Utilization of High-Energy Phosphate Compounds by Stomach

R. P. DURBIN

From the Cardiovascular Research Institute, University of California School of Medicine, San Francisco, California 94122

The frog gastric mucosa in vitro secretes HCl and maintains its electrical activity for many hours. Both processes depend on the active transport of Cl ion and are inhibited under appropriate conditions by SCN ion. It has been suggested that a membrane-bound ATPase from gastric mucosa is involved, since this ATPase is also inhibited by SCN (8). It furthermore is stimulated by physiological concentrations of HCO_3, an ion which potentiates acid production (6) and reverses inhibition of electrical activity by SCN (9).

Sachs, Mitch, and Hirschowitz confirmed the sensitivity of the gastric ATPase to SCN; they pointed out, however, that ATPase from rat liver mitochondria and brush border of hamster intestine were also inhibited by SCN (15). Furthermore, HCO_3 stimulates mitochondrial ATPase to about the same extent as gastric ATPase (14). The effects of SCN and HCO_3 are thus not specific for the gastric ATPase. The significance of this lack of specificity is not clear, however. It may indicate that the gastric ATPase is of mitochondrial origin, or instead that SCN and HCO_3 act on a variety of enzymes.

Of primary importance in evaluating an ATPase theory would be proof as to whether ATP is actually an intermediate in active transport by stomach. Forte, Adams, and Davies have answered this question in the affirmative, on the basis of the stoichiometry between acid produced and ATP consumed during prolonged anoxia (3). Their experiments are open to criticism, however, since they did not measure electrical activity or follow changes in tissue phosphocreatine. In the first part of the present studies, the effect of anoxia has been reexamined.

METHODS

For analysis of labile constituents, it is necessary to freeze the mucosa as rapidly as possible at a desired time. A simple way to use the conventional Ussing-type chamber for this purpose is shown in Fig. 1. After assembly, the Parafilm gaskets were clamped together along their circumference, forming a semirigid ring. Teflon was used to prevent sticking during disassembly. The unit could be removed, blotted between filter papers, and frozen in liquid freon in 6–10 sec. The disc of mucosa actually used in the experiment was cut out with fine scissors and weighed while still frozen. Subsequent procedures in the analyses for ATP and phosphocreatine (PC) were as described by Lowry et al. (12). The method for lactate was modified from that of Loomis (11).

To ensure that lactate measured arose from tissue glycogen, glucose was excluded from the

bathing solutions. After incubation, tissue glucose was found to be negligible. Control experiments showed that mucosae under substrate-free conditions, stimulated maximally by histamine, secreted well for 2–3 hr, and then deteriorated slowly. Consequently the time between dissection and initiation of anoxia was kept at roughly 100 min.

EFFECTS OF ANOXIA

The familiar dependence of transport activity in the stomach on aerobic metabolism is illustrated in Fig. 2. In this experiment, half the mucosa was mounted in one set of

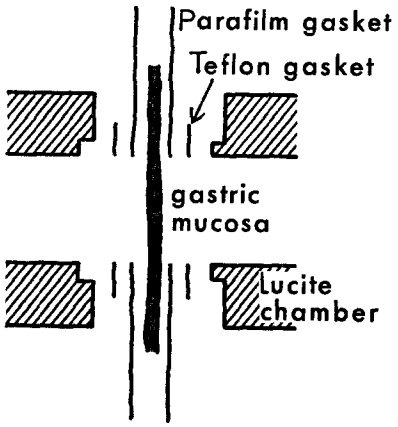

FIGURE 1. Cross-section of mucosal assembly. Teflon foil is 0.5 mil thick. Geometrical area of mucosa used is 2.8 cm².

