analyzer, separately and in admixture with the enzymic digests. The position of this peak was co-incident with peak 2 as is shown in the Figure.

Thus from this work it has been shown that the isopeptide εN (β-aspartyl) lysine occurs in native wool keratin and has probably the function of a crosslink cf. εN (γ-glutamyl) lysine. Also it appears from this work that this moiety is formed during the heating of the keratin, as is reflected in the increased amounts found in digests of heated protein.

Zusammenfassung. Chromatographisch (Ionenaustausch, Papierchromatographie, Dünnschichtchromatographie und Hochspannungselektrophorese) wird nach enzymatischer Hydrolyse von nativem und denaturiertem Wolle-Keratin ε(γ-Glutamyl)-lysin und ε(β-Aspartyl)-lysin identifiziert und so die in Proteinen postulierte anomale Peptidverknüpfung nachgewiesen.

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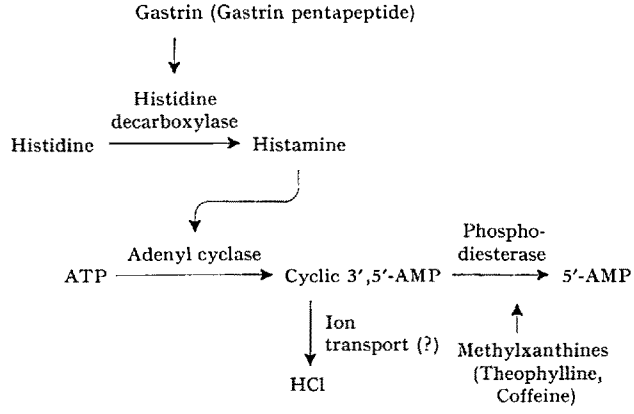
The Stimulating Action of Gastrin Pentapeptide and Histamine on Adenyl Cyclase Activity in Rat Stomach

Experimental data obtained recently[1] suggested that gastrin pentapeptide, and most probably complete gastrin also, cause transcription of DNA regions responsible for the synthesis of histidine decarboxylase. It was assumed that the enzyme induced provides a supply of histamine acting as physiological mediator of gastrin pentapeptide effect on gastric acid secretion. Increasing evidence has shown that histamine enhances adenyl cyclase activity in many tissues[2–4], and it is believed that cyclic adenosine 3′,5′-monophosphate (cyclic AMP) is directly responsible for the physiological effects of histamine. It is well known that cyclic AMP is a 'second messenger' in the action of many hormones and biogenic amines[5,6] and is able to mimic their action on target cells.

We supposed that histamine, formed due to action of gastrin (or its pentapeptide), activates adenyl cyclase in oxyntic cells and cyclic AMP in its turn affects ion transport to result in HCl secretion. The purpose of the present study was to verify this hypothesis.

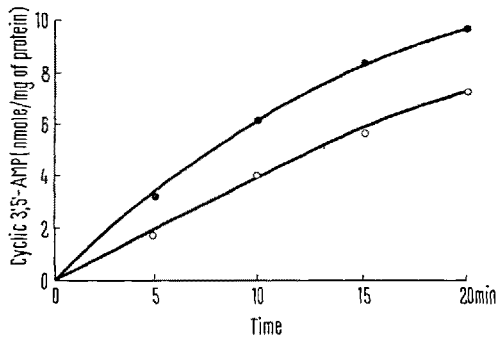
Fasted male Wistar rats (200–250 g body wt.) received s.c. gastrin pentapeptide (t-butoxycarbonyl-β-Ala-Try-Met-Asp-PheNH$_2$; ICI 50, 123) twice at 20 min intervals in a dose of 0.4 μg or histamine i.m. in a dose of 200 μg per 100 g body wt. Control rats were injected with saline. 40–60 min after the injections the rats were sacrificed and the stomachs removed, rinsed in icecold saline and homogenized in 0.25 *M* sucrose to a final concentration of 50 mg tissue per ml. The stomach tissue homogenates for adenyl cyclase estimation were prepared according to Streeto and Reddy[7].

