Br. J. Pharmac. (1969), 37, 185–197.

The action of caerulein on pancreatic secretion of the dog and biliary secretion of the dog and the rat

G. BERTACCINI, G. DE CARO, R. ENDEAN, V. ERSPAMER AND M. IMPICCIATORE

Institutes of Pharmacology, Universities of Rome and Parma, Italy, and Department of Zoology, University of Queensland, Brisbane, Australia

---

1. Caerulein displayed a potent stimulant action on pancreatic secretion in the dog. Threshold doses were 1–5 ng/kg by rapid intravenous injection, 0·25–1 ng/kg per min by intravenous infusion and 50–100 ng/kg by subcutaneous injection. There was a conspicuous increase not only in the volume flow of pancreatic juice but also in the output of solid constituents of the juice and of amylase. However, continuous stimulation of pancreatic secretion by intravenous infusion of caerulein resulted in a progressive reduction of the amylase concentration and still more of the dry residue content of pancreatic juice. The bicarbonate concentration in pancreatic juice produced by caerulein was similar to that observed in juice secreted following pancreozymin administration or following other stimuli causing the same rate of flow of pancreatic juice.

2. On a molar basis, caerulein was 25–30 times as active as human gastrin I and 3–6 times as active as cholecystokinin-pancreozymin. The presence in the molecule of caerulein of a sulphated tyrosyl residue at position 4 of the decapeptide (position 7 starting from the C-terminus) was a necessary prerequisite for the manifestation of the cholecystokinin-pancreozymin-like actions of caerulein. The C-terminal heptapeptide of caerulein retained much of the activity of the intact caerulein molecule.

3. At high dose levels (50–200 ng/kg in the dog, 1 μg/kg in the rat, by rapid intravenous injection) caerulein stimulated the flow of hepatic bile in the dog and the rat. The dry residue of the bile and the cholesterol concentration were appreciably greater in rats treated with caerulein than in control rats.

4. The activity spectrum of caerulein was identical with that of cholecystokinin-pancreozymin. This is readily explained on the basis of the almost identical structure of the C-terminal octapeptide of the two peptides.

5. Caerulein and some caerulein-like peptides may be considered as model peptides, capable of being substituted for cholecystokinin-pancreozymin in all the possible experimental and clinical uses of the duodenal hormone, with the important advantage that they are more easily available.

6. The question is raised whether cholecystokinin-pancreozymin obtained from the duodenum by acid extraction is the authentic hormone or rather a carrier polypeptide from which a smaller active peptide may be set free, when needed, into the circulation.

In preceding papers several actions of caerulein were described. These included the effect of the polypeptide on the systemic blood pressure of some experimental animals (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968a), its effect *in vivo* and *in vitro* on the smooth muscle of the gall bladder and of the gastro-intestinal tract (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968b) and its effect on gastric secretion in the dog and the rat (Bertaccini, Endean, Erspamer & Impicciatore, 1968). It would appear that caerulein is a versatile polypeptide, mimicking gastrin on one hand and cholecystokinin-pancreozymin on the other, as well as possessing some bradykinin-like activity.

The present communication describes the effects of caerulein on the secretory activity of the pancreas of the dog and on the biliary secretion of the dog and the rat. It has been found that caerulein potently stimulates the secretion of a pancreatic juice rich in dry residue and enzymes (amylase) and displays a moderate stimulant action on the flow of hepatic bile. Thus, it has become increasingly apparent that the cholecystokinin-pancreozymin-like activity of caerulein predominates over other types of activity exhibited by the polypeptide.

Methods

Pancreatic secretion

This was tested in thirty-five mongrel dogs of both sexes weighing 8–25 kg and anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously). In each case an incision, 10–15 cm in length, was made in the mid-line of the abdomen and the duodenum was lifted from the peritoneal cavity and placed on saline packs. The pancreas was gently separated from the duodenum along 2 cm and the duct of Wirsung was cannulated using a fine polythene tube. The abdomen was then closed with four or five interrupted sutures. The distal end of the polythene tube was connected to a photocell system drop timer (Basile, Milan), and the drops of secretion were recorded on a smoked drum. The drops of pancreatic juice were then collected and measured, at regular intervals, in small graduated test tubes. Determinations of the dry residue content of the juice were made by drying 0·5 ml. samples of the juice first for 6 hr by means of a heating lamp and then for 24 hr by means of an oven operating at about 100° C. The amylase activity of pancreatic juice was determined by the method of Bernfeld (1955) after diluting the samples × 4000. The activity was expressed in terms of units, each unit corresponding to 1 mg of maltose liberated by the enzyme from a known amount of soluble starch. The bicarbonate concentration in pancreatic juice was estimated according to Preshaw, Cooke & Grossman (1966) and expressed in m-equiv HCO_3/l.

Biliary secretion

Dog. Dogs were placed under pentobarbitone anaesthesia and their abdomens opened. In each case, the common bile duct was exposed and cannulated with a

polythene tube. Either the total bile or the pure hepatic bile (after exclusion of the gall bladder by tying the cystic duct) was collected and measured over a fixed period of time, before and after the administration of caerulein or other drugs. In a few experiments both hepatic and cystic ducts were cannulated simultaneously and the bile flowing from either duct was recorded by means of a photocell as described previously.

