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The effect of submaximal doses of pentagastrin on gastric acid secretion, histamine and histidine decarboxylase in rats*

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In Lai-rats gastric mucosal histamine and histidine decarboxylase were estimated after stimulation of gastric acid secretion by intravenous infusions of submaximal doses of pentagastrin for 1 or 2 h. Pentagastrin produced a dose-dependent acid response with a maximum of 26 μ equiv H^+ per 10 min at a dose of 2·56 μg kg^{-1} min^{-1}. There was a linear relation between the log dose of pentagastrin and the activation of gastric histidine decarboxylase. The highest dose of pentagastrin yielded a histidine decarboxylase activity of 200% of the unstimulated level when infused for 1 h and of 290% when infused for 2 h. No reduction of gastric mucosal histamine could be detected whatever the dose of pentagastrin or the duration of infusion. It was concluded (1) that stimulation of gastric histidine decarboxylase is a physiological function of gastrin-like peptides, (2) that a reduction of gastric mucosal histamine by gastrin or pentagastrin is a pharmacological rather than a physiological effect, and (3) that no negative feedback relation exists beween gastric mucosal histamine and the activation of histidine decarboxylase.

One of the effects of gastrin is to activate gastric histidine decarboxylase. This effect accompanies stimulation of gastric acid secretion, but it can be dissociated from the acid response by secretin (Caren, Aures & Johnson, 1969) and by antigastrin (SC-15396) (Kobayashi & Maudsley, 1968; Håkanson & Liedberg, 1971). It is still unknown whether gastrin or gastrin-like peptides, i.e. pentagastrin, stimulate gastric histidine decarboxylase directly, as suggested by Aures, Johnson & Way (1970) or by a negative feedback mechanism in which the reduction of gastric mucosal histamine by gastrin is the adequate stimulus for the activation of histidine decarboxylase, as suggested by Kahlson, Rosengren & others (1964). Furthermore it remains uncertain whether the histamine-releasing property of gastrin-like peptides is a physiological or a pharmacological effect, since most relevant reports have not made clear whether the dose of gastrin or pentagastrin was sub- or supramaximal for gastric acid secretion. We have investigated gastric mucosal histamine and histidine decarboxylase after stimulation of gastric secretion by an intravenous infusion of pentagastrin in doses submaximal for acid secretion in rats.

METHODS

The experiments were made with male Wistar rats (FW 49 Biberach, 200–400 g) in a randomized order. For 24 h before the experiments animals had free access to drinking water but no food. They were prepared according to Lai's method (1964)

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Table 1. *Composition (in ml) of and final concentrations in the incubation mediums for determination of histidine decarboxylase activity. Vessel A contains the active enzyme, vessel B the inactivated enzyme.*

	Vessel A	Vessel B	Final concentration
Main vessel			
Enzyme source	0·8	0·8	
Phosphate buffer (pH 7·0)	1·4	1·2	10^{-1} M
Aminoguanidine sulphate	0·1	0·1	$2{\cdot}5 \times 10^{-4}$ M
Nicotinamide	0·1	0·1	10^{-2} M
Pyridoxal-5′-phosphate	0·1	0·1	2×10^{-5} M
Perchloric acid	—	0·2	4×10^{-1} N
Sidearm			
L-Histidine	0·5	0·5	10^{-2} M
Final volume	3·0	3·0	

for studying gastric acid secretion. The gastric perfusate was collected in 10 min periods, total acidity was determined by automatic titration (Radiometer autoburette) to pH 7·0 and was expressed in μ equiv H^+ per 10 min.

The animals were divided into groups of 6. After three 10 min periods for estimating basal secretion, stimulation of acid secretion was achieved by intravenous infusions of 0·04–2·56 μg kg^{-1} min^{-1} of pentagastrin for 60 or 120 min into the jugular vein. The infusion volume was adjusted to 2·3 ml h^{-1}. In two control groups saline was infused in the same volume for the same length of time as in the pentagastrin-treated animals.

