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The assay of gastrin using the perfused rat stomach

G. M. SMITH, A. J. LAWRENCE, D. G. COLIN-JONES AND H. O. SCHILD

Department of Pharmacology, University College London, and University College Hospital Medical School, Gower Street, London W.C.1

Summary

1. A method is described for the bio-assay of gastric secretagogues using the perfused rat stomach in which the acid output can be readily and accurately quantified.

2. This technique increases the sensitivity of the perfused rat stomach so that 10–20 ng of synthetic human gastrin I can be detected.

3. It is possible to assay secretagogues, relative to a standard with fiducial limits of less than $\pm 30\%$.

4. The use of this system is illustrated by assays comparing synthetic human gastrin I with pentagastrin, pure hog gastrin and gastrin added to plasma.

Introduction

Since Ghosh & Schild introduced the perfused rat stomach in 1955 for measuring acid output in response to various stimuli, a number of modifications have been reported. Several workers measured the acid response by titrating regular 10–30 min samples, either by hand or using an automatic titrator (Lai, 1964; Barrett, 1966; Pissidis & Clark, 1967; Moore, Murat, Endahl, Baker & Zollinger, 1967). This technique requires careful timing and is laborious. Others have used a flow electrode to record pH changes in a dilute buffer (Ghosh & Schild, 1958; Rosenoer & Schild, 1962; Thompson & Sircus, 1967), but a drawback of methods based on the continuous measurement of pH change is that the differential record obtained necessitates the cumbersome measurement of an area in order to compute the amount of acid secreted in response to stimulant drugs. We have therefore attempted procedures in which the integral of acid secreted in response to a drug could be directly measured. After some experimentation a method was evolved which is based on reperfusion of a measured quantity of fluid through the rat stomach using the cumulative pH change as an index of acid secreted. The procedure gives acceptable confidence limits and its sensitivity is such that 10–20 ng of synthetic human gastrin I can be reliably measured.

Methods

Male black hooded rats of 180–330 g were starved for 16–24 hr with access to water, and anaesthesia was then induced by intramuscular urethane (1·5 g/kg body weight, a small additional dose being given if necessary). The body temperature fell during the experiment and was maintained at 30° C by a rectal contact thermometer operating a 25 W lamp under the table on which the animal lay and an

overhead 40 W strip light. An alternative has been used in which the body temperature is maintained at 34° C by laying the rat on a table warmed to 37° C by circulating water.

Operative technique

The trachea was exposed and cannulated. A polythene tube of 2·5 mm external diameter was passed down the oesophagus and tied in place in the neck, excluding the vagal nerves. A fine polythene cannula (Portland Plastic PP25) was used to cannulate the external jugular vein. The abdomen was opened through the linea alba and a glass cannula passed through an incision in the duodenum approximately 3 cm from the pylorus, and gently slipped into the stomach, care being taken to avoid handling the stomach. This cannula was 6 mm in external diameter with a waist 2 cm from the tip 4·5 mm in diameter, which snugly fitted the pylorus. The intragastric portion of the cannula contained numerous perforations. No ligature was required round the pylorus, thus leaving the gastric blood supply intact, but the cannula was tied in place with a ligature round the duodenum. Any gastric contents were washed out with warmed saline as a clean, freely-draining stomach was essential for good results.

