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INFLUENCE OF SOME ANAESTHETICS ON PHARMACOLOGICALLY STIMULATED GASTRIC SECRETION IN THE RAT

BY

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The antral hormones responsible for the stimulation of gastric secretion were isolated from the hog stomach by Gregory & Tracy (1964). Degradation studies have shown that the gastrins are polypeptides containing 17 amino-acids (Gregory, Hardy, Jones, Kenner & Sheppard, 1964) and their structure was subsequently confirmed by chemical synthesis (Anderson, Barton, Gregory, Hardy, Kenner, MacLeod, Preston, Sheppard & Morley, 1964). Examination of different synthetic fragments of the molecule led to the discovery that only the C-terminal tetrapeptide Try.Met.Asp.Phe.NH_2 was required for the full range of physiological actions of gastrin (Tracy & Gregory, 1964). In a series of 33 peptides related to the active tetrapeptide, one pentapeptide (*N-t*-butyloxycarbonyl-β-Ala.Try.Met.Asp.Phe.NH_2; I.C.I. 50,123) was found to be particularly active in stimulating gastric secretion in dogs provided with denervated pouches of the gastric fundus (Morley, Tracy & Gregory, 1965).

Particular interest attaches to the reported dual action of gastrin on gastric secretion (Gillespie & Grossman, 1963; Gregory & Tracy, 1964). Following the establishment of a sub-maximal secretory plateau by repeated small subcutaneous injections of gastrin, the rapid administration of a large intravenous dose produced a prompt inhibition of acid secretion. As these properties were shared by the pentapeptide I.C.I. 50,123 (Morley *et al.*, 1965), it was desirable to confirm these observations in the rat before attempting to evaluate potential stimulants and inhibitors of gastric secretion. In preliminary experiments in rats it became evident that the choice of anaesthetic had a profound effect on the acid responses of the stomach. Furthermore, in the case of urethane the route of administration also led to dramatic differences in the type of response observed.

METHODS

The rats used in these experiments were males weighing 240 to 260 g from the specific pathogen-free colony (Wistar strain) maintained at Alderley Park. The animals were allowed free access to water and a cubed diet until 18 hr before experiment when all food was withdrawn.

Rats were anaesthetized in the following ways: (a) with urethane, 1.75 g/kg, given by the intraperitoneal, intramuscular, intravenous and intraduodenal routes; (b) with barbitone sodium, 600 mg/kg, by the intraperitoneal and intramuscular routes; (c) by inhalation of halothane, 1%

in 95% O_2+5% CO_2 given by an open circuit; (d) with ether—some animals under halothane anaesthesia were temporarily changed to open ether anaesthesia. Other rats were pithed and maintained by artificial respiration.

The method used in this study was a modification of that of Ghosh & Schild (1958). A cannula was placed in the trachea of anaesthetized rats and a soft polythene tube passed down the oesophagus until the tip was approximately at the cardiac sphincter. The oesophageal tube was ligated at the neck to prevent retrograde leakage of perfusion fluid. Another cannula was inserted into the stomach via a duodenostomy and ligated close to the pylorus, taking care not to damage the blood supply to the duodenum. The stomach was gently washed out with 250 ml. of warm saline. A constant flow of 0.9% sodium chloride solution at 31° C was then instituted from a gravity feed monitored by a screw clip above the oesophageal tube. The flow rate was adjusted to 1 ml./min and the whole preparation maintained at 31° C.

After allowing a 30-min period for settling down, perfusate was collected every 10 min and titrated to pH 7.0 against $N/_{10}$ sodium hydroxide solution using a Radiometer titrator and auto-burette. The sodium hydroxide solution was protected from atmospheric CO_2.

Intravenous injections were made in volumes of 0.2 ml./100 g via a cannula placed in the jugular vein. Subcutaneous injections were made in volumes of 0.1 ml./100 g in the ventral flank area.

Solution of gastrin or I.C.I. 50,123 were prepared freshly in ammoniacal distilled water. Appropriate dilutions were made with saline. The drugs used in this study were Gastrin II (prepared by

