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Effects of Cyclic Hexapeptide Analog of Somatostatin on Pancreatic Secretion in Dogs (41985)

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Abstract. The effects of a cyclic hexapeptide analog of somatostatin, [cyclo(Pro–Phe–D-Trp–Lys–Thr–Phe)] (cyclo-SS), administered intravenously (iv) or instilled into the duodenum (id) on the pancreatic response to endogenous (meal and duodenal acidification) and exogenous (secretin, CCK) stimulants were compared in five dogs with esophageal, gastric, and pancreatic fistulae. Cyclo-SS given iv in graded doses against a constant background stimulation with secretin caused a similar and dose-dependent inhibition of pancreatic HCO_3 and protein secretion being about twice as potent as somatostatin-14 (SS-14). Cyclo-SS, whether applied topically to the duodenal mucosa in a dose of 1 μg/kg or given iv at a dose of 0.5 μg/kg-hr, resulted in a similar inhibition of pancreatic secretion induced by feeding a meat meal, sham-feeding, duodenal acidification, or infusion of secretin or CCK. The inhibition of pancreatic secretion by cyclo-SS was due in part to direct inhibitory action on the exocrine pancreas as well as to the suppression of the release of secretin, insulin, and pancreatic polypeptide. It is concluded that cyclo-SS is a more potent inhibitor of pancreatic secretion than SS-14 and that it is active when administered both parenterally and intraduodenally.

Previous studies demonstrated that 14-amino acid somatostatin (SS-14) and some of its analogs showed a similar spectrum of biological actions on gastrointestinal secretions (1, 2). These substances were found to inhibit pancreatic secretion stimulated by exogenous hormones (secretin, CCK) and by endogenous stimulants such as feeding due to the suppression of the release of gut hormones (gastrin, secretin) involved in the stimulation of the pancreatic secretion (3, 4). Recently, several analogs were obtained by replacing 9 of the 14 amino acids of SS-14 with a single proline residue (5, 6). Some of them were reported to be highly active in the inhibition of the release of islet hormones (insulin, glucagon) both after parenteral and oral administration (5, 6). This study was designed to compare the effects of SS-14 and its cyclic hexapeptide analog (cyclo-SS) [cyclo(Pro–Phe–D-Trp–Lys–Thr–Phe)], administered intravenously (iv) or into the gut lumen, on pancreatic secretion induced by various exogenous and endogenous stimulants and on the release of gut hormones involved in the control of pancreatic secretion.

Methods. Five mongrel dogs weighing 16–19 kg were prepared surgically with esophageal (EF), gastric (GF), and pancreatic fistulas (PF) as described previously (7, 8). Secretions from the GF and PF were collected continuously at 15-min intervals. Hydrogen ion concentrations in the gastric juice (in the tests with sham-feeding) and bicarbonate and protein concentrations in the pancreatic juice were measured and expressed in 15- or 30-min outputs as described before (7). Basal secretion was first collected for 30–60 min, and then the secretory stimulant for the pancreatic secretion was administered while the cyclic hexapeptide of somatostatin (cyclo-SS) was given intravenously (iv) or intraduodenally (id). During each test two infusions of 0.15 *M* NaCl were given at 80 ml/hr by a peristaltic pump through polyethylene tubes (PE-50), one being inserted into the duodenum through the hollow obturator of the pancreatic cannula and the other into a leg vein.

Studies were also carried out to compare the effect of cyclo-SS and SS-14 on pancreatic secretion induced by a constant background stimulation with iv secretin (4 U/kg-hr).

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When the pancreatic secretory rate reached a well-sustained plateau, cyclo-SS or SS-14 was added to iv infusion in graded doses, each dose being infused for 45 min and then doubled. After we established that the iv dose of cyclo-SS which reduces the pancreatic secretion by 50% was about 0.5 μg/kg-hr, this standard dose was then used in the next series of experiments with other stimulants of pancreatic secretion. For studies with intraduodenal administration of cyclo-SS, a dose of 1 μg/kg-hr was given into the gut lumen via the hollow obturator of PF. All solutions of SS-14 and cyclo-SS contained 1% of canine albumin (Sigma Chemical Co.).

To release endogenous gut hormones, studies with sham-feeding, ordinary feeding, and duodenal acidification were carried out. For tests with sham-feeding, the esophagus was totally obstructed distal to the fistula, so that the entry of food into the stomach was completely excluded. The sham-feeding period lasted 15 min. The food which fell from EF into the feeding pan was repeatedly reconsumed (9). Cyclo-SS was administered iv or id in a constant dose 30 min before, during, and after sham-feeding. Control experiments consisted of sham-feeding without cyclo-SS.

