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Relative Potencies of the Somatostatin Analogs Octreotide, BIM- 23014, and RC-160 on the Inhibition of Hormone Release by Cultured Human Endocrine Tumor Cells and Normal Rat Anterior Pituitary Cells

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ABSTRACT

In the present study we investigated the effects of the somatostatin (SS) analogs octreotide, RC-160, and BIM-23014 on GH release by cultured cells of human GH-secreting pituitary tumors, in normal rat anterior pituitary cells, and on gastrin release by cultured cells from a human gastrinoma.

In all GH-secreting adenomas and in rat anterior pituitary cells, RC-160 was the most potent compound. RC-160 significantly inhibited GH-, PRL, and/or α-subunit release by human GH-secreting pituitary adenoma cells in concentrations as low as 10^{-12}-10^{-14} M, whereas at the same concentrations, octreotide and BIM-23014 did not inhibit or were significantly less effective in inhibiting GH release ($P < 0.01$, RC-160 *vs.* octreotide and BIM-23014). In rat anterior pituitary cell cultures, the IC_{50} values for inhibition of GH release were, in rank order of potency, 0.1, 5.3, 47, 48, and 99 pM for RC-160, SS-14, BIM-23014, octreotide, and SS-28, respectively. Maximal inhibitory effects by the three analogs were the same in the human GH adenoma cell cultures and the rat anterior pituitary cell cultures (−60%). On the basis of these data, RC-160 appears to be about 500 times more potent than octreotide and BIM-23014 in inhibiting GH release by rat anterior pituitary cells *in vitro*. Forskolin (100 μM) as well as pretreatment of the cells with pertussis toxin significantly diminished the inhibitory effects of the three SS analogs and those of SS-14 and SS-28 to the same extent. The latter data suggest that octreotide, RC-160, and BIM-23014 act mainly via a pertussis toxin-sensitive G-protein and an adenylyl cyclase-dependent mechanism. In the human gastrinoma culture, RC-160 inhibited gastrin release significantly more than octreotide at 10^{-12}- and 10^{-14}-M concentrations ($P < 0.01$).

In conclusion, the SS analogs octreotide, RC-160, and BIM-23014 may have significant different potencies of inhibition of hormone release *in vitro*, with RC-160 being the most potent SS analog and octreotide and BIM-23014 having similar potencies. Depending on the pharmacokinetic properties of these three octapeptide SS analogs, these observations may have consequences for the medical therapy of patients with SS receptor-positive endocrine tumors. (*Endocrinology* **134:** 301–306, 1994)

SOMATOSTATIN-14 (SS-14) is a tetradecapeptide that has inhibitory effects in a variety of endocrine tissues (1). The effects of SS (analogs) are mediated via high affinity membrane receptors belonging to the family of G-protein-coupled receptors. For clinical use, primarily in the treatment of GH hypersecretion in patients with GH-secreting pituitary adenomas, several analogs of SS have been developed, which have a considerably longer biological half-life than SS-14 and do not cause a rebound GH hypersecretion as has been shown for SS-14 (2). Of these analogs, octreotide, BIM-23014, and RC-160, only octreotide has obtained clinical importance so far (2). Bauer *et al.* (3) originally developed the highly potent octapeptide analog octreotide. Thereafter, several analogs related to octreotide were synthesized and tested for their biological activity by Cai *et al.* (4, 5). The latter investigators suggested that RC-160, one of their analogs, might be more specific for GH inhibition and had lower activity for inhibition of insulin and glucagon release *in vivo*. In addition, Liebow and co-workers (6) demonstrated an effect of RC-160, but not octreotide, on cell growth and tyrosine phosphatase activity in the Mia-PaCa-2 human pancreatic tumor cell line. On the basis of recent data by other investigators, this discrepancy between the effects of RC-160 and octreotide can be challenged, however (7, 8).

In vitro studies have shown differential binding of SS analogs in normal rat brain and adenohypophysis (9). In certain human tumors (breast cancer, ovarian cancer, and pancreatic cancer), differences in binding characteristics have been reported between octreotide and RC-160 (10).

