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EFFECTS OF COMMON INHIBITORS OF GASTRIC ACID SECRETION ON SECRETAGOGUE-INDUCED RESPIRATION AND AMINOPYRINE ACCUMULATION IN ISOLATED GASTRIC GLANDS

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Summary

1. The effects of three inhibitors of gastric acid secretion, atropine, burimamide and thiocyanate, have been studied in isolated glands from the rabbit gastric mucosa. The glands were either resting or stimulated by carbachol, histamine or dibutyryl cyclic AMP. The effects were determined from changes in oxygen consumption and accumulation of the weak base aminopyrine. The latter gives an indirect measurement of the acid production in the glands.

2. Atropine (10^{-6} M) almost totally inhibited the transient response induced by carbachol (10^{-4} M) in both measured parameters. The histamine-induced increase in respiration was inhibited when the atropine concentration was raised to 10^{-4} M. To a lesser extent also, histamine-induced aminopyrine accumulation was reduced. The dibutyryl cyclic AMP stimulated oxygen consumption was not affected by atropine.

3. Burimamide competitively inhibited the histamine responses but was without effect on those of carbachol and dibutyryl cyclic AMP.

4. Thiocyanate (10^{-2} M) inhibited the increase in oxygen consumption induced by all three secretagogues but not down the prestimulatory level, in spite of a total abolishment of the aminopyrine accumulation.

5. In unstimulated glands, burimamide (10^{-3} M) or atropine (10^{-4} M) did not alter the normal aminopyrine ratio (aminopyrine in intraglandular water/aminopyrine in extraglandular water) of approximately 50. This may indicate the existence of preformed acid in resting parietal cells. Thiocyanate, on the other hand, lowered the aminopyrine ratio in unstimulated glands from 46 to 2.

Possible mechanisms for the thiocyanate effect are discussed in terms of an inability to separate acid and base in the secreting membrane.

Introduction

The recently developed method to prepare isolated glands from the rabbit gastric mucosa, has been shown to yield highly viable and morphologically well

preserved glandular cells [1]. Further investigations by Berglindh et al. [2] proved that the glandular parietal cells could be stimulated by histamine and dibutyryl cyclic AMP as judged by changes in oxygen consumption and accumulation of the weak base aminopyrine as well as alterations of the parietal cell morphology. The response to cholinergic stimulation did not resemble the in vivo behaviour, since it gave rise to a potent, but transient stimulation of both respiration and aminopyrine accumulation. The glands did not show any stimulatory response to pentagastrin.

Thus, the glandular behaviour in some respects resembles that of the intact mammalian stomach. The discrepancies that exist might be due to alterations or destruction of the cells and/or the receptors but could also be true signs of the obvious diversity between in vivo conditions and those in isolated gastric glands [2].

The aim of the present work was to further investigate the glandular functions by studying the effects of three well known gastric inhibitors: (a) atropine, a competitive inhibitor of the muscarinic action of acetylcholine; (b) burimamide, a histamine H_2-receptor antagonist and (c) thiocyanate, the mode of action of which has still not been clarified. The secretagogues used in connection with these inhibitors were: histamine, carbachol (carbamylcholine) and dibutyryl cyclic AMP.

Stimulation and inhibition of glandular activity were measured in terms of oxygen consumption, using a Warburg respirometer, and by indirect determination of acid production using the weak base aminopyrine, which accumulates in acid compartments.

Material and Methods

Chemicals. N^6-$O^{2'}$-Dibutyryl adenosine 3′ : 5′-cyclic monophosphoric acid (dibutyryl cyclic AMP, grade II) as sodium salt, carbamylcholine chloride (carbachol) and atropine sulfate were obtained from Sigma. Histamine-dihydrochloride was a product of Schuchardt (Munich). Sodium thiocyanate was obtained from Mallinckrodt. Burimamide was a generous gift from Dr. Parsons, Smith, Klein and French Laboratories Limited, U.K.

N-4-Dimethyl[^{14}C]aminopyrine with a specific activity of 3.9 Ci/mol was produced by NEN Chemicals.

Respiratory medium. NaCl 132.4 mM, KCl 5.4 mM, Na_2HPO_4 5.0 mM, NaH_2PO_4 1.0 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.0 mM, phenol red 10 mg/l, pH 7.4. Before use 2 mg/ml rabbit albumin (Sigma) and 2 mg/ml glucose were added.

Gland separation. For a detailed description of the preparation of isolated gastric glands see Berglindh and Öbrink [1] and Berglindh et al. [2]. Briefly, the method involves high pressure (>80 kPa) perfusion of the blood vessels of a rabbit's stomach, following which the free dissected mucosa was minced and the pieces treated with collagenase for 90 min at 37°C. The isolated glands were rinsed and finally resuspended in the respiratory medium. Each mucosa gave a large yield of gastric glands (approx. 150 mg dry weight) which made it possible to investigate several parameters in each glandular batch.

Respiratory measurements. The oxygen consumption of the glands was studied at 37°C in a Warburg respirometer, the gas phase being air. Two tech-

niques were employed, either the drugs were added to the flasks from the start of the incubation or else from a sidearm to preincubated glands [1,2]. The drugs were generally dissolved in the respiratory medium except for thiocyanate which was dissolved in distilled water to a concentration of 150 mM and added to give a final concentration of 10^{-2} M.

Aminopyrine accumulation. Aminopyrine is a weak base, which will be trapped and therefore accumulate in acid compartments. Up until now it has mainly been used for gastric blood flow determinations [3]. The theoretical background for aminopyrine accumulation in the isolated gastric glands was discussed in detail by Berglindh et al. [2].

Aminopyrine accumulation studies were performed in two different ways: (1) steady-state value determination with simultaneous respiratory measurements [2], (2) analyses of the aminopyrine accumulation kinetics.

In the first type of experiments 0.8 μCi aminopyrine/ml was added to the gland suspension. The glands were then transferred to Warburg flasks together with the drugs to be studied. After 90 min incubation at 37° C, the gland suspensions were transferred to pre-warmed (37° C) test tubes and spun down at 2000 $\times g$ for 2 min. The dried pellets were solubilized in 0.5 ml Soluene®, added to 15 ml Dimilume® and counted in a Beckman liquid scintillation counter (LS 250). 0.2 ml of each supernatant was added to Insta-Gel® and counted in the same way. The results were expressed as the ratio: aminopyrine concentration in intraglandular (intracellular) water to aminopyrine concentration in extraglandular water. The intraglandular water was determined from the finding by Berglindh and Öbrink [1] that the relationship between intraglandular water in mg and the gland dry weight in mg was fairly constant and approximately equal to 2 (see also Berglindh et al. [2]).

