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COMPARISON OF VAGAL AND MEAT STIMULATION ON GASTRIC ACID SECRETION AND SERUM GASTRIN

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SUMMARY

The gastric acid secreted per unit of serum gastrin concentration or per unit of gastrin delivered to the gastric mucosa was significantly greater during electrical vagal stimulation than during stimulation by meat extract in the pyloric antrum.

There was no significant difference in the concentrations of big, big gastrin or Components I and II in gastric venous serum during the two forms of stimulation.

There were significantly greater concentrations of Components II and IV in gastric venous serum during meat extract stimulation than during vagal stimulation.

The gastrin in gastric venous serum when meat extract was in the pyloric antrum was comprised of Component IV in addition to Component III whereas gastrin in extracts of cat antral mucosa was predominantly Component III.

Gastric acid secretion in response to vagal stimulation was greater than can be accounted for by the action of gastrin alone and cannot be explained by differences in the known gastrin variants in the circulation during the two forms of stimulation.

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INTRODUCTION

A considerable amount of evidence has accumulated in support of the concept that stimulation of the vagus nerves causes release of gastrin from the pyloric antrum (Burstall & Schofield, 1953; Pe Thein & Schofield, 1959; Emås & Fyro, 1965). In addition, increased circulating gastrin and increased gastric acid output in the dog have been reported in response to electrical stimulation of the vagus nerve (Lanciault, Bonoma & Brooks, 1971), during insulin hypoglycaemia (Jaffe, McGuigan & Newton, 1970; Korman, Soveny & Hansky, 1971) and after sham feeding (Nilsson, Simon, Yalow & Berson, 1972).

It has been shown in dogs that similar rates of acid secretion are associated with smaller increases in serum gastrin during sham feeding than during feeding (Nilsson *et al.* 1972) and it has been shown in man that a similar plasma gastrin concentration is associated with greater acid secretion in response to insulin hypoglycaemia than in response to stimulation with meat extract (Wyllie, Boulos, Lewin, Stagg & Clark, 1972). These differences might be accounted for in a number of ways. Firstly, it is possible that the sensitivity of the parietal cell is depressed during meat extract stimulation or, as has often been suggested, that the sensitivity of the parietal cell is enhanced during vagal stimulation (Uvnas, 1942). Secondly, it is possible that the total quantity of gastrin delivered to the gastric mucosa may be more important than the concentration of gastrin in the arterial blood and there is evidence for a greater mucosal blood flow per unit acid secretion during vagal stimulation than during gastrin stimulation (Harper, Reed, Sanders & Smy, 1970; Reed & Sanders, 1971*a*). Thirdly, it is possible that the gastrin variants released in response to vagal stimulation may differ from those released in response to food in the stomach and there is evidence that these variants have different biological potencies (Walsh, Debas & Grossman, 1974).

The present work investigates the last two of these hypotheses. Gastric mucosal blood flow, concentration of gastrin in arterial blood and concentration of gastrin variants in gastric venous blood were studied in anaesthetized cats during gastric acid secretion produced in response both to electrical stimulation of the vagus nerves and to the presence of meat extract in pyloric antral pouches. Abstracts of this work have been published previously (Blair, Grund, Reed, Shaw & Wilkinson, 1975; Blair, Grund, Lund, Reed & Sanders, 1974, 1975).

METHODS

The cats were starved for 36 h before the experiment, although allowed water. Anaesthesia was induced with ether, maintained by a single I.V. injection of chloralose (80 mg kg body wt.$^{-1}$) and a glass cannula was placed in the trachea. A metal cannula was inserted into the right saphenous vein. A plastic catheter with the tip directed towards the heart was inserted into a carotid artery in the neck to allow measurement of arterial blood pressure by a mercury manometer, and collection of arterial blood samples for serum gastrin, packed cell volume and amidopyrine measurements. Both vagus nerves were cut in the neck. Electrodes were attached to both the dorsal and ventral vagal trunks in the lower thorax through an incision on the right side of the chest. Respiration was maintained with a Starling Ideal Pump set at approximately 20 ml. kg. body wt.$^{-1}$ stroke and 20 strokes min^{-1}.

The abdomen was exposed through a mid line incision; the pylorus and the major veins in this region were occluded with a tape ligature. A wide bore rubber catheter (i.d. 4 mm) with a Perspex cuff attached was inserted through an incision in the cervical oesophagus, passed into the stomach and secured so that the tip lay in the pyloric antrum. An antral pouch was formed by tying a ligature around the stomach on to the Perspex cuff, taking care to avoid the major blood vessels in this region (Blair, Brown, Harper & Scratcherd, 1966). The fundus was intubated by a second wide bore catheter passed through a separate incision in the cervical oesophagus. One series of cats had whole stomach pouches intubated with a single tube. In all animals the splanchnic nerves were cut extraperitoneally on both sides through incisions in the flanks.

