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The effects of caerulein on insulin secretion in anaesthetized dogs

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Summary

1. Insulin concentration changes in pancreatico-duodenal venous plasma were studied in anaesthetized dogs injected with caerulein.

2. Rises in insulin concentration were elicited by rapid intravenous injection of caerulein, as well as by intravenous infusion. Threshold doses were 10 ng/kg and 0·5–1 (ng/kg)/min respectively.

3. At the highest dose used (500 ng/kg by rapid intravenous injection and (25 ng/kg)/min by intravenous infusion) the increase in immuno-reactive insulin release was approximately 7 to 9 times the base levels.

4. Adrenalectomy potentiated the effects of intravenous infusion of caerulein.

5. On a molar basis, caerulein was 2–3 times as active as pancreozymin.

6. It is concluded that caerulein is a potent stimulant of pancreatic islets in the dog and that it may be considered as a model peptide, capable of being substituted for pancreozymin in any experiment.

7. The mechanism of the insulin stimulating effect of caerulein is discussed. The possibility of a direct " β-cytotropic " effect of the peptide is suggested.

Introduction

Meade, Kneubuhler, Schulte & Barboriak (1967), Unger, Ketterer, Dupré & Eisentraut (1967), and Buchanan, Vance, Morgan & Williams (1968) demonstrated that the intravenous injection of pancreozymin enhanced the insulin concentration in plasma of the pancreatico-duodenal and portal veins in anaesthetized and conscious dogs.

Caerulein, the active decapeptide found in the skin of the Australian amphibian *Hyla caerulea* (Anastasi, Erspamer & Endean, 1968) resembles pancreozymin in its biological activity and chemical structure.

The present study was designed to explore the effects of caerulein on insulin secretion in the anaesthetized dog.

Methods

Fifty mongrel dogs were anaesthetized with sodium pentobarbitone, 30 mg/kg intravenously, after an overnight fast.

Laparotomy was performed by a median cut and the pancreas was gently exposed. The superior pancreatico-duodenal vein was carefully stripped from the surrounding connective tissue and cut about 3 cm from its anastomosis with the portal vein.

Polyethylene tubing (PE 260, Clay Adams) was introduced into the portal lumen through the proximal tract of the pancreatico-duodenal vein. A second tube (PE 240) was inserted in the distal tract of this vein, and the two catheters were joined. The patency of these vascular connexions was maintained by an intravenous infusion of heparin, and the venous flow was kept at a constant rate of (1 ml/kg)/min by a peristaltic pump.

Catheters were also inserted in the carotid artery for recording arterial blood pressure, in the femoral artery for collecting samples of arterial blood and in the femoral vein for administering the drugs.

Four dogs were also adrenalectomized to study the effect of caerulein on pancreatic islets in the absence of adrenal secretion.

Samples of pancreatico-duodenal venous blood as well as of femoral arterial blood were collected in polyethylene microcentrifuge tubes and immediately centrifuged to separate plasma.

Plasma was stored at −20° C until assays were performed. Immuno-reactive insulin was assayed by the radioimmunoassay method of Hales & Randle (1963). Kits for insulin radioimmunoassay were purchased from Sorin, Saluggia, Italy. Canine insulin standards were used to draw a titration curve. Caerulein was added to the blanks in order to exclude interference with insulin assay. Plasma glucose was measured by the enzymatic method of Froesch & Renold (1956).

The following drugs were used: natural caerulein, prepared at the Farmitalia Laboratories for Basic Research, Milan, and cholecystokinin-pancreozymin (3,000 u./μg) obtained through the kindness of Professor E. Jorpes, Stockholm. The drugs were dissolved in isotonic saline solution and administered intravenously either by rapid injection or by constant infusion at the rate of (1 ml/kg)/min.

Results

Rapid intravenous injection of caerulein

Immediately after the rapid intravenous injection of caerulein at a dose of 10 ng/kg a twofold rise in pancreatico-duodenal venous insulin concentration was observed. Doses of 25, 50, 100 and 500 ng/kg produced greater increments, and a clear dose response relationship was observed when the same animal received increasing doses of the drug. A typical experiment is shown in Fig. 1.

At the highest dose used (500 ng/kg), the increase in immunoreactive insulin was approximately 7-fold.

