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Dissociation between the Effects of Somatostatin (SS) and Octapeptide SS-Analogs on Hormone Release in a Small Subgroup of Pituitary- and Islet Cell Tumors

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ABSTRACT

The effects of somatostatin (SS-14 and/or SS-28) and of the three octapeptide SS-analogs that are available for clinical use (octreotide, BIM-23014 and RC-160) on hormone release by primary cultures of 15 clinically nonfunctioning pituitary adenomas (NFA), 7 prolactinomas, and 2 insulinomas were investigated. In the pituitary adenoma cultures, a comparison was made with the effects of the dopamine (DA) agonists bromocriptine and/or quinagolide. In 5 NFAs, 2 prolactinomas and 1 insulinoma somatostatin receptor (subtype) expression was determined by ligand binding studies and by *in situ* hybridization to detect sst_1, sst_2, and sst_3 messenger RNAs (mRNAs).

Four NFA cultures did not secrete detectable amounts of α-subunit, FSH, and/or LH. In the other cultures, hormone and/or subunit release was inhibited by DA-agonists (10 nM) in 9 of 11, by SS (10 nM) in 7 of 11, and by octapeptide SS-analogs (10 nM) in 3 of 10 cultures. In three NFA cultures, hormone release was sensitive to SS but not to SS-analogs. In all cultures, except for one, DA-agonists were the most effective in inhibiting hormone release. In the prolactinoma cultures, PRL release was inhibited by DA-agonists (10 nM) in 7 of 7, by SS in 4 of 4, and by octapeptide SS-analogs in 3 of 7 cultures. A dissociation between the effects of SS and SS-analogs was found in 3 cases. In the cultures sensitive to both bromocriptine and SS-28, bromocriptine was the most potent compound in 2 out of 4 cultures. In the 2 other cultures, both compounds were equally effective. In 2 insulinoma cultures, insulin release was inhibited by SS, and by octapeptide SS-analogs in only one. The presence or absence of an inhibitory effect by octreotide was in all cases in parallel with the presence or absence of the inhibitory effect by BIM-23014 and RC-160.

Autoradiographic studies using [^{125}I-Tyr^0]SS28 showed specific binding in 4 of 5 NFAs, 1 of 2 prolactinomas, and 1 of 1 insulinoma. Specific [^{125}I-Tyr^3]octreotide binding was found in 2 of 5 NFAs, in 1 of 2 prolactinomas, and in the insulinoma. Two NFAs showed binding of SS28, but not of the $sst_{2,5}$ specific ligand octreotide. The tumors showed variable sst_1 and/or sst_3 mRNA expression, whereas no sst_2 expression was found.

In conclusion, a dissociation between the inhibitory effects of SS on the one hand and of the octapeptide SS-analogs octreotide, BIM-23014 and RC-160 on the other hand, is observed in a small subgroup of NFAs, prolactinomas, and insulinomas, suggesting that novel sst subtype specific SS-analogs might be of benefit in the treatment of selected patients with somatostatin receptor positive secreting tumors not responding to octapeptide SS-analogs. However, in the majority of NFAs and prolactinomas, DA-agonists were equally or more effective than SS in the suppression of tumoral secretion products. (*J Clin Endocrinol Metab* **82:** 3011–3018, 1997)

RECENTLY, five distinct somatostatin receptor (sst) subtypes have been cloned and characterized (1–4). All sst subtypes bind native somatostatin (SS) with high affinity, whereas differences have been found between $sst_{1–5}$ in the binding of structural analogs of SS. Octreotide, which is the most widely used SS-analog, successfully controls hormonal hypersecretion in a majority of patients with neuroendocrine tumors (5). This SS-analog binds with a high and relative high affinity to sst_2 and sst_5, respectively, with low affinity to sst_3, and has no affinity to sst_1 and sst_4. Two other SS-analogs, BIM-23014 (lanreotide) and RC-160 (vapreotide), show a binding profile comparable to that of octreotide (1–4).

From several studies using different techniques, it has become clear now that the majority of the human sst positive tumors express multiple sst subtypes. While the majority of the neuroendocrine tumors carry sst with a high affinity for native SS as well as for the octapeptide SS-analog octreotide, small subgroups of tumors have been described with high affinity binding sites for SS but not for octreotide (6). Among these tumors are certain insulinomas, carcinoids, medullary thyroid carcinomas, ovarian tumors, and pituitary adenomas. Absence of the sst_2 subtype has been associated with an absent hormonal response to treatment with octreotide, suggesting that sst_2 is involved in the antihormonal actions of SS-analogs like octreotide (7, 8), whereas sst_2 seems to be involved in the inhibition of GH secretion in rats (9). So far, however, the functional role of the other sst subtypes, is unclear.

In a previous study, we have compared the effects of the three SS-analogs (octreotide, BIM-23014, and RC-160) on hormone release by pituitary GH-adenomas and a gastrinoma *in vitro* (10). In this study, we found that GH-adenomas with an absent hormonal response to octreotide were unresponsive to BIM-23014 and RC-160 as well. Still, two major questions remain. First, are there differences between the inhibitory effects of the above mentioned three clinically applicable

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SS-analogs in other types of secreting tumors? Second, is there evidence for a functional role of sst subtypes other than those with a high affinity for octapeptide SS-analogs?

In order to further investigate these questions, we have compared in the present study the effects octreotide, BIM-23014, and RC-160 with that of native SS on hormone release by the cultured cells prepared from 15 NFAs, 7 prolactinomas, and 2 insulinomas. In the pituitary adenoma cultures, a comparison with the effects of dopamine agonists was also made. Whereas several authors have described the effects of either SS or octreotide on NFAs (11, 12), a direct comparison between SS and SS-analogs on hormone release by NFAs has not been made yet.