In tests with ordinary feeding, a meat meal consisting of 500 g of cooked beef liver was fed (with the GF closed) and when the pancreatic secretion reached a steady rate, cyclo-SS was administered iv or id at a constant dose for 1 hr. In tests with duodenal acidification, a solution of 0.1 *M* HCl was instilled into the duodenum through the intestinal limb of the pancreatic cannula at a constant rate (8 mmole/hr) which induced pancreatic secretion comparable with that obtained with secretin. Cyclo-SS was given iv or id for 1 hr when the secretory response to duodenal acid reached a sustained level.

Experiments with exogenous CCK were performed using an iv infusion of CCK (2 U/kg-hr) to induce near maximal pancreatic protein secretion. Cyclo-SS was administered iv or id in the second hour of the secretory response to CCK. In tests with sham-feeding, ordinary feeding, and duodenal acidification, blood samples were taken from a peripheral vein for the determinations of plasma concentrations of gastrin (10, 11), secretin (12), pancreatic polypeptide (9, 13), and insulin (14) by radioimmunoassays. The results are presented as means ± SEM. Differences in the means for each 15-min period were compared by unpaired *t* test, *P* values of 0.05 or less being considered as statistically significant.

SS-14 as well as cyclo-SS [cyclo(Pro–Phe–D-Trp–Lys–Thr–Phe)] were synthesized by solid-phase or classical methods and repurified. Cyclo-SS was kindly donated by Professor R. Geiger, Hoechst, A. G., Frankfurt/M, West Germany. Secretin and CCK were purchased from Kabi Diagnostics, Studsvik, Sweden.

Results. *Comparison of the effects of cyclo-SS and SS-14 on pancreatic response to secretin and CCK.* Cyclo-SS and SS-14 infused iv in graded doses against a constant background stimulation with secretin caused a dose-dependent reduction in pancreatic bicarbonate and protein secretion (Fig. 1). The dose producing about 50% inhibition (ID_{50}) of bicarbonate secretion was approximately 0.5 μg/kg for cyclo-SS and 1.0 μg/kg-hr for SS-14. The time-course studies showed that the inhibition of secretin-induced pancreatic secretion started after about 15 min of infusion of cyclo-SS and SS-14. These results are not shown.

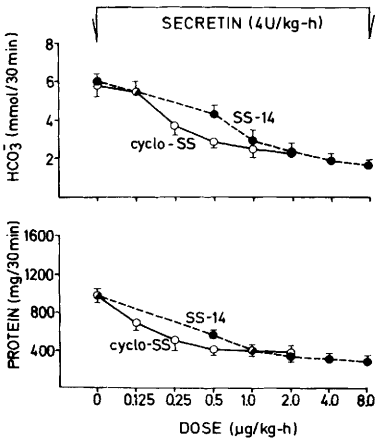

FIG. 1. Effects of graded doses of cyclo-SS and SS-14 on pancreatic bicarbonate and protein responses to secretin. In this and subsequent figures each line is mean (±SEM) of two tests on each of five dogs. Asterisks indicate significant decrease $P < 0.05$ below the control level.

After iv infusion of CCK, the pancreatic protein secretion showed a marked rise but was significantly suppressed by both iv and id instillation of cyclo-SS. After the cessation of cyclo-SS infusion, the pancreatic secretion quickly returned to the control level (Fig. 2).

Effects of cyclo-SS on pancreatic responses to endogenous stimulants. Gastric acid and pancreatic protein secretion in response to sham-feeding in control tests and in tests with iv or id administration of cyclo-SS are presented in Fig. 3. Sham-feeding alone caused a marked increase both in gastric acid and pancreatic protein outputs, which reached about 65% of CCK-induced maximal protein outputs. After iv infusion of cyclo-SS, the gastric and pancreatic responses were almost completely suppressed. Cyclo-SS given id significantly reduced the peak acid output by about 60% and the peak protein output by about 25%. Plasma PP and insulin levels, which tended to increase after sham-feeding, were almost completely suppressed by iv cyclo-SS and partly reduced by id cyclo-SS. Gastrin response to sham-feeding declined after iv and id cyclo-SS but this reduction was not statistically significant (Fig. 4).

