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Acid gastric secretory responses to histamine, crude porcine gastrin and pentagastrin in rats

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The log dose-response curves for graded doses of the secretagogues porcine gastrin (a partially purified sample; the crude and its gastrin II equivalent), histamine, and a gastrin pentapeptide (pentagastrin) on the perfused stomachs of urethanized rats are parallel. On weight basis, pentagastrin is 60 times and histamine four times more active than the crude porcine gastrin preparation. The partially purified porcine gastrin sample is six times more potent than histamine but half as potent as pentagastrin. On molar basis gastrin (as the pure porcine gastrin II) has 3000 times the activity of histamine dihydrochloride and 5000 times that of the histamine base. Gastrin is 50 times more potent than pentagastrin. Gastrin and pentagastrin are more potent and have less undesirable side-effects than histamine.

The two most important endogenous substances that control acid gastric secretion are histamine and gastrin (Amure & Ginsburg, 1964) Secretory activity in human stomach and in experimental animals is routinely tested with histamine, preceded by an antihistamine (Kay, 1953) or its pyrazole isomer (histalog) with or without antihistamine drug (Rosiere & Grossman, 1951). In spite of this, the use of histamine has its limitations and is not without dangers. The aim of the present work therefore is to compare the efficacy of the three substances, namely, histamine, crude and standardized gastrin and pentagastrin (ICI 50 123) on acid gastric secretion in rats.

EXPERIMENTAL

Materials. Histamine dihydrochloride (L. Light & Co.); pentagastrin (ICI 50 123) ICI Ltd; Crude gastrin (hog gastrin). Standardized gastrin (gift from Prof. M. Ginsburg, Chelsea College, London).

Methods. Male albino Wistar rats, 190 to 240 g, were given sugar lumps (cane sugar) instead of their normal food pellets, 24 h before the start of the experiments. This made the cleaning of the stomachs easy for the perfusion experiments. Water was given freely. Anaesthesia was induced with urethane (0·6 ml/100 g weight of a 25% w/v solution) given intramuscularly. This dose maintained a satisfactory and uniform anaesthesia for up to 13 h. Stomachs for perfusion and the continuous recording of acid secretion were prepared according to Ghosh & Schild (1958),using 0·00025–0·001N NaOH as the perfusion fluid. This fluid acted as an approximate linear buffer from pH 8·5–4·0 and pH 7·0–3·9 in 2 rats. The response metameter was the maximum fall in the pH of the gastric effluent fluid after injection. All injections were intravenous through a cannulated femoral vein in volumes of 0·05 to 0·2 ml and the cannula washed with 0·1 ml of 0·9% saline, after each injection.

Histamine doses refer to its salt in μg. Doses of pentagastrin and crude gastrin are also expressed in μg. The crude gastrin was the water soluble extract from hog antral mucosa (Blair, Harper & others, 1961). The standardized gastrin was a

partially purified porcine gastrin previously assayed by Prof. M. Ginsburg against the pure gastrin II. A laboratory unit of it is equivalent to 2 μg by weight and on assay was found to be equivalent to 50 ng of the pure gastrin II.

To facilitate comparison of the various secretagogues a standard procedure consisting of high and low doses of the standard, and high and low doses of the test drugs in randomized order was adopted. The response metameters are plotted against log dose of each secretagogue for graded doses in 4 rats as shown in Fig. 1. All the doses used were from the linear portion of the log dose-response curves. The responses for potency comparison were required to operate closely both in intensity and duration. The crude porcine gastrin extracts were assayed against the standardized gastrin.

The histamine content of the crude gastrin extracts assayed on guinea-pig ileum (Adam, Hardwick & Spencer, 1954) was less than 0·05 μg/mg of crude extract, an amount that does not interfere with acid secretory responses elicited by crude gastrin.

RESULTS

The acid secretory responses to successive injections of the same dose of a secretagogue increased up to the third or fourth dose before a steady response was reached when little or no alteration in sensitivity of the rats to the secretagogues was detected in experiments lasting over 9 h. Assays were begun when the steady state was reached. Responses to graded doses of the secretagogues are shown in Table 1.