Materials and Methods

Patients

Pituitary tumor samples were obtained by transsphenoidal operation from 18 patients with NFAs and from 7 patients with prolactinomas, as described previously (13). Tumor samples of 2 insulinomas were obtained within 30 min after surgical removal of the tumors. Diagnosis was made on the basis of clinical and biochemical characteristics of the patients, in combination with (immuno)histochemistry of the tumor samples. All patients gave their informed consent for the use of tumor material for research purposes. When sufficient adenoma tissue was obtained, pieces were frozen on dry ice and stored at −80 C.

Cell dispersion and cell culture.

Fifteen out of 18 NFAs and 7 prolactinomas were used for the cell culture studies. Single cell suspensions of the pituitary adenoma tissues were prepared by enzymatic dissociation with dispase as described in detail previously (13). Tumor tissue from the insulinoma patients was dissociated as described (14).

Short-term incubation of monolayer cultures. the dissociated cells were plated in 48-well plates (Costar, Cambridge, MA) at a density of 10^5 (prolactinomas and insulinomas) or 2×10^5 (NFAs) cells per well per 1 ml culture medium. After 3–4 days, the medium was changed, and 72-h incubations without or with SS, SS-analogs, or DA-agonists were started. At the end of the incubation, the medium was removed and centrifuged for 5 min at $600 \times g$. The supernatant was collected and stored at −20 C until analysis.

Long-term incubation studies in Transwells (15). The isolated tumor cells were plated in Transwell-COL membranes (Costar, Badhoevedorp, The Netherlands) at a density of 10^5 (prolactinomas and insulinomas) or 2×10^5 (NFAs) cells per well. The transwells were then placed into multiwell plates (24-well, Costar) containing 1 ml culture medium. After 24 h, the transwells were transferred to wells containing fresh medium (without or with test substances). Every 3–4 days, the cells were placed into fresh medium and the incubation media were collected and stored at −20 C until determination of hormone concentrations.

In all experiments SS (SS-14 and/or SS-28), octapeptide SS-analogs (octreotide, BIM-23014, RC-160) and DA-agonists (bromocriptine and/or quinagolide) were used at a concentration of 10 nM. The cells were cultured at 37 C in a CO_2-incubator. The culture medium consisted of MEM D-valine with Earle's salts supplemented with nonessential amino acids, sodium pyruvate (1 mmol/L), 10% FCS, penicillin (1×10^5 U/L), fungizone (0.5 mg/L), L-glutamine (2 mmol/L), and sodium bicarbonate (2.2 g/L). Unfortunately, generally not enough tumor material was obtained to test each tumor on its responsiveness to all of the above indicated drugs.

Hormone determinations

Human PRL, LH, and FSH concentrations in the media were determined by immunoradiometric assays (MedGenix Diagnostics, Fleurus, Belgium) as described previously (13, 15). Glycoprotein α-subunit concentrations were determined by an immunoradiometric assay from Immunotech S. A. (Marseille, France). Insulin concentrations were determined by a double antibody RIA as described (14). Dilution of PRL, LH, FSH, α-subunit and insulin in the media was in parallel to the respective standards supplied with the kits.

Test substances

Somatostatin-14 (SS-14) was obtained from Sigma (St. Louis, MO). BIM-23014 (lanreotide) and somatostatin-28 (SS-28) were obtained from Bachem (Hannover, Germany). RC-160 (vapreotide) was obtained from Peninsula (Belmont, CA). Octreotide, bromocriptine and quinagolide (CV205–502) were obtained from Sandoz Pharma (Basle, Switzerland).

Receptor autoradiography

Receptor autoradiography was carried out as described by Reubi *et al.*, (16). Ten-micrometer sections were mounted onto precleaned gelatin coated microscope slides, and stored at −80 C. Adjacent sections were used for *in situ* hybridization and processed further as described below. To wash out endogenous somatostatin, the sections were preincubated at room temperature for 10 minutes in 170 mM Tris-HCl, pH 7.4. Thereafter, the sections were incubated for 60 minutes at room temperature in 170 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 1% BSA, 40 μg/ml bacitracin in the presence of [^{125}I-Tyr^3]-octreotide (about 80–160 pmol/L) or [^{125}I-Tyr^0]SS28 (final concentration 80–160 pmol/L; ANAWA Laboratories, Wangen, Switzerland). Nonspecific binding was determined in a sequential section in the presence of excess unlabeled Tyr^3-octreotide (1 μM) or SS-28 (1 μM). The incubated sections were washed twice for 5 min in incubation buffer containing 0.25% BSA and once in incubation buffer without BSA. After a short wash with distilled water to remove salt, the sections were air dried and exposed to Kodak X-OMAT AR or Hyperfilm-^{3}H (Amersham) for 3–7 days in x-ray cassettes. Histology was performed on hematoxylin-azophloxine stained sequential cryosections.

In situ hybridization histochemistry of sst_1, sst_2, and sst_3 mRNA

The protocol and oligonucleotides used for *in situ* hybridization were the same as described by Reubi *et al.* (17). Forty-eight base pair oligonucleotides complementary to the bases coding for amino acids 2–17 of the $hsst_1$ mRNA sequence, 237–252 of the $hsst_2$ sequence, and 366–381 of the $hsst_3$ sequence (17) were synthesized and purified over a Pharmacia NAP-10 column by Pharmacia Biotech (Roosendaal, The Netherlands). 3′-end labeling by using [α-^{32}P]dATP (3000 Ci/mmol; Du Pont NEN , Boston, MA) was performed as described previously (18). Specificity of hybridization signal obtained with the above oligonucleotide probes was established previously (17). In our study, sequential cryostat sections of the tumors (see above) were incubated in duplicate with ^{32}P-labeled oligonucleotide probes without or with a 20-fold excess of unlabeled oligonucleotide to demonstrate specificity of the hybridization signal obtained. A tumor was considered positive for the respective mRNA when the hybridization signal obtained in a control section was displaced by a 20-fold excess of unlabeled oligonucleotide by more than 50%. The hybridization signals obtained were analyzed densitometrically.