Rat. Rats weighing 250–350 g were deprived of food during the night preceding the experiment, but were allowed free access to water. After urethane anaesthesia the abdomen was opened and the common bile duct was cannulated with a small needle connected to a polythene tube. Bile was collected and measured at intervals of 1 hr. The mean of values obtained in the first 2 hr of collection was considered to be the basal value. Experiments were of 4–5 hr in duration.

Generally, one group of four rats was treated with caerulein while another group injected with physiological NaCl solution acted as a control group. A total of one hundred and twenty rats was employed. As well as the quantity of biliary secretion produced, the dry residue and the cholesterol content of bile were also determined. Cholesterol was determined by the colorimetric method of Sperry & Webb (1950).

Drugs

Pure natural and synthetic caerulein (molecular weight 1,352) and synthetic desulphated caerulein (molecular weight 1272) 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 (3000 Ivy dog units/mg, molecular weight 3883) and of pure secretin (4000 clinical units/mg, molecular weight 2917), to Dr. R. C. Sheppard, The Robert Robinson Laboratories, University of Liverpool, for samples of human gastrin I (molecular weight 2177) and to Dr. T. S. Morley, Imperial Chemical Industries, Macclesfield, England, for samples of pentagastrin, the gastrin-like pentapeptide I.C.I. 50,123 (molecular weight 769).

Other drugs used were: atropine sulphate, mepyramine maleate, hexamethonium bromide, DL-propranolol hydrochloride. The doses used are given as the weights of these salts.

Results

Action on pancreatic secretion

Dog

Effects of rapid intravenous injection. In anaesthetized dogs possessing acutely cannulated pancreatic ducts, the rapid intravenous injection of caerulein regularly elicited a conspicuous increase in the volume of pancreatic juice produced, as well as an increase in the dry residue of the juice and in the output of amylase. The action of the peptide was always evident, irrespective of whether dogs presented no spontaneous secretion or had a basal secretion of a few drops per hour. The threshold dose was 1–5 ng/kg and the magnitude of the response was proportional to the dose up to 1–2 μg/kg (Fig. 1). Flow of pancreatic juice began after a latent period of 30–90 sec and, for low and medium doses, it lasted for 5 to 15 min. When very

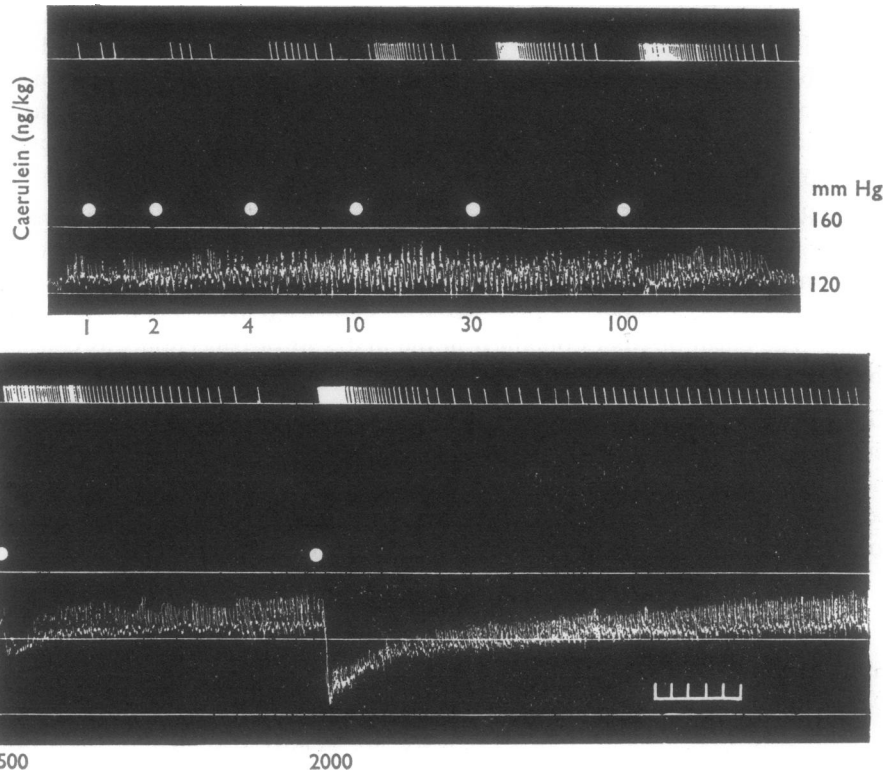

FIG. 1. Dog anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously). The effects of intravenous injections of caerulein, in ng/kg. Lower tracings, arterial blood pressure; upper tracings, pancreatic secretion. Each stroke represents one drop of pancreatic juice. Time marks, 1 min. The threshold dose of caerulein active on flow of pancreatic juice was 1 ng/kg. An increase in the dose, up to 2,000 ng/kg, caused an increase in intensity and duration of the pancreatic response. Blood pressure was affected only by doses of caerulein of 100 ng/kg and above.