The bile and pancreatic ducts were ligated close to their entry into the duodenum. To obtain samples of gastric venous blood a tributary of the gastrosplenic vein was cannulated so that the cannula tip lay near the major junction of this vein. The small veins from the pancreas were ligated and the splenic branches of the splenic artery and gastrosplenic vein were ligated close to the spleen. Gastric venous samples were taken at a rate of 1 ml. min^{-1}. The blood was immediately centrifuged and the separated red cells were returned to the animal in an equal volume of saline. The plasma was allowed to clot and the serum was stored at $-20°$ C until assayed for gastrin immunoreactivity.

An hour was allowed between completion of the surgical procedures and the beginning of each experiment. During this time when mucosal blood flow was to be measured, a priming dose of amidopyrine (30 mg kg^{-1} body wt.) was given I.V. followed by an infusion of 10 mg kg^{-1} h^{-1}. Routinely 50 ml. (25 ml. in fundic pouches) of a 1/5 (v/v) mixture of 300 m-osmole kg^{-1} solutions of glycine and mannitol adjusted to pH 3·5 by addition of 0·15 N-HCl were placed in the stomach at the beginning of each collection period and drained during the final 30 s of the collection period. Acid content was determined by electrometric titration of a sample to pH 7·0 with 0·02 N-NaOH. Arterial blood samples were collected every 20 min throughout the experiments, centrifuged, 1 ml. plasma stored for amidopyrine extraction, 1 ml. serum stored at $-20°$ C for subsequent gastrin estimation, and R.B.C.S returned to the animal via the indwelling cannula. Gastric mucosal blood flow was measured by the amidopyrine technique described for use in the anaesthetized cat by Harper *et al.* (1968).

The antral pouch was routinely drained and refilled every 20 min with 10 ml. mannitol-glycine buffer which matched the pH (5·5) and osmolality (550 m-osmole kg^{-1}) of the 7·5% suspension of meat extract (desalted OXO – a gift from Oxoid Ltd). The mannitol glycine buffer was replaced by meat extract as required. The vagus nerves were stimulated electrically at 15–20 V, 3–5 ms pulse duration and

5–10 Hz for 20 s of every minute for periods up to 3 h. Studies were made during three successive 10 min control periods before stimulation to determine the basal levels of acid output and mucosal blood flow. Arterial and gastic venous gastrin concentrations were measured during two of these resting periods. Acid output was expressed as μequiv. H^+3 or 10 min^{-1} and mucosal blood flow (MBF) as ml. 3 or 10 min^{-1}. Serum gastrin was measured by radioimmunoassay and expressed as pg $ml.^{-1} \equiv$ SHG17NS (synthetic human gastrin). Delivered gastrin was calculated from the product of arterial serum gastrin concentration and mucosal blood flow (MBF) allowing for the packed cell volume PCV because immunoreactive gastrin was not detected in saline washings of R.B.C.S,

$$\text{delivered gastrin} = \text{serum gastrin} \times \text{MBF} \times \frac{(100 - \text{PCV})}{100}.$$

The reason for concluding that any gastrin present in R.B.C.S is not readily transferred to plasma is as follows. A sample of human blood was taken into lithium sequestrene, the plasma was assayed for gastrin ($400 \pm 1{\cdot}0(12)$ pg $ml.^{-1}$), the red cells were washed with an equal volume of saline and again with half this volume. Gastrin was not detected in either sample of saline ($n = 12$ in each case). Saline added to lithium sequestrene did not interfere with the assay.

Cat antral extracts

Cats' stomachs were opened along the greater curvature between the gastro-oesophageal junction and the pyloric sphincter. The antral mucosa was dissected free, homogenized (Servall Omnimixer) and extracted with boiling water; the final product was precipitated with acetone to yield a water soluble, histamine free and gastrin containing extract. Pig and human antral extracts were similarly prepared. The method is set out by Blair, Harper, Lake, Reed & Scratcherd (1961).

Experiments

There were three groups of experiments.

Group 1. This comprised eight animals with antral pouches: in four, vagal stimulation was followed after a recovery period by stimulation with meat extract in the pyloric antrum, and in the other four the order of the experiment was reversed. Gastric acid secretion, mucosal blood flow and arterial serum gastrin concentrations were measured. Gastrin estimations were performed with Sephadex purified label (*vide infra*).

Group 2. This comprised six animals with antral pouches. Three animals were subjected to vagal stimulation and three animals to stimulation with meat extract in the pyloric antrum. Gastric acid secretion and arterial and gastric venous serum gastrin concentrations were measured. Gastrin estimations were performed with AE cellulose purified label (*vide infra*). Gastric venous samples were chromatographed on Sephadex G50 superfine columns to determine the gastrin variants present.

Group 3. This comprised four animals with whole stomach pouches in which vagal stimulation was followed after recovery by infusion of a gastrin extract prepared from cat antra. Gastric acid secretion and arterial serum gastrin concentrations were measured. Gastrin estimations were performed with Sephadex purified label (*vide infra*).

Radioimmunoassay

Antibodies

Antibodies were raised in randomly bred rabbits immunized with the two to seventeen residues of synthetic human gastrin 17NS conjugated to bovine albumin

with the carbodiimide technique (McGuigan, 1968; Blair, Grund, Reed, Sanders, Sanger & Shaw, 1975).

