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EFFECTS OF GASTRIC ACID STIMULANTS AND INHIBITORS ON THE ACTIVITIES OF HCO_3^--STIMULATED, Mg^{2+}-DEPENDENT ATPase AND CARBONIC ANHYDRASE IN RAT GASTRIC MUCOSA

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SUMMARY

The effects of gastric acid stimulants or inhibitors, *in vivo* and *in vitro*, on the activities of HCO_3^--stimulated, Mg^{2+}-dependent ATPase ((HCO_3^-–Mg^{2+})-ATPase, ATP phosphohydrolase, EC 3.6.1.3) and carbonic anhydrase in rat gastric mucosa were investigated in order to elucidate the significance of and the functional relationship between these enzymes.

1. Subcutaneous treatment with carbachol (25–400 μg/kg) produced gastric juice secretion in 3-h pylorus-ligated rats. This drug also increased the Mg^{2+}-dependent ATPase (Mg^{2+}-ATPase) and carbonic anhydrase activities of the homogenate of rat gastric mucosa.

2. Subcutaneous treatment with gastric acid stimulants, *i.e.* carbachol, tetragastrin and histamine, stimulated gastric juice secretion in 3-h pylorus-ligated rats. These reagents also increased the Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial fraction of rat gastric mucosa.

3. Pretreatment with atropine (5 mg/kg, subcutaneously) or acetazolamide (20 mg/kg, subcutaneously) prevented the carbachol-induced increase of Mg-ATPase and carbonic anhydrase activities in the mitochondrial fraction.

4. *In vitro* effects of gastric acid stimulants and inhibitors: Incubation of Mg^{2+}-ATPase or (HCO_3^-–Mg^{2+})-ATPase with histamine (10^{-3} M), carbachol (10^{-3} M) or tetragastrin (10^{-5} M) had no effect on the activity of the enzyme. These reagents did not stimulate the enzyme activity, directly. Thiocyanate (10^{-2} M) inhibited the activity of the enzyme by about 30–40%. Ouabain (10^{-2} M) had no effect on the activity.

From these results, it was obvious that Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial fraction of the gastric mucosa correlated with gastric acid secretion. It is likely that carbonic anhydrase is functionally linked to Mg^{2+}-ATPase in rat gastric mucosa.

INTRODUCTION

There are many reports suggesting that the ATPase in frog and mammalian gastric mucosal cells has an important role in gastric acid secretion[1–4]. Recently Sachs *et al.*[5,6] found HCO_3^--stimulated ATPase activity in the Mg^{2+}-dependent

ATPase of mammalian gastric mucosal cells. This finding aroused our interest in HCO_3^- and carbonic anhydrase activity in gastric mucosa.

The experiments reported here were set up to elucidate the relationship of gastric acid secretion and ATPase and carbonic anhydrase activities in rat gastric mucosal cells by using gastric acid stimulants and inhibitors; data are presented which show that gastric acid secretion correlates with Mg^{2+}-dependent ATPase (Mg^{2+}-ATPase) and carbonic anhydrase activities in mitochondrial fraction of the gastric mucosal cells.

METHODS

Procedure for in vivo measurements

Sprague–Dawley male rats (9–10 weeks old) were used. The rats were fasted for about 18 h and the pylorus was ligated under ether anesthesia. Immediately thereafter, carbachol (25–400 μg/kg), tetragastrin (50–400 μg/kg) or histamine (0.5–8 mg/kg) was injected subcutaneously. 3 h after the operation, the animals were sacrificed for the determination of gastric acid secretion and the activities of Mg^{2+}-ATPase and carbonic anhydrase in rat gastric mucosa.

Scrapings of gastric mucosa of the corpus were homogenized in 5 ml of 0.25 M sucrose solution (containing 0.25 mM EDTA adjusted to pH 7.4 with Tris) at 0 °C using a Waring blender and a teflon homogenizer. The subcellular fractionations were performed at 2 °C according to the method of Schneider and Hogeboom[7]. The protein content of each fraction was estimated using the method of Lowry *et al.*[8].

Preincubation procedures for in vitro measurements

The enzyme suspension of rat gastric mucosa, adjusted to 100 μg of protein, was exposed to various concentrations of the test drugs in 100 mM Tris–acetate buffer (pH 7.5) at 37 °C for 20 min, and the enzyme activity was assayed.