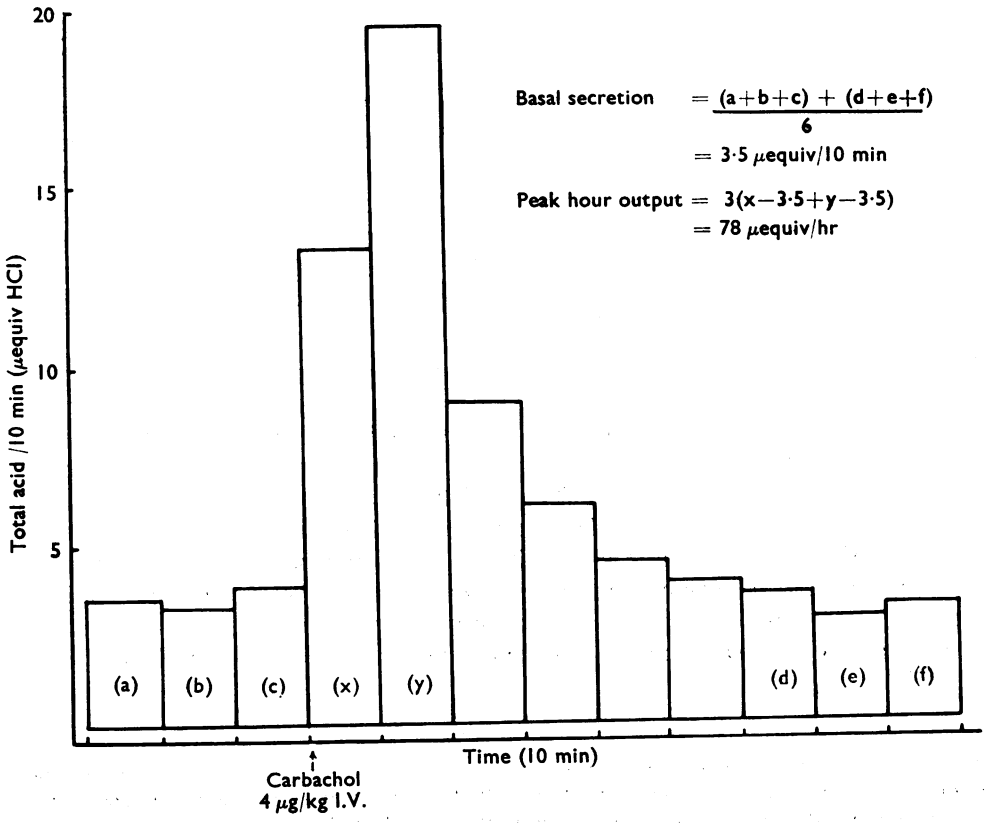

Fig. 1. Effect of single intravenous injection of carbachol on acid output from a perfused rat stomach. The three pre-injection 10-min outputs are indicated by a, b and c, and the three post-response values by d, e and f; x and y indicate the two highest 10-min outputs during the response.

Professor R. A. Gregory), I.C.I. 50,123 (prepared by Dr. J. S. Morley), histamine acid phosphate (B.D.H.), carbachol (B.D.H.) and soluble insulin (Boots). The doses of histamine and carbachol have been expressed in terms of the base.

Individual rats received up to five intravenous injections of secretory stimulant with the dose space interval never being less than 90 min. If injections were given at shorter time intervals, tachyphylaxis was observed.

The experimental results have been presented in two ways: (a) as total acid output/10 min; (b) as the peak hour output. A typical response, in this case, to the injection of carbachol is illustrated in Fig. 1 together with the method of calculation of the peak hour output.

RESULTS

Anaesthesia with intraperitoneal urethane

The stomach of the rat, anaesthetized with urethane intraperitoneally, maintains a basal secretion of acid for at least 6 hr. This basal acid output varies from animal to animal but for any given rat it is remarkably constant. The mean 10-min output for the third collection period for 48 rats, before any stimulants were given, was 4.2 ± 0.1 μ-equiv HCl with the range being from 6.2 to 2.1 μ-equiv HCl.

Single subcutaneous injections of gastrin (1 μg/kg) or I.C.I. 50,123 (4 μg/kg) produced an immediate increase in acid secretion and when these doses were repeated every 20 min a steady secretory plateau was obtained (Fig. 2 *a*, *c*). The rapid intravenous injection of ten times the dose of either agent, administered when a plateau had been established, was followed by a prompt inhibition of acid secretion which lasted for 30 min. Although acid output fell it never went below the basal secretion value (Fig. 2 *a*, *c*). A plateau of acid secretion of similar dimension was produced by injecting histamine (80 μg/kg) but there was no inhibition of this response by high doses of gastrin or I.C.I. 50,123.

The lowest dose of gastrin which would produce a significant increase in acid secretion by intravenous injection was 0.1 μg/kg. Increasing responses were observed to follow increasing doses up to 8 μg/kg. This dose produced a maximum response in the graded dose curve but doses of 32 μg/kg or higher had no effect on the acid output. Qualitatively similar results were obtained with I.C.I. 50,123 (Fig. 3). Doses of 32 μg/kg or above for either agent invariably failed to stimulate any acid secretions. At the same time there was never any reduction in the basal acid secretion following high doses of either peptide. In some animals injections of two smaller doses were followed by a larger non-stimulant dose and the subsequent administration of the original two small doses produced quantitatively similar results.

Anaesthesia with intramuscular urethane

On one occasion the urethane for anaesthesia was inadvertently given intramuscularly instead of by the usual intraperitoneal route. The animals responded to 32 μg/kg of gastrin with an acid-output larger than anything previously observed. Further increases in dose level gave yet higher acid secretions. The plateaux experiments were repeated with rats anaesthetized with urethane given intramuscularly (Fig. 2 *b*, *d*). Under these conditions rapid intravenous injections of either gastrin or I.C.I. 50,123 always produced a further increase in acid secretion.

