Biochimica et Biophysica Acta, 311 (1973) 90–97

BBA 76342

POSSIBLE ROLE OF CYCLIC AMP IN GASTRIC ACID SECRETION IN RAT

ACTIVATION OF CARBONIC ANHYDRASE

SHIGEHIKO NARUMI and YOSHITAKA MAKI

Biological Research Laboratories, Central Research Divisions, Takeda Chemical Industries, Ltd, Higashiyodogawa-ku, Osaka (Japan)

(Received January 12th, 1973)

SUMMARY

1. Administration of theophylline and gastric acid stimulants, such as histamine, tetragastrin and carbachol, increased the adenosine 3′:5′-cyclic monophosphate (cyclic AMP) level 2–3-fold as compared with the control.

2. N^6-2′-*O*-Dibutyryl cyclic AMP (dibutyryl cyclic AMP) stimulated gastric acid secretion and activity of carbonic anhydrase in rat gastric mucosa.

3. *In vitro* addition of cyclic AMP (10^{-5}–10^{-7} M) to the supernatant fraction (cytosol) of rat gastric mucosa caused a concentration dependent potentiation of carbonic anhydrase activity. It is considered that this activation was mediated through protein kinase activation.

INTRODUCTION

The function of cyclic AMP as a second messenger in the action of many hormones and biogenic amines is well known. It is proposed that cyclic AMP is responsible for the gastric acid secretion caused by histamine or gastrin. Harris *et al.*[1–6] had postulated a possible stimulatory role of cyclic AMP in gastric acid secretion of frog gastric mucose. Bersimbaev *et al.*[7] have also reported that histamine and gastrin activate adenylate cyclase in rat gastric tissue. However, Mao *et al.*[8] and Taft and Session[9] have recently indicated that the initiation of gastric acid secretion in canine and rat stomach does not depend solely upon the accumulation of cyclic AMP.

Therefore, the present study was designed to test these hypotheses by measuring the following three parameters in rat stomach: (1) cyclic AMP levels in gastric mucosa after administration of gastric acid stimulants; (2) gastric acid secretion after administration of dibutyryl cyclic AMP; (3) carbonic anhydrase activity after addition of cyclic AMP *in vitro*.

METHODS

Male Sprague–Dawley rats (8 weeks old) were used in these studies.

Measurement of gastric acid secretion

Rats were previously fasted for 17 h. Gastric perfusion and pylorus ligation methods were carried out according to Lai[10] and Shay *et al.*[11], respectively. Drugs were administered intravenously.

Measurement of cyclic AMP

Tissue preparation for determination of cyclic AMP. Cyclic AMP was isolated from tissue by the method of Walton and Garren[12] with the following minor modification. Each drug-treated rat was decapitated and the stomach (except the rumen) were removed and immediately frozen on dry ice. The frozen tissues were weighed and homogenized in 5% trichloroacetic acid at a ratio of 1 ml/100 mg of tissue. After centrifugation at $800 \times g$ for 10 min, trichloroacetic acid in the extract was removed by washing three times with 10 volumes of ether, and residual ether was expelled by heating at 90 °C in water for 3 min. 500 μl of the extracts were neutralized with 100 μl of 0.5 M Tris–HCl buffer (pH 7.5) and treated with 50 μl of 5% $ZnSO_4$ and 50 μl of 0.3 M $BaCl_2$ and kept in ice for 10 min to allow the precipitate to form. The precipitate was removed by centrifugation and an aliquot of supernatant was used for the assay of cyclic AMP.

Cyclic AMP assay. Cyclic AMP was determined by the method of Gilman[13] with the following minor modification. The binding reaction was allowed to proceed for 40 min at 4 °C in a total volume of 200 μl containing 30 μl of 1 M acetate buffer (pH 4.0). The other components of the incubation were 2 pmoles of cyclic [^{3}H]AMP (50000 dpm), 2 μg of binding protein from beef muscle and 100 μl of assay solution. Reactions were initiated by addition of binding protein. After 40 min, the reaction was terminated by adding 3 ml of cold 0.01 M potassium phosphate buffer (pH 6.0) and the reaction mixtures were passed through a millipore filter (pore size 0.45 μm) previously rinsed with the same buffer. The filter was then washed twice with 3 ml of this buffer, dried and placed in a counting vial with 1 ml of ethylcellosolve. Bray's scintillation mixture[14] was utilized for determining the radioactivity of each sample. The complex of protein kinase and the cyclic AMP binding subunit was extracted by the method of Gilman[13] from bovine and swine muscle.