Feeding of a meat meal caused a marked pancreatic secretion (Fig. 5) accompanied by a significant increase in serum gastrin, secretin, PP, and insulin concentrations (Fig. 6). In control tests, the postprandial bicarbonate secretion reached peak values at the end of the first hour after feeding and then showed a tendency to decrease. Cyclo-SS given iv or id caused about 80% reduction in both bicarbonate and 70% reduction in protein outputs (Fig. 5) accompanied by a significant suppression of plasma secretin, PP, and insulin levels (Fig. 6). Postprandial gastrin levels were not significantly affected by cyclo-SS (Fig. 6).

Duodenal acidification produced a well-sustained pancreatic bicarbonate and protein secretion accompanied by a significant increase in plasma secretin levels (Figs. 7 and 8). Cyclo-SS infused or given id decreased

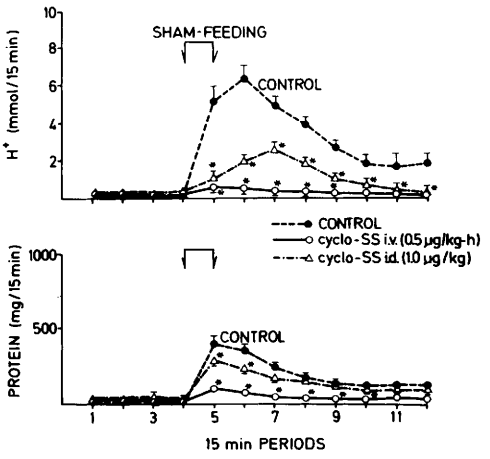

FIG. 3. Effects of cyclo-SS given iv or id on sham-feeding induced gastric acid and pancreatic protein secretion.

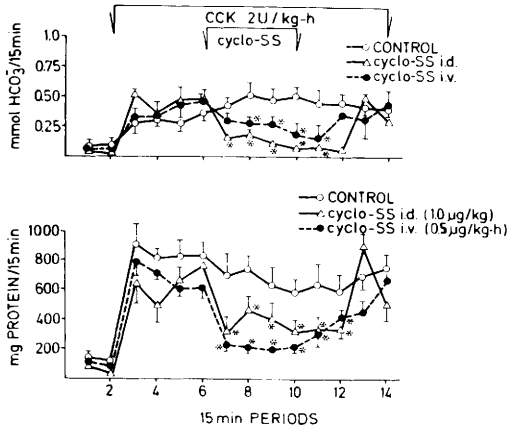

FIG. 2. Effects of cyclo-SS given intravenously (iv) or intraduodenally (id) on pancreatic bicarbonate and protein responses to CCK.

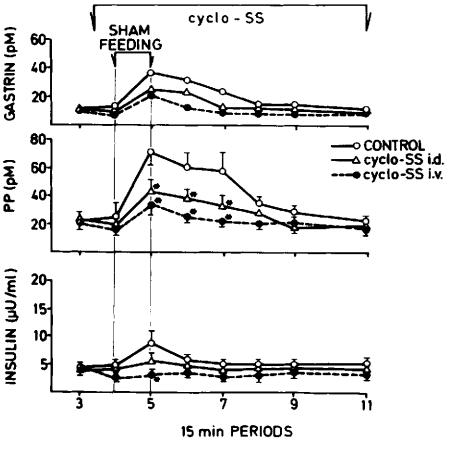

FIG. 4. Plasma gastrin, PP, and insulin levels in tests as in Fig. 3.

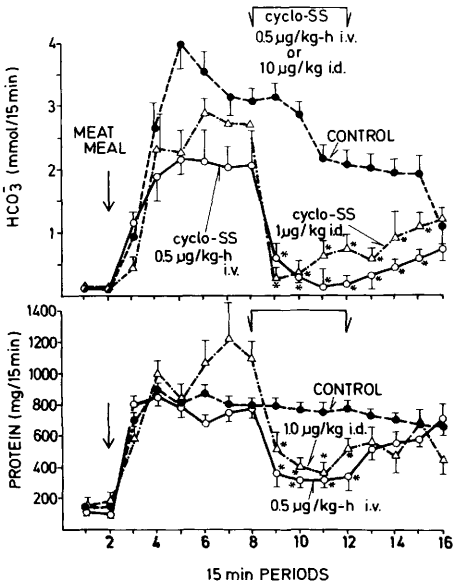

FIG. 5. Effects of cyclo-SS given iv or id on meal-induced pancreatic bicarbonate and protein secretion.

pancreatic bicarbonate and protein secretion and reduced plasma secretin. When infusion of cyclo-SS was stopped, both pancreatic secretion and plasma secretin levels tended to return to control values.

