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AN ANION-SENSITIVE ATPase IN LIZARD GASTRIC MUCOSA

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SUMMARY

1. The activity of the Mg^{2+}-activated ATPase in lizard gastric mucosa is dependent on the anion species present in the incubation medium while the (Na^+–K^+)-activated ATPase in this tissue is anion insensitive.

2. The Mg^{2+}-activated ATPase is inhibited by thiocyanate, the degree of inhibition being dependent on the other anionic species present. The (Na^+–K^+)-ATPase in the same tissue, on the other hand, is virtually unaffected by thiocyanate.

3. Anion substitution experiments lead us to postulate the presence of an anionic binding site. The relative affinity of various anions for this binding site was determined by thiocyanate inhibition experiments as well as by anion substitution experiments. The relative affinities obtained in these two ways agreed qualitatively and quantitatively.

4. For all anions investigated (glucuronate, acetate, SO_4^{2-}, Cl^-, NO_3^-, SCN^-), with the exception of HCO_3^-, the enzyme activity is inversely proportional to the logarithm of the affinity. The activity with HCO_3^- is about 2 times as high as would be expected from its affinity value.

5. These experiments suggest that the postulated anionic site is relatively specific for HCO_3^-, but that other anions can occupy this site leading to a decrease in enzyme activity.

INTRODUCTION

In many excitatory and secretory processes the (Na^+–K^+)-ATPase system plays an important role as cation pump[1]. Such a role of this enzyme system appeared to be doubtful in gastric secretion, since even the presence of this enzyme in gastric mucosa has been called into question[2,3]. Recently, however, we have established beyond doubt the presence of the (Na^+-K^+)-ATPase system in lizard gastric mucosa and determined its properties. From the effect of ouabain on the cation content of gastric mucosa and on the transmucosal potential, short circuit current and acid secretion in Ussing chamber experiments, we tentatively concluded that the (Na^+–K^+) ATPase system plays a crucial but indirect role in gastric secretion by maintaining the cation gradients essential for the functioning of this process[4].

As long as the role of the (Na^+–K^+)-ATPase system in gastric secretion was uncertain, the finding of a SCN^--sensitive Mg^{2+}-activated ATPase activity in gastric

mucosa attracted attention, since it was known that SCN^- inhibits gastric acid secretion[5]. When it was found that addition of HCO_3^- to the assay medium stimulates the enzyme, it was suggested that this SCN^--sensitive, HCO_3^--stimulated ATPase might mediate the acid secretion like the ouabain-sensitive (Na^+–K^+)-activated ATPase system mediates the cation transport in most other secretory systems[6]. Recently, a partial purification of the enzyme from gastric mucosa of Necturus, frog and dog by solubilization with Triton X-100 has been achieved[7,8]. The HCO_3^- activation of the purified enzyme was markedly increased.

Our investigations on the presence and role of the (Na^+–K^+)-ATPase system in gastric secretion have led us to a detailed study of the properties of the SCN^--sensitive HCO_3^--stimulated ATPase in lizard gastric mucosa and a comparison of its properties with those of the (Na^+–K^+)-ATPase system. The results, reported in this paper, indicate that the former ATPase appears to bind all anions leading to a lower activity the greater the affinity of this anion, but that the presence of HCO_3^- leads to about double the activity expected on the basis of its affinity for the enzyme. While a possible role of this enzyme in gastric acid secretion can not be excluded, its properties are so different from those of the (Na^+–K^+)-ATPase system that it cannot be considered as a bicarbonate or proton pump in the same sense as the latter system acts as a cation pump.

MATERIALS AND METHODS

Tissue preparation, homogenization, lyophilization and storage of the lyophilized material was carried out as described by Hansen *et al.*[4]. No attempts at fractionation or purification of the enzyme were made in view of the small amounts of tissue available to us.

