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Comparison of Somatostatin and Its Highly Potent Hexa- and Octapeptide Analogs on Exocrine and Endocrine Pancreatic Secretion (42661)

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Abstract. The effects on pancreatic responses of highly potent cyclic hexapeptide (cyclo (*N*-Me-Ala-Phe-D-Trp-Lys-Thr-Phe)) (Veber analog) and octapeptide analogs of somatostatin such as D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol (SMS 201-995), D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 (RC-121), and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160) have been compared with somatostatin tetradecapeptide (SS-14) and atropine. The parameters evaluated were pancreatic responses to secretin and meat feeding in conscious dogs with chronic pancreatic fistula and amylase release from the dispersed pancreatic acini. The analogs were administered intravenously or intraduodenally. The cyclic hexapeptide and octapeptide analogs, given iv in graded doses against a constant background stimulation with secretin, produced similar and dose-dependent inhibition of pancreatic HCO_3^- and protein secretion. Analogs RC-121, RC-160, and the Veber analog were about two to four times more active than SS-14 in suppressing HCO_3^- secretion and equipotent in reducing protein secretion, but SMS 201-995 was only about half as potent as somatostatin in inhibiting HCO_3^-. RC-160 was effective in inhibiting secretin-induced protein secretion at lower doses than other analogs. In tests with feeding, SMS 201-995, the Veber analog, RC-121, and RC-160 were more potent inhibitors of exocrine pancreatic secretion of HCO_3^- and protein and exhibited more prolonged inhibitory effects than SS-14. The Veber analog, RC-121, and RC-160 were also more effective after intraduodenal administration. Atropine also caused significant inhibition of both HCO_3^- and protein responses to secretin and meal feeding. All four analogs decreased the postprandial insulin and pancreatic polypeptide release to a similar degree as SS-14. Neither SS-14 nor the analogs tested significantly affected basal or caerulein-, gastrin-, secretin-, or bethanechol-stimulated amylase release from the dispersed canine pancreatic acini. Atropine reduced amylase release induced by bethanechol, but not that stimulated by caerulein, gastrin, or secretin. This indicated that the analogs, as somatostatin, are ineffective as secretory inhibitors *in vitro*. We conclude that cyclic hexapeptide and octapeptide analogs are more potent and longer acting inhibitors of pancreatic secretion than somatostatin-14 *in vivo*.

Somatostatins, naturally occurring polypeptides consisting of 14 (SS-14) and 28 (SS-28) amino acids, were isolated from the hypothalamus (1–3) and the gut (4) and regarded as "brain-gut" peptides which display a similar range of endocrine and gastrointestinal inhibitory effects (5). The suppression of exocrine and endocrine gastric and pancreatic functions is a well-recognized action of somatostatin and, for these reasons, somatostatin has been advocated for the treatment of peptic ulcer hemorrhage (6, 7) acute pancreatitis (8), and gastrointestinal endocrine tumors (9).

Because of the very short half-life, somatostatin-14 requires constant infusion for long-term clinical use. Therefore, many attempts have been made to synthesize biologically stable analogs of somatostatin with more specific and protracted biological actions. Veber *et al.* (10, 11) reported the synthesis of cyclic hexapeptide analogs, some of which like cyclo (*N*-Me-Ala-Phe-D-Trp-Lys-Thr-Phe) (Veber analog) were found to be much more potent inhibitors of growth hormone (GH) release than the parent molecule. Bauer *et al.* (12) synthesized a series of highly

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potent octapeptide analogs of somatostatin of which D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol (SMS 201-995) was found to be 45–70 times more potent than SS-14 in tests on the inhibition of GH release. This analog is now undergoing extensive clinical investigations.

