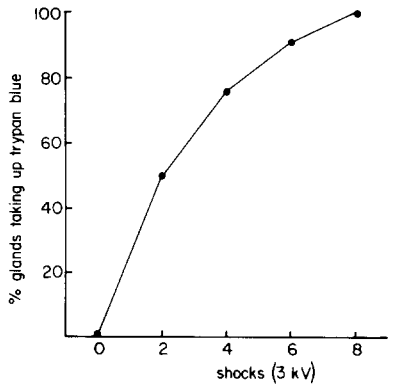


FIGURE 1 The number of glands taking up 0.1% trypan blue after 3 kV discharges in 1 ml of glands suspended in a medium containing 1.0 mM Ca^{++}.

Quantitation of H^+ Secretion

Sequestered acid was measured by determining the accumulation of the weak bases acridine orange and aminopyrine. Acridine orange has the advantage that it is an optical probe of H^+ accumulation and, thus, allows a microscopic localization of the site of H^+ secretion or accumulation and a cell-by-cell evaluation of the response (1). Measuring the uptake of [^{14}C]aminopyrine allows quantitative comparison between the H^+ gradients generated under control and experimental conditions in the gland suspension as a whole.

We visualized acridine orange, under the various conditions studied, in a Zeiss IM35 microscope using either direct fluorescence (excitation, 410 nm; emission, >510 nm) or fluorescence in combination with differential interference contrast (Nomarski) optics, as we have done previously (1). The concentration of acridine orange was 100 μM, and other conditions are detailed below.

[^{14}C]Aminopyrine uptake was measured as discussed elsewhere (22), and was expressed as the ratio of counts in glandular water to counts in the suspending medium.

Experimental Conditions

In the first series of experiments, glands suspended in normal (5.4 mM) K^+ medium at 37°C for 30 min, with or without 10^{-4} M histamine acted as controls or were shocked. [^{14}C]Aminopyrine (10^{-6} M, 84 mCi/mmol) was added, with or without 5 mM ATP, to the final suspension medium at 37°C. The medium contained 2 mM EGTA but no added Ca^{++}. After 15 min of incubation, the glands were spun down, and the radioactivity in the pellet and supernate was determined after wet and dry weight measurement as described previously (22).

In another series of experiments, the glands were suspended in high (108 mM) K^+ medium, with or without 10^{-4} M histamine, and incubated for 30 min. One group of glands served as a control, and another group was shocked. The glands were then transferred either to Ca^{++}-containing medium, with or without ATP, or to Ca^{++}-free medium with 2 mM EGTA, also with or without 5 mM ATP. In all cases, the medium contained 10^{-6} M [^{14}C]aminopyrine. Incubation was carried out as described above for 15 min, and the [^{14}C]aminopyrine distribution was determined.

In experiments in which the mitochondrial inhibitors 10^{-3} M CN^-, 10^{-3} M N_3^-, and 2×10^{-3} M Amytal were used, these substances were added at the specified concentrations to glands suspended in 108 mM K^+ medium, with or without 10^{-4} M histamine, and incubation was carried out for 30 min at 37°C. The gland suspension was then divided into a control group and an experimental group, the latter being shocked by the usual procedure. The glands were then added to a medium containing 2 mM EGTA but no added Ca^{++}, with or without 5 mM ATP. Incubation with 10^{-6} M [^{14}C]aminopyrine was carried out at 37°C for 15 min. The glands were then separated from the medium as described, and the aminopyrine distribution ratio was measured. In additional experiments, 10 mM SCN^- was added either together with ATP or 10 min later.

In all the experiments, the glands were subjected to identical experimental conditions with regard to incubation time, secretagogue, and ATP or inhibitor addition, and the only variable introduced was the shocking procedure.

To determine the time-course of the ATP-dependent response, we preincubated the glands in high K^+ medium at 37°C for 30 min, as described above, in the presence of one of the mitochondrial inhibitors. After shocking, the glands were added to two flasks, both of which contained the inhibitor and 10^{-6} M [^{14}C] aminopyrine, but only one of which contained 5 mM ATP. At predetermined times, samples were taken from the flasks, and aminopyrine distribution was measured.