Table 1. *Responses to graded doses of standardized porcine gastrin, crude porcine gastrin, pentagastrin and histamine dihydrochloride*

Stimulant	Dose (μg)	pH change (mean and standard deviation)	No. of rats used
Standardized porcine gastrin	10	2·40 ± 0·1	(10)
	20	2·75 ± 0·15	(12)
	30	2·90 ± 0·1	(8)
Crude porcine gastrin	200	2·0 ± 0·25	(12)
	400	2·2 ± 0·15	(10)
	800	2·4 ± 0·40	(14)
Histamine dihydrochloride	100	2·60 ± 0·25	(16)
	200	2·80 ± 0·10	(12)
	400	2·97 ± 0·20	(10)
	800	3·20 ± 0·05	(14)
Pentagastrin	6·25	2·10 ± 0·25	(13)
	12·5	2·30 ± 0·30	(15)
	25	2·60 ± 0·32	(12)

Responses to histamine and crude gastrin

Acid secretory responses to gastrin and histamine were typical and followed the course described by Ghosh & Schild (1958) and Amure & Ginsburg (1964). In most experiments, the maximum fall in pH of the gastric effluent was within 15 min of injection and the responses lasted about 30 min. Animals tolerated small doses of histamine (100 to 800 μg); but larger doses caused respiratory disturbances.

Responses to pentagastrin and crude gastrin

Acid secretory responses to pentagastrin started within 2 to 3½ min after the completion of the injection (2·72 ± 0·75 min, mean and standard deviation for 20 observa-

tions in 13 rats). The maximum fall was reached within 20 min and recovery was complete within 1 h. The time-course responses for histamine, crude gastrin and pentagastrin showed no marked differences and the pattern is comparable in all three secretagogues with low doses of histamine (100 to 200 μg); pentagastrin (6·25 to 12·5 μg) and high doses of crude gastrin (400 to 800 μg). The results are shown in Fig. 2.

The log dose-response curves for histamine, crude gastrin, and pentagastrin are judged paralleled as shown in Fig. 1 (slopes 0·60; 0·66 and 0·7). This indicates a common mode of action and thus allowed the comparison of these secretagogues.

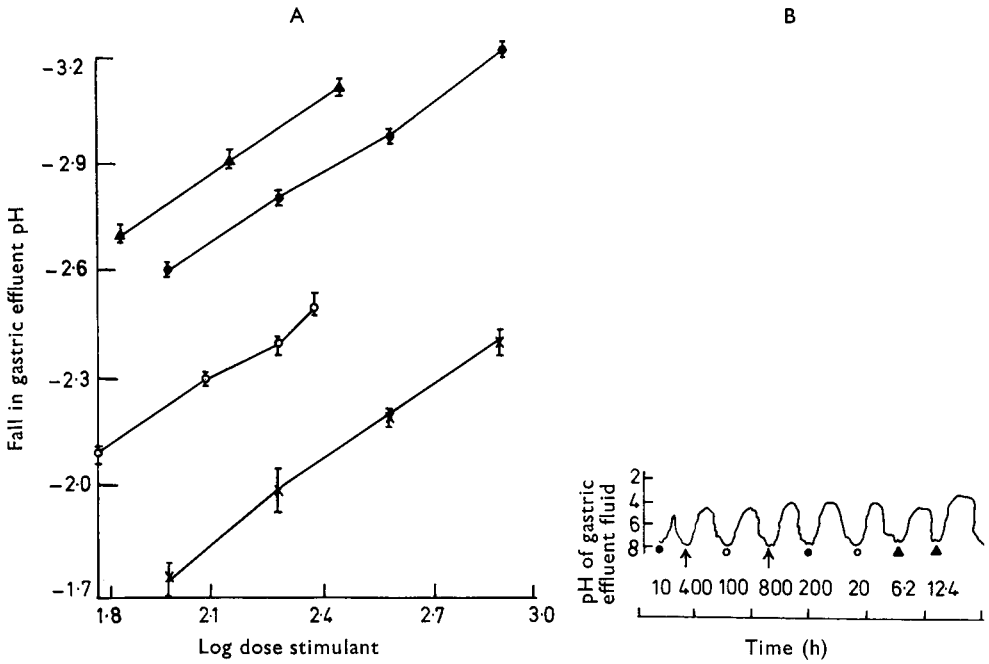

FIG. 1A. Log dose response curves for stimulants. Each point is the mean for 4 rats of maximum fall in gastric effluent pH. Vertical bars represent standard error of the mean. ▲—▲, Standardized gastrin (×10 on dose scale); ●—●, histamine dihydrochloride; ○—○, pentagastrin; (×10 on dose scale); ×—× crude porcine gastrin.