Enzyme assays

(a) Succinate dehydrogenase activities of various cell fractions were measured according to the method of King[9].

(b) ATPase activity was assayed in a medium containing 5 mM disodium ATP, 5 mM $MgCl_2$ and 100 mM Tris–acetate buffer (pH 7.5) with or without 20 mM $NaHCO_3$. This mixture, in a volume of 1 ml, was incubated at 37 °C for 20 min and the enzymatic reaction was terminated by adding 0.5 ml of an ice-cold 5% trichloroacetic acid solution. The inorganic phosphate liberated during the incubation was estimated according to the method of Takahashi[10].

(c) Carbonic anhydrase activity was assayed according to the method of Philpot and Philpot[11]. Instead of a color indicator for pH as used in the original method, the pH range of a 0.3 M Na_2CO_3 solution, containing 0.2 M $NaHCO_3$ bubbled with CO_2, was registered on a recorder connected to the pH meter.

RESULTS

Effect of carbachol on gastric juice secretion and on the Mg^{2+}-ATPase and carbonic anhydrase activities of the homogenate of rat gastric mucosa

Subcutaneous treatment of carbachol (25–400 μg/kg) produced an increase

TABLE I

Effect of carbachol on gastric juice secretion, the activities of Mg^{2+}-ATPase and carbonic anhydrase of the homogenate of rat gastric mucosa. Subcutaneous treatment with carbachol (25–400 μg/kg) produced an increase of gastric juice secretion and of both enzyme activities in a dose dependent manner in 3-h polyrus-ligated rats.

Dose of carbachol (100 μg/kg subcutaneously)	*Gastric juice secretion (ml) in 3-h pylorus-ligated rats*	*Total homogenate of gastric mucosa*	
		Mg^{2+}-ATPase (μM P_i/mg protein per h)	*Carbonic anhydrase (units/mg protein)*
0	4.0 ± 0.6	15.0 ± 0.5	63.0 ± 5
25	4.3 ± 0.9	17.1 ± 0.6	64.0 ± 6
50	7.4 ± 1.7	28.0 ± 1.2*	100.3 ± 10*
100	10.1 ± 0.3**	30.2 ± 1.6**	116.0 ± 12**
200	10.5 ± 1.9*	30.8 ± 1.5**	120.3 ± 15**
400	9.7 ± 0.4**	29.5 ± 2.0**	115.0 ± 12**

* $P < 0.05$.
** $P < 0.01$.

of gastric juice secretion in 3-h pylorus-ligated rats. The secretion induced by carbachol was dose dependent up to 200 μg/kg; a dose of 400 μg/kg decreased the secretion. This treatment also increased the Mg^{2+}-ATPase and carbonic anhydrase activities of the homogenate of rat gastric mucosa as compared with the controls (Table I). This result led us to test which subcellular component of the gastric mucosa is concerned with the increase of the enzyme activity.

The effects of carbachol at a subcutaneous dose of 100 μg/kg administered immediately after or 2 h before the surgical operation are shown in Table II. The gastric juice accumulated in these rats was sampled 3 h after the pyloric ligation. The increase in gastric juice caused by carbachol lasted until 3 h after its subcutaneous administration. The same tendency was observed for the activities of Mg^{2+}-ATPase and carbonic anhydrase of the gastric mucosa homogenate. On the basis of these results, gastric acid stimulants were administered to the rat immediately after the pyloric ligation.

Enzyme activities of the gastric subcellular fractions

Before examination of the effect of gastric acid stimulants on Mg^{2+}-ATPase and carbonic anhydrase activities in subcellular fraction of gastric mucosa, we determined distribution of these enzyme activities in subcellular components in the cells.

As shown in Table III, the subcellular distribution of Mg^{2+}-ATPase and HCO_3^--stimulated ATPase (HCO_3^--ATPase) in the rat gastric mucosal cell was as follows: microsome > mitochondria > cell debris > supernatant. In contrast, carbonic anhydrase in the same cells was mostly found in the supernatant. The activity of the mitochondrial marker enzyme, succinate dehydrogenase, was highest in

TABLE II

Effect of carbachol, in subcutaneous doses of 100 μg/kg administered immediately (0 h) or 2 h before the pylorus ligation, on gastric juice secretion, Mg^{2+}-ATPase and carbonic anhydrase activities in rats. Numbers in parentheses give the collection periods of gastric juice after the subcutaneous treatment of carbachol. All values are mean ± S.E.