A similar protocol was followed with acridine orange. Glands were suspended in 108 mM K^+, with or without 10^{-3} M CN^- or N_3^-, and incubated at 37°C in the presence of 10^{-4} M histamine or 10^{-3} M dibutyryl cyclic AMP for 30 min. After shock in the experimental series, or without shock in the control series, 100 μM acridine orange was added, and the fluorescence was observed in the Zeiss IM35 microscope. 5 mM ATP was added, and the fluorescence was observed in both control and shocked glands for 10 min after ATP addition. In some initial experiments, the morphological effects of the presence or absence of Ca^{++} in the shocking medium was studied. In the optical experiments, the morphology of both the parietal and peptic cells was observed.

Materials

Radioisotopes were obtained from New England Nuclear, Boston, Mass., collagenase from Sigma Chemical Co., St. Louis, Mo., and Amytal from Eli Lilly and Co., Indianapolis, Ind. All reagents were of the highest purity grade available.

RESULTS

Optical Observations

In earlier studies (1), it was shown that acridine orange accumulation is a sensitive indicator of acid secretion in histamine-stimulated parietal cells. Here we have used this approach to evaluate a number of different experimental manipulations. Fig. 2 *a* illustrates a gastric gland stimulated by dibutyryl cyclic AMP (db-cAMP) in the presence of 108 mM K^+ and 100 mM acridine orange. Both peptic and parietal cells can be distinguished. The parietal cells bulge out from the gland surface and display vacuoles of the intracellular canaliculus.

The vacuoles are filled with the red fluorescence characteristic of accumulated acridine orange, whereas areas that do not significantly accumulate acridine orange stain green.

When these glands were subjected to the standard shocking procedure, they routinely appeared as the glands in Fig. 2*b*. Here peptic cells have degranulated and appear shrunken so that the bulging of the parietal cells is exaggerated. The red fluorescence evident in the control glands stimulated by db-cAMP has disappeared. As is demonstrated below, this corresponds to a loss of accumulated H^+ by the gastric parietal cell. When the shock experiment was performed in zero Ca^{++} medium, as is illustrated in Fig. 2*c*, the capability for acid secretion is similarly lost, but the peptic cells, with their granules, appear to remain intact.

When shocked glands were transferred to the EGTA-containing medium to reduce medium Ca^{++} concentration and ATP was added, red fluorescence was restored to the vacuoles in the parietal cells, as is shown in Fig. 2*d*. This observation correlates well with the aminopyrine experiments, showing that the readdition of ATP to shocked glands in high K^+ medium can restore acid secretion.

It is possible to obtain similar data when mitochondrial respiration is blocked by the well-studied inhibitors CN^- and N_3^-. These reagents block the function of cytochrome oxidase and, hence, all mitochondrion-dependent oxidation reactions will be inhibited. Fig. 3*a* illustrates the appearance of a gastric gland after being shocked in the presence of 1 mM Ca^{++} and 10^{-3} M NaN_3. The uniform green fluorescence and lack of red fluorescence were consistently obtained. The addition of ATP produced the characteristic red fluorescence of secreting cells in some, but not all, parietal cells, as is seen in Fig. 3*b*. Swollen mitochondria were often easily visualized in these azide-treated preparations.