(Na^+–K^+)-ATPase and Mg^{2+}-ATPase activities were determined in the Media A and E as described by Bonting[1] (p. 262). The activity in Medium A (ATP, 2 mM; Na^+, 55 mM; K^+, 5 mM; Mg^{2+}, 2 mM; Tris, 100 mM; EDTA, 0.1 mM) represents total ATPase activity, while the activity in Medium E (ATP, 2 mM; Na^+, 60 mM; Mg^{2+}, 2 mM; Tris, 100 mM; EDTA, 0.1 mM; ouabain, 0.1 mM) represents the Mg^{2+}-ATPase (Na^+- and K^+-insensitive) activity. In the course of this investigation the anions used in these media were varied, *e.g.* in a chloride medium all salts were added as chlorides and the pH of the buffer was adjusted with HCl, while in a sulfate medium sulfates were used and the Tris buffer was brought to the desired pH with H_2SO_4.

All reagents used were of analytical grade.

RESULTS

The activities of Mg^{2+}-ATPase and (Na^+–K^+)-ATPase were measured at pH 7.5 in media in which only one anionic species (in addition to 2 mM ATP) was present in a concentration of 147 mequiv/l. The (Na^+–K^+)-ATPase activity is scarcely dependent on the anionic species present in the medium (Table I), in contrast to the activity of Mg^{2+}-ATPase (Table II). Highest Mg^{2+}-ATPase activities were found in the presence of SO_4^{2+}, acetate or glucuronate. However, in the presence of Cl^- and NO_3^- the activities were 33 and 57 % lower, respectively.

TABLE I

ACTIVITY OF (Na^+-K^+)-ATPase FROM LIZARD GASTRIC MUCOSA IN THE PRESENCE OF VARIOUS ANIONS AND OF SCN^-

Enzyme activity is given in moles ATPase hydrolyzed per h per kg dry wt at 37 °C with ± S.E. and in parentheses the number of experiments. N.S., not significant.

Anion present	*SCN^- concn in chloride medium (M)*	*Enzyme activity*	*P (vs Cl^-)*	*P (vs absence of SCN^-)*
SO_4^{2-}		0.64 ± 0.07 (4)	N.S.	
Cl^-		0.57 ± 0.04 (4)	—	
NO_3^-		0.48 ± 0.04 (4)	N.S.	
	0	0.57 ± 0.04 (4)		—
	$5 \cdot 10^{-7}$	0.59 ± 0.09 (6)		N.S.
	$5 \cdot 10^{-6}$	0.48 ± 0.05 (6)		N.S.
	$5 \cdot 10^{-5}$	0.49 ± 0.05 (6)		N.S.
	$5 \cdot 10^{-4}$	0.57 ± 0.05 (6)		N.S.
	$5 \cdot 10^{-3}$	0.54 ± 0.09 (7)		N.S.
	$5 \cdot 10^{-2}$	0.43 ± 0.05 (7)		<0.05

TABLE II

ACTIVITY OF Mg^{2+}-ATPase FROM LIZARD GASTRIC MUCOSA IN THE PRESENCE OF DIFFERENT ANIONS WITH AND WITHOUT SCN^-

Enzyme activities are expressed in moles ATP hydrolyzed per h per kg dry wt at 37 °C with ± S.E. and in parentheses the number of experiments. When SCN^- was added, the concentrations of the other anions were lowered by equivalent amounts.

Anion present	*pH*	*Mg^{2+}-ATPase activity*	
		− SCN^-	*+ SCN^- (50 mM)*
Glucuronate	7.5	3.64 ± 0.51 (2)	0.77 (1)
Acetate	7.5	3.76 ± 0.13 (4)	0.56 ± 0.01 (2)
SO_4^{2-}	7.5	3.65 ± 0.09 (10)	0.44 ± 0.05 (5)
Cl^-	7.5	2.48 ± 0.06 (15)	0.62 ± 0.01 (2)
NO_3^-	7.5	1.46 ± 0.08 (4)	0.65 ± 0.11 (2)
HCO_3^-	8.4	6.25 ± 0.21 (4)	1.05 ± 0.05 (2)
SO_4^{2-}	8.4	4.51 ± 0.11 (2)	—
Cl^-	8.4	3.55 ± 0.08 (8)	0.89 ± 0.01 (2)

To determine whether these differences in activity might be due to a shift in pH optimum upon changing the anionic species, the pH dependence of the enzyme activity was measured with either Cl^- or SO_4^{2-}. Fig. 1 shows that in both cases there is a rather flat pH–activity curve with an optimum between 8.5 and 9.0. There was no clear shift in pH optimum upon replacing Cl^- by SO_4^{2-}.