As previously reported[15], subcellular fractionation of rat gastric mucosa was carried out according to the method of Schneider and Hogeboom[16]. Protein was determined by the method of Lowry *et al.*[17].

Assay of carbonic anhydrase

Carbonic anhydrase activity was measured according to the method of Philpot and Philpot[18]. Definition of the enzyme unit: If the reciprocal of the reaction time is plotted against the amount of enzyme, the result is a straight line within a reasonable range. Thus the number of the enzyme units in solution giving a reaction time t is $K(t_0/t-1)$, where t_0 is the reaction time of the blank and K is a constant equal to 17.7.

RESULTS

Effects of gastric acid stimulants on the cyclic AMP level of rat gastric mucosa

The intravenous administration of histamine (1 mg/kg) did not have any

influence on the cyclic AMP level until 60 min after administration. Intraperitoneal treatment of theophylline (200 mg/kg) produced a 2-fold increase of the cyclic AMP level after 15–30 min, as compared with the saline-treated group. This increase was more pronounced (3-fold) in gastric mucosa treated with theophylline *plus* histamine (Fig. 1), tetragastrin (100 μg/kg, intravenously, Fig. 2) or carbachol (100 μg/kg, intravenously, Fig. 3) than after theophylline treatment alone.

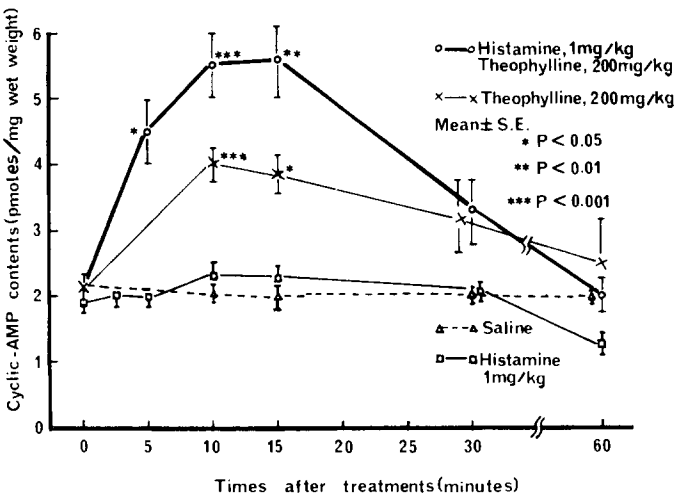

Fig. 1. Effect of histamine on the cyclic AMP levels in gastric mucosa of theophylline-treated or non-treated rats, *in vivo*. Theophylline (200 mg/kg, intraperitoneally) and histamine (1 mg/kg, intravenously) administered at the same time. Increased cyclic AMP levels of gastric mucosa were observed in rats treated with both drugs as compared with individual treatments.

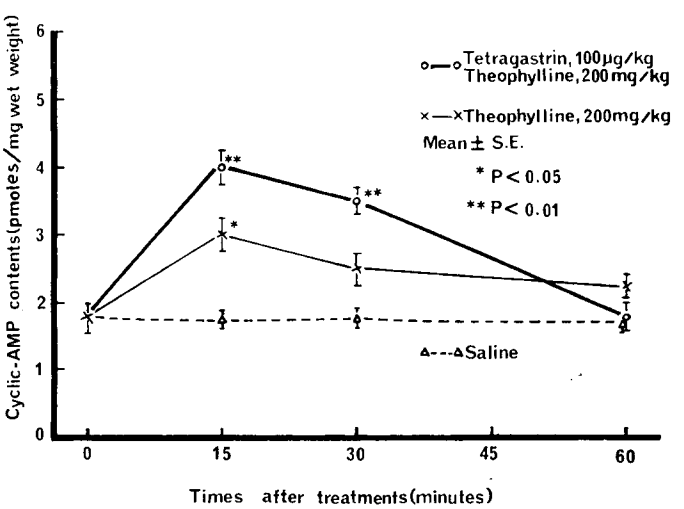

Fig. 2. Effect of tetragastrin on the cyclic AMP levels in gastric mucosa of theophylline-treated rats, *in vivo*. Theophylline (200 mg/kg, intraperitoneally) and tetragastrin (100 μg/kg, intravenously) administered at the same time.