The response to rapid intravenous caerulein injection was always short-lasting. The highest concentration of insulin was reached 60–120 s after the injection, and a sharp decline followed within a few minutes. Insulin concentration returned to pre-injection levels 5–30 min after the administration of the drug, depending on the dose used.

The results of our experiments are summarized in Table 1, where the values recorded are the mean area under the secretion curve, calculated by plotting insulin concentration against time. After rapid intravenous injection of caerulein, the increase in blood glucose concentration is either absent or negligible. Arterial blood pressure was lowered by doses greater than 25 ng/kg.

Infusion of caerulein

Caerulein infused intravenously at a rate of (0·1 ng/kg)/min for 30 min caused a 50% increase in insulin concentration in only one dog out of five. The dose of (0·5 ng/kg)/min produced a 100% increment in four dogs out of six. Increasing the rate of infusion to (1, 2, 5, 10 and 20 ng/kg)/min, however, caused an increase in insulin concentration in the pancreatico-duodenal venous plasma of all the treated animals and the response was proportional to the dose. The increase reached a peak after 10–20 min and lasted as long as the infusion was continued. At the maximum dose used (20 ng/kg)/min the increase in immuno-reactive insulin was approximately 9-fold.

A typical experiment carried out with a caerulein infusion of (5 ng/kg)/min is illustrated in Fig. 2. When the infusion was continued for periods of 80–100 min a secondary peak in insulin concentration was often observed between the 60th and 70th min of infusion, as shown in Fig. 3. After the infusion was stopped the insulin level declined, returning to the base values (Figs. 2 and 3) within 20–40 min.

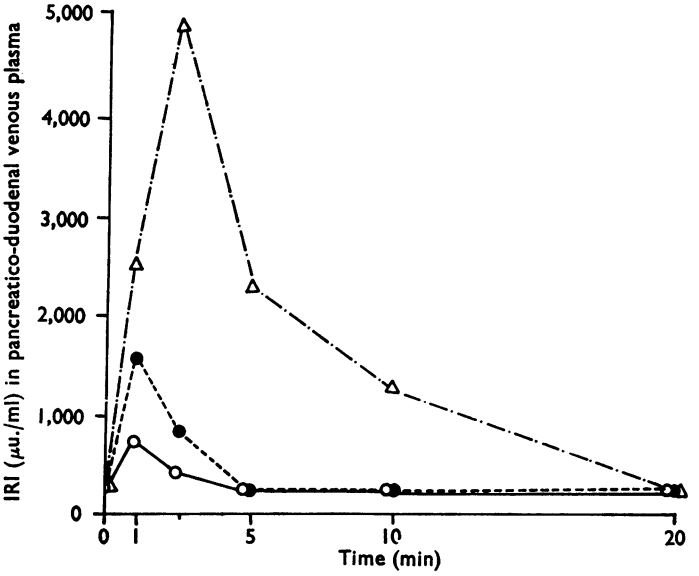

FIG. 1. Changes in pancreatico-duodenal venous immuno-reactive insulin (IRI) concentration resulting from rapid intravenous injection of caerulein at the following doses (ng/kg): 10 (○——○), 25 (●– – –●), 500 (△–·–·–△).

TABLE 1. *Stimulation of insulin secretion by rapid intravenous injections of caerulein*

Dose (ng/kg)	Number of animals	Mean (±S.E.M.) of areas under secretion curves (mm^2)	Relative insulin increase (controls=100)
Controls (saline)	15	97±25	100
10	6	300±31	310
25	5	370±50	380
100	5	410±43	420
500	5	670±52	700

Arterial blood pressure was not modified unless doses larger than (10 ng/kg)/min were used. Plasma glucose increased when the infusion rate of caerulein exceeded (2 ng/kg)/min. The observed increment was always about 100 mg% without any apparent relation to the dose.

Table 2 shows the mean area under the curves of insulin secretion during caerulein infusion.