The above data suggest that in normal tissues and in certain tumors, there may be differences in receptor binding characteristics and biological effects among the different SS analogs. However, there are as yet no *in vitro* studies that compare the effects of all three of the above-mentioned SS analogs in an established culture system and in primary cultures of SS receptor (SS-R)-positive endocrine tumors. In the present study, therefore, we compared the inhibitory effects of octreotide, BIM-23014, and RC-160 on GH release by cultured human GH-secreting pituitary adenoma cells and rat anterior pituitary cells and those of octreotide and RC-160 on gastrin release by a cultured human gastrinoma. In addition, we performed experiments dealing with the mechanism of action of inhibition of GH release by these SS analogs in the human GH adenoma and rat anterior pituitary cell cultures.

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Materials and Methods

Cell culture

Tumor tissue from eight untreated acromegalic patients (patients 1–8) was obtained by transsphenoidal operation. Single cell suspensions of the adenoma tissue were prepared by enzymatic dissociation with dispase, as described in detail previously (11).

Tumor tissue from a patient with a gastrinoma was dissociated with collagenase (type I, Sigma Chemical Co., St. Louis, MO), as described previously (12).

Dispersed rat anterior pituitary cells were prepared by enzymatic dissociation of anterior pituitaries from female Wistar rats, weighing 180–200 g, as previously described in detail (13). Animals were kept, treated, and cared for in accordance with the guidelines approved by the European Community on November 24, 1986.

The cells were plated at a density of 10^5 cells/well in 1 ml culture medium in multiwell plates (48 wells; Costar, Cambridge, MA). The human GH-secreting tumor cells and human gastrinoma cells were allowed to attach for 4 days at 37 C in a CO_2 incubator. Thereafter, the medium was changed, and 24- or 96-h incubations without or with test substances were carried out in quadruplicate. The rat anterior pituitary cells were allowed to attach for 4 days, after which the medium was changed. After another medium change on day 7 of culture, 4-h incubations without or with test substances were performed in quadruplicate. In the experiment with pertussis toxin (PT), the cells were preincubated for 2 h with PT before the 4-h incubation. At the end of the incubation, the medium was removed and centrifuged for 5 min at 600 × *g*, and the supernatant was stored at −20 C until analysis. Forskolin and PT were used in concentrations of 100 μM and 50 μg/liter, respectively.

The culture medium in all experiments was Minimum Essential Medium with Earle's salts (MEM) supplemented with nonessential amino acids, sodium pyruvate (1 mmol/liter), 10% fetal calf serum (FCS), penicillin (1×10^5 U/liter), fungizone (0.5 mg/liter), L-glutamine (2 mmol/liter), and sodium bicarbonate (2.2 g/liter). The medium was adjusted to pH 7.4 with 1 mol/liter NaOH.

Test substances

PT and SS-14 were obtained from Sigma. Forskolin was obtained from Calbiochem Corp. (La Jolla, CA). BIM-23014 (SS tumor-inhibiting analog) and SS-28 were obtained from Bissendorf Biochemicals (Hannover, Germany). RC-160 was obtained from Peninsula (Belmont, CA). Octreotide was obtained from Sandoz (Basel, Switzerland).

The structures of octreotide, BIM-23014, and RC-160 are shown in Fig. 1.

Hormone determinations

Human GH and PRL concentrations in the culture medium were determined by immunoradiometric assays, as described previously (11). Glycoprotein α-subunit concentrations in the medium were determined by a double antibody RIA, as described previously (11). Rat GH and rat PRL concentrations were determined by double antibody RIAs, using materials supplied by the NIDDK, as described in detail previously (13, 14).

Gastrin concentrations were determined by a double antibody RIA from Diagnostic Products Corp., as described previously (12).

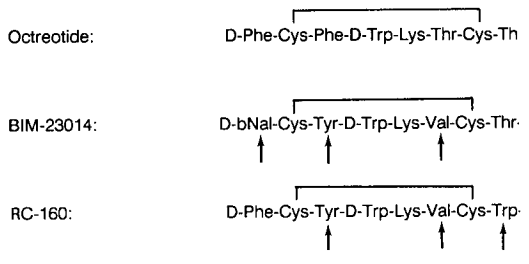

FIG. 1. Structures of the SS analogs octreotide, BIM-23014, and RC-160. *Arrows* indicate differences from octreotide.