We have synthesized over 300 octapeptide amide analogs related to SMS 201-995 (13, 14). Two of them, D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 (RC-121) and D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2 (RC-160), exhibited more than 100 times higher activity in the inhibition of GH release than SS-14 and prolonged duration of action (13). SS-14 and its potent analogs were reported to inhibit exocrine pancreatic secretion, probably by interfering with the cholinergic transmission in vagal-cholinergic stimulation of the pancreas (5). These analogs also powerfully inhibited insulin, glucagon, and gastrin releases and showed antitumor activity in animal models of prostate, breast, and pancreatic cancer (4, 13, 15).

The purpose of this study was to compare the effects of SS-14 and its potent cyclic hexapeptide and octapeptide analogs as well as atropine on exocrine and endocrine pancreatic secretion induced by various exogenous and endogenous stimulants in conscious animals and on the amylase secretion from the dispersed canine pancreatic acini *in vitro*.

Materials and Methods. Studies *in vivo* were carried out on five mongrel dogs equipped with gastric fistulas (GF) and pancreatic fistulas (PF) as described before (16). Studies were started after about 2 months of recovery from the surgery. Following a fasting period of about 18 hr, the secretions from the PF were collected in 15-min aliquots, the volume was measured, and the HCO_3^- and protein concentrations and outputs were determined as described (16). Basal secretion was first collected for 30–60 min and then the secretory stimulation with secretin (2 U/kg/hr) or meat feeding (20 g/kg) was started. During each test an infusion of 0.15 *M* NaCl was given intravenously at 80 ml/hr, through the polyethylene catheter inserted into the leg vein or instilled intraduodenally (id) through the hollow obturator of the pancreatic cannula. When the secretory rate reached a plateau, SS-14 or its cyclic minianalogs were infused iv. The solutions of SS-14 or its analogs contained 1% of canine albumin (Sigma Chemical Co., St. Louis, MO) to prevent any degradation of the peptides during infusion. For the comparison, atropine sulfate was administered iv during pancreatic stimulation by secretin and meat feeding.

In tests with secretin, the GF was opened throughout the experiment to drain the gastric content to the exterior. About 60 min from the start of stimulation, when the secretory rate reached a well-sustained plateau, SS-14 or its analogs were added to the iv infusion in graded doses (range 0.12 to 2.0 μg/kg/hr), each dose being administered for a 30-min period and then doubled.

In tests with feeding, a meat meal (20 g/kg), consisting of cooked beef meat was fed with GF being closed, and when the pancreatic secretion reached a steady state, SS-14 or its analog was infused iv or id for 60 min in a constant dose of 1 or 10 μg/kg/hr. After cessation of somatostatin infusion, the collection of the postprandial secretion was continued for the final 60-min period.

Atropine was infused iv at a standard dose (25 μg/kg/hr) during secretin or meat meal stimulation. The infusion began about 60 min after the start of secretory stimulation and lasted for a 60-min period. In control tests the secretory stimulation with secretin or meat feeding alone, without SS-14 or atropine, was examined throughout the experiment.

In tests with feeding, the blood samples were withdrawn from a peripheral vein for the determination of plasma concentrations of secretin, insulin, and pancreatic polypeptide (17–19) by specific radioimmunoassays and plasma CCK by bioassay (20).

The effects of SS-14 and its analogs on amylase release *in vitro* were studied on canine-dispersed pancreatic acini prepared from 18-hr fasted mongrel dogs. Animals were anesthetized with Nembutal and the pancreas was removed and its digestion was performed using highly purified collagenase according to the method of Amsterdam *et al.* (21). Dispersed acini were suspended in the incubation medium containing amino acid mixture, bovine serum albumin, and trypsin inhibitor. The release of amylase by the acini was determined by incubation of the acini

suspension for 60 min in the medium without any stimulant (basal release) and with caerulein, gastrin, secretin, or bethanechol in various concentrations (stimulated release), with the addition of SS-14 or its analogs in various concentrations (10^{-10}–10^{-5} *M*) and atropine (10^{-6} *M*), or without. Maximal amylase release by caerulein (10^{-10} *M*), gastrin (10^{-6} *M*), secretin (10^{-6} *M*), and bethanechol (10^{-4} *M*) was determined for a comparison. Amylase contents in the supernatant and dissolved pellet were determined separately using the method of Bernfeld *et al.* (22). Amylase release was expressed as a percentage of total amylase in supernatant and in pellet times 100.