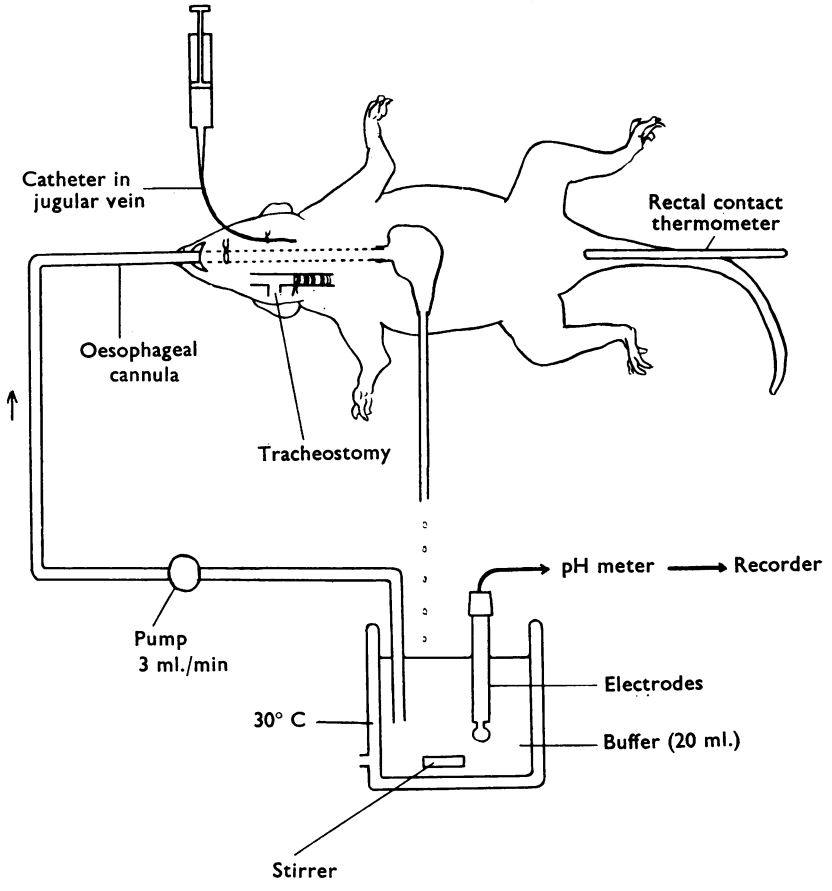

FIG. 1. Diagram of reperfusion set-up.

Gastric perfusion system

This is shown diagrammatically in Fig. 1. The stomach was continuously perfused with 20 ml. of a propionic-succinic buffer in normal saline at 30° C, recirculated at a rate of 3 ml./min by means of a Watson-Marlow roller pump, the gastric effluent dripping back into a reservoir.

A stock solution of buffer was prepared by dissolving 0·5 mole propionic acid (37 g), 0·5 mole succinic acid (59 g) with 1 mole solid sodium hydroxide (40 g) in water and diluting to 1 litre. The final pH adjustment to pH 5·5 was made with a glass electrode using N/1 NaOH or N/1 HCl. From this solution fresh buffer was made up for each experiment by diluting 5 ml. to 1 litre or for maximal sensitivity 3·33 ml. to 1 litre. This buffer has three pK values distributed over the pH range used (4·6–5·6), so that the pH change is nearly linear as H^+ ions accumulate (Fig. 2). If 80% of the scale is used, the greatest deviation from the theoretical straight line during titration is 0·9 μ-equiv HCl. Within this pH range dissolved CO_2 is no problem. The buffer was changed after each test to reduce changes due to gastric absorption.

Experimental procedure

A multichannel recorder (Multipoint Cleertrend, Leeds and Northrup) was used to record the acid output from four to six animals concurrently, which allowed a Latin square assay to be done in one day. The chart was calibrated so that a full scale deflection equalled 1·0 pH unit and, knowing the volume and concentration of the buffer, the acid secretion could be calculated in μ-equiv H^+ from the change in pH. The basal acid output was recorded for 15 min before injecting the gastric stimulant. Basal secretion was assumed to continue unchanged during the stimulation period. The acid secretion during the succeeding 40 min could be determined by extrapolating the baseline (Fig. 3) and measuring the ordinate above the extrapolated line.

The stomach was washed with warmed saline and perfusion was begun using fresh buffer. After clearing air bubbles from the stomach, a 15 min basal period was started. A stimulant, either carbachol, 0·5 μg, or pentagastrin, 0·2 μg, was given intravenously and the response recorded over 40 min to check the sensitivity of the preparation. This first dose tested gave inconsistent results and so was disregarded.

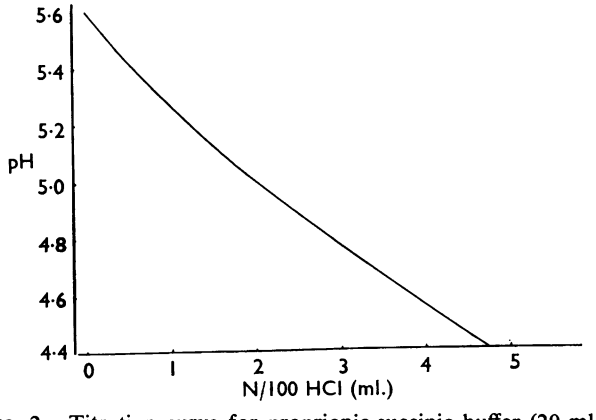

FIG. 2. Titration curve for proprionic-succinic buffer (20 ml.).

