Nucleotides Metabolism by Gastric Glands and \( H^+ - K^+ - ATPase \)-Enriched Membranes

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Rong, Qinfen, Olga Utevskaya, Marlon Ramilo, Dar C. Chow, and John G. Forte. Nucleotide metabolism by gastric glands and \( H^+ - K^+ - ATPase \)-enriched membranes. Am. J. Physiol. 271 (Cell Physiol. 40): C61–C73, 1996.] In this study we use \( \alpha \)-toxin-permeabilized gastric glands to study energy metabolism and the interplay between nucleotides to support acid secretion, as indicated by the accumulation of aminopyrine (AP). When permeabilized glands were treated with a phosphodiesterase inhibitor, the secretory response to cAMP was inhibited, whereas the secretory response to ATP was potentiated. This implied that 1) ATP provided support not only as an energy source but also as substrate for adenylate cyclase, 2) activation of acid secretion by cAMP needed ATP, and 3) ATP and cAMP exchanged rapidly inside parietal cells. To address these issues, we tested the action of adenine nucleotides in the presence and absence of oxidizable substrates. All adenine nucleotides, including AMP, ADP, ATP, and cAMP, could individually enhance the glandular AP accumulation in the absence of substrates, whereas only a high concentration of ATP (5 mM) was able to support secretory activity in substrate-free buffer. Moreover, ATP could maintain 75–80% of maximal secretory activity in phosphate-free buffer; cAMP alone could not support secretion in phosphate-free buffer. In glands and in \( H^+ - K^+ \)-adenosinetriphosphatase-rich gastric microsomes, we showed the operation of adenylate kinase, creatine kinase, and ATP/ADP exchange activities. These enzymes, together with endogenous adenylate cyclase and phosphodiesterase, provide the recycling of nucleotides essential for the viability of \( \alpha \)-toxin-permeabilized gastric glands and implicate the importance of nucleotide recycling for energy metabolism in intact parietal cells.

adenylate kinase; creatine kinase; nucleotide exchange; \( \alpha \)-toxin

The secretion of 150 mM HCl places an extraordinary energy demand on the acid-secreting parietal cell. Parietal cells are well adapted to meet these energetic requirements by virtue of their large, abundant mitochondria and functionally adapted enzymes to support high oxidative potential and nucleotide metabolism. For example, parietal cells have a high capacity to metabolize fatty acids (5, 11), their lactate dehydrogenase is predominantly of the \( H_2 \) isotype, and creatine kinase activity has been localized to the same tubulovesicles that contain the primary proton pump, the \( H^+ - K^+ \)-adenosinetriphosphatase (ATPase) (32).

Isolated gastric glands have been widely used as a model of parietal cell function, typically using the uptake of the weak base aminopyrine (AP) as an index of the secretory response to secretagogues (2). For gastric glands freshly isolated from rabbit stomach, the most potent secretagogues are those that evoke secretion via the protein kinase A (PKA) pathway, e.g., dibutyryl-adenosine 3',5'-cyclic monophosphate (cAMP) or ATP, with proven morphological and functional transition between resting and secretory states [X. Yao, S. M. Karam, M. Ramilo, Q. Rong, A. Thibodeau, and J. G. Forte. Am. J. Physiol. 271 (Cell Physiol. 40): C61–C73, 1996.] In this study we use \( \alpha \)-toxin-permeabilized rabbit gastric glands to study energy metabolism and the interplay between nucleotides to support acid secretion, as indicated by the accumulation of aminopyrine (AP). When permeabilized glands were treated with a phosphodiesterase inhibitor, the secretory response to cAMP was inhibited, whereas the secretory response to ATP was potentiated. This implied that 1) ATP provided support not only as an energy source but also as substrate for adenylate cyclase, 2) activation of acid secretion by cAMP needed ATP, and 3) ATP and cAMP exchanged rapidly inside parietal cells. To address these issues, we tested the action of adenine nucleotides in the presence and absence of oxidizable substrates. All adenine nucleotides, including AMP, ADP, ATP, and cAMP, could individually enhance the glandular AP accumulation in the absence of substrates, whereas only a high concentration of ATP (5 mM) was able to support secretory activity in substrate-free buffer. Moreover, ATP could maintain 75–80% of maximal secretory activity in phosphate-free buffer; cAMP alone could not support secretion in phosphate-free buffer. In glands and in \( H^+ - K^+ \)-adenosinetriphosphatase-rich gastric microsomes, we showed the operation of adenylate kinase, creatine kinase, and ATP/ADP exchange activities. These enzymes, together with endogenous adenylate cyclase and phosphodiesterase, provide the recycling of nucleotides essential for the viability of \( \alpha \)-toxin-permeabilized gastric glands and implicate the importance of nucleotide recycling for energy metabolism in intact parietal cells.

