The Stimulating Action of Gastrin Pentapeptide, Histamine and Cyclic Adenosine 3′,5′-Monophosphate on Carbonic Anhydrase in Rat Stomach

Experiments reported previously demonstrated that gastrin pentapeptide induces histidine decarboxylase in rat stomach. Histamine which appears as a result of this enzyme induction enhances gastric adenylcyclase activity, leading to accumulation[1–3] of cyclic adenosine 3′, 5′-monophosphate (cyclic AMP). Both gastrin pentapeptide and histamine stimulate HCl secretion, but only the gastrin pentapeptide effect is accompanied by RNA synthesis and is inhibited by aurantine[1,2]. These data led to the assumption that histamine and cyclic AMP serve as consecutively acting 'messengers' in the gastrin pentapeptide inductive effect on gastric acid secretion.

There is abundant evidence that carbonic anhydrase plays an important part in the secretion of HCl, being involved in formation of hydrogen ions and transport of hydrogen and chloride ions through gastric cell membranes[4–7]. It was tempting to suggest that cyclic AMP, being obviously a mediator of the gastrin secretagogue effect, activates carbonic anhydrase as it activates other enzymes (glycogen phosphorylase, lipase) operating as a 'second messenger' of a number of hormones (epinephrine, glucagon etc.)[8,9]. The present study was designed to verify this assumption.

Fasted Wistar rats (150–200 g body wt.) received s.c. gastrin pentapeptide (t-butoxycarbonyl-β-Ala-Try-Met-Asp-Phe NH_2; ICI 50, 123) twice at 20 min intervals in a dose of 0.4 μg per 100 g body wt. or histamine i.m. in a dose of 200 μg per 100 g body wt. Cyclic AMP, N^6-2′-O-dibutyryl cyclic adenosine 3′, 5′-monophosphate (dibutyryl cyclic AMP) and theophylline were injected i.p. in a dose of 5 mg per 100 g body wt. Gastric acid secretion was estimated by BARRET's[10] modification of the method of GHOSH and SCHILD[11] as described earlier[1,2].

To investigate the effect of inhibitors of RNA and protein synthesis, actinomycin D or its analogue aurantine were injected i.p. in a dose of 150 μg per 100 g body wt. The greater part of this dose (100 μg per 100 g body wt.) was injected 30 min before gastrin pentapeptide, histamine or cyclic AMP treatment and the rest 60 min after the first injection of the antiobiotic. Cycloheximide was injected i.p. in a dose of 70 μg per 100 g body wt. 30 min before secretagogues were injected. Control rats were injected with saline. 60 min after injections of stimulants of gastric secretion the rats were sacrificed and the stomachs removed and rinsed twice in icecold saline. The mucosa was separated and homogenized in 100 vol. of ice cold twice distilled water[12]. Mucosal homogenates were centrifuged at $4500 \times g$ for 15 min at 4 °C. The activity of carbonic anhydrase was estimated in supernatants according to ROUGHTON and BOOTH[13] and expressed in enzyme units per mg of protein. Protein was estimated by the method of LOWRY et al.[14].

Our experiments have shown that injections of cyclic AMP stimulate HCl secretion (Figure 1)[15], and the same doses of cyclic AMP and dibutyryl cyclic AMP enhance the activity of carbonic anhydrase (Figure 2). Theophylline as an inhibitor of specific phosphodiesterase which splits cyclic AMP increases the stimulating action of cyclic AMP on HCl secretion and carbonic anhydrase activity. Actinomycin D and cycloheximide do not hinder the effect of cyclic AMP (Figure 2).

We have established earlier that gastric adenyl cyclase is stimulated by histamine[3]. As seen in Table I histamine treatment also stimulates carbonic anhydrase activity in rat stomach while actinomycin D and cycloheximide fail to suppress its effect.

In addition we have shown that gastrin pentapeptide also stimulates carbonic anhydrase activity but its action

Table I. *Effect of histamine injection on carbonic anhydrase activity in rat gastric mucosa*

Conditions	Experiment No.	Carbonic anhydrase activity in enzyme units/mg protein
Control	10	11.07 ± 1.06
Histamine + Actinomycin D	9	24.64 ± 2.19
Histamine + Cycloheximide	5	19.14 ± 2.86

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[15] In our other experiments addition of cyclic AMP to perfusion saline[1,2] ($10^{-3}M$ in final concentration) also led to increase of gastric acid secretion.