Adenyl cyclase activity was determined by the method of Weiss and Costa[8] modified by Rosen and Rosen[9]. The incubation mixture (0.2 ml) contained 0.05 *M tris* – HCl buffer pH 7.8, 0.01 *M* theophylline, 0.01 *M* NaF, 3 m*M* $MgSO_4$, 0.02 *M* mercaptoethanol, 0.2 μ*M* ^{14}C–8 ATP (specific activity 0.135 μC/μ*M*) and 0.05 ml of gastric tissue suspension (0.1-0.2 mg of protein) as a source of adenyl cyclase. 1 ml of final supernatant solution obtained after precipitation of ATP, and other metabolites of ATP except the cyclic AMP[10] by $ZnSO_4$–$Ba(OH)_2$[8,9], was added to 10 ml Bray's[11] scintillation fluid and radioactivity measured with Nuclear–Chicago Mark 1 scintillation counter. The activity of adenyl cyclase was expressed as nmoles of cyclic AMP formed per mg of protein per min. Protein was estimated by the method of Lowry et al.[12].



The scheme of the regulation of HCl secretion by gastrin.

[1] R. I. Salganik, S. V. Argutinskaya and R. I. Bersimbaev, Experientia *27*, 53 (1971).
[2] T. W. Rall and S. Kakiuchi, in *Molecular Basis of Some Aspects of Mental Activity* (Ed. O. Walaas, Academic Press, New York 1966), vol. 1, p. 417.
[3] H. Shimizu, C. R. Creveling and J. W. Daly, J. Neurochem. *17*, 441 (1970).
[4] C. V. Perrier and L. Laster, J. clin. Invest. *49*, 73a (1970).
[5] E. W. Sutherland, I. Øye and R. W. Butcher, Recent Prog. Horm. Res. *21*, 623 (1965).
[6] G. A. Robison, R. W. Butcher and E. W. Sutherland, Ann. Rev. Biochem. *37*, 149 (1968).
[7] J. M. Streeto and W. J. Reddy, Analyt. Biochem. *21*, 416 (1967).
[8] B. Weiss and E. Costa, Science *156*, 1750 (1967).
[9] O. M. Rosen and S. M. Rosen, Biochem. Biophys. Res. Commun. *31*, 82 (1968).
[10] G. Krishna, B. Weiss and B. B. Brodie, J. Pharmac. exp. Ther. *163*, 379 (1968).
[11] G. A. Bray, Analyt. Biochem. *1*, 279 (1960).
[12] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).



Effect of gastrin pentapeptide injection on adenyl cyclase activity of rat gastric tissue. The incubation mixture contained 0.05 *M* Tris-HCl buffer (pH 7.8), 0.01 *M* theophylline, 0.01 *M* NaF, 3 m*M* $MgSO_4$, 0.02 *M* mercaptoethanol, 0.2 μM ^{14}C-8-ATP (specific activity 0.135 $\mu C/\mu M$) and 140 μg of protein in a final volume of 0.2 ml. Incubation was carried out at 37 °C. —●—●—, gastrin pentapeptide; —○—○—, control (saline).

Table I. Effect of histamine on the adenyl cyclase activity in rat gastric tissue

Conditions	Experiment No.	Adenyl cyclase activity, nmoles cyclic 3',5'-AMP/mg protein/min
Control	8	0.47 ± 0.03
Histamine	4	0.94 ± 0.02

The contents of incubation system were as in legend to the Figure. Incubation was carried out for 10 min at 37 °C.

