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The actions of caerulein on the smooth muscle of the gastrointestinal tract and the gall bladder

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

Institute of Pharmacology, University of Parma, Parma, Italy, and the Department of Zoology, University of Queensland, Brisbane, Australia

1. In the intact conscious dog, caerulein causes emesis and evacuation of the bowel. The mean effective dose by the intravenous route is 0.4–0.5 μg/kg, and by the subcutaneous route 3–4 μg/kg.

2. The gall bladder *in situ* or as an isolated preparation is highly sensitive to caerulein. A few ng/kg injected intravenously are sufficient to stimulate the gall bladder *in situ* and less than 1 ng/kg per min is effective when infused intravenously. The isolated gall bladder is contracted by caerulein in concentrations as low as 0.03–2 ng/ml. Krebs solution. There is no tachyphylaxis but, generally, a good dose–response relationship. Hence the gall bladder, especially that of the guinea-pig, appears to be very suitable for the bioassay of caerulein and related peptides.

3. *In situ*, the musculature of the gastrointestinal tract is also highly sensitive to caerulein. Doses as low as 1–5 ng/kg, administered intravenously, have a spasmogenic action on jejunal loops of the dog, and slightly larger doses contract the small intestine of the cat. The stomach and the large intestine seem to be somewhat less sensitive to the polypeptide. Caerulein has a considerable spasmogenic action on the rat pylorus but relaxes the sphincter of Oddi of the guinea-pig.

4. Isolated preparations of the gastrointestinal tract are relatively insensitive to caerulein and tachyphylaxis occurs readily.

5. Blockade with atropine produces different effects in different intestinal segments and in different animal species. The spasmogenic action of caerulein on the gall bladder is atropine-resistant.

6. The effects of caerulein are similar to those of cholecystokinin-pancreozymin in the organs tested *in situ* or as isolated preparations. Caerulein, however, is always more potent than cholecystokinin-pancreozymin, even on a molar basis. Compared with caerulein, human gastrin I has negligible activity.

7. The possible use of caerulein in cholecystography is discussed.

In a preceding paper the actions of caerulein on the systemic arterial blood pressure of some common laboratory animals were reported (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968). In this paper the effects of caerulein

in vivo on the motility of the gastrointestinal tract and the gall bladder are described, and its effects on a number of isolated preparations of extravascular smooth muscle.

It will be seen that caerulein has a powerful action on the musculature of the gall bladder and the gut and closely mimics the activity of the duodenal hormone, cholecystokinin-pancreozymin. This is not surprising when one considers that the molecules of the two polypeptides possess the same C-terminal pentapeptide (Mutt & Jorpes, 1967) and probably have in common other amino-acid residues, as for instance the tyrosyl *O*-sulphate residue.

The results reported here are intended to provide a basis for a thorough study of the actions of caerulein *in vivo* on the musculature of the entire gastrointestinal tract in different vertebrate species. Further, basic data from a trial of the polypeptide in man, especially in cholecystography, are given.

Methods

Intact conscious animal

Caerulein in physiological saline (0.9% NaCl solution) was injected subcutaneously or intravenously into dogs, pigeons and rats in order to study the effects of the polypeptide on gastrointestinal motility, as indicated by vomiting and bowel evacuation. Moreover, stomach motility following caerulein administration was recorded in conscious dogs with denervated fundic pouches (Bertaccini, Endean, Erspamer & Impicciatore, 1968). A balloon was introduced into the pouch and after being filled with water it was connected by a rigid plastic tube to a water manometer or to a Marey tambour writing on a smoked drum.

Intestinal loops in situ

Midline incisions of different lengths were made in the abdominal walls of dogs anaesthetized with pentobarbitone. A jejunal loop, less frequently a loop from other intestinal segments, was lifted into the wound and a small longitudinal incision was made along the free margin of the loop. A thin-walled rubber finger stall, filled with water and with a short glass tube tied into it, was gently introduced into the lumen, cranially to the incision, and the intestinal wall was fixed to the glass tube by a ligature. The loop was then replaced in the abdomen ; the edges of the wound were held together by forceps and the protruding glass tube was connected by a rubber tube, partly or totally filled with water, to a Marey tambour or a water manometer writing on a smoked drum. In order to ensure that the balloon was well stretched, a pressure of 20–30 cm H_2O was maintained in it.

With the same technique, a small number of experiments were carried out in the cat.

Gall bladder in situ

The gall bladder of the guinea-pig was prepared exactly as described by Ljungberg (1964). Guinea-pigs weighing 400–600 g were anaesthetized with urethane (2 g/kg, subcutaneously) and injections were made via a thin polythene tube inserted into the jugular vein. A midline incision about 2 cm long was made in the superior part of the abdominal wall. The gall bladder, gently separated from the adhering liver tissue, was lifted cautiously into the wound and a thin thread sewn to its free

pole. The thread ran in a caudal direction at about 45° to the horizontal plane, over a freely movable pulley, and was connected to a low-friction frontal lever giving approximately 5–7-fold magnification of the gall bladder movements.

In the dog anaesthetized with pentobarbitone, the cystic duct was ligated and a thin-walled plastic tube tied into the fundus of the gall bladder. The tube was partly filled with saline and then connected to a volume recorder or a Marey tambour. Alternatively, the common bile duct was cannulated after ligature of the hepatic duct, and bile flow was recorded by means of a drop counter.

Rat pylorus

The method of Harichaux & Thouvenot (1963) was followed, with some modifications. Rats weighing 200–250 g were fasted for 48 hr but allowed free access to water. Under urethane anaesthesia (1 g/kg, intraperitoneally) a longitudinal incision 3–4 cm long was made in the abdominal wall. A polythene tube was introduced through the mouth into the stomach and fixed by a ligature round the cardia. Another tube was placed in the duodenum at about 1 cm from the pylorus, fixed and connected to a drop counter. The stomach was perfused with warm physiological saline at a constant pressure of 4 to 8 cm H_2O. Similar experiments were carried out in which the stomach was perfused by a pump at a rate of 1 ml./min.

Guinea-pig sphincter of Oddi

Guinea-pigs weighing 500–700 g were anaesthetized with urethane (1.2 g/kg, subcutaneously). A midline abdominal incision was made and the gall bladder cannulated as near as possible to the origin of the cystic duct. A second tube was introduced into the duodenum just caudally to the opening of the common bile duct, tied in and connected to a drop counter. The system was perfused from the gall bladder with warm physiological saline at a pressure of 1 to 3 cm H_2O. Spasm of the sphincter of Oddi was elicited with morphine hydrochloride (50–200 μg/kg, intravenously).

Isolated smooth muscle preparations

The action of caerulein was assayed on the following smooth muscle preparations: the gall bladders of guinea-pig, rabbit, cat, dog, sheep and cow ; guinea-pig ileum, duodenum and large intestine ; rat stomach, duodenum, ascending colon and descending colon ; hamster stomach, duodenum, terminal ileum and large intestine ; rabbit duodenum and large intestine ; cat duodenum and large intestine ; dog duodenum and large intestine ; fowl rectal caecum and large intestine ; toad stomach and large intestine ; rat and guinea-pig uterus ; guinea-pig seminal vesicles.

The whole gall bladder of the guinea-pig was suspended in Krebs solution at 32° C gassed with air and the whole gall bladders of the rabbit and the cat were suspended in Tyrode solution at 32° C and 37° C, similarly gassed with air ; longitudinal and circular strips of the gall bladder of the dog were suspended in Tyrode solution at 32° C and 37° C ; longitudinal strips of the gall bladders of the sheep and the cow were suspended in Tyrode solution at 32° C. The volume of the bath was 5–15 ml. The contact time of the drugs with the bladder muscle was 3–50 min ; a second dose was given only after complete relaxation had occurred. Thus the dose cycle varied between 10 and 80 min.