Hours before the pylorus ligation carbachol (100 μg/kg subcutaneously)	*Gastric juice secretion (ml) in 3-h pylorus-ligated rats*	*Total homogenate of gastric mucosa*	
		Mg^{2+}-ATPase (μM P_i/mg protein per h)	*Carbonic anhydrase (units/mg protein)*
Saline	3.0 ± 0.9	15.9 ± 2.0	60.0 ± 6.0
0 (0–3)	7.8 ± 0.3**	26.0 ± 3.0*	124.2 ± 15.0*
2 (2–5)	2.8 ± 0.2	17.4 ± 1.5	72.0 ± 5.0

* $P < 0.05$.
** $P < 0.01$.

TABLE III

Subcellular distributions of Mg^{2+}-ATPase, HCO_3^--ATPase, carbonic anhydrase and succinate dehydrogenase activities in rat gastric mucosa. Cell debris, mitochondria and microsomes were fractionated at 700 × *g* for 10 min, 6000 × *g* for 10 min and 57000 × *g* for 60 min, respectively. The data are for unstimulated rat gastric mucosa. All values are mean ± S.E.

Enzymes	*Total homogenate*	*Subfractionation of gastric mucosa*			
		Cell debris	*Mitochondria*	*Microsome*	*Supernatant*
Mg^{2+}-ATPase*	16.0 ± 0.7	18.0 ± 1.0	30.2 ± 1.5	38.0 ± 2.0	5.0 ± 0.4
HCO_3^--ATPase*	3.0 ± 0.2	4.0 ± 0.5	10.5 ± 1.5	18.2 ± 3.0	1.0 ± 0.2
Carbonic anhydrase**	63.5 ± 3.5	21.2 ± 5.0	10.4 ± 2.0	17.2 ± 2.5	112.0 ± 5.0
Succinate dehydrogenase***	20.0 ± 2.5	22.7 ± 2.0	40.7 ± 2.7	30.3 ± 3.8	
Total protein contents(mg)	440	132	77	60	141

* μM P_i/mg protein per h
** units/mg protein
*** μmoles succinate oxidized/mg protein per min

the mitochondrial fraction. The microsomal and cell debris fraction activities were about 75% and 50%, respectively, of the mitochondrial activity. Protein was mostly found in the cell debris and supernatant fractions.

In vivo effects of gastric acid stimulants on the activities of Mg^{2+}-ATPase and carbonic anhydrase in rat gastric mucosa

As shown in Fig. 1, subcutaneous treatment of carbachol (25–400 μg/kg) produced an increase of gastric juice secretion in a dose-dependent fashion. Dose–response curves of Mg^{2+}-ATPase (Fig. 1, middle) and carbonic anhydrase activities

(Fig. 1, left) in the mitochondrial fraction of gastric mucosa were similar to those of gastric juice secretion, while those of other fractions lost dose dependence. The activity of HCO_3^--ATPase in every fraction was increased less than that of Mg^{2+}-ATPase.

As shown in Fig. 2, subcutaneous treatment of tetragastrin (50–400 μg/kg) produced an increase of gastric juice secretion in 3-h pylorus-ligated rats. A dose of

