0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 5
Printed in U.S.A.

Effect of 17β-Estradiol on Somatostatin Receptor Expression and Inhibitory Effects on Growth Hormone and Prolactin Release in Rat Pituitary Cell Cultures

D. DJORDJIJEVIC, J. ZHANG, M. PRIAM, C. VIOLLET, D. GOURDJI, C. KORDON, AND J. EPELBAUM

INSERM U-159, Centre Paul Broca, 75014 Paris, France

ABSTRACT

In the present study, we tested whether 17β-estradiol (E_2)-induced PRL sensitivity to somatostatin-14 (SRIF) involves selective up-regulation of discrete somatostatin receptor subtypes (ssts) in primary cultures of female rat pituitary cells. The efficacy of the endogenous peptide SRIF to inhibit GH and PRL secretion and cAMP accumulation was compared with those of octreotide (OCT), BIM-23052, BIM-23056, and BIM-23268, which have been reported to be relatively selective for rat sst2, sst3, and sst5. Experiments were performed in steroid-depleted media supplemented or not with 1 nM E_2 for 96 h. SRIF, OCT, and BIM-23052 inhibited cAMP accumulation and GH release independently of E_2. In contrast, all three agonists affected PRL release in E_2-treated cultures only. Inhibition of cAMP accumulation by SRIF, OCT, and BIM-23052 was enhanced by exposure of cells to E_2. The rank of potency of the agonists, OCT = SRIF > BIM-23052, was similar for GH and PRL inhibition. BIM-23268 was a weak agonist on GH, but not on PRL, secretion. BIM-23056 had no effect on the release of either hormone, but slightly inhibited cAMP formation in E_2-treated cells. To verify whether SRIF receptor gene expression correlated with these observations, messenger RNA (mRNA) transcripts corresponding to the five ssts were measured by quantitative RT-PCR in the presence or absence of E_2. Control cells expressed predominantly sst2 and sst3 transcripts; sstl mRNA was present in moderate amounts, whereas sst4 and sst5 were only weakly expressed. E_2 had a differential effect on distinct ssts; it increased mRNA concentrations corresponding to sst2 and sst3, and decreased that of sst1. These results indicate that sst2 and sst3 receptors are the major somatostatin receptors expressed in the female rat pituitary, and that both of them are positively regulated by estradiol. However, the capacity of lactotropes to respond to SRIF after exposure to E_2 seems to depend more upon E_2-induced up-regulation of the sst2 than of the sst3 receptor subtype. (*Endocrinology* **139:** 2272–2277, 1998)

PREVIOUS studies have shown that somatostatin-14 (SRIF) is able to inhibit GH, TSH, and PRL secretion from female rat anterior pituitary cells, being more potent on GH and TSH than on PRL release (1). Unlike inhibition of GH release by SRIF, effects of the peptide on PRL release have been shown to depend upon prior exposure of the gland to estrogens. This was unambiguously demonstrated *in vivo* (2) as well as *in vitro* on pituitaries from castrated male or estrogen- and progesterone-treated rats (3), or after exposure of the cells to estradiol (4). In parallel, chronic administration of 17β-estradiol (E_2) was shown to up-regulate somatostatin receptor sites expressed by mammotropes (4). Culture of pituitary cells in the presence of estradiol also provided evidence that the rat pituitary expresses both E_2-dependent and -independent somatostatin receptor subtypes (5).

Five different somatostatin receptor subtypes (sst1 to sst5) have recently been cloned and characterized in various species, including human, rat and mouse (for review, see Refs. 6 and 7). However, the functional significance of these multiple isoforms has not yet been clarified. The anterior pituitary is one of the few tissues in which transcripts of all five subtypes have been detected (8). *In situ* hybridization studies demonstrated that discrete pituitary cell types could express several isoforms of the somatostatin receptors, but whether the profile of sst receptor expression is cell specific is still debated. High levels of sst4 and sst5 receptor messenger RNA (mRNA) have been reported in somatotropes, whereas thyrotropes may predominantly express sst2 mRNA (9). According to another study, sst5 and sst2 transcripts are preferentially expressed in somatotropes and thyrotropes compared with the other cell types; sst5 mRNA is more broadly expressed than sst2 (10). Finally, it was reported recently that in the 7315b rat prolactinoma model, sst2 and sst3 expression is primarily dependent upon the presence of estrogens (11).

Although no fully selective agonist has been developed to date for the sst receptors, several synthetic compounds were reported to exhibit a relative specificity for discrete rat subtypes when transfected in various cell lines. For example, octreotide (OCT) was reported to be more active on sst2, whereas BIM-23052, BIM-23056, and BIM-23268 exhibited a higher potency for sst3 and sst5 (12–14).

In the present study, we investigated whether the same sst receptor subtypes were involved in somatostatin inhibition of GH and PRL secretion and of cAMP accumulation in the female rat pituitary by comparing the potency ratios of SRIF and its agonists on these parameters. We also used a recently described method for measuring sst mRNA levels by quantitative RT-PCR (15) to determine whether basal and E_2-induced pituitary levels of all five sst mRNA subtypes are correlated to the hormonal responses obtained under both conditions.