In the experiments with HCO_3^- the buffer was prepared with Tris brought to pH 8.4 with CO_2, because at pH 7.5 the attainable HCO_3^- concentration was too low for our purpose. In these experiments the anion concentration was 97 mequiv/l. For purposes of comparison the activities of Mg^{2+}-ATPase with Cl^- and SO_4^{2-} were also determined at this pH. The ratio between the activities with SO_4^{2-} and Cl^- at pH 8.4 was not significantly different from that at pH 7.5. The activity with

HCO_3^- was significantly higher (40 %) than the activity with SO_4^{2-} at pH 8.4 (Table II).

In another experiment we tested the influence of the ionic strength or the tonicity on the activity. Both at pH 7.5 and at pH 8.4 we added graded amounts of NaCl to the medium containing Tris–HCl buffer 100 mM; ATP 2 mM; Mg^{2+} 2 mM;

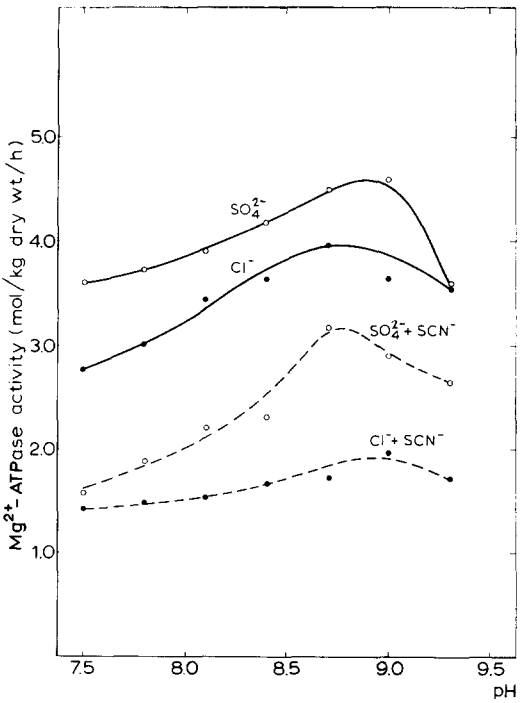

Fig. 1. Mg^{2+}-ATPase activity from gastric lizard mucosa as a function of pH. Activity measured in the presence of SO_4^{2-} (○—○), Cl^- (●—●), SO_4^{2-} + 1 mM SCN^- (○---○) and Cl^- + 10 mM SCN^- (●---●). The pH of the incubation media was adjusted with H_2SO_4 and HCl, respectively. When NaSCN was added, the concentrations of Na_2SO_4 and NaCl, respectively, were lowered by equivalent amounts.

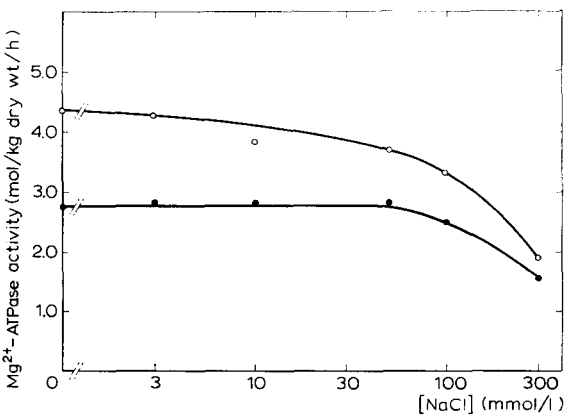

Fig. 2. Mg^{2+}-ATPase from lizard gastric mucosa as a function of addition of NaCl to the incubation medium. Activity was measured at pH 8.4 (○—○) and at pH 7.5 (●—●) with 100 mM Tris–HCl as buffer.

EDTA 0.1 mM and ouabain 0.1 mM. Fig. 2 shows that the enzyme activity decreases with increasing salt concentration. This effect is somewhat more pronounced at pH 8.4 than at pH 7.5. In order to exclude effects of either ionic strength or tonicity all further experiments were carried out at constant total anionic concentration (in mequiv/l).