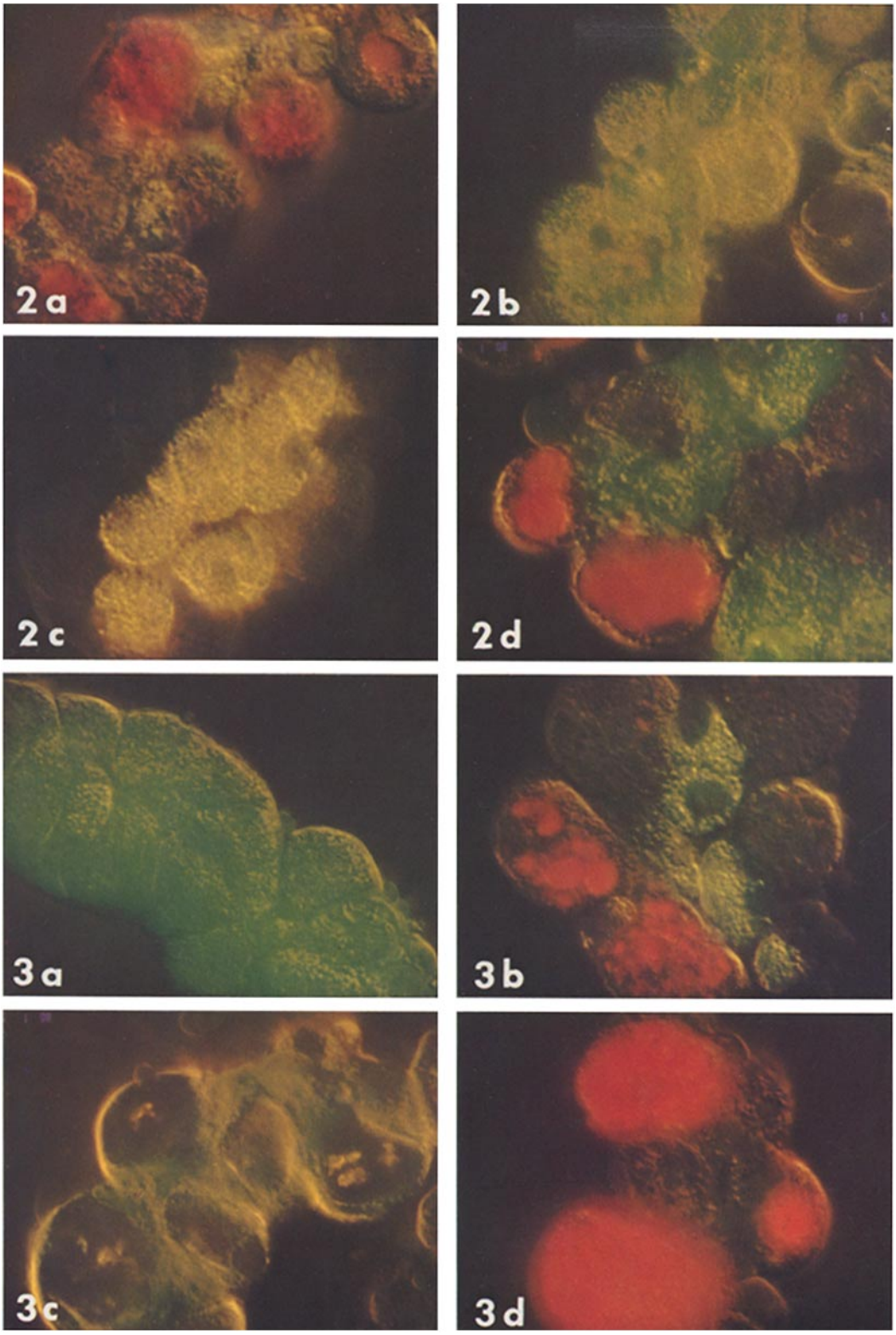
Similar data were obtained from the use of CN^-. Fig. 3*c* illustrates a gland shocked after treatment with 10^{-3} M CN^- in normal (1 mM) Ca^{++} medium. The degranulation of peptic cells and the loss of red fluorescence from parietal cells were routinely seen. As illustrated in Fig. 3*c*, particles were found within the cellular vacuoles; these particles, unlike the cytoplasmic contents, exhibited Brownian motion. The addition of ATP, with CN^- as with N_3^- inhibition, resulted in the restoration of red fluorescence within parietal cell vacuoles (Fig. 3*d*).