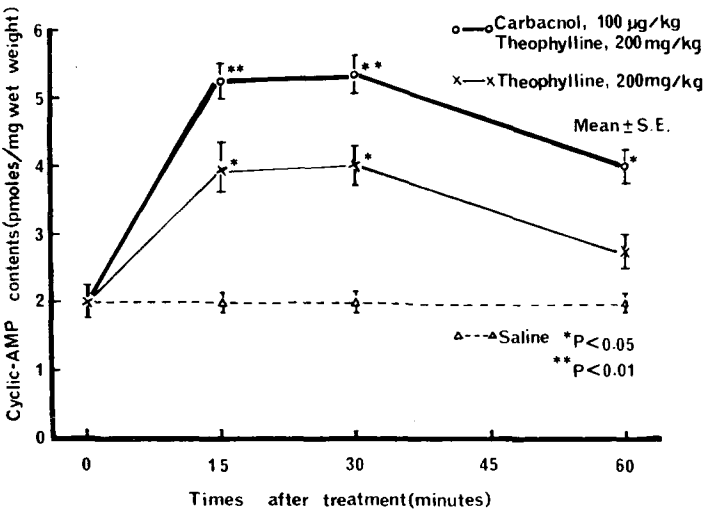

Fig. 3. Effect of carbachol on the cyclic AMP levels in gastric mucosa of theophylline-treated rats, *in vivo*. Theophylline (200 mg/kg, intraperitoneally) and carbachol (100 μg/kg, intravenously) administered at the same time.

Effect of dibutyryl cyclic AMP on gastric acid secretion and carbonic anhydrase activity in rat gastric mucosa

Gastric perfusion method. Dibutyryl cyclic AMP (25 mg/kg, intravenously) increased gastric acid secretion in the urethane-anesthetized rats and this increase was potentiated by pretreatment with theophylline (200 mg/kg, intraperitoneally), as shown in Fig. 4.

Pylorus-ligation method. The dose response curves of dibutyryl cyclic AMP

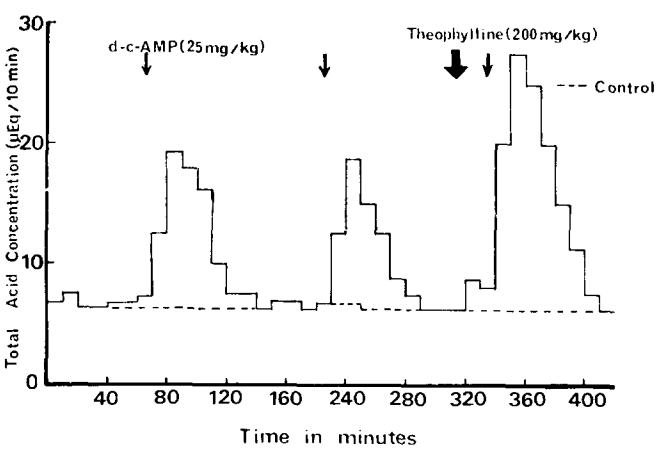

Fig. 4. Effect of dibutyryl cyclic AMP (25 mg/kg, intravenously) and theophylline (200 mg/kg, intraperitoneally) on gastric acid secretion in urethane-anesthetized rats using gastric-perfusion method. Dibutyryl cyclic AMP produced an increase of gastric acid secretion and theophylline potentiated this increase 2-fold.

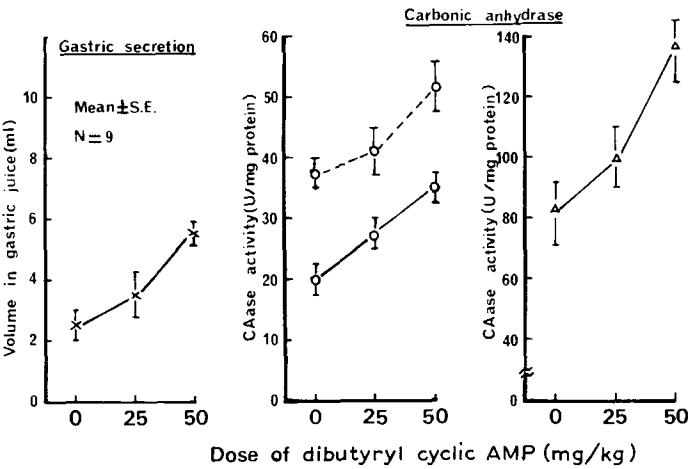

Fig. 5. Dose response of dibutyryl cyclic AMP on gastric acid secretion and carbonic anhydrase activity in the mitochondrial (○—○), microsomal (○---○) and supernatant (△—△) fractions of gastric mucosal cells using pylorus-ligated rats. Dibutyryl cyclic AMP (25 and 50 mg/kg, intravenously) stimulated gastric acid secretion and carbonic anhydrase activity in these fractions in a dose dependent manner.

for the gastric acid secretion and carbonic anhydrase activity in the mitochondria, microsome and supernatant fractions show that cyclic AMP is likely to stimulate gastric acid secretion and the activity of carbonic anhydrase in rat gastric mucosa (Fig. 5).