Labelled gastrin

Gastrin was labelled with ^{125}I with the Chloramine T technique. The purification procedure varied in that the label used in groups 1 and 3 was separated by gel filtration (Sephadex G10) and the label used in group 2 was purified by ion exchange chromatography(AE cellulose).

The technique for the preparation of the label used in groups 1 and 3 has been published in detail elsewhere (Blair *et al.* 1975) and the preparation of the second label was based on the method of Stadil & Rehfeld (1972). The carrier-free sodium iodide (^{125}I) was stored in a glass vial at 4° C until used. About 0·2 mc was dispensed into a 30 × 5 mm polystyrene tube and measured with an ionizing chamber (AERE 1383A, Elliot Process Automatic Ltd) connected to a Vibron Electrometer (model 33C-2, EIL Ltd), and mixed with 4 μg SHG17NS in 20 μl. 0·05 M ammonium hydrogen carbonate at 4° C. To this was added 10 μl. 0·25 M phosphate buffer solution pH 7·4 containing 5 μg chloramine T. The mixture was agitated with a fine polystyrene rod for 2 min and the reaction was stopped by the addition of 60 μg sodium metabisulphite in 25 μl. 16% (w/v) sucrose solution. The reaction mixture was diluted with 0·2 ml. 1% (w/v) potassium iodide solution in 0·05 M phosphate buffer pH 7·4 and immediately applied to an AE41 cellulose column (10 × 150 mm), as described by Stadil & Rehfeld (1972). The individual fractions were counted on a Wallac 80000 gamma counter.

The concentration of the labelled gastrin was estimated by comparing two standard curves, one with increasing amounts of unlabelled SHG and the second with increasing amounts of labelled gastrin.

Separation of antibody bound and free hormone

After incubation for 16 h at 4° C, separation was achieved by adding 20 mg Amberlite CG4B in 0·2 ml. to each tube. The contents of the tubes were mixed for 5 s, centrifuged at 4° C and decanted. Both supernatant, which contained antibody bound gastrin, and precipitate, which contained non-antibody bound gastrin, were counted.

Protocol of immunoassay

Buffer standard curves were set up with quadruplicate estimations at each concentration of unlabelled gastrin (SHG17NS). Each standard curve consisted of 0, 5, 10, 15, 20, 30, 50 and 80 pg for the assays of the samples from groups 1 and 3. In the group 2 experiments, concentrations of gastrin (SHG) were 0, 2·5, 5, 7·5, 10, 15, 20, 30, 50, 75 and 100 pg. The group 1 and 3 assays all contained 2 pg ^{125}I-labelled gastrin and the group 2 assays contained 6 pg labelled gastrin. The veronal acetate buffer (pH 7·4) contained 350 mg bovine serum albumin 100 ml.$^{-1}$ as described previously (Blair *et al.* 1975). Standard gastrin, labelled gastrin, antibody dilutions and amberlite suspensions were made in this buffer. The antiserum was diluted 1/100,000 when using the Sephadex purified label and 1/400,000 when using the AE cellulose purified label. The effective equilibrium constant of the assay reaction when using the AE cellulose purified label was 4×10^{11} l. mole^{-1}.

A porcine antral extract (Blair *et al.* 1961) which had previously been shown to have no loss of biological activity over several years, was used as an internal laboratory standard for comparison with SHG17NS at regular intervals. To date all batches of SHG17NS used have been of equal immunoreactivity.

Accuracy of assay

With Sephadex purified label in the assay, 1 mole SHG17NS was ≡ 186 mole of gastrin pentapeptide (I.C.I.). With the AE cellulose purified label 1 mole of SHG17NS was ≡ 86 mole of the pentapeptide, whereas synthetic cat gastrin 17NS, natural human gastrin 34NS and synthetic human gastrin 13NS were equally active compared with SHG17NS. Crossreactivity of the assay with Sephadex purified label is detailed elsewhere (Blair *et al.* 1975). (SHG13NS was the C-terminal 13 amino acid sequence of gastrin. Recent characterization indicates that mini gastrin has an additional tryptophan residue at the N-terminus (Professor R. A. Gregory, personal communication).)

The sensitivity of the assay (95% confidence limits about zero) with Sephadex purified label was 21–41 pg ml.$^{-1}$ serum (Blair *et al.* 1975) whereas with AE cellulose purified label it was 9 pg ml.$^{-1}$ serum or 3·6 pg ml.$^{-1}$ column eluate. The interassay variability of the assay (coefficient of variation) with Sephadex purified label was 18%, about a mean value of 180 pg ml.$^{-1}$ serum (Blair *et al.* 1975) whereas with AE cellulose purified label it was 9·6%, about a mean value of 110·6 pg ml.$^{-1}$ serum.