SS-14 and cyclic hexapeptide and octapeptides were synthesized by solid-phase or classical methods and repurified. Secretin and CCK33 were purchased from Kabi Diagnostica, Studvisk, Sweden. Caerulein was obtained from Farmitatlia, Milano, Italy. The comparison of the activity of the analogs was made on weight basis.

The results are presented as means ± SEM. Statistical analysis for the comparison of the means in 15- or 30-min secretory outputs was carried out by analysis of variance. Probability values of less than 0.05 were considered statistically significant.

Results. Intravenous infusion of secretin at a constant dose (2 U/kg/hr) for 210 min resulted in a marked and well-sustained pancreatic HCO_3^- secretion throughout the test period. Pancreatic protein response to secretin was rather small, but relatively well sustained during the control experiments for 210 min (Figs. 1 and 2). Addition of SS-14 and its hexapeptide or octapeptide analogs to the iv infusion resulted in a dose-dependent inhibition of both HCO_3^- and protein responses to secretin. The doses of SS-14, Veber analog, SMS 201-995, RC-121, and RC-160 reducing secretin-induced pancreatic HCO_3^- secretion by 50% were 0.78, 0.48, 2.1, 0.51, and 0.52 μg/kg/hr, respectively. Thus, RC-121, RC-160, and Veber analog were equipotent (on weight basis) as inhibitors of HCO_3^- secretion, being about twice as active as SS-14. SMS 201-995 also inhibited dose-dependently the secretin-induced secretion, but its inhibitory potency was only about half that of SS-14.

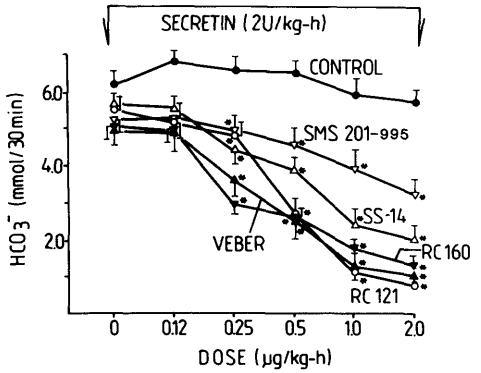

FIG. 1. Effects of SS-14 and its cyclic hexapeptide and octapeptide analogs given iv in gradually increasing doses on secretin-induced pancreatic HCO_3^- secretion. Means ± SEM of five tests on five dogs with chronic PF. Asterisks indicate significant ($P < 0.05$) decreases below the control value with secretin alone.

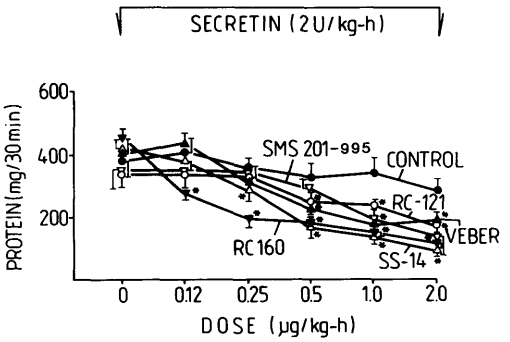

FIG. 2. Pancreatic protein outputs in tests as in Fig. 1.

SS-14 and its cyclic analogs also inhibited the protein response to secretin, but this inhibition was not dose-related and was significant only at the higher doses (0.5–2.0 μg/kg/hr), except for RC-160 which reduced significantly protein secretion at all the doses used (Fig. 2). Atropine (25 μg/kg/hr), administered during secretin-induced pancreatic secretion, caused a small but significant reduction (about 20%) of both pancreatic HCO_3^- and protein secretion (Table I).