Table II. Effect of gastrin pentapeptide injection on carbonic anhydrase activity in rat gastric mucosa

Conditions	Experiment No.	Carbonic anhydrase activity in enzyme units/mg protein
Control	7	9.81 ± 0.22
Gastrin pentapeptide	7	19.96 ± 0.51
Gastrin pentapeptide + Actinomycin D	6	8.82 ± 0.42
Gastrin pentapeptide + Aurantine	4	7.91 ± 0.80
Gastrin pentapeptide + Cycloheximide	4	8.78 ± 0.74

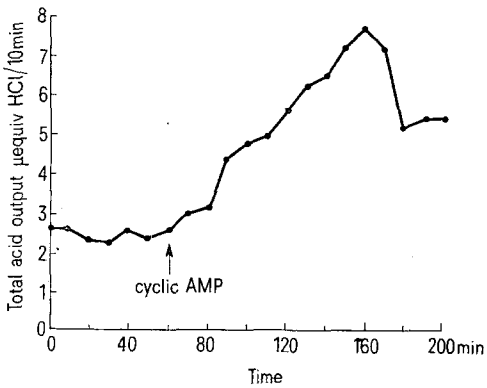

Fig. 1. The secretion of HCl by the rat stomach after injection of cyclic AMP. Each point on the curve represents the mean 10 min acid output for 20 consecutive collection periods for 4 rats.

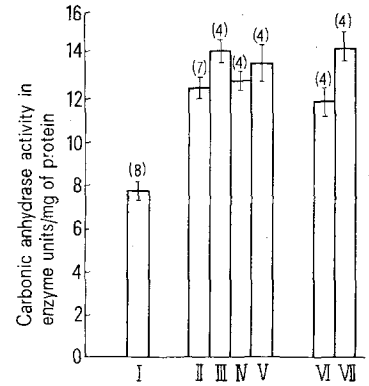

Fig. 2. Effect of cyclic AMP and dibutyryl cyclic AMP injection on carbonic anhydrase activity in rat gastric mucosa. Mean values ± standard deviation are presented. Number of experiments in parentheses. I, control; II, cyclic AMP; III, cyclic AMP + theophylline; IV, cyclic AMP + actinomycin D; V, cyclic AMP + cycloheximide; VI, dibutyryl cyclic AMP; VII, dibutyryl cyclic AMP + theophylline.

may be inhibited by actionomycin D and cycloheximide (Table II).

These results are compatible with our earlier findings indicating that the stimulating action of histamine on gastric acid secretion does not depend on DNA-directed synthesis of RNA and proteins while the effect of gastrin pentapeptide is concerned with induction of transcription[1–3].

The data obtained support the previously suggested scheme of regulation of gastric acid secretion[3,16] according to which gastrin (or gastrin pentapeptide in experiments) evokes transcription of DNA regions responsible for the synthesis of histidine decarboxylase, and the enzyme provides a supply of histamine in target cells of stomach mucosa. Histamine, in turn, activates adenyl cyclase and the cyclic AMP which is formed enhances carbonic anhydrase activity.

It is well known that cyclic AMP mediates the action of a number of hormones by activation of protein kinases of target cells[17,18]. The protein kinases, in turn, phosphorylate definite enzymes[19,20] or other proteins[21,22] providing physiological effects of these hormones. It is likely that changes in carbonic anhydrase activity produced by cyclic AMP may also arise as a result of the enzymic protein phosphorylation.

Выводы. Введение крысам пентапептида гастрина, гистамина и 3′,5′-АМФ усиливают активность карбоангидразы в слизистой желудка крыс. Установлено, что актиномицин Д и циклогексимид тормозят только активацию карбоангидразы, вызываемую пентапептидом гастрина, и не влияют на этот процесс, если он был стимулирован гистамином или 3′,5′-АМФ.

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Effect of Local Anaesthetics on the Accumulation of [^{3}H]-Metaraminol by Rabbit Atria and Vasa Deferentia

The potentiation of responses of peripheral tissues to noradrenaline produced by cocaine is most generally thought to result from the inhibition by cocaine of the uptake of noradrenaline into adrenergic nerves, with a consequent increase in the concentration of amine at the adrenergic receptors[1,2]. Procaine[3,4], lidocaine and prilocaine[5] have also been reported to either potentiate or prolong responses of peripheral tissues to noradrenaline and related amines. In view of these reports, the effect of these agents on the accumulation of [^{3}H]-metaraminol by isolated rabbit atria and vasa deferentia has been examined.

Methods and materials. Pieces of rabbit atria and vasa deferentia were prepared as described previously[6] and preincubated at 37°C for 30 min in a physiological salt solution[6]. [^{3}H]-metaraminol (1×10^{-8} *M*) was then added to the media and the incubation continued for a further 30 min. At the end of this period, the total [^{3}H] content