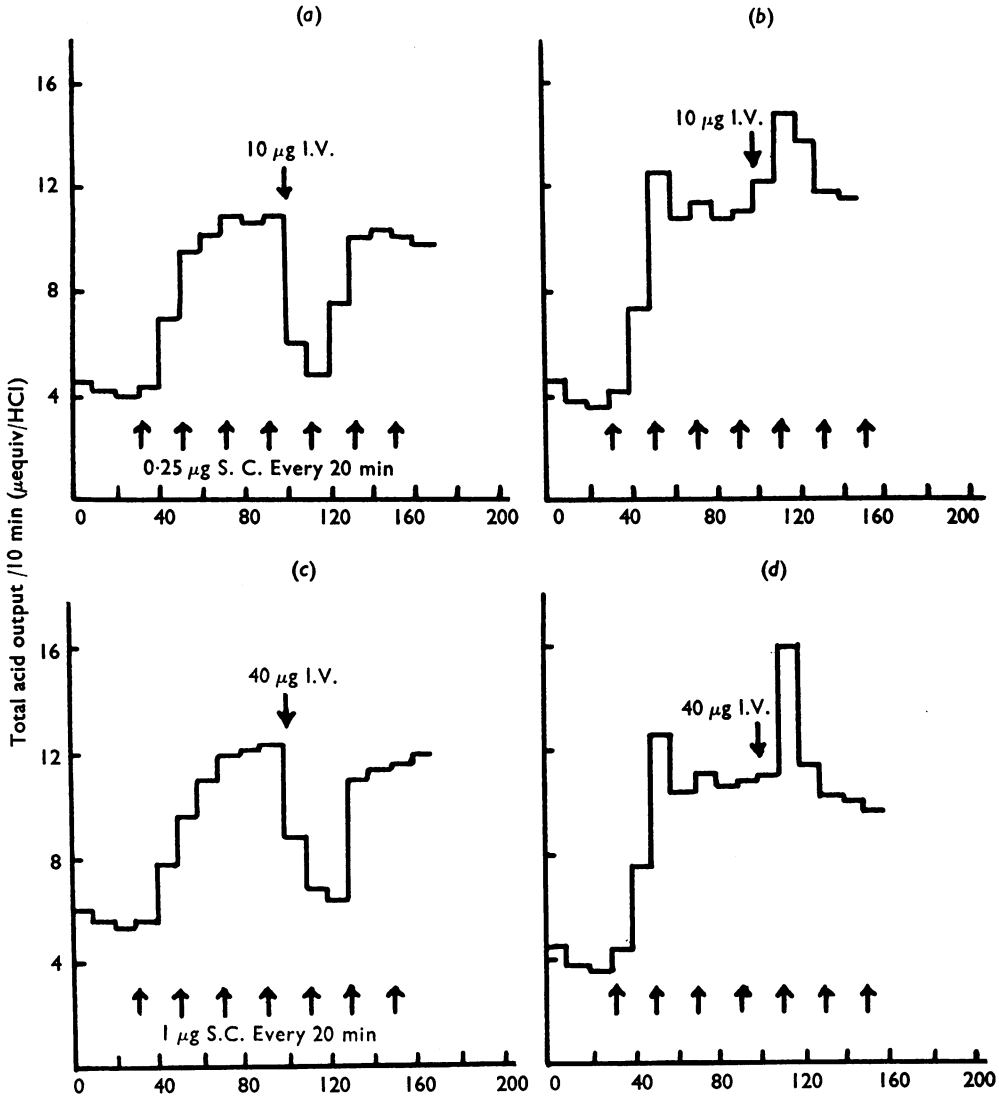

Fig. 2. Effects of single large intravenous injection of gastrin (*a*) (*b*) and I.C.I. 50,123 (*c*) (*d*) on acid secretory plateau produced by repeated subcutaneous injections of small doses of gastrin and I.C.I. 50,123. The animals were anaesthetized with urethane by intraperitoneal (*a*) (*c*) or intramuscular (*b*) (*d*) routes. Horizontal lines represent mean total acid output for four rats/10 min collection period.

Dose-response curves were constructed for single intravenous injections of gastrin and I.C.I. 50,123 in rats anaesthetized with urethane given by both intraperitoneal and intramuscular routes (Fig. 4). There was a striking similarity in the overall pattern of response for the two secretory stimulants. In the intraperitoneally injected animals the response was biphasic: for the intramuscularly injected it was monophasic reaching a maximum output. The slopes of the curves in the intramuscularly anaesthetized animals

