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The actions of caerulein on gastric secretion of the dog and the rat

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1. Caerulein, as expected from its amino-acid composition and sequence, has a potent stimulant action on gastric secretion in the dog, the rat and the frog.

2. In the denervated fundic pouch of the dog, caerulein increases the rate of flow of gastric juice and the outputs of acid and pepsin. Acid concentration and pepsin concentration in caerulein-produced juice are generally greater than in control juice. The threshold subcutaneous dose of caerulein is 0.15–0.5 μg/kg and the threshold rate of intravenous infusion 0.25–0.5 μg/kg per hr. Rapid intravenous injection is ineffective. On a molar basis, caerulein is approximately twice as active as human gastrin I on volume and acid output of the gastric pouch and 4 times as active on pepsin output.

3. Sustained acid secretion of the fundic pouch produced by histamine infusion is inhibited by caerulein, administered either intravenously or subcutaneously. In turn, acid secretion elicited by caerulein is inhibited by atropine.

4. In the rat, the activity ratio of caerulein to human gastrin I is 7–30, calculated on a molar basis, and is thus considerably greater than in the dog. Further, caerulein is 3 times more active than cholecystokinin-pancreozymin. Tested on the perfused stomach preparation of the rat, the threshold dose of caerulein by rapid intravenous injection is 25 ng/kg, by intravenous infusion 0.25 μg/kg per hr, and by subcutaneous injection 0.25 to 0.5 μg/kg.

5. The activity of caerulein is sharply reduced by pretreatment of the rats with the histamine liberator 48/80 and potentiated by pretreatment with the diamine oxidase inhibitor aminoguanidine. When caerulein is given by rapid intravenous injection during a priming infusion of histamine its effect is enhanced and considerably prolonged.

6. The isolated mucosa of the frog stomach is extremely sensitive to caerulein which, in a concentration of a few pg/ml., stimulates active transport of chloride.

7. Qualitative and quantitative differences in the action of gastrin and caerulein are pointed out, and particular emphasis is laid on the importance of esterification of the tyrosyl residue for the biological activity of caerulein.

It was shown in a preceding paper (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968a) that caerulein had a moderate but relatively long-lasting hypotensive action in the dog and the rabbit. A hypotensive action which was less

marked and somewhat irregular was produced in certain other species. *In vivo*, the polypeptide was a potent stimulant of the gall bladder and the upper part of the small intestine. On the isolated gall bladder musculature caerulein had a very powerful spasmogenic action, but isolated preparations of the gastrointestinal tract were relatively insensitive to the polypeptide (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968b).

The present communication describes the effects of caerulein on the secretory activity of the stomach of the dog and the rat. It has been found that caerulein possesses very intense secretagogue activity, exceeding that of gastrin or cholecystokinin-pancreozymin.

Methods

Denervated fundic pouch of the dog

Thirteen dogs weighing 10–18 kg were each provided, by prior aseptic surgery, with a completely denervated pouch of the gastric fundus having a fistula permanently opened on the abdomen, as described by Gregory (1958). Fourteen days after operation the dogs were ready for the study of gastric secretion. Gastric juice was collected and measured every 30 min, 1 hr before and 2–3 hr, or more, after injection of the drugs under examination. Hydrochloric acid was titrated with 0.1 NaOH with methyl orange as indicator. Pepsin was estimated by the method of Hunt (1948); the blue colour developed by the Folin–Ciocalteu reagent was read in a Beckman spectrophotometer at 750 mμ. Each dog could be used twice a week for several months.

Perfused rat stomach preparation

The procedure described by Mantegazza & Piccinini (1962) for continuous recording of acid gastric secretion was followed. Animals weighing 200–300 g were fasted for 36 hr before use but allowed free access to water. Under urethane anaesthesia (1.2 g/kg, intraperitoneally) the trachea was exposed and cannulated. A polythene tube was passed via the mouth and oesophagus to the stomach, the abdomen opened and the tube tied near the cardia. Then a small rubber tube was introduced through a cut in the duodenum up to the pylorus and secured firmly by a ligature tied round the pylorus. Care was taken to avoid inclusion of blood vessels within the ligature. The stomach was then washed by slow injection of 10 ml. of tepid physiological saline (0.9% NaCl solution) through the oesophageal tube. The rat was placed on an operating table and maintained at a constant temperature by means of a heating lamp. The oesophageal tube was connected to a Braun infusion pump which injected warm physiological saline at a rate of 1.2 ml./min into the stomach. The perfusion fluid collected from the duodenal tube was titrated automatically to pH 8.5 with 0.01 N-NaOH solution. A pH-stat Radiometer TTT 1*a* titrator was used and the changes in gastric acid secretion were recorded for 8 min periods by means of a Radiometer recorder connected to an automatic burette. Drugs were given by different routes. Intravenous injections (0.2 ml./100 g) or infusions were made via a cannula in a jugular vein. The average values obtained during five 8 min periods following administration of physiological saline were considered to be basal values.

Rat stomach with pylorus ligated

A modification of the method of Antonsen (1965) was followed. Female rats weighing 150–200 g were used. In order to ensure that each animal had an empty stomach and was adequately hydrated, the rats had been placed in wire cages 36 hr before the experiment and had free access to a solution containing 5% glucose and 0.4% sodium chloride. At the time of the experiment, the animals were given, by stomach tube, 5 ml. of boiled water of 37° C. The water was aspirated quantitatively and then a further 2 ml. was given which was also aspirated. Subsequently, the animals were laparotomized under light ether anaesthesia and the duodenum was ligated as near to the pylorus as possible. After the abdomen had been closed with two interrupted sutures, 1 ml./100 g of tepid tap water was given to each rat by stomach tube. Drugs to be examined were dissolved in 0.5 ml. of physiological saline and administered subcutaneously. Control rats received the same amount of saline solution. After 1 hr the rats were killed by decapitation and in each case the gastric contents, plus 2 ml. of distilled water used to wash out the stomach, were filtered and titrated with 0.01 N-NaOH with a 0.2% solution of bromothymol blue as indicator.

Drugs

Pure natural and synthetic caerulein (molecular weight 1,352) and synthetic desulphated caerulein (molecular weight 1,272), prepared at the Farmitalia Laboratories for Basic Research, Milan, were used. We are greatly indebted to Professor E. Jorpes, Kemiska Institutionen II, Karolinska Institutet, Stockholm, for samples of pure cholecystokinin-pancreozymin (1,500 and 3,000 Ivy dog units/mg, molecular weight 3,883), to Dr. R. C. Sheppard, The Robert Robinson Laboratories, University of Liverpool for samples of human gastrin I (molecular weight 2,177), and to Dr. J. S. Morley, Imperial Chemical Industries, Macclesfield, for samples of pentagastrin, the gastrin-like pentapeptide I.C.I. 50,123 (molecular weight, 769).

Other drugs used were: aminoguanidine sulphate (British Drug Houses), histamine liberator 48/80 (Imperial Chemical Industries), histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), carbamylcholine chloride (carbachol), atropine sulphate and hexamethonium bromide. The doses of the drugs are given as the weights of the salts.

Results

Dog

Effects of subcutaneous injection. In conscious dogs with denervated fundic pouches, single subcutaneous injections of caerulein produced, as a rule, a conspicuous increase in the volume of gastric juice and an increase in the output of acid and pepsin. The threshold dose was 0.15–0.5 μg/kg. The magnitude of the response was proportional to the dose up to 2 μg/kg (Fig. 1). Vomiting generally interfered with experiments in which larger doses were given. The response to an injection of caerulein began after 10–15 min, reached a maximum at 30–90 min and then declined. The duration of the effect was 2–3 hr, depending on the dose administered.