The hamster terminal ileum and large intestine were mounted in Tyrode solution at 32°–35° C, the rat stomach and the hamster stomach and duodenum in a modified Krebs solution at 37° C gassed with air. This solution had the following composition (g/l.): NaCl 6.9, KCl 0.39, $CaCl_2$ 0.5, NaH_2PO_4 0.16, $MgSO_4$ 0.29, $NaHCO_3$ 2.1, glucose 1 (Ferreira & Vane, 1967).

The other smooth muscle preparations were prepared exactly as described in a preceding communication and the bath fluids had the same composition (Erspamer & Falconieri Erspamer, 1962).

Drugs

Both pure natural and synthetic caerulein prepared at the Farmitalia Laboratories for Basic Research, Milan, were used throughout the present experiments. Synthetic desulphated caerulein, synthetic eledoisin and synthetic physalaemin were prepared at the same laboratories. We are greatly indebted to Professor E. Jorpes, Kemiska Institutionen II, Karolinska Institutet, Stockholm, for samples of pure cholecystokinin-pancreozymin (1,500–3,000 Ivy dog units/mg), to Dr. R. C. Sheppard, The Robert Robinson Laboratories, University of Liverpool, for samples of human gastrin I and des-glutamyl human gastrin I, to Dr. J. S. Morley, Imperial Chemical Industries, Macclesfield, England, for samples of the gastrin-like pentapeptide I.C.I. 50,123 (pentagastrin), and to Messrs. Sandoz, Basel, for samples of synthetic bradykinin. Other drugs used were N,N-dibenzyl-β-chloroethylamine (Dibenamine), hexamethonium bromide, mepyramine maleate, atropine sulphate, morphine hydrochloride, acetylcholine chloride, histamine dihydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), (−)-adrenaline base. The doses are given as the weights of these salts and bases.

Results

General effects in unanaesthetized animals

Dog. Experiments were carried out on forty-three mongrel dogs weighing 8–25 kg. In order to avoid the establishment of conditioned reflexes, only one or, at most, two experiments were performed on any one animal.

Caerulein (1 μg/kg) given intravenously to three dogs produced in each vomiting and discharge of formed stools within 30–60 sec of injection. Episodes of violent emesis accompanied by evacuation of diarrhoeic stools and profound prostration recurred several times in the following 5 min. Recovery took 20–30 min. A dose of 0.5 μg/kg produced vomiting (1–4 episodes) and diarrhoea (several episodes) in six of nine dogs used. In addition, one animal which did not vomit had diarrhoea. As in the preceding group, recovery took 15–30 min. When 0.2 μg/kg was injected, vomiting and diarrhoea occurred in only one of six dogs.

Subcutaneous injection of caerulein 5 μg/kg into six dogs was followed, after 3–5 min, by prostration and then by vomiting which was frequently heralded by violent retching. Vomiting recurred 2–5 times, at intervals of 5–10 min, until 30–60 min after the injection. In four of the six animals used, vomiting was accompanied by diarrhoea and tenesmus. Recovery was complete after 2–4 hr. Subcutaneous injection of caerulein 2 μg/kg produced vomiting, prostration and discharge of formed stools in two of the six dogs used. A smaller dose (1 μg/kg) produced depression in ten of the fourteen dogs tested but vomiting or diarrhoea,

or both, in only three dogs. With 0.5 μg/kg none of six dogs showed vomiting or diarrhoea, the only effect being a mild depression. Finally, with 0.2 μg/kg no appreciable signs of any action of caerulein were apparent in four dogs.

Seven dogs were each given atropine 0.2 mg/kg subcutaneously and, after 30 min, caerulein 5 μg/kg by the same route. All the animals showed depression, followed 20–75 min later by retching and repeated vomiting but not diarrhoea and only two animals discharged formed stools.

From the above experiments it appeared that the doses of caerulein which produced vomiting in the majority of dogs were 0.4–0.5 μg/kg by the intravenous route and 3–4 μg/kg by the subcutaneous route. It appeared, too, that emesis and diarrhoea were provoked by approximately the same dose levels, vomiting, however, being somewhat more easily induced. The experiments involving pretreatment with atropine were only preliminary and must be extended considerably before definite conclusions can be drawn. It seems, however, that atropine retarded vomiting and reduced bowel stimulation considerably.

Pigeon. Five animals injected intravenously with caerulein 10 μg/kg displayed no obvious effects. One of four animals injected with caerulein 50 μg/kg showed several episodes of emesis 10–120 min after the injection but the other three were unaffected.

Rat. No obvious effects on bowel motility were observed in rats injected subcutaneously with doses of caerulein ranging from 20 to 1,000 μg/kg. A moderate antidiuretic effect was seen in hydrated animals, however, especially in those which had received the largest doses. A more detailed description of this action of caerulein will be given elsewhere, together with an interpretation of its mechanism. Caerulein antidiuresis is mentioned here because it is possibly due, totally or in part, to a pylorospasm induced by the polypeptide. The delay in the passage of the water load from the stomach into the intestine and the consequent retardation of the absorption of the water administered could readily explain the apparent antidiuretic effect of caerulein.

Man. In preliminary experiments it has been shown that caerulein could be tolerated by man, with no untoward effects, when injected intravenously in doses up to 10–20 ng/kg. With larger doses the majority of subjects experienced, for 5 to 10 min, nausea, abdominal discomfort with borborygmi and awareness of intestinal movements. When caerulein, dissolved in physiological saline, was given by intravenous infusion (1 ml./min for 30 min), it could be tolerated in doses up to 4–5 ng/kg/min, with larger doses the majority of subjects complained of nausea (Torsoli, personal communication). These side-effects were similar to those described after administration of cholecystokinin-pancreozymin (Bandwell, Northam & Cooke, 1967).

General effects in the anaesthetized dog

Vomiting was not observed, although doses of caerulein as large as 100–1,000 μg/kg were administered intravenously. Frequently borborygmi were heard and, occasionally, evacuation of formed stools was noted.

Action on the gastric musculature in vivo

After a short latent period, the subcutaneous injection of caerulein usually caused more or less evident episodes of increased tone and motility in the denervated gastric pouch of the conscious dog, interposed between periods of reduced activity (Fig. 1A). The threshold dose was of the order of 0.2–1 μg/kg. Atropine, given intramuscularly in doses of 0.1–0.3 mg/kg, reduced the spasmogenic action of caerulein (Fig. 1B). An intense but brief stimulation of movements could be elicited by caerulein 50 ng/kg administered intravenously.

The denervated gastric pouch of the anaesthetized dog responded to intravenous injection of caerulein in much the same way as that of the conscious animal. There were increases in tone and rhythmic movements which, within certain limits, were related to the dose administered (Fig. 2). With large doses tachyphylaxis was evident. The threshold dose was usually 30–50 ng/kg, with an exceptionally low value of 5 ng/kg, observed in only one dog.

In two experiments, human gastrin I was 20–50 times less effective than caerulein on the dog gastric musculature.