Data analysis

The statistical significance of the differences between mean values was determined using two-way analysis of variance. When significant overall effects were obtained by analysis of variance, multiple comparisons were made using the Newman-Keuls test (15). Differences were considered significant when $P < 0.05$. Each experiment with rat anterior pituitary cells is representative of at least two independent experiments.

Results

Effects of SS analogs on GH-, PRL-, and α-subunit release by cultured human GH-secreting pituitary adenoma cells

Table 1 shows the effects of octreotide, BIM-23014, and RC-160 on GH release by five cultures of human GH-secreting pituitary adenoma cells. In all cultures, GH release was significantly inhibited by the three analogs in a dose-dependent fashion. Although the maximal inhibitory effects of the analogs were comparable in all cultures, there were considerable differences in the concentration range over which the analogs inhibited GH release. RC-160 significantly inhibited GH release in concentrations up to 10^{-12} M in four cultures (patients 1–4; $P < 0.01$ *vs.* control) and in a concentration up to 10^{-14} M in one culture (patient 5), whereas at these concentrations, octreotide and BIM-23014 did not inhibit or were significantly less effective ($P < 0.01$ *vs.* effect of RC-160 in cultures of patients 1, 2, 3, and 5; $P < 0.05$ *vs.* effect of RC-160 in culture of patient 4) in inhibiting GH release in five cultures (patients 1–5) and four cultures (patients 2–5), respectively. Although in most cultures, a 24-h incubation with the SS analogs was performed (except in the culture of patient 3, which represents a 96-h incubation), we found a comparable difference in the potencies of GH inhibition between RC-160, octreotide, and BIM-23014 in cultures that were incubated for 4 or 96 h (data not shown), suggesting that the differences in the potencies between the analogs are not due to degradation of the drugs. In four cultures (patients 1, 3, 5, and 6) that secreted PRL and/or α-subunit as well as GH, the effects of octreotide and RC-160 on all three hormones were comparable. Again, RC-160 was significantly more potent than octreotide in inhibiting hormone release (GH, PRL, and α-subunit). As an example, Fig. 2 shows that in the culture from patient 6 (not shown in Table 1), RC-160 was significantly more effective (in concentrations of 10^{-12} and 10^{-14} M) than octreotide ($P < 0.01$ *vs.* effect of octreotide) in inhibiting GH, PRL, and α-subunit release. Two other cultures (patients 7 and 8) that secreted GH and PRL simultaneously showed a low sensitivity of PRL release to the inhibitory effects of the somatostatin analogs, whereas GH release was significantly inhibited at the same time (Table 2). This demonstrates the specificity of the effects of the SS analogs. To study the involvement of a G-protein in the inhibitory effects of octreotide, BIM-23014, and RC-160, the cells from two cultures were pretreated with PT. In both cultures (patients 4 and 5), PT significantly reduced the inhibitory effect of the three SS analogs on GH release, although this was most evident in the culture from patient 5. This is shown in Table 3.

TABLE 1. The effects of octreotide (OCTR), RC-160, and BIM-23014 on GH release by cultured human GH-secreting pituitary tumor cells