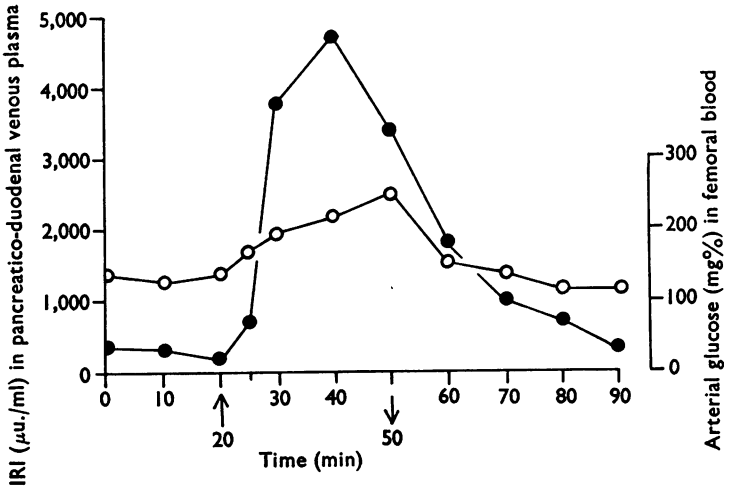

FIG. 2. Changes in pancreatico-duodenal venous immuno-reactive insulin (IRI) concentration (●——●) and in arterial glucose levels (○——○) resulting from intravenous infusion of caerulein at the rate of (5 ng/kg)/min. ↑, Infusion started; ↓, infusion stopped.

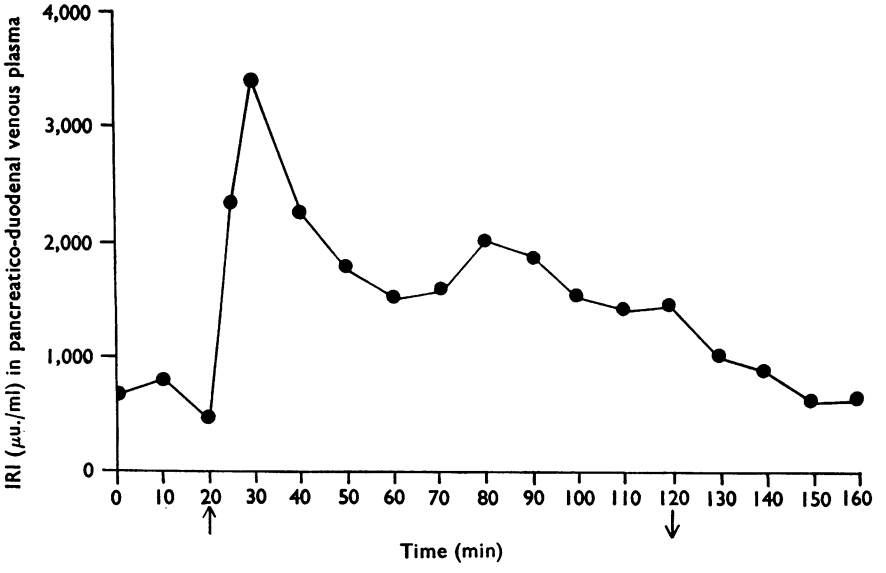

FIG. 3. Changes in pancreatico-duodenal venous immuno-reactive insulin (IRI) resulting from intravenous infusion of caerulein at the rate of (5 ng/kg)/min lasting 100 min. ↑, Infusion started; ↓, infusion stopped.

Effects of caerulein infusion in adrenalectomized dogs

Four dogs were adrenalectomized approximately 2 h before the administration of caerulein. The peptide was infused at a rate of (25 ng/kg)/min.

The pancreatico-duodenal venous insulin concentration increased during infusion and the values obtained were larger than those observed in sham-operated dogs. Arterial blood glucose concentration was affected to the same extent in adrenalectomized and non-adrenalectomized animals (Fig. 4).

TABLE 2. *Stimulation of insulin secretion by the intravenous infusion of caerulein*

Dose (ng/kg)/min	Number of animals	Mean (±S.E.M.) of areas under secretion curves (mm^2)	Relative insulin increase (controls=100)
Controls	30	98±30	100
0·1	5	130±40	130
0·5	6	190±16	190
1	8	250±22	260
2	5	470±120	480
5	5	700±80	720
10	5	820±110	840
20	5	860±92	880