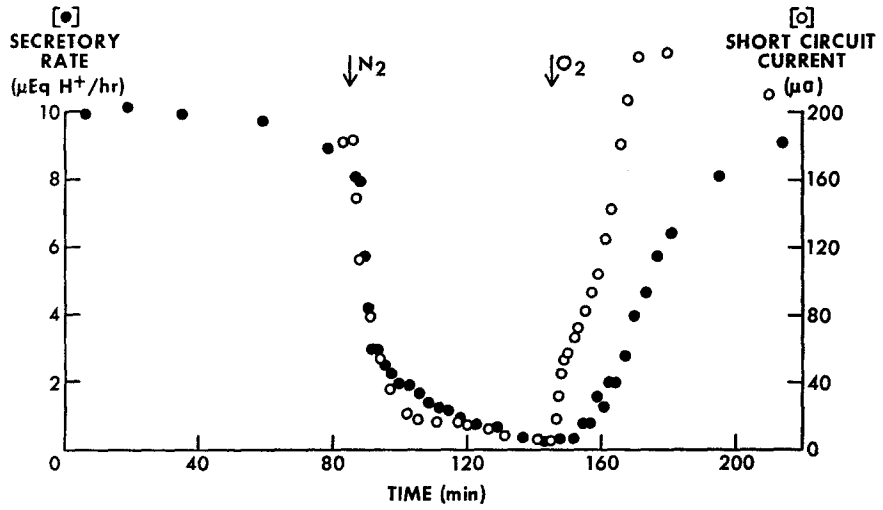

FIGURE 2. Onset and reversal of anoxia. Paired halves of gastric mucosa were initially monitored for acid production (not shown for one half); this was continued for one half, and the other was kept short-circuited from 80 min throughout the remainder of the experiment. Histamine (10^{-4} M) was present in all nutrient solutions. The latter were substrate free until O_2 was restored, at which time 11 mM glucose was added to facilitate recovery.

chambers and acid production was followed throughout; the other half was kept short-circuited in another set of chambers. Both were stimulated to secrete acid with 10^{-4} M histamine.

The sharp drop in acid production with the onset of anoxia was paralleled by a similar decrease in short circuit current. Upon restoration of oxygen in the bathing solutions, the short circuit current rapidly builds up, and this is followed a few minutes later by an increase in acid production toward its initial level.

At the onset of anoxia, solutions prebubbled with N_2 (secretory) or $N_2 + CO_2$ (nutrient) were instilled on the mucosa. Tests with an oxygen electrode showed that

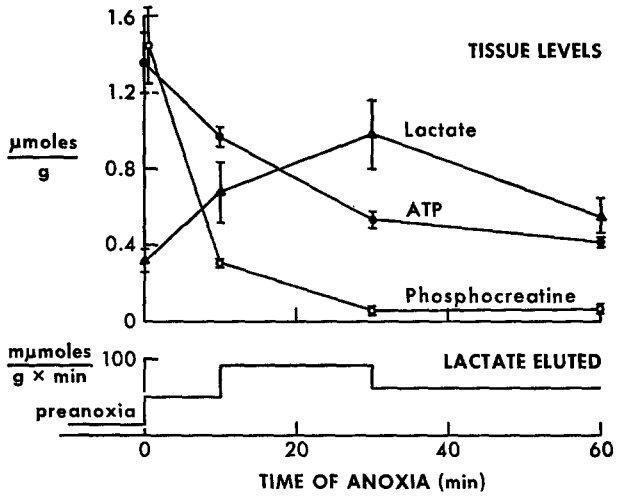

FIGURE 3. Tissue lactate, ATP, and phosphocreatine per gram wet weight, and lactate eluted into bathing solutions over the given intervals. Results ± standard error of means are given.

the chambers were free of O_2 within a few minutes. Transport does not then disappear, but dwindles slowly toward zero in the ensuing hour (Fig. 2). The observation that these residual levels are not constant indicates that they are not artifacts of measurement.

ROLE OF ANAEROBIC GLYCOLYSIS

In analyzing the decline after onset of anoxia, intervals of time were arbitrarily selected. Paired mucosae were monitored either for short circuit current or for acid production and frozen at 0, 10, 30, or 60 min of anoxia for later measurement of tissue ATP, PC, and lactate. These results, with those for lactate released into the bathing solutions, are given in Fig. 3.