Statistical analysis of the data

All data on hormone release are expressed in mean ± SE, n = 4 wells per treatment group. All data were analyzed by ANOVA to determine overall differences between treatment groups. When significant differences were found by ANOVA, a comparison between treatment groups was made using the Newman-Keuls test.

Results

Clinically nonfunctioning pituitary adenomas

Table 1 shows basal release of α-subunit, FSH, and LH by cultured cells of NFAs 1–6. Five out of 6 NFA cultures secreted detectable amounts of α-subunit *in vitro*, 5 out of 6 secreted FSH, whereas only 2 of 6 (no. 1 and 2) secreted LH. Hormone release was not inhibited by SS-28 (10 nM), oct-

TABLE 1. Characteristics of hormone release *in vitro* by clinically nonfunctioning pituitary adenomas (NFA)

Culture no.	α-SU release (mU/well·72 h)	FSH release (mU/well·72 h)	LH release (mU/well·72 h)
NFA 1	6.1 ± 0.1	18.4 ± 1.7	1.3 ± 0.1
NFA 2	5.3 ± 0.5	16.1 ± 0.6	2.0 ± 0.1
NFA 3	0.5 ± 0.0	4.6 ± 0.2	Not detectable
NFA 4	2.6 ± 0.2	Not detectable	Not detectable
NFA 5	4.6 ± 0.3	77.5 ± 2.8	Not detectable
NFA 6	Not detectable	10.2 ± 1.0	Not detectable

200,000 cells per well were plated in 24-well multiwell plates in 1 ml of MEM-Dval + 10% FCS. On day 4 of the culture, the medium was changed and a 72-h incubation was performed in quadruplicate. α-Subunit (α-SU), FSH, and LH concentrations in the media were determined as described in the *Materials and Methods* section. No detectable amounts of GH, PRL, and ACTH were found in the media.

reotide (10 nM), RC-160 (10 nM), or BIM-23014 (10 nM) in a 72-h exposure of the cells in 3 out of 6 cultures (nos. 2, 4, and 5, data not shown). In culture no. 5, however, the DA-agonist quinagolide (10 nM) significantly inhibited α-subunit and FSH release by 48 and 30%, respectively ($P < 0.01$ *vs.* control cells, data not shown). The effect of SS-28, SS-analogs, and quinagolide (all used at a concentration of 10 nM) on α-subunit or FSH release by NFA cultures 1, 3, and 6 is shown in Fig. 1. Quinagolide significantly inhibited α-subunit or FSH release during a 72-h exposure in all cultures ($P < 0.01$ *vs.* control cells), whereas in cultures no. 1 and 3 both SS-28 and octreotide significantly inhibited hormone release ($P < 0.01$ *vs.* control cells). In culture no. 3, SS-28, octreotide, RC-160, and BIM-23014 were equally effective. However, in culture no. 6, which was sensitive to the inhibitory effect of SS-28, none of the three octapeptide SS-analogs were effective in inhibiting FSH release. In NFA culture no. 1, the response of FSH and LH release was comparable with that of α-subunit release, whereas in culture no. 3, α-subunit release was not inhibited (data not shown).

In nine NFA cultures (nos. 7–15), the effects of bromocriptine (10 nM), SS-28 (10 nM), and the three SS-analogs (10 nM) were investigated during prolonged exposure in Transwell tissue culture inserts (15). Four of these cultures (nos. 12–15) did not secrete detectable amounts of LH, FSH, or α-subunit (data not shown). Figure 2 shows the effects of the above-mentioned drugs on α-subunit release by cultured cells from NFA no. 7 and no. 10. In the cells of patient 7, α-subunit release was progressively inhibited to the same extent by SS-28 and the three SS-analogs. The degree of inhibition after 2 weeks of exposure *in vitro* was comparable to that of bromocriptine. In NFA culture, no. 10 bromocriptine and SS-28 also increasingly inhibited α-subunit release, although bromocriptine was most effective. After drug withdrawal on day 14, these inhibitory effects recovered, suggesting that the increasing inhibitory actions of bromocriptine, SS-28 and SS-analogs, represented inhibition of hormone release and or production and not inhibition of cell proliferation. Table 2 shows the effects of bromocriptine, SS-28, and SS-analogs on α-subunit-, FSH and/or LH release by NFAs no. 7–11, at day 14 of incubation. In all NFA cultures (7–11) bromocriptine (10 nM) significantly inhibited hormone release. SS-28 significantly inhibited hormone release in cultures 7, 9, 10, and 11. Octreotide, BIM-23014, and RC-160 significantly inhibited hormone release in culture no. 7.

Overall, hormone release was sensitive to DA-agonists in 9 out of 11, to SS-28 in 7 out of 11, and to SS-analogs in 3 out of 10 cultures. In three cases (nos. 6, 9, and 11) hormone release was sensitive to SS, but not to SS-analogs. In all cultures, except for one (no. 7), bromocriptine was the most effective compound.