Table II. Effect of gastrin pentapeptide and histamine in vitro on adenyl cyclase activity in rat gastric tissue

Additions	Adenyl cyclase activity, nmoles cyclic 3',5'-AMP/mg protein/min
Control	0.64 ± 0.02 (3)
Gastrin pentapeptide (10 μg)	0.60 ± 0.01 (3)
Histamine ($1 \times 10^{-3} M$)	1.16 ± 0.03 (4)

Data are the mean values ± S.D. of 3–4 (parentheses) separate experiments. Gastric tissue suspension was preincubated with gastrin pentapeptide (10 μg/sample) 30 min at 37 °C and then incubation was carried out for 10 min at 37 °C with the standard incubation mixture. Histamine was added directly to the incubation mixture without any preincubation with gastric tissue suspension.

Table III. Effect of coffeine and theophylline on adenyl cyclase activity

Conditions	Adenyl cyclase activity, nmoles cyclic 3',5'-AMP/mg protein/min
Complete system	0.60
+coffeine (0.02 *M*)	1.00
—theophylline	0.12

Data are the mean values of duplicate experiments. Complete incubation system (0.2 ml) contained 0.05 *M* *Tris*-HCl buffer pH 7.8, 0.01 *M* NaF, 0.02 *M* mercaptoethanol, 0.01 *M* theophylline, 3 m*M* $MgSO_4$, 0.2 μM ATP and 100–120 μg of protein. Incubation was carried out for 10 min at 37 °C.

Our experiments have shown that injection of gastrin pentapeptide or histamine to rats in doses stimulating HCl secretion[1] increases adenyl cyclase activity in gastric tissues (Figure, Table I); when pentapeptide or histamine were added to the incubation mixture containing gastric tissues homogenate, only histamine enhanced the activity of adenyl cyclase (Table II).

The results obtained in these experiments support the suggested pattern of the regulation of gastric acid secretion (Scheme). All the main components of this scheme: gastrin or its pentapeptide, histamine and, finally, cyclic AMP act as stimulants of HCl secretion.

It seems reasonable to suggest that pentapeptide is unable to activate adenyl cyclase in homogenates of gastric tissues because its action needs transcription and translation and undamaged cell structures are prerequisite to provide it. On the other hand, histamine is active not only in vivo, but also in vitro, probably because it interacts directly with adenyl cyclase without complicated intermediate processes.

It was found earlier that stimulants of gastric acid secretion like coffeine and theophylline[13,14] are inhibitors of specific phosphodiesterase that inactivates cyclic AMP by transformation into 5'-AMP[6].

It was tempting to assume that coffeine and theophylline stimulate gastric acid secretion increasing the accumulation of cyclic AMP in gastric cells. The data presented confirm this suggestion. Coffeine added to incubation mixture increases the amount of cyclic AMP (Table III). When theophylline was excluded from the complete incubation mixture, the amount of formed cyclic AMP decreased.

Many lines of evidence indicate that cyclic AMP activates protein kinases[15–17] which probably influence various processes and, among them, ion transport[18]. The findings imply that, presumably, such a mechanism may produce HCl as the last step in this pathway of regulation.

ВЫВОДЫ. Введение крысам пентапептида гастрина или гистамина повышает активность аденилциклазы в тканях желудка, катализирующей образование 3',5'-АМФ, В опытах in vitro только гистамин активирует этот фермент. Высказано предположение, что гастрин, гистамин и 3',5'-АМФ являются последовательными звеньями в регуляции секреции HCl в желудке.

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[13] C. R. ROBERTSON, C. E. ROSIERE, D. BLICKENSTAFF and M. I. GROSSMAN, J. Pharmac. exp. Ther. *99*, 362 (1950).
[14] S. KRASNOW and M. I. GROSSMAN, Proc. Soc. exp. Biol. med. *71*, 335 (1949).
[15] T. A. LANGAN, Science *162*, 579 (1968).
[16] E. MIYAMOTO, J. F. KUO and P. GREENGARD, J. biol. CHEM. *244*, 6395 (1969).
[17] J. F. KUO, B. K. KRUEGER, J. R. SANES and P. GREENGARD, Biochim. biophys. Acta *212*, 79 (1970).
[18] J. ORLOFF and J. S. HANDLER, J. clin. Invest. *41*, 702 (1962).
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