MATERIALS AND METHODS

Preparation of Gastric Glands

Gastric glands were prepared from New Zealand White rabbits as described by Berglindh and Obrink (2). After isolation, gastric glands were subjected to washing three times with minimum essential medium (GIBCO) and maintained in the resting condition in the same buffer containing 10 µM cimetidine.

Permeabilization of Glands

Freshly isolated glands were washed once in a permeabilization buffer rich in \( K^+ \) (K medium), including (in mM) 100 KCl, 20 NaCl, 1.2 MgSO\(_4\), 1 NaH\(_2\)PO\(_4\), 40 mannitol, 20 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, and 10 tris(hydroxymethyl)aminomethane (Tris), pH 7.4. Glands were...
then resuspended in K medium at 10% cytocrit and incubated with Staphylococcus α-toxin at the concentration of 0.11 mg/ml for 45 min at 37°C. Staphylococcus α-toxin was a generous gift from Dr. S. Bhakdi, purified from S. aureus (4, 25). After permeabilization, the gland suspension was diluted to a 5% cytocrit in K medium in which 10 mM succinate and 1 mM pyruvate were added as oxidative substrates (35). Functional activity of glands was characterized by AP uptake assay. AP accumulation is reported either as the AP accumulation ratio, as described by Berglindh and Obrink (2), or as the AP index, which is the percentage of maximum AP accumulation for a given preparation (35).

Preparation of H⁺-K⁺-ATPase-Enriched Subfraction of Microsomes

H⁺-K⁺-ATPase-containing microsomes were prepared from rabbit stomach as previously reported (8, 28). New Zealand White rabbits (2–3 kg) were injected with cimetidine (20 mg/kg body wt) 1 h before the animals were euthanized with pentobarbital sodium (Nembutal). The stomach was then removed and washed with ice-cold isotonic saline. The mucosa from the fundus and body region was scraped, minced, and homogenized in a Potter-Elvehjem homogenizer with 15 passes in a hypotonic solution containing ice-cold (in mM) 113 mannitol, 37 sucrose, 0.4 EDTA, and 5 piperazine-bis(2-ethanesulfonic acid) (PIPES), pH 6.7. The homogenate was centrifuged at 14,500 g for 10 min at 4°C. The supernatant was further centrifuged at 100,000 g for 1 h. The resulting pellet was resuspended in a suspending medium (in mM; 300 sucrose, 5 Tris, and 0.2 EDTA, pH 7.4). The crude microsomal suspension was brought up to 43% sucrose and overlaid with successive gradient layers of 30 and 10% sucrose (wt/vol) in 5 mM Tris and 0.2 mM EDTA, pH 7.4, and centrifuged for 4 h at 90,000 g using a Beckman SW27 rotor. H⁺-K⁺-ATPase-enriched rabbit gastric microsomes were collected as the flotation layer at the interface between the 10 and 30% layers and stored in aliquots at −20°C until use.

Probing Nucleotide Interchange by TLC

Permeabilized glands were bathed in K medium containing 0.2–0.5 µCi of [8-14C]adenosine di- or triphosphate and additional additives, as specified by the conditions in each experiment. The reaction was stopped by 0.6 N perchloric acid (PCA), and nucleotides were extracted by adsorption onto charcoal (14) and finally eluted from the charcoal with 2% NH₃ in 50% ethanol. Samples were assayed for individual nucleotides by thin-layer chromatography (TLC).

Enzymatic characterization of H⁺-K⁺-ATPase-enriched microsomes was assessed by a modified method of Reenstra et al. (29). Isolated gastric microsomes (10 µg protein) were incubated with various substrates at room temperature (23–24°C) in buffer containing 1 mM MgSO₄, 20 mM NaCl, 15 mM PIPES (pH 6.8), and 0.1 µCi/100 µl [8-14C]ADP with 0.1 mM ATP. Aliquots were taken at assigned time intervals, and the reaction was stopped by 20 mM EDTA. Protein was precipitated with 0.6 N PCA followed by neutralization with ice-cold KOH. Samples were clarified by centrifugation, and the supernatant was saved for assay by TLC.