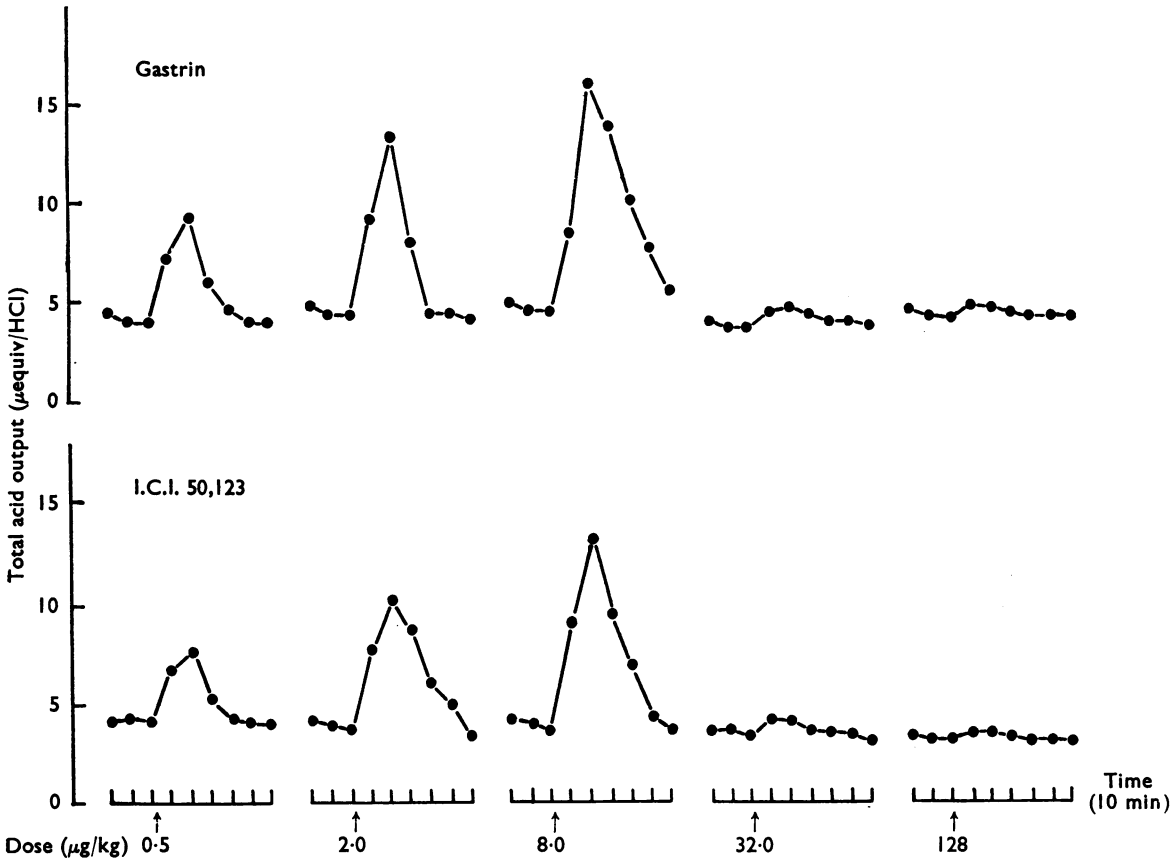

Fig. 3. Effect of intravenous injection of various doses of gastrin and I.C.I. 50,123 on total acid output from perfused rat stomachs. The animals were anaesthetized with urethane given by intraperitoneal route. Each point represents mean acid content of 10-min sample of perfusate from four rats.

were shallow, with that of the pentapeptide being flatter than that of gastrin (Fig. 7). This difference in slope was due to the shorter duration of action of I.C.I. 50,123. When the maximum 10-min outputs for both stimulants were plotted against the logarithm of the dose, two parallel lines were obtained. The maximum peak hour output to I.C.I. 50,123 was only 80% of that for gastrin but the differences, although reproducible, were not statistically significant.

Anaesthesia by other routes using urethane

Other routes of administration for urethane have been employed and the responses to gastrin and I.C.I. 50,123 determined. No treatment other than intraperitoneal urethane led to a biphasic dose-response curve. The routes used were oral, intravenous and intraduodenal (after induction with ether). In another series of experiments rats which had been anaesthetized with urethane intramuscularly were perfused with isotonic

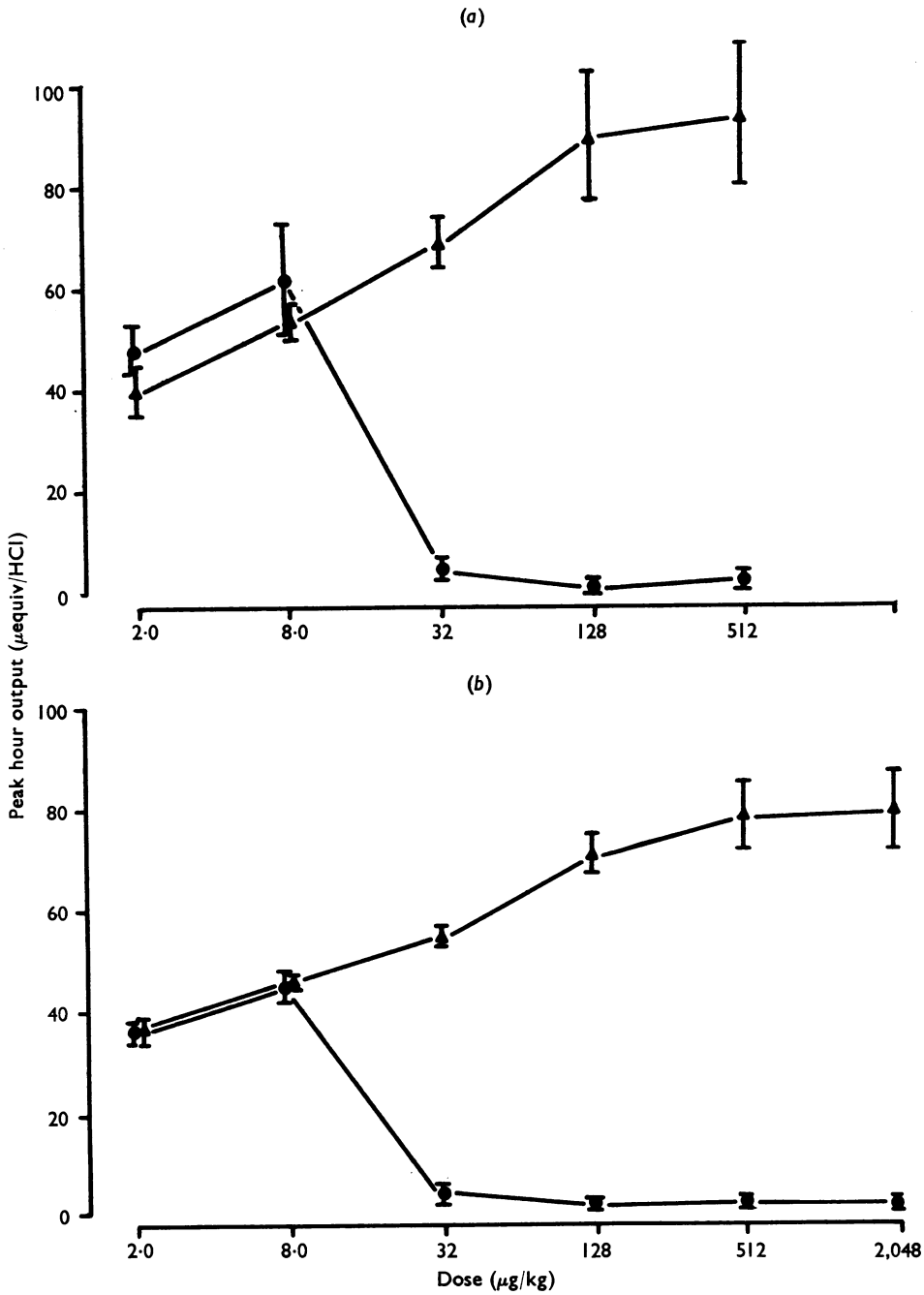

Fig. 4. Effect of intravenous injections of different doses of gastrin (*a*) and I.C.I. 50,123 (*b*) on acid secretion of perfused rat stomach. The animals were anaesthetized with urethane by either intraperitoneal (●——●) or intramuscular (▲——▲) routes. Each point represents mean peak hour output (μequiv HCl) for four rats with vertical lines indicating standard errors.