Drug	Conc. (log M)	Patient 1 (24 h)	Patient 2 (24 h)	Patient 3 (96 h)	Patient 4 (24 h)	Patient 5 (24 h)
OCTR	0	1663 ± 47 (100)	8083 ± 321 (100)	1468 ± 35 (100)	48 ± 1 (100)	1149 ± 29 (100)
	−6	783 ± 49 (47)[a]	2899 ± 146 (36)[a]	454 ± 5 (31)[a]	—	—
	−8	585 ± 25 (35)[a]	2664 ± 102 (33)[a]	403 ± 10 (27)[a]	35 ± 2 (73)[a]	400 ± 8 (35)[a]
	−10	707 ± 27 (43)[a]	3047 ± 80 (38)[a]	442 ± 8 (30)[a]	40 ± 2 (83)[b,d]	415 ± 5 (36)[a]
	−12	1303 ± 43 (78)[b,c]	7627 ± 316 (94)[c]	848 ± 44 (58)[a,c]	48 ± 2 (100)[d]	773 ± 29 (67)[a,c]
	−14	—	—	—	—	1024 ± 38 (89)[b,c]
RC-160	−6	597 ± 20 (36)[a]	2267 ± 205 (28)[a]	527 ± 12 (36)[a]	—	—
	−8	553 ± 25 (33)[a]	2133 ± 192 (26)[a]	437 ± 26 (30)[a]	33 ± 2 (69)[a]	377 ± 19 (33)[a]
	−10	593 ± 28 (36)[a]	2000 ± 29 (25)[a]	413 ± 15 (28)[a]	37 ± 1 (77)[a]	401 ± 15 (35)[a]
	−12	810 ± 62 (49)[a,d]	2967 ± 148 (37)[a,c]	470 ± 25 (32)[a]	41 ± 2 (85)[b]	523 ± 40 (46)[a,c]
	−14	—	—	—	—	530 ± 7 (46)[a]
BIM-23014	−6	—	2200 ± 29 (27)[a]	427 ± 12 (29)[a]	—	—
	−8	—	2000 ± 29 (25)[a]	405 ± 25 (28)[a]	35 ± 2 (73)[a]	520 ± 16 (45)[a]
	−10	—	2633 ± 192 (33)[a]	465 ± 5 (32)[a]	47 ± 1 (98)[c]	642 ± 21 (56)[a,c]
	−12	—	4533 ± 219 (56)[a,c]	765 ± 15 (52)[a,d]	46 ± 2 (96)	825 ± 26 (72)[a,c]
	−14	—	—	—	—	1011 ± 34 (88)[b,c]

n = 4 wells/treatment group. —, Not tested. Numbers in parentheses refer to the percentage of control release.
[a] $P < 0.01$ *vs.* control.
[b] $P < 0.05$ *vs.* control.
[c] $P < 0.01$ *vs.* previous lower concentration of SS analog.
[d] $P < 0.05$ *vs.* previous lower concentration of SS analog.

Effects of SS analogs on gastrin release by human gastrinoma cells

Figure 3 shows the effects of octreotide and RC-160 on gastrin release by cultured cells from a human gastrinoma. Both analogs significantly inhibited gastrin release in a dose-dependent manner in concentrations as low as 10^{-14} M (octreotide: 10^{-14} *vs.* 10^{-12} M, $P < 0.05$; 10^{-12} *vs.* 10^{-10} M, $P < 0.01$; RC-160: 10^{-14} *vs.* 10^{-12} M, $P < 0.05$). However, at 10^{-12} and 10^{-14} M, RC-160 inhibited gastrin release significantly more than octreotide ($P < 0.01$ *vs.* effect of octreotide). Again, the maximal inhibitory effects of octreotide and RC-160 were similar. Insufficient cells were isolated from this tumor to study the effect of BIM-23014.

Effects of SS analogs, SS-14, and SS-28 on GH release by cultured rat anterior pituitary cells

In Fig. 4, the effects of octreotide, BIM-23014, RC-160, SS-14, and SS-28 on GH release by cultured rat anterior pituitary cells are shown. All SS (analogs) inhibited GH release maximally and to the same extent (by ~60%) at 10^{-8} M. However, the dose-response curve of RC-160 was significantly different from those of SS-14, BIM-23014, octreotide, and SS-28. Although RC-160 significantly inhibited GH release up to 10^{-14} M, no other somatostatin (analogs) significantly inhibited GH release at 10^{-12} or 10^{-14} M. IC_{50} values for inhibition of GH release were, in rank order of potency, 0.1, 5.3, 47, 48, and 99 pM for RC-160, SS-14, BIM-23014, octreotide, and SS-28, respectively. On the basis of these data, RC-160 appears to be about 500 times more potent than octreotide and BIM-23014 in inhibiting GH release by rat anterior pituitary cells *in vitro*. In 24- and 96-h incubations, this significant difference in the potency of inhibition of GH release among RC-160, octreotide, and BIM-23014 was still present (data not shown), but at the same time the IC_{50} value of inhibition of GH release by SS-14 was significantly lower (500 and 8700 pM, respectively), pointing to degradation of SS-14, but not of the SS analogs.