Prolactinomas

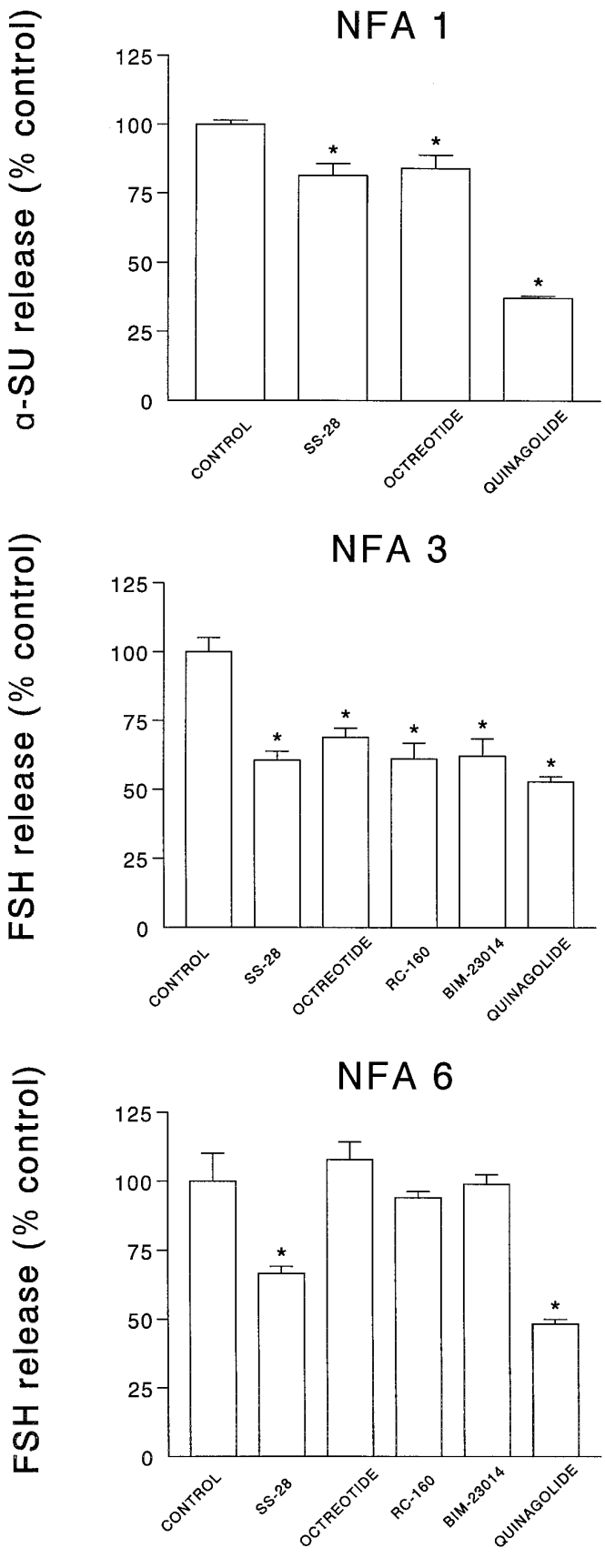
Quinagolide (10 nM) significantly inhibited PRL release during a 72-h incubation in three prolactinoma cultures (nos. 1, 2, and 3) by 25, 33, and 50% respectively (data not shown). In these three cultures, octreotide (10 nM) did not significantly inhibit PRL release. At the same time, 10 nM RC-160 (in culture nos. 2 and 3) or 10 nM BIM-23014 (in culture no. 3) were also ineffective (data not shown).

In 4 prolactinoma cultures (nos. 4–7), the effects of SS-28, octreotide, RC-160, BIM-23014, and bromocriptine (all at a concentration of 10 nM) were studied during prolonged incubation of the cells in Transwells. Figure 3 shows that PRL release by the cells of adenoma no. 4 was slightly sensitive to octreotide (statistically significant inhibition of PRL release at days 3, 10, and 14) but responded very well to SS-28 and bromocriptine (90 and 84% inhibition, respectively, day 14 of culture). In addition, we found that PRL release by the cells of patient 5 was also increasingly inhibited by bromocriptine and SS-28 (by 40 and 40%, respectively, day 14 of culture; $P < 0.01$ *vs.* control cells), in contrast to the slight (approximately 17%, day 14 of culture), but significant inhibition by the three SS analogs. Comparable to the NFA cultures, drug withdrawal on day 14 in culture no. 5, led to a recovery of PRL release. It is also important to note that the maximal inhibition of PRL release by both SS-28 and bromocriptine was considerably less in the culture of adenoma no. 5 in comparison with culture no. 4. Patient no. 5 had become insensitive to DA-agonist therapy, whereas patient no. 4 was operated because of intolerance to DA-agonist therapy. Table 3 shows the effects of the above compounds on PRL release by culture nos. 6 and 7, at day 14 of incubation. PRL release by adenoma cells from patients no. 6 and 7 (operated because of intolerance to DA-agonist therapy) was significantly inhibited by bromocriptine, and slightly by SS-28. Octreotide significantly inhibited PRL release in culture no. 6 only. The cells of patient 7 were unresponsive to octreotide, BIM-23014, and RC-160.

Overall, hormone release was sensitive to DA-agonists in all 7 prolactinoma cultures, to SS-28 4 of 4, and to octreotide in 3 of 7. In 2 cultures (nos. 4 and 5) hormone release was significantly inhibited by SS-28 and only slightly by octreotide, BIM-23014, and RC-160. In those cultures sensitive to both bromocriptine and SS-28, bromocriptine was the most potent compound in 2 out of 4 cultures (nos. 6 and 7). In the two other cultures, both compounds were equally effective.