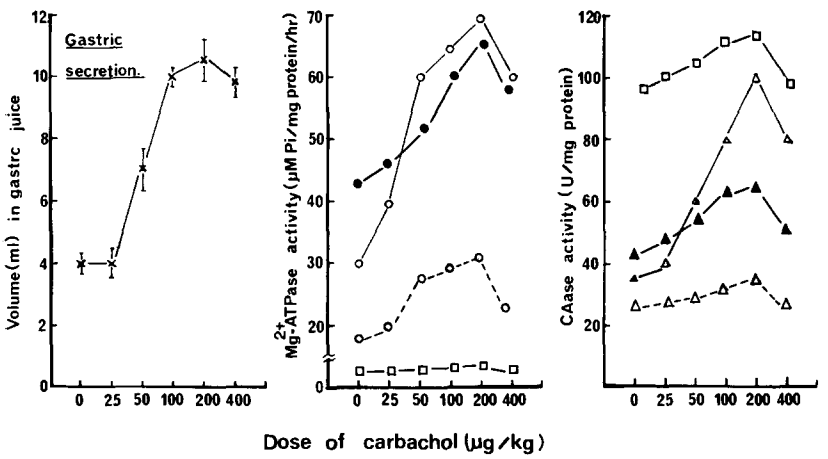

Fig. 1. Interrelationship between gastric acid secretion (left), Mg^{2+}-ATPase (middle) and carbonic anhydrase (CAase) (right) activities in cell debris (○--○, △—△), mitochondrial (○—○, △—△), microsomal (●—●, ▲—▲) and supernatant (□—□) fractions by treatment with carbachol. Subcutaneous treatment with carbachol (25–400 μ/kg) produced gastric juice secretion in 3-h pylorus-ligated rats, then the activities of ATPase and carbonic anhydrase in various fractions in rat gastric mucosa were estimated.

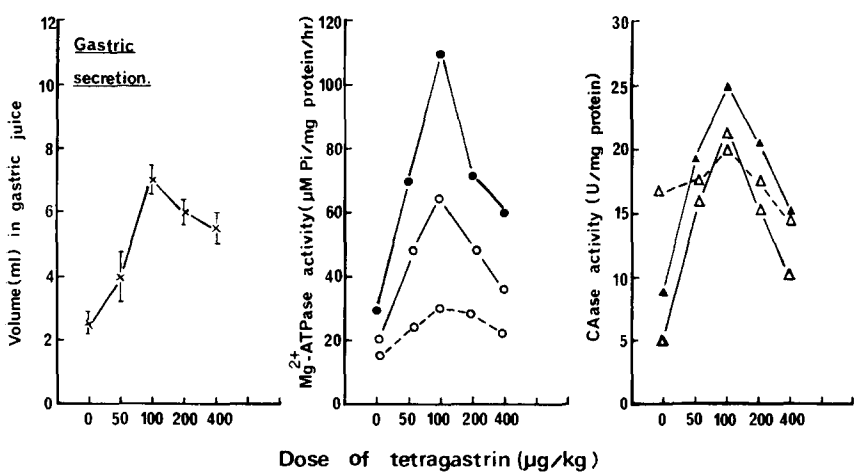

Fig. 2. Interrelationship between gastric acid secretion (left), Mg^{2+}-ATPase (middle) and carbonic anhydrase (CAase) (right) activities by treatment with tetragastrin. Tetragastrin stimulated both enzyme activities, not only of the mitochondrial (○—○, △—△) but also of the microsomal (●—●, ▲—▲) fraction. ○--○, △--△, cell debris fraction.

100 μg/kg of tetragastrin produced the maximum activity in the gastric juice secretion, Mg^{2+}-ATPase and carbonic anhydrase activities of the mitochondrial and microsomal fractions; the dose dependence of gastric juice secretion was the same as that of both enzyme activities in the two fractions.

Histamine (0.5–8 mg/kg) treatment produced the same results as mentioned above (Fig. 3). A dose of 1 mg/kg of histamine produced maximum gastric juice secretion, and maximum Mg^{2+}-ATPase and carbonic anhydrase activities of the mitochondrial fraction. Histamine doses higher than 1 mg/kg produced a decrease in secretion and the activities of the the enzymes as compared with the effects of 1 mg/kg histamine.