As reference for oxygen consumption and aminopyrine accumulation, unstimulated glands were treated in the same way.

In the second type of experiments the gland suspension from one preparation was divided into two 10—15 ml portions which were transferred to separate flasks. The flasks, left open to the air, were put in a 37° C waterbath and the suspensions were gently stirred with magnets. After a 30-min preincubation period, 0.4 μCi aminopyrine/ml suspension was added to the flasks and the glands were allowed to accumulate aminopyrine for 15—45 min. During this period, aliquots of 0.2 ml were repeatedly taken and the glands rapidly spun down in a centrifuge. 0.1 ml of the supernatant was transferred to a liquid scintillation vessel containing 10-ml Insta-Gel®. This whole procedure took less than 2 min.

Secretagogues and inhibitors alone or in combination, were then added to the flasks and their effects on aminopyrine accumulation were studied for a further 30—45 min as described above. This was generally followed by new drug additions for one or two consecutive periods with the same sampling technique. At the end of some experiments thiocyanate (10^{-2} M) was added and its effects studied for a final 30-min period.

The aminopyrine content in the glands was not examined in these kinetic studies. The figures of the aminopyrine concentration in the extracellular fluid will, however, indicate the aminopyrine accumulation since the glandular aminopyrine accumulation is of such a high magnitude [2], that it will be

possible to detect even small increments in the glandular accumulation as a decrease in the extracellular fluid, and vice versa. The small sample volumes made it possible to extend the experiments considerably and to take frequent samples. For comparative purposes, a 1.0-ml sample was taken at the end of each investigating period and transferred to a prewarmed test tube then processed as described for the steady-state type of accumulation study (see above).

In the investigation of the effect of atropine on carbachol-induced stimulation, which needed a shorter incubation time, the technique described by Berglindh et al. [2] for carbachol studies was adopted, where the aminopyrine ratio was measured in each sample.

Results

Respiratory studies

Burimamide. In unstimulated glands burimamide slightly decreased the oxygen consumption. At $2.4 \cdot 10^{-5}$ M this effect was not significant (—1.7%) whereas 10^{-4} M (Table I) and 10^{-3} M (Table IV) caused significant decreases of 7.7% and 14.1% respectively, below the control value.

To investigate the specific histamine receptor blocking properties which are

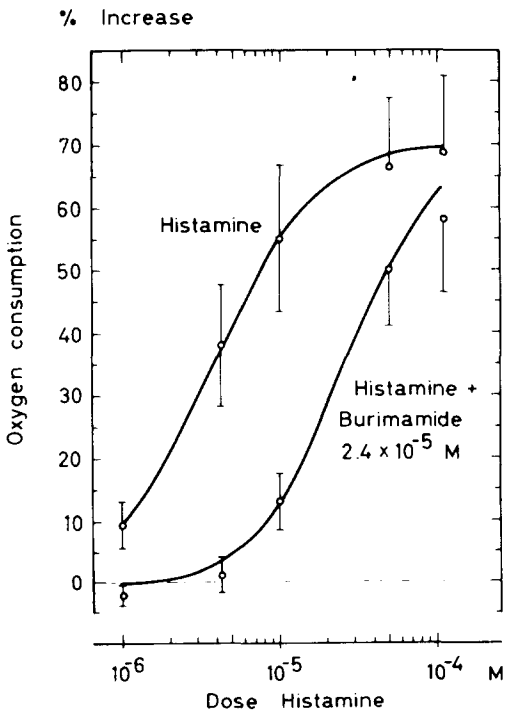

Fig. 1. Dose-response relationship between histamine concentration and oxygen consumption in isolated glands, with or without burimamide $2.4 \cdot 10^{-5}$ M. The vertical bars represent ± S.E. (n = 5). The untreated glands (the reference) had an oxygen consumption of 11.9 µl. O_2/mg dry wt. in 90 min. Burimamide per se lowered respiration by 1.7 ± 1.0%. The ED-50 (K_m) values were for histamine $3.9 \cdot 10^{-6}$ M and for histamine plus burimamide $2.6 \cdot 10^{-5}$ M. The curves are drawn according to the integral of the normal probability curve obtained by probit analysis.

TABLE I

EFFECTS OF ATROPINE AND BURIMAMIDE UPON DIBUTYRYL CYCLIC AMP-INDUCED GLANDULAR RESPIRATION

Each experiment was performed on glands from the same population, $n = 6$. The control gland preparation (the reference) had an oxygen consumption of 9.7 μl O_2/mg dry wt in 60 min. The figures show mean ± S.E. $P > 0.05$ is considered not significant (n.s.).

	1	2	3	4	5
Drugs added	Dibutyryl cyclic AMP $5 \cdot 10^{-4}$ M	Atropine 10^{-4} M	Burimamide 10^{-4} M	Dibutyryl cyclic AMP $5 \cdot 10^{-4}$ M + atropine 10^{-4} M	Dibutyryl cyclic AMP $5 \cdot 10^{-4}$ M + burimamide 10^{-4} M
% increase in oxygen consumption per 60 min ($n = 6$)	61.0 ± 5.1 $P < 0.01$	−7.0 ± 0.9 $P < 0.01$	−7.7 ± 1.6 $P < 0.01$	48.5 ± 6.0 $P < 0.01$	48.4 ± 2.7 $P < 0.01$
Statistical significance:	1—4, n.s.; 1—5, n.s.; (1+2) — 4, n.s.; (1+3) — 5, n.s.				

ascribed to burimamide, increasing doses of histamine were added to glands from the same population with or without $2.4 \cdot 10^{-5}$ M burimamide. The dose-response curves obtained are presented in Fig. 1.

From the figure it is obvious that the histamine curve is shifted to the right in the presence of burimamide. Since this curve did not reach its V with the histamine concentrations used, the V for each curve was determined from a linear transformation, $D/v = [(D/V) + (K_m/V)]$. (D, dose; v, response; K_m is a constant). The maximal response, V, calculated in this way did not significantly differ between the two curves. The curves in Fig. 1 are recalculated from probit analysis based on identical V. The K_m values for the curves were $3.9 \cdot 10^{-6}$ M and $2.6 \cdot 10^{-5}$ M for histamine and histamine plus burimamide respectively.

Even if the burimamide concentration was increased to 10^{-4} M no significant effect upon dibutyryl cyclic AMP ($5 \cdot 10^{-4}$ M) respiration was obtained. The small percentage decrease seen for dibutyryl cyclic AMP plus burimamide is probably explained by the inhibitory influence of burimamide per se upon unstimulated glands, see Table I.