The buffered reperfusion solution was then changed and the routine repeated with the secretagogue to be investigated. The experiment then started with the second dose administered.

Results

Constancy of the base line

As the response of the rat to a secretagogue was measured by the acid secreted in excess of the basal rate, a constant base line was necessary. The basal rate was observed in each of the four rats over 2 hr to assess any spontaneous changes in

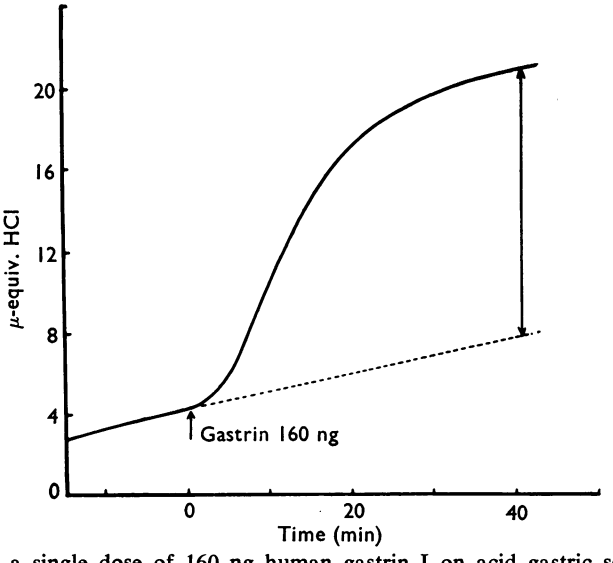

FIG. 3. Effect of a single dose of 160 ng human gastrin I on acid gastric secretion. Reperfused rat stomach, this and subsequent tracings.

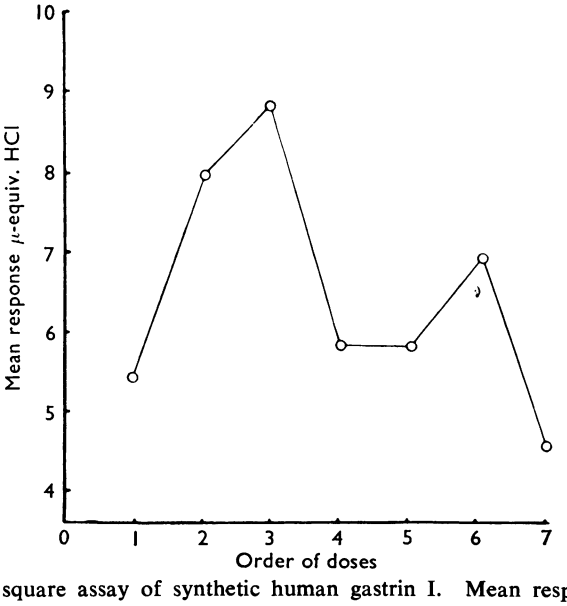

FIG. 4. 7 × 7 latin square assay of synthetic human gastrin I. Mean responses in successive injections.

secretion. The basal rates of acid secretion were 0·1, 0·3, 0·4 and 2·1 μ-equiv HCl/10 min for the four rats and spontaneous changes were random about each mean, except one in which there was a gradual increase in secretion from 0·4 to 1·4 μ-equiv/10 min.

Constancy of response to gastrin

Figure 4 shows successive mean responses in a gastrin assay designed as a 7×7 latin square. Seven rats were used, each receiving all seven doses. The variation in successive mean responses appears to be random, with no obvious trend. In this particular assay analysis of variance showed significant differences arising from dose order ($P = 0{\cdot}01$). In shorter $2+2$ assays arranged as 4×4 latin square there has been no significant variation with the order of doses in the course of over a dozen assays.

Dose-response curve to synthetic human gastrin I

From the above mentioned 7×7 latin square experiment, a dose response curve was constructed which is shown in Fig. 5. It can be seen that the lowest detectable dose of gastrin was 10–20 ng; only five of the seven animals responded to 10 ng. The steepest part of the curve, and hence the most suitable for assay, is from 40 to 200 ng.