Received September 18, 1997.
Address all correspondence and requests for reprints to: Dr. J. Epelbaum, INSERM U-159, 2ter rue d'Alésia, 75014 Paris, France. E-mail: epelbaum@broca.inserm.fr.

Materials and Methods

Cultures of dispersed anterior pituitary cells

Anterior pituitaries were collected from Wistar female rats (175–200 g; Charles River Breeding Laboratories, France) promptly after decapitation, and cells were enzymatically and mechanically dispersed as previously described (1, 16). In brief, cells from 10–15 pituitaries/experiment were dispersed using trypsin and deoxyribonuclease I (Sigma-Aldrich Chimie, France), suspended in DMEM culture medium (Sigma), with no phenol red, containing 10% charcoal-dextran-stripped FCS (Boehringer Mannheim, Meylan, France) and seeded at a density of 10^5 cells/ml·well in 24-well plates (Nunc, Roskilde, Denmark) for hormone and cAMP measurements or at a density of 10^6 cells/3 ml·35-mm diameter culture dish (Nunc) for measurement of sst mRNA levels. Cells were precultured as monolayers for 4 days at 37 C in a water-saturated 6% CO_2-94% air, with medium renewal after 2 days. Cultures were conducted in parallel in the presence or absence of 1 nM E_2 (Sigma), as previously reported (4, 5, 17).

GH and PRL release

After removal of the culture medium, the monolayers were rinsed with DMEM and exposed to forskolin (1 μM) alone or in combination with different concentrations of SRIF or somatostatin receptor agonists at 37 C for 3 h in DMEM-HEPES (15 mM; pH 7.4)-buffered medium containing BSA (0.1%). At the end of the treatment, media were collected, aliquoted, and stored frozen for hormone assay. GH and PRL levels in the medium were measured by competitive enzyme immunoassays using rat GH (rGH) or rat PRL (rPRL) coupled to acetylcholinesterase as tracers and highly specific polyclonal immune sera obtained from goats for rGH and from rabbits for rPRL (18). Dose-response curves established using NIAMDD RP-2 rGH or RP-3 rPRL as standards displayed ED_{50} values of 3.3 and 2.5 ng/ml, respectively. OCT and AcNH-4-NO_2-Phe-c(D-Cys-Tyr-D-Trp-Lys-Thr-Cys)-D-Tyr-NH_2 were gifts from Novartis (Basel, Switzerland), and BIM-23052, BIM-23056, and BIM-23268 were obtained from Biomeasure (Milford, MA).

Intracellular [^{3}H]cAMP accumulation

After removal of the culture medium, cells were labeled with [^{3}H]adenine (2 μCi/ml) in serum-free DMEM-HEPES-buffered medium at 37 C for 2 h. The labeling medium was aspirated, and cells were incubated with different test substances diluted in DMEM-HEPES containing 1 mM 3-isobutyl-1-methylxanthine (Sigma). After 2 h, the supernatant was eliminated, and the reaction was terminated by the addition of ice-cold 5% trichloroacetic acid (Sigma) containing 1 mM cAMP (Boehringer Mannheim) and 1 mM ATP (Boehringer Mannheim). Isolation of [^{3}H]cAMP was performed by sequential chromatography on Dowex (Pharmacia, Uppsala, Sweden) and alumina columns according to the technique described by Salomon *et al*. (19). Radioactivity was evaluated by liquid scintillation counting (LKB-Pharmacia), and the data were expressed as [^{3}H]cAMP/[^{3}H]ATP ratios, which permits estimates of adenylate cyclase activity by the conversion of [^{3}H]ATP to [^{3}H]cAMP.

Assay of sst mRNAs levels by quantitative RT-PCR

mRNA levels for each somatostatin receptor isoform were quantified by means of the competitive RT-PCR assay described by Viollet *et al*. (15). The principle of the method is to coamplify an internal standard together with the mRNA species of interest in each assay. The internal standard consists of an artificial RNA containing the sequences required for specific amplification of each sst. Coamplification was permitted by using pairs of RT-PCR primers consisting of a 5′-primer specific for a given sst1 to sst5 mRNA and a 3′-primer common to all five species.

Total RNA was extracted and purified from control and E_2-treated cells via the guanidium/CsCl gradient method (20). One microgram of total RNA was mixed with serial dilutions of the synthetic RNA standards. The mixture was denatured and reverse transcribed in a 20-μl volume containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 625 μM of each deoxy-NTP (Boehringer Mannheim), 100 μM random hexamers (Pharmacia), 20 U RNasin (Promega Biotech, Madison, WI), and 200 U Moloney murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY). Samples were incubated for 90 min at 37 C, followed by 10 min at 70 C and chilled on ice. Two types of controls were performed. For the absence of genomic DNA contamination during the preparation of RNA samples, aliquots of each total RNA sample were submitted to the RT reaction in the absence of reverse transcriptase. Additionally, the RT reaction was performed in the absence of RNA sample.