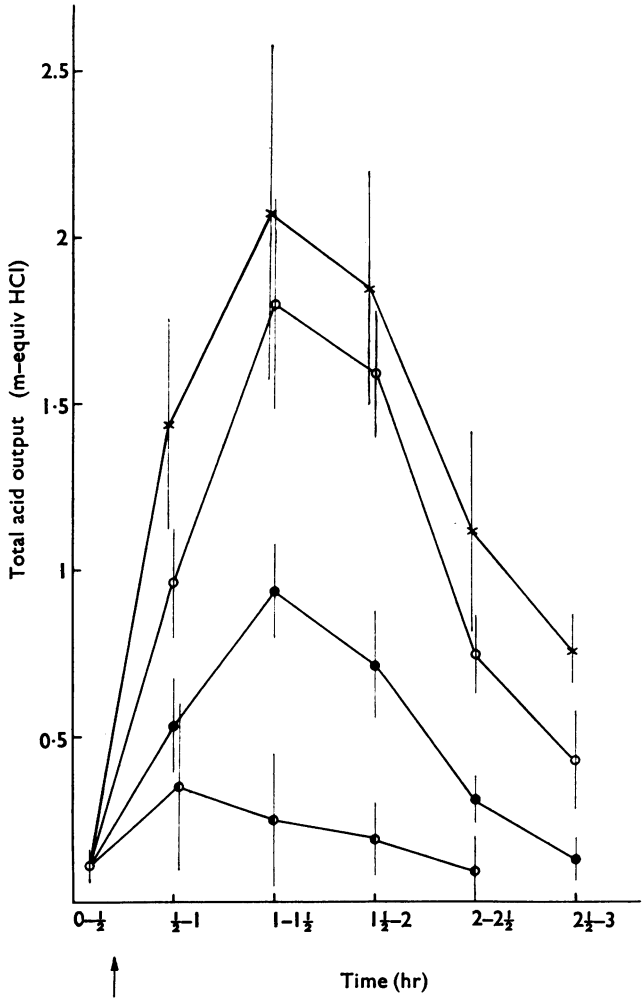

FIG. 1. Denervated gastric pouches of six dogs. Total acid output (m-equiv HCl) following the subcutaneous injection, at arrow, of different doses of caerulein. ◑, 0.25 μg/kg; ●, 0.5 μg/kg; ○, 1 μg/kg; ×, 2 μg/kg. The points are the means of five–twenty experiments, the vertical bars being standard errors.

TABLE 1. *Effects of subcutaneous injection of caerulein (1 μg/kg) on the volume of gastric juice, acid secretion (fifty experiments) and pepsin secretion (nine experiments) of the denervated fundic pouch of the dog*

Observation periods	Volume of gastric juice (ml.)	Total acid output (m-equiv HCl)	Total pepsin output (units)
Pre-injection period			
30– 0 min	1·48±0·47	0·14 ±0·05	36·4± 12
		(0·096±0·004)	(24·6± 3·4)
Post-injection periods			
0– 30 min	6·20±0·60	0·76 ±0·07	313·4± 77·5
		(0·123±0·0003)	(50·6± 5·8)
30– 60 min	9·95±1·5	1·55 ±0·15	560·5±117·3
		(0·156±0·002)	(56·3± 4·1)
60– 90 min	8·61±0·78	1·23 ±0·12	532·1± 93
		(0·143±0·01)	(61·8± 6·2)
90–120 min	3·74±0·61	0·46 ±0·08	224·4± 57·2
		(0·124±0·008)	(60·0± 5·9)
120–150 min	1·77±0·42	0·19 ±0·13	
		(0·107±0·01)	

The values given are the means and S.E. In parentheses are values per ml. of gastric juice.

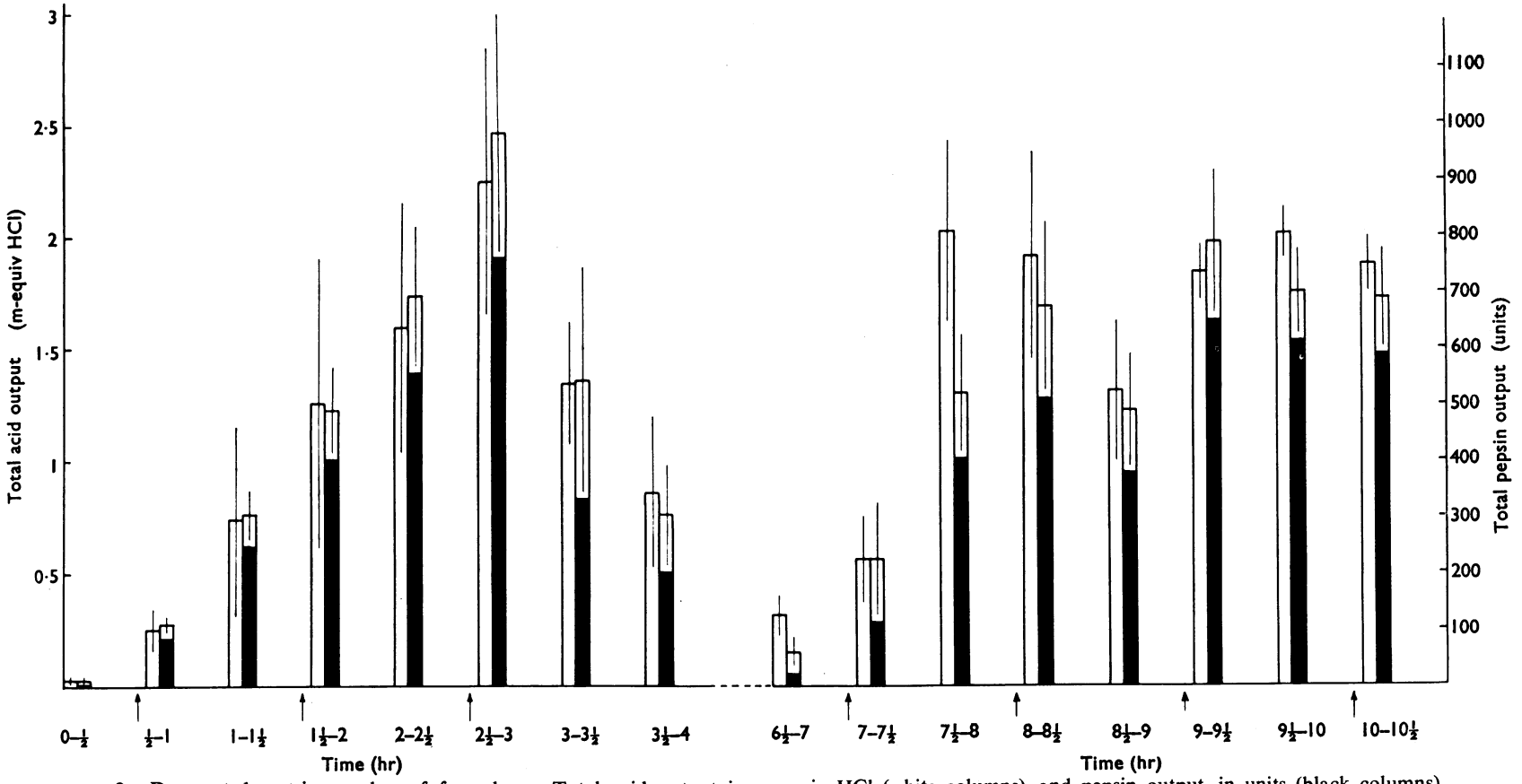

FIG. 2. Denervated gastric pouches of four dogs. Total acid output in m-equiv HCl (white columns), and pepsin output, in units (black columns), following subcutaneous injections of caerulein 1 μg/kg repeated at hourly intervals, at arrows. Three injections were given in the morning, and four in the afternoon. Vertical bars are standard errors. In the morning, injections produced a progressively increasing response ; in the afternoon the maximum response was obtained with the first injection.

