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ACTIVE TRANSPORT AND ATP IN FROG GASTRIC MUCOSA

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SUMMARY

Measurement of adenine nucleotides in isolated frog gastric mucosae showed that ADP levels were elevated by stimulating acid secretion in resting preparations. The ratio, ATP/ADP, was thereby decreased upon stimulation. These changes could be prevented by blocking acid secretion, using Cl-free solutions to bathe the mucosa. Treatment of histamine-stimulated mucosae with ouabain, K-free solutions or acetazolamide significantly depressed acid secretion and increased ATP/ADP. These results are consistent with the hypothesis that ATP is the immediate substrate for acid secretion and they suggest that secretagogues act by increasing ATP utilization.

INTRODUCTION

ATP has been shown to be the immediate energy donor in a number of biological processes. Its role can be verified by direct addition, for example in glycerinated muscle fibers, or by injection in large cells such as the squid giant axon. The analogous procedure of exposing epithelial tissue to exogenous ATP would require that this anion readily penetrate the intact, functioning cell. Such is not the case in gastric mucosa [1].

The presence of the well-defined $(Na^+ + K^+)$-activated ATPase in epithelia responsible for active transport of Na^+ and K^+ is in itself strong evidence for the involvement of ATP. In stomach, a transport ATPase has not been conclusively identified, but the contribution of the $(Na^+ + K^+)$-dependent ATPase in the gastric mucosa from several species is relatively minor (cf. ref. 2). For this and other reasons the role of ATP in acid secretion by stomach is controversial [3]. Various proponents [4, 5] of the redox theory of acid secretion have suggested that the H^+ component arises directly from electron flow associated with respiration, thereby eliminating any dependence on ATP or other high energy phosphate compound.

In the present study we have measured levels of endogenous ATP, ADP, creatine phosphate and creatine in isolated bullfrog gastric mucosa under various secretory conditions. The underlying assumption was that if ATP is the intermediate in acid secretion, the mucosal level of ATP or ADP (or both) should vary in some consistent way with stimulation and inhibition of acid secretion.

METHODS

The stomach was removed from a double-pithed bullfrog (*Rana catesbeiana*) and the gastric mucosa freed of outer smooth muscle. The mucosa was bisected longitudinally, and the paired segments mounted in two separate sets of plastic chambers, using Parafilm gaskets [6]. The nutrient (serosal) solution contained, in mM: 89.4 NaCl, 18 $NaHCO_3$, 4 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$ and 1.0 NaH_2PO_4; the secretory (mucosal) solution was 120 mM NaCl. The nutrient solution also contained 11 mM glucose, except as indicated. Nutrient and secretory solutions were oxygenated with O_2–CO_2 (95 : 5, v/v) and 100 % O_2, respectively, and contained penicillin (143 units/ml) and streptomycin (0.1 mg/ml). Cl-free solutions were prepared by the use of 89.4 mM sodium isethionate, 2 mM K_2SO_4 and 1.8 mM $CaSO_4$ for the corresponding Cl^- salts in the nutrient solution; 120 mM sodium isethionate was substituted for the secretory solution. The rate of H^+ secretion was followed by maintaining the secretory solution at pH 7.7 with an automatic buret and pHstat (Radiometer ABU 11 and TTT 1c). Nutrient and secretory solutions were renewed hourly, and the mucosa kept electrically short-circuited throughout the experiment [7].

The experiments to be reported fall into two classes. The first kind was performed on resting mucosae obtained by the procedure of Kasbekar [8] as subsequently modified [9]. After both secretory rates of the pair dropped below 0.5 μEq H^+/cm^2 per h, one of each pair was stimulated by changing its nutrient solution to one containing 10^{-4} M histamine and 10^{-2} M theophylline; the other was subjected to a change of nutrient without secretagogues and kept as a resting control. One hour after, both halves were rapidly frozen in liquid Freon (cooled with liquid N_2) for later analysis by fluorometric, enzymatic techniques for ATP, ADP, creatine phosphate and creatine [6, 10].