TABLE 1. *Effects of intravenous infusion of caerulein* (20 *ng/kg per min in* 0·9% *NaCl solution,* 0·25 *ml./1 min*) *on volume of pancreatic juice, amylase secretion and dry residue of the juice in four dogs*

Observation period	Volume of pancreatic juice (ml.)	Total amylase output (u.)	Total dry residue (mg)
0–1 hr	4·25±1·59	884±93	322·2±23
		(208±19)	(75·8±6·1)
1–2 hr	4·62±1·0	979·5±37	307·2±36
		(212±7·7)	(66·5±7·3)
2–3 hr	6·92±2·2	1370·2±45	248·4±49
		(198±6)	(35·9±7·1)
3–4 hr	6·07±1·3	1171·5±46	171·2±39
		(193±6)	(28·2±5·4)
4–5 hr	5·2 ±1·0	946·4±41	129·5±23
		(182±6·7)	(24·9±4)
5–6 hr	5·9±1·9	991·2±37	151·6±37
		(168±5·6)	(25·7±5·9)
6–7 hr	5·0±1·3	760±65	99±22
		(152±14)	(19·8±4·7)
7–8 hr	4·7±0·9	635·1±92	80·1±31
		(136±24)	(19·3±6·3)
8–9 hr	5·1±1·0	634·9±49	47·6
		(124±31)	9·3
9–10 hr	4·8±1·1	586·7±99	42·9
		(123±31)	9·0

The values given are the means ± S.E. In parentheses are values per ml. of pancreatic juice.

large doses of caerulein (10 μg/kg or more) were injected pancreatic stimulation often persisted for hours. Tachyphylaxis was absent and the constancy and repeatability of the response was satisfactory during a period of at least 2 hr.

The effect of caerulein on pancreatic secretion was largely independent of the level of systemic blood pressure. Even at levels as low as 30–40 mm Hg, caused by bleeding or seen towards the end of a prolonged experiment as a consequence of progressive deterioration of the animal, response to caerulein was only little affected.

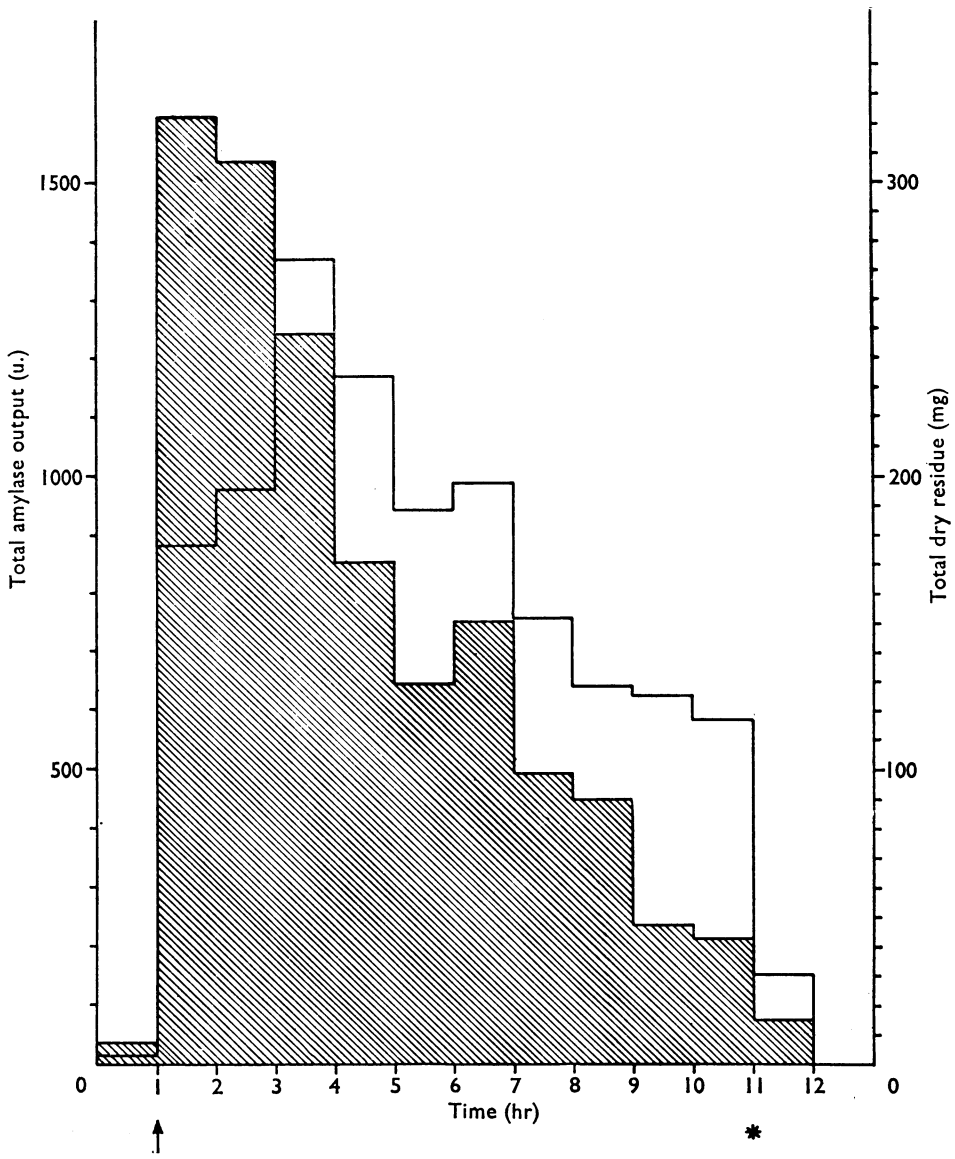

FIG. 2. Pancreatic secretion in four dogs anaesthetized with pentobarbitone. Caerulein was infused intravenously at a rate of 20 ng/kg min. White columns represent the average total output of amylase, in units, hatched columns the average total dry residue of the pancreatic juice, in mg. ↑, Infusion started ; *, infusion stopped. The amylase output increased at first and then declined ; the dry residue of the pancreatic juice, on the contrary, showed from the beginning a continuous, progressive decrease.

