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BLOCK OF ACID SECRETION BY AMYTAL AND ITS PARTIAL REVERSAL BY MENADIONE WITH ASCORBATE IN THE GASTRIC MUCOSA OF THE GUINEA-PIG

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Summary

The effect of amytal on energy metabolism and acid secretion in an isolated gastric mucosa of the guinea-pig were studied. Determination of adenine nucleotides, creatine phosphate, pyruvate and lactate in the gastric mucosa showed that amytal depressed the levels of ATP, creatine phosphate and energy charge with elevation of the AMP and pyruvate levels. This treatment inhibited concomitantly acid secretion and active chloride transport detected by short circuit current. The addition of menadione with ascorbate to the medium in the presence of amytal partially restored ATP and energy charge levels and also induced a partial recovery of acid secretion and active chloride transport. These results suggest that ATP is a direct energy donor for acid secretion in the gastric mucosa of the guinea-pig.

Introduction

The secretion of HCl by the gastric mucosa of the frog is inhibited by uncouplers of oxidative phosphorylation such as dinitrophenol and by inhibitors of mitochondrial F_1-ATPase such as aurovertin [1]. These results suggest that acid secretion depends on ATP as energy source. Amytal, which is an inhibitor of mitochondrial respiratory chain at the coupling site I, has also been shown to inhibit acid secretion and oxygen consumption and to reduce ATP level in the frog gastric mucosa [2,3]. Menadione (vitamin K-3) that bypasses

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an amytal block in mitochondria [4] has been found to be active to restore oxygen consumption [5] and electron flow [3] when added in the presence of amytal. The addition of menadione with ascorbate restored ATP and creatine phosphate levels and induced a partial recovery of short circuit current, but did not reverse inhibition of acid secretion in the amytal-treated gastric mucosa of the bullfrog [6]. These results suggest that ATP is not a sufficient source of energy for acid secretion in the frog gastric mucosa.

This paper describes a partial restoration of ATP and concomitant recoveries of acid secretion and chloride active transport by the addition of menadione with ascorbate in the amytal-blocked gastric mucosa of the guinea pig.

Materials and Methods

Animals. Female guinea pigs weighing 350 g used in the experiment were anesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg). The stomach was removed after decapitation and immediately thereafter the outer smooth muscle layer was removed. The mucosa obtained was mounted in a lucite chamber. The whole procedure from the removal of stomach until mounting the mucosa took less than 5 min.

Media. The serosal bathing medium contained in mM: NaCl, 110; KCl, 5; $CaCl_2$, 3.6; $MgCl_2$, 1.2; $NaHCO_3$, 26; and glucose, 16.7, and was gassed with 95% O_2/5% CO_2. The mucosal bathing medium contained in mM: NaCl, 136; KCl, 5; $CaCl_2$, 3.6; $MgCl_2$, 1.2; and glucose, 16.7 and was gassed with 100% O_2. Amytal, menadione and ascorbate were added to serosal solution to reach a final concentration of 4.0, 0.5 and 5 mM, respectively. Amytal was dissolved in 100% ethanol and, therefore, to the control was added the same volume of 100% ethanol.

Assay of acid secretion. Acid secretion was measured by pH stats (TOA Electronics, Ltd., Tokyo) and the end point was set at pH 4.5.

Assay of potential difference and application of short circuit current. The transtissue potential difference was measured with a pair of matched calomel electrodes (TOA Electronics, Ltd., Tokyo). Short circuit current was applied via zinc-zinc acetate electrodes and agar-Ringer bridge from an external source [7]. During the short circuit experiment, the tissue was maintained at zero potential. Potential difference and short circuit current as well as acid secretion were recorded on a Polygraph (WATANABE Electronics, Ltd., Tokyo).

Assays of metabolites. After appropriate treatments as described above, the tissues were rapidly frozen in liquid nitrogen. The frozen tissue, approximately 0.3 g in wet weight, was transferred to a centrifuge tube containing 5 ml of 40% ethanol and 8% $HClO_4$, and homogenized with Polytron 20ST (Kinematica GmbH, Switzerland) at 0°C, followed by centrifugation for 10 min at 18 000 $\times g$ and −20°C. The supernatant was collected and the pellet was subjected to reextraction in the same procedure as the first extraction. The supernatants combined, approximately 10 ml, were neutralized with 2.3 ml of 3 M K_2CO_3 and 0.5 M triethanolamine at −20°C, and then centrifuged as described above. The clear supernatant was collected and adenine nucleotides [9,10], creatine phosphate [11], pyruvate [12] and lactate [13] were determined. The pellet obtained after reextraction was suspended in 1 N NaOH to

analyze protein concentration by the method of Lowry et al. [8].