In some of the experiments with resting mucosae, secretagogues were added while the mucosa was exposed to Cl-free or 10 % Cl solutions. In both cases, normal Cl solutions were replaced 2–3 h after mounting by Cl-free nutrient and secretory solutions. The mucosa was bathed in Cl-free solutions for 2 h, with frequent changes of solution, to reduce mucosal Cl; at this time, 10 % Cl solutions (made by mixing Cl and Cl-free solutions 1 : 9, v/v) were introduced if desired. One paired half was stimulated with histamine and theophylline for 1 h as above, and the other half used as control.

The second class of experiments used mucosae stimulated from the time of mounting with 10^{-4} M histamine in the nutrient solution. After 1–2 h to allow stabilization of electrical and secretory activities, one half was treated with an inhibitor and the other kept as a secreting control. Inhibition was prolonged until the secretory rate (in the case of acetazolamide, the short-circuit current) dropped to less than half of the corresponding parameter for the control mucosa. Both halves were then frozen for later analysis as discussed above.

The *t*-test for paired differences between control and experimental halves was used as a test of significance. $P > 0.05$ was considered not significant (N.S.).

RESULTS

Maximal stimulation of resting mucosae with histamine and theophylline gave

TABLE I

EFFECTS OF SECRETAGOGUES

Paired mucosae were incubated without exogenous secretagogue until both secretory rates dropped to a resting level (cf. Methods). One of each pair was then stimulated with 10^{-2} M theophylline + 10^{-4} M histamine for 1 h. In (A) ($n = 11$), normal Cl^- solutions were used; in (B) ($n = 9$), zero Cl^-; in (C) ($n = 7$), 10% normal Cl^-. Means ±S.E. are given. N.S., not significant. *SR*, secretory rate and I_{sc}, short-circuit current, both measured just prior to freezing mucosae.

	SR (μEq/cm^2 per h)	I_{sc}	ATP (nmoles/g)	ADP (nmoles/g)	ATP/ADP	Creatine phosphate (nmoles/g)	Creatine (nmoles/g)	Creatine phosphate/ creatine	*K'*
A. Resting	0.4±0.1	2.3±0.3	1115± 87	167±15	6.88±0.52	880± 66	806±99	1.21±0.12	0.18±0.02
Stimulated	3.7±0.2	2.5±0.2	1032± 89	211±18	5.00±0.32	1065±101	657±69	1.75±0.22	0.35±0.03
P	< 0.001	N.S.	N.S.	< 0.005	< 0.005	< 0.05	< 0.02	< 0.02	< 0.001
B. Resting	0 ±0	−0.1±0.1	942± 89	160±17	6.06±0.28	866± 71	394±36	2.30±0.19	0.38±0.03
Stimulated	0.2±0.1	−0.5±0.2	861± 51	147±13	6.04±0.34	802± 87	462±53	1.86±0.24	0.31±0.04
P	N.S.	< 0.005	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	< 0.02
C. Resting	0 ±0	1.1±0.1	1126±139	169±20	6.68±0.24	969± 56	564±71	1.85±0.21	0.28±0.04
Stimulated	2.5±0.4	−0.1±0.1	1079±105	213±17	5.06±0.21	1107± 75	554±45	2.04±0.16	0.41±0.04
P	< 0.001	< 0.005	N.S.	< 0.02	< 0.001	N.S.	N.S.	N.S.	< 0.001

the results shown in Table IA. Acid secretion increased by nearly 10-fold, without significant change in short-circuit current. As Kasbekar [8] found previously, stimulation did not affect the mucosal ATP level. That of ADP was significantly increased by stimulation, however, resulting in a significant drop in the ATP/ADP ratio.

