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A POSSIBLE ROLE FOR CYCLIC ADENOSINE 3′,5′-MONOPHOSPHATE IN THE REGULATION OF ACID SECRETION IN THE ISOLATED STOMACH OF GUINEA-PIG

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1 The rate of acid secretion and mucosal cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content have been measured on the same guinea-pig isolated stomach preparation in response to histamine, theophylline and ICI 63197, a potent phosphodiesterase inhibitor.

2 Unstimulated control tissues had a spontaneous rate of acid secretion of 74.41 ± 9.06 μmol H^+/g wet wt. of mucosa per hour (s.e. mean, $n=20$) and a cyclic AMP content of 0.517 ± 0.058 nmol/g wet weight.

3 Each of the three drugs caused an increase in both the mucosal cyclic AMP content and the rate of acid secretion. These increases were linearly related to the logarithm of drug concentration for each drug.

4 There were no statistically significant differences between the three regression coefficients obtained for acid on drug and for cyclic AMP on drug.

5 There was a significant correlation between the rate of acid secretion and mucosal cyclic AMP content in stimulated preparations ($P < 0.001$) and also in control preparations which received no drug ($P < 0.05$).

6 These results are discussed in relation to the possible role of cyclic AMP in the mediation of acid secretory responses in the mammalian stomach.

Introduction

Cyclic adenosine 3′,5′-monophosphate (cyclic AMP) has been implicated as the intracellular mediator of a diverse range of physiological responses. Its possible role as a mediator of acid secretion in the mammalian stomach has been recently reviewed (Amer, 1972; 1974; Kimberg, 1974). Amer concluded that increased cyclic AMP in the stomach was associated with a decrease in acid secretion. He explained the stimulatory effects of phosphodiesterase inhibitors in terms of changes in the microcirculation to the mucosa and alterations in the calcium ion permeability of the cells. However, Kimberg concluded that, in some mammalian species at least, acid secretion was associated with an increase in cyclic AMP. More recent publications (Ruoff & Sewing, 1974; 1975; Katsumata & Glick, 1975) have added further support to the hypothesis that increased acid secretion is associated with increased cyclic AMP in the rat stomach. If acid secretion is regulated by the cyclic AMP content of the mucosa one would expect there to be a quantitative relationship between mucosal cyclic AMP and the rate of acid secretion. This paper describes experiments in which the rate of acid secretion and the mucosal cyclic AMP content have been measured on the same guinea-pig isolated stomach preparation. The use of an *in vitro* preparation has the advantage that the results are not complicated by changes in either mucosal blood flow or circulating hormone levels which might occur in an intact animal. Some preliminary results have been communicated to the Physiological Society (Canfield, Curwain & Spencer, 1976).

Methods

The preparation and general procedures are those described previously by Holton & Spencer (1976). The stomach was divided into two halves along the greater curvature and each half was tied over the end of a short perspex tube, mucosal surface facing the lumen. The muscle coat was not removed. The serosal surface was bathed with a bicarbonate buffered saline and gassed with 95% O_2 and 5% CO_2 whilst the mucosal surface was bathed with a similar but unbuffered saline gassed with 100% O_2. In the current

series of experiments the volume of serosal bathing fluid was 30 ml and the rate of acid secretion was determined by titration with 20 mM NaOH. Following dissection, the preparations were set up in the tissue bath and gassed for 1 h before addition of drugs. The mucosal saline was changed every 15 min and the rate of spontaneous acid secretion determined. After the addition of drugs, acid secretion was measured for four further 15 min periods. The rate associated with the drug was taken as the average of the last two such periods. Tissues were then taken from the bath and placed in ice cold saline before extraction for cyclic AMP assay.

Tissue extraction and cyclic AMP assay

Each tissue was pinned out on a board that had been cooled to −18°C. The tissue was blotted to remove surface moisture and the mucosal layer quickly scraped off, transferred to a glass cover slip and kept at 4°C. The scraped mucosa was quickly weighed and transferred to a plastic centrifuge tube in an ice-water bath. The remaining procedures were carried out at 0°C. The tissue was homogenized in the tube with 0.5 ml of 0.5 M perchloric acid and then neutralized with 0.5 ml of 0.5 M K_2CO_3. The mixture was centrifuged for 30 min at 2000 *g* at 0°C; 0.5 ml of the supernatant was added to another tube containing 0.1 ml of cold 0.5 M Tris/HCl buffer, pH 7.5, and stored at −18°C until assayed. Cyclic AMP was usually measured within two days by an isotope dilution technique. This was obtained in kit form and had a limit of detection of 0.05 pmol/50 µl of sample. The coefficient of variation for repeated measurements given by manufacturers is less than 7% over the range 0.5–14 pmol/50 µl sample. (The cyclic AMP assay. Radiochemical Centre, Amersham, 1975). Tritium was counted on a Hewlett-Packard Tricarb liquid scintillation counter using a methanol/toluene scintillator.