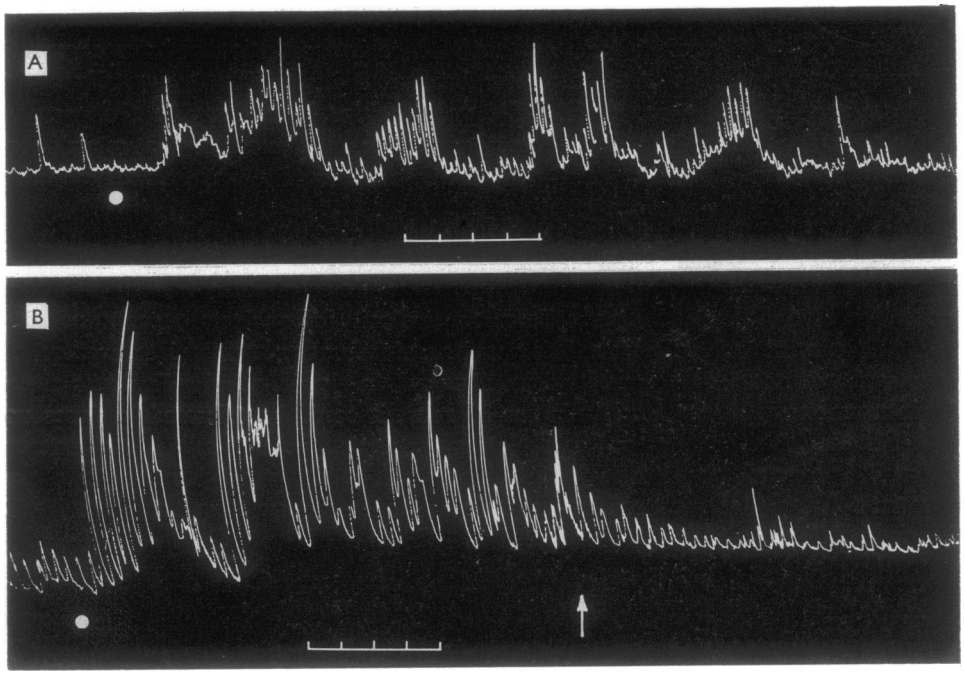

FIG. 1. Motor activity of denervated gastric pouches in two conscious dogs. Time marks, 1 min. Dog A: at ●, caerulein 0.5 μg/kg, intravenously. Dog B: at ●, caerulein 1 μg/kg, intravenously; at arrow, atropine sulphate 0.2 mg/kg, intramuscularly. Note the increase of motor activity elicited by caerulein and the inhibitory action of atropine.

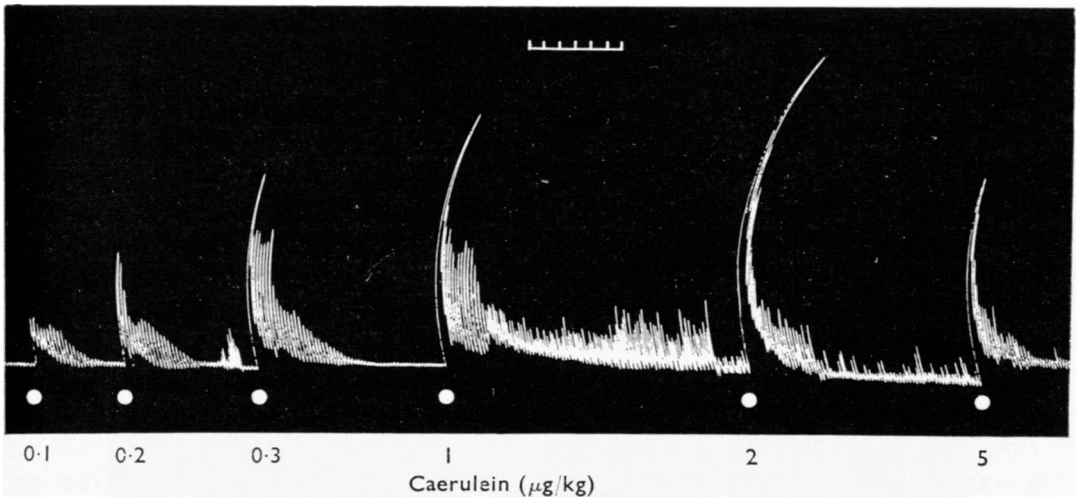

FIG. 2. Motor responses to graded intravenous doses of caerulein of a denervated gastric pouch in a dog anaesthetized with sodium pentobarbitone (30 mg/kg, intravenously). Time marks, 1 min. There was a good dose–response relationship from 0.1 to 1 μg/kg; after 2 μg/kg the increase in tone was greater but less sustained than after 1 μg/kg and the stimulation of rhythmic activity was less intense. With 5 μg/kg tachyphylaxis was evident.

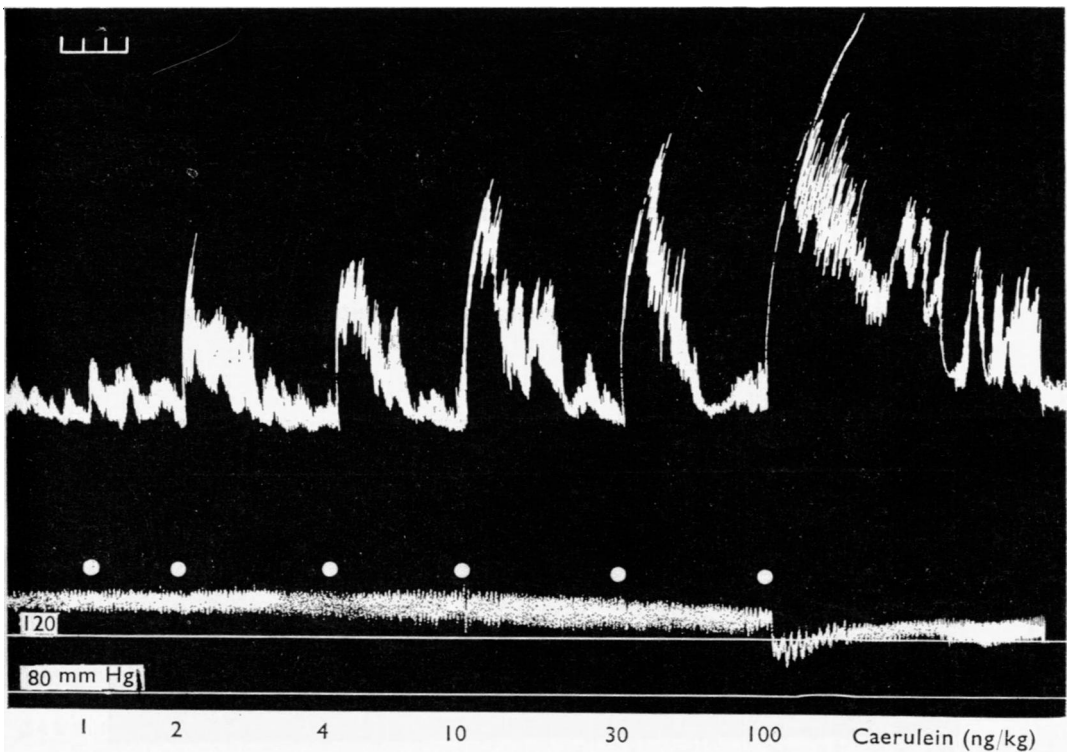

FIG. 3. Dog anaesthetized with sodium pentobarbitone. Upper tracing, motor activity of a jejunal loop *in situ*; lower tracing, arterial blood pressure. Time marks, 1 min. Responses to intravenous injections of caerulein. The threshold dose was 1 ng/kg. An increase in the dose caused an increase in both size and, partially, duration of the response. Blood pressure was affected only by doses of caerulein above 30 ng/kg.

Effects on tone and motility of the intestine in situ

The intravenous injection of caerulein into anaesthetized dogs produced a prompt and intense stimulation of movements and tone of the jejunal loops. The threshold dose ranged between 1 and 5 ng/kg, and the responses were proportional to the doses, up to 100 ng/kg. Small doses usually produced an increase in movements only, larger doses produced an increase in tone as well. The duration of the response (5–10 min) remained unchanged up to a dose of 100 ng/kg, when a prolongation of the effect became evident (Fig. 3). Tachyphylaxis was either lacking or moderate. Atropine, given intravenously in doses of 30–100 μg/kg, produced 75% inhibition of the spasmogenic action of caerulein (40–200 ng/kg).

Loops of the large intestine were much less sensitive to caerulein (threshold dose, 20–100 ng/kg) than jejunal loops and the response was preceded by a latent period of longer duration.

It was observed, in preliminary experiments, that the small intestine of the cat responded to caerulein like that of the dog. The threshold intravenous dose of caerulein was of the order of 5–10 ng/kg, the responses again being proportional to the doses up to 100 ng/kg. Atropine (50 μg/kg, intravenously) produced a complete inhibition of the response to the polypeptide, however, even when it was administered in doses up to 1 μg/kg.