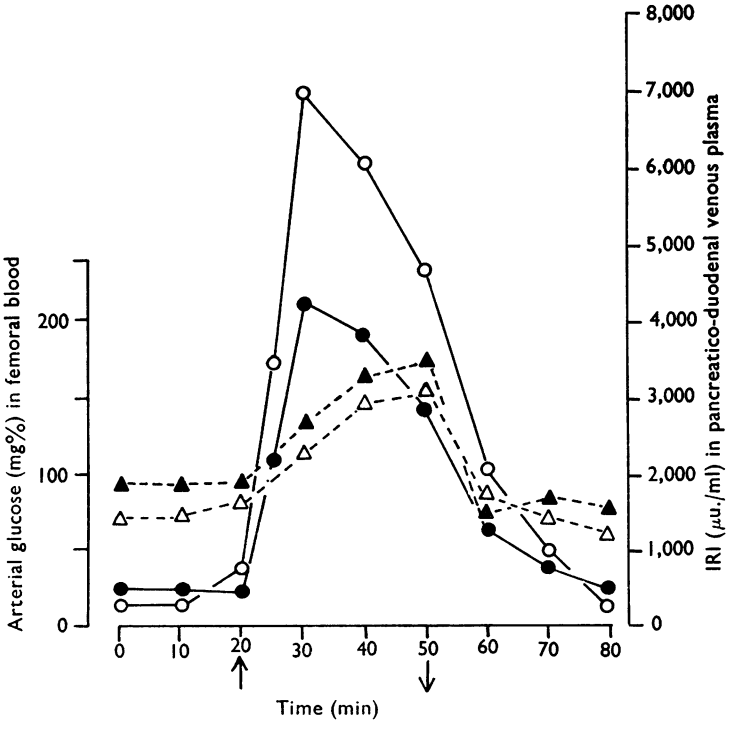

FIG. 4. Effects of caerulein infusion at the rate of (25 ng/kg)/min on pancreatico-duodenal venous immuno-reactive insulin (IRI) concentration and on arterial glycaemia in adrenalectomized and sham-operated dogs. ○ and △, IRI and glucose concentration, respectively, in adrenalectomized dogs; ● and ▲, IRI and glucose concentration, respectively, in sham-operated dogs.

Comparative potency of caerulein and pancreozymin

Because caerulein closely resembles pancreozymin both in structure and biological activity, the insulin stimulating effect of the two peptides was compared.

Both peptides were administered by rapid intravenous injection: caerulein in doses of 10, 25 and 50 ng/kg, pancreozymin in doses of 50, 100 and 200 ng/kg. These experiments showed that caerulein was 7–9 times as potent as pancreozymin on a weight basis, and 2–3 times as potent on a molar basis (Fig. 5). The pattern of insulin secretion curves after caerulein and after pancreozymin was very similar.

Discussion

In a previous paper (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1969) it was demonstrated that caerulein is one of the most potent stimulants of the external pancreatic secretion of the dog.

Present results show that caerulein is also able to stimulate insulin secretion in the dog and that the threshold dose is of the same magnitude as that found active on the exocrine gland: 5–10 ng/kg by rapid intravenous injection and (0·5–1 ng/kg)/min by intravenous infusion (Bertaccini *et al.*, 1969). Thus, caerulein appears to be the most potent stimulant of insulin secretion so far known, even more active than the gastrointestinal hormone cholecystokinin-pancreozymin. The relative potency of caerulein and pancreozymin in stimulating the endocrine pancreas of the dog parallels the relative potency of the two peptides on exocrine pancreatic