Uptake of [^{14}C]Aminopyrine by Gastric Glands

Before discussing our observations on the effect of ATP on aminopyrine accumulation in inhibited glands, we will consider three other variables: the effect of shocking alone, the effect of medium K^+, and the effect of medium Ca^{++}.

EFFECT OF SHOCKING: At the voltage levels used, the gastric glands were made highly permeable to trypan blue. Although some cells continued to exclude the dye, this does not mean that these cells were not leaky to solutes of lower molecular weights. Evidently, not only did this procedure result in the ability to allow the entry of solute into cells, but it also resulted in the loss of solute from the cells. It might be expected, therefore, that no matter whether shocking is carried out in the presence or absence of secretagogue, a loss in the ability of the glands to accumulate aminopyrine will be found. This is illustrated in Fig. 4. Here gastric glands in normal K^+ medium, with or without histamine, are compared with gastric glands in identical medium but subjected to the voltage discharge procedure. It can be seen that, under all experimental conditions, shocking significantly reduced aminopyrine accumulation. In the case of glands suspended in normal K^+ medium, the basal ratio was reduced from a value of 33.5 to 3.6. Although histamine raised the control aminopyrine accumulation ratio significantly, after shock in normal K^+ medium, the ratio is similar to the ratio found in shocked glands in the absence of added secretagogue (i.e., 10.0). This can be explained by cellular damage induced by the shock procedure and by the loss of various solutes. One possibility, associated with major leakiness of plasma membrane, is the loss of cell K^+.

EFFECT OF K^+: When glands not treated with secretagogue were incubated in high K^+ medium, the aminopyrine ratio increased two- to three-fold (Fig. 5). This has been interpreted as demonstrating a K^+ limitation on pump activity (1, 27), alleviated by high medium K^+. When these glands were shocked, there was also a depression of aminopyrine accumulation, but to a level significantly higher than the level under the same conditions but in normal K^+ medium. The presence of secretagogue, such as histamine, further stimulated aminopyrine accumulation, and shock reduced accumulation to the same level as that found in the absence of secretagogue. High K^+, therefore, had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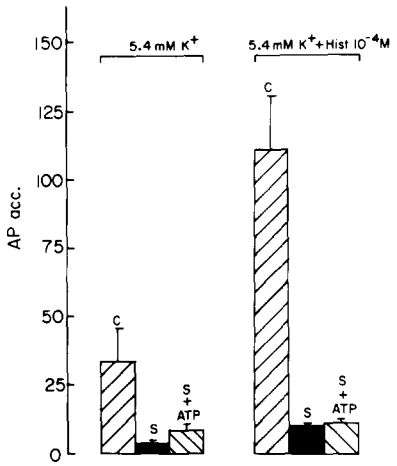

FIGURE 4 The effect of shock and ATP on aminopyrine accumulation in control and histamine (10^{-4} M)-stimulated gastric glands in 5.4 mM K^+. The aminopyrine ratio was reduced by shock and not restored by ATP. *C*, control; *S*, shocked. The bars show the mean ±SEM, $n = 3$.

a significant effect on acid secretion in both control and shocked glands.

EFFECT OF Ca^{++}: When shocking was carried out in the presence of 1 mM Ca^{++}, as is mentioned above, a uniform permeation of the cells comprising the glands was observed. If this level of Ca^{++} was maintained in the incubation medium, the suppression by shock of aminopyrine accumulation was similar to that found with no added Ca^{++} (Fig. 5). As is discussed below, high medium Ca^{++} inhibits the ATP effect in aminopyrine accumulation. This result led to the choice of zero Ca^{++} medium after shock for the weak base uptake experiments.