On addition of $1.4 \cdot 10^{-4}$ M carbachol to glands preincubated with 10^{-4} M burimamide, no effect on the transient respiratory response was seen.

Atropine. Carbachol-induced stimulation was extremely sensitive to atropine. Thus the transient stimulation following addition of $1.4 \cdot 10^{-4}$ M carbachol was almost totally abolished, when the glands were preincubated with atropine 10^{-6} M as is seen in the histogram in Fig. 2. Due to the transient nature of the carbachol response, no investigation of the dose-dependent relationship between atropine and carbachol was attempted.

Atropine to some extent also inhibited the histamine-induced increase in respiration, but a concentration of 10^{-4} M had to be used. The degree of inhibition was, however, dependent on the histamine concentration. Thus, the response after $4.2 \cdot 10^{-6}$ M histamine was significantly decreased whereas that

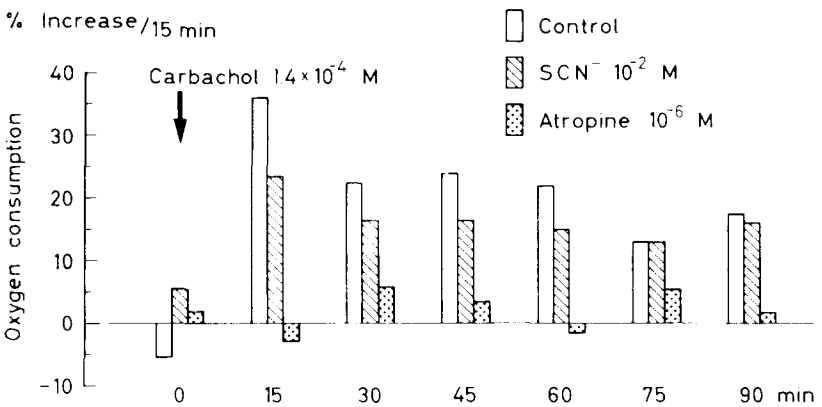

Fig. 2. Example of the carbachol effect upon the glandular respiration in the presence of SCN^- or atropine studied on the same gland population. Four flasks of each kind containing medium, thiocyanate or atropine respectively were incubated for 45 min. To two of each type, carbachol was added as indicated by the arrow, whereas the other two served as internal control. Each bar represents the mean of the two results as compared with their respective controls, which were recorded at the same time. A typical example from a series of 4 experiments is shown.

after $1.1 \cdot 10^{-5}$ M was not, see Table II.

Finally, atropine (10^{-4} M) was used together with $5 \cdot 10^{-4}$ M dibutyryl cyclic AMP. The stimulated oxygen consumption was not significantly affected, although atropine lowered the basal glandular respiration (Table I).

Thiocyanate. Thiocyanate (10^{-2} M) per se had an inhibitory effect on the unstimulated gland respiration, which in most cases was significant (Tables IIIa, b, c and Table IV). In respect to secretagogue stimulated increase in oxygen consumption, SCN^- (10^{-2} M) significantly inhibited the response independent of its drug origin. The effects of thiocyanate upon histamine-, dibutyryl cyclic AMP- and carbachol-induced respiration are listed in Tables IIIa, b, c. In all

TABLE II

EFFECT OF ATROPINE ON HISTAMINE STIMULATED GLANDULAR OXYGEN CONSUMPTION

Each experiment was performed on glands from the same population, $n = 8$. The control gland preparation (the reference) had an oxygen consumption of 13.3 μl O_2/mg dry wt. in 90 min. The figures show mean ± S.E. $P > 0.05$ is considered not significant (n.s.).

Drugs added	1	2	3	4	5
Drugs added	Histamine $4.2 \cdot 10^{-6}$ M	Histamine $1.1 \cdot 10^{-5}$ M	Atropine 10^{-4} M	Histamine $4.2 \cdot 10^{-6}$ M + atropine 10^{-4} M	Histamine $1.1 \cdot 10^{-5}$ M + atropine 10^{-4} M
% increase in oxygen consumption per 90 min ($n = 8$)	36.3 ± 6.1 $P < 0.01$	45.1 ± 4.8 $P < 0.01$	−5.9 ± 5.9 $P < 0.01$	17.9 ± 5.4 $P < 0.01$	36.0 ± 5.7 $P < 0.01$
Statistical significance:	1—4, $P < 0.01$; 2—5, $0.01 < P < 0.02$ (1+3) — 4, $0.02 < P < 0.05$; (2+3) — 5, n.s.				

TABLE IIIa

EFFECT OF SCN^- ON HISTAMINE INDUCED GLANDULAR OXYGEN CONSUMPTION

Each experiment was performed on glands from the same population, $n = 8$. The control gland preparation (the reference) had an oxygen consumption of 12.4 μl O_2/mg dry wt. in 90 min. The figures show mean ± S.E. $P > 0.05$ is considered not significant (n.s.).

	1	2	3	4	5
Drug added	Histamine $4.2 \cdot 10^{-6}$ M	Histamine $1.1 \cdot 10^{-5}$ M	SCN^- 10^{-2} M	Histamine $4.2 \cdot 10^{-6}$ M + SCN^- 10^{-2} M	Histamine $1.1 \cdot 10^{-5}$ M + SCN^- 10^{-2} M
% increase in oxygen consumption per 90 min ($n = 8$)	56.0 ± 13.0 $P < 0.01$	69.0 ± 16.4 $P < 0.01$	−6.9 ± 3.5 $P < 0.05$	8.0 ± 5.6 n.s.	17.4 ± 8.4 $P < 0.01$
Statistical significance:	1—4, $P < 0.01$; 2—5, $P < 0.01$; 4—3, $P < 0.01$ (1+3) — 4, $P < 0.01$; (2+3) — 5, $P < 0.01$				

TABLE IIIb

EFFECT OF SCN^- ON DIBUTYRYL CYCLIC AMP-STIMULATED GLANDULAR OXYGEN CONSUMPTION

The control gland preparation (the reference) had an oxygen consumption of 12.1 μl O_2/mg dry wt. in 90 min. For details see text Table IIIa.