Amplification conditions were the same for all receptors. One tenth of the RT sample was amplified in a 50-μl volume in 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each deoxy-NTP (Boehringer Mannheim), 25 pmol of each sense and reverse primer, 0.5×10^6 cpm 5′-end ^{32}P-labeled reverse primer, and 1.5 U *Taq* polymerase (Promega Biotech, Madison, WI). The amplification included an initial denaturation step at 94 C for 30 sec, 29–32 cycles (denaturation at 94 C for 30 sec, annealing at 60 C for 1 min, elongation at 72 C for 30 sec), and a final step at 72 C for 10 min. Amplification was performed in an automatic thermocycler (Hybaid, Teddington, UK). In each experiment, a control including the PCR mixture with no complementary DNA template was added to check possible external contamination.

A 5-μl aliquot of each PCR reaction was electrophoresed in a 5% or 8% polyacrylamide gel (Bio-Rad, Hercules, CA). In the case of sst1 detection, amplification products were digested for 1 h at 37 C with *Xba*I restriction enzyme (Boehringer Mannheim) before migration to distinguish both standard and target PCR products. After migration, the gel was dried and exposed to X-Omat autoradiographic films (Eastman Kodak, Rochester, NY). Autoradiogram bands corresponding to the amplified products were cut and counted in a β-scintillation counter. The amount of radioactivity recovered from the excised gel was plotted as the internal standard RNA/target mRNA ratio against the initial internal standard RNA amount. Linear regression of the curve was calculated. The number of target molecules was obtained by extrapolating the number of internal standard RNA molecules corresponding to an isomolar ratio of both species. Results were expressed as molecules per μg total RNA.

Statistical analysis

Each experiment was repeated at least three times using triplicate or quadruplicate independent culture dishes per experimental condition. Data obtained from independent experiments were normalized and pooled as indicated in the figure legends. The IC_{50} of dose-response analyses were calculated using the GraphPad Prism program. Data were submitted to ANOVA and parametric F tests. A two-tailed *t* test was used for RT-PCR data analysis. Results are expressed as the mean ± SEM.

Results

GH and PRL release

Added to estrogen-depleted culture at 0.1-μM concentrations, SRIF, OCT, and BIM-23052 were equally effective in inhibiting forskolin-stimulated GH release, whereas BIM-23056 was ineffective (Fig. 1, *upper panel*). Addition of 1 nM E_2 to the culture for 96 h induced a moderate decrease ($P = 0.09$) in basal and a significant decrease ($P < 0.001$) in forskolin-stimulated GH secretion with respect to those recorded in estrogen-depleted cultures, but did not affect the hormonal response to SRIF agonists. Analysis of dose-response curves obtained on E_2-treated cells (Fig. 2, *upper panel*) allowed them to be ranked in a SRIF = OCT ≫ BIM-23052 > BIM-23268 decreasing order of potency, with similar maximal inhibition for all four analogs (Table 1).

E_2 treatment did not affect basal PRL release, but forskolin stimulation was increased ($P < 0.005$) compared with that in controls. In the absence of E_2, PRL release was not affected by SRIF analogs (Fig. 1, *lower panel*). In contrast, an inhibitory response to SRIF, OCT, and BIM-23052 was detected after pretreatment of the cells with the steroid.

Dose-response plots (Fig. 2, *lower panel*) suggest that in steroid-supplemented medium, the order of potency of the

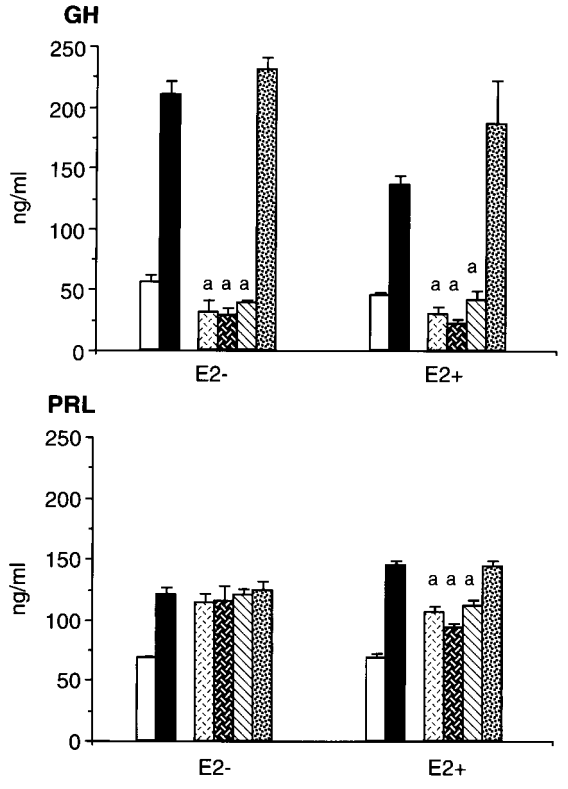

FIG. 1. Effect of E_2 on SRIF- and SRIF agonist-induced inhibition of forskolin-stimulated GH (*top*) and PRL (*bottom*) release from anterior pituitary cells in culture. Cells were exposed to 1 nM E_2 for 96 h as described in *Materials and Methods*. □, Basal hormone release; ■, forskolin (1 μM)-stimulated hormone release; ▨, forskolin- plus 0.1 μM SRIF-stimulated hormone release; ▩, forskolin- plus 0.1 μM OCT-stimulated hormone release; ▧, forskolin- plus 0.1 μM BIM-23052-stimulated hormone release; ▨ forskolin- plus 0.1 μM BIM-23056-stimulated hormone release. Values are the mean ± SEM of 6–10 independent determinations. a, $P < 0.001$ *vs.* forskolin-stimulated release.