It may be seen from Table 1 that caerulein (1 μg/kg) produced not only an absolute increase in acid and pepsin output, but also an increase in HCl concentration (from 0.096 to 0.156 m-equiv/ml.) and an even greater increase in pepsin concentration (from 24.6 to 61.8 units/ml.). Further, it appeared that stimulation of peptic cells lasted longer than stimulation of oxyntic cells; whereas in the period between 90 and 120 min after caerulein administration the HCl concentration was 26% higher than in the control period, the pepsin concentration was 140% higher. The maximum values observed in a particularly sensitive dog were 5 m-equiv HCl/30 min and 1,800 units pepsin/30 min after injection of caerulein 1 μg/kg.

Dogs showing a large basal secretion of gastric juice were generally less sensitive to caerulein. In a few dogs with a basal output of gastric juice of 5–20 ml./30 min, caerulein 1 μg/kg caused, instead of the usual increase, a decrease in gastric secretion.

In four dogs, three subcutaneous injections of caerulein 1 μg/kg were given at intervals of 1 hr and then, after an interval at noon of 2.5 hr, four further injections were made, again at hourly intervals. It can be seen (Fig. 2) that in the morning experiment the second and third injections produced marked increases in the acid and pepsin outputs caused by the preceding injection. In the afternoon, however, injections subsequent to the first one maintained the high acid and pepsin outputs produced by the first injection but did not increase them any further. Thus a very intense secretory response could be maintained for as long as 7 hr. In two dogs, increasing doses of caerulein (0.5, 1, 2 and 4 μg/kg) were injected subcutaneously at hourly intervals. The results were similar to those obtained with repeated doses of 1 μg/kg; vomiting and diarrhoea occurred after the injection of 4 μg/kg.

TABLE 2. *Effects of intravenous infusion of caerulein (1 μg/kg per hr) on volume of gastric juice, acid and pepsin secretion of the denervated gastric pouch in four dogs*

Observation periods	Volume of gastric juice (ml.)	Total acid output (m-equiv HCl)	Total pepsin output (units)
Pre-infusion period (0·9% NaCl solution, 22 ml./hr)			
30–0 min	2·13±0·86	0·25 ±0·10 (0·117±0·006)	57·5± 20 (27·0± 9·5)
Infusion period (0·9% NaCl solution, 22 ml./hr+caerulein, 1 μg/kg per hr)			
0– 30 min	10·5±3·0	1·39 ±0·50 (0·133±0·007)	490·9±172 (46·8± 6·0)
30– 60 min	14·5±2·6	2·25 ±0·52 (0·15 ±0·002)	822·9±112 (56·8± 8·0)
60– 90 min	19·0±4·7	2·85 ±0·80 (0·15 ±0·003)	1,149·5±205 (60·5± 10·0)
90–120 min	15·0±3·2	2·25 ±0·52 (0·15 ±0·003)	1,008·8±191 (67·3± 11·0)
120–150 min	11·0±1·9	1·62 ±0·34 (0·15 ±0·003)	877·3±152 (79·8± 11·0)
150–180 min	9·0±3·5	1·32 ±0·28 (0·14 ±0·004)	714·0±132 (79·3± 13·5)
Post-infusion period (0·9 NaCl solution, 22 ml./hr)			
180–210 min	1·75±0·86	0·18 ±0·16 (0·1 ±0·002)	101·9± 66 (58·3± 0·9)

The values given are the means and S.E. In parentheses are values per ml. of gastric juice.

Effects of rapid intravenous injection. Doses of caerulein ranging from 20 ng to 2 μg/kg were given by rapid intravenous injection in twelve experiments. The polypeptide caused either no change in the basal secretion or a decrease if the rate of basal secretion was high.

Effects of intravenous infusion. The threshold dose of caerulein, given by intravenous infusion, capable of producing an appreciable increase in gastric secretion ranged from 0.25 to 0.5 μg/kg per hr. The infusion was continued for 1–3 hr. The effects of the infusion of caerulein (1 μg/kg per hr) into four dogs are summarized in Table 2.

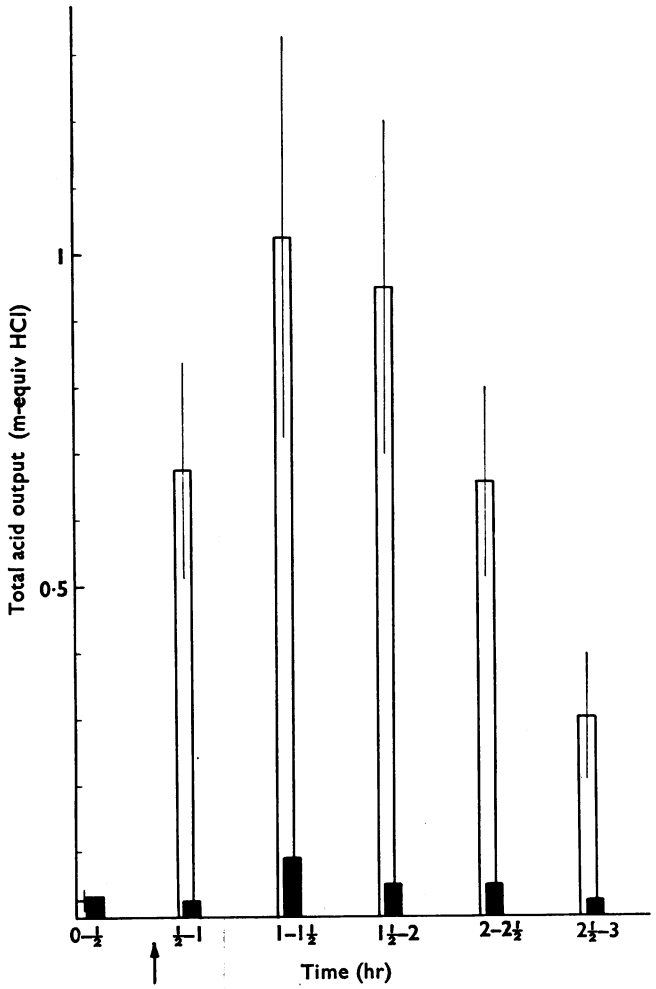

FIG. 3. Denervated gastric pouches of five dogs. Total acid output (m-equiv HCl) produced by a subcutaneous injection of caerulein 1 μg/kg in non-premedicated animals (white columns) and in the same animals injected subcutaneously with atropine sulphate 0.2 mg/kg 30 min before injection of caerulein (black columns). Vertical bars are standard errors.