Insulinomas

Table 4 shows the effect of SS-14, SS-28, and octreotide and RC-160 (72 h incubation) on insulin release by cultured cells of insulinoma no. 1. Both SS-14 (10 nM) and SS-28 (10 nM)



significantly inhibited insulin release by 29 and 32%, respectively ($P < 0.01$ *vs.* control). Octreotide (10 nM) and RC-160 (10 nM) were ineffective, however.

Cells of insulinoma no. 2 were cultured in Transwells in the absence or presence of SS-14, SS-28, and SS-analogs. Figure 4 shows that SS-14, SS-28, and the SS-analogs octreotide, BIM-23014, and RC-160 progressively inhibited insulin release to the same extent.

Somatostatin receptor subtype expression

Table 5 shows the results of the autoradiographic and *in situ* hybridization experiments. It should be mentioned that not sufficient material was obtained from each adenoma to perform both the receptor and cell culture studies.

[^{125}I-Tyr^0]SS-28 binding was found in 4 out of 5 NFAs, in 1 out of 2 prolactinomas, and in insulinoma no. 2. Binding of the sst_2- and sst_5- subtype selective ligand [^{125}I-Tyr^3]octreotide was parallel in all cases except for 2 NFAs (nos. 9 and 18), which showed no binding of [^{125}I-Tyr^3]octreotide. In the majority of the NFAs and prolactinomas, receptor density was low. The two NFAs that showed binding of [^{125}I-Tyr^3]octreotide did not express the sst_2 subtype, however. In addition, no sst_2 mRNA expression was found in the other NFAs and prolactinomas. One pheochromocytoma and one carcinoid, which were already known to express sst_2 mRNA, were included as a positive controls for sst_2 mRNA expression (Table 5). One NFA (no. 11), which showed binding of both radioligands, did not express sst_1, sst_2, or sst_3 mRNA. Two other adenomas (NFA no. 17 and prolactinoma no. 6) showed sst_3 expression, whereas no ligand binding was detected, possibly due to low levels of expression of the receptor protein. Furthermore, the expression of sst_1 and sst_3 was variable. Insulinoma no. 2, which had a high density of receptors, expressed sst_1 and sst_3 mRNA only (Fig. 5).

Discussion

Experimental and human tumors of different cellular origin express multiple sst subtypes. By various methods including RT-PCR, RNAse-protection, and *in situ* hybridization, it has been demonstrated that sst_1, sst_2, sst_3, sst_4, and sst_5 mRNA can be expressed in different human pituitary adenomas and insulinomas at the same time (7, 8, 19–21). The expression of multiple sst subtypes in tumors makes it difficult to establish their functional role in mediating antihormonal and/or antiproliferative effects. A subgroup of neuroendocrine tumors, including pituitary adenomas and insulinomas, express ssts with high affinity binding for SS-28 but not for octreotide (6). In the present study, we have investigated the effects of native SS (SS-14 and/or SS-28) and of the three octapeptide SS-analogs octreotide, BIM-23014, and RC-160, on hormone release by cultured NFAs, prolacti-

FIG. 1. Effects of SS-28, octapeptide SS-analogs, and the DA-agonist quinagolide on hormone release by cultures of three NFAs (nos. 1, 3, and 6). Pituitary adenoma cells were incubated in MEM + 10% FCS during 72 h in quadruplicate without or with 10 nM of the drugs indicated. Values are expressed as the percentage of hormone release by control cells and are the mean ± SE; *, $P < 0.01$ *vs.* control cells.

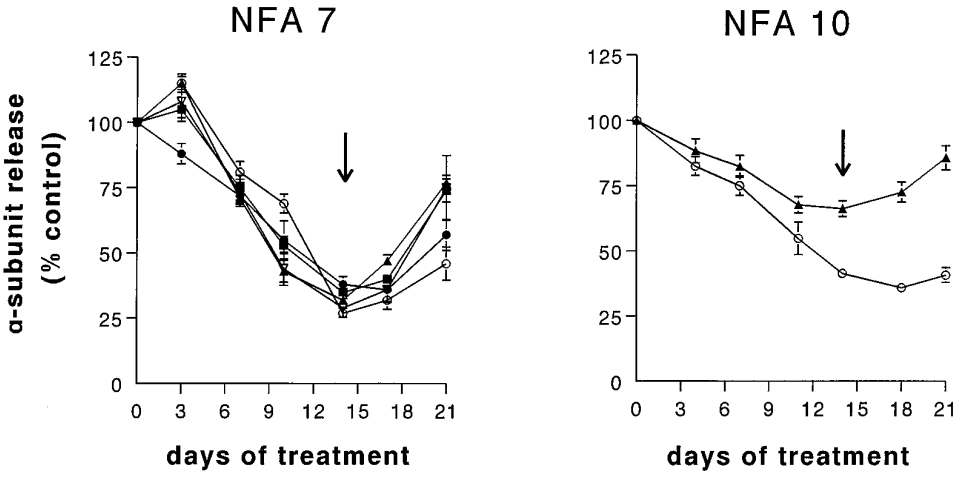


FIG. 2. The effect of long-term incubation with SS-28, octapeptide SS-analogs, and the DA-agonist bromocriptine on hormone release by cultured cells of NFA nos. 7 and 10. The pituitary adenoma cells were incubated in Transwell tissue culture inserts for 3 weeks without or with 10 nM of the drugs indicated. Medium was changed every 3 or 4 days and stored at −20 C until determination of hormone concentrations. Values are expressed as the percentage of hormone release of control cells at each time-point indicated and are the mean ± SE of four wells per treatment group. Symbols represent: (○) bromocriptine, (▲) SS-28, (●) octreotide, (▽) BIM-23014, (■) RC-160. The *arrow* at day 14 of culture indicates the time-point of drug withdrawal.

