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SPECIFIC INHIBITION OF RAT PANCREATIC INSULIN OR GLUCAGON RELEASE BY RECEPTOR-SELECTIVE SOMATOSTATIN ANALOGS

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A group of new peptide ligands displaying high selectivity for binding to somatostatin receptor subtypes 2, 3 or 5 have been used to characterize somatostatin receptor involvement in the inhibition of glucagon secretion in rats. It was found that NC-8-12 and DC-25-100, which have high affinity for SSTR2 and much less affinity for the type 5 receptor, were by far the most potent inhibitors of glucagon secretion with EC_{50}s of 48 and 18 nmole, respectively, relative to somatostatin itself (EC_{50} 131 nmole). These two analogs were actually much less potent than somatostatin in inhibiting glucose-stimulated insulin release. In contrast, DC-23-99 (a type 5 receptor selective analog), which was previously found to be a more potent inhibitor of insulin secretion than somatostatin, had considerably less potent (EC_{50} 410 nmole) effects on glucagon release. The SSTR3-specific ligands, DC-25-12 and DC-25-20, were not effective at the doses tested. The differing spectra of activities of these analogs suggest that inhibition of insulin and glucagon secretion in rats is mediated by entirely different somatostatin receptor populations.

Thus far, five sub-types of somatostatin (SS) (1) receptors have been isolated and cloned (2-5). All receptor types were found widely throughout the various regions of the brain but with discrete patterns of distribution. Peripherally, type 1 and 2 receptor mRNA was found in the pituitary, spleen, heart and intestine with particularly high concentrations of receptor 2 in pituitary, pancreas and stomach. Patterns of expression of the type 3 and 4 receptors in heart, liver, stomach, intestine, kidney, spleen and pituitary were similar. However, the type 5 receptor [in the present paper, the type 5 receptor refers to that of O'Carroll et al. (5) and the SSTR4 to that described by Bruno et al. (4)] was found mainly in the pituitary with detectable levels in spleen and intestine. The individual transfection of these receptors into stable cell lines recently allowed the binding affinities of a library of synthetic analogs to be calculated and compared to their known biological potencies (6,7). Cyclic octapeptide analogs related to octreotide (8) were found to have particularly high affinity for type 2 and considerably lower affinity for type 3 and 5 receptors. Type 2 affinity exhibited a linear correlation with *in vitro* inhibition of pituitary cell growth hormone release (6). Also, a number of linear

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SS analogs were high affinity binders to type 2, 3, and 5 receptors and, among them, several were found to display reasonable selectivity for type 3 and 5 receptors (6,7). In a subsequent study (9), five of these specific peptides were examined for their abilities to inhibit pancreatic insulin release in rats and it was concluded from their relative potencies that this important physiological process is mediated by type 5 receptors. Recently we have found that inhibition of gastrin-stimulated gastric acid secretion is mediated by type 2 receptors while bombesin-stimulated amylase secretion appears to be mediated by type 5 receptors (10). In the present study we have extended this approach to the examination of the relative potencies of the same group of analogs for lowering of radioimunoassayable glucagon levels in fasted, anesthetized rats relative to the native hormone.

MATERIALS AND METHODS

Animals. Male Charles River CD rats weighing 350-400 g were used for all experiments. The rats were housed under standard conditions and kept in an artificial 12 h light cycle while receiving standard Purina rat chow and water *ad libitum*. Eighteen hours before experiments rats were deprived of food but not water. Rats were anesthetized with pentobarbital (50 mg/kg) by the intraperitoneal (i.p.) route. Femoral veins were exposed and cannulated for continuous infusion of 0.9% NaCl. After a 1 h stabilization period, 1 ml of blood was withdrawn into siliconized tubes containing EDTA and leupeptin (200 μg), for basal glucose and glucagon/insulin measurements. Continuous intravenous (i.v.) infusion of peptide/ peptide+glucose/ glucose or 0.9% NaCl was then started and continued for the next 2 hours. Two hours after i.v. infusion was begun, successive 1 ml blood samples were drawn as described above. Pancreatic glucagon concentrations in the plasma were measured using double antibody radioimmunoassay kits (Linco Research, Inc., St Louis, MO) specifically designed for measuring pancreatic hormone in several species, including rats. Insulin concentrations in the plasma were measured using double antibody RIA kits (Binax, Inc., South Portland, Maine).