The rate of gastric secretion increased at first, reached a peak between 60 and 90 min and thereafter gradually declined, attaining half the maximum value after 2.5–3 hr. The rise in acid concentration was well maintained and was of approximately the same magnitude throughout the experiment. This increase was never more than 32%, however, and the acid concentration rapidly dropped to basal levels after interruption of the infusion. The pepsin concentration showed a continuous increase throughout the experiment, attaining a maximum of up to 230% increase after 120–180 min of infusion. The decline in pepsin concentration after discontinuing the infusion was considerably slower than the decline in the volume of the gastric juice or in the acid concentration. Again it was apparent that the action of caerulein on peptic cells was considerably stronger than its action on oxyntic cells.

Effects of atropine and hexamethonium. Seven dogs were injected with caerulein 1 μg/kg subcutaneously and gastric secretion was recorded. Two days later, the same dose of caerulein was given to the same dogs after intramuscular injection of atropine 200 or 500 μg/kg which caused an inhibition of more than 90% of the acid output stimulated by caerulein (Fig. 3). Similar results were obtained when 50 or even 20 μg/kg of atropine were administered. Intramuscular injection of hexamethonium (5 mg/kg) was without effect on caerulein-stimulated gastric secretion.

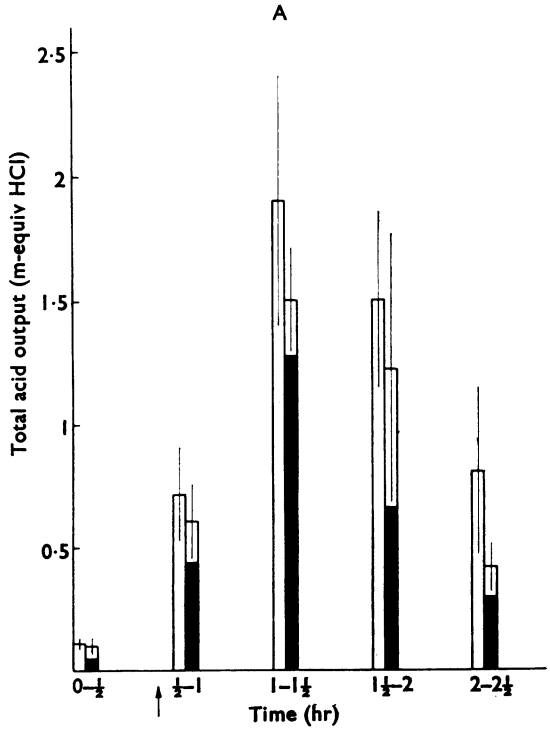

FIG. 4A. (See next page for Fig. 4B and legend.)

Potency of caerulein relative to human gastrin I, pentagastrin and desulphated caerulein. Caerulein was compared with human gastrin I in six dogs. With regard to acid output, it was found that caerulein 1 μg/kg given by the subcutaneous route was equivalent to approximately 3 μg/kg of human gastrin I administered by the same route (Fig. 4A). With regard to pepsin output, however, the potency of caerulein relative to gastrin was considerably greater (Fig. 4B). In further experiments it was shown that human gastrin I 6 μg/kg had less effect on pepsin output than caerulein 1 μg/kg, and human gastrin I 3 μg/kg had approximately the same effect as caerulein 0.5 μg/kg. This indicates that caerulein was about 6 times more active than human gastrin I, on a weight basis.

Des-glutamyl human gastrin I was as active as human gastrin I. The gastrin-like pentapeptide, pentagastrin, in turn, was 20 to 30 times less active than caerulein on volume of gastric juice and acid output. Particular emphasis should be laid on the fact that desulphated caerulein had barely 3–5% of the potency of caerulein.

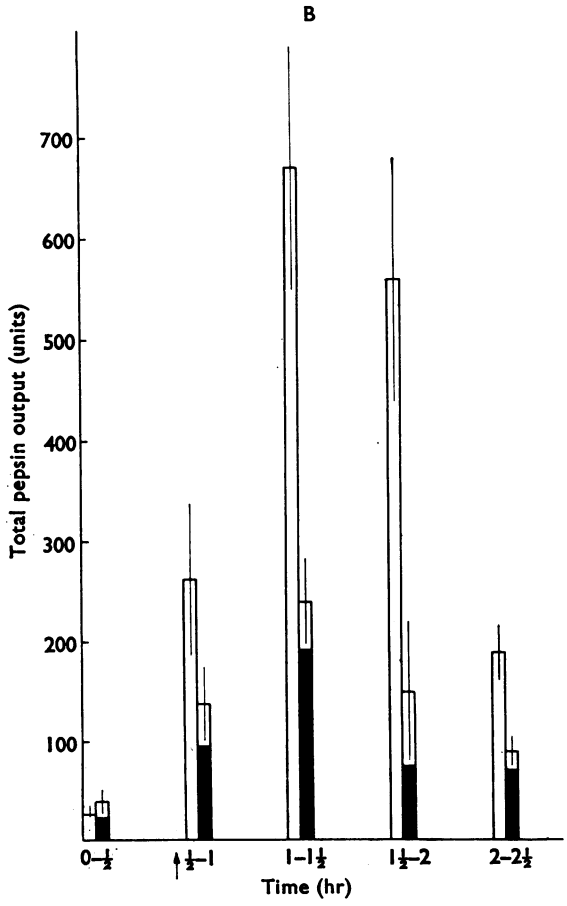

FIG. 4B. Denervated gastric pouches of six dogs. A: Total acid output (m-equiv HCl) produced by subcutaneous injection of caerulein 1 μg/kg (white columns, six experiments) and human gastrin I 3 μg/kg (black columns, five experiments). Vertical bars are S.E. of the means. B: Total pepsin output (units) produced by the same injections. Caerulein stimulated more effectively the pepsin output than gastrin.

Interaction between cærulein and histamine. Histamine given to conscious dogs by intravenous infusion at a rate of 0.5 mg/kg per hr produced the usual increase in acid output, as shown in Fig. 5. When, 75 min after the start of histamine infusion, caerulein 1 μg/kg was administered to two dogs by subcutaneous injection and to two other dogs by rapid intravenous injection, there was, in spite of the continuing infusion of histamine, in all cases a sharp reduction in the volume of gastric juice and acid output. Intravenous injection produced a more prompt effect and basal pre-histamine values were attained within 30–45 min. This inhibitory effect recalls the inhibition occasionally observed in non-premedicated dogs with unusually high basal levels of gastric secretory activity.

Some preliminary experiments were carried out on five dogs injected intravenously with the histamine liberator, 48/80 (0.15 mg/kg). The effect of caerulein (1 μg/kg, subcutaneously) appeared to be enhanced by 30–50%.

Rat

The perfused stomach preparation secreted a small amount of acid throughout the whole period of perfusion. The mean basal secretion as established in one hundred rats was 3.32 ± 0.18 μ-equiv HCl/kg per min.

Effects of rapid intravenous injection. Given by rapid intravenous injection, caerulein produced, at all dose levels above the threshold, an increase in acid secretion generally accompanied by pylorospasm which lasted 3–6 min (Fig. 6).

