Induction of Transcription in the Stimulating Action of a Gastrin Pentapeptide on Gastric Acid Secretion

The hormone gastrin has been isolated from the gastric antral mucosa of several species, including man, in the form of heptadecapeptide amides of almost identical constitution[1]. It has been shown that the C-terminal tetrapeptide amide possesses all of the biological activities displayed by the total molecule, though its potency is considerably lower[2]. Many analogues of the tetrapeptide amide have been synthesized and their activity compared with that of the parent structure[3,4]. The C-terminal pentapeptide sequence of the gastrin is in all species so far studied Gly.Trp.Met.Asp.Phe.NH_2; a synthetic analogue of this structure[3] (t-butoxycarbonyl-β-alanine-Trp.Met.Asp.Phe.NH_2) is commercially available ('Peptavlon', I.C.I. 50, 123; Imperial Chemical Industries Ltd.) for clinical trial as a powerful stimulant of gastric acid secretion and was used in this study. It will be referred to as 'pentapeptide'. We are indebted to Dr. J. S. MORLEY of I.C.I. Ltd. for generous supplies of this material.

It has become evident in recent years that many hormones exert their effects by switching on or enhancing the transcription of definite DNA regions, programming the synthesis of RNA molecules and corresponding enzymes, which fulfil the functions of the hormone in the target cells. Such a mechanism has been established for some steroid hormones, as well as for those of peptide nature[5-9]. The aim of the experiments to be described was to examine such a possibility in regard to the stimulation of gastric acid secretion by the gastrin pentapeptide.

Fasted male Wistar rats (200–250 g body weight) were anaesthetized with urethane and gastric acid secretion followed by BARRETT's[10] modification of the method of GHOSH and SCHILD[11], the stomach being perfused with saline (31 °C) at 1 ml per min and 10-min collections of perfusate titrated for HCl content with 0.005 N NaOH. In these circumstances there was a constant basal secretion of acid which was augmented 2–3-fold by giving 4 s.c. injections at 20-min intervals of pentapeptide in a dose of 0.4 μg per 100 g body weight (Figure 1a).

However, if aurantine, an actinomycin analogue which inhibits DNA-dependent RNA synthesis[12,13], was injected in a dose of 100 μg/100 g body weight 30 min before the first of the pentapeptide injections and followed by a further dose of 50 μg per 100 g body weight 1 h later, then the pentapeptide had no significant effect, although the basal secretion of acid was unaffected (Figure 1b). The basal secretion was also unaffected in control experi-

[1] R. A. GREGORY, Gastroenterology *51*, 953 (1966).
[2] H. J. TRACY and R. A. GREGORY, Nature, Lond. *204*, 935 (1964).
[3] J. S. MORLEY, H. J. TRACY and R. A. GREGORY, Nature, Lond. *207*, 1356 (1965).
[4] J. S. MORLEY, Proc. R. Soc., B *170*, 97 (1968).
[5] P. KARLSON, Perspect. Biol. Med. *6*, 203 (1963).
[6] F. T. KENNEY, W. D. WICKS and D. L. GREENMAN, J. Cell. comp. Physiol., Suppl. 1, *66*, 125 (1965).
[7] A. SOLS, A. SILLERO and J. SALAS, J. Cell. comp. Physiol., Suppl. 1 *66*, 23 (1965).
[8] G. WEBER, R. L. SINGHAL, N. B. STAMM and S. K. SRIVASTAVA, Fedn Proc. *24*, 745 (1965).
[9] N. A. JUDAYEV, Problemy Endocr. *13*, 112 (1967).
[10] A. M. BARRETT, J. RAVENTOS and R. A. SIDDAL, Br. J. Pharmac. Chemother. *28*, 51 (1966).
[11] M. N. GHOSH and H. O. SCHILD, Br. J. Pharmac. Chemother. *13*, 54 (1958).
[12] E. REICH, Science *143*, 684 (1964).
[13] JU. O. SAZYKIN, in *Antibiotiki kak ingibitory biokhimicheskikh processov* (Moskwa 1968), p. 336.