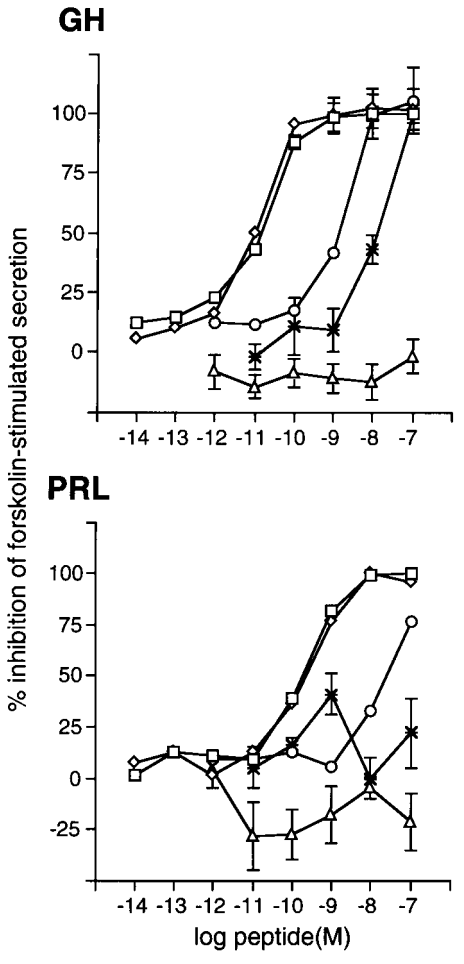

FIG. 2. Dose-dependent inhibition by SRIF and SRIF agonists of forskolin (1 μM)-stimulated GH (*top*) and PRL (*bottom*) secretion from E_2-treated rat anterior pituitary cells in culture. Data are presented as a percentage of maximal inhibition induced by SRIF (0.1 μM; mean ± SEM of four independent determinations from one representative experiment). Basal release, 45.1 ± 2.3 and 74.5 ± 1.4 ng/ml for GH and PRL, respectively. Forskolin-increased secretion, 127.0 ± 2.0 ng/ml and 145.9 ± 1.6 ng/ml, respectively. SRIF (0.1 μM) inhibition of forskolin-stimulated secretion, 23.7 ± 1.0 and 100.2 ± 1.1 ng/ml, respectively. □, SRIF; ◇, octreotide; ○, BIM-23052; △, BIM-23056; *, BIM-23268.

agonists on PRL and GH release is identical. However, the potencies of SRIF, OCT, and BIM-23052 were 1 order of magnitude higher for GH than for PRL inhibition. BIM-23268, which was weakly active on GH secretion, appeared inactive on PRL secretion. Although maximal SRIF, OCT, and BIM-23052 inhibition of forskolin-stimulated GH secretion reached values lower than basal levels, this was not the case for inhibition of PRL secretion.

BIM-23056 did not have any effect by itself on the release of either hormone under all conditions tested (Figs. 1 and 2). As the analog has been proposed as a SRIF antagonist on the human recombinant sst5 receptor (21), we tested its action in the presence of SRIF (0.01 μM) on GH or PRL release. BIM-23056 (0.1 μM) weakly potentiated the inhibitory effect of SRIF on PRL release in E_2-treated cultures only (Table 2). Another somatostatin analog, AcNH-4-NO_2-Phe-c(D-Cys-Tyr-D-Trp-Lys-Thr-Cys)-D-Tyr-NH_2, which was reported to have antagonistic properties on the sst2 receptor (compound 4 in Ref. 22), was not effective as such on SRIF inhibition of GH and PRL release under our experimental conditions (Table 2). However, it weakly potentiated the inhibitory effect of SRIF on GH release in E_2-treated cultures.

TABLE 1. Relative potencies of SRIF agonists on the inhibition of GH and PRL release from E_2-treated anterior pituitary cells

	IC_{50} (nmol)	
	GH	PRL
SRIH-14	0.019 ± 0.012	0.21 ± 0.12
Octreotide	0.015 ± 0.011	0.24 ± 0.13
BIM-23052	2.00 ± 1.22	28.1 ± 14.9
BIM-23268	19.93 ± 1.39	>100
BIM-23056	>100	>100

Effect of E_2 on forskolin-induced cAMP accumulation

In control cells, SRIF, OCT, and BIM-23052 (1 μM) reduced forskolin-induced cAMP accumulation by 39 ± 5%, 49 ± 3%, and 21 ± 3%, respectively (Fig. 3). Treatment with E_2 resulted in a 2-fold increase in the amplitude of the responses, to 75 ± 1%, 76 ± 2%, and 62 ± 1%, respectively. BIM-23056 was

TABLE 2. Lack of effect of putative human sst5 and rat sst2 antagonists on GH and PRL secretion from E_2-treated rat pituitary cells in culture