TLC was carried out on 20-cm² polyethyleneimine-cellulose plastic plates (Selecto Scientific, Norcross, GA). Aliquots of samples were applied 3 cm from the bottom of the plate. Unlabeled nucleotides were applied as a standard for identification. Chromatograms were developed in different solvent systems, depending on the purpose of the specific nucleotide separation (14, 17, 27). To separate ATP, ADP, and AMP the chromatogram was first developed with 2 M sodium formate (pH 3.4) up to 6 cm from the bottom and then with 4 M sodium formate (pH 3.4) to the top of the plate (14). To separate cAMP from other nucleotides the chromatogram was developed in distilled water to the top of the plate, followed by 0.25 M LiCl after air drying (adapted from Ref. 17). The position of nucleotides was identified under ultraviolet light. Radioactivity of nucleotides was evaluated by radioautography and liquid scintillation counting of eluted spots.

Statistics

Results from different experiments were averaged and presented as the means ± SE. Differences between experiments were evaluated by Student’s t-test, with P < 0.05 being considered significant.

RESULTS

Support of AP Accumulation in α-Toxin-Permeabilized Gastric Glands

Phosphodiesterase inhibitors block stimulatory effects of cAMP in permeabilized gastric glands. The effects of a phosphodiesterase inhibitor on the AP accumulation ratio of permeabilized gastric glands are shown in Table 1. Without IBMX the glands responded briskly to the addition of 0.1 mM cAMP, and in this case there was no measurable potentiation when 0.1 mM ATP was included. Similar to our previous results (35, 38), even without added cAMP AP accumulation was also increased if 1 mM ADP (Table 1) or ATP (cf. Fig. 1) was added. When 50 µM IBMX was included, the cAMP-elicted secretory activity was inhibited by about 50%, whereas no significant difference was found in AP ratios mediated by either 1 mM ADP or 0.1 mM cAMP-0.1 mM ATP. The inhibitory effect of IBMX and its analog is just the opposite of what has been seen in intact glands (1, 33). IBMX blocks the degradation of cAMP to AMP, and in normal intact gastric glands with a constitutively active adenylate cyclase, IBMX results in the elevation of intracellular cAMP, stimulating acid secretion itself and/or potentiating the action of secretagogues like histamine. These latter effects of IBMX in intact tissue and glands have long been used to support the cAMP-PKA pathway for parietal cell activation. However, the inhibition by IBMX of cAMP-elicted acid

Table 1. cAMP-elicted acid secretion in α-toxin-permeabilized glands is inhibited by inhibiting phosphodiesterase activity

<table>
<thead>
<tr>
<th>Secretagogue</th>
<th>Without IBMX</th>
<th>With 50 µM IBMX</th>
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<tbody>
<tr>
<td>0.1 mM cimetidine</td>
<td>11.1 ± 2.0 (7)</td>
<td>15.8 ± 6.6 (4)</td>
</tr>
<tr>
<td>0.1 mM cAMP</td>
<td>117.7 ± 15.9 (7)</td>
<td>49.0 ± 8.1 (7), P &lt; 0.01</td>
</tr>
<tr>
<td>0.1 mM cAMP/0.1 mM ATP</td>
<td>109.0 ± 4.3 (3)</td>
<td>92.4 ± 1.2 (3)</td>
</tr>
<tr>
<td>1.0 mM ADP</td>
<td>81.0 ± 21.7 (2)</td>
<td>77.2 ± 13.4 (2)</td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of experiments is shown in parentheses. α-Toxin-permeabilized glands were incubated with indicated secretagogues for 20 min at 37°C. P value compares results with and without 3-isobutyl-1-methylxanthine (IBMX). Aminopyrine (AP) ratios for intact glands of parallel experiments were 26.6 ± 3.0 (cimetidine) and 190.7 ± 20.9 (histamine + IBMX).
secretion in the α-toxin model was unexpected and required some alternative explanation.