Chemicals. Amytal, menadione (sodium bisulfate form) and ascorbate were purchased from Sigma Chemical Co. ATP, ADP, AMP, creatine phosphate, pyruvate, lactate, creatine kinase, hexokinase, glucose-6-phosphate dehydrogenase, myokinase, pyruvate kinase and lactate dehydrogenase were purchased from Boehringer Mannheim GmbH. Other chemicals used were of analytical grade.

Results

Effect of amytal on short circuit current and acid secretion

When short circuit current and acid secretion reached a steady state level of 6.49 ± 0.27 μequiv. · cm^{-2} · h^{-1} and 1.71 ± 0.08 μequiv. · cm^{-2} · h^{-1}, respectively, amytal was added to the serosal solution. This addition resulted in a rapid decrease of short circuit current by approximately 2.5 ± 0.05 μequiv. · cm^{-2} · h^{-1} in 30 min and then reached a stationary level of 1.23 ± 0.07 μequiv. · cm^{-2} · h^{-1} (Fig. 1). Acid secretion also decreased to 0.84 ± 0.03 μequiv. · cm^{-2} · h^{-1} in 30 min and was further reduced gradually (Fig. 2). The addition of thiocyanate inhibited the residual acid secretion, indicating that the acid secreted was HCl. When menadione and ascorbate were added to the serosal solution 90 min after amytal treatment, both short circuit current and acid secretion were restored immediately after the addition (Figs. 1 and 2). The level of short circuit current was partially recovered from 1.23 ± 0.07 to 2.87 ± 0.19 μequiv. · cm^{-2} · h^{-1}, and acid secretion from 0.66 ± 0.10 to 1.20 ± 0.13 μequiv. · cm^{-2} · h^{-1} in 30 min. The addition of menadione alone to the serosal solution did not induce restoration of both acid secretion and short circuit current. The addition of menadione and ascorbate in the absence of amytal as a control did not affect the level of short circuit current and acid secretion (data not shown).

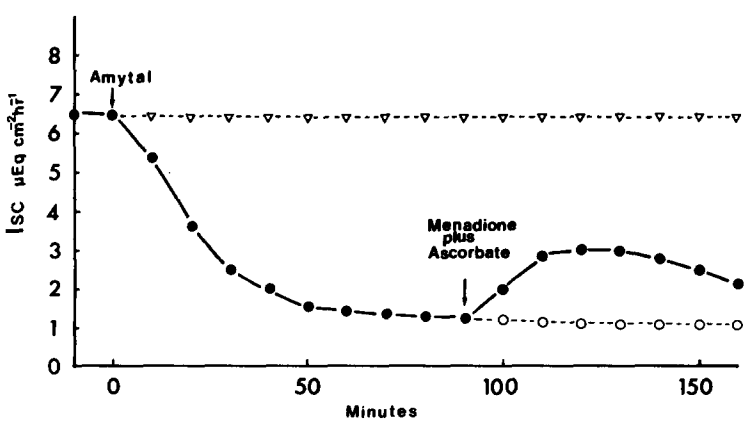

Fig. 1. Effect of the addition of menadione and ascorbate on short circuit current (i_{sc}) in the amytal-treated gastric mucosa: •———•, with 0.5 mM menadione and 5 mM ascorbate in the presence of amytal; o- - - - -o, with 4 mM amytal alone; ▽- - - - -▽, without amytal. Menadione and ascorbate were added to the serosal site 90 min after the addition of 4 mM amytal, when the decreased short circuit current reached a steady state.

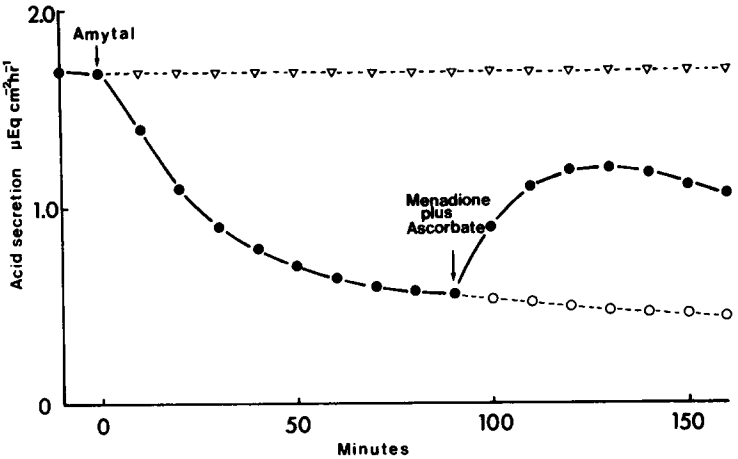

Fig. 2. Effect of the addition of menadione and ascorbate on the rate of acid secretion in the amytal-treated gastric mucosa. ●———●, with 0.5 mM menadione and 5 mM ascorbate in the presence of amytal; ○- - - - -○, with 4 mM amytal alone; ▽- - - - -▽, without amytal. Experimental conditions were identical to those described in the legend of Fig. 1.