Drugs and chemicals

The following were used as stimulants; theophylline hydrate (BDH), 2 amino-6-methyl-5-oxo-4-*n*-propyl-4,5-didhydro-*s*-triazolo (1,5-a) pyrimidine (ICI 63197), histamine acid phosphate (1 mg/ml; Macarthys) and dibutyryl cyclic AMP (Sigma). These were always added to the serosal bathing fluid.

Experimental design

The tissue bath accomodated 6 half-stomach preparations from 3 animals. Two were used as controls (O), two for one drug dose (A) and two for another (B). This provided three 'dose pairs'; (OA), (OB) and (AB) which were randomly allocated to the 3 animals. The allocation of the dose pair within the half-stomach pair was also randomized.

This design was adopted to facilitate a comparison between the two half-stomachs from the same animal. However, examination of the results indicated that the within animal variance was not any smaller than that between animals. Consequently, each half-stomach has been treated as an individual observation for the purposes of statistical analysis.

In a few experiments, accurate estimates of the acid secretory rate were not obtained. This was due to leakage of bicarbonate from the serosal bathing fluid resulting from physical damage to the preparation by the tube used to remove the mucosal bathing fluid.

The rate of acid secretion is expressed as µmol H^+/g wet wt. mucosa per h and the cyclic AMP content as nmol/g wet wt. of mucosa.

Results

Unstimulated control tissues

All the tissues secreted acid spontaneously, the mean value for controls during the second hour was 74.41 ± 9.06 µmol $H^+ g^{-1} h^{-1}$ (s.e. mean, $n=20$). This was equivalent to 2.79 ± 0.63 µmol $H^+ cm^{-2} h^{-1}$. The mean cyclic AMP content of these tissues was 0.517 ± 0.058 nmol/g (s.e. mean, $n=20$), comparable to the values found by Karppanen, Neuvonen, Bieck & Westermann (1974) using isolated mucosa from the guinea-pig.

Phosphodiesterase inhibitors

Figure 1a shows graphs of the rate of acid secretion as a function of the logarithm of drug concentration for the two drugs, theophylline and ICI 63197 and Figure 1b shows similar graphs for the mucosal cyclic AMP content. There are more values for cyclic AMP than acid because accurate estimates of acid secretion were not obtained from some tissues for reasons described in the Methods section. The inclusion of those 'extra' points for cyclic AMP is justified when one is estimating the effects of drug concentrations on a single parameter. Both the rate of acid secretion and cyclic AMP content were linearly related to the logarithm of the drug concentration over the range used for both drugs ($P<0.05$ in all four cases). The values of the regression coefficients of acid on drug concentration were not significantly different for the two drugs, nor were those for cyclic AMP on drug concentration. However, ICI 63197 is more potent than theophylline on a molar basis.

Reversal of the effect of theophylline

The effect of theophylline on the rate of acid secretion is readily reversible (Holton & Spencer, 1976). Table 1 shows the results of experiments in which all