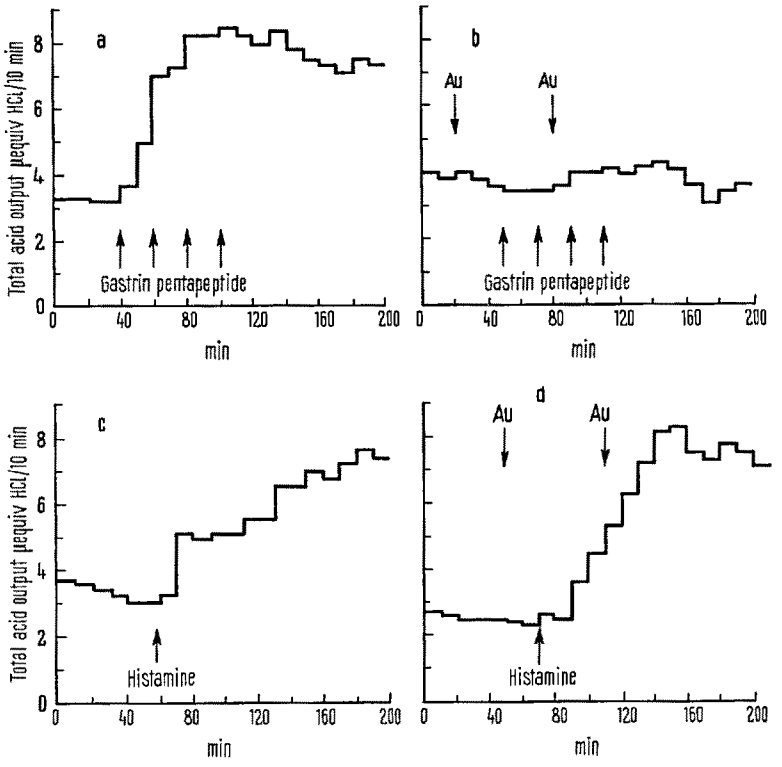

Fig. 1. The secretion of HCl by the anaesthetized rat stomach after injection of: a) Gastrin pentapeptide (0.4 μg/100 g at each arrow). b) Gastrin pentapeptide (0.4 μg/100 g) with aurantine (100 μg/100 g and 50 μg/100 g). c) Histamine (200 μg/100 g). d) Histamine (200 μg/100 g) with aurantine (100 μg/100 g and 50 μg/100 g). Each graph represents the mean response of 4 animals.

ments in which aurantine was given without pentapeptide.

In similar experiments in which histamine was used instead of pentapeptide, a single i.m. injection of 200 μg/100 g body weight stimulated gastric acid secretion (Figure 1c), but injection of aurantine as before failed to inhibit the effect (Figure 1d).

From these results it is concluded that aurantine does not suppress the ability of the gastric mucosa to secrete acid in response to histamine, but specifically inhibits the stimulant action of pentapeptide. This suggests that the physiological effect of the latter involves DNA-dependent RNA and protein synthesis, while histamine stimulates acid secretion in some other way.

The following experiments show that RNA synthesis in gastric mucosa is increased by pentapeptide, but not by histamine. ^{14}C-labelled adenine (specific activity 48 mC/g) was administered i.p. to rats anaesthetized with urethane in a dose of 25 μC/100 g body weight 30 min before the injection of pentapeptide or histamine. The animals were sacrificed 60 min after the administration of adenine, and the specific activity of RNA extracted from the gastric mucosa by the method of SCHMIDT and TANNHAUSER[14] was determined. The results are shown in Table I. It is evident that the increased RNA synthesis induced by pentapeptide (though not by histamine) is inhibited by aurantine.

Table I. Effect of gastrin pentapeptide and histamine on RNA-synthesis in rat gastric mucosa

Experiment No.	^{14}C-adenine incorporation in cpm/mg RNA			
	Control	Pentapeptide	Pentapeptide + aurantine	Histamine
1	89	146	–	–
2	151	212	72	–
3	195	228	122	–
4	117	175	102	–
5	128	–	–	127
6	128	–	–	119
7	71	–	–	72

Each value is the result of measurements obtained from a group of 2–3 animals.