We also investigated the effect of a 2-h preincubation with 50 μg/liter PT. Table 4 shows that pretreatment of the cells with PT significantly reduced the inhibitory effects of the three SS analogs, SS-14, and SS-28 on GH release. In addition, simultaneous exposure of the cells to a maximally active concentration of forskolin (100 μM) resulted in significantly less inhibition of GH release by all SS analogs tested (Table 4; $P < 0.01$ *vs.* percent effect on control untreated cells).

Discussion

The present study shows for the first time that the SS analogs octreotide, BIM-23014, and RC-160 have different potencies of inhibition of GH release by primary cultures of human GH-secreting pituitary tumor cells and normal rat anterior pituitary cells, and of inhibition of gastrin release by a primary culture of a human gastrinoma *in vitro*. In all cases, RC-160 was clearly the most potent in its inhibitory action. Although the *in vivo* and *in vitro* effects of octreotide, BIM-23014, and RC-160 have been studied extensively, no studies have been performed so far comparing simultaneously the three SS analogs available for clinical use in primary cultures of human endocrine tumor cells and rat anterior pituitary cells.

Bauer *et al.* (3) originally developed a series of highly potent octapeptide analogs of SS-14, of which octreotide was the most active. Thereafter, several other analogs, related to octreotide, were synthesized and tested for their biological activity by Cai and co-workers (4, 5). Cai *et al.* found that substitution of Phe and Thr by Tyr and Val, respectively, at positions 3 and 6 resulted in a much higher potency for

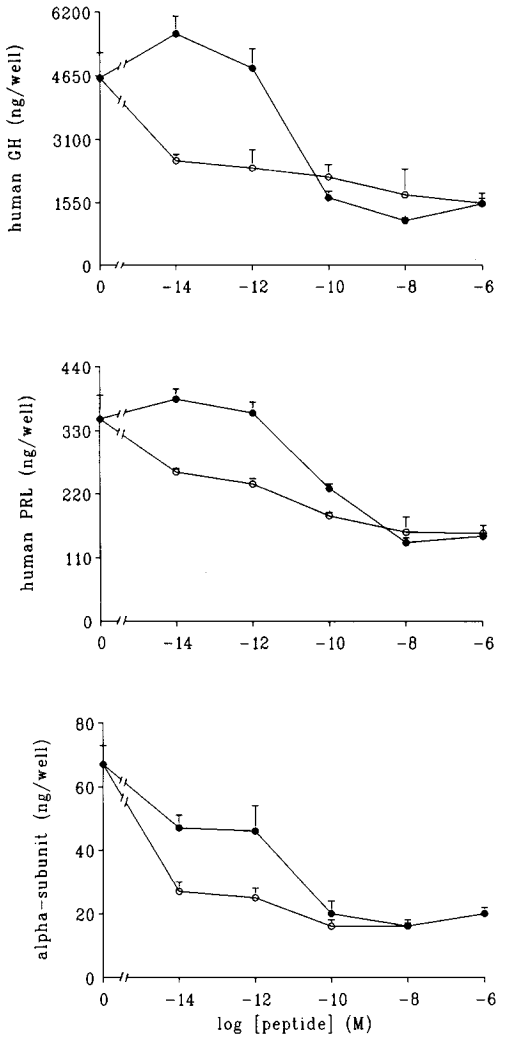

FIG. 2. Dose-response relationship of the effects of the SS analogs octreotide and RC-160 on hormone release by a cultured mixed GH-, PRL-, and α-subunit-secreting human pituitary adenoma (patient 6). Cells (10^5) were cultured for 4 days in MEM-10% FCS. On day 4 of culture, the medium was changed, and a 24-h incubation without or with octreotide (●) and RC-160 (○) was performed in quadruplicate. Values are the mean ± SE.