	1	2	3
Drug added	Dibutyryl cyclic AMP $5 \cdot 10^{-4}$ M	SCN^- 10^{-2} M	Dibutyryl cyclic AMP $5 \cdot 10^{-4}$ M + SCN^- 10^{-2} M
% increase in oxygen consumption per 90 min ($n = 6$)	83.0 ± 16.9 $P < 0.01$	−10.2 ± 2.2 $P < 0.01$	27.7 ± 12.1 $P < 0.01$
Statistical significance:	1—3, $P < 0.01$ (1+2) — 3, $P < 0.01$		

TABLE IIIc

EFFECT OF SCN^- ON CARBACHOL-STIMULATED GLANDULAR OXYGEN CONSUMPTION

The control gland preparation (the reference) had an oxygen consumption of 11.6 μl O_2/mg dry wt. in 90 min. For details see text Table IIIa.

	1	2	3
Drug added	Carbachol $6.9 \cdot 10^{-5}$ M	SCN^- 10^{-2} M	Carbachol $6.9 \cdot 10^{-5}$ M + SCN^- 10^{-2} M
% increase in oxygen consumption per 90 min ($n = 5$)	24.6 ± 6.4 $P < 0.01$	−5.5 ± 3.1 n.s.	6.7 ± 3.6 $0.02 < P < 0.05$
Statistical significance:	1—3, $P < 0.01$ (1+2) — 3, $0.01 < P < 0.02$		

cases but for the low histamine concentration the results were significantly above the unstimulated control level. The influence of thiocyanate on the carbachol-induced transient response is presented in Fig. 2, where carbachol was added from the side-arm to glands preincubated with SCN^-. Thiocyanate decreased the transient respiratory pattern, and in all experiments the inhibition appeared to be most pronounced for the initial peak response (the first 15 min).

Aminopyrine accumulation

In a previous study unstimulated glands were shown to accumulate aminopyrine at a high rate giving a final accumulation ratio of 30—50 [2]. Since this could be due to a spontaneous secretion, the aminopyrine ratio in unstimulated glands was also investigated after treatment with atropine, burimamide or thiocyanate. The results obtained are presented in Table IV.

Neither atropine nor burimamide, both in high concentrations, affected the spontaneous aminopyrine accumulation. NaSCN, 10^{-2} M, on the other hand, totally abolished the accumulation, lowering the ratio from 46 to 2. For comparison the aminopyrine ratio in glands incubated for approx. 10 min at room temperature is also listed in Table IV. This ratio of 5.6 did not increase appreciably upon prolonged incubation, probably due to the low metabolic rate at room temperature. In spite of the divergent accumulation results both burimamide and thiocyanate inhibited oxygen consumption significantly and roughly to the same extent, and to a higher degree than did atropine.

In the kinetic studies, aminopyrine was normally added after 30 min preincubation (except for carbachol plus atropine, see Material and Methods). The decrease in aminopyrine content in the extracellular fluid (i.e. increase in glandular accumulation) was followed for 15—45 min. In most cases the spontaneous accumulation did not fully reach a steady state within 45 min.

TABLE IV

AMINOPYRINE ACCUMULATED AND OXYGEN CONSUMPTION IN GLANDS TREATED WITH THIOCYANATE, BURIMAMIDE OR ATROPINE

The results are obtained from paired samples of glands from the same populations. The figures show mean ± S.E. $P > 0.05$ is considered not significant (n.s.). The ratio ICW/ECW means aminopyrine in intracellular water/aminopyrine in extracellular water.

Condition	Control	SCN^- 10^{-2} M	Control	Burimamide 10^{-3} M	Control	Atropine 10^{-4} M	Control before incubation
	(n = 8)	(n = 8)	(n = 6)	(n = 6)	(n = 5)	(n = 5)	(n = 16)
% change in oxygen consumption per 60 min	0	−17.2 ± 3.3 $P < 0.01$	0	−14.1 ± 6.4 $P < 0.01$	0	−6.5 ± 1.6 $P < 0.01$	—
Aminopyrine accumulation ratio ICW/ECW	45.8 ± 6.1	2.0 ± 0.1 $P < 0.01$	49.3 ± 6.6	51.0 ± 5.9 n.s.	32.8 ± 1.2	29.8 ± 1.5 n.s.	5.6 ± 0.(

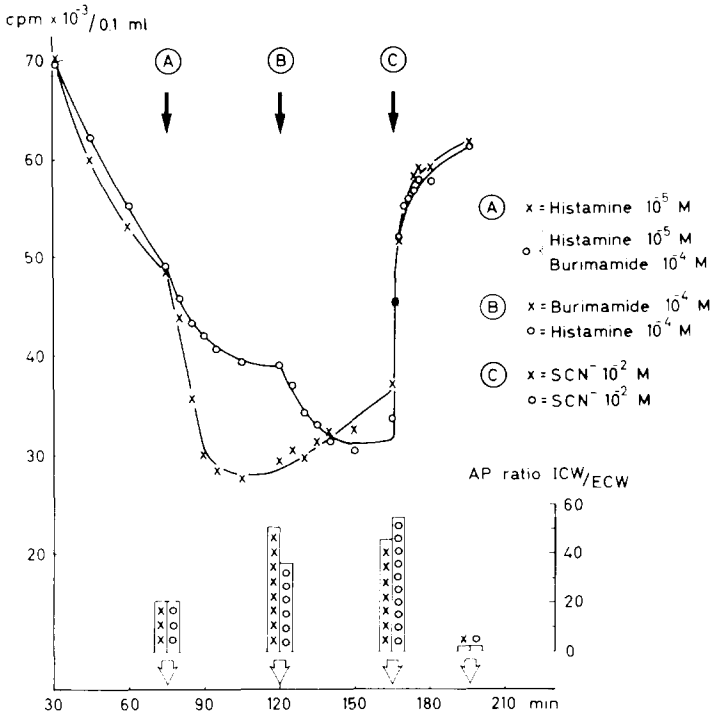

Fig. 3. The influence of histamine, histamine plus burimamide and SCN^- upon aminopyrine (AP) accumulation, determined from changes in the aminopyrine content of 0.1 ml extraglandular fluid. After 30 min preincubation aminopyrine was added to both flasks. The aminopyrine ratios, aminopyrine in extracellular water/aminopyrine in intracellular water (ICW/ECW) were determined 1 min before each drug addition (A, B, C) and at the end of the experiments as indicated by the bars. In this experiment the concentration of glands in each flask was 6.5 mg dry wt./ml suspension. A typical example from a series of 5 experiments is shown.

Upon addition of the different secretagogues, however, the accumulation rapidly increased, resulting in a much steeper slope in the extracellular aminopyrine disappearance curve. For histamine and dibuturyl cyclic AMP, accumulation reached a maximum after 30—40 min and then remained roughly constant, whereas carbachol as described before [2] showed a transient course in the accumulation. The influence of the inhibitors upon the accumulation courses were as follows.