Peptides. **SS** (Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys); **DC-25-100** (D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2); **NC-8-12** (D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Nal-NH_2); **DC-25-20** (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2); **DC-25-12** (D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2); **DC-23-99** (D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2) were synthesized by standard solid phase methods and, after hydrogen fluoride cleavage and cyclization of Cys-containing peptides, purified by HPLC. They were characterized by amino acid analysis and molecular weight determinations by matrix-assisted laser desorption mass spectrometry (Finnegan). Peptides were studied in doses 0.1, 1, 10, 100 and 250 nmoles/kg/h and were i.v. infused in sterile 0.9% saline solution containing 0.1% bovine serum albumin (BSA, Fraction V, Sigma Chem.Comp., St. Louis, MO).

Calculations. Dose-effect curves were constructed from data obtained at five different concentrations of the peptides. Statistical comparisons of means within a group were made using Student's paired t test. Comparisons between means of different groups were made by analysis of variance followed by Newman-Keuls post hoc procedures. Differences were accepted as significant at $p<0.05$. EC_{50} values were calculated by means of non-linear regression analysis of the concentration response curves using the GraphPad computer program.

RESULTS AND DISCUSSION

Somatostatin, has been shown to potently inhibit insulin and glucagon secretion from the endocrine pancreas of several species including humans (11). It was suggested also that some SS analogs could preferentially inhibit glucagon more than insulin release (12,13), observations in retrospect possibly indicating a greater degree of type 2 receptor specificity for the analogs employed. It has been impossible up until now, however, to specifically attribute SS-inhibited pancreatic secretions to any receptor subtype since the peptide analogs used could not be characterized in this fashion until very recently.

The development of a panel of SS analogs with differing selectivities for the recently cloned receptor types SSTR2, SSTR3 and SSTR5 offered a unique opportunity for relating specific receptors to a particular biological activity. We have already reported that insulin release appears to be primarily regulated by type 5 SS receptors (9) and gastric acid and pancreatic exocrine secretions by SS receptor subtypes 2 and 5, respectively (10). These studies have now been extended to the glucagon system using similar peptide infusion protocols which compensate for the differing pharmacokinetic properties of the various peptides used. In fact, the time course of activity of the linear peptide, DC-23-99, compares favorably with those of octreotide and lanreotide (DC-25-100) after i.v. injection into rats (unpublished observations). Dose responses (% inhibition relative to control animals) of SS and the five analogs with respect to pancreatic glucagon inhibition are shown in Figure 1a and are compared directly to insulin release in Figure 1b. The results at each dose level were the average of between 6-8 separate experiments and EC_{50} values calculated for each analog from the data in Figures 1a and 1b are shown in Table 1 where they are compared to previously acquired

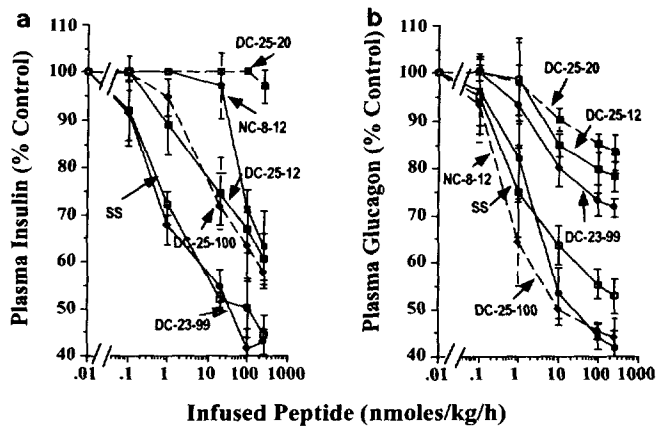

Figure 1. Dose-dependent inhibition of pancreatic glucagon secretion (a) and glucose-stimulated insulin secretion (b) produced by i.v. infusion of various doses of SS-analogues. The results at each dose level are the average of 6-8 separate experiments ± SEM.