The effect of SCN^- was tested by replacing equimolar amounts of the sodium salt of the chosen anion by equimolar amounts of NaSCN keeping the total anionic concentration (excluding 2 mM ATP) of the medium constant at 147 mequiv/l at pH 7.5 and at 97 mequiv/l at pH 8.4. Table I shows that at pH 7.5 SCN^- in concentrations up to 5 mM has no effect on the $(Na^+–K^+)$-ATPase activity, while a 50 mM concentration has only a very small inhibitory effect. SCN^- in concentrations of 1 mM and above has a strongly inhibitory effect on the Mg^{2+}-ATPase activity. The magnitude of this inhibitory effect depends on the other anionic species present in the incubation medium. Some typical examples of this effect are plotted in Fig. 3. In this figure the ATPase activity is plotted as a function of the negative logarithm of the molar SCN^- concentration. Apart from the fact that the activities in the absence of the inhibitor differ considerably, there is a large difference in the values for the half-inhibitory SCN^- concentration. Although no concentrations above 50 mM were used, the shape of the curves suggests that complete inhibition of the enzyme activity cannot be reached.

The residual activity seems to be quite independent of the other anionic species present (Table II).

The half-inhibition concentrations for SCN^-, although obtainable from the curves shown in Fig. 3, were determined with higher accuracy from modified Dixon plots[9], in whichs $1/v_{i(corr)}$ was plotted *vs* $[SCN^-]$. Here $v_{i(corr)}$ is the activity in the

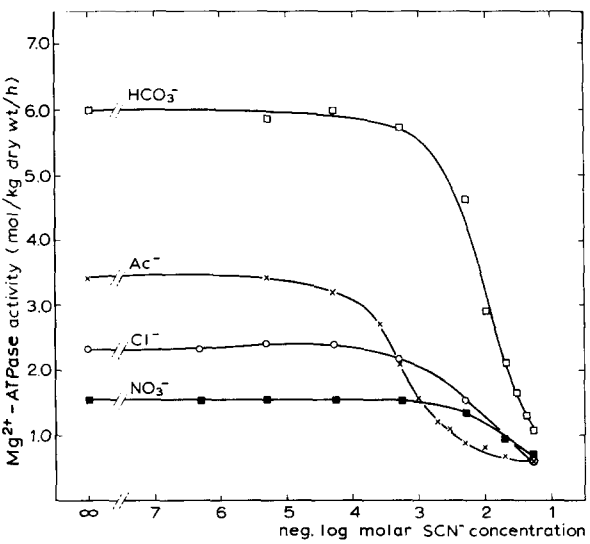

Fig. 3. Mg^{2+}-ATPase activity from lizard gastric mucosa as a function of the negative logarithm of the molar SCN^- concentration. In every experiment only one other anion was present: HCO_3^- (□—□), acetate (×—×), Cl^- (○—○) or NO_3^- (■—■). Experiments with HCO_3^- were carried out at pH 8.4, the others at pH 7.5. When NaSCN was added, the sodium salts of the other anions were lowered by equivalent amounts.

presence of inhibitor minus the residual activity at maximal thiocyanate concentration. Such modified Dixon plots gave straight lines, which intersect the x axis at $-K_i$ (Fig. 4), as is the case for inhibition which is non-competitive[3] or incompetitive[2] with regard to the substrate. Values thus obtained for K_i in the presence of various anionic species are listed in Table III (Column 2). The ratio between the concentration of SCN^- and the other anion present, at which 50% inhibition occurs, was also calculated (Table III, Column 5). This ratio increased in the order glucuronate, acetate, SO_4^{2-}, Cl^-, NO_3^- at pH 7.5. HCO_3^- and also Cl^- were tested at pH 8.4. Since the ratio for Cl^- was not much different at these