Effect of cyclic AMP and protein kinase on the activity of carbonic anhydrase in rat gastric mucosa, in vitro

We have already reported[15] that 75–90% of the subcellular activity of carbonic anhydrase in rat gastric mucosa was present in the supernatant fraction (cytosol).

TABLE I

EFFECT OF CYCLIC AMP ON THE ACTIVITY OF CARBONIC ANHYDRASE IN THE RAT GASTRIC MUCOSA, *IN VITRO*

The addition (37 °C, incubation for 20 min) of cyclic AMP (10^{-5}–10^{-7} M) increased carbonic anhydrase activity in the supernatant fraction in proportion to the concentration used, however, the enzyme activities of other fractions were not influenced.

Concentration of of cyclic AMP (M)	*Activity of carbonic anhydrase (units/mg protein)*		
	Supernatant fractions	*Microsome fractions*	*Mitochondria fractions*
0	145 ± 9	2.8 ± 1.0	4.8 ± 2.9
10^{-5}	327 ± 48*	8.9 ± 4.1	7.6 ± 3.8
10^{-6}	225 ± 27*	3.8 ± 2.0	5.0 ± 4.4
10^{-7}	120 ± 7	6.3 ± 3.2	7.2 ± 5.3

* $P < 0.05$ Significant difference with untreated group. Each number indicates the mean of three experiments and standard error of the mean.

TABLE II

INFLUENCE OF PROTEIN KINASE ON THE ACTIVATION OF CARBONIC ANHYDRASE BY CYCLIC AMP IN THE RAT GASTRIC MUCOSA

The addition (37 °C, incubation for 20 min) of protein kinase from swine muscle; ATP and Mg^{2+}, under the conditions cited below, potentiated the increased activity of carbonic anhydrase caused by cyclic AMP (10^{-6} M). +, addition; − no addition, Figures are the mean ± S.E.

Supernatant fraction (1 mg protein)	*Cyclic AMP (10^{-6} M)*	*Protein* kinase (200 µg protein)*	*BSA** (200 µg protein)*	*ATP+ Mg^{2+}****	*Activity of carbonic anhydrase (units/mg protein)*
+	−	−	−	−	145 ± 9
+	−	−	−	+	175 ± 13
+	+	−	−	−	225 ± 27†
+	+	−	+	+	250 ± 17†
+	+	+	−	+	325 ± 37††
−	−	+	−	−	0 ± 0

* Originated from swine muscle.
** Bovine serum albumin.
*** ATP 10^{-6} M, $MgCl_2$ $2 \cdot 10^{-3}$ M
† $P < 0.05$.
†† $P < 0.01$.

The addition (preincubation at 37 °C for 20 min) of cyclic AMP (10^{-5}–10^{-7} M) increased carbonic anhydrase activity of the supernatant fraction in proportion to the concentration used; however, the enzyme activities of other fractions were not influenced (Table I).

The simultaneous addition of protein kinase (from swine muscle), ATP (10^{-6} M) and Mg^{2+} ($2 \cdot 10^{-3}$ M) to the supernatant fraction potentiated the increase of carbonic anhydrase activity caused by cyclic AMP (10^{-6} M) alone (Table II). The addition of the same concentration of bovine serum albumin in place of protein kinase did not potentiate the increase.

DISCUSSION

That gastric acid stimulants stimulate the activity of adenyl cyclase of rat gastric mucosa is suggested by our results that histamine, tetragastrin or carbachol increased the cyclic AMP levels of the gastric mucosa in theophylline-treated rats. The observation of Bersimbaev *et al.*[7] that pentagastrin or histamine treatment produced an increase of adenyl cyclase activity in rat gastric mucosa is in agreement with our data. However, we could not detect any increase of the cyclic AMP levels induced by these stimulants in conscious, non-theophylline-treated rats, although Okura *et al.*[19] observed an increase of the cyclic AMP levels induced by histamine or gastrin in anesthetized, non-theophylline-treated rats. The reason for this discrepancy cannot be explained at this moment, but our data may indicate that newly formed cyclic AMP will be promptly destroyed by phosphodiesterase.