Table 1. Comparison of binding affinities of cyclic and linear SS octapeptides for 5 SS receptors on transfected cells and *in vivo* inhibition of rat insulin, glucagon, gastric acid, and amylase release

	IC_{50} (nM) SS Receptor [a]					EC_{50} (nM) *in vivo*			
Peptide	1	2	3	4	5	Insulin[b]	Glucagon	H+[c]	amylase[c]
SS	0.1	0.28	0.07	1.2	0.86	2.91	131	1.65	1.08
DC-25-100	>1000	1.60	4.9	>1000	0.1	55.7	18.2	1.44	0.47
NC-8-12	>1000	<0.01	11	>1000	6.0	183	48.0	1.26	1.19
DC-25-12	>1000	46	0.03	77	1.2	109	>1000	52.0	8.04
DC-23-99	23	32	0.42	18	0.002	1.53	410	>1000	1.08
DC-25-20	>1000	>1000	0.02	158	43	>1000	>1000	63.0	>1000

[a]Taken from refs. 6,7; [b]Taken from ref. 9; [c]Taken from ref. 10.

K_d values derived from binding experiments with cells transfected with the five known SS receptor sub-types (6,7), IC_{50} values derived from binding to rat pancreatic acinar cells (10) and EC_{50} values derived from *in vivo* inhibition of insulin (9), and gastric acid and pancreatic amylase secretion (10).

The data presented in Table 1 and Figure 1a indicate that the cyclic octapeptides, NC-8-12 and DC-25-100, were more potent than SS in inhibiting glucagon release *in vivo*, a reversal of the situation with respect to insulin (Figure 1b). Therefore, as with GH (6) and gastric acid (10), the most potent analogues for glucagon inhibition are the type 2-receptor selective ligands. In binding studies, NC-8-12 displays extremely high SSTR2 affinity relative to other cyclic SS octapeptides such as DC-25-100. In the present study, this extremely high SSTR2 affinity for transfected cells is not accompanied by higher biological activity since NC-8-12 is actually less potent than DC-25-100 on glucagon release. This has also been the case with respect to inhibition of rat gastric acid release where no real differences in the potencies of these two analogs was seen (Table 1). Another dramatic difference between analog effects on glucagon compared to insulin release is observed for DC-23-99 which has minimal effects on release of the former but very potent effects on insulin (Figures 1a and 1b) (9). This analog has very high affinity for SSTR5 (Table 1) and significant affinity for SSTR3 so that the involvement of these receptors in glucagon release appears unlikely. Also, their involvement of SSTR3 appears even more unlikely since the high affinity, SSTR3-selective analog (DC-25-20) had only slight activity on glucagon release at the highest doses tested. In a final experiment, the possibility that alterations in glucagon levels might result from the potent inhibition of insulin release by some analogs *via* some undefined feedback process was examined. Infusion of insulin antibodies in order to completely eliminated insulin from circulating blood had no

Table 2. Effect of somatostatin agonist, DC-25-100, and anti-insulin serum (AIS) infusion on plasma glucagon and insulin level

	Time (min)	Control (NaCl+AIS) (n=6)	DC-25-100 (100 nM)+AIS (n=6)	DC-25-100 (100 nM)+NS (n=6)
Glucagon (pg/ml)	0	21.6 ± 2.8	21.9 ± 1.3	25.8 ± 2.9
	120	48.6 ± 2.3	16.3 ± 0.9	14.6 ± 0.8
Insulin (μU/ml)	0	20.6 ± 3.2	18.3 ± 3.0	21.5 ± 1.6
	120	0.0	0.0	16.6 ± 3.0

observable effect (Table 2) on the inhibition of glucagon secretion by lanreotide (DC-25-100), the most potent analog on this process.

Thus the present data offers the first proof that SS inhibition of release of the insulin and glucagon is under the control of separate receptor subtype populations on pancreatic endocrine cells. The accumulating data on the type-5 selective linear analog, DC-23-99, reveals it to be uniquely effective in lowering insulin levels, at least in the rat. Assuming that this phenomenon extrapolates fully to humans, it is possible that this type of analog, having simple structures based on little more than a central fragment of SS, could prove useful in the specific relief of hyperinsulinemic conditions.

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