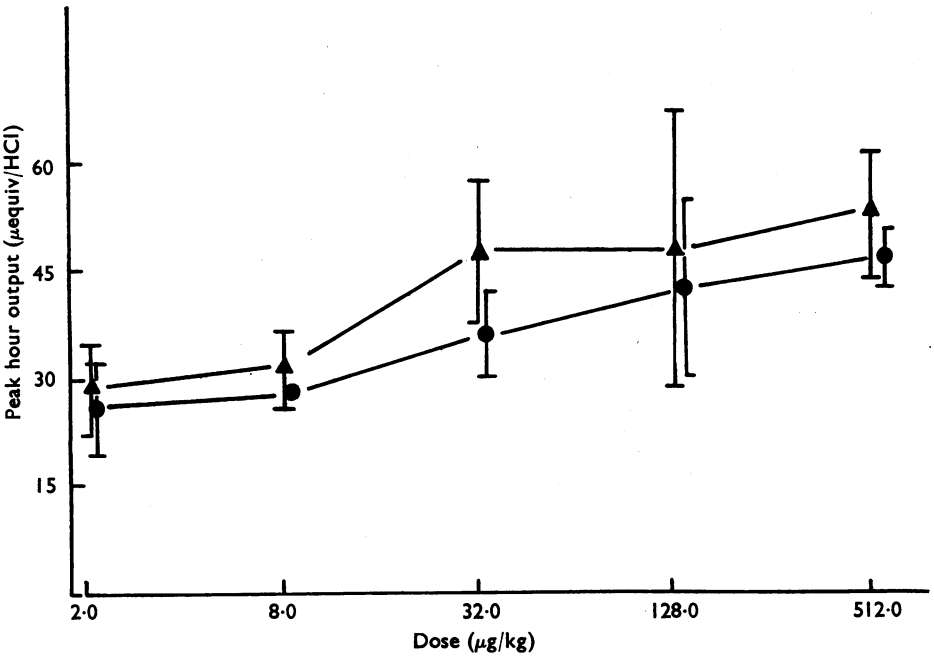

Fig. 5. Effect of intravenous injections of different doses of I.C.I. 50,123 on acid secretion of perfused rat stomach. The animals were anaesthetized with barbitone by either intraperitoneal (●——●) or intramuscular routes (▲——▲). Each point represents mean peak hour output (μequiv HCl) for four rats with vertical lines indicating standard errors.

urethane solution in place of sodium chloride solution. The sensitivity of the preparation to gastrin and I.C.I. 50,123 was reduced but the response pattern was unmodified.

Anaesthetics other than urethane

The dose-response curve to I.C.I. 50,123 has been studied in rats anaesthetized with other anaesthetics. The choice of other agents was limited by the desire to avoid variations in depth of anaesthesia and the necessity for additional dosage during the experimental period of 6 to 8 hr. Barbitone sodium was found to meet all requirements and at a dose level of 600 mg/kg by either intramuscular or intraperitoneal routes provided even, uncomplicated anaesthesia for up to 8 hr. Unlike urethane, the route of administration of barbitone did not affect the type of response to I.C.I. 50,123 (Fig. 5). The slopes of the curves were much flatter and the maximum response only 60% of the value obtained in rats anaesthetized with urethane intramuscularly. Similar curves were obtained in animals anaesthetized with halothane and in pithed rats although the peak outputs were even lower than with barbitone (Table 1). There was still a steady basal secretion in rats which were anaesthetized with barbitone or halothane and in pithed rats. Some rats which were anaesthetized with halothane and showed a relative insensitivity to I.C.I. 50,123 were changed over to open ether anaesthesia. In most cases the responses to similar doses of I.C.I. 50,123 given under ether were much larger than under halothane but it was difficult to maintain a uniform level of anaesthesia throughout the experiment.

TABLE 1

PEAK HOUR OUTPUTS (μequiv HCl) OF ACID FROM PERFUSED RAT STOMACH PREPARATIONS FOLLOWING VARIOUS DOSES OF I.C.I. 50,123

The animals were anaesthetized with urethane or barbitone intramuscularly, halothane, or were pithed. Each value is the mean obtained from four rats together with the standard error.

Dose of I.C.I. 50,123 (μg/kg I.V.)	Urethane	Barbitone	Halothane	Pithed
2·0	37·1±3·3	29·3± 6·7	7·9±1·6	8·2±1·4
8·0	46·1±0·9	31·6± 5·0	15·3±2·6	14·2±3·9
32·0	55·0±1·8	47·7±10·1	17·5±6·3	19·1±4·7
128·0	71·4±4·2	47·9±19·8	20·1±3·5	24·7±5·1
512·0	78·2±6·9	52·1± 7·5	31·1±7·1	32·1±6·1

Hypertonic solutions given intraperitoneally

The concentration of urethane solution used to produce anaesthesia (17.5%) is considerably hypertonic (2.93% is isotonic).

It was thought that the intraperitoneal injection of strongly hypertonic urethane solution might itself bring about the biphasic response to gastrin. However, the injection of solutions of urea, glucose or sucrose iso-osmotic with 17.5% urethane, into the peritoneal cavity of rats anaesthetized with urethane intramuscularly did not alter the response pattern for the intramuscular urethane preparation. It was noted that there was an appreciable fluid accumulation (12 ml.) after glucose and sucrose injection but not following urea or urethane.