Measurement of gastrin variants

Solutions of antral extracts or gastric venous serum samples were applied at room temperature to columns of Sephadex G50 superfine (1900 × 15 mm or 2000 × 10 mm). Fractions were eluted with the veronal buffer used in the radioimmunoassay without bovine serum albumin but with 0·6 mM ethyl mercuric thiosalicylate. With the wider column, samples of 3–10 ml. were applied, the columns were eluted at 8·5 ml. h^{-1} and 3·5 ml. fractions were collected. With the narrower column, samples of 1–3 ml. were applied, the columns were eluted at 5 ml. h^{-1} and 1·7 ml. fractions were collected. The columns were routinely calibrated after every three runs with bovine serum albumin, haemoglobin, cytochrome C, myoglobin, [^{125}I] insulin (Amersham), [^{125}I] SHG17NS and potassium dichromate.

Gastrin activity in the eluates from the column was estimated by the radioimmunoassay method which incorporated AE cellulose purified label. The elution zone of a column eluate is characterized by reference to its K_{av} value which lies between 0 and 1, where 0 represents the peak albumin elution zone (V_0) and 1 represents the peak salt elution zone (V_t). Big, big gastrin was considered to be eluted in the void volume region between K_{av} −0·05 and +0·15 (Yalow & Wu, 1973). Component I is eluted in the same region as pro-insulin, mol. wt. ≏ 9000 (Rehfeld & Stadil, 1973) which from the column calibrations in the present work corresponds to K_{av} 0·2–0·3. The elution zone for Component II (K_{av} 0·3–0·6) was confirmed by calibration with natural human gastrin 34NS and for Component III (K_{av} 0·6–0·85) with porcine gastrin 17S (M.R.C. research standard A, 66/138) and SHG17NS. Component IV was assumed to be eluted between the elution zone for Component III and the salt elution zone (Rehfeld, Stadil & Vikelsøe, 1974), i.e. between K_{av} 0·85 and K_{av} 1·00. The concentration of the gastrin variants in gastric venous serum was calculated from a knowledge of the total gastrin concentration in the serum and the quantity of variant eluted following chromatography of a known volume of the serum, assuming 100% recovery.

Expression of results

All results are expressed as the mean ± S.E. of mean (n). Significance of difference between means was tested by the method of Student's t or Cochran's modification.

The types of gastrin are referred to as gastrin no. (e.g. 34) where the number refers to the amino acid content. Where the content is not known and the only evidence

as to molecular weight is from Sephadex chromatography, the type of gastrin is referred to as Component I, II, III or IV (Rehfeld *et al.* 1974). The non-sulphated forms of gastrin are referred to as NS rather than roman numeral I to avoid confusion with the Component numbers.

RESULTS

Group 1. Comparison of the effects of vagal and meat extract stimulation on gastric acid secretion, mucosal blood flow and arterial serum gastrin concentration

In this group of eight animals, gastric acid secretion and mucosal blood flow were measured in 3 min collection periods during vagal and meat extract stimulation. Arterial serum gastrin was assayed in blood samples collected before stimulation, from 15 to 30 min of vagal stimulation and from 70 to 100 min of meat extract stimulation.

TABLE 1. Comparison of basal data for experiments where (*a*) vagal stimulation preceded stimulation with meat extract and (*b*) when stimulation with meat extract preceded vagal stimulation. Results shown in Table 2

(*a*)

	Vagal stimulation			Stimulation with meat extract		
	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*
H^+ (μequiv 3 min^{-1})	1·3	0·9	4	4·1	3·5	4
MBF (ml. 3 min^{-1})	2·7	0·4	4	2·4	0·6	4
Serum gastrin (pg $ml.^{-1}$)	0	0	4	20	11·6	4
Delivered gastrin (pg 3 min^{-1})	0	0	4	39·5	25·3	4

(*b*)

	Stimulation with meat extract			Vagal stimulation		
	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*
H^+ (μequiv 3 min^{-1})	2·3	1·4	4	4·1	2·9	4
MBF (ml. 3 min^{-1})	2·4	0·4	4	2·6	0·8	4
Serum gastrin (pg $ml.^{-1}$)	5	5	4	25	13·2	4
Delivered gastrin (pg 3 min^{-1})	8·8	8·8	4	52·3	35·8	4

The resting data are shown in Table 1. The data from those experiments in which vagal stimulation preceded stimulation with meat extract suspension in the pyloric antrum are presented in Table 2*a*, and the data from the experiments in which the order of stimulation was reversed are presented in Table 2*b*. The mean acid output and mucosal blood flow during vagal stimulation were significantly greater than during meat extract stimulation, irrespective of the order in which the stimuli were administered in the experiment, whereas the serum gastrin concentration was significantly less during vagal stimulation. The calculated rate of delivery of gastrin to the

gastric mucosa was not significantly different during the two methods of stimulation; nevertheless, the acid output per unit gastrin concentration, and the acid output per unit delivered gastrin were significantly greater during vagal stimulation.