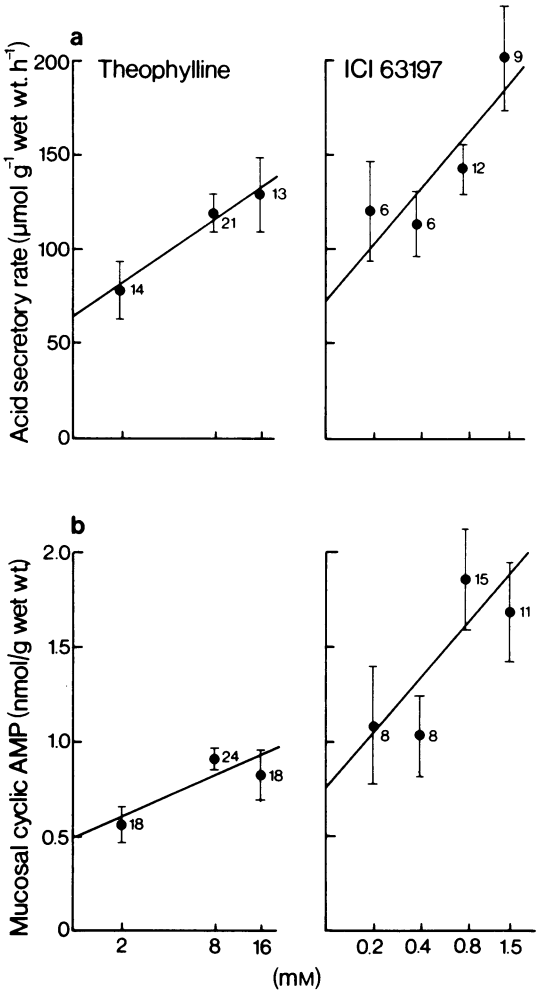

Figure 1 The relationships between the concentrations of phosphodiesterase inhibitors (theophylline or ICI 63197) plotted on a logarithmic scale and (a) the rate of acid secretion (μmol H^+ g^{-1} h^{-1}) and (b) mucosal cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content (nmol/g) in the guinea-pig isolated stomach. Vertical lines show s.e. means. Number of preparations is given beside each point.

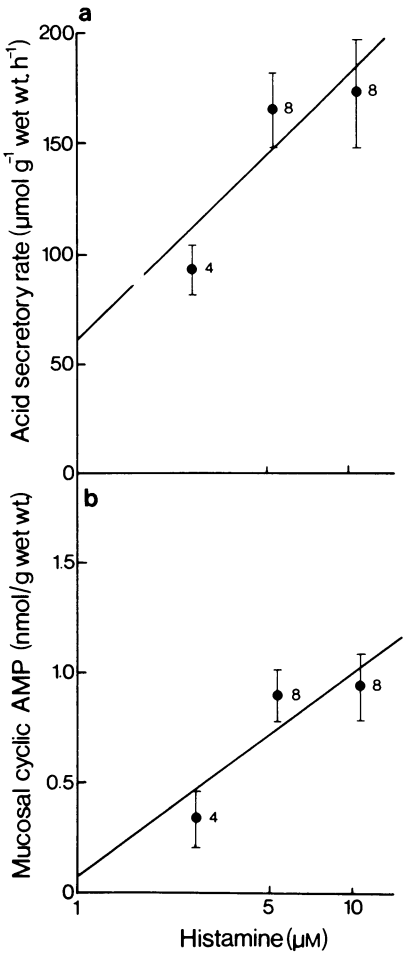

Figure 2 The relationship between the concentration of histamine plotted on a logarithmic scale and (a) the rate of acid secretion (μmol H^+ g^{-1} h^{-1}) and (b) mucosal cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content (nmol/g) in the guinea-pig isolated stomach. Vertical lines show s.e. means. Number of preparations is given beside each point.

tissues received 8 mM theophylline during the first hour of the experiment. This was withdrawn from one member of each pair during the second hour. The tissues were then extracted for cyclic AMP assay as before. During the first hour, the mean rate of acid secretion for the two groups (2 h drug and 1 h drug + 1 h recovery) were not significantly different but there was a significant difference ($P < 0.02$) during the second hour as expected. The recovery group had a significantly ($P < 0.02$) lower cyclic AMP content than the group continuously exposed to the drug. The values for the rate of acid production and cyclic AMP content for the group from which the theophylline was removed were not significantly different from those described above under control tissues.

Histamine

Figure 2 shows the relation between the rate of acid production and the logarithm of histamine concentra-

tion and cyclic AMP content and the logarithm of histamine concentration. Both parameters were linearly related to the logarithm of drug concentration and the regression coefficients for acid on histamine and cyclic AMP on histamine were not significantly different from those obtained with the phosphodiesterase inhibitors. This is summarized in Table 2.

Dibutyryl cyclic AMP

The rate of acid secretion of 5 preparations was increased by an average of 25% following application of 1 mM dibutyryl cyclic AMP. This increase was not statistically significant. Addition of 1 mM dibutyryl cyclic AMP during a plateau response to 1 mM theophylline caused a further 50% increase in the rate of acid secretion above the theophylline plateau. This increase was significant ($P < 0.01$).

Relationship between mucosal cyclic AMP and rate of acid secretion

An analysis of covariance between cyclic AMP and acid secretion was performed on the paired values from the same tissue, pooling the results for all three drugs. A correlation coefficient (r) of 0.3944 with 96 degrees of freedom was obtained indicating a very significant correlation between them ($P < 0.001$). A significant correlation ($r = 0.4828$, d.f. = 18, $P < 0.05$) was also obtained when the paired values from control tissues which had not received any drug were analysed. The linear regression coefficient of the rate of acid secretion on the cyclic AMP content of the mucosa obtained from the covariance analysis of the control data did not differ significantly from that obtained from the analysis of the stimulated tissues.