Because of the possibility that hypertonic solutions given intraperitoneally—i.e., 17.5% urethane—might damage the vagal innervation of the stomach, acid responses to insulin were studied after intraperitoneal and intramuscular urethane and also under halothane anaesthesia (Table 2). It was necessary to give rather large doses of insulin (1.6 u/kg I.V.) in order to reduce the blood sugar by 60%, since urethane itself produces a relative hyperglycaemia. However, the response under either route of anaesthetic medication was very similar. In contrast, under halothane, insulin was devoid of any secretory action. If atropine (5 mg/kg I.V.) was given to either type of urethane-anaesthetized rat the acid secretion fell to base line values within 30 min.

TABLE 2

EFFECT OF INTRAVENOUS INSULIN (1·6 u/kg) ON TOTAL ACID OUTPUT FROM PERFUSED RAT STOMACH

The animals were anaesthetized with urethane intraperitoneally or intramuscularly and with halothane. Each value is the mean of the total acid output per 10 min (μequiv HCl) for three rats

Time (min)	Urethane (intraperitoneal)	Urethane (intramuscular)	Halothane (inhalation)
10	4·8	5·1	4·1
20	4·2	4·9	4·2
30	4·7	5·0	4·2
Insulin injected			
40	5·1	5·5	3·9
50	5·1	5·4	4·1
60	5·0	5·9	4·0
70	5·7	6·1	3·9
80	7·3	7·8	3·9
90	8·4	9·0	4·1
100	11·3	14·3	4·1
110	15·5	17·6	4·2
120	17·0	21·3	2·9
130	18·8	20·9	3·6
140	20·6	24·2	3·7
150	21·2	26·5	3·6
160	24·2	25·8	2·9

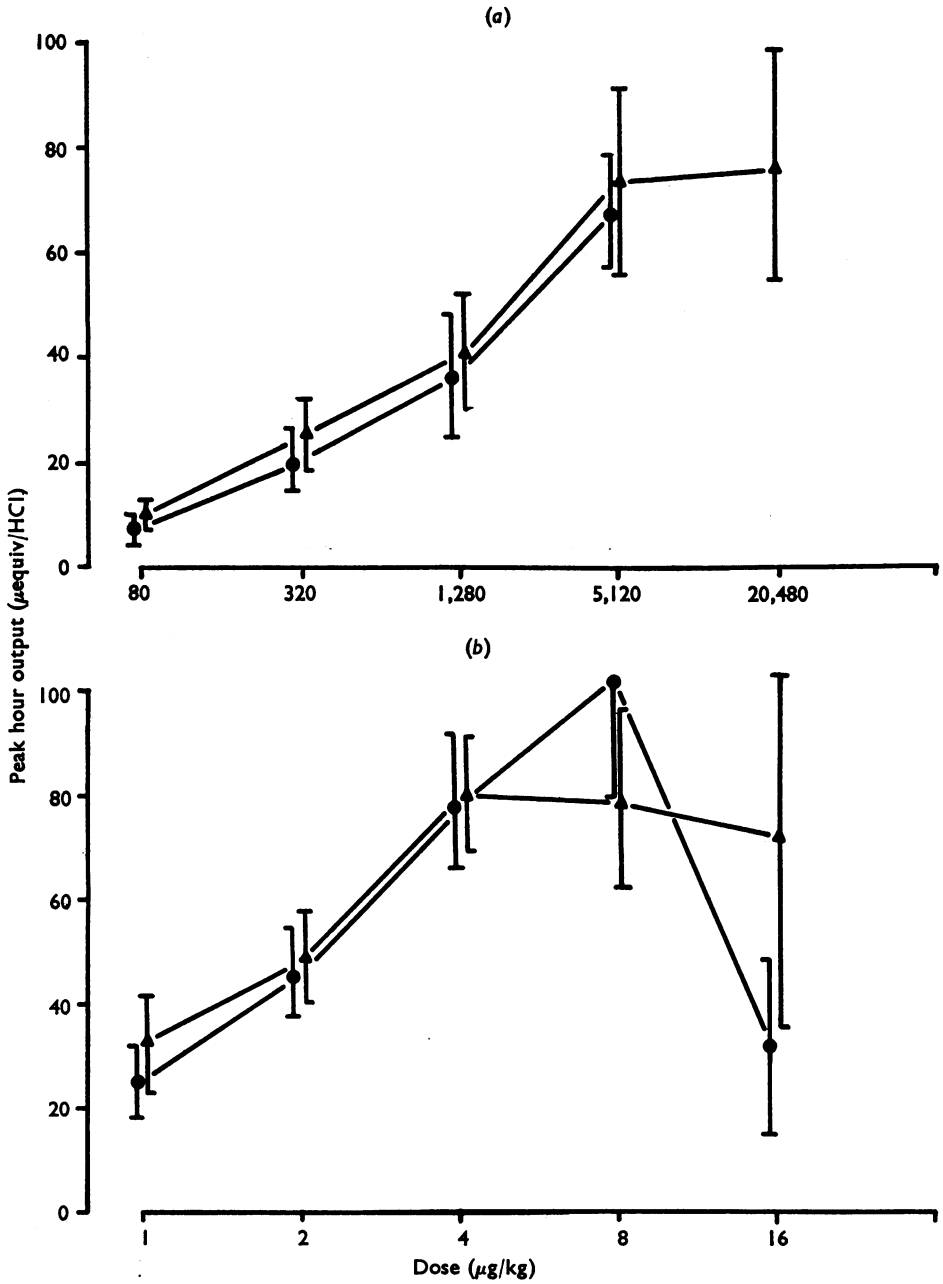

Fig. 6. Effect of intravenous injections of different doses of histamine (*a*) and carbachol (*b*) on acid secretion of perfused rat stomach. The animals were anaesthetized with urethane by either intraperitoneal (●——●) or intramuscular (▲——▲) routes. Each point represents mean peak hour output (μequiv HCl) for four rats with vertical lines indicating standard errors.