At the end of infusion the stomach was removed, opened along the lesser curvature, rinsed with demineralized water and blotted. The mucosa of the glandular part (400–700 mg) was scraped off and homogenized in 7 volumes of demineralized water and centrifuged at 1800 *g* for 5 min. The supernatant served as enzyme source which was prepared at 4°. The composition of the incubation media is given in Table 1. Both media were equilibrated for 30 min under N_2 at 37° in a Warburg apparatus, L-histidine remaining in the sidearm. After the components were mixed, the reaction media were incubated under preincubation conditions for 60 min. Immediately afterwards the enzymatic process in vessel A containing the intact enzyme was stopped by adding 0·5 ml of 3 N perchloric acid, vessel B, containing the inactivated enzyme, was filled to the same volume with 0·5 ml of demineralized water. Histamine in both vessels was extracted and estimated by the method of Shore, Burkhalter & Cohn (1959) as modified by Burkhalter (1962). The histidine decarboxylase activity, expressed as μg histamine formed g^{-1} h^{-1} was calculated from the difference of the histamine contents in vessels A and B. The histamine concentration of B was taken as the histamine content of the mucosa and is expressed in μg g^{-1} wet tissue.

MATERIALS

Pentagastrin (Merck, Darmstadt),* aminoguanidine sulphate and *o*-phthaldialdehyde (Fluka, Buchs), nicotinamide and L-histidine (Schuchardt, Munich), pyridoxal-5′-phosphate (EGA-Chemie, Steinheim). *O*-Phthaldialdehyde was freshly prepared for each experiment, all other compounds were kept as stock solutions at −16° and were renewed every 4 weeks.

* Pentagastrin was kindly supplied by Dr. Wendt, Fa. E. Merck, Darmstadt.

RESULTS

Gastric acid secretion (Fig. 1). The basal secretion before any infusion was begun varied between 0·5–4·0 μ equiv H^+ per 10 min. In some animals the infusion of saline caused a slight increase of the secretion rate without seriously affecting the basal secretion. Pentagastrin produced a dose-dependent acid response forming a plateau which was reached within 20–30 min at lower doses and within 50–60 min at higher doses. The height of the plateau was independent of the duration of infusion. The acid responses covered almost the total range of the dose response curve. The highest dose yielded a secretion rate of about 26 μ equiv H^+ per 10 min, which is above the 90% level of the maximal response calculated by a linear transformation of the Michaelis-Menten-kinetics (Johnson & Grossman, 1969) as described by the equation $V = -K \cdot V/S + V_{max}$, in which V = observed response, V_{max} = calculated maximal response, S = dose and K = calculated constant numerically equal to the dose required to elicit half maximal response.

Histidine decarboxylase (Figs 2 and 3). The histidine decarboxylase activity of the control animals was $13 \pm 3{\cdot}2$ $\mu g\ g^{-1}\ h^{-1}$ after 1 h of infusion and $10{\cdot}1 \pm 1{\cdot}9$ $\mu g\ g^{-1}\ h^{-1}$ after 2 h of infusion. With increasing doses of pentagastrin the histidine decarboxylase activity rose to 200% of the controls after 1 h of infusion and up to 290% after 2 h of infusion. The differences between adjacent doses of pentagastrin were statistically insignificant. However, in linear regressions calculated from the single values, the regression coefficients differed significantly from zero for both groups of experiments ($b/s_b = 2{\cdot}19$, $P < 0{\cdot}05$ resp. $b/s_b = 3{\cdot}86$, $P > 0{\cdot}001$) indicating a clear dose response relation.

Histamine content. In all experiments the mean values of the histamine content varied between 60–74 $\mu g\ g^{-1}$ wet tissue. None of the pentagastrin doses infused for 1 or 2 h were able to reduce significantly the histamine content in the gastric mucosa.

DISCUSSION

We chose an intravenous infusion of pentagastrin to maintain a circulating amount of pentagastrin in quantities submaximal for gastric acid secretion over a longer period of time. Comparing our dose response relation with that of Kowalewski (1971), who used conscious rats with gastric fistulas, our maximal response is about 50% and the dose eliciting it about 5 times that he reported. Stimulation of gastric histidine decarboxylase by gastrin or pentagastrin is now generally accepted (for references see Johnson, 1971). However, in most reports dealing with dose response relations the gastrin or pentagastrin dose was not correlated with both the acid response and histidine decarboxylase activity. From the available data it is not possible to decide whether a pentagastrin dose that is maximal for stimulating gastric acid secretion is also maximal for activating gastric histidine decarboxylase. Our results indicate that submaximal doses of pentagastrin, which may be regarded as "physiological", are able to stimulate gastric histidine decarboxylase.