TABLE 2. The effect of long-term incubation with bromocriptine, SS-28, and octapeptide SS-analogs on hormone release by cultured clinically nonfunctioning pituitary adenoma cells (NFA)

Culture no.	Hormone release (day 10–14 of culture)		
	α-SU (mU/well)	FSH (mU/well)	LH (mU/well)
NFA 7			
Control	6.7 ± 0.2 (100)	12.5 ± 0.4 (100)	1.0 ± 0.1 (100)
Bromocriptine (10 nM)	1.8 ± 0.1 (27)[a]	6.7 ± 0.2 (53)[a]	0.6 ± 0.1 (60)[a]
SS-28 (10 nM)	2.2 ± 0.2 (33)[a]	7.9 ± 0.2 (63)[a]	0.8 ± 0.1 (80)[a]
Octreotide (10 nM)	2.5 ± 0.2 (37)[a]	8.5 ± 0.4 (68)[a]	0.7 ± 0.1 (70)[a]
BIM-23014 (10 nM)	1.9 ± 0.3 (28)[a]	7.7 ± 0.3 (62)[a]	0.7 ± 0.1 (70)[a]
RC-160 (10 nM)	2.3 ± 0.2 (34)[a]	8.5 ± 0.3 (68)[a]	0.7 ± 0.1 (70)[a]
NFA 8			
Control	1.0 ± 0.1 (100)	15.6 ± 0.6 (100)	Not detectable
Bromocriptine (10 nM)	0.3 ± 0.1 (30)[a]	4.0 ± 0.4 (26)[a]	
SS-28 (10 nM)	1.0 ± 0.1 (100)	13.7 ± 1.2 (88)	
Octreotide (10 nM)	0.8 ± 0.1 (80)	13.8 ± 1.0 (88)	
NFA 9			
Control	0.6 ± 0.1 (100)	22.2 ± 0.9 (100)	Not detectable
Bromocriptine (10 nM)	0.3 ± 0.0 (50)[a]	9.3 ± 0.5 (42)[a]	
SS-28 (10 nM)	0.6 ± 0.0 (100)	16.7 ± 0.5 (76)[a]	
Octreotide (10 nM)	0.6 ± 0.1 (100)	19.6 ± 1.4 (90)	
NFA 10			
Control	3.6 ± 0.2 (100)	1.8 ± 0.1 (100)	0.5 ± 0.0 (100)
Bromocriptine (10 nM)	1.5 ± 0.1 (42)[a]	1.0 ± 0.1 (56)[a]	0.4 ± 0.0 (80)[a]
SS-28 (10 nM)	2.4 ± 0.1 (67)[a]	1.4 ± 0.1 (78)[a]	0.5 ± 0.0 (100)
NFA 11			
Control	0.4 ± 0.0 (100)	7.5 ± 0.1 (100)	Not detectable
Bromocriptine (10 nM)	0.3 ± 0.1 (75)[a]	3.5 ± 0.1 (47)[a]	
SS-28 (10 nM)	0.4 ± 0.0 (100)	5.2 ± 0.3 (69)[a]	
Octreotide (10 nM)	0.4 ± 0.0 (100)	6.5 ± 0.4 (87)	

The cells were cultured during 14 days without or with the test substances in Transwell tissue culture inserts as described in the *Materials and Methods* section. Medium and test-substances were changed every 3–4 days. Values in *brackets* are the percentages of control hormone release from days 10–14 of culture.

[a] $P < 0.01$ *vs* control cells. No detectable amounts of GH, PRL, and ACTH were found in the media.

nomas, and insulinomas to further establish whether tumors lacking sst subtypes with high affinity for octapeptide SS-analogs (sst_2 and sst_5) can be responsive to native SS. This may give information whether sst subtypes other than sst_2 and sst_5 may be involved in the antihormonal actions of SS in such tumors.

We found that, in a small subgroup of NFA cultures (3/10), prolactinomas (3/4), and insulinomas (1/2), hormone release *in vitro* was insensitive or showed a very low sensitivity to the inhibitory effects of the octapeptide SS-analogs octreotide, RC-160 and BIM-23014, while at the same time hormone release was inhibited by SS-14 and/or SS-28. In line

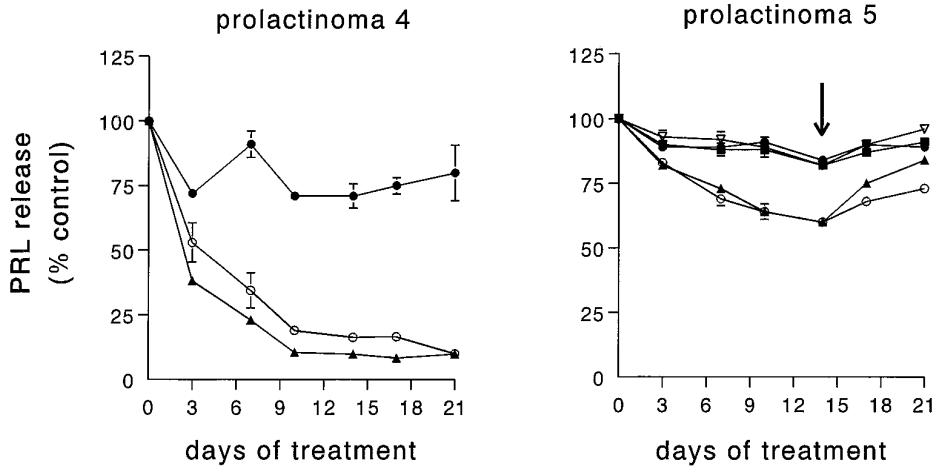


FIG. 3. The effect of long-term incubation with SS, SS-analogs, and the DA-agonist bromocriptine on PRL release by cultured cells of prolactinomas nos. 4 and 5. The cells were cultured in Transwells as described in the legend to Fig. 2. The drug concentration was 10 nM. Values are the mean ± SE of four wells per treatment group and expressed as the percentage of PRL release by control cells at each time-point. Symbols represent: (○) bromocriptine, (▲) SS-28, (●) octreotide, (▽) BIM-23014, (■) RC-160. The *arrow* at day 14 of culture (culture no. 5) indicates the time-point of drug withdrawal.