Dibutyryl cyclic AMP has been known to be more effective in producing

biological activity in several intact tissues than cyclic AMP[20,21]. It was hoped that the use of an effective form of cyclic AMP would facilitate the entry of cyclic AMP into the parietal cell. In the present experiments, dibutyryl cyclic AMP caused gastric acid secretion in rats, as reported by Okura *et al.*[22]. These results will support the view that cyclic AMP plays an intermediary role in gastric acid secretion in rats.

We reported that gastric acid stimulants increased Mg^{2+}-dependent ATPase and carbonic anhydrase activities in rat gastric mucosa and it was concluded that the activation of both enzymes correlated with gastric acid secretion[15]. In this study, the incubation of the supernatant fraction (cytosol) with cyclic AMP (10^{-5}–10^{-7} M) produced an increase in carbonic anhydrase activity in proportion to the concentration used. This increased activity is thought to be induced not by direct activation of carbonic anhydrase, but through the activation of protein kinase for the following reasons: (1) the addition of protein kinase (originated from swine muscle) system potentiated the increase in carbonic anhydrase activity of rat gastric mucosa caused by cyclic AMP (10^{-6} M); (2) protein kinase activity found in the supernatant fraction of rat gastric mucosa[23]; (3) the protein kinase system and cyclic AMP (10^{-5} M) activated pure (commercial) carbonic anhydrase (originated from bovine erythrocyte) (unpublished data).

From these results, we now propose that gastric acid stimulants increase the cyclic AMP levels which allow protein kinase to stimulate carbonic anhydrase activity and finally the H^+ formed is secreted. Our results are supported by recent finding indicating that pentagastrin and histamine activate adenylate cyclase and the cyclic AMP which is formed enhance carbonic anhydrase activity in rat stomach[24].

ACKNOWLEDGMENTS

The authors thank Dr K. Kikuchi of this laboratory, and Dr E. Miyamoto of Nakamiya Mental Hospital, for their valuable advice and criticism.

REFERENCES

1 Harris, J. B. and Alonso, D. (1963) *Fed. Proc.* 22, 623
2 Alonso, D. and Harris, J. B. (1965) *Am. J. Physiol.* 208, 18–23
3 Harris, J. B. and Alonso, D. (1965) *Fed. Proc.* 24, 1368–1376
4 Alonso, D., Park, O. H. and Harris, J. B. (1965) *Am. J. Physiol.* 208, 1183–1190
5 Nigon, K. and Harris, J. B. (1968) *Am. J. Physiol.* 215, 1299–1303
6 Alonso, D., Park, O. H. and Harris, J. B. (1968) *Am. J. Physiol.* 215, 1305–1309
7 Bersimbaev, R. I., Argutinskaya, S. V. and Salganik, R. I. (1971) *Experientia* 27, 1389–1390
8 Mao, C. C., Shanbour, L. L., Hodgins, D. S. and Jacobson, E. D. (1972) *Gastroenterology* 63, 427–438
9 Taft, R. C. and Session, Jr, J. T. (1972) *Clin. Res.* 20, 43
10 Lai, K. S. (1964) *Gut* 5, 327–333
11 Shay, H., Sun, C. H. and Gruenstein, M. (1954) *Gastroenterology* 26, 906–913
12 Walton, G. M. and Garren, L. D. (1970) *Biochemistry* 9, 4223–4229
13 Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. U.S.* 67, 305–312
14 Bray, G. A. (1960) *Anal. Biochem.* 1, 279–285
15 Narumi, S. and Kanno, M. (1973) *Biochim. Biophys. Acta* 311, 80–89
16 Schneider, W. C. and Hogeboom, G. H. (1950) *J. Biol. Chem.* 183, 123–128
17 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–274

18 Philpot, F. J. and Philpot, J. L. (1936) *Biochem. J.* 30, 2191–2193
19 Okura, H., Kitamura, T., Yamamura, T. and Sakoda, M. (1972) *Igaku No Ayumi* (in Japanese) 82, 205–206
20 Posternak, T. H., Sutherland, E. W. and Henion, W. F. (1962) *Biochim. Biophys. Acta* 65, 558–560
21 Field, M. (1971) *Am. J. Physiol.* 221, 992–997
22 Okura, H., Kitamura, T., Yamamura, T. and Sakoda, M. (1972) *Igaku No Ayumi* (in Japanese) 81, 819–820
23 Kitamura, T. (1971) *Fol. Endocrinol. Jap.* 47, 1068–1069
24 Salganik, R. I., Argutinskaya, S. V. and Bersimbaev, R. I. (1972) *Experientia* 28, 1190–1191