Action on the gall bladder in situ

Guinea-pig. Caerulein had a powerful stimulant action on the gall bladder of the anaesthetized guinea-pig. The intravenous threshold dose ranged between 0.3 and 1.5 ng/kg. Not infrequently the maximum sensitivity to caerulein was attained some hours after the commencement of an experiment. Tachyphylaxis was completely lacking even in experiments of 24–36 hr duration, and an excellent dose–response relationship was always noted. When the dose was raised above threshold, both an increase and a prolongation of the gall bladder contraction were observed initially. A further increase in the dose caused only prolongation of the spasm, as shown in Fig. 4. It could be seen that the contractions produced by 60 ng/kg of caerulein lasted for 8 min, and those elicited by 120 and 250 ng/kg for 12 and 15 min, respectively.

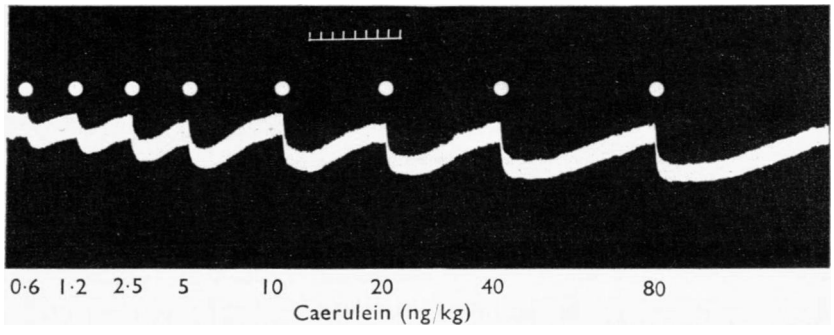

FIG. 4. Guinea-pig anaesthetized with urethane (2 g/kg, subcutaneously). The effect of intravenous injections of caerulein on the gall bladder *in situ*; contractions downward. Time marks, 1 min. Note the excellent dose–response relationship.

No known biogenic substance, except cholecystokinin-pancreozymin, compares with caerulein in its stimulant action on the gall bladder. Approximate equivalents to 1 ng caerulein were as follows: human gastrin I 2–5 μg (Fig. 5), eledoisin >1 μg, physalaemin >1 μg, bradykinin 0.1–1 μg, 5-HT >2 μg, histamine 0.2–0.5 μg. Because of its more rapid onset and its shorter duration, the contraction obtained after the administration of histamine differed considerably from that caused by the administration of caerulein (Fig. 6). For example, 1 ng of caerulein was equiactive with 200 ng of histamine with regard to intensity of action and equiactive with 3,000 ng of histamine with regard to duration of action. Simultaneous injection of histamine and caerulein produced a summation of effects.

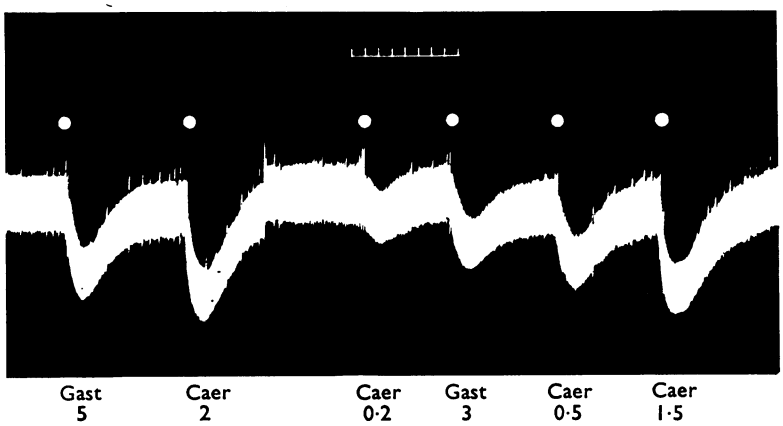

FIG. 5. Guinea-pig anaesthetized with urethane. Responses of the gall bladder *in situ* to intravenous injections of caerulein (Caer, ng/kg) and human gastrin I (Gast, μg/kg). Time marks, 1 min. The shapes of the records of the contractions produced by the two polypeptides were similar, but human gastrin I was 3,000 to 6,000 times less active than caerulein.

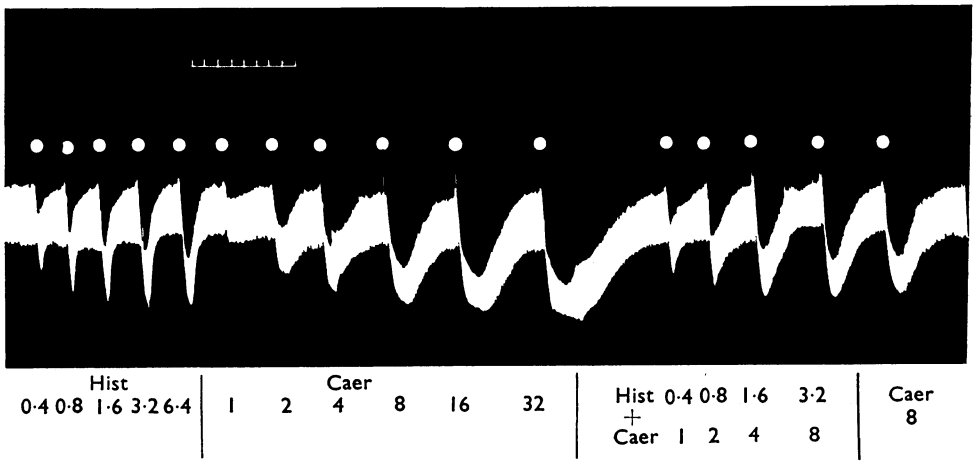

FIG. 6. Guinea-pig anaesthetized with urethane. Responses of the gall bladder *in situ* to intravenous injections of histamine dihydrochloride (Hist, μg/kg), caerulein (Caer, ng/kg) and histamine plus caerulein. Time marks, 1 min. The shapes of the contraction curves produced by histamine differed sharply from those produced by caerulein.

Cholecystokinin-pancreozymin was also less active than caerulein because 1 Ivy dog unit, corresponding to 300 ng of the pure polypeptide, was equiactive with 25–30 ng of caerulein (Fig. 7). This would imply that, even on a molor basis, caerulein was at least 3 times more active than pure cholecystokinin-pancreozymin.

Subcutaneous injection of caerulein produced a long-lasting contraction of the gall bladder, again proportional to the dose with respect to the intensity and duration of action. The threshold dose was 150–200 ng/kg. The spasm produced by 0.4 μg/kg of caerulein lasted approximately 20 min, that caused by 75 μg/kg more than 90 min.

When administered by intravenous infusion, the threshold dose of caerulein was 0.2–0.3 ng/kg per min ; the contraction lasted for as long as the infusion was continued. In the experiment shown in Fig. 8, the contraction was maximal with 50 ng/kg per min. A ten-fold increase in the rate of infusion produced a contraction of similar magnitude but there was a greater delay in the onset of relaxation of the gall bladder musculature after the infusion was discontinued.

Intravenous injection of atropine 0.5–1 mg/kg did not affect the gall bladder contraction produced by caerulein injected intravenously. Hexamethonium (5 mg/kg, intravenously), Dibenamine (2 mg/kg, intravenously) and mepyramine (0.1 mg/kg, intravenously) all produced moderate reduction of the response to caerulein. (−)-Adrenaline 10 μg/kg did not affect the spasmogenic action elicited by caerulein 30 ng/kg given simultaneously by intravenous injection. (−)-Adrenaline 20 μg/kg, however, reduced the height of the contraction by 25%, and 40 and 60 μg/kg by 50 and 70%, respectively.