TABLE 2. Comparisons of results of vagal and meat extract stimulation when (*a*) vagal stimulation preceded stimulation with meat extract and (*b*) when stimulation with meat extract preceded vagal stimulation

(*a*)

	Vagal stimulation			Stimulation with meat extract			
	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*	
H^+ (μequiv 3 min^{-1})	60·1	7·3	16	6·1	1·6	16	$P < 0{\cdot}001$
MBF (ml. 3 min^{-1})	11·4	1·5	16	2·7	0·5	16	$P < 0{\cdot}001$
Serum gastrin (pg ml.$^{-1}$)	19·3	4·4	15	187·8	20·5	16	$P < 0{\cdot}001$
Delivered gastrin (pg 3 min^{-1})	148·3	46·1	15	196·7	61·0	16	$P > 0{\cdot}5$
n-equiv. H^+/ pg serum gastrin^{-1}	2726	645	14	29	6	16	$P < 0{\cdot}005$
n-equiv. H^+/ pg delivered gastrin^{-1}	436	98	14	41	9	16	$P < 0{\cdot}005$

(*b*)

	Stimulation with meat extract			Vagal stimulation			
	$\bar{x}$	S.E.	*n*	$\bar{x}$	S.E.	*n*	
H^+ (μequiv 3 min^{-1})	18·0	2·4	16	47·2	9·5	15	$P < 0{\cdot}01$
MBF (ml. 3 min^{-1})	3·7	0·5	16	10·1	1·7	15	$P < 0{\cdot}005$
Serum gastrin (pg ml^{-1})	76·6	7·3	16	51·3	7·1	15	$P < 0{\cdot}025$
Delivered gastrin (pg 3 min^{-1})	166·3	23·2	16	337·3	91·5	15	$P > 0{\cdot}05$
n-equiv H^+/ pg serum gastrin^{-1}	256	38	16	896	110	15	$P < 0{\cdot}001$
n-equiv H^+/ pg delivered gastrin^{-1}	116	10	16	202	29	15	$P < 0{\cdot}025$

Group 2. Comparison of the effects of vagal stimulation and meat extract stimulation on the gastrin variants in gastric venous serum

In this group of six animals, three were subjected to electrical stimulation of the vagus nerves and three were stimulated by meat extract suspension in the antral pouch. Gastric venous blood samples were taken between 25 and 85 min after the start of vagal stimulation and between 45 and 105 min after the first instillation of meat extract into the antral pouch. The mean gastric venous serum gastrin concentration of these samples was $140 \pm 24{\cdot}7$ (3) pg ml.$^{-1}$ during vagal stimulation and $698 \pm 115{\cdot}4$ (3) pg ml.$^{-1}$

during meat extract stimulation, and these were significantly greater than the prestimulation values of 37·1 ± 10·7 (3) pg ml.$^{-1}$ and 95·0 ± 21·8 (3) pg ml.$^{-1}$ respectively ($P < 0·05$).

The three gastric venous serum samples collected during each form of stimulation were chromatographed. Those collected during vagal stimulation contained three major variants, big big and Components III and IV. In addition some immunoreactive gastrin activity was present in the elution zones of Component I and II (Fig. 1). In the gastric venous serum samples collected during meat extract stimulation, the major proportions of gastrin immunoreactivity were Components III and IV (Fig. 2). The concentrations of these variants during meat extract stimulation were significantly greater than during vagal stimulation (Fig. 3*A* and *B*, $P < 0·05$), whereas concentrations of the other gastrin variants were not significantly different (Fig. 3).

Group 3. Comparison of the effect of vagal stimulation and cat 'gastrin' infusion on gastric acid secretion and arterial serum gastrin concentrations

In this group of four animals the vagus nerves were stimulated for 30 min. After a recovery period of 60–90 min cat antral extract (Blair *et al.* 1961) was infused I.V. (10 mg kg^{-1} h^{-1} in saline). When results obtained during the period from 15 to 30 min after the start of both types of stimulation are compared, the acid secretory response to vagal stimulation (185·0 ± 28·2 (16) μequiv H^+ 3 min^{-1}) is significantly greater ($P < 0·001$) than the response to 'gastrin' (45·1 ± 2·8 (12) μequiv H^+ 3 m^{-1}). By contrast the arterial serum gastrin concentration is significantly lower ($P < 0·001$) in the vagal stimulation experiments (87·0 ± 12·7 (16) pg ml.$^{-1}$) when compared with samples taken over the same time course in the cat 'gastrin' infusion experiments (522·5 ± 43·3 (12) pg ml.$^{-1}$).

Gastrin components of cat antral extracts

In two extracts of cat antra (extracts 1 and 2) the gastrin immunoreactivity was 23 and 132 ng g mucosa^{-1} respectively. Three similar extracts of human antral mucosa had considerably greater activity of between 1·85 and 6·5 μg.g mucosa^{-1} as did a porcine antral extract (1·37 μg.g mucosa^{-1}).

Extracts 1 and 2 were chromatographed on G50 superfine columns and the elution profiles are shown in Fig. 4. The major proportion of the gastrin activity is in the elution zone for Component III and, by comparison all other elution zones contain only traces of gastrin.