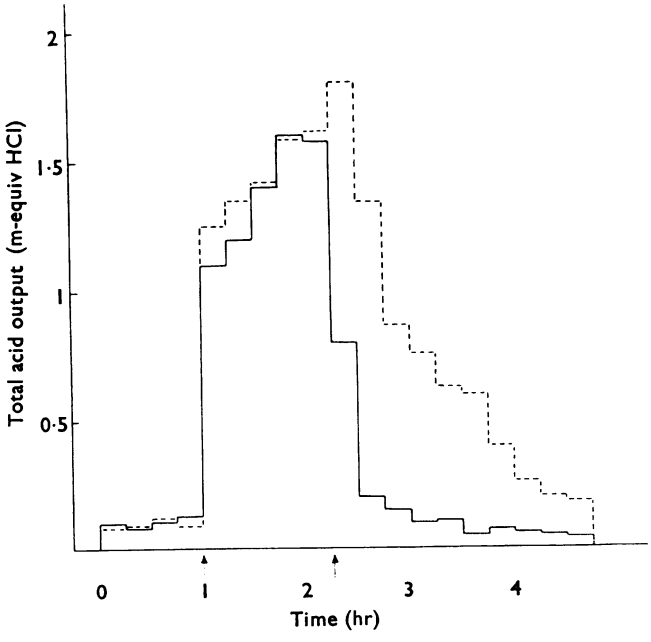

FIG. 5. Denervated gastric pouches of four dogs. At the first arrow an intravenous infusion of histamine dihydrochloride at the rate of 0.5 mg/kg per hr was started and continued until the end of the experiment; at the second arrow, caerulein 1 μg/kg was injected intravenously (continuous line, two dogs) or subcutaneously (broken line, two dogs). In all cases, but more rapidly after the intravenous injection, the polypeptide produced an inhibition of the histamine-induced acid secretion.

During this period, passage of the perfusate from the stomach into the duodenum was, of course, reduced. The threshold dose of caerulein was approximately 25 ng/kg for acid secretion and 50 ng/kg for pylorospasm. At dose levels up to 500 ng/kg there was always a good dose-response relationship.

The pattern of responses to different doses of caerulein is shown in Table 3. With the dose of 0.1 μg/kg used in the majority of experiments, the response lasted 20–25 min and the total excess acid output during this period was about 60 μ-equiv HCl/kg, with a maximum between 8 and 16 min after the injection. Dose-response curves for mean and peak HCl outputs (μ-equiv/kg per min) against log dose of caerulein are shown in Fig. 7.

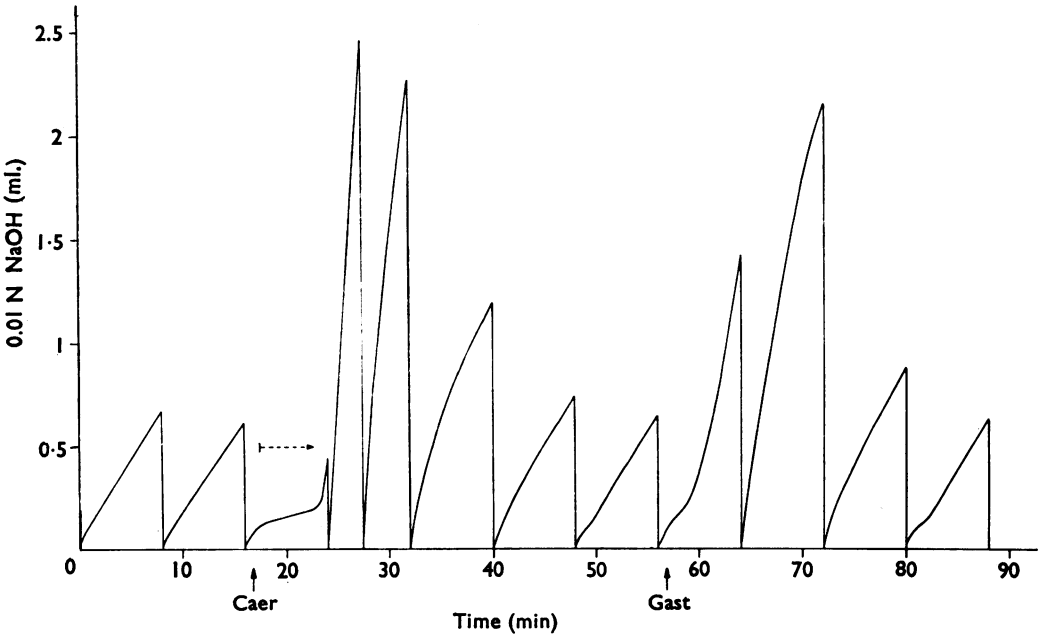

FIG. 6. Perfused rat stomach preparation. Abscissa, time (min); ordinate, amount of 0.01 N NaOH required (ml.) to titrate the effluent from the stomach to pH 8.5. Caer, intravenous injection of caerulein (0.5 μg/kg); Gast, intravenous injection of human gastrin I (7.5 μg/kg). Caerulein produced 63 μ-equiv and gastrin 44.8 μ-equiv of HCl. ⟶, Spasm of pylorus.

TABLE 3. *Acid output of the perfused stomach preparation of the rat following intravenous injection of different doses of caerulein*

Dose of caerulein (ng/kg)	Number of experiments	Mean increase (%)	Peak increase (%)
25	10	19·0± 6·1	34·9± 9·3
50	15	38·1± 5·6	66·3± 9·0
100	30	77·2± 8·6	139·1±13·1
200	10	98·4±10·6	211·6±26·0
500	10	146·1±24·0	283·5±39·0
2,000	10	157·5±13·0	286·6±28·0
10,000	8	130·0±39·5	267·8±62·0

"Mean increase" is the mean of the increases over and above the basal level of 3·32±0·18 μ-equiv HCl/kg per min during three consecutive post-injection periods of 8 min each; "peak increase" is the increase during period of 8–16 min after injection. The values given are the means and S.E.

In contrast to the intensity of the response, its duration was little affected by the dose of caerulein. With very large doses (2–10 μg/kg), however, a prolongation of the secretory response (up to 30–45 min) and of the pylorospasm could be observed. Table 3 shows that caerulein 10 μg/kg produced a smaller increase in the acid output than 2 and 0.5 μg/kg. This may be due, at least in part, to local or systemic circulatory effects of caerulein.

Effects of intravenous infusion. The threshold dose of caerulein was 0.25 μg/kg per hr. With 0.5 μg/kg per hr, two phases could be distinguished. A peak of secretion with an increase of 100 to 120% was reached in 15–20 min ; thereafter there was a steady state of secretion which showed an increase of only 40 to 70% and persisted for as long as the infusion of caerulein was continued, namely up to a maximum of 2 hr (Fig. 8). With 5 μg/kg per hr of caerulein the peak increase was 300% and the increase during the steady state was 100%. Pylorospasm was observed only with large doses of caerulein.