These events were accompanied by an additional phosphorylation of creatine, so that mucosal creatine phosphate (CrP) increased at the expense of creatine (Cr) and the ratio, CrP/Cr, increased. All these changes were significant. The results for the two ratios can be combined by calculating their ratio, K':

$$K' = \frac{\text{CrP/Cr}}{\text{ATP/ADP}} \tag{1}$$

K' is formally equivalent to the apparent equilibrium constant defined previously [11] for the creatine kinase reaction:

$$\text{ATP} + \text{Cr} \rightleftharpoons \text{ADP} + \text{CrP} \tag{2}$$

Since the present measurements are not performed at equilibrium in a single homogenous compartment, K' as calculated here is only an empirical ratio. Since stimulation increased the numerator and decreased the denominator in Eqn 1, K' nearly doubled in value (Table IA). Possible interpretations of changes in K' will be considered later in conjunction with the experiments using acetazolamide.

If secretagogues produce the increase in mucosal ADP level and other changes due to stimulation of active transport, they should exert negligible effects under conditions where transport cannot take place. In bullfrog gastric mucosa, the latter can be conveniently accomplished by replacing Cl^- of the bathing solutions with isethionate [12] or glucuronate [7].

Paired mucosae were bathed with Cl-free solutions for at least 2 h, as described in the Methods. One of each pair was then stimulated with histamine and theophylline, and the other used as a control, with the results shown in Table IB. The first two columns here, in comparison with those of Table IA, confirm the expectation that Cl^- removal would block effectively the normal secretory and electrical activities. The small, reversed short-circuit current induced by the secretagogues is probably associated with a minimal transport of H^+ without accompanying anion [13]. Under these conditions, the secretagogues failed to elevate the mucosal level of ADP, or to produce any other significant change in high energy intermediates. K' was decreased, rather than increased (Table IA) by stimulation; the reason for this is not clear.

To verify that the inhibition of acid secretion and other effects of Cl-free solutions were not due to the presence of isethionate or SO_4^{2-}, but to the absence of Cl^-, we stimulated another set of mucosae bathed in 10% Cl solutions (cf. Methods). Mucosae bathed with Cl^- levels in this range cannot maintain both a normal acid secretion and short-circuit current [14]. This is exemplified in the first two columns of Table IC, where secretagogues increased acid secretion at the expense of short-circuit current. It is likely that these two forms of active transport compete for intracellular Cl^-, limited in supply due to the reduced exogenous level. Stimulation of acid secretion was accompanied by an enhanced rate of ATP breakdown, as judged by the increase in ADP and decrease in ATP/ADP. These changes, and the increase in K', closely parallel those seen in normal Cl^- solutions.

The experiments reported thus far utilized nutrient solutions containing 11 mM glucose. Addition of various fatty acids and ketones, including β-hydroxybutyrate, to this solution has been reported to stimulate acid secretion by the isolated mucosa [15]. While the latter experiments were not performed with a resting mucosal preparation, they raise the question whether the primary action of secretagogues could be to increase substrate availability at the site of active transport. Since this did not appear to be consistent with the drop in ATP/ADP with stimulation as found in the present study, we initiated some experiments to explore further the role of substrate.

Two pairs of mucosae were mounted at the same time, in an attempt to reduce variability due to the nutritional status of the bullfrog. The nutrient solution for one pair contained no added substrate; in the other, it included 11 mM glucose and 10 mM β-hydroxybutyrate. After each pair attained resting levels of acid secretion, as defined above, one of each pair was stimulated with histamine and theophylline and the other kept as resting control. These experiments were terminated with four pairs in either group, since the results accumulated to that point showed little difference from the results quoted for glucose in the nutrient solution (Table IA). For example, in the absence of exogenous substrate, ATP/ADP dropped from 6.3 to 3.6 upon stimulation, and with both substrates present, from 7.0 to 4.8. The effects of stimulation on acid secretion in the two groups were not significantly different. These results indicate that the isolated gastric mucosa has sufficient endogenous substrate to perform adequately through the experiment.

The most important aspect of the substrate experiments was the fact that addition of β-hydroxybutyrate and glucose to the nutrient solution did not keep the mucosa from reaching a resting level of acid secretion. The range of times for the latter to occur was 4–12 h, indistinguishable from the times required in the absence of exogenous substrate.

We saw above that Cl^- removal could block the effects of stimulation, and this observation prompted us to investigate other modes of inhibition. For these experiments, each paired half was stimulated with 10^{-4} M histamine from the outset of the experiment: later, one half was subjected to inhibition and the other kept as secreting control.