DISCUSSION

It is clear from the results of the experiments in groups 1 and 3 that in the anaesthetized cat the gastric acid secreted per unit of serum gastrin concentration is significantly greater during electrical vagal stimulation than during stimulation by meat extract or the injection of an extract of pyloric

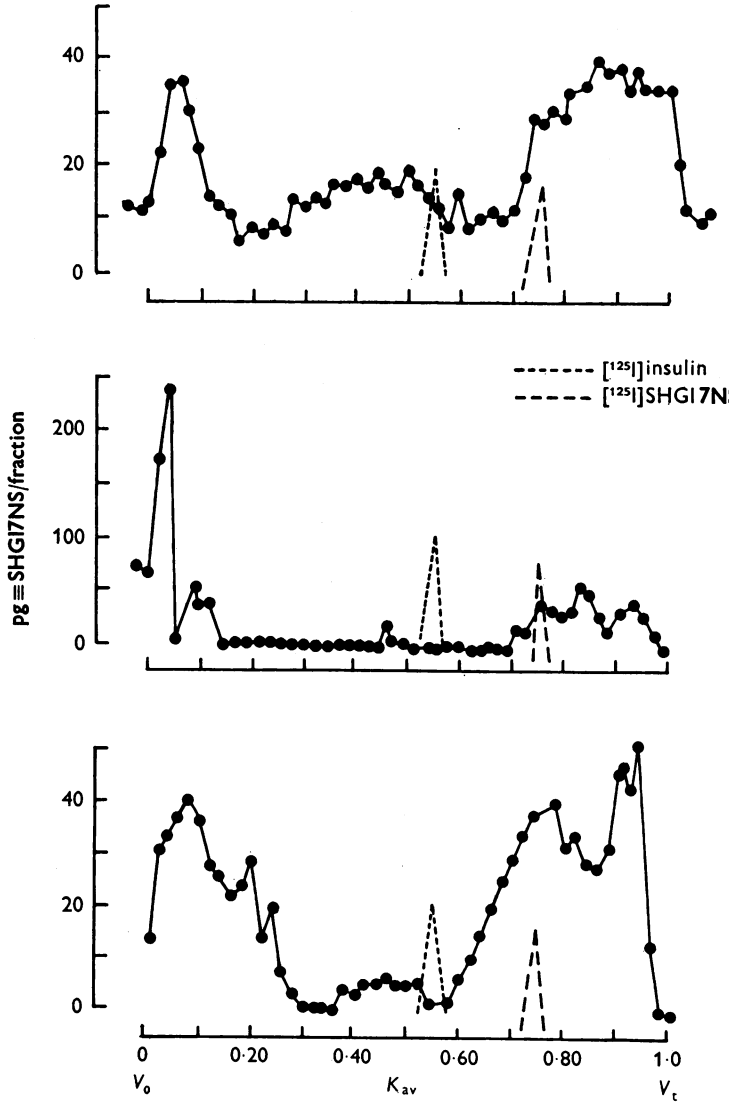

Fig. 1. The gastrin elution profiles of gastric venous serum from three animals after gel chromatography on Sephadex G50 superfine. Samples taken during electrical vagal stimulation.

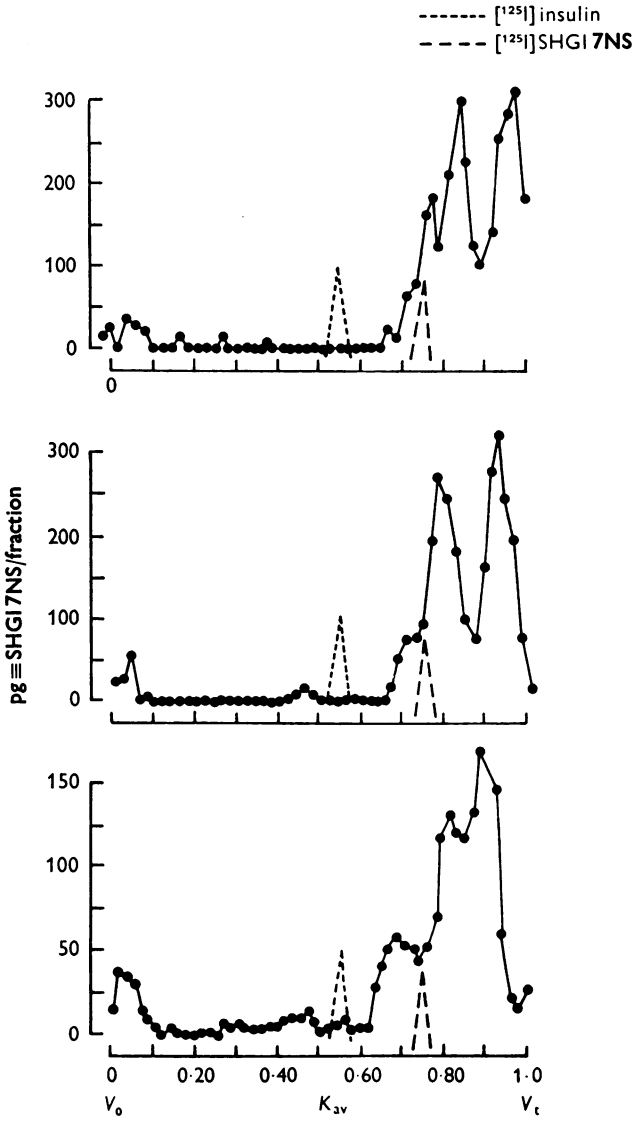

Fig. 2. The gastrin elution profiles of gastric venous serum from three animals after gel chromatography on Sephadex G50 superfine. Samples taken during stimulation with meat extract suspension in antral pouches.