Pancreatic secretion in response to a meat feeding was characterized by high HCO_3^- outputs, reaching about 50% of those achieved with exogenous secretin, and high protein outputs that were five to six times higher than those obtained with secretin (Figs. 3 and 4). SS-14 or its cyclic analogs

TABLE I. EFFECT OF ATROPINE GIVEN IV IN A DOSE OF 25 μg/kg/hr ON PANCREATIC HCO_3^- AND PROTEIN RESPONSES TO SECRETIN (2 U/kg/hr) OR MEAT FEEDING (10 g/kg)

	HCO_3 (μmole/hr)	Protein (mg/hr)
Secretin alone (control)	11840 ± 1240	724 ± 122
Secretin + atropine	92359 ± 940*	521 ± 94*
Feeding alone (control)	6480 ± 578	2860 ± 304
Feeding + atropine	2320 ± 370	918 ± 226*

Note. Means ± SEM of five tests on five dogs. Asterisks indicate significant ($P < 0.05$) decreases below control value obtained with feeding alone without atropine.

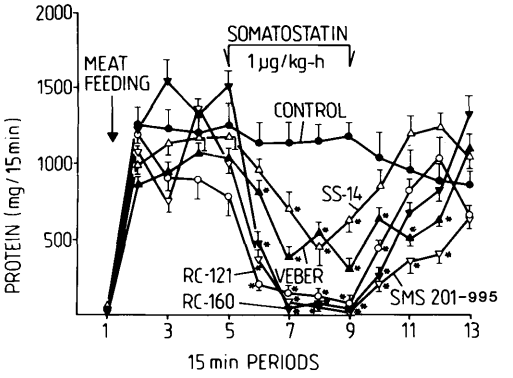

FIG. 4. Pancreatic protein outputs in tests as in Fig. 3.

given iv caused an immediate and profound inhibition of pancreatic HCO_3^- and protein secretion lasting throughout the period of infusion. The most potent inhibitors were RC-121, RC-160, and SMS-201-995, whereas SS-14 was somewhat less effective in this model of pancreatic secretion. Following the cessation of somatostatin-14 infusion there was an immediate return of the secretory response toward the control level, whereas in tests with the somatostatin analogs, the suppression of the pancreatic secretion continued for 30–60 min after the withdrawal of the peptide administration (Figs. 3 and 4). Intraduodenal installation of somatostatin peptides caused a significant decrease in pancreatic HCO_3^- and protein outputs only when Veber analog, RC-121, and RC-160 were used. SS-14 and SMS-201-995 were ineffective in this system (Table II). Atropine (25 μg/kg/hr) given iv reduced significantly the postprandial HCO_3^- secretion by about 64% and protein secretion by about 68%. This reduction was observed during and after administration of atropine (Table I).

Meat feeding resulted in a significant increase in plasma concentrations of secretin and CCK as measured by radioimmunoassay or bioassay, respectively (Figs. 5 and 6). SS-14 and its analogs significantly sup-

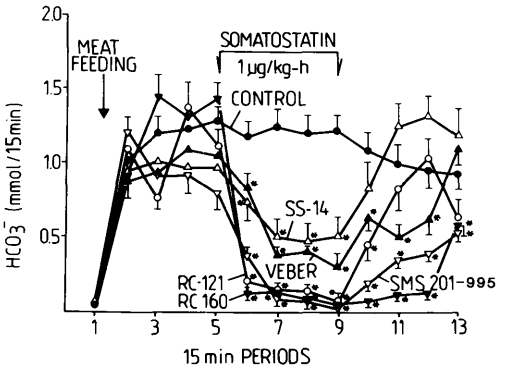

FIG. 3. Effects of SS-14 and its cyclic hexapeptide or octapeptide analogs given iv in a standard dose (1 μg/kg/hr) on pancreatic HCO_3^- response to meat feeding. Means ± SEM of five tests on five dogs with chronic PF. Asterisks indicate significant ($P < 0.05$) decreases below the control value with meat feeding alone.