ATP supports AP accumulation as an energy source and as a source of cAMP. The dose response to ATP for AP accumulation by α-toxin-permeabilized glands is shown in Fig. 1 in the absence and presence of IBMX. AP accumulation increased gradually as ATP concentration increased, and over the entire range the accumulation of AP was enhanced in the presence of 50 µM IBMX, with the potentiating effect of IBMX being significant (P < 0.05) at 0.2 mM ATP and higher. These data are consistent with our previous explanation that ATP may serve both as an energy source and as a precursor for cAMP through endogenous adenylate cyclase activity (35, 38). Accordingly, as predicted from results on intact preparations, IBMX would presumably operate to preserve the generated cAMP. The plausibility of this latter explanation is supported by our experiments measuring the distribution of 14C among nucleotides in α-toxin-permeabilized glands treated with [14C]ATP. Figure 2 shows that there is a low level of [14C]cAMP produced in conditions where glands were treated with 0.1 mM forskolin and 0.1 mM [14C]ATP, and that the [14C]cAMP spot became more intense in preparations treated with IBMX, consistent with IBMX blocking the degradation of cAMP. It is also apparent from the autoradiographs that the level of [14C]AMP was quite high in both conditions, with or without IBMX, suggesting a high flow path from ATP to AMP. (With this solvent system to specifically separate AMP and cAMP, ATP could not be separated from ADP.)

Functional oxidative metabolism is essential for AMP and cAMP to support nucleotide interchange and AP accumulation. To test the ability of AMP to serve as a source of nucleotide in α-toxin-permeabilized glands, AP accumulation was measured in response to exogenous AMP. Figure 3 shows that AP accumulation was progressively increased as AMP was raised from 0.01 to 1 mM AMP. The AP ratios produced by 1 mM AMP were comparable to those produced by 1 mM ATP or ADP. Figure 3 also shows that the addition of 50 µM IBMX greatly diminishes the stimulatory effect of cAMP but does not appreciably alter the responses to AMP or ADP. In earlier studies we demonstrated that AP accumulation by α-toxin-permeabilized glands could be supported by either ATP or ADP (35), but that in the case of ADP, oxidative substrates and functional mito...
chondria were necessary for optimal secretory activity. Without functional mitochondrial oxidation, stimulation of AP accumulation by either cAMP or ADP diminished and, as shown in Fig. 3, only relatively high concentrations of ATP could recover the activation. In the case of activation by AMP, the AP ratio was totally abolished (from 60.5 ± 4.0 to 5.9 ± 0.5) in the absence of oxidative substrates (Fig. 3). Thus, with intact oxidative phosphorylation, ATP, ADP, AMP, and even cAMP, will all serve as a source of adenine nucleotide to support energy metabolism and the H⁺ pump, suggesting that phosphate interchange among the adenine nucleotides is very efficient and is essential to maintain parietal cell function.

Pi is essential for nucleotide exchange. To further address nucleotide interchange, we investigated the effect of Pi on AP accumulation. In Pi-free buffer, ATP (both 0.1 and 1.0 mM) retained about 80% of the AP accumulation observed in phosphate buffer (Fig. 4). The stimulation elicited solely by cAMP was completely abolished, and the large stimulation ordinarily produced by 0.1 mM ATP plus 0.1 mM cAMP was greatly attenuated (60–70%) in Pi-free buffer. Synthesis of ATP from the mono- or diphosphate requires a source of Pi; in the buffer. The addition of sufficient ATP provides a direct energy source for proton transport, as well as a source of phosphate for nucleotide exchange and recycling. Nevertheless, in Pi-free buffer, ATP could not support the maximum AP accumulation, which is probably due to the trapping of phosphate in other stores, such as protein phosphorylation.

The conclusion we draw thus far is that there is a rapid flow of adenine nucleotide from AMP or cAMP to ATP, sufficiently rapid to keep up with the demand set by proton transport associated with AP accumulation. In each of the cases shown above, AP accumulation was completely inhibited by the H⁺-K⁺-ATPase inhibitor omeprazole (data not shown). The purpose of the next group of experiments was to test for the operation of a variety of enzymes associated with nucleotide metabolism and exchange, using both the permeabilized cell model and isolated H⁺-K⁺-ATPase-rich microsomal vesicles.