When histamine was added at 50 μM to the serosal solution under the same condition as the addition of menadione and ascorbate, restoration of both short circuit current and acid secretion was not observed. Histamine added in the absence of amytal was found to cause a maximum stimulation in acid secretion in the isolated gastric mucosa of the guinea pig (data not shown).

TABLE I

EFFECT OF AMYTAL AND OF THE FOLLOWING ADDITION OF MENADIONE AND ASCORBATE ON METABOLITE CONCENTRATIONS IN THE GASTRIC MUCOSA

Values are expressed as the mean ± S.E.

	Control (n = 6)	Amytal [a] (n = 6)	Amytal + menadione + ascorbate [b] (n = 6)	P_1 [d]	P_2 [e]
ATP (nmol/mg protein)	13.80 ± 0.91	6.95 ± 1.05	10.78 ± 0.66	<0.005	<0.02
ADP (nmol/mg protein)	3.16 ± 0.83	3.30 ± 0.52	2.61 ± 0.13	n.s.	n.s.
AMP (nmol/mg protein)	2.33 ± 0.64	7.93 ± 1.13	4.95 ± 0.62	<0.005	<0.05
Creatine phosphate (nmol/mg protein)	5.99 ± 0.43	0.21 ± 0.06	0.93 ± 0.35	<0.001	n.s.
Total adenine nucleotides (nmol/mg protein)	19.29 ± 1.79	18.17 ± 1.12	18.34 ± 1.13		
ATP/ADP	5.78 ± 1.51	1.74 ± 0.11	4.17 ± 0.92	<0.01	<0.001
Energy charge [c]	0.80 ± 0.03	0.47 ± 0.05	0.66 ± 0.02	<0.001	<0.05
Pyruvate (nmol/mg protein)	2.04 ± 0.22	3.04 ± 0.30	2.36 ± 0.66	<0.05	n.s.
Lactate (nmol/mg protein)	67.12 ± 8.23	129.25 ± 21.99	79.21 ± 7.69	n.s.	n.s.

[a] Samples were taken 90 min after the addition of 4 mM amytal.

[b] Samples were taken 40 min after the addition of 0.5 mM menadione and 5 mM ascorbate following the amytal treatment for 90 min.

[c] Energy charge = (0.5[ADP] + [ATP])/([AMP] + [ADP] + [ATP]).

[d] P_1 refers to the t-test for differences between the control and amytal-treated mucosa.

[e] P_2 refers to the t-test for differences between the amytal-treated mucosa and that treated with menadione and ascorbate following the addition of amytal.

Levels of high energy compounds and metabolites

To investigate the relationship between acid secretion and cellular levels of metabolites, adenine nucleotides, creatine phosphate, pyruvate and lactate in the gastric mucosa were determined under conditions described in Methods. Levels of ATP, creatine phosphate, ratio of ATP to ADP, and energy charge [14] were reduced by the addition of amytal, while AMP and pyruvate were enhanced (Table I). After the addition of menadione and ascorbate followed by the amytal treatment, ATP, ratio of ATP to ADP, and energy charge were significantly enhanced and AMP decreased. Pyruvate and lactate were not significantly recovered under the same conditions (Table I).

The addition of menadione alone to the serosal solution did not induce restoration of ATP as well as changes in the levels of the other metabolites (data not shown).

Discussion

It remains unsolved whether or not the H^+ pump is energized by ATP or redox [15,16]. In order to investigate the role of ATP in acid secretion in the gastric mucosa of the guinea pig, short circuit current and acid secretion as well as levels of metabolites in energy metabolism were determined in the presence of amytal, an inhibitor of mitochondrial electron transport. ATP, creatine phosphate and energy charge were found to decrease with increase in AMP and pyruvate levels. Short circuit current and acid secretion in the amytal-blocked mucosa were also reduced with the decrease of ATP and creatine phosphate. The subsequent addition of menadione and ascorbate partially but significantly recovered ATP level, ATP/ADP ratio and energy charge. Menadione and ascorbate probably increase ATP levels by restoring oxido-reduction reactions in the mitochondrial respiratory chain beyond the amytal-blocked site.

The recovery of short circuit current and ATP by the addition of menadione and ascorbate shown in Fig. 1 and Table I is consistent with the results reported by Hersey using the frog gastric mucosa [6], but the restoration of acid secretion shown in the gastric mucosa of the guinea pig (Fig. 2) was not detected in the frog [6]. On the basis of his results Hersey postulated that ATP was not a sufficient energy source for acid secretion and suggested that oxido-reduction reactions in the region of the amytal sensitive site were important. Such conclusions are not consistent with our data from guinea pig mucosa.

The results presented in this paper suggest a possible role of ATP directly coupled to acid secretion in the guinea pig gastric mucosa. However, these results do not rule out a redox mechanism completely, an another possibility for acid secretion.

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