TABLE II. EFFECTS OF INTRADUODENAL ADMINISTRATION OF SS-14 OR ITS ANALOGS AT A DOSE OF 10 μg/kg/hr ON THE POSTPRANDIAL PANCREATIC HCO_3^- AND PROTEIN SECRETION

	HCO_3 (μmole/hr)	Protein (mg/hr)
Feeding alone (control)	5720 ± 610	2874 ± 460
Feeding + SS-14	4724 ± 592	2472 ± 380
Feeding ± SMS 201-995	4890 ± 618	2300 ± 510
Feeding + Veber analog	3720 ± 286*	2017 ± 284*
Feeding + RC-121	4120 ± 572*	2344 ± 720*
Feeding + RC-160	4214 ± 534*	2240 ± 380

Note. Asterisks indicate significant ($P < 0.05$) decreases below the values obtained in control tests without administration of SS-14 or its analogs.

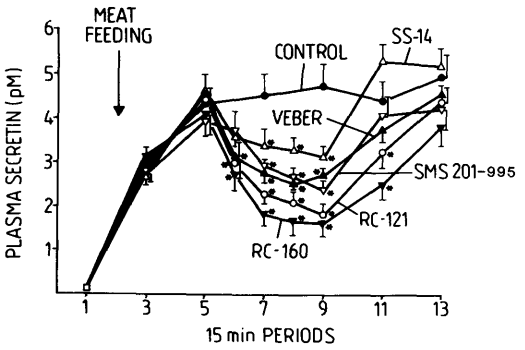

FIG. 5. Plasma secretin levels in tests as in Fig. 3.

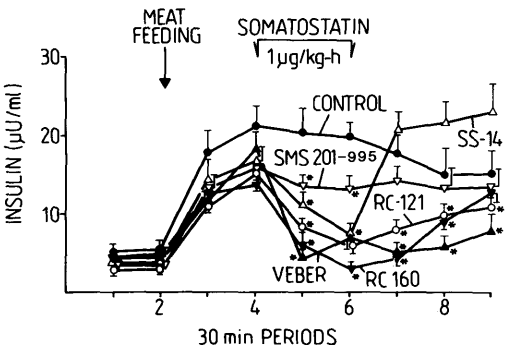

FIG. 7. Plasma insulin levels in tests as in Fig. 3.

pressed plasma levels of secretin and CCK in tests with postprandial secretion. Plasma levels of insulin and PP, were also increased after meat feeding, but SS-14 and its analogs significantly reduced them (Figs. 7 and 8). Atropine also reduced significantly the postprandial increment in plasma secretin or CCK as well as plasma insulin and PP levels (Table III).

The dispersed canine pancreatic acini released (during the standard 60-min period) under basal conditions only about 3% of the total amylase output. The acini responded to the maximal stimulation of caerulein (10^{-10} *M*), gastrin (10^{-6} *M*), and bethanechol (10^{-4} *M*) with an increase in amylase release to about 25–30% of the total output. Secretin (10^{-6} *M*) failed to affect the amylase release at the concentrations used. Neither SS-14 nor its analogs, added to the incubation medium in gradually increasing concentrations (10^{-10}–10^{-5} *M*), affected basal amylase release (Table IV). When caerulein (10^{-12} *M*), gastrin (10^{-8} *M*), and bethanechol (10^{-5} *M*) were added to the incubation medium at a concentration that resulted in about half-maximal stimulation of amylase release, somatostatin and its analogs at concentrations of 10^{-10}–10^{-5} *M* failed to inhibit this release. SS-14 and its analogs also did not change the amylase release when secretin (10^{-6} *M*) was added to the medium. Atropine added to the incubation medium at 10^{-6} *M* did not affect basal amylase release and that stimulated by submaximal concentrations of caerulein and gastrin but caused a significant reduction in bethanechol stimulated amylase secretion (Table IV).