antral mucosa. It might be, in those experiments in which meat extract in the pyloric antrum or intravenous 'gastrin' injection was the second stimulus in an experiment, that there was a smaller acid output per unit gastrin concentration because of a progressive reduction in the sensitivity of the acid secretory mechanism as the experiment proceeded. This may

have arisen as a result, for example, of tachyphylaxis (Blair, 1967) or a decreasing arterial P_{CO_2} (Reed & Sanders, 1971*b*). A significantly greater mean rate of acid secretion per unit of serum gastrin concentration was, however, observed during vagal stimulation compared with meat extract stimulation, whether vagal stimulation occurred first or second in the experiments (Table 1).

The gastric mucosal blood flow must influence the rate of delivery of gastrin to the gastric mucosa and this blood flow was significantly greater during vagal stimulation than during meat extract stimulation (Table 1). If there is a sufficiently high rate of gastrin destruction in the region of the parietal cell, then the rate at which gastrin is delivered to the gastric mucosa, rather than the arterial gastrin concentration alone, could conceivably be a limiting factor causing a relatively smaller rate of acid secretion during meat extract stimulation. The mean quantity of gastrin delivered to the gastric mucosa was not, however, significantly different with the two forms of stimulation (Table 1) and the mean gastric acid secreted per unit of delivered gastrin was greater during electrical vagal stimulation than during stimulation by meat extract, whether this came first (Table 1*a*) or second (Table 1*b*) in the experiments.

The results of the group 2 experiments (Fig. 3) do not support the hypothesis that the greater acid secretion per unit of gastrin observed during vagal stimulation is due to differences in the concentration of the known normal forms of gastrin variants in the circulation. The fact that gastric mucosal blood flow is greater during vagal stimulation will, however, also affect the quantities of the different gastrin variants delivered to the gastric mucosa. It appears that the concentration of gastrin variants in the systemic circulation reflects their concentration in portal blood (Dencker, Håkanson, Liedberg, Norryd, Oscarson, Rehfeld & Stadil, 1973). Of these gastrin variants there is no direct evidence about the biological activity of big, big gastrin or Component I but there is little evidence at present to suggest they have a significant role in the physiological stimulation of gastric acid secretion. It has been shown, in the dog at least, that compared with gastrin 34 on a molar basis, gastrin 14 and 17 are respectively approximately twice and four times more effective in stimulating gastric acid secretion (Walsh *et al.* 1974). When account is taken of these facts, the concentration of gastric variants in gastric venous blood (Fig. 3) and the evidence that gastric mucosal blood flow is three or four times greater during vagal stimulation (Table 1) it is unlikely that differences in the rate of delivery of the various forms of gastrin to the gastric mucosa can explain the observed differences in the effects of vagal and meat extract stimulation.

There is evidence that in the dog the sensitivity of the parietal cell to gastrin is enhanced during vagal stimulation (Grossman, 1974). This may

be the case in the cat, or it may be that the parietal cell sensitivity is depressed during meat extract stimulation. During vagal stimulation, for example, the secretory response to gastrin may be potentiated by the

