Nucleotides metabolism by gastric glands and H⁺-K⁺-ATPase-enriched membranes

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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), forskolin, or histamine, operating through a Gs-coupled H₂ receptor, plus phosphodiesterase inhibitors (6, 7). Earlier studies from our laboratory demonstrated the utility of the α-toxin-permeabilized gastric gland model to evaluate second messenger pathways in parietal cell secretion (35, 38), and these have essentially been confirmed by the recent report of Miller and Hersey (24). When bathed in high K⁺ buffer containing a supply of oxidizable substrates, the α-toxin-permeabilized glands were responsive to the addition of 0.1 mM cAMP, and this was potentiated by a background of 0.1 mM ATP, the latter presumably as a supporting energy substrate. Our studies also showed that α-toxin-permeabilized glands responded to ATP alone at concentrations of 0.1 mM or higher. We interpreted these data to generally support the cAMP-PKA pathway of parietal cell activation. In the absence of cAMP, when ATP concentration was sufficient, we presumed that endogenous adenylate cyclase activity generated the cAMP required for the activation. Thus we were surprised by our more recent observation that cAMP activation of α-toxin-permeabilized gastric glands was blocked by the addition of phosphodiesterase inhibitors, such as 3-isobutyl-1-methylxanthine (IBMX). The present experiments were undertaken to more fully evaluate cAMP involvement in parietal cell activation and provide a more complete picture of nucleotide metabolism in the system.

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 MgSO₄, 1 NaH₂PO₄, 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-ATPase-Enriched Subfraction of Microsomes

H-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, 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-N,N’-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-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-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

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<th>Secretagogue</th>
<th>Without IBMX</th>
<th>With 50 µM IBMX</th>
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<tr>
<td>0.1 mM cimetidine</td>
<td>11.1 ± 2.0 (7)</td>
<td>15.8 ± 6.6 (4)</td>
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<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>
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<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>
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<tr>
<td>1.0 mM ADP</td>
<td>81.0 ± 21.7 (2)</td>
<td>77.2 ± 13.4 (2)</td>
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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.

P_i is essential for nucleotide exchange. To further address nucleotide interchange, we investigated the effect of P_i on AP accumulation. In P_i-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 P_i-free buffer. ATP, sufficiently rapid to keep up with the demand set by proton transport, as well as a source of phosphate for nucleotide exchange and recycling, 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.

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 AMP via ADP phosphohydrolase reactions, which we generically indicate as ADP ⇌ AMP + P_i. 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 diphosphate 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 microsomes.

![Figure 4](image-url)  
**Fig. 4.** Influence of P_i on AP accumulation supported by cAMP or ATP in α-toxin-permeabilized gastric glands. Glands were isolated, permeabilized with α-toxin, and incubated in K buffer containing oxidative substrates, with or without P_i. Medium was also supplemented with nucleotides (in mM) as indicated. Values are means ± SE, n = 3.

![Figure 5](image-url)  
**Fig. 5.** Nucleotide metabolism and interchange in α-toxin-permeabilized gastric glands. Permeabilized glands were incubated in K buffer in absence of oxidative substrates and with indicated concentrations of nucleotides or phosphocreatine (PCr), as well as 0.5 µCi [14C]ADP. After 5 min incubation at 37°C reaction was stopped by 0.6 N PCA. Medium was also supplemented with 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 diphosphate exchange reaction, NTP + ADP ⇌ ATP + NDP (26, 29).
somalous membranes that are rich in $\text{H}^+\cdot\text{K}^+\cdot\text{ATPase}$. In many of the subsequent experiments a potent inhibitor of $\text{H}^+\cdot\text{K}^+\cdot\text{ATPase}$, 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 AMP and ATP. 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 14C-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, P1, P5-Di(adenosine-5')pentaphosphate (Ap5A; 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 Ap5A is included in the medium.