TABLE 3. The effect of long-term incubation with bromocriptine, SS-28, and octapeptide SS-analogs on PRL release by cultured human prolactinoma cells

	PRL release (day 10–14 of culture: ng/well)	
	Prolactinoma 6	Prolactinoma 7
Control	2404 ± 82	4501 ± 142
Bromocriptine (10 nM)	85 ± 7 (4)[a]	1190 ± 115 (26)[a]
SS-28 (10 nM)	1680 ± 57 (70)[a]	3359 ± 60 (75)[b]
Octreotide (10 nM)	1738 ± 77 (72)[a]	3805 ± 225 (85)
BIM-23014 (10 nM)	Not investigated	4324 ± 222 (96)
RC-160 (10 nM)	Not investigated	4190 ± 169 (93)

The cells were cultured during 14 days without or with the test substances in Transwell tissue culture inserts as described in the *Materials and Methods* section. Medium and test-substances were changed every 3–4 days. Values in *brackets* are the percentages of control prolactin release from days 10–14 of culture.

[a] $P < 0.01$ and [b] $P < 0.05$ *vs.* control cells.

TABLE 4. Effect of somatostatin and the somatostatin analogs octreotide and RC-160 on insulin release by human insulinoma cells (no. 1)

	Insulin release (μU/well·72 h)
Control	687 ± 10
SS-14 (10 nM)	490 ± 9 (71)[a]
SS-28 (10 nM)	464 ± 6 (68)[a]
Octreotide (10 nM)	690 ± 15 (100)
RC-160 (10 nM)	672 ± 22 (98)

Human insulinoma cells were incubated in quadruplicate in MEM + 10% FCS during 72 h with or without the compounds indicated. Thereafter the media were collected and stored at −20 C until determination of the insulin concentration by RIA. Values in *brackets* are the percentages of insulin release by control cells.

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

with the observation that the three octapeptide SS-analogs show a comparable binding profile to the five sst subtypes, we found no differences between the maximal inhibitory effects of octreotide, BIM-23014, and RC-160. Comparable results were found in GH-secreting adenomas in a previous study (10). In this study, we also found that those adenoma cell cultures that did not respond to octreotide did not respond to BIM-23014 and RC-160 as well (10).

Octreotide, RC-160, and BIM-23014 bind with a high and relative high affinity to sst_2 and sst_5, respectively, with low

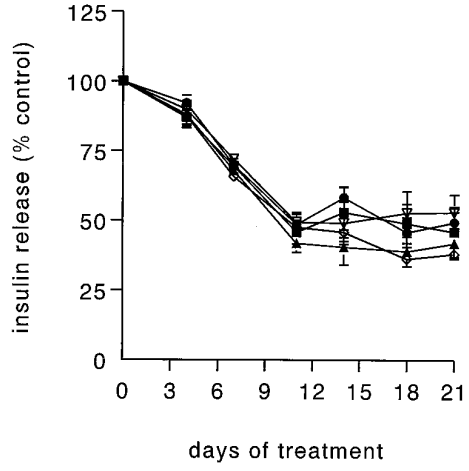


FIG. 4. The effect of long-term incubation with SS and octapeptide SS-analogs on insulin release by cultured human insulinoma cells (no. 2). The cells were cultured in Transwells as described in Fig. 2. The drug concentration was 10 nM. Values are the mean ± SE of four wells per treatment group and expressed as the percentage of insulin release by control cells at each time-point. Symbols represent: (▲) SS-28, (◇) SS-14, (●) octreotide, (▽) BIM-23014, (□) RC-160.

affinity to sst_3, and display no binding to sst_1 and sst_4 (1–4). This suggests that the tumors mentioned above lack the expression of sst_2, sst_5, and possibly also sst_3. In a few selected cases, sufficient tissue was obtained to perform both cell culture studies and ligand binding and *in situ* hybridization studies. In one NFA, which showed a high density of [^{125}I-Tyr^0]SS28 binding sites, and sst_1 mRNA expression only, hormone release was only slightly sensitive to SS-28 and not to octreotide, suggesting that activation of sst_1 is not important in mediating an antihormonal effect. However, βLH and βFSH secretion was not measured in our study. Previously, Klibansky *et al.* (12) showed that SS significantly inhibited βLH and βFSH secretion *in vitro* in 53% of patients with NFAs, whereas α-subunit secretion was inhibited in only 17% of these tumors. Further studies are needed to elucidate this point. Two prolactinomas that showed a low sensitivity to SS-28 *in vitro* expressed sst_1 and sst_3 mRNA, respectively. Prolactinoma no. 6, which responded to both octreotide and SS-28, did not express sst_2, suggesting that sst subtypes other than sst_2 are involved in the regulation of

TABLE 5. sst expression in human pituitary adenomas and one insulinoma, as determined by SSR-autoradiography and *in situ* hybridization

Tumor type	SSR-autoradiography		SSR-subtype mRNA		
	[^{125}I-Tyr^0]-SS-28	[^{125}I-Tyr^3]-octreotide	sst_1	sst_2	sst_3
NFA no. 9	++	−	+	−	−
NFA no. 11	+	+	−	−	−
NFA no. 16	+	++	+	−	−
NFA no. 17	−	−	−	−	+
NFA no. 18	+	−	−	−	+
Prolactinoma no. 6	−	−	−	−	+
Prolactinoma no. 7	+	+	+	−	−
Insulinoma no. 2	++	++	+	−	+
Pheochromocytoma	++	++	nd	+	nd
Carcinoid	++	++	nd	+	nd

nd, Not determined. Grading: −, negative; +, positive; ++, strongly positive.