B. Effects of intravenous injection of standardized porcine gastrin (●), crude porcine gastrin (↑), histamine dihydrochloride (○) and pentagastrin (▲). The signs indicate the points of injection. Doses (μg) are shown below the signs.

On weight to weight basis, pentagastrin was found to possess about 60 times the activity of the crude porcine gastrin, while histamine (expressed as the dihydrochloride) has four times the potency of crude porcine gastrin. The standardized gastrin (partially-purified porcine gastrin) was six times more potent than histamine (95% confidence limit 5·1–6·9) but it is half as potent as pentagastrin (1·5–2·5). After conversion of the crude porcine gastrin to its pure porcine gastrin II equivalent, gastrin was found on a weight basis to be 270 times more potent than histamine (95% confidence limit 231–309) and 18 times more potent than pentagastrin (16·6–19·6). On molar basis the differences became more pronounced, when pure gastrin was found to be 3000 times more potent than histamine (95% confidence limit 2600–3500) and 50 times more potent than pentagastrin (45·1–53·5). Pentagastrin was, on weight basis, 15 times more potent than histamine (95% confidence limit 14·2–15·7 and 60 times more potent on molar basis (59–66). The index of precision of assays

calculated from two log dose–response curves for graded doses was 0·18 and 0·25. Thus the assays may be regarded as reliable.

In the doses used, pentagastrin had a slight but consistent pressor effect on rat blood pressure (4·37 $\pm$ 1·75 mm Hg rise, mean and standard deviation for 8 rats). Histamine in doses used in the assays exhibited pronounced and consistent depressor effects on blood pressure (37·7 $\pm$ 8·3 mm Hg fall in 10 rats). In rats in which simultaneous recordings of blood pressure and gastric secretion were made, the hypotensive effect of histamine started before the gastric secretory effect and the recovery of blood pressure occurred long before the recovery of the gastric secretion. Crude porcine gastrin had no consistent effect on blood pressure except occasionally when slight pressor effects occurred with large doses and these were significantly lower when compared with the depressor effects of histamine ($P < 0{\cdot}001$).

DISCUSSION

The increase in the sensitivity of the rat stomach to the injected secretagogues with the first three or more doses of an assay confirms the observation of Rosenoer & Schild (1962) for carbachol in rats. The necessity for stabilizing the sensitivity of the rat stomach before beginning an assay is thus seen. Also with histamine there was a difference in the time for the responses to develop in the 'stabilized' and 'non-stabilized' stomachs. The differences were not apparent with crude gastrin, standardized gastrin or pentagastrin. In stabilized stomachs the onset of responses to histamine is as short as those of gastrin and pentagastrin. The short latency of response to extracts containing gastrin confirms the finding of Amure & Ginsburg (1964) in rats and Makhlouf, McManus & Card (1964) in man. The over-all time course of responses to gastrin confirms in its entirety the findings of Amure & Ginsburg (1964) but only part of the confirmation of their findings and those of Ghosh & Schild (1958) were obtained for histamine.

The crude porcine gastrin was less pure than the purified gastrin, thus explaining the lower potency of the crude material compared with histamine and pentagastrin.

Barrett, Raventos & Siddal (1966) using a modification of the method of Ghosh & Schild (1958) found that gastrin possessed on molar basis 6000 times the activity of histamine base and about ten times the activity of pentagastrin. On a weight for weight basis, Hansky & Eu (1967), using subcutaneous injection in pyloric and oesophageal ligated rats, found pentagastrin seven times less potent than gastrin. We found histamine (as the dihydrochloride) to be about 3000 times less potent than gastrin on a molar basis and 5000 times less active as the histamine base. Gastrin is 50 times more potent than pentagastrin on molar basis.

The findings confirm that gastrin is the most potent of the three secretagogues.

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