Discussion. This study provides strong evidence that cyclic hexapeptide and octapeptide analogs of somatostatin exhibit a more powerful and protracted inhibitory action on hormonally stimulated or postprandial pancreatic secretion than native SS-14. Previous studies on analogs obtained by substitutions

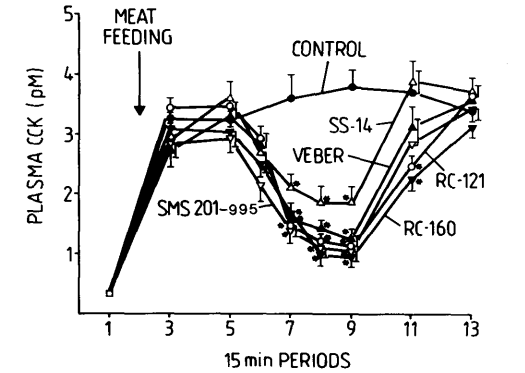

FIG. 6. Plasma CCK levels in tests as in Fig. 3.

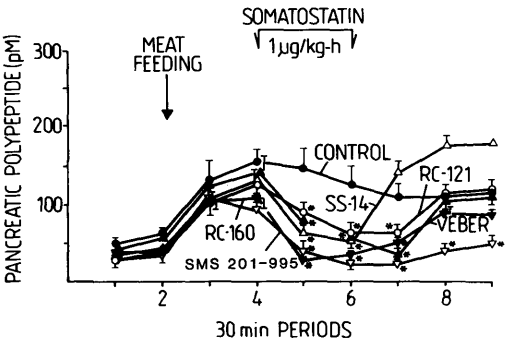

FIG. 8. Plasma PP levels in tests as in Fig. 3.

TABLE III. EFFECTS OF ATROPINE GIVEN IV AT A DOSE OF 25 μg/kg/hr ON PLASMA CONCENTRATION OF SECRETIN, CCK, INSULIN, AND PP IN TESTS WITH FEEDING AS IN TABLE I

Plasma hormone levels	Feeding alone (control)	Feeding + atropine
Secretin (p*M*)	3.9 ± 0.6	2.7 ± 0.4*
CCK (p*M*)	4.8 ± 0.7	2.2 ± 0.6*
Insulin (μU/ml)	21.4 ± 3.4	12.4 ± 3.1*
PP (p*M*)	147.8 ± 22.1	63.9 ± 14.0*

Note. Means ± SEM of five tests on five dogs. Asterisks indicate significant ($P < 0.05$) decreases below the control value obtained with feeding alone without atropine.

of certain residues in SS-14 showed that their spectrum of biological actions on the gastrointestinal secretion was similar to that of SS-14 (23). The gastric or pancreatic inhibitory activity of some analogs such as D-Trp-8-somatostatin and D-Cys-14-somatostatin was smaller than their potency for suppressing GH release *in vitro*. Previously we reported that the cyclohexapeptide of Veber *et al.* (11) was about twice as potent as SS-14 in tests on the inhibition of pancreatic secretion induced by secretin, CCK, meat feeding, sham-feeding, or duodenal acidification (24). The results of the present study confirm and reinforce our previous conclusion (24) that Veber analog (9) is a more potent and longer acting inhibitor of pancreatic secretion than SS-14 and that this inhibition is partly due to the suppression of the gut hormones stimulating exocrine pancreas such as secretin or CCK.

Our two new analogs tested in this study, RC-121 and RC-160, are structurally related to the octapeptide of Bauer *et al.* (12), but contain a C-terminal amide. Previously we determined in rats that these analogs are about 100 times more potent than SS-14 in tests for the inhibition of GH release *in vivo* (13). Their GH release suppressing activity was thus about two times greater than that reported for octapeptide of Bauer *et al.* (12). Like the analog of Bauer *et al.* (12), analogs RC-121 and RC-160 were also more selec-

TABLE IV. EFFECTS OF SS-14 AND ITS ANALOGS IN VARIOUS CONCENTRATIONS AND ATROPINE ON BASAL AND STIMULATED (CAERULEIN, GASTRIN, SECRETIN, AND BETHANECHOL) AMYLASE RELEASE (EXPRESSED AS PERCENTAGES OF TOTAL RELEASE) FROM ISOLATED PANCREATIC ACINI