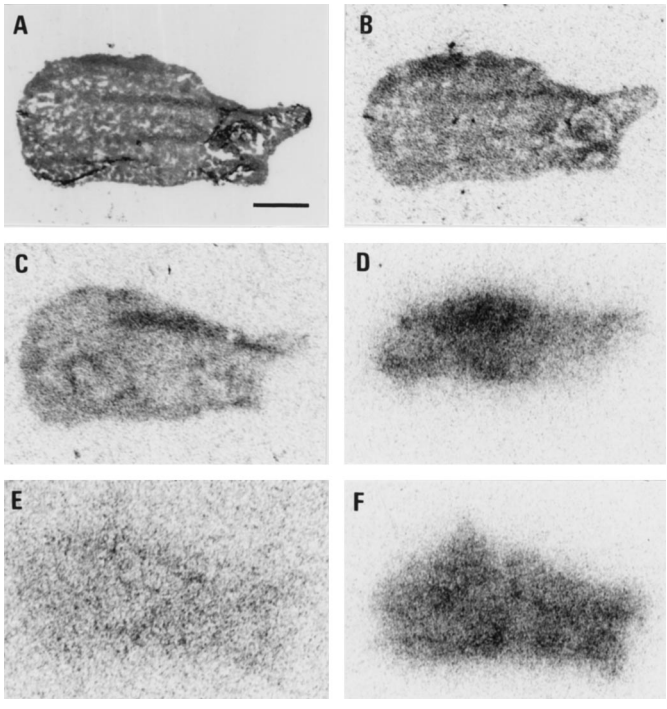


FIG. 5. Photomicrograph of the results of SSR-autoradiography and of *in situ* hybridization of sst_1, sst_2, and sst_3 mRNAs of insulinoma no. 2. Note the absence of sst_2 mRNA. A, Hematoxylin-azophloxine stained section; B, autoradiogram showing total binding of [^{125}I-Tyr^0]SS-28; C, autoradiogram showing total binding of [^{125}I-Tyr^3]octreotide; D, autoradiogram showing a high amount of sst_1 mRNA; E, autoradiogram showing the absence of sst_2 mRNA; F, autoradiogram showing a high amount of sst_3 mRNA. B and C, Total binding was displaced with more than 50% by excess (1 μM) unlabeled SS-28 or octreotide, respectively; D and F, the hybridization signal was displaced with more than 50% by a 20-fold excess unlabeled sst_1 or sst_3 oligonucleotide probe, respectively; E, the very low hybridization signal was not displaced by excess sst_2 oligonucleotide probe. *Bar*, 1 mm.

hormone secretion in this particular case. On the other hand, the low density of SS binding sites may also account for their low sensitivity to SS *in vitro*. The insulinoma, which was highly sensitive to the inhibitory effects of SS-28, SS-14, as well as to the three octapeptide SS-analogs octreotide, BIM-23014 and RC-160, did not express sst_2 mRNA, suggesting that other sst subtypes (*i.e.* sst_5) are also important in mediating the inhibition of insulin release in this case. The involvement of sst_3, which is also expressed in this case, cannot be fully excluded, however. Moreover, disregulation of receptor/signaling systems, like density of receptors, desensitization, signaling, or gene regulation, can be involved in determining the response of hormone secretion to SS and SS analogs as well.

In conclusion, sst subtypes other than sst_2 and sst_5 may mediate an antihormonal effect because, in particular tumors that were not responsive to the three octapeptide SS-analogs, native SS was effective. Therefore, it seems reasonable to develop sst_1, sst_3, and/or sst_4- subtype selective SS-analogs for the treatment of this small subgroup of patients.

Our observations seem well in agreement with the results of studies by Greenman and Melmed (7, 19). These investigators demonstrated by RNAse protection and RT-PCR assays the absence of sst_2 mRNA in prolactinomas, whereas the majority of the tumors expressed sst_1 (4 out of 5), sst_3 (3 out of 3), and sst_5 (3 out of 3) mRNA. It is also known for some time that the vast majority of the patients with prolactinomas do not respond to treatment with octreotide (22–23). However, data concerning sst subtype mRNA expression in pituitary adenomas are conflicting. Other groups demonstrated sst_2 mRNA expression by RT-PCR analysis in prolactinomas in 100, 67, and 100% of the cases, respectively (20, 21, 24). The high sensitivity of the RT-PCR technique may be an explanation for this observation but also for the discrepancy between clinical studies using octreotide in prolactinoma patients on the one hand and the high incidence of sst_2 mRNA positive prolactinomas when evaluated by RT-PCR.

In conclusion, our study demonstrates that, in a subgroup of NFAs, prolactinomas, and insulinomas, hormone release is inhibited by native SS, but not by the octapeptide SS-analogs octreotide, RC-160, or BIM-23014. This suggests that, in selected neuroendocrine tumors, sst-subtypes other than sst_2 may be involved in the inhibition of hormone release and that novel sst-subtype specific SS-analogs might be of value in the treatment of selected patients harboring tumors with ssts with low affinity for octapeptide SS-analogs.

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