	Fk (1 μM)	Fk + SRIF (0.01 μM)	Fk + SRIF + BIM-23056 (0.1 μM)	Fk + SRIF + compound 4[a] (0.1 μM)
GH (ng/ml)	130.9 ± 3.4	50.7 ± 8.6[b]	36.9 ± 1.4[b]	32.7 ± 6.8[b,c]
PRL (ng/ml)	147.2 ± 2.5	96.3 ± 3.5[b]	79.2 ± 5.4[b,c]	96.7 ± 5.6[b,d]

Data are the mean ± SEM of four independent determinations.
[a] According to the nomenclature of Bass *et al.* (22).
[b] $P < 0.001$ *vs.* Fk.
[c] $P < 0.05$ *vs.* Fk + SRIF.
[d] $P < 0.05$ *vs.* Fk + SRIF + Bim-23056.

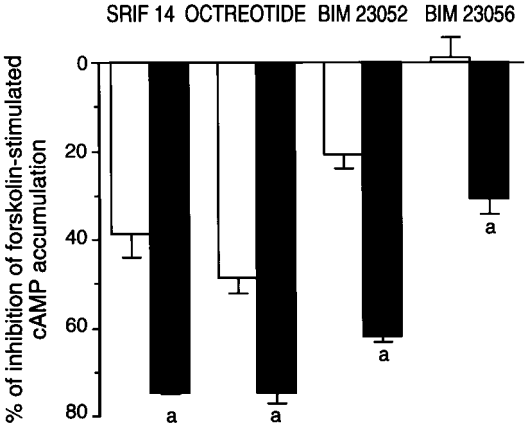

FIG. 3. Comparison of the effects of SRIF and analogs (1 μM) on forskolin (1 μM)-induced cAMP accumulation in control (□) and in E_2-treated cells (■). Values are the mean ± SEM of threee independent determinations from three experiments. a, $P < 0.001$, E_2 *vs.* control.

ineffective under control conditions (0.5 ± 1% activation), but induced a moderate, but significant, inhibition of cAMP accumulation in cells exposed to E_2.

Effect of E_2 on expression of somatostatin receptor subtypes

Using quantitative RT-PCR, all five somatostatin receptor mRNAs were identified in primary cultures of pituitary cells (Fig. 4). No difference in PCR efficiency occurred between wild-type and standard mRNA, as shown by the parallelism of both amplification curves illustrated for sst2 (Fig. 4, *inset*). Highest mRNA levels in control cells correspond to sst2 and sst3 subtypes (31% and 34% of total ssts, respectively). sst1 mRNA was moderately expressed (19% of the total ssts), and sst4 and sst5 mRNAs were weakly expressed (9% and 6%, respectively).

Treatment with estradiol significantly increased the levels of expression of sst2 and sst3 by 60% and 43%, respectively. In contrast, the same treatment induced a significant decrease in sst1 mRNA level expression and a discrete inhibition of mRNA levels corresponding to sst4 and sst5 receptor subtypes.

Discussion

The fact that E_2 pretreatment of pituitary cells is a prerequisite for inhibition of PRL by SRIF confirms previous reports (2–4) that showed that the peptide interferes with PRL release only after estradiol priming of lactotropes.

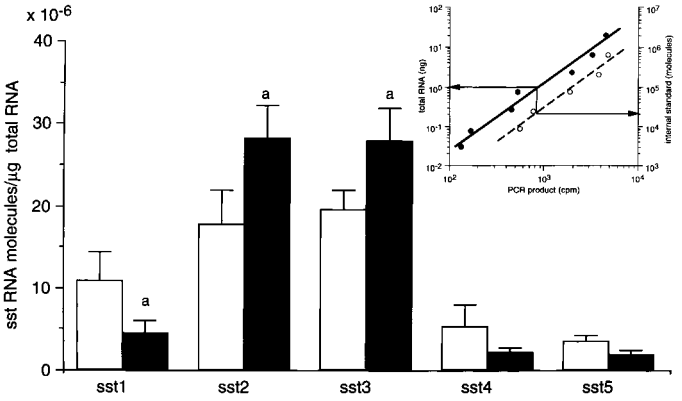

FIG. 4. Effect of E_2 treatment on distribution of SRIF receptor mRNAs in anterior pituitary cells in culture. □, Control; ■, E_2-treated cells. Each value represents the mean ± SEM from three independent cultures. a, $P < 0.01$, E_2 *vs.* control. *Inset*, Quantitative RT-PCR standard curve. The standard curve is obtained by amplifying serial dilutions of internal standard RNA (○) and total ssts RNA (●) under the same conditions.

To test whether E_2-induced PRL sensitivity to SRIF involves selective up-regulation of discrete somatostatin receptor subtypes, we compared the pattern of expression of pituitary sst receptor mRNA, measured by quantitative RT-PCR, with the efficacy of SRIF and of relatively selective SRIF analogs (OCT, BIM-23052, BIM-23056, and BIM-23268) in inhibiting GH and PRL secretion and cAMP accumulation (for the first three analogs) in anterior pituitary cells. We used a phenol red-free defined culture medium, because this frequently used pH indicator exhibits a weak estrogenic activity and by itself is able to stimulate cell content and release of PRL (23, 24).