Effects of other stimulants of gastric secretion

The effects of other stimulants of acid secretion in rats anaesthetized with intraperitoneal and intramuscular urethane have been studied. There was no difference in the pattern of response for either histamine or carbachol with the two routes of anaesthetic medication (Fig. 6). Both substances stimulated acid secretion proportionally to the dose injected. The response to intravenous histamine reached a plateau at 5.12 mg/kg. There was no change in the peak hour output at 20.48 mg/kg despite the fact that at this dose mean arterial blood pressure fell from 110 to 35 mm Hg in four experiments in which it was measured. The response to intravenous carbachol was maximal at 8 μg/kg. Higher doses produced less acid but there were overt signs of toxicity (bradycardia, hypotension and respiratory distress with profuse salivation). In passing it may be noted that salivation and chromodacryorrhea which occurred with carbachol at 4 μg/kg were never observed with even the highest doses of gastrin or I.C.I. 50,123.

Finally, to facilitate comparison of the secretory stimulants used in this investigation the peak hour outputs have been plotted against the log-dose on a molar basis, for rats anaesthetized with intramuscular urethane (Fig. 7).

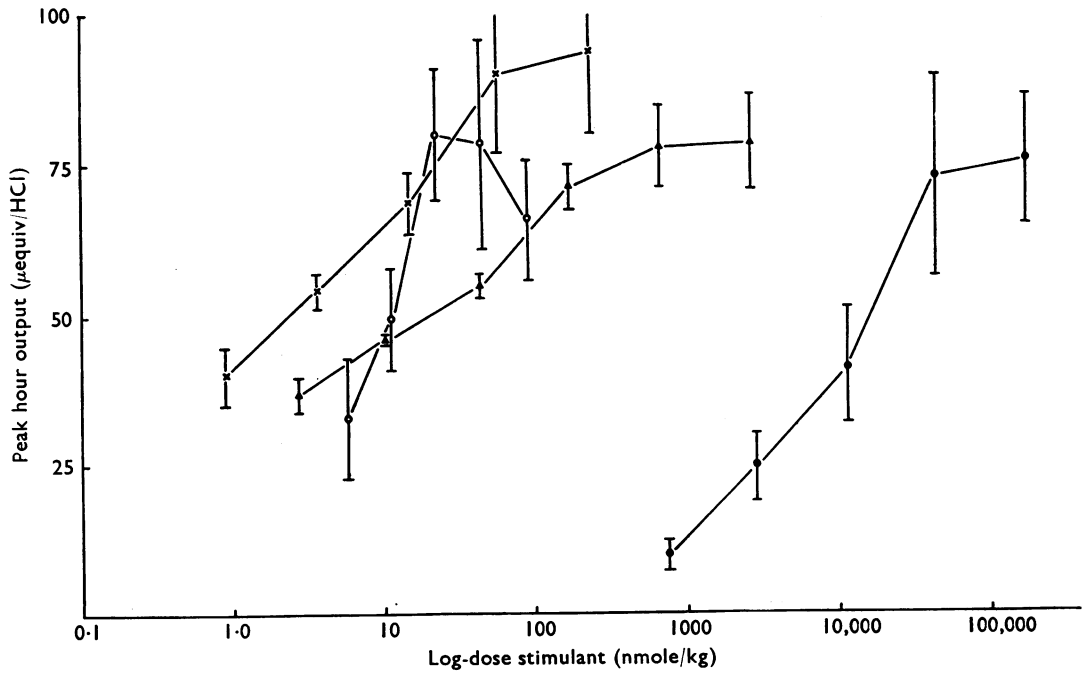

Fig. 7. Comparison of stimulants of acid secretion in rats anaesthetized with intramuscular urethane. Each point represents mean peak hour output (μequiv HCl) for four rats, with vertical lines indicating standard errors. Acid response has been plotted against logarithm of dose of agonist expressed in n-mole/kg injected intravenously. (×——× gastrin ; ▲——▲ I.C.I. 50,123 ; O——O carbachol ; ●——● histamine.)

In the light of these results it appears that, in the rat, gastrin is the most powerful stimulant of acid secretion. On a molar basis it has about ten times the activity of I.C.I. 50,123 and 6,000 times that of histamine. The ratio of activity of carbachol in terms of gastrin is somewhat difficult to establish because of the different slope of the dose-response curve. The slopes of the curves for gastrin, I.C.I. 50,123 and histamine are fairly parallel, suggesting a common mode of action.

DISCUSSION

The administration of urethane to conscious rats provided with chronic gastric fistulae reduces the basal acid secretion (Lane, Ivy & Ivy, 1957; Komarov & Bralow, 1960). Urethane also diminishes the secretory response to insulin (Bralow, Komarov & Shay, 1961). Comparison of our results with the literature shows that greater responses are obtained with histamine and gastrin in the conscious preparation (Lane *et al.*, 1957; Adashek & Grossman, 1963; Ganguli, 1966, personal communication) than in the anaesthetized animals of the present experiments. However, under appropriate conditions it is clear that readily detectable changes in acid-output occur in response to all stimulants used in this study in the urethane anaesthetized rat. The route of administration of urethane has varied considerably in the investigations cited above—e.g., intravenous (Schachter, 1949); subcutaneous (Lane *et al.*, 1957); intramuscular (Ghosh & Schild, 1958); intracolonic (Bralow *et al.*, 1961); part intraperitoneal and part subcutaneous (Lai, 1964). In our laboratories we avoid use of intravenous urethane because of the ensuing haemolysis and have had very constant anaesthesia with the intraperitoneal route on a fixed weight/dose basis. Both Ghosh & Schild (1958) and Lai (1964) comment on the variation of rats in their sensitivity to urethane. That we have not experienced this problem may derive from the use of specific pathogen-free animals. In retrospect the choice of the intraperitoneal route for 2 ml. of a solution some ten times the osmotic strength of body fluids, for the study of gastric secretion, was perhaps unwise but it has led to some intriguing results.