Nucleotide Metabolism and Interchange in Permeabilized Glands

In addition to functional oxidative metabolism, several enzymatic pathways are involved in the cycling of nucleotides, and we used [14C]ADP as a precursor to assess the operation of these enzymes in the permeabilized gastric gland model. Figure 5 shows the results of experiments in which glands were incubated with 0.1 mM ADP containing tracer [14C]ADP. These experiments were performed in the absence of oxidative substrates to eliminate the formation of labeled ATP from ADP through oxidative phosphorylation. When the incubation included 0.1 mM ADP alone there was a great loss of [14C]ADP with a large increase in [14C]AMP, suggesting the net degradation of ADP via ADP phosphohydroxylase reactions, which we generically indicate as ADP ⇄ AMP + Pi. On the basis of data shown below, the modest production of ATP was most likely the result of adenylate kinase activity, 2ADP ⇄ ATP + AMP. When the incubation included 1 mM phosphocreatine (PCr) there was a large production of ATP and much of the [14C]ADP was preserved, indicating the operation of creatine kinase activity, PCr + ADP ⇄ ATP + creatine. The inclusion of several nucleoside triphosphate substrates (NTP), such as ATP (not shown), ITP, or GTP, produced qualitatively similar results as PCr, i.e., the synthesis of 14C-labeled ATP and the preservation of ADP. These latter results are consistent with a nucleoside phosphate exchange reaction, NTP + ADP ⇄ ATP + NDP (26, 29).

Nucleotide Metabolism and Interchange by Isolated Gastric Microsomes

Adenylate kinase activity. We next undertook a study of nucleotide reactions within purified gastric micro-
somal membranes that are rich in H\(^+\)-K\(^+\)-ATPase. In many of the subsequent experiments a potent inhibitor of H\(^+\)-K\(^+\)-ATPase, Sch-28080, was used to prevent this enzyme from dominating the data. For example, one experiment tested the ability of the microsomes to metabolize \([14C]\)ADP, similar to what we did with the permeabilized glands above. Figure 6A shows a representative time course of the relative amounts of label from \([14C]\)ADP that appear in ATP and ADP. After the reaction was initiated, there was an exponential decrease in \([14C]\)ADP. The initial rate of \([14C]\)ADP decrease was accompanied by a rise in \(^{14}C\) label appearing as ATP and an equivalent increase appearing as AMP; the sum of increased \([14C]\)ATP and \([14C]\)AMP was stoichiometrically equivalent to the loss of \([14C]\)ADP. These data are consistent with the presence of adenylate kinase activity in the microsomal fraction. The feasibility of this conclusion was tested by running the same conditions of reaction but in the presence of an inhibitor of adenylate kinase, P\(_1\), P\(_5\)-Di(adenosine-5\')pentaphosphate (Ap\(_5\)A; Ref. 21). As demonstrated in Fig. 6B, no ATP is formed from \([14C]\)ADP, and only the slow appearance of \([14C]\)AMP from dephosphorylating \([14C]\)ADP is seen when Ap\(_5\)A is included in the medium. Table 2 provides summary data for adenylate kinase activity measured as the initial rate of \([14C]\)ATP formation on three microsomal preparations.

Creatine kinase activity. Sistermans et al. (32) reported the operation of efficient creatine kinase activity in hog gastric microsomes. We tested for microsomal creatine kinase activity in much the same manner as for adenylate kinase. Figure 7 shows the results of incubating microsomes with 1.25 mM phosphocreatine and 0.1 mM ADP (plus tracer \([14C]\)ADP). In the absence of the adenylate kinase inhibitor Ap\(_5\)A (Fig. 7A), the synthesis of ATP occurs both by creatine kinase and adenylate kinase, the latter occurring with the greatest velocity. When adenylate kinase was inhibited by Ap\(_5\)A (Fig. 7B) there was a steady production of \([14C]\)ATP from \([14C]\)ADP, presumably driven by phosphocreatine. The initial rate of \([14C]\)ATP production was taken as creatine kinase activity. A summary of creatine kinase activity measured on several preparations is given in Table 2.