Substance		Basal	Caerulein (10^{-12} *M*)	Gastrin (10^{-8} *M*)	Secretin (10^{-6} *M*)	Bethanechol (10^{-5} *M*)
Control		3.4 ± 0.6	14.1 ± 1.6	13.7 ± 1.6	4.2 ± 0.7	14.3 ± 1.8
SS-14	10^{-10} *M*	3.5 ± 0.5	13.2 ± 1.8	12.7 ± 1.4	4.5 ± 0.6	15.2 ± 2.1
	10^{-8} *M*	3.6 ± 0.6	13.8 ± 1.5	13.2 ± 1.7	3.8 ± 0.7	14.7 ± 2.0
	10^{-5} *M*	3.2 ± 0.6	12.3 ± 2.2	12.4 ± 1.6	3.9 ± 0.5	14.4 ± 1.8
SMS 201-995	10^{-10} *M*	4.1 ± 0.8	13.9 ± 1.7	14.2 ± 2.2	4.7 ± 0.6	15.2 ± 1.8
	10^{-8} *M*	3.9 ± 0.6	15.2 ± 1.8	13.8 ± 1.5	3.9 ± 0.6	14.2 ± 1.9
	10^{-5} *M*	3.5 ± 0.6	14.7 ± 1.7	14.6 ± 1.8	4.2 ± 0.8	14.8 ± 2.0
Veber analog	10^{-10} *M*	3.2 ± 0.7	15.1 ± 1.8	14.7 ± 0.9	4.0 ± 0.6	15.3 ± 1.9
	10^{-8} *M*	4.1 ± 0.8	13.9 ± 2.0	15.0 ± 1.3	2.4 ± 0.5	14.8 ± 1.6
	10^{-5} *M*	3.9 ± 0.6	15.4 ± 1.6	14.8 ± 1.6	3.1 ± 0.8	16.5 ± 1.9
RC-121	10^{-10} *M*	2.9 ± 0.5	14.8 ± 1.2	16.3 ± 2.0	4.6 ± 0.8	14.8 ± 1.7
	10^{-8} *M*	3.8 ± 0.6	15.2 ± 1.4	14.8 ± 1.6	3.9 ± 0.6	14.1 ± 1.6
	10^{-5} *M*	3.3 ± 0.5	14.8 ± 1.6	13.8 ± 1.9	4.1 ± 0.8	15.2 ± 1.5
RC-160	10^{-10} *M*	2.9 ± 0.4	15.2 ± 1.6	12.8 ± 1.6	3.8 ± 0.6	14.3 ± 1.2
	10^{-8} *M*	4.2 ± 0.8	14.5 ± 1.2	13.9 ± 1.5	3.6 ± 0.4	14.0 ± 1.6
	10^{-5} *M*	3.6 ± 0.6	13.8 ± 1.5	12.7 ± 0.9	4.3 ± 0.6	15.1 ± 1.8
Atropine	10^{-6} *M*	3.4 ± 0.5	14.1 ± 1.6	14.8 ± 1.2	4.1 ± 0.7	4.7 ± 0.6*

Note. Means ± SEM of six separate experiments. Asterisk indicates significant ($P < 0.05$) decrease below the control value.

tive, inhibiting GH release much more than insulin and glucagon secretion (13). Our present study shows that also in dogs RC-121 and RC-160 are, indeed, more potent and longer acting inhibitors of the pancreatic secretion induced by secretin or meat feeding and more effective inhibitors of the release of secretin and CCK from the gut than SS-14 or the octapeptide of Bauer *et al.* (12). Unlike SS-14 or SMS-201-995, the analogs RC-121 and RC-160 were also active after intraduodenal administration. This indicates that these peptides are resistant to the action of pancreatic proteases and that they may be absorbed from the intestine to reach the target cells in the pancreas. This may be of importance when considering the therapeutic use of these peptides. Because of the marked selectivity in actions, the inhibitory effect of these peptides on exocrine pancreatic secretion is, however, within the same order of magnitude as that of SS-14 and the Bauer octapeptide. Also, no major differences between SS-14 and cyclohexapeptide or octapeptide analogs were found in their effects on endocrine pancreatic secretion (insulin and PP). Although the inhibitory effect of RC-121 and RC-160 on insulin or PP release was more profound and longer acting than that of SS-14, the activity was much smaller than that for the suppression of the release of GH. This, again, shows a more selective inhibitory action of these analogs on the release of GH than on the pancreatic hormones.