EFFECT OF ADDED ATP: The action of added ATP on control and shocked glands was studied at 15 min after shock and with regard to its time-

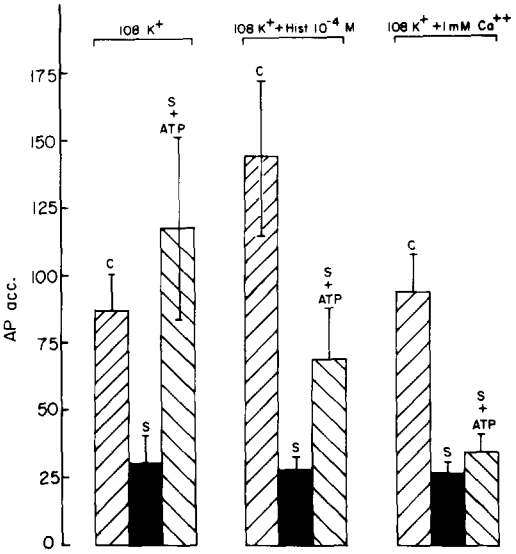

FIGURE 5 The effect of shock and subsequent ATP addition in control and histamine (10^{-4} M)-stimulated glands incubated in 108 mM K^+ and transferred to zero Ca^{++} medium with 2 mM EGTA before ATP and aminopyrine addition. Shock significantly reduced aminopyrine accumulation, and ATP restored it ($P < 0.05$). The continued presence of 1 mM Ca^{++} prevents a significant increase in aminopyrine accumulation due to ATP addition. *C*, control; *S*, shocked. The bars show the mean ±SEM, $n = 3$.

FIGURE 2 (*a*) An isolated living gastric gland incubated with dibutyryl cAMP in the presence of 100 μM acridine orange, showing the accumulation of red fluorescence in the parietal cells when viewed by a combination of Nomarski and fluorescent illumination. × 730. (*b*) A gland from the suspension described in *a* after shock in 1 mM Ca^{++} medium, showing the maintenance of some vacuoles but the loss of the red fluorescence characteristic of acridine orange accumulation in low pH regions. (*c*) A gland from the suspension described in *a* shocked in the absence of Ca^{++}. The maintenance of prominent peptic granules and the loss of acridine orange red fluorescence is demonstrated. (*d*) A gland after shock as described in *b*. Red fluorescence due to the addition of 5 mM ATP within the exposed secretory canaliculus is also demonstrated.

FIGURE 3 (*a*) An isolated gastric gland after exposure to 10^{-3} M NaN_3 and shock, showing uniform green fluorescence in the presence of 100 μM acridine orange. (*b*) A gland from the suspension described in *a* after the addition of 5 mM ATP, which results in the reappearance of the red fluorescence in the secretory canaliculus and reexpansion of the canaliculus. (*c*) An isolated gastric gland in the presence of 100 μM acridine orange and 10^{-3} M NaCN after shock, showing the presence of expanded particle-containing secretory canaliculi but the absence of the red fluorescence characteristic of accumulated acridine orange. (*d*) The same glands shown in *c* after the addition of 5 mM ATP. The restoration of red fluorescence due to the addition of the nucleotide is seen.

course. As is also shown in Fig. 4, the addition of ATP to glands treated in normal K^+ medium had no effect on aminopyrine accumulation under any conditions studied, even after shock. These data are in contrast to the effects observed in high K^+ medium, in which ATP completely reversed the suppression of aminopyrine accumulation induced by shock when secretagogue was absent (Fig. 5). ATP also increased the ratio in shocked glands in which aminopyrine accumulation had been stimulated by high K^+ and histamine, but the accumulation ratio of stimulated unshocked glands was not reached. In the presence of 1 mM medium Ca^{++}, however, this effect of ATP was not seen (Fig. 5), which is consistent with the inhibition of the H^+:K^+ exchange ATPase by these high Ca^{++} levels.[2]

When mitochondrial respiration was inhibited by 10^{-3} M CN^-, for example (N_3^- and Amytal give similar data), the aminopyrine ratio dropped almost to one, as is shown in Fig. 6. With the addition of ATP to these glands, even without shock, in contrast to all the previous experiments, some elevation of the aminopyrine ratio occurred. This might be due to the 45-min exposure to cyanide rendering some cells permeable to ATP. Following shock, however, there was a substantial increase in the aminopyrine ratio in the CN^--treated glands when ATP was added (Fig. 6), reaching a mean of 47 ± 0.8 ($n = 3$). SCN^- blocked this response.