NTP/NDP exchange activity. Gastric microsomes also display potent ATP/ADP exchange activity, and this has been a proposed partial reaction of the H\(^+\)-K\(^+\)-ATPase (26), analogous to what has been seen for the Na\(^+\)-K\(^+\)-ATPase (10). Accordingly, we have measured active ATP/ADP exchange activity in gastric microsomes. As shown in Fig. 8A, when 1.25 mM ATP was included in the medium along with 0.1 mM ADP containing tracer \([14C]\)ADP there was a relatively prompt exchange of \(^{14}C\)-labeled nucleotide from ADP to

### Table 2. Rates of nucleotide reaction and interaction for gastric microsomal vesicles

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Rate (µmol ATP produced·mg microsomal protein(^{-1})·h(^{-1}))</th>
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<tr>
<td>Adenylate kinase</td>
<td>2.62 ± 0.23 (3)</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>1.68 ± 0.35 (6)</td>
</tr>
<tr>
<td>NTP/ADP exchange</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>10.73 ± 0.20 (3)</td>
</tr>
<tr>
<td>ITP</td>
<td>11.33 ± 0.54 (3)</td>
</tr>
<tr>
<td>CTP</td>
<td>4.61 ± 0.57 (4)</td>
</tr>
<tr>
<td>GTP</td>
<td>2.21 ± 0.23 (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE (given in µmol ATP produced·mg microsomal protein\(^{-1}\)·h\(^{-1}\)); no. experiments is shown in parentheses. All reactions were measured as the initial rate of \([^{14}C]\)ATP production from \([^{14}C]\)ADP by rabbit gastric microsomes at 23–24°C. For adenylate kinase reaction conditions included 0.1 mM ADP and 10 µM Sch-28080 to inhibit H\(^+\)-K\(^+\)-ATPase activity; the decline in \([^{14}C]\)ADP was comparable to the summed appearance of \([^{14}C]\)ATP and \([^{14}C]\)AMP. For creatine kinase reaction, conditions included 0.1 mM ATP, 1.25 mM creatine phosphate, 10 µM Sch-28080, and 0.2 mM AP\(_5\)A to inhibit adenylate kinase activity. For NTP exchange reactions, indicated nucleoside triphosphate (NTP) was 1.25 mM, with 0.1 mM ADP and 10 µM Sch-28080 and 0.2 mM AP\(_5\)A to inhibit H\(^+\)-K\(^+\)-ATPase and adenylate kinase activities, respectively.
Gastric parietal cells are highly active metabolizing systems, requiring intense oxidative activity to sustain the energy demands for the secretory product of ~0.16 N HCl. To meet the energy requirements the parietal cell has many large mitochondria, occupying a larger portion of the parietal cell volume (25–45%) than any other vertebrate cell type (18, 39). The requirement for a fluid supply of ATP as the primary energy source for the proton pump, the H^+)-K^+-ATPase, has been known for many years (12, 13, 30). Thus it has been no surprise that various permeabilized parietal cell models have a requirement for supplementary ATP (16, 23, 35).

Our earlier studies with the α-toxin-permeabilized parietal cell model demonstrated that either cAMP or ATP was capable of supporting histamine-stimulated AP accumulation, although maximal stimulation was achieved with combined cAMP plus ATP (35, 38). We reasoned that the "ATP effect" was mediated by endogenous adenylate cyclase activity to provide an activating level of cAMP. However, no clear view was formulated as to why cAMP alone would be an effective stimulant, except to postulate some residual level of ATP. The present data support the former conclusion and provide a clear explanation for the latter effect. In α-toxin-permeabilized glands, AP accumulation stimulated by increasing levels of ATP is potentiated by adding low levels of cAMP or by inhibiting glandular phosphodiesterase activity. This action of phosphodiesterase inhibitors supports the role of cAMP in the activation pathway along with the present demonstration of cAMP synthesis through endogenous adenylate cyclase activity. Furthermore, the previously observed inhibition of AP accumulation and cAMP-stimulated protein phosphorylation by H-89, a PKA inhibitor (35, 38), reinforce the cAMP-PKA pathway for activating HCl secretion, although other signals (e.g., Ca^{2+}) may have a role.

**DISCUSSION**

Gastric parietal cells have a high degree of specificity that has been reported for H^+-K^+-ATPase (30). We reasoned that the "ATP effect" was mediated by endogenous adenylate cyclase activity to provide an activating level of cAMP. However, no clear view was formulated as to why cAMP alone would be an effective stimulant, except to postulate some residual level of ATP. The present data support the former conclusion and provide a clear explanation for the latter effect. In α-toxin-permeabilized glands, AP accumulation stimulated by increasing levels of ATP is potentiated by adding low levels of cAMP or by inhibiting glandular phosphodiesterase activity. This action of phosphodiesterase inhibitors supports the role of cAMP in the activation pathway along with the present demonstration of cAMP synthesis through endogenous adenylate cyclase activity. Furthermore, the previously observed inhibition of AP accumulation and cAMP-stimulated protein phosphorylation by H-89, a PKA inhibitor (35, 38), reinforce the cAMP-PKA pathway for activating HCl secretion, although other signals (e.g., Ca^{2+}) may have a role.