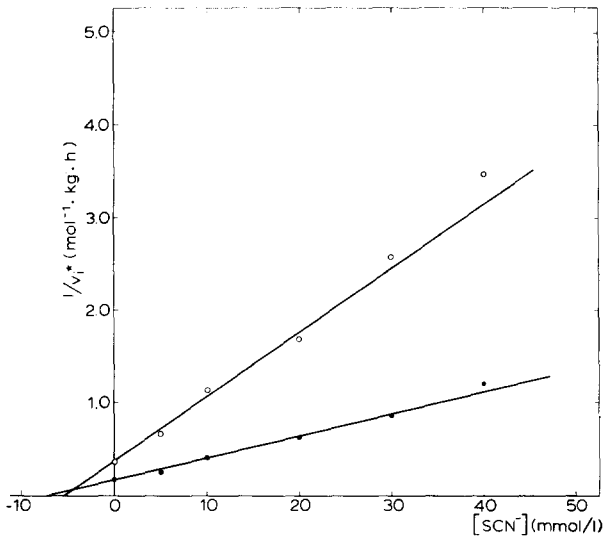

Fig. 4. Modified Dixon plot, in which the reciprocal ($1/v_i^*$) of the Mg^{2+}-ATPase activity (corrected for the residual activity in the presence of 50 mM NaSCN, see Table II) was plotted against the SCN^- concentration. The pH in this experiment was 8.4. Either HCO_3^- (●—●) or Cl^- (○—○) was present as the only other anion. When NaSCN was added, the concentrations of $NaHCO_3$ and NaCl, respectively, were lowered by equivalent amounts.

TABLE III

HALF-MAXIMAL INHIBITION CONDITIONS FOR Mg^{2+}-ATPase FROM LIZARD GASTRIC MUCOSA BY SCN^-

Total anion concentration 147 mequiv/l at pH 7.5; 97 mequiv/l at pH 8.4.

Anion	*pH*	*Concns at 50% inhibition (mM)*			
		$[SCN^-]$	$[Anion]$	*Ratio*	$\frac{[SCN^-]}{[anion]} \times 10^3$
Glucuronate	7.5	0.35	146.65	2.4	
Acetate	7.5	0.5	146.5	3.4	
SO_4^{2-}	7.5	0.6	73.2	8.2	
Cl^-	7.5	6.6	140.4	47	
NO_3^-	7.5	20	127	157	
Cl^-	8.4	5.5	92	52	
HCO_3^-	8.4	8.0	89	90	

two pH values (47 at pH 7.5 and 52 at pH 8.4), the place of HCO_3^- in the sequences appears to be between Cl^- and NO_3^-. The small differences for the Cl^- ratio at the two pH values and in the pH–activity curves in the presence of SCN^- (Fig. 1) indicate that the degree of inhibition for Cl^-, and very likely also for the other anions, is virtually independent of the pH of the medium.

It appears that these anion effects are due to differences in the affinity of the various anions to an anion-binding site on the enzyme. Assuming that at concentrations, at which 50 % inhibition occurs, equivalent amounts of SCN^- and the other anion are bound to the enzyme, this means that the calculated ratio is a measure for the affinity of the anion for the enzyme relative to that of SCN^-. The larger the ratio, the larger is the relative affinity of the anion.

TABLE IV

INHIBITION OF Mg^{2+}-ATPase FROM LIZARD GASTRIC MUCOSA BY SCN^- IN DIFFERENT CONCENTRATIONS BUT IN A CONSTANT RATIO RELATIVE TO THE OTHER ANION PRESENT

The enzyme activity was compared to the activity in the same medium in which SCN^- was replaced by Cl^- and acetate, respectively. For each value the standard error of the mean and in parentheses the number of experiments are given. N.S., not significant.

Expt.	*Anion concns (mM)*			*Relative enzyme activity* (%)	*P**
	[*SCN*$^-$]	[*Cl*$^-$]	[*Acetate*]		
a	—	147	—	100	
b	10	137	—	48.1 ± 2.2 (3)	—
c	5	68.5	—	46.7 ± 5.0 (3)	N.S.
d	2.5	39.25	—	51.7 ± 5.4 (2)	N.S.
e	—	—	147	100	
f	1.0	—	146	53.2 ± 1.4 (4)	—
g	0.5	—	73	62.5 ± 5.5 (4)	N.S.
h	0.25	—	36.5	60.0 ± 3.3 (4)	N.S.

* Expts c and d were compared with b; Expts g and h with f.