The effects of 10^{-4} M ouabain and those of K-free solutions were to some extent comparable, as shown in Table IIA and IIB. Both kinds of inhibition significantly reduced acid secretion and short-circuit current, and significantly elevated ATP/ADP in comparison to the normally secreting control. Neither had much effect on the creatine phosphate : creatine couple.

Ouabain differed from K-free treatment, however, in its effect on short-circuit current (Table II). This difference became more obvious when the time course of inhibition was examined. After ouabain had been present for 1 h, short-circuit current was abolished or reversed, while acid secretion was reduced by less than half. K-free solutions, on the other hand, inhibited acid secretion and short-circuit current gradually and to about the same extent.

The effects of fluoride (10 mM) and thiocyanate (10 mM) were different from those described so far, and the two inhibitors have been somewhat arbitrarily grouped in Table IIIA and IIIB. Both strongly inhibited acid secretion, and F^- inhibited short-circuit current as well. Neither had any significant effect on the ATP : ADP couple. This result is in agreement with previous findings in the case of SCN^- (ref. 16). The

TABLE II

EFFECTS OF OUABAIN AND K-FREE SOLUTIONS

Paired mucosae were stimulated from the beginning with 10^{-4} M histamine. One of each pair was either treated with 10^{-4} M ouabain for 2–5 h, or bathed with K-free solutions for 1.5–4 h. Means ±S.E.; $n = 8$ for ouabain and $n = 6$ for K-free solutions. –, secretory rate; I_{sc}, short-circuit current.

	SR (μEq/cm^2 per h)	I_{sc}	ATP (nmoles/g)	ADP (nmoles/g)	ATP/ADP	Creatine phosphate (nmoles/g)	Creatine (nmoles/g)	Creatine phosphate/ creatine	*K'*
A. Control	2.8±0.3	2.2±0.1	884± 57	213±13	4.22±0.26	1100± 85	737±105	1.67±0.19	0.39±0.04
Ouabain	1.0±0.3	−0.2±0.1	897± 63	172±12	5.30±0.35	1003± 91	536±86	2.19±0.37	0.44±0.11
P	< 0.001	< 0.001	N.S.	< 0.005	< 0.01	N.S.	< 0.05	N.S.	N.S.
B. Control	2.6±0.2	2.6±0.3	1101±102	214±32	5.41±0.52	1438±164	638±92	2.50±0.45	0.51±0.13
K-free	0.7±0.1	0.9±0.3	1304±135	173±23	7.89±0.92	1546±190	680±37	2.25±0.19	0.31±0.06
P	< 0.001	< 0.005	< 0.01	N.S.	< 0.05	N.S.	N.S.	N.S.	N.S.

TABLE III

EFFECTS OF FLUORIDE AND THIOCYANATE

Paired mucosae were stimulated from the beginning with 10^{-4} M histamine. One of each pair was treated with either 10 mM F^- or 10 mM SCN^- in the nutrient solution. Duration of treatment was 2–4 h for F^-, and 2 h for SCN^-. Means ±S.E.: $n = 8$ for F^- and $n = 7$ for SCN^-. *SR*, rate of H^+ secretion; I_{sc}, short-circuit current.

	SR (μEq/cm^2 per h)	I_{sc}	ATP (nmoles/g)	ADP (nmoles/g)	ATP/ADP	Creatine phosphate (nmoles/g)	Creatine (nmoles/g)	Creatine phosphate/ creatine	*K'*
A. Control	2.9±0.3	2.5±0.3	877±65	196±17	4.76±0.59	1268± 65	614±105	2.62±0.56	0.58±0.13
F^-	0.9±0.2	0.5±0.1	783±77	193±16	4.25±0.50	623±111	1028±163	0.67±0.11	0.16±0.03
P	< 0.001	< 0.001	N.S.	N.S.	N.S.	< 0.001	< 0.01	< 0.01	< 0.02
B. Control	2.7±0.2	2.2±0.2	1125±98	247±28	4.73±0.50	1198±105	691±105	1.96±0.31	0.42±0.06
SCN^-	0.6±0.1	2.6±0.2	1093±89	206±25	5.52±0.42	814±65	875±105	1.04±0.18	0.20±0.04
P	< 0.001	N.S.	N.S.	N.S.	N.S.	< 0.01	< 0.05	< 0.005	< 0.001