Pancreatic juice produced under the influence of caerulein yielded a conspicuous dry residue and was rich in amylase.

Effects of intravenous infusion. The threshold dose of caerulein, given by intravenous infusion, capable of producing an appreciable increase of pancreatic secretion ranged from 0·25 to 1 ng/kg per min. The response was proportional to the dose up to 100 times the threshold dose. The infusion was continued up to 30 hr.

The results of an experiment prolonged for 10 hr at an infusion rate of 20 ng/kg per min of caerulein are shown in Table 1 and illustrated in Fig. 2. Basal values were difficult to determine owing to the paucity of secretion. Those presented in Table 1 are average values.

It may be seen that whereas the volume of pancreatic juice remained approximately constant throughout the experiment, the dry residue per ml. showed a prompt and sharp reduction. Indeed, the value obtained during the third hour was only 45–50%, and that obtained during the ninth hour as little as 12% of the value obtained during the first hour. Reduction of amylase activity was considerably more gradual, as much as 60% of the activity present in the juice produced during the first hour being retained in the juice secreted at the end of the experiment.

In another dog, 10 ng/kg per min of caerulein in 0·25 ml./min of physiological saline were infused for 19 hr. Flow rate was again constant during the experiment (2·6–3·3 ml./hr) and the total volume of pancreatic juice was 55 ml. The dry residue was 75–84 mg/ml. during the first 3 hr, then it declined rapidly, attaining 19 mg/ml. after 16 hr. Amylase activity remained virtually unchanged for 10 hr (200–220 u./ml.) then it decreased to 136 u./ml. at the end of the experiment.

In a third typical experiment infusion of 1·5 ng/kg per min of caerulein for two hours produced approximately 1 ml. of pancreatic juice with a dry residue of 78 mg/ml.

In two experiments also the bicarbonate concentration was estimated following an intravenous infusion of 20 ng/kg per min of caerulein. In the first experiment in which the rate of secretion of pancreatic juice was 2–4 ml./hr, the bicarbonate concentration ranged from 58 to 87 m-equiv./l. throughout the 11 hr of infusion; in the second experiment with a secretion of fluid of 3–5 ml./hr the bicarbonate concentration was 60 to 90 m-equiv./l. throughout the 29 hr of infusion.

These results are similar to those obtained by Henrikson (1968) with pancreozymin-cholecystokinin and are in accordance with those reported by Birnbaum & Hollander (1965) who found that, depending on the rate of secretion, the bicarbonate concentration in pure pancreatic juice varied widely, from 20 to 150 m-equiv./l. or even higher.

Effects of subcutaneous injection. The threshold dose of caerulein by subcutaneous route was of the order of 50–100 ng/kg. The response was proportional to the dose up to at least 1 μg/kg.

With 100 ng/kg the response appeared after a latent period of 2–4 min and lasted 30–60 min; with 1 μg/kg the latent period was of 1–2 min and the duration of action of 3 to 5 hr, with a maximum effect during the first two hours.

It may be noted that the threshold subcutaneous dose of caerulein influencing the pancreatic secretion was 30–40 times less than the subcutaneous dose of the polypeptide capable of producing emesis in conscious animals (Bertaccini *et al.*, 1968b).

These data can be probably transferred to human beings. In fact, preliminary clinical experiments have demonstrated that subcutaneous doses of caerulein are well tolerated up to 0·5–1 μg/kg, and that pancreatic secretion is stimulated by subcutaneous doses as low as 25–50 ng/kg.

In the present study only a limited number of experiments was carried out on the effects of the subcutaneous administration of caerulein on the pancreatic secretion. An extension of these studies seems to be opportune, as Vagne & Grossman (1968) have shown that subcutaneously injected secretin stimulated the secretion of more pancreatic juice than the same dose of polypeptide injected intravenously. Perhaps the same occurs with caerulein.

Potency relative to secretin, pancreozymin-cholecystokinin, human gastrin I and other polypeptides. The potency of caerulein was compared with that of secretin in four dogs, with that of cholecystokinin-pancreozymin in three dogs and with that of human gastrin I in four dogs.

With regard to the volume of pancreatic juice it was found that the potency of caerulein relative to that of secretin varied with the dose. A total dose of 100 ng of caerulein given by rapid intravenous injection was equivalent to approximately 0·1–0·3 clinical units of secretin and a dose of 500 ng of caerulein to 0·2–0·4 clinical units of secretin. This would indicate that in different experimental conditions