This ratio, rather than the concentration of SCN^-, determines the degree of inhibition as shown by the experiments described in Table IV. In these experiments the ratio of SCN^- and the other anion was kept constant (1:13.7 in the case of Cl^- and 1:146 in the case of acetate). The total anion concentration was reduced successively to 50 and 25 % of the original value by equiosmolar replacement with sucrose. Both with acetate and with Cl^- as the other anion, the degree of inhibition was independent of the final SCN^- concentration.

When anion binding to the enzyme is the cause of the differences in inhibitory characteristics of SCN^- in the presence of different anions, the same phenomenon could cause the differences in absolute activity in the presence of those anions alone (Table I). We studied this by gradually replacing in a Cl^- medium the latter anion by equimolar amounts of an other anion, keeping the total anion concentration constant and equal to 147 at pH 7.5 and to 97 mequiv/l at pH 8.4. The results of a typical experiment are plotted in Fig. 5. This figure shows that after replacement of 120 mM Cl^- by acetate the activity begins to increase. This indicates that the enzyme has a much higher affinity for chloride than for acetate. The ratio of the Cl^- concentration

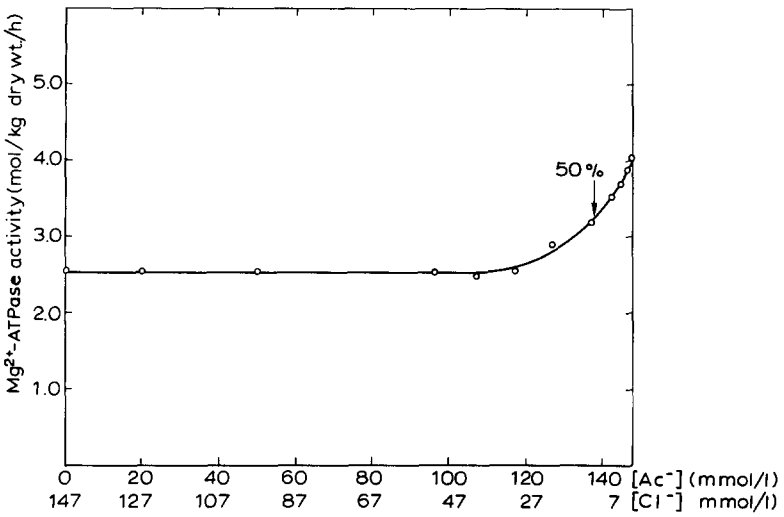

Fig. 5. Influence of replacement of Cl^- by acetate ions on the Mg^{2+}-ATPase activity. The pH of the incubation medium was 7.5.

TABLE V

EFFECT OF ANION MIXTURES ON Mg^{2+}-ATPase FROM LIZARD GASTRIC MUCOSA

Total anion concn 147 mequiv/l at pH 7.5; 97 mequiv/l at pH 8.4.

Anion	*pH*	*Concn at 50% activity change*		*Ratio [Cl⁻]/[anion] at 50% activity change*
		Cl^- (mM)	*Anion (mM)*	
Glucuronate	7.5	5	142	0.035
Acetate	7.5	8	139	0.058
SO_4^{2-}	7.5	9	69	0.13
NO_3^-	7.5	117	30	3.9
HCO_3^-	8.4	70	27	2.6

TABLE VI

RELATIVE ANION AFFINITY OF Mg^{2+}-ATPase FROM LIZARD GASTRIC MUCOSA

The figures in the second column were derived from Table V, Column 5 and in the third column obtained by transforming those in Table III, Column 5 to a ratio of 1 for Cl^-.

Anion	*From anion substitution*	*From thiocyanate inhibition*	*Average*
Glucuronate	0.035	0.051	0.043
Acetate	0.058	0.072	0.065
SO_4^{2-}	0.13	0.17	0.15
Cl^-	1.0	1.0	1.0
HCO_3^-	2.6	1.4	2.0
NO_3^-	3.9	3.4	3.7
SCN^-	—	21.3	21.3

to that of acetate, at which the activity was midway between the activities for each anion alone, was taken as a measure of the relative affinity of the acetate anion for the enzyme. Table V lists the results for five different anions.

The two sets of relative affinities obtained from SCN^- inhibition studies and from the anion substitution experiments can be compared by transforming the first set to a relative affinity for Cl^- of 1. This can be done by dividing the first 5 values in Column 5 of Table III by 47 and the last 2 values by 52. The resulting two sets of relative affinities, listed in Table VI, show good agreement, both qualitatively and quantitatively.