Effects of subcutaneous injection. The threshold dose was 0.25–0.5 μg/kg. An increase in the dose produced an increase in both intensity and duration of the gastric secretory response. After 30 μg/kg (4 experiments) the effect lasted for 2 hr with a peak increase in acid output of 100 to 200% between 20 and 40 min ;

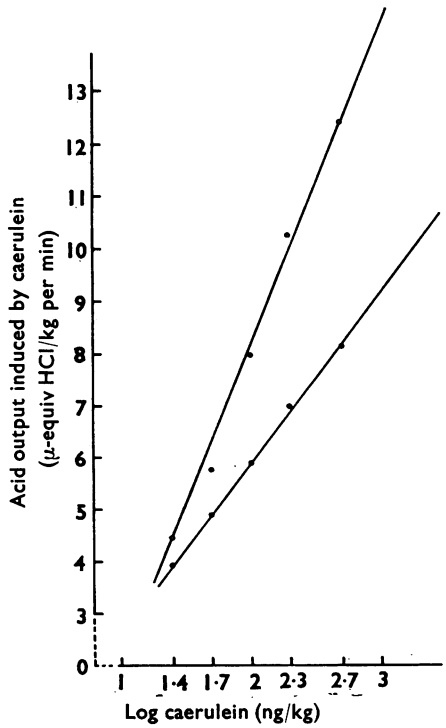

FIG. 7. Perfused rat stomach preparation. Dose–response curve of acid output induced by caerulein. ●, Mean of responses during period 0–24 min ; ○, peak of responses during period 8–16 min after injection of caerulein.

after 10 μg/kg (3 experiments) the effect lasted for 1 hr with a peak increase of 50–80% between 30 and 40 min; after 5 μg/kg (3 experiments) the duration of the effect was 45 min with a maximum increase of 30–40% between 25 and 35 min; after 1 μg/kg (3 experiments) the duration of the effect was 40–50 min with a maximum increase of 15–30%; finally after 0.5 μg/kg the effect lasted for 30–40 min with a maximum increase of 15%.

Effect of gastric administration. Caerulein added to the perfusion fluid of the stomach in a concentration of 1–2 μg/ml. for 30 min was completely inactive.

Potency of caerulein relative to desulphated caerulein, human gastrin I, pentagastrin and cholecystokinin-pancreozymin. Desulphated caerulein retained 8–13% of the activity of caerulein.

Comparison of the potency of caerulein with that of gastrin was frequently difficult owing to the appearance, during the course of an experiment, of a mutual potentiation of effects. Potentiation of the effects of gastrin by caerulein was more frequent and intense than potentiation of the effects of caerulein by gastrin. If all our experiments were considered, however, it could be established that on a weight for weight basis human gastrin I was 10–40 times less active than caerulein. In some experiments there was also an autopotentiation of the effects of gastrin, the effects of successive equal doses increasing by 100–200%. A similar but slightly lower activity ratio was observed for pentagastrin.

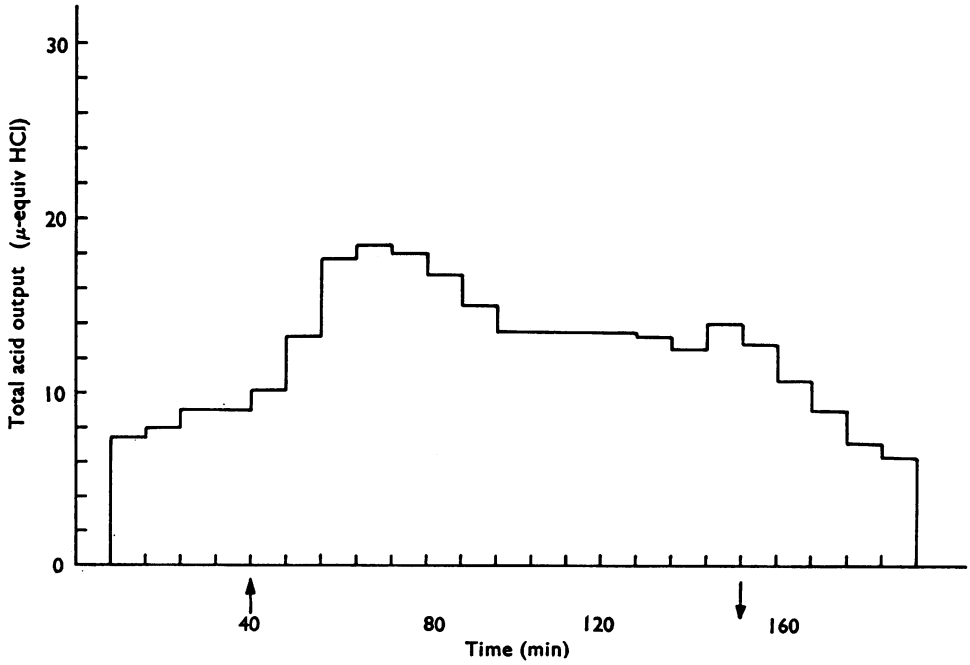

FIG. 8. Perfused rat stomach preparation. Acid output (μ-equiv HCl) following the intravenous infusion of caerulein at a rate of 0.5 μg/kg per hr. ↑, Infusion started; ↓, infusion stopped.

Pure natural cholecystokinin-pancreozymin proved to be considerably more potent than human gastrin I, but less potent than caerulein. In fact, 3 Ivy dog units of cholecystokinin-pancreozymin (= 1 μg pure polypeptide) given intravenously produced the same acid output as 0.1 μg of caerulein, given intravenously. Comparison of the patterns of the responses to the above polypeptides showed no appreciable differences.

Potency of caerulein relative to carbachol, histamine and 5-hydroxytryptamine. On a weight for weight basis, intravenously injected carbachol had 10–20% of the activity of caerulein. As much as 1 mg of histamine had to be injected intravenously to produce the same effect as 100 ng of caerulein. 5-Hydroxytryptamine had no effect on the acid secretion of the stomach preparation injected intravenously in doses up to 500 μg/kg.

Intravenous infusion of histamine followed by rapid intravenous injection of caerulein. When histamine was infused at the rate of 1 mg/kg per hr, there was either no or only a moderate increase of 30–50% in the basal acid secretion. Rapid intravenous injection of 50–100 ng/kg of caerulein, 30–45 min after the histamine infusion was started, produced an increase in acid output over and above the histamine-induced secretion ; this increase was only slightly greater than the increase in the basal secretion caused by caerulein alone.

An increase in the infusion rate of histamine to 2.5–10 mg/kg per hr produced very variable effects. In a few cases the basal secretion remained unchanged, but generally it rose by 30 to 200% with no regular dose–response relationship. The effects of caerulein, given by rapid intravenous injection 30–45 min after the histamine infusion had commenced, were, however, considerably potentiated. Not only

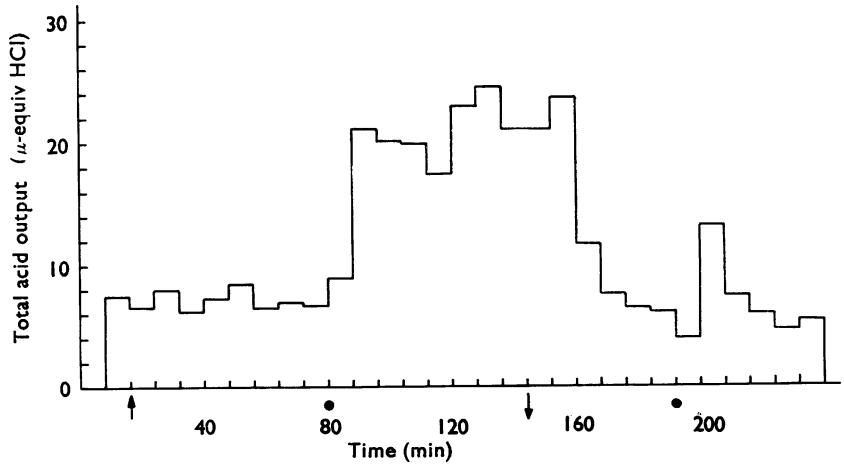

FIG. 9. Perfused rat stomach preparation. Acid output in μ-equiv HCl. Between arrows intravenous infusion of histamine dihydrochloride (5 mg/kg per hr). At ●, intravenous injection of caerulein 200 ng/kg. Infusion of histamine produced no appreciable change in basal acid output. Caerulein, given during the histamine infusion, elicited a considerable increase in acid output, which lasted as long as the infusion of histamine was continued. After interruption of the histamine infusion the acid output returned to basal values, and caerulein now produced the usual response.

was the intensity of the response increased, but the duration of the effect was also prolonged markedly. Indeed, the effect was usually sustained for as long as the infusion of histamine continued. The effect rapidly subsided, however, when the histamine infusion was discontinued. The threshold dose of caerulein was 25–50 ng/kg.