Burimamide 10^{-4} M did not affect the spontaneous aminopyrine accumulation in the glands (see Fig. 4). When added together with histamine (A in Fig. 3) it caused a pronounced inhibition of the normal histamine response. If the histamine concentration was raised from 10^{-5} M to 10^{-4} M the inhibition was clearly suppressed. Addition of burimamide to the histamine flask reduced accumulation (B in Fig. 3).

The transient type of accumulation after carbachol was not influenced by the presence of burimamide 10^{-4} M, see Fig. 4.

Atropine at a concentration of 10^{-4} M but not 10^{-6} M to some extent inhibited the spontaneous rate of accumulation (see Fig. 6).

As seen in Fig. 5, the peak accumulation response following addition of 1.4 ·

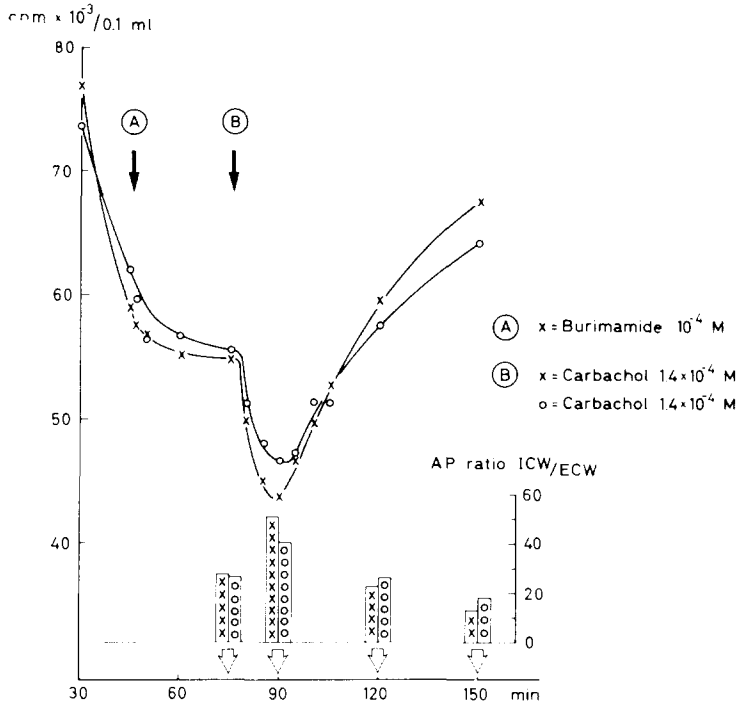

Fig. 4. The influence of burimamide, carbachol and carbachol plus burimamide upon aminopyrine (AP) accumulation, determined as changes in the aminopyrine content of 0.1 ml extraglandular fluid. The aminopyrine ratios were measured at times indicated by the bars. The concentration of glands in each flask was 6.5 mg dry wt./ml suspension. A typical example from a series of 4 experiments is shown.

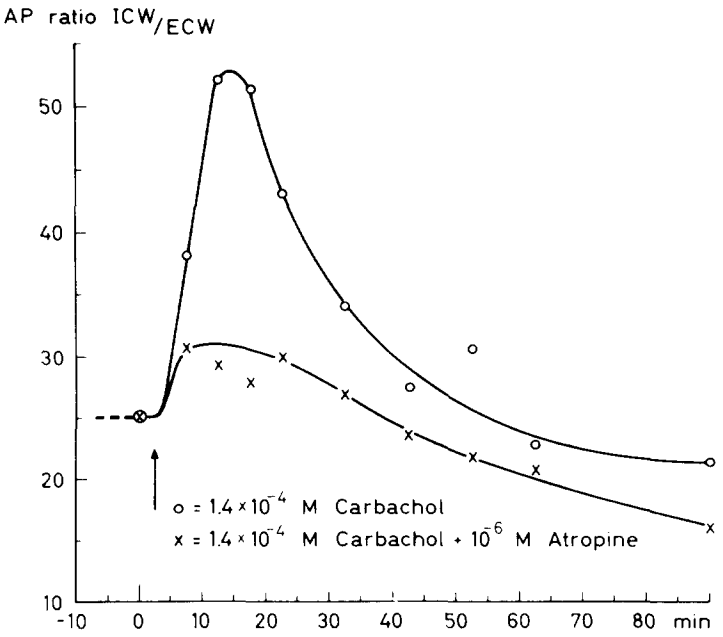

Fig. 5. The effect of atropine on carbachol stimulated aminopyrine (AP) accumulation, determined as aminopyrine ratios. The aminopyrine was added from the start to the two flasks and one determination was made after 30 min, just before addition of the drugs as indicated by the arrow. A typical example from a series of 5 experiments is shown.

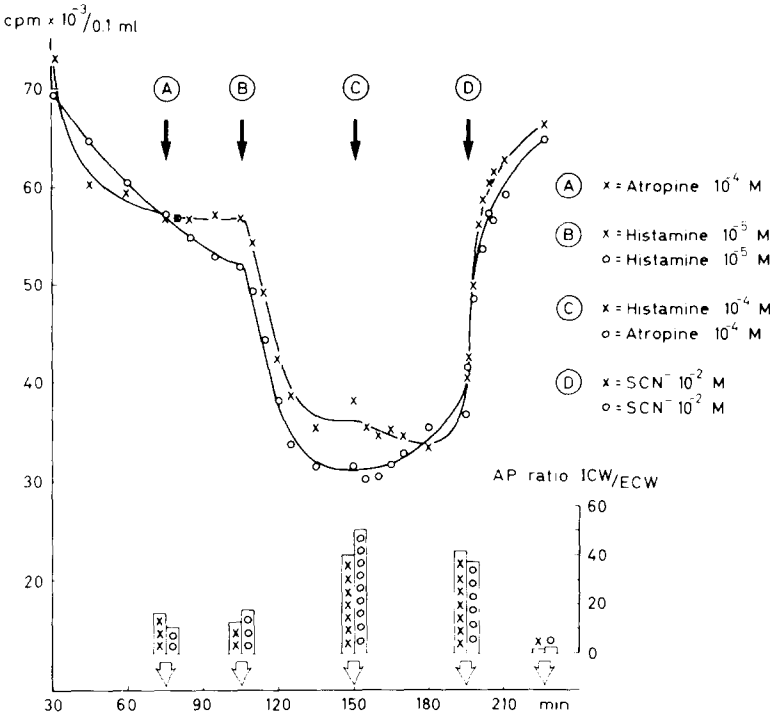

Fig. 6. The influence of atropine, histamine, histamine plus atropine and SCN^- upon aminopyrine (AP) accumulation, determined as changes in the aminopyrine content of 0.1 ml extraglandular fluid. The aminopyrine ratios were measured 1 min before addition of each drug (A, B, C, D) and at the end of the experiment, as indicated by the bars. The amount of glands was 9.5 dry wt./ml suspension. A typical example from a series of 6 experiments is shown.