Under the present experimental conditions, exposure of cells to E_2 did not affect somatostatin inhibition of forskolin-stimulated GH release, in agreement with previous data obtained on female Sprague-Dawley pituitary cells grown for 72 h in the presence of 0.1 μM E_2 (25). The fact that E_2 did not affect the amplitude of the GH response suggests that receptor numbers on somatotropes are already maximal under basal conditions. At the same time E_2-induced sensitivity of lactotropes resulted in a significant inhibition of forskolin-stimulated PRL secretion by SRIF, OCT, and BIM-23052, but not by BIM-23056 or BIM-23268.

Using quantitative RT-PCR assay we determined the absolute amounts of somatostatin receptor mRNAs expressed in pituitary cultures. The RT-PCR quantitation was always achieved within the range of parallel amplification of synthetic standard and sample mRNAs. Under these conditions, all five known sst subtypes were expressed after 4 days in culture. Major forms of mRNAs coding for SRIF receptors corresponded to isoforms sst2 and sst3, whereas mRNAs coding for the other subtypes were present in low amounts only. In addition, our data indicate that sst5 represents a minor component only of total pituitary sst receptor mRNA, although this subtype appears more strongly expressed in the pituitary than in most other tissues such as the brain or the gastrointestinal tract. Our results are in agreement with those of Bruno *et al.*, who reported a similar pattern of expression in adult male rat Sprague-Dawley pituitaries

using a semiquantitative hybridization/nuclease protection method (8). *In situ* hybridization studies have also shown that all sst subtypes seem to be present in all pituitary cell types and do not exhibit clear-cut colocalization with given subtypes (9). In another study, sst2 and sst5 transcripts were found to be more abundant in somatotropes and thyrotropes than in the other cell types (10). sst3 mRNA-containing cells were also frequently observed in the intermediate lobe of the pituitary, and sst2 and sst3 mRNAs were found in scattered cells of the neural lobe (9).

Treatment of the cells with estradiol for 4 days in culture resulted in selective overexpression of sst2 and sst3 subtypes. This observation agrees with the report by Visser-Wisselaar *et al.* (11, 26) that addition of E_2 for 7 or 14 days *in vitro* induces the expression of sst2 and sst3 receptor subtypes in the 7315b rat prolactinoma cells, whereas sc administration of estradiol *in vivo* to 7315b tumor-bearing rats provokes sst2 mRNA expression only. In the GH_4C_1 cell line, 24-h treatment with estrogen was reported to increase not only sst2 and sst3, but also sst1 expression (27). Other conclusions were drawn concerning the gonadal steroid regulation of somatostatin receptor expression. *In situ* hybridization studies suggested that pituitary sst2 mRNA levels were unaffected by gonadal steroids in Sprague-Dawley adult rats (28). In contrast, in the same study, pituitary sst1 and sst3 mRNA expression appeared sexually dimorphic, with higher levels of sst1 in the female and higher levels of sst3 in the male.

As far as the other receptor subtypes are concerned, we observed lower levels of sst1 receptor mRNAs after estradiol treatment, whereas the decrease in the expression of sst4 and sst5 receptor mRNAs did not reach statistical significance. This suggests the existence of a differential regulation of sst5 with respect to that of sst2 and sst3. Such differential regulation of SRIF receptor subtypes has also been reported under different experimental conditions, in particular between sst1 and sst3, on the one hand, and sst5, on the other, in the pituitaries of food-deprived rats (29).

As the turnover of subtype-specific mRNAs is not known, any extrapolation of our data to corresponding receptor proteins should be interpreted with caution. To evaluate functional receptors, we thus analyzed the pharmacology of GH and PRL release in response to OCT, BIM-23052, BIM-23056, and BIM-23268, four subtype-selective SRIF analogs.

Involvement of sst2 receptors in somatostatin inhibition of GH secretion was already indicated by previous data for rat (30, 31), sheep (31), and fetal human (14) pituitary cultures, whereas sst5 appeared involved in the last two species only. Our data indicate that E_2 pretreatment does not modify the pharmacology of SRIF inhibition of GH secretion, as only sst2 active agonists (SRIF itself and OCT) displayed high potency compared with sst3 and sst5 preferential agonists (BIM-23052 and BIM-23268). A recently reported sst2-selective antagonist (22) did not display antagonistic properties, but weakly potentiated the actions of SRIF on GH secretion. It might be recalled that at the dose that we used, it slightly inhibited cAMP accumulation from GH_4C_1 cells (22).

Our pharmacological results suggest that inhibition of lactotropes by SRIF is also preferentially mediated by sst2. After exposure to E_2, OCT, and BIM-23052, but not BIM-23268 and BIM-23056, induced a dose-dependent inhibition of PRL release with IC_{50} values 1 order of magnitude higher than those corresponding to GH inhibition. This rank of potency for SRIF agonist is in keeping with 1) the 20 times lower membrane binding affinity of OCT in cells transfected with rat sst3 compared with cells transfected with rat sst2 (32) (Meyerhof, W., personal communication), 2) the 25- to 40-fold higher affinity of BIM-23052 (13) and BIM-23268 (Taylor, J. E., personal communication) for rat sst3 and sst5 than for rat sst2 in transfected cells, and 3) the absence of effects of the weak sst5 agonist BIM-23056 (13). These data are strongly suggestive that sst2 is the major receptor controlling GH and PRL inhibition. On the other hand, however, increased efficacy of OCT and BIM-23052 to inhibit cAMP accumulation after treatment with E_2 may result from a combined action of sst2 and sst3. Finally, the failure of a putative antagonist (22) to show the same effect in our system suggests that data obtained from transfected cells cannot be easily extrapolated to normal pituitary cells.