Figure 9 illustrates such an experiment in which histamine infusion had no appreciable effect on the background secretion. In another experiment intravenous infusion of histamine 10 mg/kg per hr doubled the acid output and subsequent rapid intravenous injection of caerulein 200 ng/kg caused a further increase of 170%. Thus the pre-histamine basal acid output was increased by 435% and this very large response was maintained for the 3 hr during which the priming infusion of histamine was continued. Thereafter, the original basal level was regained in 20–30 min. When the dose of caerulein was raised to 500 ng/kg, however, the even larger increase in the response, up to 950%, could be sustained for only 30 min. Thereafter, in spite of continuing histamine infusion, acid output slowly decreased to the level before caerulein administration.

Intravenous infusion of caerulein followed by rapid intravenous injection of histamine. Infusion of caerulein at a rate of 0.5 μg/kg per hr did not affect the secretory response to a subsequent rapid intravenous injection of a threshold dose of histamine (0.5 mg/kg).

Intravenous infusion of histamine followed by rapid intravenous injection of human gastrin I. On the whole the effect of human gastrin I was similar to that of caerulein, but gastrin 5–10 μg/kg was required to reproduce the effect elicited by caerulein 0.1 μg/kg.

Effects of caerulein in rats pretreated with aminoguanidine. Intravenous injection of the diamine oxidase inhibitor aminoguanidine (50 mg/kg) caused an increase of 15–30% in the basal acid secretion of the perfused stomach. When caerulein 100–200 ng/kg was injected intravenously 10–15 min after aminoguanidine administration, the response to the polypeptide was 1.5 to 3 times larger than that observed in non-premedicated animals.

Effects of caerulein in rats pretreated with compound 48/80. Intravenous injection of the histamine liberator 48/80 (0.2 mg/kg) led to a stimulation of acid secretion similar to that caused by caerulein 50 ng/kg. Caerulein, given intravenously in doses up to 1 μg/kg 30–90 min after administration of compound 48/80, was, however, completely ineffective. A similar loss of activity was observed when 5–10 μg of human gastrin I was injected. The dose of compound 48/80 used seemed to be critical; half the dose did not inhibit but increased slightly the response to caerulein 100 ng/kg and twice the dose had severe toxic, often fatal, effects.

Effects of atropine, bilateral vagotomy and hexamethonium. Atropine 5–10 mg/kg given by intravenous injection produced either no or a small reduction (10–30%) in the secretory response to intravenous administration of caerulein 100 ng/kg. Similarly, acid output produced by caerulein remained unchanged after bilateral vagotomy in the neck.

Hexamethonium, injected intravenously in doses up to 30 mg/kg, was without effect on the secretory response to caerulein. Pylorospasm, however, was abolished.

Rat stomach with pylorus ligated. A limited number of experiments gave results comparable with those obtained in the perfused stomach preparation. In the 60 min period of observation the stomach of sixteen control rats gave a total acid output of 60 μ-equiv HCl; the stomach of sixteen rats given caerulein 10 μg/kg by subcutaneous injection had a total acid output of 400 μ-equiv HCl. The gastrin-like pentapeptide pentagastrin had 1.5% of the activity of caerulein.

Frog

In experiments which will be published in detail elsewhere (Pesente & Erspamer, unpublished) it was shown that caerulein increased the active transport of chloride in the isolated gastric mucosa of *Rana esculenta*. The threshold concentration was of the order of 0.002 ng/ml. or less, and the maximum effect (50% increase) was obtained with 0.25 ng/ml. The effect was prompt and lasted for 10–20 min, the peak of the response being reached within 5 min. The stimulation of chloride transport was followed by a long-lasting depression of 10–15%. On a weight basis, human gastrin I was at least 500 times less active than caerulein.

Discussion

It has been shown in previous papers that caerulein has a moderate hypotensive action in the dog and rabbit and a powerful stimulant action on the musculature of the gall bladder *in situ*. This paper shows that caerulein stimulates the flow of gastric juice and the acid and pepsin outputs. In a forthcoming paper a powerful pancreozymin-like action of caerulein on the pancreas will be reported. Thus caerulein is a very versatile polypeptide, mimicking gastrin on the one hand and cholecystokinin-pancreozymin on the other, and causing some bradykinin-like effects.

The gastrin-like activity of caerulein is readily explainable on the basis of its amino-acid composition and sequence. As shown by Anastasi, Erspamer & Endean (1968), caerulein has in common with the gastrins the C-terminal pentapeptide which possesses the entire range of physiological activity displayed by the natural gastrins (Gregory & Tracy, 1964, 1966).

$$\overset{1}{\text{Pyr}}\text{-}\overset{2}{\text{Gly}}\text{-}\overset{3}{\text{Pro}}\text{-}\overset{4}{\text{Trp}}\text{-}\overset{5}{\text{Leu}}\text{-}(\overset{6\text{-}10}{\text{Glu}})_5\text{-}\overset{11}{\text{Ala}}\text{-}\overset{12}{\text{Tyr}}\text{-}\overset{13}{\text{Gly}}\text{-}\overset{14}{\text{Trp}}\text{-}\overset{15}{\text{Met}}\text{-}\overset{16}{\text{Asp}}\text{-}\overset{17}{\text{Phe}}\text{-}NH_2$$

Human gastrin I

$$-\overset{12}{\text{Tyr}}(SO_3H)-$$

Human gastrin II

$$\overset{1}{\text{Pyr}}\text{-}\overset{2}{\text{Gln}}\text{-}\overset{3}{\text{Asp}}\text{-}\overset{4}{\text{Tyr}}(SO_3H)\text{-}\overset{5}{\text{Thr}}\text{-}\overset{6}{\text{Gly}}\text{-}\overset{7}{\text{Trp}}\text{-}\overset{8}{\text{Met}}\text{-}\overset{9}{\text{Asp}}\text{-}\overset{10}{\text{Phe}}\text{-}NH_2$$

Caerulein

It has clearly emerged from the present study, however, that the existing structural differences between the two polypeptides have a profound influence on their activities; in this respect the length of the molecule and the amino-acid composition

and sequence of the remaining part of the molecule are of importance. This topic will be discussed in detail elsewhere; here we wish to emphasize some similarities and dissimilarities in the activities of caerulein and gastrin on gastric secretion.

Points of similarity are as follows:

(1) Like gastrin, caerulein is ineffective when administered to dogs by rapid intravenous injection, but both polypeptides are active when administered to rats by this route. The absence of a secretory action of gastrin and its eventual inhibitory effect when given by quick intravenous injection to dogs has been emphasized by Gregory (1966) and by Grossman (1966).

(2) The secretory response to caerulein, like that to gastrin, is strongly inhibited by atropine in the dog, but to a much less extent, or not at all, in the rat.

(3) As is the case with gastrin (Amure & Ginsburg, 1964; Thayer & Martin, 1967), caerulein is inactive in rats depleted of histamine by compound 48/80 and has an increased effect in rats pretreated with the diamine oxidase inhibitor aminoguanidine. These observations point to the possibility that the action of caerulein on gastric secretion in the rat is mediated, at least in part by histamine, and in the dog by acetylcholine.