Discussion. This study provides evidence

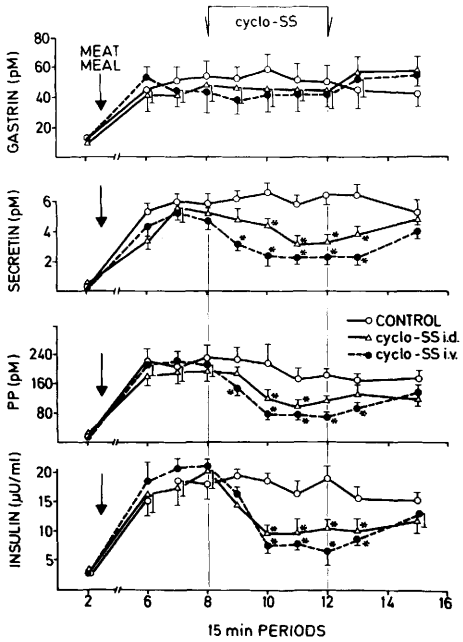

FIG. 6. Plasma gastrin, secretin, PP, and insulin levels in tests as in Fig. 5.

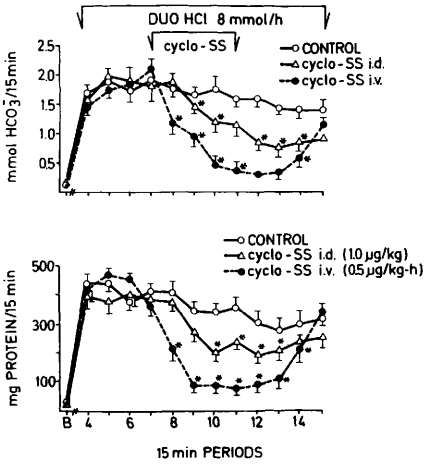

FIG. 7. Effects of cyclo-SS given iv or id on pancreatic bicarbonate and protein responses to duodenal acidification.

that the cyclic form of mini-somatostatin analog, obtained by replacing 9 of the 14 amino acids of somatostatin (SS) with a single proline (5, 6), is a highly active inhibitor of exocrine and endocrine pancreatic secretion induced by various stimulants.

The cyclo-hexapeptide was found to be at least twice as potent in inhibiting secretin-induced pancreatic bicarbonate and enzyme secretion as compared with SS-14 after an iv infusion. This high inhibitory potency of cyclo-SS could be attributed to its reduced susceptibility to degradation by peptidases. The relatively high potency and intraduodenal activity of this analog is supported by our observation that intraduodenal application of the analog in a dose only twice as large as that given intravenously resulted in similar

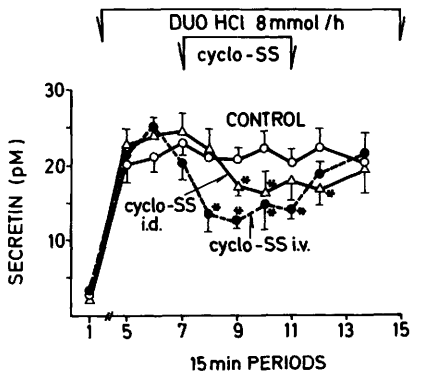

FIG. 8. Plasma secretin levels in tests as in Fig. 7.

inhibition of pancreatic secretion induced by exogenous and endogenous stimulants. Such an inhibitory action after luminal application was previously observed with SS-14 but an intraduodenal dose four to eight times larger than intravenous was required to elicit this inhibition (15).

The spectrum of biological action of cyclo-SS on exocrine pancreatic secretion appears to be similar to that of SS-14. However, the cyclo-SS may differ from SS-14 in its action on the release of certain gastrointestinal hormones. Unlike SS-14, which is a potent inhibitor of gastrin release induced by various stimulants (11, 16), cyclo-SS does not cause significant reduction in gastrin release caused by powerful physiological stimulants such as feeding of a meat meal or sham-feeding. Cyclo-SS resembles SS-14 as a suppressor of the release of secretin (4), pancreatic polypeptide (17), and insulin (18). The influence of cyclo-SS on the release of hormones that are directly involved in the control of exocrine pancreatic secretion explains its profound inhibitory effect on pancreatic responses to endogenous stimulants such as sham-feeding, ordinary feeding, or duodenal acidification.