10^{-4} M carbachol was strongly suppressed by atropine 10^{-6} M. The final part of both curves showed an aminopyrine ratio which was well below the initial value, an effect of carbachol which was also observed by Berglindh et al. [2].

The pretreatment of the glands with atropine 10^{-4} M, had only a small inhibiting effect upon histamine-induced accumulation (B in Fig. 6). This inhibition could be partly overcome by increasing the histamine concentration from 10^{-5} M to 10^{-4} M (C in Fig. 6). Simultaneous addition of atropine to the histamine flask somewhat decreased the control histamine induced accumulation.

Thiocyanate exhibited a strong inhibiting effect upon the aminopyrine accumulation in unstimulated glands as shown in Table IV. The inhibitory effect of SCN^- was, however, equally powerful when added to stimulated glands irrespective of what secretagogue was used. SCN^- (10^{-2} M), was usually added towards the end of the accumulation period. In all cases, as indicated by Figs. 3 and 6, aminopyrine accumulation decreased very rapidly, usually reaching an aminopyrine ratio around 2, 30 min after the addition of SCN^-.

In Fig. 7, the effect of a lower SCN^- concentration (10^{-3} M), is presented. The spontaneous aminopyrine accumulation in unstimulated glands was arrested and almost totally reversed upon SCN^- addition (A in Fig. 7), giving an aminopyrine ratio at 75 min of 2.9 as compared with 30.8 for the control. The addi-

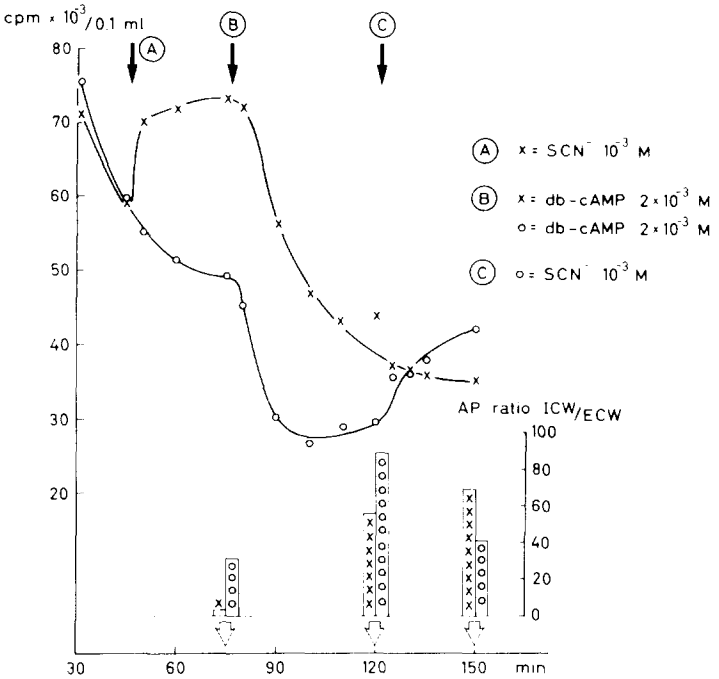

Fig. 7. Effect of a lower concentration of SCN^- (10^{-3} M) upon spontaneous- and dibutyryl cyclic AMP-(db-cAMP) stimulated aminopyrine (AP) accumulation. The aminopyrine ratios were measured at times indicated by the bars. The concentration of glands in each flask was 7.1 mg dry wt./ml suspension. A typical example from a series of 3 experiments is shown.

tion of dibutyryl cyclic AMP ($2 \cdot 10^{-3}$ M) to both flasks (B in Fig. 7) induced a substantial accumulation which for the SCN^- flask unlike the control, needed a long time (more than 70 min) to reach a steady state. Finally, addition of 10^{-3} M SCN^- to the control dibutyryl cyclic AMP stimulated preparation (C in Fig. 7) caused the glands to lose some aminopyrine, but not to the extent as seen after 10^{-2} M SCN^-.

Discussion

The indirect methods used were chosen to reflect changes occurring in the parietal cells. The parietal cells amount to approximately 50% of the total cell volume of the glands [1] and due to their very high mitochondrial content (about 40% of the cell volume [4]) any major change in the glandular respiratory rate must be of parietal cell origin [1]. Aminopyrine accumulates solely due to pH gradients; the molecule being ionized and less membrane permeable in an acid environment [2,3]. In a pre-study tissue slices from heart, liver, kidney, gastric antrum and gastric fundus were tested. As expected only the fundic (acid secreting) part of the stomach had the ability to accumulate aminopyrine (Berglindh, T., unpublished results).

All experiments were carried out in the absence of CO_2. Since in the frog gastric mucosa exogenous CO_2 is necessary for optimal acid secretion [5], the

glandular parietal cells most probably work below their maximal capacity [1].

Results obtained from previous works with isolated gastric glands [1,2] have given information about some of their "normal" properties. The present investigation was designed to further explore the receptor specificity for histamine and carbachol using the specific inhibitors burimamide and atropine and to study some mechanisms concerning the inhibition of HCl production.

The results obtained from the intact mucosa do not directly support the specificity of these inhibitors. Burimamide, when first presented by Black et al. [6], was shown to inhibit both histamine- and pentagastrin-induced secretion in rat, cat and dog and food-induced secretion in the dog, whereas vagal- or carbachol-stimulation was not inhibited. In the frog gastric mucosa, however, mecholyl-stimulated secretion was counteracted [7]. Atropine, besides being a potent inhibitor of cholinergic stimuli, also diminish pentagastrin stimulation in the dog and, to a lesser degree, histamine stimulation [8]. To clarify these complex responses the isolated glands may be of help. As was discussed in previous papers [1,2], the isolated glands should be expected only to respond to the direct action of secretagogues or inhibitors. Thus, it is possible that the transient nature of the carbachol stimulation and the inability of pentagastrin to stimulate the isolated glands are consequences of such a "reduced" system [2]. The hypothesis that gastrin-stimulated secretion is mediated via release of histamine (cf. Kahlson et al. ref. 9), would be consistent with the experimental findings with burimamide in vivo and with the inability of pentagastrin to stimulate the isolated glands [2]. The aminopyrine accumulation method is of a quantitative type, which reflects the product of acidity and volume of some acid intaglandular fluid. Thus changes in the accumulation ratio can occur due to changes in pH, in volume or in both. Aminopyrine is freely permeable in the unionized form, at a neutral pH, and will rapidly enter through the blood-side membrane of the parietal cells, the large surface of which is exposed directly to the surrounding medium. Inside the cell, aminopyrine will be concentrated within acid compartments and transported into the lumen as part of the secretion. Thus in a situation of brisk secretion, concentrated aminopyrine would be found in the secretory canaliculi of the parietal cells and in the glandular lumen, whereas at low secretory rates only minor accumulation in the lumen is to be expected due to dilution and rapid diffusion out of the lumen [2].