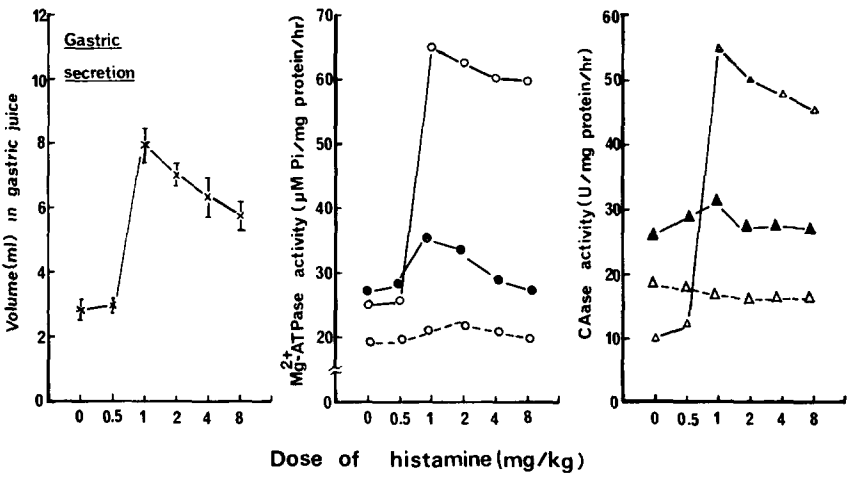

Fig. 3. Interrelationship between gastric acid secretion (left), Mg^{2+}-ATPase (middle) and carbonic anhydrase (CAase) (right) activities in the cell debris (○--○, △--△), mitochondrial (○—○, △—△) and microsomal (●—●, ▲—▲) fractions by treatment with histamine. See legend to Fig. 1 for details.

These results indicated that gastric acid stimulants, such as carbachol, tetragastrin and histamine, increased gastric juice secretion, Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial or microsomal fraction of rat gastric mucosa.

Effects of gastric acid inhibitors on the increase of the activities of Mg^{2+}-ATPase and carbonic anhydrase induced by carbachol

As shown in Fig. 4 (left), in the case of rat gastric mucosa, subcutaneous pretreatment with atropine (5 mg/kg) prevented the increase of Mg^{2+}-ATPase and carbonic anhydrase in the mitochondrial fraction induced by treatment of carbachol (200 μg/kg). However, the atropine treatment did not prevent the increase of carbonic anhydrase activity in the supernatant fraction induced by carbachol.

As shown in Fig. 4 (right), in the case of rat whole kidney, subcutaneous treatment of carbachol produced a decrease of Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial fraction but an increase of carbonic anhydrase activity in the supernatant fraction. Treatment with atropine did not affect the action of carbachol in the kidney.

In the case of gastric mucosa, Fig. 5 shows that subcutaneous pretreatment of acetazolamide (20 mg/kg), known as a carbonic anhydrase inhibitor, prevented the increase of Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial

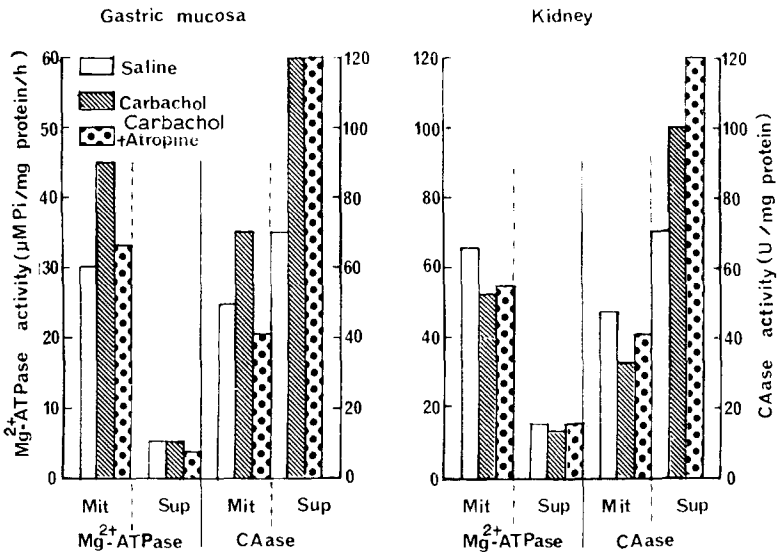

Fig. 4. Effect of atropine on the increase of the activities of Mg^{2+}-ATPase and carbonic anhydrase (CAase) induced by carbachol (200 μg/kg). Subcutaneous pretreatment (30 min) of atropine (5 mg/kg) prevented the increase of Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial fraction (Mit) induced by treatment with carbachol (left figure in the case of gastric mucosa). However, the same treatment with atropine did not have any effect on the effects of carbachol in kidney (right figure). Sup, supernatant.