The time-course of aminopyrine accumulation after either CN^- or amytal treatment is illustrated in Fig. 7. Without ATP addition, there was no change in the aminopyrine ratio with time. After ATP addition, the ratio rapidly rose to a maximum of 40 within 5 min in the case of the Amytal- or CN^--treated glands. N_3^- gave similar data but a lower maximum ratio.

CELL VOLUME: Cell volume was measured as the water space in the gland pellet that excluded [^{14}C]inulin. The results are detailed in Table I. The water space impermeable to inulin decreased as a result of shock under all conditions. This is readily explained as being the result of an increase in inulin penetration, at least in some cells, due to the shocking procedure and/or loss of peptic cell volume. ATP, in all cases, reduced the inulin-impermeable space even further, although there was a variation in the statistical significance of the change. ATP did not change the number of glands taking up trypan blue. It must be concluded that ATP increases the inulin-permeable space or that it induces a reduction of glandular volume. The

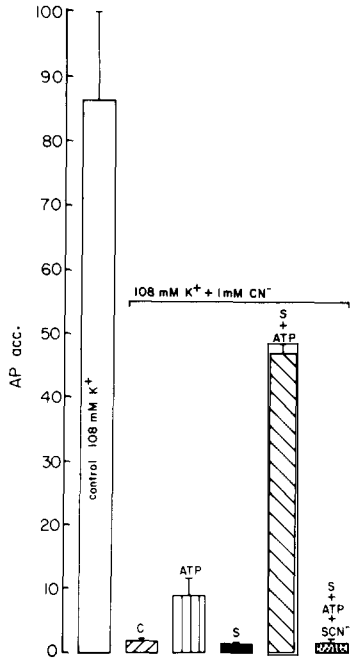

FIGURE 6 The effect of ATP on the aminopyrine ratio of glands incubated in the presence of 108 mm K^+ and 10^{-3} M CN^-, with or without shock. ATP raises the aminopyrine accumulation only slightly in unshocked glands but raises it very significantly ($P < 0.05$) in shocked glands. 10 mM SCN^- blocked the ATP effect in shocked glands. Bars show mean ±SEM, $n = 3$.

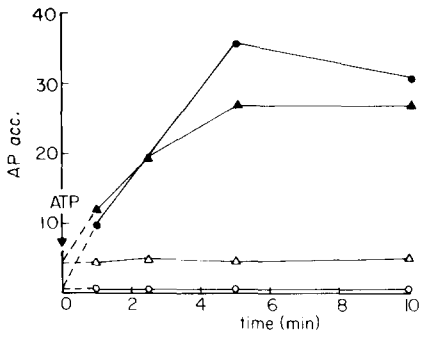

FIGURE 7 The time-course of aminopyrine accumulation in shocked glands incubated in 108 mM K^+ and 10^{-3} M CN^- or 1.6×10^{-3} M Amytal, with or without the addition of 5×10^{-3} M ATP. Without ATP, no time-dependent changes were seen. With ATP, a time-dependent accumulation of aminopyrine in the presence of either CN^- or Amytal was seen. (●) 1 mM NaCN with 5 mM ATP; (▲) 1.6 mM Amytal with 5 mM ATP; (△) 1.6 mM Amytal; (○) 1 mM NaCN.

[2] Saccomani, G., and G. Sachs. Unpublished observations.