**ATP**

ATP, i.e., ATP + [1^{14}C]ADP → [1^{14}C]ATP + ADP. Figure 8B shows the CTP/ADP exchange reaction in gastric microsomes, i.e., CTP + [1^{14}C]ADP → [1^{14}C]ATP + CDP. It is important to account for, or inhibit, adenylate kinase activity for an accurate measurement of nucleotide exchange rate. For the experiments shown here we included 0.2 mM ApoA as an inhibitor of adenylate kinase, and it is clear that rather little [1^{14}C]AMP is formed over the time course of reaction. We also included the H^+)-K^+-ATPase inhibitor Sch-28080 (10 µM), which is especially important for ascertaining the initial rate of [1^{14}C]ATP production from nucleoside triphosphates other than ATP. Control experiments established that NTP/ADP exchange activity was not significantly altered by Sch-28080 (data not shown). Sch-28080 is known to prevent the turnover of the H^+)-K^+-ATPase by inhibiting K^+-stimulated dephosphorylation of the phosphoenzyme intermediate (37), and thus it is not surprising to find that the K^+-independent formation of phosphoenzyme intermediate from ATP and the catalyzed transfer of γ-phosphate to ADP remains unaltered, as has been shown for another K^+-site inhibitor of H^+)-K^+-ATPase, AHR-9294 (29). Furthermore, our data summarized in Table 2 suggest that the exchange reaction is in fact a general nucleoside triphosphate/diphosphate (NTP/NDP) exchange reaction, catalyzing the transfer of γ-phosphate from a variety of nucleotides to ADP. The specificity of nucleotides for the rate of γ-phosphate exchange was ATP > ITP > CTP > GTP. The relative lack of specificity among nucleotides for the triphosphate/diphosphate exchange reaction is in contrast to the high degree of specificity that has been reported for H^+ transport by the H^+)-K^+-ATPase (30).

**DIAGRAM**

Fig. 9. Representation of nucleotide metabolism in parietal cell. Bold arrows, major pathways; dashed arrows, interchangeable nucleotide pool. Enzymatic reactions include protein kinase A (PKA, subscripts i and a indicate inactive and active forms, respectively), H^+)-K^+-ATPase (H/K), adenylate cyclase (AC), phosphodiesterase (PDE), adenylate kinase (AK), and creatine kinase (CK). Transformation of H^+)-K^+-ATPase from functionally inactive vesicular form to apical plasma membrane presumably occurs by protein phosphorylations (Prot-P) mediated via PKAa. Channels for K^+ and Cl^- at apical plasma membrane promote, respectively, recycling of K^+ through H^+)-K^+-exchange pump and net flow of Cl^- to follow H^+ as HCl.
have a potentiating role. The addition of cAMP to permeabilized glands, without exogenous ATP, provides an adequate stimulus of AP accumulation as long as phosphodiesterase remains active, suggesting that the product AMP participates in the reaction. It is now clear that the parietal cell can effectively utilize AMP as a nucleotide source to synthesize nucleoside triphosphate as long as oxidative metabolism is intact. Adenylate kinase is the principal reaction for recycling AMP, and relatively high levels of adenylate kinase activity were demonstrated in isolated gastric glands as well as in H$^+\cdot$K$^+\cdot$ATPase-enriched microsomes derived therefrom. Thus the adenylate kinase reaction, AMP + ATP $\rightarrow$ 2ADP, coupled with functioning mitochondria, i.e., ADP $\rightarrow$ P$_i$ $\rightarrow$ ATP, produces a redistribution of added nucleoside monophosphate to nucleoside triphosphate. Because of the virtual autocatalytic production of ATP by these reactions, the initial level of ATP can be extremely low.