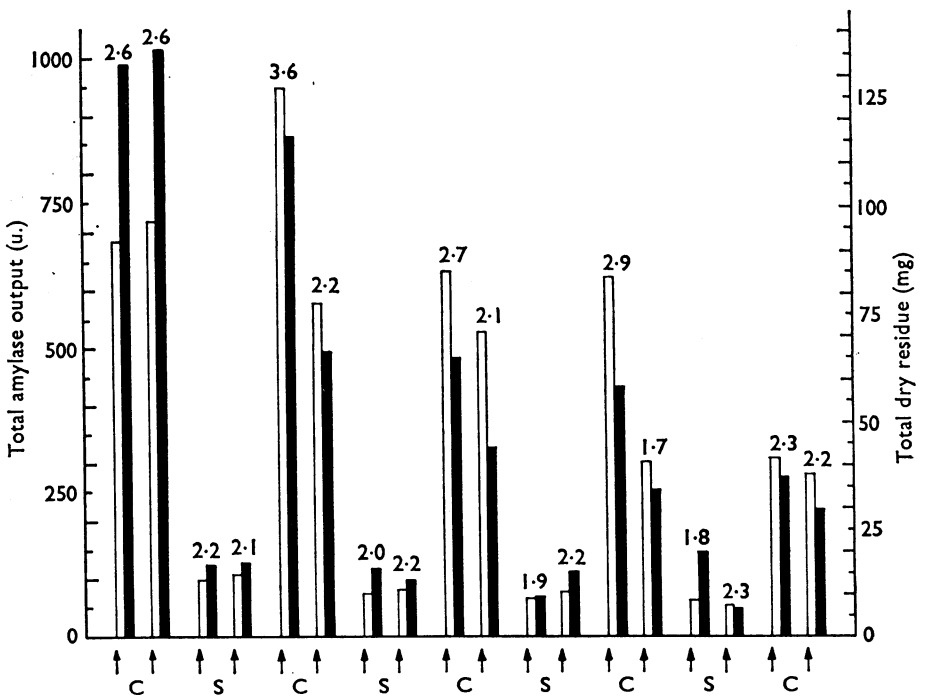

FIG. 3. Pancreatic secretion of a dog with acutely cannulated pancreatic duct. Pentobarbitone anaesthesia. Total amylase output, in units (white columns), and total dry residue of the pancreatic juice, in mg (black columns) following repeated intravenous injections of 50 ng/kg caerulein (C) and 0·075 u./kg secretin (S) given at intervals of 15 min. Figures above the columns indicate the total volume of pancreatic fluid, in ml. The considerably less effect of secretin on amylase output and dry residue output is obvious, as is the decreasing effect of successive doses of caerulein.

1 μg of caerulein may be equivalent to 0·4–3 clinical units of secretin—that is, to 0·1–0·8 μg of pure polypeptide. Thus, on a molar basis, secretin was 2·5 to 20 times more potent than caerulein.

Figure 3 shows the response of the pancreas to repeated intravenous injections of 50 ng/kg of caerulein alternated with injections of 0·075 u./kg of secretin. Injections were given at intervals of 10–15 min. It may be seen that whereas the volume of pancreatic juice remained constant for both polypeptides throughout the experiment, the dry residue and the amylase activity of the juice secreted in response to caerulein showed a progressive reduction. This was less appreciable in the case of secretin. At the beginning of the experiment the juice produced by caerulein was 7–8 times richer in dry residue and in amylase activity than the juice produced by secretin ; at the end of the experiment the difference was less marked.

One μg of caerulein produced a pancreatic response of the same intensity as that caused by 25–50 Ivy dog units of cholecystokinin-pancreozymin (=8-16 μg of pure polypeptide) and the pancreatic juice showed, per ml., a similar dry residue and a similar amylase activity. On a molar basis, caerulein was 3 to 6 times more potent than cholecystokinin-pancreozymin (Fig. 4).

Human gastrin I possessed approximately 2% of the activity of caerulein. For example, 2·5 μg/kg of human gastrin I given by rapid intravenous injection produced 2 ml. of a pancreatic juice having a dry residue of 83 mg/ml. and an amylase activity of 180 u./ml. In its turn, 50 ng/kg of caerulein produced 2·2 ml. of a pancreatic juice having a dry residue of 90 mg/ml. and an amylase activity of 216 u./ml.

If the activity of caerulein on volume output of pancreatic juice equals 100, the activity of other peptides related to caerulein was as follows on a molar basis:

Pentagastrin (ICI 50,123)	1
Desulphated caerulein	2–4
Tyr (SO_3H)-Gly-Trp-Met-Asp-Phe-NH_2 (C-terminal hexapeptide of gastrin II)	5–8
Tyr-Gly-Trp-Met-Asp-Phe-NH_2 (C-terminal hexapeptide of gastrin I)	1–2
Tyr (SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 (C-terminal heptapeptide of caerulein)	45–60
Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2 C-terminal heptapeptide of desulphated caerulein)	0·9–2
Tyr (SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (C-terminal heptapeptide of cholecystokinin-pancreozymin)	50–55
Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2 (C-terminal heptapeptide of desulphated cholecystokinin-pancreozymin)	0·7–2
Physalaemin	1

See also Anastasi, Bernardi, Bertaccini, Bosisio, De Castiglione, Erspamer, Goffredo & Impicciatore (1968).

Effects of autonomic blocking drugs. Atropine and propranolol given intravenously in doses of 0·1 mg/kg and 1 mg/kg, respectively, did not affect the action of caerulein on pancreatic secretion. However, hexamethonium (0·5–2·5 mg/kg,

intravenously) reduced the action of 5–20 ng/kg of caerulein, given intravenously, by 50%. Mepyramine (10 mg/kg, intravenously) reduced the effect of the same dose of caerulein by 10–20%.