TABLE I

Apparent Intracellular Water in Glands Shocked under Various Conditions and Trypan Blue Uptake after 4 × 3 kV (n = 3)

Medium	Inulin excluding space	Glands taking up trypan blue
	μl/mg dry wt	*%*
5.4 mM K^+ + S	1.75 ± 0.01	51.6 ± 10.0
5.4 mM K^+ + 5 mM ATP + S	1.32 ± 0.14 ($P < 0.05$)	
5.4 mM K^+ + Hist 10^{-4} M + S	2.05 ± 0.17	48.9 ± 4.0
5.4 mM K^+ + Hist 10^{-4} M + 5 mM ATP + S	1.04 ± 0.07 ($P < 0.05$)	
108 mM K^+ + S	1.90 ± 0.13	43.2 ± 3.3
108 mM K^+ + 5 mM ATP + S	1.41 ± 0.18 ($P < 0.05$)	
108 mM K^+ + Hist 10^{-4} M + S	1.84 ± 0.13	45.3 ± 17.2
108 mM K^+ + Hist 10^{-4} M + 5 mM ATP + S	1.42 ± 0.12 ns	
108 mM K^+ + 1 mM Ca^{++} + S	2.15 ± 0.10	34.5 ± 9.5
108 mM K^+ + 1 mM Ca^{++} + 5 mM ATP + S	1.74 ± 0.24 ns	
108 mM K^+ + 1 mM CN^- + S	1.86 ± 0.25	30.0 ± 8.4
108 mM K^+ + 1 mM CN^- + 5 mM ATP + S	1.40 ± 0.06 ns	
108 mM K^+	2.70 ± 0.08 $n = 2$	
5.4 mM K^+	2.01 ± 0.05 $n = 2$	

ns, not significant. S, shocked. The Ca^{++} group was shocked and maintained in Ca^{++}, in contrast to the other groups, which were shocked in Ca^{++} medium and then transferred to zero Ca^{++} with 2 mM EGTA.

increased inulin-permeable space is unlikely to be the intracellular canaliculus because ATP decreases the apparent glandular volume in 5.4 mM K^+ medium without activating H^+ secretion. Moreover, no change was seen in the cell diameter.

DISCUSSION

From the data presented, it can be seen that dielectric discharge over a rabbit gastric gland suspension is a reproducible method for making the plasma membrane of the parietal cell permeable to ATP without destroying the ability of the intracellular canaliculus to secrete acid into its lumen. The contents of the parietal cell can, therefore, be manipulated at will. The effects of this manipulation can be assessed in the suspension in general by observation of the uptake of [^{14}C]aminopyrine. The effects on individual cells can be determined by microscope observation of acridine orange fluorescence. This should, therefore, prove to be a suitable model with which to explore a complicated process, the HCl secretory capacity of the parietal cell.

On the basis of the properties of the H^+:K^+ exchange ATPase present in hog (13) or rabbit gastric membrane (27), when isolated in the form of relatively ion-impermeable vesicles, we expected a high K^+ requirement for H^+ transport by this enzyme. This was borne out by the inhibition of H^+ secretion by ouabain in the isolated rabbit gastric glands and by the relief of this inhibition by elevation of medium K^+ (1, 27). It is, therefore, to be expected that, in 5.4 mM K^+ medium, generation of a leaky plasma membrane will drastically reduce H^+ secretion. That high K^+ alone is not sufficient under these conditions to maintain an acid transfer rate is further shown by the inhibition of secretion produced by shock, even in 108 mM K^+ medium.

An additional factor that evidently is lost in the shock procedure is ATP inasmuch as the addition of the nucleotide restores secretion to control levels, as visualized by acridine orange or as measured by [^{14}C]aminopyrine accumulation. The data obtained with acridine orange show that the acid compartment is the structure also responsible for acid accumulation in stimulated, unshocked glands, namely, the secretory canaliculus. In unstimulated glands in 108 mM K^+ medium, the acid space could not be visualized with acridine orange, in spite of very high aminopyrine accumulation ratios. Thus, in that case, the acid site could be tubulovesicles or collapsed canaliculi.