inhibition of GH secretion, but lower activity for inhibition of insulin and glucagon release *in vivo* in rats. They also suggested that replacing Thr at position 8 by Trp may result in increased receptor affinity. This suggests that the Tyr^3/Val^6-containing series of analogs might be more specific for GH inhibition, and there may be selectivities in the biological actions of SS octapeptide analogs. Both RC-160 and BIM-23014 are Tyr^3/Val^6-containing analogs, whereas RC-160 also has Trp at position 8. On the basis of the results from our present study, we can confirm this high potency of inhibition of GH secretion by RC-160 in cultures of human GH-secreting pituitary adenomas and in one culture of a human gastrinoma. In GH-secreting pituitary adenomas, octreotide and BIM-23014 inhibited GH release to the same extent, whereas RC-160 was clearly the most active compound. In cultured rat anterior pituitary cells, RC-160 was about 500 times more potent than octreotide and BIM-23014.

TABLE 2. Effects of SS analogs on GH and PRL release by cultured GH-secreting pituitary adenoma cells

Patient no.	Treatment	GH (μg/liter)	PRL (μg/liter)
7	Control	1583 ± 75	165 ± 3
	Octr (10^{-8} M)	808 ± 51 (51)[a]	177 ± 6 (107)
	BIM (10^{-8} M)	950 ± 33 (60)[a]	190 ± 6 (115)
	RC (10^{-8} M)	938 ± 17 (59)[a]	157 ± 7 (95)
8	Control	269 ± 17	51 ± 3
	Octr (10^{-10} M)	138 ± 4 (51)[a]	43 ± 2 (84)[b]
	RC (10^{-10} M)	138 ± 6 (51)[a]	43 ± 2 (84)[b]

n = 4 wells/treatment. The incubation times were 24 and 96 h for cultures from patients 7 and 8, respectively. Octr, Octreotide; BIM, BIM-23014; RC, RC-160. Values are the mean ± SE. Numbers in parentheses refer to the percentages of control values.

[a] $P < 0.01$ *vs.* control cells.

[b] $P < 0.05$ *vs.* control cells.

TABLE 3. Effects of pretreatment with PT on the inhibitory effects of octreotide (Octr), RC-160 (RC), and BIM-23014 (BIM) on GH release by cultured human GH-secreting pituitary adenoma cells

Patient no.	Treatment	GH release (ng/well)	
		− PT	+ PT
4	Control	48 ± 1	59 ± 1[a]
	Octr (10^{-8} M)	35 ± 2 (73)[b]	49 ± 1 (83)[b,c]
	BIM (10^{-8} M)	35 ± 2 (73)[b]	49 ± 1 (83)[b,c]
	RC (10^{-8} M)	33 ± 2 (69)[b]	48 ± 1 (81)[b,c]
5	Control	1149 ± 29	1641 ± 59[a]
	Octr (10^{-9} M)	398 ± 7 (35)[b]	1452 ± 47 (88)[b,d]
	BIM (10^{-9} M)	608 ± 6 (53)[b]	1521 ± 59 (93)[d,e]
	RC (10^{-9} M)	388 ± 12 (34)[b]	1284 ± 22 (78)[b,d]

n = 4 wells/treatment. Values are the mean ± SE. The incubation time was 24 h for cultures from patients 4 and 5; before this 24-h incubation, the cells were preincubated for 2 h with 50 μg/liter PT. Numbers in parentheses refer to the percentages of control values.

[a] $P < 0.01$ *vs.* control without PT.

[b] $P < 0.01$ *vs.* control cells.

[c] $P < 0.05$ *vs.* effect of SS analog on cells without PT.

[d] $P < 0.01$ *vs.* effect of SS analog on cells without PT.

[e] $P < 0.05$ *vs.* control cells.