Action on bile flow

Dog

When administered by rapid intravenous injection, the threshold dose of caerulein producing an increase in the flow of bile from the cannulated common bile duct or from the cannulated cystic duct was of the order of 1–3 ng/kg. For example, with 5 ng/kg a 50–100% increase of bile flow lasting for 5–10 min was observed, with

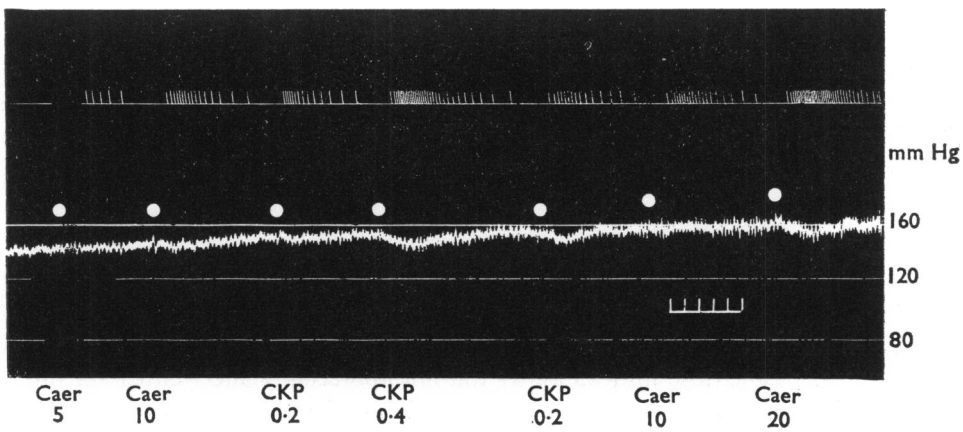

FIG. 4. Dog anaesthetized with sodium pentobarbitone. Upper tracing, pancreatic secretion; lower tracing, arterial blood pressure. Time marks, 1 min. The effects on pancreatic secretion of different doses of caerulein (Caer, ng/kg) were compared with those of different doses of cholecystokinin-pancreozymin (CKP, Ivy dog units), both injected intravenously. In this experiment 0·2 u. of cholecystokinin-pancreozymin was equivalent to approximately 8 ng caerulein.

TABLE 2. *Effects of intravenous injections of caerulein (single injections, in twenty-two rats, of 1 μg/kg of caerulein at 0 min and respectively four injections, in ten rats, of the same dose of caerulein at 0', 60, 120 and 180 min) on volume, cholesterol concentration and dry residue of bile*

Observation periods	Volume of bile (ml./kg)	Cholesterol concentration (μg/ml.)	Dry residue (mg/ml.)
Pre-injection period			
60–0 min controls	2·24±0·15	56·0±8·8	29·8±2·9
caerulein×1	2·55±0·04	52·0±9·8	31·5±3·5
caerulein×4	2·58±0·07	50·5±9·5	22·2±2·9
Post-injection period			
0–60 min controls	2·17±0·16	51·7±7·0	25·7±2·9
caerulein×1	2·94±0·05	58·0±6·3	33·3±2·8
caerulein×4	3·04±0·11	50·0±5·5	21·5±2·5
60–120 min controls	2·12±0·16	49·0±7·8	22·8±3·0
caerulein×1	2·85±0·05	49·8±3·3	27·5±3·3
caerulein×4	3·12±0·11	54·2±7·3	23·3±3·0
120–180 min controls	2·04±0·17	45·1±6·6	21·1±2·5
caerulein×1	2·57±0·06	50·7±3·5	25·9±3·0
caerulein×4	3·02±0·18	48·0±4·5	20·8±3·0
180–240 min controls	2·08±0·19	34·9±3·9	19·4±3·1
caerulein×1	2·56±0·07	48·7±4·7	27·7±0·4
caerulein×4	3·15±0·20	54·2±6·8	22·5

Forty rats served as controls. After the pre-injection period all the animals received intravenously, at hourly intervals, 0·5 ml. of a 0·9% NaCl solution, with or without caerulein. The values given are the means ± S.E.

10 ng/kg a 300–600% increase lasting for 10–15 min. Basal values of bile flow were 0·12–0·17 ml./min. However, if the cystic duct was tied before the injection of caerulein the intravenous dose of the polypeptide required to increase the flow of bile rose to 50–200 ng/kg. Increase in flow was generally preceded by a transient decrease coinciding with, and possibly caused by, the fall of systemic blood pressure. Response was satisfactorily proportional to the dose up to 3 μg/kg.

In one experiment in which the cystic duct was tied and bile collected from the common duct, 200 ng/kg of caerulein produced first a reduction of bile flow (from 0·14 ml./min to 0·1 ml./min and lasting for 5 min) and then an increase of bile flow up to 0·2 ml./min and lasting for 25 min. Similarly, 2 μg/kg of caerulein produced a reduction of bile flow from 0·15 to 0·1 ml./min during the first 10 min, followed by an increase in flow up to a maximum of 0·28 ml./min which occurred between 30 and 40 min after injection. Thereafter the flow slowly decreased and the basal level was reached 60 min after injection.

By the subcutaneous route the threshold dose of caerulein producing an increase of bile flow from the common bile duct with non-ligated cystic duct was of the order of 100 ng/kg.