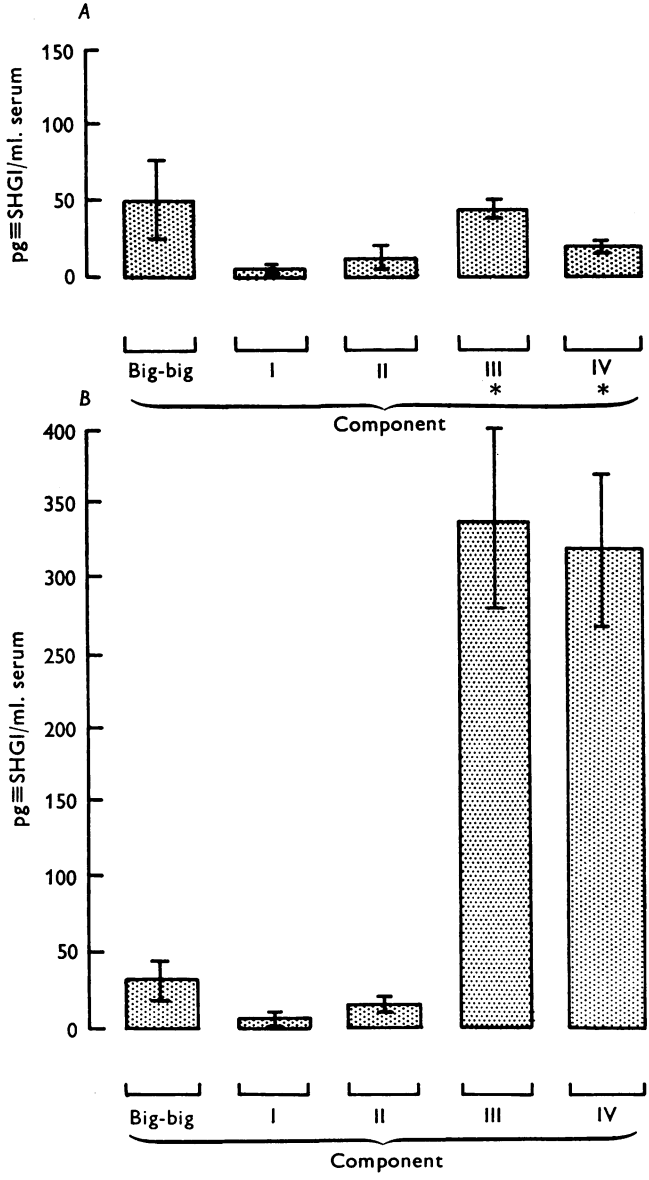

Fig. 3. The calculated concentrations of gastrin variants in gastric venous serum during (*A*) vagal stimulation and (*B*) meat extract stimulation. The stars indicate significance of difference at the 5% level; vertical bars represent ± 1 S.E. of mean.

simultaneous release of cholinergic transmitter in the region of the parietal cell (Grossman, 1974) or conceivably there may be stimulation of release of an additional, as yet unidentified hormone or suppression of release of a chalone. It is also possible that in cats, as in dogs, vagal stimulation causes direct stimulation of the parietal cell (Pevsner & Grossman, 1955; Tepperman, Walsh & Preshaw, 1972).

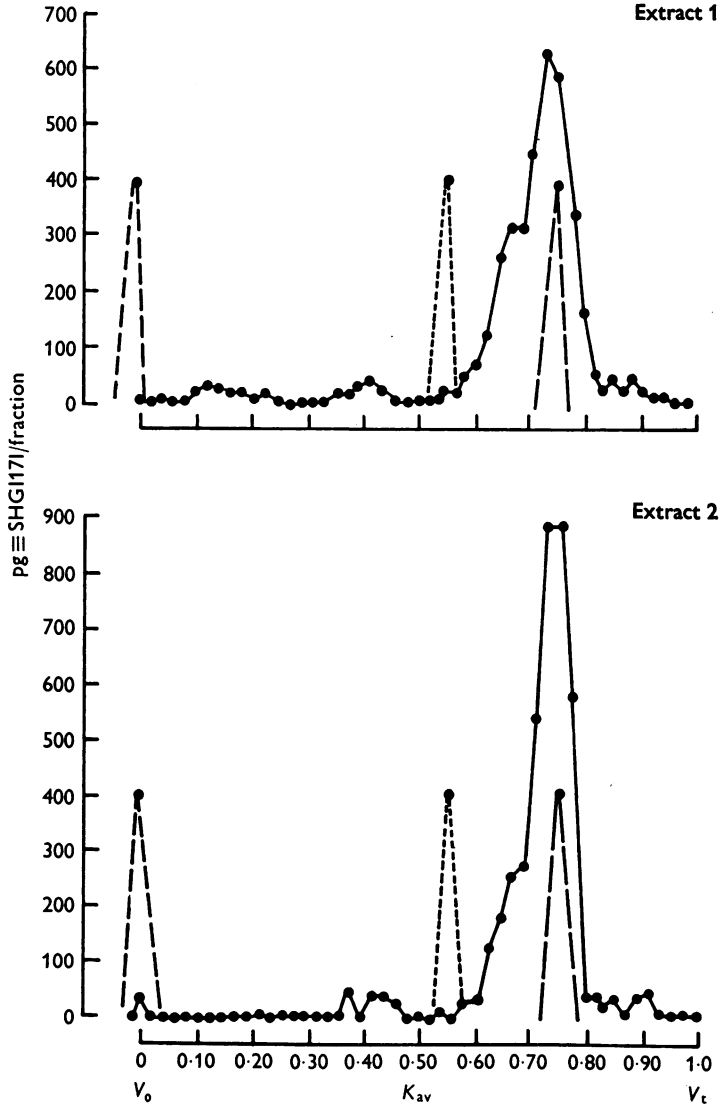

Fig. 4. The gastrin elution profile of two preparations of cat antral extract on Sephadex G50 superfine. The dotted lines, from left to right represent the elution zones of ^{125}I-labelled albumin, [^{125}I] insulin and [^{125}I] SHG17NS.

In man after feeding (Yalow & Berson, 1973) and in dog after the instillation of meat extract into the pyloric antrum (Dockray, 1975) the predominant gastrin variants found in the circulation are Component II, and to a lesser extent III. This contrasts with the observations in the present work that after the instillation of meat extract into the pyloric antrum of the cat, Component III and Component IV are the major gastrin variants in gastric venous blood which contains relatively little Component II (Fig. 3). Despite these differences in the circulating gastrin between cat on the one hand and man and dog on the other, the major proportion of immunoreactive gastrin in extracts of pyloric antral mucosa in cat is Component III (Fig. 4) just as it is in man (Berson & Yalow, 1971), dog (Dockray, 1975) and hog (Praissman & Berkowitz, 1972). These observations may assist in the eventual elucidation of the biological significance of the different forms of gastrin.

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