Lower potency of all three active SRIF analogs on PRL than on GH secretion cannot be explained by differential receptor involvement, because their potency ratio was found homothetic in both cases. Alternative splicing of the sst2 isoform (33), which was reported to exhibit different coupling properties (34), may be one of the possible mechanisms responsible for the lower potency of analogs in lactotropes.

Participation of the rat sst5 isoform in GH inhibition or in E_2-induced PRL inhibition by SRIF appears of minor importance, as BIM-23056, an analog with highest (although rather weak) affinity for rat sst5 (13), was ineffective as either an agonist or antagonist despite reports (21) that the analog acted as a potent antagonist (0.1 μM) in CHO-K1 cells transfected with the human recombinant sst5 receptor. In fact, BIM-23056 was only able to slightly potentiate the effect of SRIF on PRL secretion. In addition, sst5 mRNA appeared more down-regulated than up-regulated by exposure to estradiol. Nevertheless, inhibition of cAMP accumulation by BIM-23056 after pretreatment of cells with estradiol suggests that the agonist may also be recognized by cell types other than lactotropes and somatotropes. On the other hand, this observation is also consistent with recurrent doubts about the extent of direct cAMP involvement in the regulation of hormone secretion.

In summary, our results indicate that estrogens selectively increase pituitary mRNA levels corresponding to sst2 and sst3 receptor subtypes in the female rat. In addition, they confirm that sst2 is the most relevant somatostatin receptor for rat GH inhibition regardless of the steroid environment. However, there appears to be species differences in the receptors involved, as sst5 may also play a significant role in the regulation by SRIF of sheep (31) and human fetal (14) GH secretion. sst2 is also the major receptor responsible for rat PRL inhibition after priming of lactotropes with estrogens as also found in human fetal pituitary cells (14). Although participation of the sst3 subtype cannot be formally excluded, our pharmacological data suggest that its role in GH and PRL control is relatively minor. The pituitary functions of lowly expressed sst1 and sst4 receptor transcripts remain to be established.