Man. Caerulein also proved to be a powerful stimulant of the gall bladder musculature in man, as assessed by cholecystography. Preliminary experiments carried out in the Institute of Radiology of the University of Parma by Braibanti, Bertaccini & Uva (1968) showed that the intravenous threshold dose of caerulein capable of reducing the size of the gall bladder shadow was of the order of 0.5–1 ng/kg. With 10 ng/kg there was a very marked reduction in the volume of the gall bladder ; this effect persisted for 60–90 min and was frequently associated with an

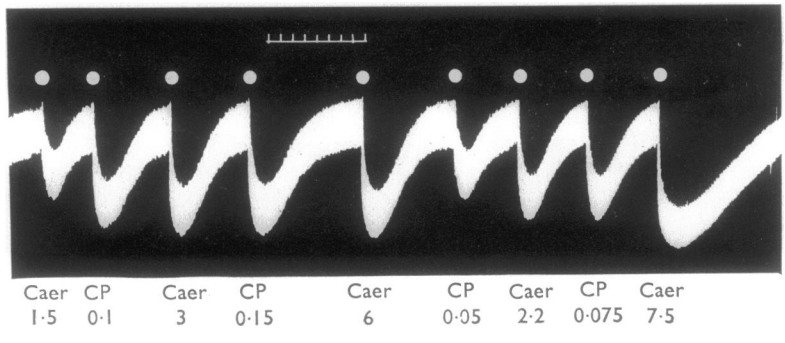

FIG. 7. Guinea-pig anaesthetized with urethane. Responses of the gall bladder *in situ*. The effects of different doses of caerulein (Caer, ng/kg) were compared with those of different doses of cholecystokinin-pancreozymin (CP, Ivy dog units), both injected intravenously. Time marks, 1 min. The shapes of the contraction curves produced by the two polypeptides were similar. In this experiment 0.1 unit CP was equivalent to 3 ng caerulein.

exceptionally good visualization of the extrahepatic bile ducts. The reduction in the size of the gall bladder shadow produced by the usual test meal of milk or egg yolk was of approximately the same magnitude as that produced by caerulein 3–4 ng/kg.

Dog. The contraction of the gall bladder in the anaesthetized dog in response to intravenous injections of caerulein was demonstrated by recording the pressure in the cannulated organ and by recording the flow of bladder bile through the cannulated bile duct with the hepatic duct ligated. By the second, more sensitive, method the threshold dose of caerulein was found to be of the order of 0.5–1 ng/kg.

This is in full agreement with the recent data of Vagne & Grossman (1968), who found that caerulein was 47 times more potent than cholecystokinin-pancreozymin on a weight basis and 16 times on a molar basis, and that porcine gastrin I and porcine gastrin II, which had the same activity, were approximately 550 times less potent than caerulein on a weight basis and 350 times less potent on a molar basis.

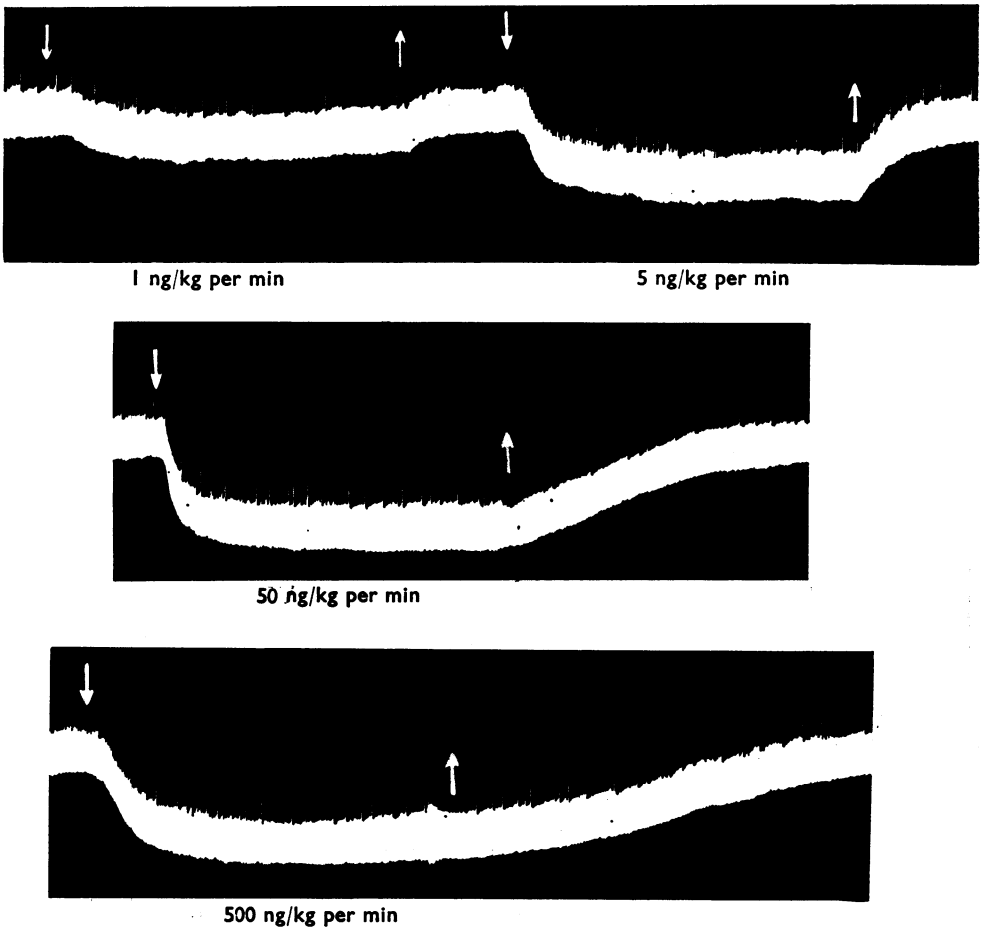

FIG. 8. Guinea-pig anaesthetized with urethane. Response of the gall bladder *in situ* to intravenous infusion of caerulein. ↓, Infusion started; ↑, infusion stopped. Each infusion period lasted for 60 min. Note the good dose–response relationship for infusion rates ranging from 1 to 50 ng/kg per min. The response to 500 ng/kg per min was similar in its intensity to the response to 50 ng/kg per min, but relaxation was more gradual.

Action on the rat pylorus

On the pyloric sphincter of the rat studied by the method of Harichaux & Thouvenot (1963), caerulein had a marked spasmogenic action. The threshold dose when injected intravenously was of the order of 5–15 ng/kg and the effects were well correlated with the doses, up to 100–200 ng. The spasm lasted, depending on the dose, from 2 to 20 min and was not appreciably affected by either atropine (up to 10 mg/kg) or hexamethonium (up to 20 mg/kg). Tachyphylaxis was sometimes evident after two or three injections of caerulein. It has previously been pointed out that the antidiuresis observed in hydrated rats following caerulein administration is probably due, in part at least, to pylorospasm.

Action of the sphincter of Oddi

Caerulein caused a relaxation of the sphincter of Oddi. This effect was sometimes observed in non-premedicated guinea-pigs but was particularly striking when the sphincter was first contracted by morphine. The threshold intravenous dose of caerulein was 5–20 ng/kg. The spasm produced by morphine (100 μg/kg) was abolished by 50–100 ng/kg of the polypeptide.

A dose of caerulein (100 ng/kg) was always more effective than 20 Ivy dog units/kg of cholecystokinin-pancreozymin. This means that 1 μg caerulein is more effective than 200 Ivy dog units or 60–65 μg of pure cholecystokinin-pancreozymin.