Rat

The threshold intravenous dose causing a significant increase in the flow of hepatic bile was 1 μg/kg. This dose was 40 times larger than the threshold dose (25 ng/kg) producing an increase of acid output in the perfused stomach preparation. Increasing the dose up to 3 μg/kg did not appreciably affect either the duration or the intensity of the response. Larger doses (5 μg/kg or more) sometimes caused death. However, duration of the response could be prolonged by repeated administration of caerulein, for example four doses given at hourly intervals.

The percentage increase of bile flow over the basal values which was produced by caerulein was not great, a maximum of 15–17% being attained after a single injection and a maximum of 20–22% after repeated injections. These values might be somewhat raised in view of the small but definite reduction of bile flow shown by control rats during the course of an experiment.

In rats treated with caerulein values for the dry residue and cholesterol contents of bile remained virtually unchanged during the whole period of action of the polypeptide. However, these values declined progressively in control rats. For example, the dry residue content of bile from control rats dropped within 4 hr from $29{\cdot}8 \pm 2{\cdot}9$ to $19{\cdot}4 \pm 3{\cdot}1$ mg/ml. and the cholesterol content from $56 \pm 8{\cdot}8$ to $35 \pm 3{\cdot}9$ μg/ml. Hence, the total dry residue and total cholesterol content of bile was consistently greater in rats treated with caerulein than in control rats.

The above experimental data are presented in greater detail in Table 2.

Human gastrin I given intravenously in three doses of 25 μg/kg repeated at hourly intervals produced an effect which was less intense than that caused by 1 μg/kg of caerulein. Desulphated caerulein was inactive even at intravenous doses of 20 μg/kg.

Discussion

The results reported in this and in previous papers reveal that caerulein displays a number of actions on vascular and extravascular smooth muscle and on external

secretions of the gut: (*a*) a moderate hypotensive effect in the dog and rabbit and biphasic or erratic responses in other animal species; (*b*) a potent stimulant action on the gall bladder *in vivo* and *in vitro* and on the gastrointestinal musculature *in vivo*; (*c*) a relaxing action on the sphincter of Oddi; (*d*) a conspicuous stimulant action on gastric secretion in the dog, the rat and the frog; (*e*) a potent stimulant action on pancreatic secretion and (*f*) a moderate stimulant action on the flow of hepatic bile.

To these effects should be added a stimulant action on Brunner's gland secretion in dogs and cats, as demonstrated by Stening & Grossman (1968).

It has been repeatedly pointed out that caerulein has many actions in common with the two hormones of the gut gastrin and cholecystokinin-pancreozymin. From our experimental results it appears now that the activity spectrum of caerulein is consistently different from that of gastrin, but is very similar to, or virtually identical with, that of cholecystokinin-pancreozymin. However, this does not exclude the possibility that there is a partial overlapping of the caerulein spectrum with that of gastrin.

Gastrin is a potent stimulant of gastric secretion, a moderate stimulant of pancreatic secretion and a still more moderate stimulant of the motility of the gall bladder and small intestine. In their turn, both cholecystokinin-pancreozymin and caerulein are formidable stimulants of the musculature of the gall bladder and the small intestine, powerful stimulants of pancreatic secretion and relatively moderate stimulants of gastric secretion. By intravenous infusion, threshold doses for the cholecystokinin-pancreozymin-like activities of caerulein are of the order of 0·25–0·5 ng/kg per min while threshold doses for the gastrin-like activity are of the order of 4–10 ng/kg per min.

It has been shown in a preceding paper (Bertaccini *et al.*, 1968b) that although caerulein possessed approximately twice the activity of human gastrin I on the denervated gastric pouch of the dog, it had an action considerably more potent than gastrin on the perfused rat stomach preparation or on the isolated gastric mucosa of the frog. It was suggested that rats and amphibians may possess specific gastrins, differing in their amino acid composition and/or sequence from known gastrins.

Like cholecystokinin-pancreozymin, caerulein differs sharply from secretin, which possesses a powerful action on the volume flow and bicarbonate output of pancreatic juice, but a poor action on enzyme output.

The complete or partial structural formulae of the three gastrointestinal polypeptides and of caerulein are listed below. It is evident that caerulein, cholecystokinin-pancreozymin and gastrin show a marked resemblance in chemical structure and that the chemical structure of each of these three polypeptides differs radically from that of secretin.

Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2

Caerulein

Pyr-Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr(SO_3H)-Gly-Trp-Met-Asp-Phe-NH_2

Human gastrin II

Ala_1, Arg_3, Asp_2, Glu_1, Gly_1, His_1, Ile_2, Leu_2, Lys_2, Met_1, Pro_2, Ser_5, Val_1, -Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

Porcine cholecystokinin-pancreozymin

His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-Leu-Gln-Arg-Leu-Gln-Gly-Leu-Val-NH_2
Porcine secretin

Whereas the characteristic spectrum of activity of gastrin seems to depend largely on the C-terminal pentapeptide, the spectrum of activity of both caerulein and cholecystokinin-pancreozymin depends on the C-terminal heptapeptide, a necessary prerequisite for activity being the presence at the N-terminus of the heptapeptide of an *O*-sulphated tyrosyl residue.