Table II. Effect of gastrin pentapeptide injection on histidine decarboxylase activity of rat gastric mucosa

Experiment No.	Histidine decarboxylase activity in nmoles CO_2/mg tissue/h			
	Control	Pentapeptide (1 day treatment)	Pentapeptide (1 day treatment + aurantine)	Pentapeptide (30–50 days treatment)
1	3.38 ± 0.12	8.00 ± 0.60	–	3.12 ± 0.10
2	2.95 ± 1.02	9.48 ± 1.11	–	3.25 ± 0.02
3	2.50 ± 1.02	8.15 ± 0.50	–	0.78 ± 0.02
4	3.28 ± 0.15	6.10 ± 0.10	3.27 ± 0.17	–
5	2.57 ± 0.34	8.16 ± 0.50	2.88 ± 0.40	–
6	1.64 ± 0.58	11.65 ± 1.10	2.71 ± 0.27	–

Each value shown represents the mean of 3–4 parallel determinations of enzyme activity in pooled mucosa homogenates from 3 animals.

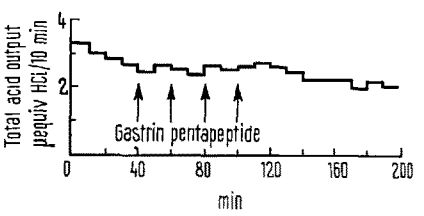

Fig. 2. Effect of prolonged (30–50 days) injections of gastrin pentapeptide on HCl secretion by the anaesthetised rat stomach.

It has been shown[15] that gastrin increases histidine decarboxylase activity (histamine-forming capacity) in gastric mucosa. We have found that a similar effect produced by pentapeptide is also inhibited by aurantine (Table II). In these experiments histidine decarboxylase activity was estimated by a combination of the techniques of KOBAYASHI[16] and BUHLER[17], in which the amount of radioactive CO_2 released by decarboxylation of ^{14}C-histidine is measured. It is concluded that the pentapeptide, and most probably the total gastrin molecule also, causes transcription of the DNA regions (genes) which are responsible for the synthesis of histidine decarboxylase. It seems possible that the newly-formed enzyme may provide a supply of histamine which increases the formation of cyclic 3′,5′-AMP, and this in turn stimulates the secretion of HCl[18].

Other experiments in this laboratory have shown that prolonged treatment of animals with certain hormones of inductive type (e.g., hydrocortisone, insulin) leads to a decrease in the ability of the target cells to respond to such hormones by an increase in DNA-dependent synthesis of RNA and corresponding protein[19]. Such an effect was sought using pentapeptide in the following manner. Rats were injected daily with pentapeptide (0.4–0.8 μg/100 g body weight 4 h before feeding) for 30–50 days. The acid secretion and changes in histidine decarboxylase in response to pentagastrin injections were then studied in acute experiments as described earlier in this paper. It was found that, after such pretreatment, injections of pentapeptide in the acute experiments did not increase histidine decarboxylase activity (Table II) and did not stimulate the secretion of gastric HCl (Figure 2).

Выводы. Введение крысам синтетического аналога пентапептида гастрина («Пентавлон», I. C. I. 50, 123) усиливает синтез РНК и активность гистидиндекарбоксилазы в слизистой желудка, стимулирует секрецию соляной кислоты. Введение животым аналога актиномицина Д – аурантина, тормозит все эти эффекты пентапептида гастрина. Введение гистамина животным стимулирует секрецию соляной кислоты в желудке; этот процесс не сопровождается усилением синтеза РНК и не тормозится аурантином, Предполагается, что гастрин и его активные дериваты, индуцируя гистидиндекарбоксилазу, стимулируют накопление гистамина в клетках слизистой желудка и гистамин является посредником в действии гастрина.

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[14] G. SCHMIDT and S. J. THANNHAUSER, J. biol. Chem. *161*, 83 (1945).
[15] D. AURES and R. HAKANSON, Experientia *24*, 666 (1968).
[16] Y. KOBAYASHI, Analyt. Biochem. *5*, 284 (1963).
[17] D. R. BUHLER, Analyt. Biochem. *4*, 413 (1962).
[18] R. W. RAMWELL and J. E. SHAW, J. Physiol., Lond. *195*, 34P (1968).
[19] R. I. SALGANIK, S. V. ARGUTINSKAYA, N. P. MERTVETSOV and N. B. KHRISTOLYUBOVA Molekulyarnaya biolgiya *2*, 721 (1968).