TABLE IV

EFFECTS OF ANOXIA

Paired mucosae were stimulated from the beginning with 10^{-4} M histamine. In one of each pair, 5 % CO_2–95 % N_2 was substituted for 5 % CO_2–95 % O_2 in the serosal solution, and N_2 for O_2 in the mucosal solution. Duration of anoxia was either 10 min or 20 min. Means ±S.E.; $n = 5$. *SR*, rate of H^+ secretion; I_{sc}, short-circuit current.

	SR (μEq/cm² per h)	I_{sc}	ATP (nmoles/g)	ADP (nmoles/g)	ATP/ADP	Creatine phosphate (nmoles/g)	Creatine (nmoles/g)	Creatine phosphate/ creatine	*K'*
Control	2.8±0.4	2.3±0.2	1088±114	227±23	4.92±0.62	1259±107	712±106	1.90±0.26	0.39±0.05
Anoxia	0.9±0.1	1.2±0.2	726±64	342±45	2.19±0.16	214±42	1650±261	0.13±0.02	0.06±0.01
P	< 0.02	< 0.005	< 0.01	N.S.	< 0.025	< 0.005	<0.05	< 0.005	< 0.005

TABLE V

EFFECTS OF ACETAZOLAMIDE

Paired mucosae were stimulated from the beginning with 10^{-4} M histamine. One of each pair was treated with 10 mM acetazolamide in the nutrient solution for 50 min. Means ±S.E.; $n = 7$; *SR*, rate of H^+ secretion; I_{sc}, short-circuit current.

	SR (μEq/cm² per h)	I_{sc}	ATP (nmoles/g)	ADP (nmoles/g)	ATP/ADP	Creatine phosphate (nmoles/g)	Creatine (nmoles/g)	Creatine phosphate/ creatine	*K'*
Control	2.9±0.3	2.0±0.1	1009±56	272±25	3.79±0.17	1176±47	536±82	2.50±0.34	0.66±0.09
Acetazolamide	2.2±0.1	0.8±0.1	1051±50	228±17	4.70±0.26	1162±115	648±63	1.87±0.20	0.41±0.05
P	< 0.05	< 0.001	N.S.	N.S.	< 0.01	N.S.	N.S.	N.S.	< 0.05

two inhibitors did affect the creatine phosphate : creatine couple, however, producing a similar and significant dephosphorylation.

A decrease in the level of creatine phosphate can signal the interruption of oxidative phosphorylation, as in anoxia [6, 10]. To reconfirm this for the conditions of the present study, we ran a short series of experiments in which mucosae stimulated by histamine were exposed to a brief period of anoxia (10 or 20 min). The results are given in Table IV. In the present context, anoxia constitutes a third class of inhibition, since both ATP : ADP and creatine phosphate : creatine couples were affected. The effect on the latter was more drastic, however, since creatine phosphate was reduced to 17 % of the control, and ATP to 67 % of the control. This result may be compared to the previous finding [16] that anoxia, prolonged enough to abolish acid secretion, reduced mucosal ATP by only a half. It appears that part of the ATP store is compartmentalized so that it is relatively inaccessible to the transport machinery.

The finding that stimulation under normal conditions changed ATP/ADP and creatine phosphate/creatine in opposite directions (Table IA) is puzzling, and demands further consideration. One possible explanation is that the increase in acid secretion led to an alkalinization of the oxyntic cell cytoplasm [17]. This would shift the equilibrium catalyzed by creatine kinase [18] towards the right:

$$MgATP^{2-} + Cr^{\pm} \rightleftharpoons MgADP^{-} + CrP^{2-} + H^{+} \quad (3)$$

In agreement with this reaction the apparent equilibrium constant, K', was found to increase with pH (ref. 11).