(4) When in the dog a steady and maintained increase in gastric secretion has been established by intravenous infusion of histamine, both gastrin (Gregory & Tracy, 1964) and caerulein, given by intravenous or subcutaneous injection, reduce the flow of gastric juice and acid output. The events caused by the two polypeptides in the perfused rat stomach differ from those observed in the dog. When caerulein or gastrin is administered by rapid intravenous injections to rats given a priming infusion of histamine, either polypeptide elicits an intense and sustained increase of acid output, over and above the increase caused by histamine. This effect, which seems to be more intense with caerulein than with gastrin is very difficult to explain.

Dissimilarities between caerulein and gastrin are as follows:

(1) Caerulein and gastrin differ in their relative potencies on the oxyntic and peptic cells of the dog stomach. In fact, if the activity of human gastrin I is considered to be 100, the activity of an equimolar dose of caerulein is approximately 170–180 on acid output and 350–380 on pepsin output.

(2) Whereas caerulein has a potent stimulant effect on acid secretion in all three animal species examined in this study, the activity of human gastrin I is comparable with that of caerulein in the dog only. The activity ratios of caerulein to gastrin, on a molar basis, are 1.8 in the dog, 6–24 in the rat and >200 in the frog. It has been pointed out repeatedly (Grossman, 1967) that, so far, no differences in the potency or the spectrum of biological activity have been found between gastrins from hog, man, dog and sheep. From the present data, which show that human gastrin I is a moderate or poor stimulant of gastric secretion in rats and amphibians, one is tempted to speculate that rats and amphibians may possess specific gastrins, differing from the hitherto known gastrins in their amino-acid composition and sequence. Compared with the gastrins, the smaller molecule of caerulein appears to be a key suitable for a greater number of locks.

(3) According to Gregory & Tracy (1964), gastrin I and gastrin II, which latter contains a tyrosyl *O*-sulphate residue instead of a tyrosyl residue, have apparently identical qualitative and quantitative effects on gastric secretion and, more generally,

on alimentary tract functions. This fact indicates that sulphation of the tyrosyl residue is of little or no importance for the specific effects of the gastrins. On the other hand, desulphated caerulein, obtained either directly by synthesis or by gentle acid hydrolysis of caerulein, possesses barely 3–5% of the effect of caerulein on the acid output of the denervated fundic pouch of the dog and 8–13% of the effect of caerulein on the acid output of the perfused stomach preparation of the rat. A similar decrease in activity after desulphation has been observed for the C-terminal heptapeptide of caerulein and for other synthetic sulphated caerulein-like polypeptides (Anastasi, Bernardi, Bertaccini, Bosisio, de Castiglione, Erspamer, Goffredo & Impicciatore, 1968).

Gastric secretion is strongly stimulated not only by the gastrins and caerulein, but also by cholecystokinin-pancreozymin. Present observations are in agreement with the data of Heitmann, Mjungreis & Janowitz (1967) and of Cooke (1967). In our experiments on the acid output of the perfused rat stomach preparation, the duodenal hormone was approximately 3 times less potent than caerulein, on a molar basis, but 2 to 8 times more potent than human gastrin I. This result is not surprising when it is recalled that cholecystokinin-pancreozymin has in common with both caerulein and the gastrins the C-terminal pentapeptide (Mutt & Jorpes, 1967), and probably has in common with caerulein other essential amino-acid residues, such as the tyrosyl *O*-sulphate residue.

A more thorough discussion of the results described in this paper, together with a broader comparison of the activity spectra of gastrin, caerulein and cholecystokinin-pancreozymin will be presented in a forthcoming paper. It is obvious that data collected in the present study must be completed and extended in several directions.

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REFERENCES

AMURE, B. & GINSBURG, M. (1964). Inhibitors of histamine catabolism and the action of gastrin in the rat. *Br. J. Pharmac. Chemother.*, **23**, 476–485.

ANTONSEN, S. (1965). Gastric secretion in rats after test meals. *Acta pharmac. tox.*, **23**, 165–178.

ANASTASI, A., BERNARDI, L., BERTACCINI, G., BOSISIO, G., DE CASTIGLIONE, R., ERSPAMER, V., GOFFREDO, O. & IMPICCIATORE, M. (1968). Synthetic peptides related to caerulein. Note I. *Experientia*, **24**, 771–773.

ANASTASI, A., ERSPAMER, V. & ENDEAN, R. (1968). Isolation and amino acid sequence of caerulein, the active decapeptide of the skin of *Hyla caerulea*. *Arch. Biochem. Biophys.*, **125**, 57–68.

BERTACCINI, G., DE CARO, G., ENDEAN, R., ERSPAMER, V. & IMPICCIATORE, M. (1968a). The action of caerulein on the systemic arterial blood pressure of some experimental animals. *Br. J. Pharmac. Chemother.*, **33**, 59–71.

BERTACCINI, G., DE CARO, G., ENDEAN, R., ERSPAMER, V. & IMPICCIATORE, M. (1968b). The actions of caerulein on the smooth muscle of the gastrointestinal trace and the gall bladder. *Br. J. Pharmac. Chemother.*, **34**, 291–310.

COOKE, A. R. (1967). Effect of pancreozymin preparations on gastric secretion. *Nature, Lond.*, **214**, 729.

GREGORY, R. A. (1958). Gastric secretory responses after portal venous ligation. *J. Physiol., Lond.*, **144**, 123–137.

GREGORY, R. A. (1966). Discussion. In *Gastrin*, ed. Grossman, M. I., p. 164. Berkeley and Los Angeles: University of California Press.

GREGORY, R. A. & TRACY, H. J. (1964). The constitution and properties of two gastrins extracted from hog antral mucosa. *Gut*, **5**, 103–117.

GREGORY, R. A. & TRACY, H. J. (1966). Studies on the chemistry of gastrins I and II. In *Gastrin*, ed. Grossman, M. I., pp. 9–26. Berkeley and Los Angeles: University of California Press.

GROSSMAN, M. I. (1966). After the Conference: review and perspective. In *Gastrin*, ed. Grossman, M. I., pp. 325–331. Berkeley and Los Angeles: University of California Press.

GROSSMAN, M. I. (1967). Some aspects of gastric secretion. *Gastroenterology*, **52**, 882–892.

HEITMANN, P., MJUNGREIS, A. & JANOWITZ, H. D. (1967). Effect of acetazolamide and cholecystokinin on the secretion of pepsin from histamine-stimulated Heidenhain pouches. *Gastroenterology*, **52**, 211–215.

HUNT, J. N. (1948). A method for estimating peptic activity in gastric contents. *Biochem. J.*, **42**, 104–109.

MANTEGAZZA, P. & PICCININI, F. (1962). Titolazione continua della secrezione acida dello stomaco di ratto ed influsso di stimoli diversi. *Arch. Sci. biol.*, **46**, 347–355.

MUTT, V. & JORPES, J. E. (1967). Isolation of aspartyl-phenylalanine amide from cholecystokinin–pancreozymin. *Biochem. Biophys. Res. Commun.*, **20**, 392–397.

THAYER, W. R., Jr. & MARTIN, H. F. (1967). Histidine decarboxylase inhibition and gastric secretion. *Am. J. dig. Dis.*, **12**, 1050–1061.

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