Gregory & Tracy (1964) demonstrated that in denervated canine pouches repeated small doses of gastrin produced a continuous hypersecretion of acid. The rapid injection of a larger dose of gastrin intravenously brought about a prompt fall in acid output. Our initial results in the rat, anaesthetized with urethane intraperitoneally (Fig. 2 *a*, *c*) afforded excellent confirmation of these findings and showed that I.C.I. 50,123 behaved similarly. Preparation of a dose-response curve for both gastrin and the pentapeptide revealed a curious biphasic pattern. Following the accidental use of the intramuscular route for the urethane anaesthetic, quite different results were seen and a fairly typical dose-response curve was obtained of a monophasic pattern (Fig. 4) for both gastrin and I.C.I. 50,123. Similarly, when the plateaux experiments were repeated with intramuscular urethane, large intravenous doses of stimulants merely produced a further increase in acid-output (Fig. 2 *b*, *d*). Experiments in man have failed to produce clear evidence of inhibition of acid output by intravenous injection of gastrin following the establishment of a submaximal plateau to subcutaneous gastrin (Mahklouf, 1965). In addition the dose-response curve for gastrin in conscious rats with a chronic gastric fistula follows the same pattern as seen here in rats given urethane intramuscularly (Ganguli, 1966, personal communication).

All other routes of administration of urethane have given responses comparable to the intramuscular preparation. It was thought that the arrival of high concentrations of urethane in the liver via the portal drainage after intraperitoneal administration might give rise to some different metabolite, or a higher blood level thereof, capable of altering the responses to gastrin and I.C.I. 50,123. This is unlikely, since the response to gastrin after intraduodenal urethane conforms to the intramuscular pattern. Exposure of the mucosal surface of the stomach to hypertonic urethane completely destroyed all responsiveness, although when isotonic urethane solution was used in place of isotonic saline as a perfusion medium in rats anaesthetized with urethane intramuscularly there was no change in the pattern of gastric response.

Another possible explanation was that exposure of the nerve endings in the peritoneal cavity to a strongly hypertonic solution might bring about loss of conduction properties. The intraperitoneal preparation might then be held to resemble the denervated canine pouch preparation. However, since the principal pathway involved is vagal, the similarity of the response to insulin in animals anaesthetized by either route would argue against such an effect. While there may be some doubt about the mechanism by which insulin induces gastric secretion, the prompt inhibition of this response by atropine underlines the predominantly vagal role. The fact that insulin did not produce any increase in the gastric secretion in rats anaesthetized with halothane confirms this point of view, since in previous experiments in cats it has been shown that halothane inhibits the secretion produced by stimulation of the vagi (Raventós, 1961). The effect of atropine also shows that adequate arrival of intravenously injected agents occurs at the mucosal area regardless of the presence of highly hypertonic agents in the peritoneum. That the effect is not due to the hypertonicity of the urethane solution is confirmed by the use of other hypertonic solutions in combination with intramuscular urethane. No change in the intramuscular pattern of response was seen.

The biphasic response of gastric secretion to gastrin and I.C.I. 50,123 was not seen with other anaesthetics or in pithed rats. However, the maximum output of acid in rats anaesthetized with barbitone was only two-thirds that obtained in urethane-treated animals. With halothane the maximum output was less than half that for urethane anaesthesia. The response in pithed rats was almost identical with that in halothane-anaesthetized rats. Despite these differences in sensitivity to secretory stimulants the background secretion of acid was similar in rats anaesthetized with urethane, barbitone and halothane as in pithed animals. The results suggest that the basal secretion is not modified by the vagus whereas the response to gastrin and I.C.I. 50,123 is potentiated by vagal tone.

On no occasion have we observed any biphasic response to histamine. The peak response with histamine was reached at a dose of 5 mg/kg. The highest doses of carbachol did produce sub-maximal acid outputs but there were clear signs of acute toxicity. It would appear that the disparate effects of the peptides and other stimulants may afford some clue as to the mechanism of action of the former.

SUMMARY

1. The secretion of hydrochloric acid by the rat stomach has been studied, using a continuous perfusion of the stomach in anaesthetized animals. Acid output was estimated

by titration of successive 10-min samples of perfusate.

2. The secretory stimulants studied were gastrin, a synthetic peptide (I.C.I. 50,123), histamine, carbachol and insulin.

3. The anaesthetics used were urethane, barbitone, halothane and ether. Some experiments were done in pithed rats.

4. The dose-response curves for gastrin and I.C.I. 50,123 were biphasic in rats anaesthetized with urethane given intraperitoneally but monophasic responses were found in rats anaesthetized with urethane given intramuscularly, intravenously or intraduodenally, with barbitone given intraperitoneally or intramuscularly, with halothane and in pithed rats.

5. The dose-response curves for histamine and carbachol were monophasic in all conditions studied except where toxic symptoms supervened.

6. Attempts to explain the differences have been made and discussed.

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