The normal, aerobic value for tissue ATP (at 0 min) is comparable to that reported by Forte et al. (3). Normal phosphocreatine is of the same order of magnitude, much as Sharp and Leaf found in toad bladder (16).

Phosphocreatine levels drop sharply after onset of anoxia, and lactate production rises (Pasteur effect) as the tissue attempts to maintain a flow of metabolic energy.

These changes minimize but do not prevent the decay in ATP level. The pattern observed here, and the size of the Pasteur effect, are comparable to what Lowry et al. have reported for decapitated mouse brain (12).

Enough O_2 is dissolved initially in the mucosa to render the 0–10 min interval unsuitable for calculations. The data for 10–30 min and 30–60 min are summarized in Table I.

Short circuit current (i_{sc}) and acid produced during a given interval have been numerically integrated to give the respective columns in Table I. These have been added to yield total transport. Implicit here is the assumption that the metabolic requirement for production of a mole of HCl is the same as that for net transport of an equivalent of Cl ion as current. In justification, it should be noted that similar amounts of oxygen are consumed per mole of HCl or equivalent of Cl ion transported (4, 17).

TABLE I

ACTIVE TRANSPORT AND ~ P CONSUMED DURING ANOXIA

Period of anoxia	Transport			Lactate appearing in		
	Acid	i_{sc}	Sum	Solutions	Tissue	Sum
min		*mμEq*			*mμmoles*	
10–30 (6 exp.)	570	254	824	260	+50	310
30–60 (5 exp.)	247	147	394	305	−65	240
			10–30 min		30–60 min	
ATP from glycogen			465		360	
Decrease in tissue ~P			89		18	
			554		378	
Transport/~P consumed			1.48		1.04	

The lactate measured in the bathing solutions[1] has been corrected for changes in tissue lactate from Fig. 3 to obtain lactate production. The latter, multiplied by 3/2, yields ATP generated in the breakdown of tissue glycogen. Adding this figure to the decrements of ATP and PC for the particular interval gives the total expenditure of high-energy phosphate (~P) compounds.

As the ratios indicate, the transport and ~P consumption are of the same order of magnitude. Though neither is trivial with respect to the other, it must be emphasized that both are derived from rates which are much smaller than the normal, aerobic transport or energy flux. It is therefore not surprising that the final ratios (1.48 and 1.04) appear to reflect a considerable experimental error. A ratio of 1.3 ± 0.3 would probably describe this preliminary set of data.

Forte et al. obtained 1.46 for the ratio of acid produced to ~P consumed, 30–60 min after onset of anoxia (3). They did not measure concomitant short circuit current,

[1] The nutrient (serosal) solution contained 90–100% of the lactate eluted, in agreement with previous observation (3). The serosal surface of toad bladder also is much more permeable to lactate than is the mucosal surface (10).

and reference to Table I indicates that the ratio of total transport to ~P consumed in their experiments must have exceeded 2.

Much lower values for this ratio can be inferred from experiments under aerobic conditions. Simultaneous measurement of oxygen consumption, acid production, and short circuit current yield total transport per mole of O_2 of 3.2–3.8 (4, 17). Assuming 6 moles of ATP are produced per mole of O_2, we find the ratio of total transport to ATP consumed to be about 0.6.

The present ratios of 1.48 and 1.04 lie midway between the values of Forte et al. and those from aerobic experiments. Possibly during anoxia energy is diverted from else-