References

1. **Epelbaum J, Enjalbert A, Krantic S, Musset F, Bertrand P, Rasolonjanahary R, Shu C, Kordon C** 1987 Somatostatin receptors on pituitary somatotrophs, thyrotrophs, and lactotrophs: pharmacological evidence for loose coupling to adenylate cyclase. Endocrinology 121:2177–2185
2. **Cooper GR, Shin SH** 1981 Somatostatin inhibits prolactin secretion in the estradiol primed male rat. Can J Physiol Pharmacol 59:1082–1088
3. **Vale W, Rivier C, Brazeau P, Guillemin R** 1974 Effects of somatostatin on the secretion of thyrotropin and prolactin. Endocrinology 95:968–977
4. **Kimura N, Hayafuji C, Konagaya H, Takahashi K** 1986 17β-Estradiol induces somatostatin (SRIF) inhibition of prolactin release and regulates SRIF receptors in rat anterior pituitary cells. Endocrinology 119:1028–1036
5. **Kimura N, Hayafuji C, Kimura N** 1989 Characterization of 17β-estradiol-dependent and -independent somatostatin receptor subtypes in rat anterior pituitary. J Biol Chem 264:7033–7040
6. **Reisine T, Bell GI** 1995 Molecular biology of somatostatin receptors. Endocr Rev 16:427–442
7. **Viollet C, Prevost G, Maubert E, Faivre-Bauman A, Gardette R, Kordon C, Loudes C, Slama A, Epelbaum J** 1995 Molecular pharmacology of somatostatin receptors. Fund Clin Pharmacol 9:107–113
8. **Bruno JF, Xu Y, Song JF, Berelowitz M** 1993 Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat. Endocrinology 133:2561–2567
9. **O'Carroll AM, Krempels K** 1995 Widespread distribution of somatostatin receptor messenger ribonucleic acids in rat pituitary. Endocrinology 136:5224–5227
10. **Day R, Dong WJ, Panetta R, Kraicer J, Greenwood MT, Patel YC** 1995 Expression of mRNA for somatostatin receptor (sstr) types 2 and 5 in individual, rat pituitary cells. A double labeling *in situ* hybridization analysis. Endocrinology 136:5232–5235
11. **Visser-Wisselaar HA, Van Uffelen CJ, Van Koetsveld PM, Lichtenauer-Kaligis EG, Waaijers AM, Uitterlinden P, Mooy DM, Lamberts SW, Hofland LJ** 1997 17β-Estradiol-dependent regulation of somatostatin receptor subtype expression in the 7315b prolactin secreting rat pituitary tumor *in vitro* and *in vivo*. Endocrinology 138:1180–1189
12. **Bruns C, Raulf F, Hoyer D, Schloos J, Lubbert H, Weckbecker G** 1996 Binding properties of somatostatin receptor subtypes. Metabolism 45:17–20
13. **Viollet C, Bodenant C, Prunotto C, Roosterman D, Schaefer J, Meyerhof W, Epelbaum J, Vaudry H, Leroux P** 1997 Differential expression of multiple somatostatin receptors in the rat cerebellum during development. J Neurochem 68:2263–2272
14. **Shimon I, Taylor JE, Dong JZ, Bitonte RA, Kim S, Morgan B, Coy DH, Culler MD, Melmed S** 1997 Somatostatin receptor subtype specificity in human fetal pituitary cultures. Differential role of SSTR2 and SSTR5 for growth hormone, thyroid-stimulating hormone, and prolactin regulation. J Clin Invest 99:789–798
15. **Viollet C, Faivre-Bauman A, Zhang J, Llorens-Cortes C, Loudes C, Kordon C, Epelbaum J** 1995 Differential expression of somatostatin receptors by quantitative PCR in the rat brain. C R Acad Sci [D] (Paris) 318:851–857
16. **Hopkins CR, Farquhar MG** 1973 Hormone secretion by cells dissociated from rat anterior pituitaries. J Cell Biol 59:276–303
17. **Bertrand P, Drouva S, Krantic S, Epelbaum J, Kordon C, Enjalbert A** 1988 Modulation of receptivity of lactotroph cells by estradiol. In: Genazzani AR, Montemagno U, Nappi C, Petraglia F (eds) The Brain and Female Reproductive Function. Parthenon, Park Ridge, pp 137–144
18. **Duhau L, Grassi J, Grouselle D, Enjalbert A, Grognet JM** 1991 An enzyme immunoassay for rat prolactin: application to the determination of plasma levels. J Immunoassay 12:233–250
19. **Salomon Y, Londos C, Rodbell M** 1974 A highly sensitive adenylate cyclase assay. Anal Biochem 58:541–548
20. **Maniatis T, Fritsch EF, Sambrook J** 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor
21. **Wilkinson GF, Thurlow RJ, Sellers LA, Coote JE, Feniuk W, Humphrey PP** 1996 Potent antagonism by BIM-23056 at the human recombinant somatostatin sst5 receptor. Br J Pharmacol 118:445–447
22. **Bass RT, Buckwalter BL, Patel BP, Pausch MH, Price LA, Strnad J, Hadcock JR** 1996 Identification and characterization of novel somatostatin antagonists. Mol Pharmacol 50:709–715
23. **Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS** 1986 Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. Proc Natl Acad Sci USA 83:2496–2500
24. **Hofland LJ, van Koetsveld P, Koper JW, den Holder A, Lamberts SW** 1987 Weak estrogenic activity of phenol red in the culture medium: its role in the study of the regulation of prolactin release *in vitro*. Mol Cell Endocrinol 54:43–50
25. **Fukata J, Martin JB** 1986 Influence of sex steroid hormones on rat growth hormone-releasing factor and somatostatin in dispersed pituitary cells. Endocrinology 119:2256–2261
26. **Visser-Wisselaar HA, Hofland LJ, Vanuffelen CJC, Vankoetsveld PM, Lamberts SWJ** 1996 Somatostatin receptor manipulation. Digestion 57:7–10
27. **Xu Y, Berelowitz M, Bruno JF** 1995 Dexamethasone regulates somatostatin receptor subtype messenger ribonucleic acid expression in rat pituitary GH_4C_1 cells. Endocrinology 136:5070–5075
28. **Senaris RM, Lago F, Dieguez C** 1996 Gonadal regulation of somatostatin receptor 1, 2 and 3 mRNA levels in the rat anterior pituitary. Brain Res Mol Brain Res 38:171–175
29. **Bruno JF, Xu Y, Song JF, Berelowitz M** 1994 Pituitary and hypothalamic somatostatin receptor subtype messenger ribonucleic acid expression in the food-deprived and diabetic rat. Endocrinology 135:1787–1792
30. **Raynor K, Murphy WA, Coy DH, Taylor JE, Moreau JP, Yasuda K, Bell GI, Reisine T** 1993 Cloned somatostatin receptors: identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides. Mol Pharmacol 43:838–844
31. **Briard N, Dutour A, Epelbaum J, Sauze N, Slama A, Oliver C** 1997 Species differences between rat and sheep pituitary somatostatin receptors involved in the inhibition of GH secretion. Eur J Endocrinol 137:545–555
32. **Coy DH, Taylor JE** 1996 Receptor-specific somatostatin analogs: correlations with biological activity. Metabolism 45:21–23
33. **Vanetti M, Kouba M, Wang X, Vogt G, Hollt V** 1992 Cloning and expression of a novel mouse somatostatin receptor (SSTR2B). FEBS Lett 311:290–294
34. **Vanetti M, Vogt G, Hollt V** 1993 The two isoforms of the mouse somatostatin receptor (mSSTR2A and mSSTR2B) differ in coupling efficiency to adenylate cyclase and in agonist-induced receptor desensitization. FEBS Lett 331:260–266