Quantitative assays

Assay of pentagastrin (I.C.I.) against synthetic human gastrin I

This was carried out as a $2+2$ assay using a latin square design. The responses to 40 and 160 ng of each stimulant are shown in Fig. 6. Calculated on a weight basis synthetic human gastrin was 1·23 times as active as pentagastrin; on a molar basis it is 3·5 times as active. The analysis of variance and 95% fiducial limits (Colquhoun, 1969) for the assay are shown in Table 1. There is no significant deviation from parallelism and no significant effect of dose order; there are, however, significant variations in sensitivity between the four rats employed for the assay.

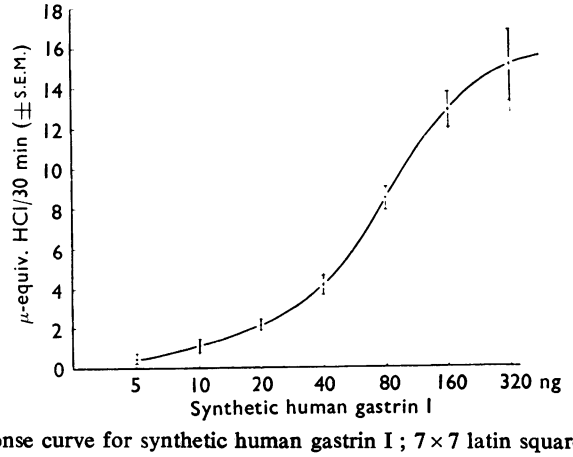

FIG. 5. Dose-response curve for synthetic human gastrin I; 7×7 latin square. Mean responses and standard errors.

A 3+3 assay of synthetic human gastrin I and pure hog gastrin

The latter preparation was kindly supplied by the Medical Research Council. Doses of 40, 80 and 160 ng of each gastrin were employed in a latin square design. No significant difference in activity was found between the two gastrins; the calculated potency ratio human gastrin/hog gastrin was 0·85; unity was included in the 95% fiducial limits, which were 0·61 and 1·18.

Assay of gastrin added to plasma

In order to test whether plasma influences the response to gastrin, a 2+2 assay was done comparing synthetic human gastrin I in saline (50 and 150 ng) with the

TABLE 1. *Analysis of variance of* 2+2 *assay*

Synthetic human gastrin I (SHG) v. pentagastrin (I.C.I.)
40 ng and 160 ng 40 ng and 160 ng

Source of variation	Degrees of freedom	"Sum of squares"	Mean square	*F* Ratio	*P*
Linear regression	1	256	256	285	<0·001
Deviation from parallelism	1	0·7	0·7	0·8	>0·2
Between S.H.G. and pentagastrin	1	5·7	5·7	6·3	<0·05
Between the four rats	3	20·9	6·9	7·7	<0·05
Between the order of doses	3	5·2	1·7	1·9	>0·2
Error	6	5·5	0·9		
Total	15	294			

Potency ratio (weight for weight) and 95% fiducial limits

$$\frac{\text{Synthetic human gastrin I}}{\text{Pentagastrin}} = 1{\cdot}23 \begin{cases} 1{\cdot}52 \\ 0{\cdot}998 \end{cases}$$

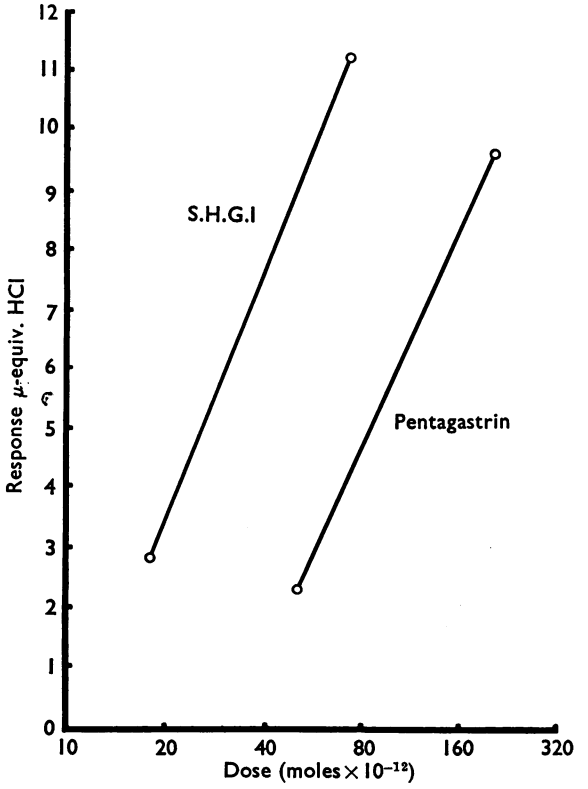

FIG. 6. 2+2 assay of synthetic human gastrin I (mol. wt. 2,200) and pentagastrin (mol. wt. 768).

same doses of gastrin added to 1 ml of normal fasting human plasma. The potency ratio saline gastrin/plasma gastrin was 1·18, with 95% limits of 0·92 and 1·55. It would thus appear legitimate to assay gastrin-like activity in plasma of patients.