DISCUSSION

The presence of an anion-sensitive ATPase in gastric mucosa was first reported by Kasbekar and Durbin[2] and by Sachs *et al.*[3] in a microsomal fraction of frog gastric mucosa. This enzyme is inhibited by SCN^- and maximally activated by HCO_3^- anions, but is it not inhibited by ouabain and not activated by Na^+ and K^+. We have found that in lizard gastric mucosa homogenates at pH 7.4 only 12 % of the total ATPase is activated by Na^+ and K^+ and inhibited by ouabain. Therefore we concluded that in this tissue at least two different ATPases are present, and we decided to study the effect of anions on both enzymes.

Our method of investigation was quite different from those of the authors mentioned above. They used media of relatively low ionic strength (20 mM Tris buffer (pH 8.4); 2–3 mM ATP, 1–3 mM Mg^{2+}) and added the anions to be investigated to the media in concentrations up to 150 mM. We used media of constant and more physiological ionic strength (100 mM Tris buffer (pH 7.5 and 8.4), 2 mM ATP, 2 mM Mg^{2+}, 60 mM Na^+) replacing Cl^- as the anionic species present in the control medium by the anionic species to be investigated without changing the Na^+, Mg^{2+} and Tris concentration. The advantage of our method is that the cation composition of the medium does not change and that only the effect of the anion and not of the accompanying cation is investigated. The importance of maintaining constant ion concentrations is proven by Fig. 2 which shows the inhibitory effect of NaCl concentrations over 50 mM. This may explain the fact that Kasbekar and Durbin[2] always found inhibition of enzyme activity when high amounts of the tested anions were added. These authors found that upon adding increasing amounts of $NaHCO_3$ the activity first increased and thereafter decreased again. Maximal activity was found with 25 mM $NaHCO_3$. That this maximal activity concentration is the same as the HCO_3^- concentration in blood seems purely accidental in the light of our findings.

We established that the (Na^+–K^+)-ATPase present in lizard gastric mucosa is virtually insensitive to anions and is not inhibited by SCN^- in concentrations below 50 mM (Table I). Therefore this enzyme activity was excluded in all further experiments by the omission of K^+ and the addition of 10^{-4} M ouabain to the assay medium.

The Mg^{2+}-ATPase activity is strongly anion sensitive as shown in Table II. This anion sensitivity is not due to an effect of the anionic species on the position of the pH optimum (Fig. 1). The enzyme is also inhibited by SCN^-, although complete inhibition could not be obtained, which may be due to the presence of a third SCN^--insensitive ATPase possibly a mitochondrial enzyme.

The anion sensitivity can be expressed in a quantitative way by assuming the presence of an anionic binding site on the enzyme. Starting from this hypothesis we could calculate the relative affinities of a series of anions for the enzyme by SCN^- inhibition experiments (Table III) as well as by anion substitution experiments (Table V). The relative affinities calculated from both types of experiments summarized in Table VI show good agreement, both qualitatively and quantitatively.

Comparison of these relative affinity constants with the activities of the enzyme in the presence of these anions shows an interesting relationship (Fig. 6). The logarithm of the relative affinity varies linearly with the relative enzyme activity (expressed as percent of the activity for Cl^-). This linear relationship applies for all anions tested with the exception of HCO_3^-. It appears that rather than an anion stimulation of the enzyme, there is an inhibition of its activity by anions, which is stronger the higher the affinity of the anion for the enzyme.