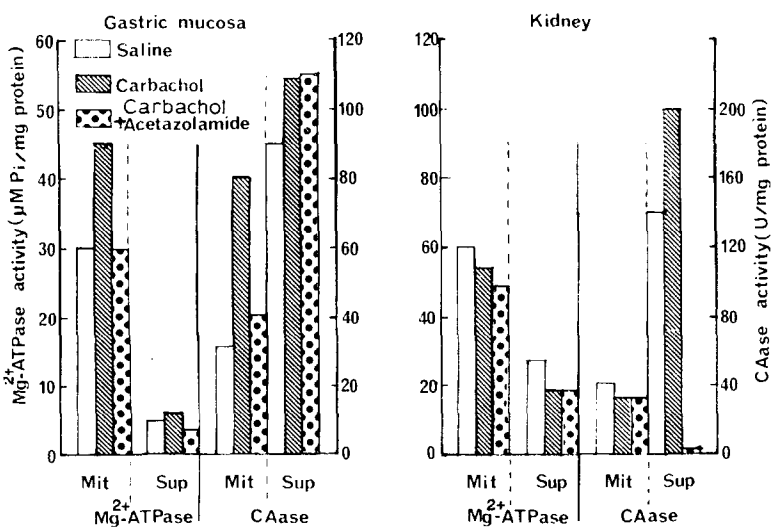

Fig. 5. Effect of acetazolamide (20 mg/kg, subcutaneous treatment) on the increase of the activities of Mg^{2+}-ATPase and carbonic anhydrase (CAase) induced by treatment with carbachol (200 μg/kg). See legend Fig. 4 for details.

fraction induced by treatment with carbachol. In the case of whole kidney, the treatment of acetazolamide abolished carbonic anhydrase activity of the supernatant and mitochondrial fractions and produced a small decrease of Mg^{2+}-ATPase of the same fractions.

From these results, it was obvious that Mg^{2+}-ATPase and carbonic anhydrase activities of the mitochondrial fraction in gastric mucosa correlated with gastric acid secretion. In addition, the carbonic anhydrase inhibitor (acetazolamide) prevented the increase of Mg^{2+}-ATPase in gastric mucosa and kidney, which suggested that carbonic anhydrase and Mg^{2+}-ATPase were coupled in their actions.

In vitro effects of gastric acid stimulants and inhibitors on the activities of Mg^{2+}-ATPase, HCO_3^--ATPase and carbonic anhydrase in rat gastric mucosa

Histamine (10^{-3} M), carbachol (10^{-3} M) and tetragastrin (10^{-5} M) had no effect on the activities of Mg^{2+}-ATPase, HCO_3^--ATPase and carbonic anhydrase in the cell debris, mitochondrial, microsomal and supernatant fractions. As shown in Fig. 6, thiocyanate (10^{-2} M) inhibited the activity of Mg^{2+}-ATPase in the microsomal fraction by about 30–40% and that of HCO_3^--ATPase in the same fraction by about 20–30%. The same results were found in the case of the cell debris and mitochondrial fractions. Ouabain (10^{-2}–10^{-4} M), known as an inhibitor of (Na^+–K^+)-ATPase, had little effect on the activities of Mg^{2+}-ATPase and HCO_3^--ATPase in the microsomal fraction.

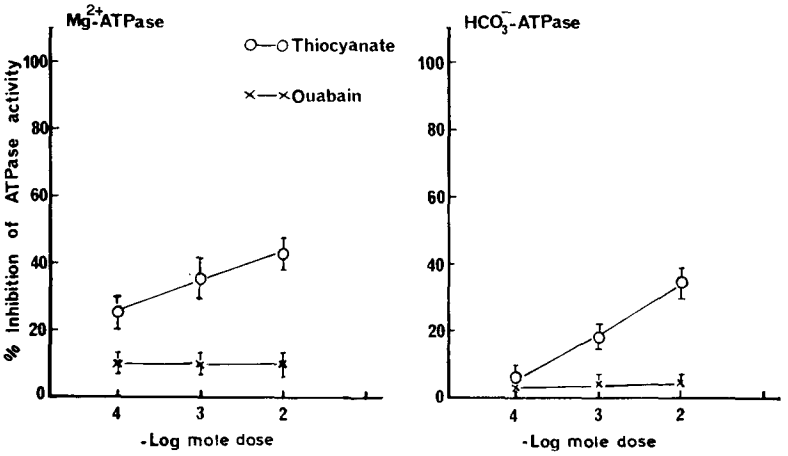

Fig. 6. Effect of thiocyanate and ouabain on the ATPase activities in the microsomal fraction of rat gastric mucosa, *in vitro*. The mixture of enzyme suspension (microsomal fraction) was exposed to various concentrations of thiocyanate and ouabain in 100 mM Tris-acetate buffer (pH 7.5) at 37 °C for 20 min and ATPase activity was assayed.