Discussion

The modifications which we have introduced were designed to increase the speed and accuracy of the measurement of acid secretion and hence to facilitate the quantitative bioassay of gastrin and related polypeptides. A succinic-propionic buffer was used which gave a near-linear change in pH when hydrogen ions were secreted. The buffer was continuously recirculated through the rat's stomach so that hydrogen ions accumulated and the response was integrated as it occurred. A simple linear measurement gave the amount of acid secreted in a given time and the time course of secretion could be continuously followed by a glass electrode.

In order to obtain a steady baseline and reproducible results it is essential to have a clean stomach with no obstruction to the outflow through the pyloric cannula. The length of the cannula was designed to fill the body of the stomach and the perforations allowed free flow of the buffer.

The preparation is sensitive to small amounts of gastrin, so that 10–20 ng of synthetic human gastrin I can be detected and 20 ng can be used as the lower dose of a block assay. Our threshold dose of synthetic human gastrin I was less than that found by other workers. Thus Blair & Wood (1968) found that 50 ng could be detected in their cat preparation, while Barrett (1966) in the rat used about 500 ng as the standard. The present system is sufficiently sensitive to measure gastrin-like activity in the plasma and tumour extract of a patient with the Zollinger–Ellison syndrome (Colin-Jones, Gibbs, Copping & Sharr, 1969).

Although not as sensitive for the assay of gastrin as a recently developed radioimmunoassay (McGuigan, 1968 ; McGuigan & Trudeau, 1968) a bioassay measures the physiologically active secretagogue rather than an immunologically reactive substance. Thus ideally the two techniques should be used together to measure gastric secretagogues in the body fluids of man. In this respect the rat has an advantage over cat and guinea-pig in being about 1,000 times less sensitive to histamine than to gastrin.

Lai (1964) observed that in the anaesthetized rat assay the first response was variable, being too unreliable to include in an assay, and that the fifth and subsequent response showed an increase in sensitivity which prevented him from completing a block assay. We have also found the first response to be unreliable and therefore have rejected it in the assay, but otherwise the variability with successive doses was usually small with no systematic trend in responsiveness, so that we were able to do complete block assays. In a number of 2+2 assays done, there has been no significant variation due to the order of doses. The ability to use this technique for a complete block assay increased the accuracy and reduced the fiducial limits, but as in all pharmacological assays the best results were obtained when the known and the unknown samples were of similar potencies, and when the difference between the high and low doses was large so as to give a steep slope to the regression line. A 2+2 assay was used regularly as it gave reasonable confidence limits and could be completed in a working day. Doses of 40–200 ng synthetic human gastrin I

produced effects on the steep part of the log dose-response curve and gave the best bioassay results.

This technique has also been used for other pharmacological studies. Lawrence, Smith & Schild (unpublished) investigated urogastrone, an inhibitor of acid secretion found in the urine, and were able to use as a measure of the activity of extracts the reduction it caused in the secretory response to carbachol. Colin-Jones & Himsworth (1969) used it to investigate the characteristics determining the gastric acid response to hypoglycaemia.

A similar potency, on a weight basis, between pentagastrin and synthetic human gastrin I has been found. This gives a greater potency for the pentapeptide than found by Barrett (1966), but the difference is not large, and as our confidence limits include a potency ratio of one, it suggests that for practical purposes, as a gastric stimulant, the pentapeptide is equipotent, weight for weight, with synthetic human gastrin I. Hog gastrin, purified by the method of Gregory & Tracy (1964), is equipotent with synthetic human gastrin I in the rat, a finding previously reported for the dog (Gregory, Tracy & Grossman, 1966). Although several workers have used a bioassay to detect gastrin-like activity in the serum of patients with the Zollinger–Ellison syndrome (Moore *et al.*, 1967; Thompson & Sircus, 1967), no previous studies have been done to show that the acid response of the rat to gastrin is unaffected by plasma.

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