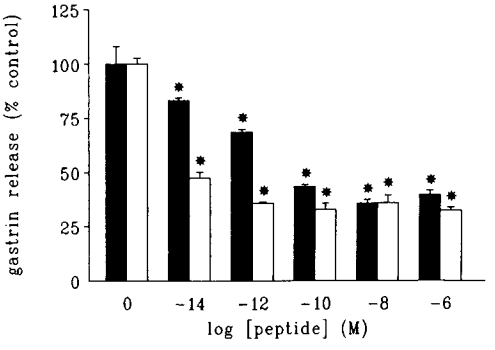

FIG. 3. Dose-response relationship of the effect of the SS analogs octreotide and RC-160 on gastrin release by cultured cells of a human gastrinoma. Cells (10^5) were cultured for 4 days in MEM-10% FCS. On day 4 of culture, the medium was changed, and a 24-h incubation without or with octreotide (■) and RC-160 (□) was performed in quadruplicate. Values are the mean ± SE; basal gastrin release values are 13 ± 1 and 15 ± 0.5 ng/well, respectively. *, $P < 0.01$ *vs.* control.

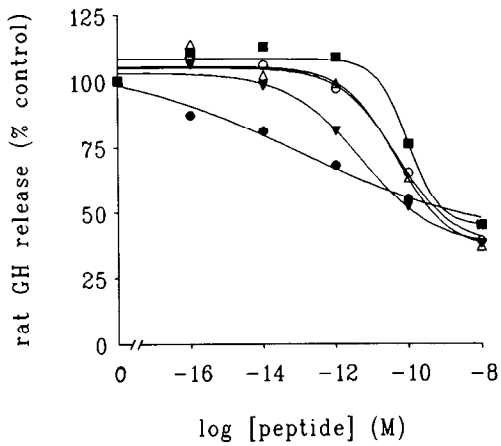

FIG. 4. Dose-response relationship of the effects of SS and SS analogs on GH release by cultured normal rat anterior pituitary cells. On day 7 of culture, 4-h incubations were performed in quadruplicate. Values are expressed as a percentage of control GH release and represent the mean of two independent experiments. ●, RC-160; ▼, SS-14; △, BIM-23014; ○, octreotide; ■, SS-28.

TABLE 4. Effects of forskolin and PT on the inhibitory effects of SS and SS analogs on GH secretion by cultured rat anterior pituitary cells

Treatment	Control	Forskolin (100 μM)	PT (50 μg/liter)
Control	100 ± 4	100 ± 6	100 ± 2
Octr (10^{-8} M)	41 ± 3[a]	79 ± 1[a,b]	81 ± 1[a,b]
RC-160 (10^{-8} M)	44 ± 3[a]	78 ± 2[a,b]	83 ± 2[a,b]
BIM (10^{-8} M)	43 ± 2[a]	69 ± 3[a,b]	92 ± 2[a,b]
SS-14 (10^{-8} M)	41 ± 2[a]	68 ± 3[a,b]	76 ± 2[a,b]
SS-28 (10^{-8} M)	31 ± 3[a]	70 ± 4[a,b]	74 ± 4[a,b]

Rat anterior pituitary cells were cultured for 7 days. On day 7 of culture, 4-h incubations without or with test substances were performed in quadruplicate. Before this 4-h incubation, a 2-h preincubation with PT was performed. All effects are expressed as a percentage of the respective control value. Forskolin stimulated basal GH release to 345 ± 8% of control GH release, and PT slightly stimulated GH release to 117 ± 5%. n = 4 wells/treatment group. Values are the mean ± SE. Octr, Octreotide; BIM, BIM-23014.

[a] $P < 0.01$ *vs.* control.

[b] $P < 0.01$ *vs.* effect of SS (analog) in control cells.