We sought to test the possibility that K' increased with stimulation due to an alkaline shift in the cytoplasm, by examining the effect of acetazolamide (Diamox). This drug is reported to increase intracellular pH in the isolated gastric mucosa [19].

Table V gives the results of treating histamine-stimulated mucosae with acetazolamide (10 mM). As previously reported [20], acetazolamide inhibited short-circuit current to a greater extent than acid secretion. This was accompanied by a significant increase in ATP/ADP, as if the drug had inhibited utilization of ATP. Contrary to what would have been expected if the cytoplasm had become alkaline, however, K' did not increase; instead it decreased significantly.

DISCUSSION

The principal result of the present study is the finding that stimulation of acid secretion increased the mucosal level of ADP. In turn this led to a decrease in the ATP/ADP ratio, since ATP levels were unaffected. A ratio such as ATP/ADP should provide a more reproducible index of metabolism than either numerator or denominator, since it is independent of errors in weight due to ice or mucus adhering to the frozen tissue.

The increased mucosal ADP in the secreting state may serve to accelerate oxidative phosphorylation by gastric mitochondria [21]. This could provide the mechanism whereby histamine increases O_2 consumption of gastric mucosa [22]. At the same time, histamine would promote increased utilization of ATP by the secretory mechanisms, through a link which is not presently understood.

In isolated mitochondria, an increase in ADP level in the suspending medium is associated with oxidation of certain members of the respiratory chain, provided

substrate is present [21]. In contrast, histamine has been reported to produce a general reduction of the respiratory chain components in the intact gastric mucosa [23]. Isolated mitochondria and gastric mucosa may not be directly comparable, however. For example, substrate may be relatively unavailable to the respiratory chain in the resting mucosa, and secretagogues may act to remedy this lack.

Substrate mobilization cannot be the sole or primary action of secretagogues, that being inconsistent with the decrease in ATP/ADP which these agents induce. Moreover we found that provision of abundant, utilizable substrate did not prevent the mucosa from reaching a resting state.

The results indicate that the initiation of acid secretion is associated with a transfer of phosphate from ATP to creatine, leading to an increase in creatine phosphate/creatine. It is most unlikely that creatine phosphate could serve as the immediate source of energy for active transport, however. It undoubtedly constitutes an energy store, analogous to its role in skeletal muscle. Accordingly the increase in creatine phosphate with stimulation of acid secretion reflects a transient flow of metabolic energy, minor in comparison with that represented by the steady-state respiration.

At least two explanations seem plausible at the moment to account for the simultaneous increase in creatine phosphate/creatine and decrease in ATP/ADP upon stimulation. From Eqn 3 (Results), a relatively large decrease in cytoplasmic acidity [17] could overcome the small increase in mucosal ADP to favor the phosphorylation of creatine. Our failure to observe such a shift in the case of acetazolamide treatment does not negate the possibility of its occurrence in the normal transition from the resting to the secreting state.

Alternatively, some degree of compartmentalization within the oxyntic cell could account for these results. For this to occur, ATP/ADP would have to reflect principally levels in the vicinitiy of ATP-utilizing mechanisms (ATPase), and creatine phosphate/creatine, levels near sites of ATP production (mitochondria). The hypothesis would seem to demand that oxyntic cell mitochondria be rich in creatine kinase, as was shown for mitochondria from muscle and brain [24].

The multiplicity of cell types in the isolated gastric mucosa requires that we examine carefully the assumption that the oxyntic cells are the main site of changes in ADP level and respiration. The arguments here are somewhat circumstantial. Oxyntic cells comprise 28 % by volume of frog gastric mucosa [25]. A primary index of oxyntic cells in the electron microscope is their abundance of mitochondria. These organelles occupy 43 % of the cytoplasmic space in adult rat oxyntic cells, but only 4.8 % in zymogen cells [26]. This figure is about 40 % in dog oxyntic cells, and 22 % in frog oxyntic cells [25]. The levels of dipyridine nucleotides and nicotinic acid were found to be much greater (by at least a factor of 3) in cat oxyntic cells than in cat mucous cells, smooth muscle cells or other gastric cells [27]. Blum et al. [28] reported that respiration per mg protein was significantly greater in isolated oxyntic cells of Necturus than in the intact mucosa. Especially pertinent is the finding that respiration of isolated bullfrog oxyntic cells is considerably enhanced by dibutyryl cyclic AMP, a substance which is a potent secretagogue in intact mucosa (Michelangeli, F., unpublished results).