From these results, gastric acid stimulants had no *in vitro* effect on the activities of Mg^{2+}-ATPase, HCO^-_3-ATPase and carbonic anhydrase.

DISCUSSION

We found that subcutaneous treatment with carbachol produced an increase of Mg^{2+}-ATPase and carbonic anhydrase activities in the homogenate of rat gastric

mucosa. This fact is supported by the finding[12] that carbonic anhydrase activity of dog gastric mucosa homogenate was stimulated by cutting the sympathetic nerve.

We also found that addition of thiocyanate inhibited the activities of Mg^{2+}-ATPase and HCO_3^--ATPase in the cell debris, mitochondrial and microsomal fractions of rat gastric mucosa. This agrees with frog gastric mucosa presented by Sachs *et al.*[13]. In the mammalian gastric mucosa, the relationship between the activities of (HCO_3^-–Mg^{2+})-ATPase, carbonic anhydrase and gastric acid secretion remains to be settled. In the present study, (HCO_3^-–Mg^{2+})-ATPase in every fraction of rat gastric mucosa was not activated by the addition of histamine (10^{-3} M), tetragastrin (10^{-5} M) or carbachol (10^{-3} M). Kasbekar and Durbin[3] showed that histamine (10^{-3} M) had no effect on the activity of Mg^{2+}-ATPase of frog gastric mucosa.

However, subcutaneous treatment with gastric acid stimulants, especially parasympathomimetic drugs such as insulin (unpublished data) and carbachol, produced an increase of Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial or microsomal fraction of rat gastric mucosa. In the cell debris and supernatant fractions of rat gastric mucosa, the same tendency was obtained, but the increase of the enzyme activities was slight compared with the mitochondrial fraction. Kasbekar and Durbin[3] suggested that (HCO_3^-–Mg^{2+})-ATPase in the microsomal fraction of frog gastric mucosa was related to the mechanism of gastric acid secretion. However, in our results, the enzyme activities in the microsomal fraction of rat gastric mucosa was increased only by carbachol or tetragastrin treatment. Further investigation is needed to clarify the role of Mg^{2+}-ATPase and carbonic anhydrase in the microsomal fraction, in view of cross-contamination with the mitochondrial fraction.

Kikuchi[14] showed that oxidative phosphorylation in mitochondria of dog gastric mucosa was stimulated by cutting the cervical sympathetic nerve. This supports our results. We support the hypothesis[4] that ATPase supplied the high energy required for gastric acid secretion, because treatment with gastric acid stimulants produced an increase in Mg^{2+}-ATPase activity in the mitochondrial fraction of rat gastric mucosa.

Subcutaneous pretreatment with atropine prevented the increase of Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial fraction induced by treatment with carbachol, but this pretreatment did not prevent the increase of carbonic anhydrase activity in the supernatant fraction.

From these results, we concluded that Mg^{2+}-ATPase and carbonic anhydrase activities in the mitochondrial fraction of rat gastric mucosa correlated with gastric acid secretion and that carbonic anhydrase activity in the supernatant fraction correlated with the maintenance of the gastric mucosal barrier, as previously reported[15]. In addition, the carbonic anhydrase inhibitor acetazolamide prevented the increase of Mg^{2+}-ATPase activity in the mitochondrial fraction of gastric mucosa, which suggested that carbonic anhydrase produced HCO_3^- and that the anion stimulated Mg^{2+}-ATPase activity. It is likely that carbonic anhydrase is functionally linked to the Mg^{2+}-ATPase of gastric mucosa.

Gastric acid stimulants increased the activities of the enzymes indirectly; addition of these reagents *in vitro* had no effect. Recently, we found that dibutyryl cyclic AMP increased gastric acid secretion and the Mg^{2+}-ATPase and carbonic

anhydrase activities of rat gastric mucosa. This result may suggest that gastric acid stimulants increased the enzyme activities *via* the formation of cyclic AMP[16].

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