On the basis of many studies it has become evident that the SS-R shows both pharmacological and molecular heterogeneity, either within a particular SS-R-positive organ or between SS-R-positive organs (*i.e.* brain, pituitary, adrenal, and exocrine pancreas). With respect to the anterior pituitary gland, Patel *et al.* have shown, by cross-linking of SS-R proteins and using different SS-R ligands, the presence of three receptor proteins of 80, 58, and 27 kilodaltons in size (1, 16), whereas other investigators reported two subtypes of the receptor in the rat anterior pituitary gland which differ in size (82 and 94 kilodaltons) and sensitivity to estrogen regulation (17). Recently, five different SS-R genes have been cloned. Cultured cell lines that stably express these five recombinant SS-R genes have been established, and it is clear that these different genes are encoding for receptor proteins with approximately the same affinity for SS-14 and SS-28, but different affinities for the SS analogs octreotide and MK-678, demonstrating that there is a molecular basis for the observed pharmacological and molecular heterogeneities of SS-R (18). Finally, in a very small subgroup of human pituitary adenomas, Reubi *et al.* (19) found, using autoradiographic studies, SS-R with low affinity to octreotide and high affinity to SS-28, whereas the vast majority of the tumors showed high affinity to both ligands. At this moment we cannot exclude that the difference between the GH inhibitory effects of the SS analogs is caused by interaction with different SS-R subtypes. Comparative binding studies using the three SS analogs in cultured cell lines that stably express the somatostatin receptor subtypes might help to resolve this question. There are, however, several lines of evidence that argue against this hypothesis. First, we found comparable differences among the effects of octreotide, RC-160, and BIM-23014 in all GH-secreting pituitary adenomas, whereas SS-R with a low affinity for octreotide are found in only a small subgroup of the adenomas. Secondly, the maximal inhibitory effects of octreotide, RC-160, and BIM-23014 were the same in three culture systems we used in our study. Thirdly, a maximal concentration of forskolin as well as pretreatment of the cells with PT significantly reduced the inhibitory effects of the three SS analogs, SS-14, and SS-28 to the same extent. In our experiments we performed a 2-h pretreatment with PT. A longer preincubation with PT might have completely blocked the inhibitory effect of the SS analogs. The latter data suggest that octreotide, RC-160, and BIM-23014 all act mainly via a PT-sensitive G-protein and adenylyl cyclase-dependent mechanism. Therefore, we suggest that the major differences among octreotide, BIM-23014, and RC-160 in their hormone release inhibitory effects are caused by differences in affinity for the same receptor.

Although it is clear from our study that in all GH-secreting pituitary adenomas and in the gastrinoma studied, RC-160 is clearly more potent in its inhibitory action on hormone release, the clinical significance of this finding has yet to be established. In previous studies comparing receptor binding characteristics with bioactivity *in vivo* and *in vitro*, several investigators have shown that with certain SS analogs there may be a dissociation between binding characteristics and bioactivity (9, 20, 21). The dissociation constant of the SS-R is approximately 1 nM (22, 23). In normal men, sc administration of 100 μg octreotide was reported to result after 30–60 min in circulating octreotide levels of between 1.6–2.5 nM (24). In this nanomolar concentration range, however, we did not observe differences in the GH and gastrin release inhibitory effects among the three SS analogs studied. Only at significantly lower concentrations (picomolar range) did the differences between the analogs became evident. If this difference in the potency of inhibition of hormone release among RC-160, octreotide, and BIM-23014 is related to a difference among these SS analogs in affinity for the SS-R, it may have implications in the treatment of those tumors that have low affinity for octreotide but high affinity for SS-14 and SS-28, as has been reported in a subgroup of insulinomas, medullary thyroid carcinomas, carcinoids, and a small subgroup of GH-secreting pituitary adenomas (25). Moreover, Srkalovic *et al.* (10) previously showed that RC-160 had a significant higher affinity for SS-14-binding sites,

compared to that of octreotide, in human ovarian, breast, and pancreatic cancers.

In conclusion, this study shows for the first time in primary cultures of human GH-secreting pituitary tumor cells and human gastrinoma cells that three SS analogs, available for clinical use, *i.e.* octreotide, RC-160, and BIM-23014, may have significant different potencies of inhibition of hormone release *in vitro*, with RC-160 being the most potent SS analog. It is suggested that in GH (adenoma) cells these analogs all act mainly via a PT-sensitive G-protein and adenylyl cyclase-dependent mechanism. Depending on the pharmacokinetic properties of these three octapeptide SS analogs, these observations may have consequences for the medical therapy of patients with SS-R-positive endocrine tumors.

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