Another indication that decreased ATP/ADP upon stimulation reflects the activity of oxyntic cells is the finding that it could be prevented or reversed by several

inhibitors of acid secretion. These included Cl-free solutions, ouabain, K-free solutions and acetazolamide.

Cl^- is, of course, necessary for the maintenance of normal acid secretion and electrical activity in bullfrog gastric mucosa [7, 29]. Reduced mucosal Cl^- is associated with significant acid secretion but zero or reversed short-circuit current (Table IC), and it is of interest that ouabain and acetazolamide also restrict short-circuit current more severely than acid secretion. This correspondence suggests the possibility that these two inhibitors act to reduce intracellular Cl^-. This could be due to interference with Cl^- entry at the Cl–HCO_3 exchange site on the serosal surface [30].

Ouabain is also known to reduce mucosal levels of K^+ (ref. 31), like K-free solutions [32, 33]. The role of K^+ in mucosal transport is unknown, but it might relate to the function of the K^+-stimulated ATPase described by Ganser and Forte [2].

While the above four modes of inhibition (Cl^--free and K^+-free solutions, ouabain and acetazolamide) reduce ATP utilization, as judged by the increase in ATP/ADP they produced, their effects on the creatine phosphate/creatine couple were varied. Cl^--free solutions had the most pronounced action, yielding a decrease in creatine phosphate/creatine as opposed to the increase seen in normal solutions with stimulation. The other three treatments did not affect creatine phosphate/creatine significantly, perhaps because inhibition of active transport was not sufficiently extensive.

In contrast, the remaining inhibitors, F^- and SCN^-, had well defined actions on the creatine phosphate/creatine couple. Both decreased acid secretion and creatine phosphate/creatine. Quantitatively their effects were different, however. The significant inhibition of short-circuit current and severe depression of creatine phosphate/creatine produced by F^- suggest that this agent interferes with ATP production (compare with anoxia, Table IV). F^- is a well known inhibitor of the glycolytic enzymes, phosphoglucomutase and enolase. SCN^-, on the other hand, appears to induce a quasi-resting state (compare Tables IIIB and IA). It should be kept in mind, however, that large concentrations of SCN^- inhibit oxidative phosphorylation by isolated mitochondria [34–36].

ATP utilization, as well as production, may be affected by F^- and SCN^-. F^- was found to inhibit the K-stimulated components of a gastric microsomal ATPase (Forte, J., private communication) and a *p*-nitrophenyl phosphatase [37], and SCN^- inhibited the basal level of the ATPase described by Ganser and Forte [2] as well as the microsomal ATPase purified by Sachs et al. [38]. Thus the lack of effect of F^- and SCN^- on the ATP/ADP ratio may represent a temporary balance between impaired supply and demand for ATP in the mucosa.

The present results with inhibitors as complex as F^- can offer only slight evidence for or against the hypothesis that ATP provides the immediate energy for acid secretion. Instead the approach might illuminate the action of the inhibitors. A definitive study of this kind will require the measurement of the relevant metabolic intermediates at various levels of inhibitor and stages of action.

It is fortunate that at least one of the modes of inhibition used here is (in principle) straightforward, that being treatment with Cl-free solutions. The latter effectively prevented the usual increase in ADP and decrease in ATP/ADP due to secretagogues. While such results do not unambiguously prove that ATP is the immediate energy donor for acid secretion, they conform well to this view. In addition

the direction of the change in ATP/ADP with stimulation should afford a valuable clue to the mechanism of secretagogue action.

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