The failure of pentagastrin to reduce the histamine content of the gastric mucosa contrasts with other reports describing a histamine release by gastrin or pentagastrin (Kahlson & others, 1964; Haverback, Tecimer & others, 1964; Haverback, Stubrin & others, 1965; Stubrin, Dyce & others, 1965; Johnson & Aures, 1970; Lin & Evans, 1970; Garparg & Halpern, 1971; Håkanson & Liedberg, 1971). We have found that in rats a single intraperitoneal injection of 125 $\mu g\ kg^{-1}$ pentagastrin reduced gastric

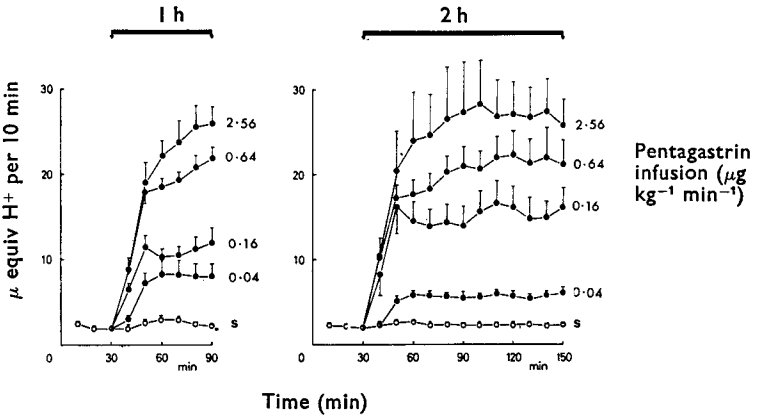

FIG. 1. Effect of submaximal doses of pentagastrin infused for 1 h or 2 h on gastric acid secretion in Lai-rats. In this and subsequent figures each point is the mean value of 6 experiments. The vertical bars represent the standard error of mean. S=saline.

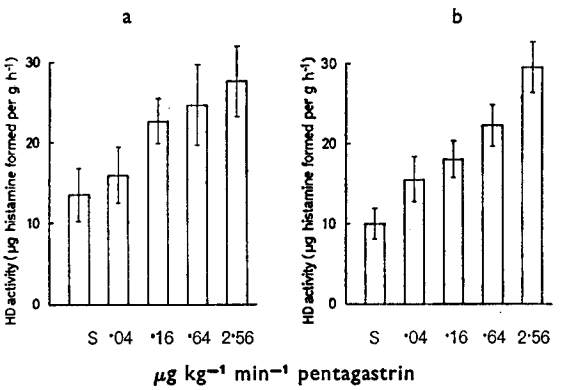

FIG. 2. Gastric histidine decarboxylase (HD) activity after different doses of pentagastrin infused for 1 h (a) or 2 h (b). For further explanations see Fig. 1.

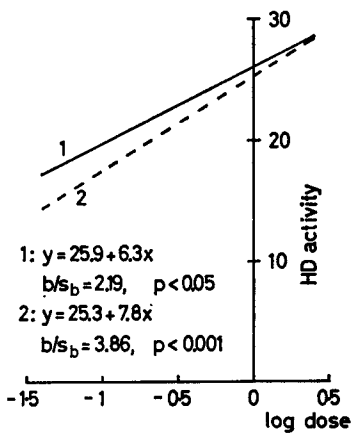

FIG. 3. Regression lines and equations to demonstrate the linear relation between the log dose of pentagastrin infused for 1 h (1) or 2 h (2) and the activation of gastric histidine decarboxylase (HD).

mucosal histamine within 90 min significantly ($P < 0{\cdot}001$) by 30%. However, in all these studies gastrin or pentagastrin was administered as a single injection (s.c. or i.p.,) and most of the doses of gastrin or pentagastrin seem to be supramaximal with regard to acid stimulation so that the histamine-releasing potency of gastrin or pentagastrin may be regarded as a pharmacological rather than a physiological effect. The finding that those doses of pentagastrin that activate gastric histidine decarboxylase fail to reduce the histamine content of the gastric mucosa do not favour Kahlson's concept of a negative feedback mechanism. The negation of such a feedback control is supported by Kim & Glick (1968) who found a reduction of gastric histamine by ACTH and bethanechol without an increased histidine decarboxylase activity. Furthermore Kobayashi & Maudlsey (1971) were able to stimulate gastric histidine decarboxylase by pentagastrin at histamine levels far above normal, and Håkanson & Liedberg (1971) showed that gastric histidine decarboxylase can be stimulated independently of the histamine level. That gastric mucosal histamine is initially reduced by pentagastrin and that this reduction triggers the activation of histidine decarboxylase cannot be completely excluded. However such a possibility is not supported by the literature.

In conclusion the present study is in agreement with Johnson's (1971) view that gastric secretion stimulated by gastrin and gastrin-like peptides is not causally linked with the release or biosynthesis of histamine.

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