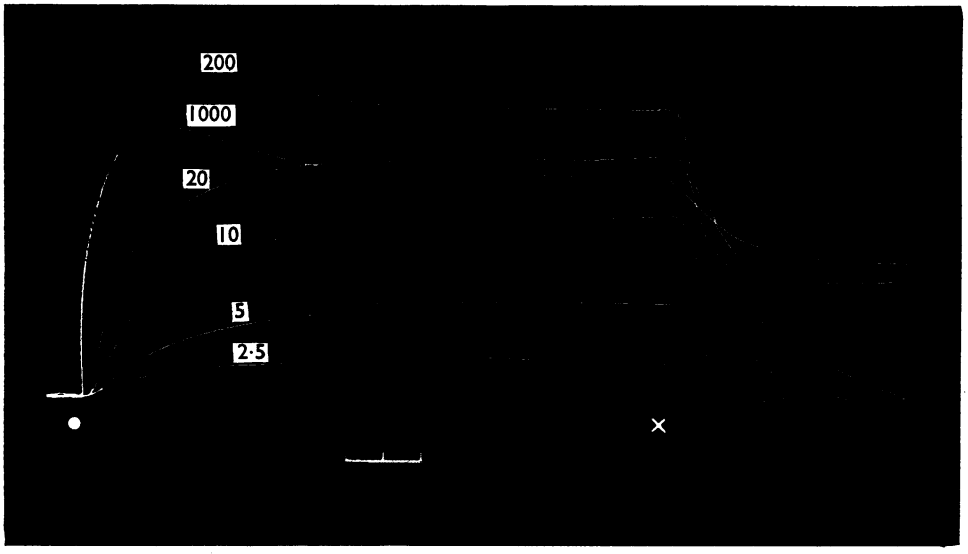

FIG. 9. Guinea-pig gall bladder suspended in 5 ml. Krebs solution at 32° C. Contraction produced by increasing doses of caerulein (ng). Time marks, 3 min. At ×, washing. Up to 200 ng there was a good dose–response relationship. With 1,000 ng the contraction was smaller, but relaxation was slower than with 200 ng.

Action on the isolated gall bladder

Guinea-pig. Of all the isolated smooth muscle preparations tested in this study, the guinea-pig isolated gall bladder proved to be the most suitable preparation for the bioassay of caerulein. It responded with an increase in tone which was proportional to the concentration of caerulein (Fig. 9); there was no tachyphylaxis. The increase in tone was maintained as long as the polypeptide remained in contact with the preparation which, depending on the dose, relaxed more or less rapidly after washing with fresh Krebs solution. The threshold concentration was of the order of 0.5–2 ng/ml.

As was the case with the gall bladder *in situ*, the isolated preparation was relatively insensitive to most biogenic substances known to stimulate smooth muscle. Because of differences in the shape of the contraction curves obtained, comparison of the effects elicited by caerulein with those elicited by other active compounds often presented difficulties. Bradykinin, for example, produced a prompt contraction of the gall bladder but the spasm was not maintained and the musculature began to relax even in the continuing presence of the polypeptide (Fig. 10).

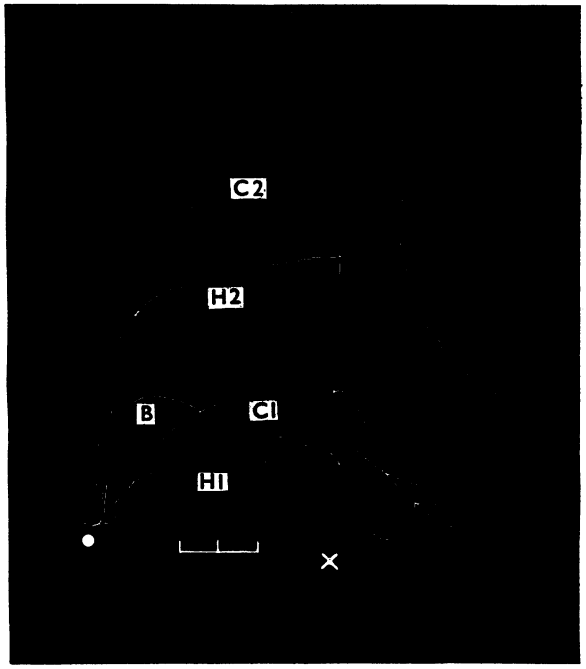

FIG. 10. Guinea-pig gall bladder suspended in 5 ml. of Krebs solution at 32° C. Time marks, 3 min. At ×, washing. Contractions produced by histamine dihydrochloride (H1, 200 ng; H2, 400 ng), bradykinin (B, 1,500 ng) and caerulein (C1, 10 ng; C2, 20 ng). Note the difference in the contraction curves caused by the three compounds.

One nanogram of caerulein was approximately equiactive with 0.1–0.5 μg of eledoisin, >2 μg of physalaemin, 0.05–0.15 μg of bradykinin, >2 μg of human gastrin I, 0.02–0.05 μg of acetylcholine and 0.1–0.2 of 5-hydroxytryptamine. Of the compounds tested, histamine was the most active, being only 5 to 15 times less potent than caerulein, weight for weight.

Atropine (0.01–1 μg/ml.) did not affect the response to caerulein; 1 μg of (−)-adrenaline almost abolished the action of 10 ng of caerulein and reduced the action of 20 ng by 50%.

Rabbit. The rabbit gall bladder was even more sensitive to caerulein, the threshold concentration being 0.1–0.25 ng/ml.; a good dose-response relationship was obtained (Fig. 11). Contraction and particularly relaxation were, however, slower and more sluggish than in the guinea-pig. Tested on the rabbit gall bladder, 1 ng of caerulein was equiactive with 0.2–1 μg of eledoisin, >10 μg of physalaemin, 1–2 μg of bradykinin, >5 μg of human gastrin I, >2 μg of acetylcholine, >10 μg of 5-HT and >2 μg of histamine. Thus the action of caerulein on the rabbit gall bladder was very selective.

Cat. The threshold concentration of caerulein for the cat gall bladder was 1–2 ng/ml. and the effect was fairly proportional to the dose. Relaxation after washing with fresh Tyrode solution was slow. Caerulein was about 5,000 times more active than either eledoisin or physalaemin and 200 times more active than acetylcholine.

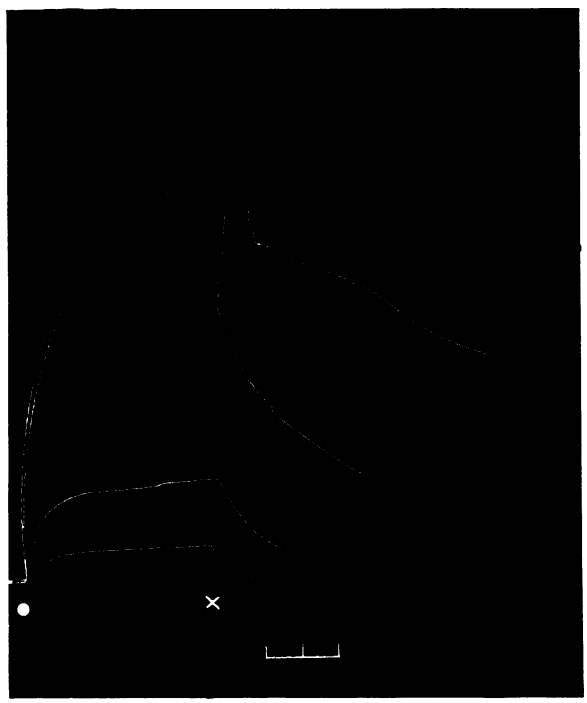

FIG. 11. Rabbit gall bladder suspended in 10 ml. of Tyrode solution at 37° C. Time marks, 5 min. At ×, washing. The spasmogenic action of increasing doses of caerulein is shown (from bottom to top 5, 10, 50 and 500 ng).

Sheep. The threshold concentration of caerulein for the sheep isolated gall bladder was very low, 0.1–0.2 ng/ml., but the dose-response relationship was not completely satisfactory. The concentration was sluggish, as was the relaxation after washing with fresh Tyrode solution.

Cow. Of the preparations used, the musculature of the cow isolated gall bladder was among the most sensitive, the threshold concentration of caerulein being 0.03–0.1 ng/ml. The exceedingly slow relaxation after washing out caerulein, however, made this preparation unsuitable for routine use. Physalaemin, in concentrations up to 1 μg/ml., was inactive. Eledoisin, on the other hand, although not very potent (threshold concentration 0.2 μg/ml.) led to a contraction of the preparation and rhythmic movements. After caerulein, the effects of eledoisin were potentiated.