Discussion

Interpretation of these results is complicated by the fact that the gastric mucosa is a heterogeneous tissue. The extraction technique used in this work does not selectively measure the cyclic AMP content of the parietal cells which are responsible for acid secretion. Katsumata & Glick (1975) have shown that histamine causes an increase in the cyclic AMP content only in the parietal–mucus neck cell layer of the rat gastric mucosa whilst theophylline has a similar effect and also causes a smaller increase in the cyclic AMP content of the chief cell layer. As there are no statistically significant differences between our results for histamine and theophylline we feel it is reasonable to assume that most of the changes in cyclic AMP we have observed have taken place in the parietal–mucus neck cell layer but must bear in mind possible species differences between rat and guinea-pig. The action of theophylline and histamine on acid secretion confirm earlier findings (Holton & Spencer, 1976) whilst the results with ICI 63197 contrast with those of Wan (1976) who found that this drug caused only a slight stimulation of acid secretion in the mouse isolated

Table 1 Recovery of the rate of acid secretion and mucosal cyclic adenosine 3′,5′-monophosphate (cyclic AMP) to control levels following treatment with 8 mM theophylline for one hour

	Drug for 2 h			*Drug 1 h + 1 h recovery*		
	Acid secretion (μmol H^+ g^{-1} h^{-1})		*cyclic AMP (nmol/g)*	*Acid secretion (μmol H^+ g^{-1} h^{-1})*		*cyclic AMP (nmol/g)*
	1st h	*2nd h*		*1st h*	*2nd h*	
Mean	129.3	109.6	0.858	118.4	77.6	0.542
s.e. mean	13.7	9.7	0.061	13.5	9.1	0.066
n	19	19	20	19	18	18

Table 2 Summary of the linear regression data for the rate of acid secretion and cyclic adenosine 3′,5′-monophosphate (cyclic AMP) content on the logarithm of drug concentration

	Rate of acid secretion			*cyclic AMP content*		
Drug	n	*Correlation coefficient*	*Regression coefficient*	n	*Correlation coefficient*	*Regression coefficient*
Theophylline	48	0.3630	58.56	47	0.3362	375.00
ICI 63197	33	0.4519	96.71	41	0.3396	963.63
Histamine	20	0.4664	117.41	20	0.5259	873.35

stomach but did potentiate the action of histamine and pentagastrin. The effect of histamine on the guinea-pig mucosal cyclic AMP content is similar to the results of Domschke, Domschke, Classen & Demling (1973) obtained from the intact rat.

The phosphodiesterase inhibitors might be stimulating acid secretion via a mechanism involving calcium as suggested by Amer (1972) with a concentration-dependent increase in cyclic AMP as a side effect. However, there is no evidence that histamine changes calcium permeability but it has been reported to stimulate adenyl cyclase activity in the gastric mucosa of rat and rabbit. (Bersimbaev, Argutinskaya & Salganik, 1971; Sung, Jenkins, Burns, Hackney, Spenney, Sachs & Wiebelhaus, 1973). Thus, an increase in the mucosal cyclic AMP content brought about by two different means, phosphodiesterase inhibition and adenyl cyclase activation, is associated with an increase in the rate of acid secretion in our experiments. Both the rate of acid secretion and the mucosal cyclic AMP content increased linearly with the logarithm of the drug concentration for all three drugs and the respective linear regression coefficients were not significantly different between the drugs (see Table 2). This does not mean that all three drugs are acting on a common mechanism nor does it prove a causal relationship between cyclic AMP content and the rate of acid secretion. However, it does suggest that there is no reason to treat the results from the three drugs seaparately and they were pooled for the purpose of the analysis of covariance. Again a significant correlation is not proof of a causal relationship between the parameters. We cannot, for example, completely exclude the possibility that the cyclic AMP content of the mucosa is affected by the concentration of acid in the mucosal bathing fluid which is a function of the drug concentration.

The non-significant increase in acid secretion in response to exogenous dibutyryl cyclic AMP in this preparation contrasts with findings on the rat (Brennan, Arbakov, Stefankiewicz & Groves, 1975) and rabbit (Fromm, Schwartz & Quijano, 1975) stomachs, but this may be due to a species difference in the metabolism of cyclic nucleotides. The potentiating effect of theophylline suggests that the rate of entry of dibutryryl cyclic AMP in this preparation may be so low that it is largely destroyed by the phosphodiesterase and a significant increase in acid secretion is only obtained when there is partial inhibition of this enzyme. However, Main & Whittle (1974) have shown that the stimulatory effect of dibutyryl cyclic AMP in the rat is potentiated by a threshold dose of histamine. We have not tested this with the guinea-pig preparation but it is of interest that both theophylline and histamine stimulation are associated with an increase in mucosal cyclic AMP content. It may be that the action of exogenous dibutyryl cyclic AMP is potentiated in some way by a simultaneous increase in endogenous cyclic AMP.