The present study indicates that cyclo-SS suppresses pancreatic enzyme secretion induced by direct excitation of acinar cells with CCK or by a meal stimulus, which probably acts by both neural and hormonal pathways.

1. Konturek SJ, Tasler J, Krol R, Dembinski A, Coy DH, Schally AV. Effect of somatostatin analogs on gastric and pancreatic secretion. Proc Soc Exp Biol Med **155**:519–522, 1977.
2. Meyers CA, Coy DH. Somatostatin, enkephalins and endorphins. In: Glass GB, ed. Gastrointestinal Hormones. New York, Raven Press, p363, 1980.
3. Konturek SJ, Tasler J, Obtulowicz W, Coy DH, Schally AV. Effect of growth hormone release inhibiting hormone on hormone stimulating pancreatic secretion. J Clin Invest **58**:1–6, 1976.
4. Boden G, Sivitz M, Owen OE, Essa-Koumar N, Landor JH. Somatostatin suppresses secretin and pancreatic exocrine secretion. Science (Washington, DC) **190**:163–165, 1975.
5. Veber DF, Holly FW, Nutt RF, Bergstrang SJ, Brandy SF, Hirschman R, Glitzer MS, Saperstein R. Highly active cyclic and bicyclic somatostatin analogues or reduced ring size. Nature (London) **280**:512–515, 1979.
6. Veber DF, Freidinger RM, Perlow DS, Paleveda WJ, Holly FW, Strachan RG, Nutt RF, Arison BH, Honinick C, Randall WC, Glitzer MS, Saperstein R, Hirschmann R. A potent cyclic hexapeptide analogue of somatostatin. Nature (London) **292**:55–58, 1981.
7. Konturek SJ, Pucher A, Radecki T. Comparison of vasoactive intestinal peptide and secretin in stimulation of pancreatic secretion. J Physiol (London) **255**:487–509, 1976.
8. Konturek SJ, Jaworek J, Cieszkowski M, Schally AV. Effect of thyrotropin-releasing hormone on gastrointestinal secretions in dogs. Life Sci **29**:2289–2298, 1981.
9. Swierczek JS, Konturek SJ, Tasler J, Jaworek J, Cieszkowski M. Pancreatic polypeptide and vagal stimulation of gastric and pancreatic secretion in dogs. Hepatogastroenterology **28**:206–209, 1981.
10. Yalow RS, Berson SA. Radioimmunoassay of gastrin. Gastroenterology **58**:1–14, 1970.
11. Konturek SJ, Tasler J, Cieszkowski M, Coy DH, Schally AV. Effect of growth hormone release-inhibiting hormone on gastric secretion, mucosal blood flow and serum gastrin. Gastroenterology **70**:737–741, 1976.
12. Chey WB, Konturek SJ. Plasma secretin and pancreatic secretion in response to liver extract meal with varied pH and exogenous secretin in the dog. J Physiol (London) **324**:263–272, 1982.
13. Chance RE, Cieszkowski M, Jaworek J, Konturek SJ, Swierczek J, Tasler J. Effect of pancreatic polypeptide and C-terminal hexapeptide on meal and secretin-induced pancreatic secretion in dogs. J Physiol (London) **314**:1–9, 1981.
14. Berson SA. Methods in Investigative and Diagnostic Endocrinology. New York, American Elsevier, 58, 1–14, 1972.
15. Konturek SJ, Tasler J, Cieszkowski M, Jaworek J, Arimura A, Schally AV. Studies on the inhibition of pancreatic secretion by luminal somatostatin. Amer J Physiol **241**:G109–G115, 1981.
16. Bloom SR, Mortimer CH, Thorner MO, Besser GM, Hall R, Gomez-Pan A, Ray VM, Russel RC, Coy DH, Kastin AJ, Schally AV. Inhibition of gastrin and gastric acid secretion by growth hormone release-inhibiting hormone. Lancet **2**:1106–1109, 1981.
17. Floyd JC, Fajans SS, Pek S, Chance RE. Newly recognized pancreatic polypeptide plasma levels in health and disease. Rec Prog Horm Res **33**:519–570, 1977.
18. Alberti KG, Christensen SE, Iversen J, Seyer-Hansen K, Christensen NJ, Prange Hansen A, Lundbaeck K, Orskov H. Inhibition of insulin secretion by somatostatin. Lancet **2**:1299–1301, 1973.

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