Action on isolated preparations of the gastrointestinal tract

Caerulein usually had a spasmogenic action on these preparations but the effect was not very intense and tachyphylaxis occurred frequently. Occasionally, repeated stimulation of the smooth muscle with adequate doses of acetylcholine or eledoisin interposed between two successive doses of caerulein, 15 min or more apart, led to some restoration of the original responsiveness of the preparation to caerulein but this restoration was never complete. Accordingly, isolated preparations of the gastrointestinal tract appeared unsuitable for the bioassay of caerulein.

Guinea-pig. Threshold concentrations of caerulein were approximately 100 ng/ml. for the duodenum, 10–30 ng/ml. for the ileum and 5–20 ng/ml. for the large intestine. In every case the polypeptide produced an increase in tone and ultimately rhythmic movements appeared. After having reached its maximum the contraction frequently declined spontaneously in the continuing presence of caerulein. Tachyphylaxis was always evident (Fig. 12). Bradykinin was 2 to 10 times more potent in the ileum and 40 times more potent in the large intestine than caerulein; physalaemin was 10 to 50 times more potent.

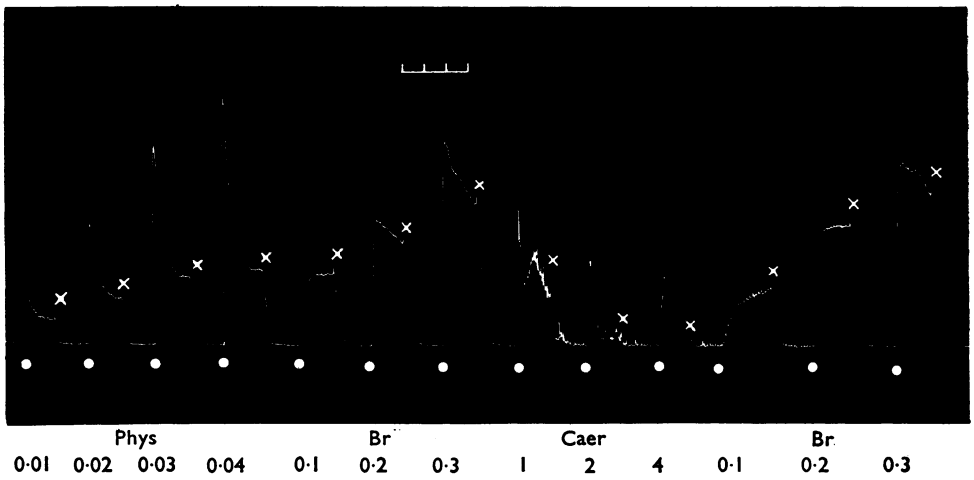

FIG. 12. Guinea-pig ileum suspended in 10 ml. of Krebs solution at 32° C. Time marks, 2 min. At ×, washing. The effect of different doses (μg) of physalaemin (Phys), bradykinin (Br) and caerulein (Caer). Note the good dose–response relationship for physalaemin and bradykinin, and the prompt appearance of tachyphylaxis after administration of caerulein.

Rat. The threshold concentrations of caerulein were approximately 0.1 μg/ml. for the duodenum, 0.5–1 μg/ml. for the descending colon and 1 μg/ml. for the ascending colon. All segments of the gastrointestinal tract were stimulated by the polypeptide. On the descending colon, caerulein was 10–25 times less active than human gastrin I.

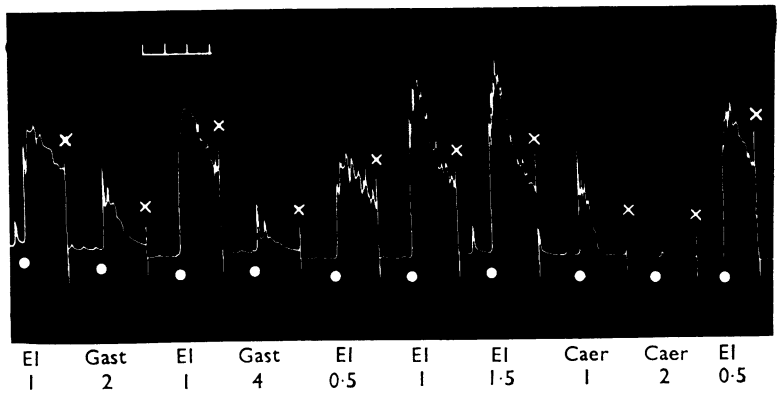

FIG. 13. Hamster stomach suspended in 10 ml. of Ferreira–Vane solution at 37° C. Time marks, 2 min. At ×, washing. Responses to eledoisin (El), human gastrin (Gast) and caerulein (Caer). All doses in μg. The poor spasmogenic action of caerulein was similar to that elicited by gastrin. With both polypeptides tachyphylaxis was pronounced.

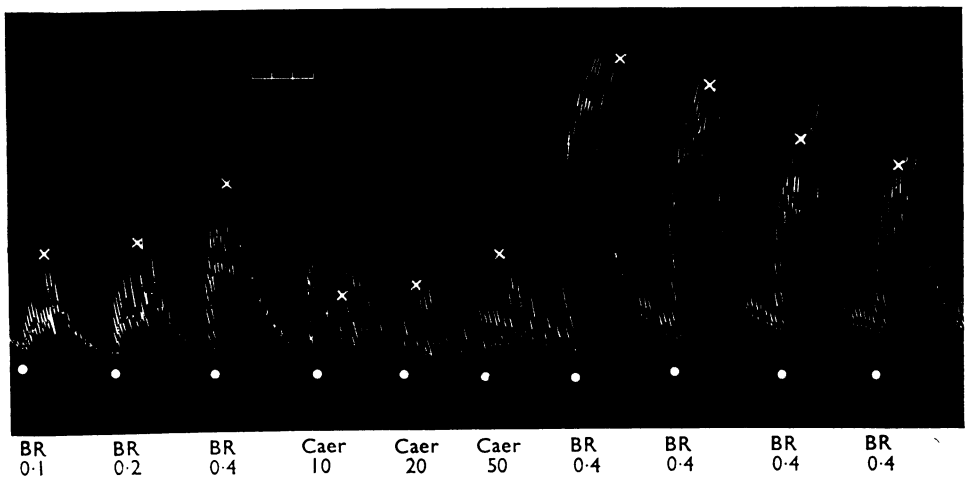

FIG. 14. Cat large intestine suspended in 10 ml. of Tyrode solution at 37° C. Time marks, 2 min. At ×, washing. Responses to caerulein (Caer) and bradykinin (BR). All doses in μg. Note the low sensitivity to caerulein and the prompt appearance of tachyphylaxis. The spasmogenic action of bradykinin was considerably enhanced when the preparation had been first exposed to caerulein.

Hamster. Threshold concentrations of caerulein were approximately 50–100 ng/ml. for the stomach and the duodenum, 5–20 ng/ml. for the terminal ileum and 1 μg/ml. for the large intestine. After reaching its maximum, the moderate contraction declined in the continuing presence of the polypeptide. Tachyphylaxis was marked. The effect of human gastrin I was indistinguishable from that produced by caerulein (Fig. 13).

Rabbit. The duodenum responded to caerulein (threshold concentration 0.2–1 μg/ml.) with a slight increase in tone which declined spontaneously ; tachyphylaxis was prominent. Physalaemin was 500–2,000 times more potent than caerulein. The large intestine was even less sensitive than the duodenum and did not respond to a concentration of 1 μg/ml.

Cat. The duodenum was insensitive to 2 μg/ml. of caerulein but responded to concentrations of bradykinin which were 100 to 200 times smaller. For the large intestine the threshold concentration of caerulein varied between 30 and 300 ng/ml. The contraction was followed by a rapid relaxation in the continuing presence of caerulein ; washing with fresh Tyrode solution often produced spontaneous movements or increased them if already present. If bradykinin was added to the bath at this point its spasmogenic activity was considerably enhanced (Fig. 14).