In histamine-stimulated glands, burimamide behaved as a potent competitive inhibitor. In vitro, histamine has been shown to activate adenylate cyclase from gastric tissue of guinea pig [10] and rabbit [11]. This stimulation could be inhibited by H_2-receptor blockers [11]. Karppanen and Westermann [12] measuring cyclic AMP content in minced pieces of gastric tissue from guinea pig, obtained similar types of dose-response curves after stimulation with histamine and histamine plus burimamide (in the presence of theophylline), as presently found for the respiratory response. This seems to indicate that the H_2-receptor is firmly associated to the adenylate cyclase of gastric mucosa.

In the present and in a previous study [2] unstimulated glands were shown to accumulate aminopyrine 30—50 times, indicating the presence of acid. Although the unstimulated parietal cells did appear resting [2], a small spontaneous secretion could not be excluded. It was therefore of interest to try to determine the secretory state of these parietal cells. Burimamide (10^{-3} M)

has been shown to totally inhibit both spontaneous acid secretion in the frog gastric mucosa [7], as well as histamine stimulated rabbit gastric adenylate cyclase activity [11]. The high aminopyrine accumulation found in unstimulated glands was, however, not affected by burimamide and the same observation was made after atropine treatment (Table IV). There was however a small decrease in oxygen consumption, which could be a sign of secretory inhibition. If so, a morphological transformation of the parietal cells into a more resting would have occurred without changes in the aminopyrine accumulation, which is theoretically possible (see above). Alternatively, the respiratory response could be unspecific and due to the high drug concentrations. In either case, the results indicate the existence of an acid formation in presumably resting parietal cells, which is not susceptible to receptor antagonists, and as discussed by Berglindh et al. [2] the only conceivable production site for such an acid pool would be the tubulo-vesicles of the non-secreting parietal cell.

The high intraglandular aminopyrine concentration in spite of an expected low adenylate cyclase activity, raises the question of the function of cyclic AMP. It indicates the possibility that the primary function of the cyclic AMP is not in the formation of HCl, but in the morphological transformation from tubulo-vesicles to secretory canaliculi. Dibutyryl cyclic AMP has been shown to distinctly alter the parietal cell morphology of the isolated glands into a secretory pattern [2].

Burimamide did not inhibit the stimulation induced by carbachol or dibutyryl cyclic AMP. For carbachol this is in agreement with the findings of Black et al. [4] and thus confirms the specificity of burimamide. Metiamide (a second generation, more potent H_2-receptor blocker [13]) has, however, been shown to inhibit cholinergic stimulation in vivo, one fact which led Grossman and Konturek [14] to suggest that histamine-, cholinergic- and gastrin-receptors interact on the parietal cell, making one specific antagonist interfer with the other receptors. Metiamide has not yet been tried on the gland preparation, but the inability of burimamide to inhibit carbachol stimulation and the negative results of pentagastrin [2] do not support that hypothesis.

Atropine was a potent inhibitor of carbachol stimulation. The type of inhibition was not investigated, due to the transient response pattern which did not give any steady-state values. It has been stated, however, that in frog gastric mucosa, atropine gives a competitive inhibition of acethylcholine stimulation [15]. From the present investigation it seems clear that the cholinergic responses measured here are true receptor mediated ones. Furthermore, the parallel behavior of oxygen consumption and aminopyrine accumulation both for the normal [2] and the atropine inhibited response, strongly favours the parietal cells as the main source of the transient stimulation.

In contrast, the histamine stimulation was much less sensitive to atropine. The type of inhibition is not quite obvious, but at least an indication was obtained from a linear transformation of the results which showed that the inhibition could be of a competitive nature. The aminopyrine studies seem to support such an assumption.

In the frog gastric mucosa Thorpe and Durbin [15] found that a very large concentration of atropine (greater than 10^{-3} M) had to be applied before histamine stimulation was inhibited. At that concentration, however, they

stated that atropine might exhibit some degree of competition with histamine. In dogs with Pavlov pouches or fistulae, both Linde [16] and Hirschowitz et al. [17] found atropine to be a largely competitive inhibitor of histamine stimulation. Those results must however be interpreted with some caution, since in the innervated stomach there is a normal cholinergic background, which is missing in the isolated frog gastric mucosa and in the isolated glands. Thus at this stage it is not clear, what degree of specificity lies behind the inhibitory action of atropine on histamine stimulated secretion.

Finally, atropine did not significantly affect dibutyryl cyclic AMP-induced respiration. The possibility remains however that atropine would inhibit at lower dibutyryl cyclic AMP concentrations (in parallel to the histamine behavior) but this has not yet been investigated. In fact Nakajima et al. [18] found an inhibitory effect of atropine on dibutyryl cyclic AMP stimulated *Necturus* gastric secretion.

Thiocyanate in a concentration of 10^{-2} M abolished both the normal spontaneous as well as the secretagogue-induced aminopyrine accumulation. Thus the aminopyrine accumulation data for SCN^- brings us one step further, i.e. not only as in the case of burimamide and atropine was the stimulated acid secretion inhibited, but the very intracellular H^+ accumulation was affected. Since the inhibition was independent of the type of secretagogue, thiocyanate seems to act at a very critical point in HCl production, which ought to be reflected in the oxygen consumption. Several investigators have found, however, that the respiratory changes are usually relatively small. In the frog gastric mucosa, Forte and Davies [19] and Bannister [20] found a diminishing oxygen consumption with decreasing HCl secretion after SCN^- addition, whereas Hersey [21] found no changes or a small increase in the respiration. In a segment of the dog stomach with intact blood supply no decrease was obtained in histamine-stimulated respiration in spite of almost total inhibition of HCl secretion [22].

Forte and Davies [19] found 10^{-3} M SCN^- to give an 80% reduction in HCl secretion with only marginal inhibition of respiration, whereas 10^{-2} M or more was needed for total inhibition of HCl production. They suggested that high concentrations could interfere with parts of the over-all metabolism other than those directly concerned with acid secretion.