Several other reactions associated with nucleotide metabolism and phosphate metabolism were evident in parietal cells, including the reaction promoting phosphate exchange between NTP/NDP exchange and creatine kinase (Cr$'$). Sistermans et al. (32) demonstrated the colocalization of creatine kinase in isolated gastric tubulovesicles, and that Cr$'$ + ADP were capable of supporting proton transport by the pump in those vesicles. Our experiments show that NTP/NDP exchange colocalizes with H$^+\cdot$K$^+\cdot$ATPase-rich tubulovesicles, most likely the ATP/ADP exchange that is a recognized partial reaction of P-type ATPases (10, 26). As with the creatine kinase, we have found that NTP/NDP exchange activity is capable of providing sufficient ATP, e.g., GTP + ADP $\rightarrow$ ATP + GDP, to power the proton pump in isolated tubulovesicles (unpublished observation).

In the skeletal and cardiac muscle literature a large body of evidence has demonstrated that distinctive isoenzymes of creatine kinase (36) and adenylate kinase (40) operate at sites of ATP utilization in addition to those mitochondrial isoenzyme forms that function within sites of ATP generation. It has been proposed that these enzymes function to transfer or shuttle high-energy phosphoryl groups from sites of production to sites of energy consumption, serving as spatial and temporal energy buffers in cells that consume large amounts of ATP (3). Moreover, functional interactions between creatine kinase and adenylate kinase systems have been suggested (9, 31). Apart from the studies on muscle, a functional coupling has been shown to occur between the creatine kinase system and various P-type ATPases, including Na$^+\cdot$K$^+\cdot$ATPase (5), Ca$^{2+}\cdot$ATPase (20), and H$^+\cdot$K$^+\cdot$ATPase (32). The present results, as well as those of Sistermans et al. (32), show that several of the enzymes associated with nucleotide and phosphate metabolism are present at membrane sites in the periphery of parietal cells, at or near the site of ATP utilization. Because the parietal cell has a regulated secretory cycle with high-energy demand, it is reasonable to propose that creatine kinase and adenylate kinase in the vicinity of energy consumption may help to buffer the ATP level and ATP-to-ADP ratio under differing functional secretory states.

We suggest at least one additional function for high activity adenylate kinase, which is to prevent a depletion of the cellular nucleotide pool. Accumulation of AMP leads to deamination of AMP and degradation to hypoxanthine derivatives (19), with a potential loss to the adenine nucleotide pool. It is clear from this and earlier studies that parietal cells maintain relatively high activity phosphodiesterase, even in a vegetative state, e.g., inhibitors of phosphodiesterase produce 50–100% activation of parietal cells (1, 33). Thus the regulated flow of nucleotide from ATP $\rightarrow$ cAMP $\rightarrow$ AMP may be considerable and represents a potential loss from the ATP/ADP energy pool, in addition to other leakage pathways that produce AMP. Results here show that parietal cell adenylate kinase can effectively recycle AMP to the ATP/ADP pool. The equilibrium constant for the adenylate kinase reaction is close to 1.0 (34) and therefore as cellular metabolism normally maintains a high ATP-to-ADP ratio, AMP will be driven toward ATP (AMP + ATP $\rightarrow$ 2ADP and ADP + P$_i$ $\rightarrow$ ATP). When cells are maintained in an anaerobic or metabolically stressful environment, there can be a serious depletion of the nucleotides, and in many tissues such treatment can appear irreversible as the resynthesis of adenine nucleotides from IMP and hypoxanthine derivatives requires large amounts of energy expenditure (22).

Data presented in this study provide a comprehensive view of nucleotide metabolism in the parietal cell that is schematically represented in Fig. 9. The pool of ATP is modulated by balancing the productive pathways (principally mitochondrial oxidative phosphorylation) and the major (H$^+\cdot$K$^+\cdot$ATPase) and minor (protein phosphorylation) consumptive pathways. The high-energy demand of acid secretion is met by a steady flow of oxygen and substrates through the large mitochondrial pool. Extramitochondrial sources of creatine kinase and adenylate kinase help to buffer ATP level and the ATP-to-ADP ratio throughout the cytoplasm. ATP also serves an essential role in the process of activation, both in the production of the intracellular message and as a substrate for protein phosphorylation. Adenylate cyclase and phosphodiesterase modulate the level of cAMP, hence cell activation, through receptor-regulated pathways. The resulting AMP is recycled back into the high-energy nucleotide pool via adenylate kinase.

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REFERENCES


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