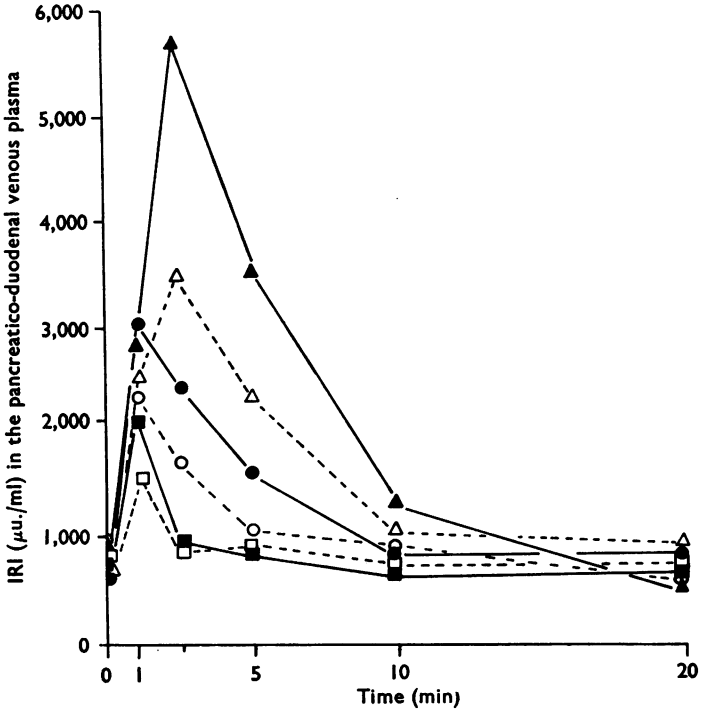

FIG. 5. Effects of rapid intravenous injection of caerulein (continuous line) and pancreozymin (dotted line) at the dose of 10 (■——■), 25 (●——●), and 50 (▲——▲) and of 50 (□- - -□), 100 (○- - -○) and 200 ng/kg (△- - -△), respectively.

secretion (Bertaccini *et al.*, 1969), gall bladder contraction and intestinal motility (Bertaccini, De Caro, Endean, Erspamer & Impicciatore, 1968).

Unger *et al.* (1967) proposed the theory of a physiological control of insulin secretion by gastrointestinal hormones. The present research, carried out with a natural peptide possessing the same active C-terminal heptapeptide as pancreozymin and displaying the same spectrum of biological effects, offers strong support to this fascinating theory.

During the course of long-lasting caerulein infusion two peaks of maximum insulin concentration were observed. A similar secretion pattern was described *in vivo* by Kanazawa, Kuzuya & Ide (1968) in dogs treated with glucose, and *in vitro* by Curry, Bennett & Grodsky (1968) with the perfused rat pancreas. It has been suggested that the first peak depended on a simple insulin release, while the secondary peak was the sign of hormone neosynthesis. We are investigating whether such an interpretation is consistent with our results.

Dorigotti & Glässer (1968) demonstrated that caerulein produced a marked increase in blood flow in the canine pancreatic vascular bed. The possibility that insulin stimulation by caerulein might depend, at least in part, on these changes in blood flow through the pancreas can be excluded under our experimental conditions because pancreatico-duodenal blood flow was kept constant by a peristaltic pump.

Adrenalectomy potentiated the effects of intravenous infusion of caerulein on pancreatico-duodenal venous immuno-reactive insulin.

We cannot explain this effect, because our data were obtained in a limited number of animals. Unger *et al.* (1967) demonstrated that during the infusion of adrenaline, secretin and glucagon failed to produce the expected increase in pancreatico-duodenal venous immuno-reactive insulin. Possibly in the present experiments withdrawal of the adrenaline supply from the adrenal caused a higher sensitivity of Langerhans islets to caerulein.

It has been shown that caerulein infusion at doses greater than (2 ng/kg)/min produced hyperglycaemia. The mechanism of this effect is not clear. It is possible that hyperglycaemia depends on release of adrenal catecholamines; however, adrenalectomy did not modify the hyperglycaemic effect of caerulein. Another possibility is that caerulein may affect glucose metabolism either directly or, more probably, indirectly. In fact, preliminary studies have demonstrated that the peptide greatly increased glucagon concentration in the pancreatico-duodenal venous blood.

It is well known that hyperglycaemia may stimulate the Langerhans islets to produce insulin. According to Kanazawa *et al.* (1968) a glucose concentration of 150–200 mg% blood elicits a 2–3 fold increase in pancreatico-duodenal insulin. However, the peak of insulin concentration elicited by caerulein infusion always occurred about 10–15 min before the maximum glucose increase. On the other hand, the rapid intravenous injection of the peptide did not cause any evident modification of blood sugar levels. Moreover, preliminary research carried out *in vitro* suggests the possibility that caerulein has a direct action on pancreatic islets. Studies in progress with synthetic caerulein-like peptides have demonstrated the possibility of a sharp dissociation (up to 50–100 times) between the action of

these peptides on exocrine pancreas and the action on endocrine pancreas, with selective stimulation of the latter.

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