Table 2 provides summary data for adenylate kinase activity measured on several 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 Ap5A (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 Ap5A (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$^+\cdot$K$^+\cdot$ATPase (26), analogous to what has been seen for the Na$^+\cdot$K$^+\cdot$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 14C-labeled nucleotide from ADP to

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<th>Table 2. Rates of nucleotide reaction and interaction for gastric microsomal vesicles</th>
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<tr>
<td>Adenylate kinase</td>
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<tr>
<td>Creatine kinase</td>
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<td>NTP/ADP exchange</td>
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<td>ATP</td>
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<td>CTP</td>
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<td>GTP</td>
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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 [14C]ATP production from [14C]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$^+\cdot$K$^+\cdot$ATPase activity; the decline in [14C]ADP was comparable to the summed appearance of [14C]ATP and [14C]AMP. For creatine kinase reaction, conditions included 0.1 mM ADP, 1.25 mM creatine phosphate, 10 µM Sch-28080, and 0.2 mM Ap5A to inhibit adenylate kinase activity. For nucleotide exchange reactions, incubated nucleoside triphosphate (NTP) was 1.25 mM, with 0.1 mM ADP and 10 µM Sch-28080 and 0.2 mM Ap5A to inhibit H$^+\cdot$K$^+\cdot$ATPase and adenylate kinase activities, respectively.

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![Fig. 6. Adenylate kinase activity is expressed in H$^+\cdot$K$^+\cdot$ATPase-enriched gastric microsomes. Gastric microsomes were incubated in 0.2 ml buffer containing 0.1 mM ADP, 0.2 µCi [14C]ADP, and 10 µM Sch-28080 as an inhibitor of H$^+\cdot$K$^+\cdot$ATPase activity. Samples were taken at times indicated for nucleotide extraction and analysis by TLC, similar to Fig. 5. Radioactivity was measured in spots corresponding to ATP (●), ADP (●), and AMP (▲). A: in absence of adenylate kinase inhibitor P1, P5-Di(adenosine-5')pentaphosphate (Ap5A). B: in presence of 200 µM Ap5A.](http://ajpgi.physiology.org/)

![Fig. 7. Creatine kinase activity is expressed in H$^+\cdot$K$^+\cdot$ATPase-enriched gastric microsomes. Gastric microsomes were incubated in 0.2 ml buffer containing 1.25 mM PCr, 0.1 mM ADP, 0.2 µCi [14C]ADP, 10 µM Sch-28080 as an inhibitor of H$^+\cdot$K$^+\cdot$ATPase activity and in absence (A) and presence (B) of 200 µM adenylate kinase inhibitor Ap5A. Samples were taken at times indicated for nucleotide extraction and analysis by TLC. Radioactivity was measured in spots corresponding to ATP (●), ADP (●), and AMP (▲).](http://ajpgi.physiology.org/)
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 adenylyl 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 adenylyl 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., Ca²⁺) may.

ATP, i.e., ATP + [¹⁴C]ADP → [¹⁴C]ATP + ADP. Figure 8B shows the CTP/ADP exchange reaction in gastric microsomes, i.e., CTP + [¹⁴C]ADP → [¹⁴C]ATP + CDP. It is important to account for, or inhibit, adenylyl kinase activity for an accurate measurement of nucleotide exchange rate. For the experiments shown here we included 0.2 mM Ap5A as an inhibitor of adenylyl kinase, and it is clear that rather little [¹⁴C]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 [¹⁴C]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⁺-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 activity, catalyzing the transfer of γ-phosphate from a variety of nucleotides to ADP. The specificity of the 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).

**DISCUSSION**

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).

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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^+·K^-ATPase-enriched microsomes derived therefrom. Thus the adenylate kinase reaction, AMP + ATP ↔ 2ADP, coupled with functioning mitochondria, i.e., ADP + P_i → 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 + ATP ↔ PCr + ADP). Sistermans et al. (32) demonstrated the colocalization of creatine kinase in isolated gastric tubulovesicles, and that PCr + ADP were capable of supporting proton transport by the pump in those vesicles. Our experiments show that NTP/NDP exchange colocalizes with H^+·K^-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 → 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^+·K^-ATPase (5), Ca^2+·ATPase (20), and H^+·K^-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 → cAMP → 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 ↔ 2ADP and ADP + P_i → 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^+·K^-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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G110 NUCLEOTIDE METABOLISM IN GASTRIC PARIETAL CELLS