In the isolated glands 10^{-2} M SCN^- was needed for total inhibition of the stimulated aminopyrine accumulation whereas 10^{-3} M was sufficient for the spontaneous accumulation (Fig. 7). 10^{-2} M caused a pronounced inhibition of the secretagogue-induced respiration to roughly the same degree as reported in some investigations of the amphibian gastric mucosa [19,20]. The inhibition, which was dependent on the dose of SCN^- and independent of the sequence of drug addition was, however, never complete. Thus in spite of the divergent results obtained from different investigations (see above) indicating a possible unspecific effect on the respiration due to SCN^- treatment, it seems possible to obtain a total inhibition of the glandular acidity (the aminopyrine ratio) without the corresponding influence on oxygen consumption.

Moody [22] suggested that the SCN^--inhibition occurred beyond the point of supply and utilization of substrates of the acid secretory process. Hersey [21] explained the lack of correlation between respiration and acid secretion in

terms of an uncoupling effect of SCN^-. Since there is no reduction in the high energy phosphate compounds after SCN^- [23], Hersey suggested that SCN^- could be a specific uncoupler of the respiration needed for acid production [21].

The apparent acidity seen in both resting and stimulated isolated gastric glands is presumably created by the membranes forming the tubulo-vesicles and secretory canaliculi. For each H^+ secreted on the mucosal side of the stomach there is a HCO_3^- appearing on the serosal side, as shown by Teorell [24]. Thus somewhere in the secretory process there must be a separation of hydrogen ion and base. The hydrogen ion is secreted into the vesicles or the canaliculi, while the base is delivered to the cytoplasm. This latter process is reflected by an increase in the cytoplasmic pH after histamine stimulation [25]. The thiocyanate inhibition of hydrogen ion accumulation could then be interpreted as an inability to keep acid and base separated in or by the secretory membrane. The expected lower cytoplasmic pH has been shown by Hersey [25]. The small effect on respiration could mean that energy is still needed for the production of H^+ in spite of the immediate neutralization of the product, due to inadequate separation of acid and base.

How such a "reunion" could arise is at present completely unknown, since we do not known the mechanism behind hydrogen ion production. Sachs et al. [26] have presented a mixed redox-ATPase mechanism, involving the HCO_3^--stimulated, SCN^--inhibited ATPase found in parietal cells. They suggested that this ATPase was responsible for the handling of the base, if inhibited by SCN^- the acid and the base could reunite. Doubts have, however, arisen about the importance of this enzyme [21,27].

From the kinetic type of experiments (Fig. 3, 6) it is evident that the glandular aminopyrine content decreases very drastically upon addition of SCN^-. Thus the concentration of aminopyrine in the extracellular fluid increased from 34 000 cpm/0.1 ml to 52 000 cpm/0.1 ml within 3 min (Fig. 3). Thus to understand how SCN^- exerts its inhibitory effect we must now look for an extremely rapid mechanism for the destruction of the H^+ gradient.

Conclusion

The histamine- and cholinergic-receptors on the parietal cell appear to be regulatory devices for the increase in HCl production without being connected to the basal activity (the "resting" H^+ formation). Thiocyanate affects a crucial point in the very formation of the hydrogen ions.

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References

1 Berglindh, T. and Öbrink, K.J. (1976) Acta Physiol. Scand. 96, 150—159
2 Berglindh, T., Helander, H.F. and Öbrink, K.J. (1976) Acta Physiol. Scand. 97, 401—414
3 Jacobsen, E.D., Linford, R.H. and Grossman, M.I. (1966) J. Clin. Invest. 45, 1—13
4 Helander, H.F. (1969) Gastroenterology 56, 35—52
5 Kidder, G.W. and Montgomery, C.W. (1974) Am. J. Physiol. 227, 300—304
6 Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R. and Parsons, E.M. (1972) Nature 236, 385—390
7 Shoemaker, R.L., Buckner, E., Spenney, J.G. and Sachs, G. (1974) Am. J. Physiol. 226, 898—902
8 Hirschowitz, B.I. and Sachs, G. (1969) Gastroenterology 59, 693—702
9 Kahlson, G., Rosengren, E. and Svensson, S.E. (1973) in International Encyclopedia of Pharmacology and Therapeutics, Sect. 39 (a) Pharmacology of Gastrointestinal Motility and Secretion (Holton, P., ed.), Vol. 1, pp. 41—102, Pergamon Press, Oxford
10 Perrier, C.V. and Laster, L. (1970) J. Clin. Invest. 49, 73a
11 Sung, C.P., Jenkins, B.C., Burns, L.R., Hackney, V., Spenney, J.G., Sachs, G. and Wiebelhaus, V.D. (1973) Am. J. Physiol. 225, 1359—1363
12 Karppanen, H.O. and Westermann, E. (1973) Naunyn Schmiedeberg's Arch. Pharmacol. 279, 83—87
13 Black, J.W., Duncan, W.A.M., Emmett, J.C., Ganellin, C.R., Hesselbo, T., Parsons, M.E. and Wyllie, J.H. (1973) Agents and Actions 3, 133—137
14 Grossman, M.I. and Konturek, S.J. (1974) Gastroenterology 66, 517—521
15 Thorpe, C.D. and Durbin, R.P. (1972) Gastroenterology 62, 1153—1158
16 Linde, S. (1950) Acta Physiol. Scand. 21, suppl. 74
17 Hirschowitz, B.I., Hutchison, G. and Sachs, G. (1972) Am. J. Physiol. 222, 1316—1321
18 Nakajima, S., Shoemaker, R.L., Hirschowitz, B.I. and Sachs, G. (1970) Am. J. Physiol. 219, 1259—1262
19 Forte, J.G. and Davies, R.E. (1964) Am. J. Physiol. 206, 218—222
20 Bannister, W.H. (1964) Nature 203, 978—979
21 Hersey, S.J. (1974) Biochim. Biophys. Acta 344, 157—203
22 Moody, F.G. (1968) Am. J. Physiol. 215, 127—131
23 Forte, J.G., Adams, P.H. and Davies, R.E. (1965) Biochim. Biophys. Acta 104, 25—38
24 Teorell, T. (1951) J. Physiol. 114, 267—276
25 Hersey, S.J. (1971) Phil. Trans. R. Soc. Lond. B 262, 261—275
26 Sachs, G., Shah, G., Strych, A., Cline, G. and Hirschowitz, B.I. (1972) Biochim. Biophys. Acta 266, 625—638
27 Ganser, A.L. and Forte, J.G. (1973) Biochim. Biophys. Acta 307, 169—180