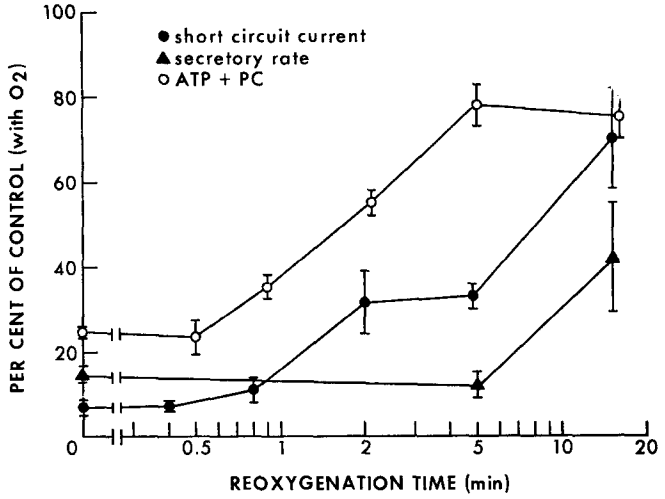

FIGURE 4. Terminal short circuit current or secretion rate upon reoxygenation, plotted with mucosal ATP + phosphocreatine (PC). The tissue was frozen about 0.1 min after transport had been measured, so that tissue levels and transport rates were not simultaneous. Increments in ATP and PC upon reoxygenation were roughly comparable, hence are not plotted separately. Results ± standard error of means are given.

where, e.g., smooth muscle, to transport. Alternatively, high values for the ratio may reflect a continued oxygen contamination, or O_2 which is bound and slowly dissociating. The latter seems unlikely, since oxygen bound to myoglobin or (vertebrate) hemoglobin dissociates with a half-time of a fraction of a second (see 18).

EFFECTS OF REOXYGENATION

The prompt rise in short circuit current seen in Fig. 2 would imply that the mucosa can regenerate its store of ATP at least as rapidly, if ATP is an intermediate. If it is not, the demand of electrical activity on metabolic energy might be expected to inhibit the restoration of normal ATP levels. Thus ion transport across mitochondrial membranes competes with phosphorylation of ADP for energy derived from substrate oxidation (for review see reference 13).

In reoxygenation experiments, glucose, as well as histamine, was included throughout the experiment for optimal recovery. Mucosae were incubated aerobically for

about 100 min, exposed to 30 min anoxia to lower ATP and PC, and finally reoxygenated for various times. After terminal measurement of short circuit current or acid production, the mucosae were rapidly frozen in the usual manner. The results are indicated in Fig. 4. The log scale for time has been chosen simply as a convenience.

The rapid increase in short circuit current is remarkably parallel to the restoration of ATP and PC, especially in the first 2 min of reoxygenation. Acid production, on the other hand, continues to decline during the first 5 min. The delay in its restoration is too long to be ascribed to diffusion of HCl from the site of production. The source of the delay is unknown.

The time sequences observed are consistent with the view that ATP is an intermediate in both kinds of transport. In particular, the electrical activity of the mucosa seems to reflect the tissue content of high-energy compounds. The state of the mucosa after a few minutes of reoxygenation is reminiscent of its normal, resting condition (7).

DISCUSSION

Evidence, other than that presented here, points toward ATP as an intermediate in active transport by stomach. For example, well-established inhibitors of ATP synthesis by mitochondria also inhibit acid production. Of these, uncoupling agents such as 2,4-dinitrophenol increase mucosal respiration, whereas oligomycin, which inhibits respiration linked to phosphorylation in mitochondria, reduces mucosal respiration (1). Both effects are therefore consistent with the conventional view of mitochondrial behavior. It should be pointed out, however, that Bannister has advanced a different explanation of these effects (1).

In an important paper, Forte, Forte, Gee, and Saltman have verified directly that mitochondria from rabbit stomach carry out conventional oxidative phosphorylation (5). Respiratory control for these mitochondria did not differ greatly from that for mitochondria from rabbit kidney cortex. Thiocyanate ion was relatively ineffective as an inhibitor, in agreement with the view that its action in blocking gastric acid production is on the utilization of ATP, not its synthesis (2).

The present experiments indicate that anaerobic glycolysis can supply energy via ATP for small, transient amounts of active transport by stomach. High-energy phosphate levels decrease with transport following the onset of anoxia, and increases in these levels accompany or precede the restoration of transport upon reoxygenation.

Though the experimental evidence does not support a simple redox system, in which electron flow to O_2 would be directly coupled to H ion production, it of course does not preclude redox mechanisms for active transport driven by ATP.

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