Dog. The fresh duodenum was insensitive to concentrations of caerulein up to 1.5 μg/ml., but when the organ had been kept in Tyrode solution at 4° C for 24 hr it was stimulated by concentrations above 0.1 μg/ml. Tachyphylaxis was striking. Physalaemin was 500–5,000 times more potent. The large intestine behaved in a similar fashion, the threshold concentration being 0.05–0.2 μg/ml.

Fowl. Concentrations of 0.1–0.2 μg/ml. of caerulein produced an increase in tone and enhancement of movements of the caecum and the large intestine. Relaxation after washing was sluggish and tachyphylaxis always occurred.

Toad (Bufo bufo bufo). The large intestine contracted in response to caerulein and showed rhythmic movements. The threshold concentration was 0.1–0.2 μg/ml. and there was some dose-response relationship. Physalaemin was 1,000 times more potent than caerulein. Similar behaviour was exhibited by the toad stomach, but the threshold concentration was higher and physalaemin was 50,000 times more potent than caerulein.

Discussion

The present investigation shows that caerulein has a very potent stimulant action on the musculature of the gastrointestinal tract *in situ,* but different segments of the gut vary markedly in their responses to the polypeptide. This stimulation of the gastrointestinal tract is readily observed in the conscious intact animal. Vomiting and diarrhoea occur in the dog, abdominal discomfort, borborygmi and awareness of intestinal movements in man, and pseudoantidiuresis due to pylorospasm in the rat.

It remains to be shown whether nausea, vomiting and even diarrhoea are due, in part, to some direct or reflex central effect of caerulein rather than to the peripheral actions of the polypeptide on the intestinal wall. The potent actions of caerulein on the denervated fundic pouch of the dog and on the isolated gall bladder indicate, however, that the main actions of caerulein are on the smooth muscle directly or on the neuronal structures in the intestinal wall, or on both.

In the anaesthetized animal all the parts of the gastrointestinal tract examined were stimulated by caerulein. It is impossible at present to provide a reliable estimate of the sensitivity of the different intestinal segments to caerulein. Moreover, a considerable amount of work will be necessary before information on the actions of caerulein on the motility of the gastrointestinal tract in an adequate number of different animal species is available. Part of the work required is in progress; this includes a study of the effects of caerulein on the motor activity of the human gut.

In spite of these reservations, it is apparent that the musculature of the gall bladder and of the jejunum are particularly sensitive to caerulein. Indeed, the exceptionally high sensitivity of the gall bladder musculature is beyond dispute and has been observed in man, dog, and guinea-pig. The threshold doses of intravenously injected caerulein are of the order of 1 ng/kg and less. For the jejunum the threshold doses are of approximately the same order in the dog but somewhat larger in the cat. It is of interest that, in the rat, pylorospasm is elicited by doses of caerulein as low as 5–10 ng/kg.

Stimulation of the gastric musculature of denervated gastric pouches or of the musculature of the colon appears to require larger doses of caerulein (>10 ng/kg).

In sharp contrast to the action of caerulein on the musculature of the gall bladder and probably of the other segments of the biliary tree, the sphincter of Oddi was relaxed by the polypeptide, especially if the sphincter had first been contracted by morphine.

The location of the receptor sites for caerulein in the gastrointestinal wall have not been elucidated in the present study. Indeed, they are probably different in the different segments of the gastrointestinal tract and the mechanism of action of the polypeptide may be different in the different animal species examined. This possibility emerges from the different effects of premedication with atropine which does not affect, for example, the response to caerulein of the guinea-pig gall bladder and the rat pylorus but depresses markedly the responses of jejunal loops in the dog and the cat. When the responses are sensitive to atropine it may be assumed that cholinergic mechanisms are involved in the action of caerulein. Similar striking discrepancies in the effects of atropine have been described for isolated preparations of gastrointestinal muscle stimulated by gastrin (Mikos & Vane, 1967) and for the cat gall bladder and jejunum stimulated by intravenous administration of cholecystokinin-pancreozymin (Hedner, Persson & Rorsman, 1967).

Contrasting sharply with the great sensitivity to caerulein of the intestinal musculature *in situ* is the very low sensitivity of isolated preparations of the gastrointestinal tract. All isolated preparations of the gut were, except in rare cases, relatively insensitive to caerulein and tachyphylaxis was prompt and striking. The gall bladder musculature, however, provides a remarkable exception. In fact, apart from a few cases which may possibly be attributed to methodological defects, isolated preparations of the gall bladder were highly sensitive to caerulein. The spasmogenic action was proportional to the dose and there was no sign of tachyphylaxis.

From a pharmacological point of view it appears that the gall bladder *in situ* or as an isolated preparation is very suitable for the bioassay and the qualitative and quantitative evaluation of the spasmogenic action of caerulein and caerulein-like

polypeptides. Because of its ready availability, the excellent dose-response relationship and the consistency of the results obtained, the gall bladder of the guinea-pig is the preparation of choice. No known substance is more potent than caerulein as far as its stimulant action on the gall bladder and the small intestine and its relaxing action on the sphincter of Oddi are concerned.

The only polypeptide which warrants comparison with caerulein is cholecystokinin-pancreozymin. Caerulein closely mimics the effects of this duodenal hormone, but, even on a molar basis, is more potent than the hormone in all gastrointestinal preparations examined so far. On a weight for weight basis, caerulein is about 10 times more potent than cholecystokinin-pancreozymin on the gall bladder of the anaesthetized guinea-pig (Jorpes & Mutt found an activity ratio 8 : 1, personal communication), 13 to 20 times more potent on the jejunal loop of the dog, 47 times more potent on the gall bladder of the conscious dog (Vagne & Grossman, 1968), and at least 50 times more potent as a relaxant of the sphincter of Oddi contracted by morphine. These calculations are based on the assumption that 1 μg of pure cholecystokinin-pancreozymin equals 3 Ivy dog units. The molecular weight of cholecystokinin-pancreozymin (3883) is approximately 3 times that of caerulein (1352), so the potency ratios calculated on a molar basis must be reduced by this factor.

As far as gastrin is concerned, its action on the motility of the gastrointestinal tract is considerably weaker than that of cholecystokinin-pancreozymin, and hence of caerulein. Yet the three polypeptides possess the same C-terminal pentapeptide. In the experiments reported now, human gastrin I had less than 0.1% of the activity of caerulein on the guinea-pig gall bladder and 2–5% of the activity of caerulein on the gastric and jejunal musculature of the dog.

Our results are in accordance with those of Vagne & Grossman (1967, 1968) who found that porcine gastrin I and porcine gastrin II had, on a weight basis, about 7–8% of the potency of cholecystokinin-pancreozymin on the motility of the dog gall bladder, and with those of Connell & Logan (1967) and Smith & Hogg (1966) who observed that in man porcine gastrin II increased motility of the stomach, small intestine and large intestine only when 0.25 μg/kg or more was injected intravenously. These doses are considerably larger than the doses of caerulein (1–20 ng/kg) which cause contraction of the gastrointestinal tract of the dog, cat and rat.

As regards the possible clinical use of caerulein in cholecystography, it is probable that caerulein may compete satisfactorily with cholecystokinin. Jorpes, Mutt & Olbe (1959) believe that the optimal clinical dose of cholecystokinin to be given intravenously for cholecystography is 1 Ivy dog unit/kg—that is, approximately 300 ng/kg of pure polypeptide. Braibanti *et al.* (1968) found in man that 10 ng/kg of caerulein produced a marked contraction of the gall bladder; thus 0.5–0.7 μg of caerulein would be the approximate dose required for an adult patient.

It is obvious that the present results are only a basis for the work which remains to be done to complete the study of the actions of caerulein on the motility of the musculature of the gastrointestinal tract. Caerulein must be considered not merely as an active polypeptide from the amphibian skin, but as a model polypeptide mimicking closely both gastrin and cholecystokinin-pancreozymin. It is therefore probable that the experimental results obtained with caerulein may be useful in the investigation of the mode of action of cholecystokinin-pancreozymin and gastrin.

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