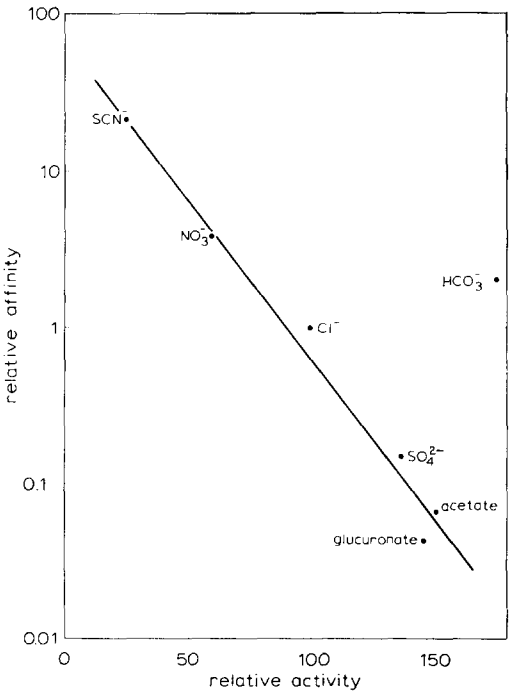

Fig. 6. Relationship between relative anion affinity of Mg^{2+}-ATPase (data from Table VI) and the relative enzyme activity (data from Table II; the activity with Cl^- at both pH values was set at 100) in the presence of each anion.

HCO_3^- is an exception in so far as the activity with HCO_3^- is about twice as high as would be excepted from its affinity to the enzyme. It is in the first instance attractive to postulate from these results a role of the enzyme in HCO_3^- transport and thus in acid secretion as suggested by Durbin and Kasbekar[6]. These authors found not only an activation of the enzyme by HCO_3^-, but also a small (8 %) activation by 5 mM Cl^- relative to the activity in 20 mM Tris glucuronate. On the other

hand, we obtained 33 % inhibition by Cl^- relative to the activity in media containing anions, like glucuronate and acetate, with a low affinity for the enzyme. Durbin and Kasbekar[6] postulated that this Mg^{2+}-ATPase would act in transmembrane HCO_3^-–Cl^- exchange in gastric mucosa, like the (Na^+–K^+)-ATPase system acts in active Na^+–K^+ exchange in most tissues. Their hypothesis was further elaborated by assuming a Cl^--activated phosphorylation, followed by a HCO_3^--activated dephosphorylation. SCN^- would act as inhibitor of the last step in the Mg^{2+}-ATPase reaction, just as ouabain is an inhibitor of the last step in the (Na^+-K^+)-ATPase reaction.

However, there are several differences between the two ATPases which plead against such a role of the Mg^{2+}-ATPase in anion transport. First, the (Na^+–K^+) ATPase system requires the presence of both Na^+ and K^+ in order to display any activity. The anion-sensitive Mg^{2+}-ATPase, on the other hand, shows activity in the presence of any single anion. The higher activity in the presence of HCO_3^- represents a single ion stimulation.

Secondly, whereas the (Na^+–K^+)-ATPase has a Na^+-activated phosphorylation step and a K^+-activated dephosphorylation step in its mechanism (ref. 1, p. 341), the presence of only a single anion-binding site for the Mg^{2+}-ATPase pleads against a Cl^--activated phosphorylation and a HCO_3^--activated dephosphorylation as suggested by Durbin and Kasbekar[6].

Thirdly, the inhibition of (Na^+–K^+)-ATPase by ouabain differs also considerably from that of Mg^{2+}-ATPase by SCN^-. The half-inhibitory concentration of ouabain for (Na^+–K^+)-ATPase is about three orders of magnitude lower than that of SCN^- for Mg^{2+}-ATPase. In (Na^+–K^+)-ATPase there are strong indications for the existence of a particular ouabain binding site, while the inhibition of SCN^- seems to be due to a high affinity of this anion for the general anion-binding site on the Mg^{2+}-ATPase.

Fourthly, another difference between the two ATPases is the ratio between ion transport and ATP hydrolysis. While for the (Na^+–K^+)-ATPase system a fixed molar ratio of three exists between cations transported and ATP hydrolyzed for a large variety of transport processes[1] (ref. 1, p. 272), the ratio between H^+ transported and ATP hydrolyzed by the Mg^{2+}-ATPase is only 0.06–0.17, as calculated from the data given by Kasbekar and Durbin[2] and Hansen *et al.*[4].

These points, while not excluding a role of this ATPase in gastric acid secretion, indicate that the enzyme cannot function in a similar manner as the (Na^+–K^+)-ATPase system operates in active cation transport. The experiments described in this paper, in particular the additional activation of the enzyme by HCO_3^- anions, support the suggestion that this enzyme has some relationship to HCO_3^- transport, although the mechanism is unclear.

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