ATP is able to restore the aminopyrine accumulation ratio to close to control levels in glands that have been stimulated by a secretagogue, such as histamine, in the presence of high K^+, but there is a statistically significant shortfall. Because in the presence of secretagogue, but not with high K^+ alone, there is a significant expansion of the secretory canaliculus (1), a factor contributing to the lower aminopyrine levels after shock and the addition of ATP could be voltage-induced leak in the expanded canalicular membrane. Alterna-

tively, additional factors necessary for maximum acid secretion could leak from the cell.

Nevertheless, the ability of ATP to restore the acid secretory parameters under these conditions conforms to the expectations derived from gastric vesicle studies, namely, that ATP can energize H^+ gradients in the parietal cell. The ultimate source of energy is, however, still a matter of dispute (3, 23).

An alternative to the H^+:K^+ exchange ATPase model illustrated in Fig. 8*a* is a redox hypothesis quite similar to the protonmotive loop concept that forms a cornerstone of the chemiosmotic theory (24). This is illustrated in Fig. 8*b*. According to this model, there is no role for ATP in H^+ transport by the gastric mucosa. This form of the redox hypothesis is disproved by the data presented in this study.

The gastric parietal cell contains numerous mitochondria, which account for 34% by volume of the cell (25). They could function as a source of ATP, or the mitochondrial respiratory chain could be linked to a plasma membrane redox pump to reoxidize reduced substrate. Amytal, a complex I inhibitor, and azide or cyanide, inhibitors of the terminal oxidase, block mitochondrial respiration, which results in loss of cellular ATP (5, 6), and inhibit acid secretion in the rabbit gland preparation. ATP is able to partially reverse this blockade. Evidently, these are highly toxic agents, and it is not surprising that total reversal is not obtained. The accumulation space of aminopyrine is only 10% of glandular volume, as the use of acridine orange as a monitor of the volume of the acid compartments indicates. The measured ratio of 100 would correspond to a pH of 2 in control canaliculus and to a pH of 2.4 after the addition of CN^- with ATP, when the ratio reaches 40. This relatively small difference in pH tends to exclude a more complex H^+ pump model, namely, that of mitochondrially dependent redox and ATP pumps in series, each providing about half of the overall energy required (26). SCN^-, although a relatively ineffective inhibitor of H^+ transport in vesicles isolated from rabbit (27), was highly inhibitory to the ATP-dependent secretion in the permeable gland. Some factor essential for SCN^- action such as local high concentration of base formed during acid production must have been lost in vesicle isolation.

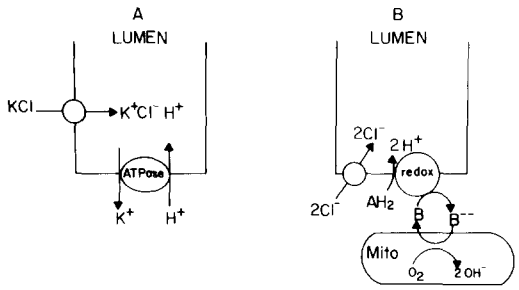

FIGURE 8 Two models for HCl production. *A* represents a model utilizing an H^+:K^+ exchange ATPase in which KCl leaks from cytosol to the canalicular lumen and K^+ is exchanged for H^+ by the ATPase. *B* shows a redox-driven pump in which electrons and H^+ are separated across the canalicular membrane by an oriented redox reaction. The electron acceptor is shown as being reoxidized by the mitochondrial respiratory chain. The data presented in this paper seem to rule out model *B*.

An additional point of interest is the apparent role of Ca^{++} in mediating degranulation of the peptic cell. It has been observed that gastrin in a Ca^{++}-containing medium degranulated the peptic cell (28). Here, dielectric breakdown of the plasma membrane in the presence of Ca^{++}, but not in its absence, resulted in massive discharge of peptic granules, suggesting that Ca^{++} is the second messenger for exocytosis by this cell. The same level of Ca^{++} in medium, in contrast, inhibited acid secretion.

Further exploitation of this model should give more detailed information on the cytoplasmic and luminal membrane factors involved in acid secretion by the parietal cell and on the Ca^{++} mediation of pepsinogen release.

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