It is of interest that, in spite of the profound inhibitory effect of SS-14 and its highly potent analogs on exocrine or endocrine pancreas *in vivo,* none of these peptides was effective in the inhibition of amylase release from the dispersed pancreatic acini. These acini responded with increased amylase secretion to caerulein, gastrin, or bethanechol, but were completely resistant to the inhibitory action of somatostatin or its analogs. Secretin failed to affect amylase release from canine pancreatic acini, and SS-14 or its analogs combined with secretin did not change this release significantly. The failure of SS-14 to affect the pancreatic secretion *in vitro* was reported recently (25), but this could be attributed to the degradation of the hormonal peptide in the incubation medium. Using somatostatin analogs, which are highly resistant to enzymatic inactivation, we expected a strong inhibitory effect of these peptides on amylase release from the dispersed pancreatic acini. The lack of effect of both native SS-14 and its potent analogs on amylase release from the pancreatic acini is difficult to explain, especially since the effects of somatostatin on the pancreas has been attributed to the competition for receptor occupancy (26) or to the alteration of cyclic AMP production (27). Moreover, the binding sites for somatostatin in the isolated acinar cells have been described (28). The possibility exists that the preparation of the pancreatic acini destroys somatostatin receptors or somehow nullifies the mechanisms that mediate the inhibitory action of somatostatin on exocrine and endocrine pancreatic cells. Such mechanisms could be related to the release of acetylcholine from the cholinergic nerve terminals in the pancreas. Somatostatin was previously reported to suppress the release of acetylcholine from the cholinergic nerves (29) and it is likely that its major mechanism of action is the interference with the cholinergic transmission in the pancreas. This is supported by our results showing close similarity of the effects of atropine and somatostatin or its analogs on exocrine (HCO_3^- and protein) and endocrine (insulin and PP) pancreatic responses to meat feeding and on exocrine pancreatic response to secretin. Atropine, like somatostatin peptides, also significantly suppressed the release of secretin and CCK induced by meat feeding.

The preparation of dispersed pancreatic acini may eliminate inhibitory effects of somatostatin mediated by cholinergic mechanisms. Indeed, our results obtained from the isolated acini demonstrate that atropine, which is known to block the muscarinic receptors, inhibited the cholinergically stimulated amylase release whereas SS-14 or its analogs were completely ineffective. The effect of atropine was specific, as it failed to affect amylase release stimulated by caerulein, gastrin, or secretin but reduced only cholinergically stimulated secretion from the dispersed pancreatic acini. The fact that somatostatin and its potent analog were completely devoid *in vitro* of their well-known inhibitory action on hormonally or cholinergi-

cally stimulated pancreatic secretion could be interpreted that *in vivo* these peptides interfere with the cholinergic mechanisms involved in the action of extrinsic and intrinsic cholinergic nerves and in the release and action of gut hormones (CCK, secretin) on pancreatic secretion. The preparation of the isolated pancreatic acini may abolish the cholinergic mechanisms responsible for the *in vivo* stimulation of exocrine and endocrine pancreas, leaving only intact muscarinic receptors at the secretory cells so that atropine but not somatostatin is capable of inhibiting the secretory activity of these cells. It is of interest that other neuropeptides including endorphins or neurotensin that were reported to affect the pancreatic secretion *in vivo* but not *in vitro* may also act via the cholinergic transmission that is abolished by the preparation of pancreatic acini or pancreatic cells (30).

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