Desulphation of the tyrosyl residue always produced a drastic reduction of activity while a shift of the tyrosyl residue towards the C-terminus, as in the case of gastrin, produced a complete abolition of the cholecystokinetic activity and caused a loss of 85–92% of the stimulant action on the pancreas.

On a molar basis, the C-terminal heptapeptide of caerulein retained 20 to 60% of the total spectrum of activity of caerulein while the C-terminal heptapeptide of cholecystokinin-pancreozymin possessed 50 to 80% of the activity of caerulein, being several times more potent that cholecystokinin-pancreozymin itself.

These observations raise the question of whether cholecystokinin-pancreozymin is the true hormone released by the duodenal mucosa following passage of the chyme from the stomach to the intestine. Cholecystokinin-pancreozymin with its thirty-three amino acid residues and a molecular weight of 3900 could be simply a carrier polypeptide from which a smaller hepta- or octapeptide with a molecular weight of 1,028 or 1,143 is split off and liberated into the circulation when flow of bile and pancreatic juice as well as intestinal movements are necessary for digestive processes.

It is probable that only immunological or radioimmunological methods of assay will facilitate the solution of the above problem since the amount of hormone circulating under normal conditions in the blood seems too small to be traced by other methods.

The action of caerulein on flow of hepatic bile is very moderate, as is that of cholecystokinin-pancreozymin, and it is probable that this effect has no physiological significance. Moreover, it is debatable whether the mechanisms responsible for the action of caerulein on flow of hepatic bile are located in the intrahepatic bile ducts or ductules rather than in the hepatocytes. The predominant role of bile ducts in secretin choleresis in the dog has, for example, been emphasized by Wheeler & Mancusi-Ungaro (1966), and Jonson, Svartengren & Thulin (1967) point out that the effect of cholecystokinin-pancreozymin preparations on hepatic bile output is largely related to the motor and secretory activity of the stomach and the duodenum during the experiment.

From this paper and the preceding ones it may be concluded that caerulein and some caerulein-like hepta- and octapeptides have all the prerequisites to be considered excellent substitutes for cholecystokinin-pancreozymin in all the clinical and experimental uses of the duodenal hormone.

To mention only a couple of possibilities in the field of pancreatic pathology, caerulein seems to deserve clinical trial on the one side in digestive disorders attributable to defect of pancreatic secretion and on the other side in pancreatic scintiscanning. It is in fact possible that a prolonged infusion of caerulein causes

also in human patients, as in dogs, an exhaustion of enzymes in the pancreatic acini and thereafter their accelerated re-synthesis, starting from amino acids which may be labelled.

A promising way for administering caerulein is nasal insufflation, which has already proved to be effective in producing a contraction of the gall bladder (Agosti & Bertaccini, 1969).

This work was supported by grants from the Consiglio Nazionale delle Ricerche, Rome.

REFERENCES

AGOSTI, A. & BERTACCINI, G. (1969). Nasal absorption of caerulein. *Lancet*, **1**, 580–581.

ANASTASI, A., BERNARDI, L., BERTACCINI, G., BOSISIO, G., DE CASTIGLIONE, R., ERSPAMER, V., GOFFREDO, O. & IMPICCIATORE, M. (1968). Synthetic peptides related to caerulein. Note 1. *Experientia*, **24**, 771–773.

BERNFELD, P. (1955). Amylase α and β. In: *Methods in Enzymology*, vol. 1, ed. Colowick, S. P. & Kaplan, N. O., p. 149. New York: Academic Press.

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 action of caerulein on the smooth muscles of the gastrointestinal tract and the gall bladder. *Br. J. Pharmac.*, **34**, 291–310.

BERTACCINI, G., ENDEAN, R., ERSPAMER, V. & IMPICCIATORE, M. (1968). The actions of caerulein on gastric secretion of the dog and the rat. *Br. J. Pharmac.*, **34**, 311–329.

BIRNBAUM, D. & HOLLANDER, F. (1965). Relation between bicarbonate concentration and rate of canine pancreatic secretion. *Am. J. Physiol.*, **209**, 966–972.

HENRIKSEN, F. W. (1968). Effect of pancreozymin on the canine pancreatic secretion of fluid and bicarbonate. *Scand. J. Gastroenterol.*, **3**, 637–640.

JONSON, G., SVARTENGREN, G. & THULIN, L. (1967). The effect of cholecystokinin-pancreozymin preparations on hepatic bile output in fasting and digesting dogs. *Acta physiol. scand.*, **69**, 23–28.

PRESHAW, R. M., COOKE, A. R. & GROSSMAN, M. I. (1966). Quantitative aspects of response of canine pancreas to duodenal acidification. *Am. J. Physiol.*, **210**, 629–634.

SPERRY, W. M. & WEBB, M. (1950). A revision of the Schönheimer-Sperry method for cholesterol determination. *J. biol. Chem.*, **187**, 97–103.

STENING, G. F. & GROSSMAN, M. I. (1968). Stimulating Brunner's gland secretion. *Lancet*, **1**, 1435.

VAGNE, M. & GROSSMAN, M. I. (1968). Comparison of intravenous and subcutaneous secretin in dogs. *Gastroenterology*, **54**, 907–912.

WHEELER, O. H. & MANCUSI-UNGARO, P. L. (1966). Role of bile ducts during secretin choleresis in dogs. *Am. J. Physiol.*, **210**, 1153–1159.

(*Received February* 17, 1969)