Our results indicate that acid secretion in control preparations is related to the mucosal cyclic AMP content. The stimulatory action of histamine and phosphodiesterase inhibitors may be due to their action in increasing the cyclic AMP content. This provides further evidence to support the hypothesis that cyclic AMP could be one of the factors in the regulation of acid secretion.

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References

AMER, M.S. (1972). Cyclic AMP and gastric secretion. *Amer. J. dig. Dis.*, **17**, 945–953.

AMER, M.S. (1974). Cyclic GMP and gastric secretion. *Amer. J. dig. Dis.*, **19**, 71–74.

BERSIMBAEV, R.I., ARGUTINSKAYA, S.V. & SALGANICK, R.I. (1971). The stimulating action of gastrin pentapeptide and histamine on adenyl cyclase activity in rat stomach. *Experientia.* **27**, 1389–1390.

BRENNAN, F.T., ARBAKOV, D., STEFANKIEWICZ, J.S. & GROVES, W.G. (1975). Acid secretory effects of pentagastrin, histamine, urecholine, DBcAMP and cGMP in isolated stomachs of fed and fasted rats. *Proc. Soc. exp. Biol. Med.*, **149**, 725–730.

CANFIELD, S.P., CURWAIN, B.P. & SPENCER, JAN (1976). A quantitive relationship between acid secretion and mucosal 3,5 cyclic AMP in the isolated guinea-pig stomach. *J. Physiol., Lond.* **260**, 61–62P.

DOMSCHKE, W., DOMSCHKE, S., CLASSEN, J. & DEMLING, L. (1973). Histamine and cyclic 3′, 5′-AMP in gastric acid secretion. *Nature, Lond.*, **241**, 454–455.

FROMM, D., SCHWARTZ, J.H. & QUIJANO, R. (1975). Effects of cyclic adenosine 3′:5′ monophosphate and related agents on acid secretion by isolated rabbit gastric mucosa. *Gastroenterology*, **69**, 453–462.

HOLTON, PAMELA & SPENCER, JAN (1976). Acid secretion by guinea-pig isolated stomach. *J. Physiol., Lond.*, **255**, 465–479.

KARPPANEN, H.O., NEUVONEN, P.J., BIECK, P.R. & WESTERMANN, E. (1974). Effect of histamine, pentagastrin and theophylline on the production of cyclic AMP in isolated gastric tissue of the guinea-pig. *Naunyn-Schmiedebergs Arch. Pharmac.*, **284**, 15–23.

KATSUMATA, Y. & GLICK, D. (1975). Effects of drugs influencing gastric secretion on the quantitative histological distribution of cyclic adenosine 3′:5′ monophosphate in the rat stomach. *Gastroenterology*, **67**, 409–415.

KIMBERG, D.V. (1974). Cyclic nucleotides and their role in gastrointestinal secretion. *Gastroenterology.* **67**, 1023–1064.

MAIN, I.H.M. & WHITTLE, B.J.R. (1974). Prostaglandin E_2 and the stimulation of rat gastric acid secretion by dibutyryl cyclic 3′5′ AMP. *Eur. J. Pharmac.*, **26**, 204–211.

RUOFF, H.J. & SEWING, K. Fr. (1974). Rat gastric mucosal c-AMP following cholinergic and histamine stimulation. *Eur. J. Pharmac.*, **28**, 338–343.

RUOFF, H.J. & SEWING, K. Fr. (1975). Influence of atropine, metiamide and vagotomy on c-AMP of resting and stimulated gastric mucosa. *Eur. J. Pharmac.*, **32**, 227–232.

SUNG, C.P., JENKINS' B.C., BURNS, L.R., HACKNEY, V., SPENNEY, J.G., SACHS, G. & WIEBELHAUS, V.D. (1973). Adenyl and guanyl cyclase in rabbit gastric mucosa. *Amer. J. Physiol.*, **255**, 1359–1363.

WAN, B. (1976). Effects of dibutryl cyclic AMP and phosphodiesterase inhibitors on acid secretion by mouse stomach *in vitro*. *Br. J. Pharmac.*, **56**, 357P.

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