Species differences between male rat and ram pituitary somatostatin receptors involved in the inhibition of growth hormone secretion

N Briard, A Dutour, J Epelbaum1, N Sauze, A Slama1 and C Oliver

Laboratoire de Neuroendocrinologie Experimementale, INSERM U297, Institut Fédératif Jean Roche, 13916 Marseille Cedex 20, France
and 1INSERM U159, Centre Paul Broca, 2 Ter rue d’Alesia, 75014 Paris Cedex, France

(Correspondence should be addressed to A Dutour, Laboratoire de Neuroendocrinologie Experimementale, INSERM U297, Institut Fédératif Jean Roche, 13916 Marseille Cedex 20, France)

Abstract

The sheep is a valuable model in which to study GH neuroregulation as its pattern of GH secretion is very close to that in humans. Furthermore, important differences in somatostatin (SRIH) action between rats and sheep have been found previously. Our goal was to compare in male rat and ram pituitaries the binding characteristics of somatostatin receptors and the effect of SRIH and 17 analogues on GH release. Using radioautography, SRIH binding was seen to be evenly distributed over the anterior pituitary of both species. In the binding assay, binding sites were three times more concentrated in rats than in sheep. Important interspecies differences in the action of SRIH and its analogues were found: they inhibited GH at lower concentrations in rats than in sheep. Seven peptides displayed greater inhibitory ability in sheep than in rats while three were more potent in rats. Agonistic potencies to inhibit GH release in rats were correlated with somatostatin receptors subtype 2 (sst2) affinities. Our data confirm and extend the quantitative differences between rat and sheep in SRIH inhibitory action on GH secretion and confirm that ligand-binding properties of a given receptor subtype cannot be extrapolated across species.

European Journal of Endocrinology 137 545–555

Introduction

Somatostatin (somatotropin release-inhibiting hormone or SRIH) is a cyclic tetradecapeptide that was originally isolated from ovine hypothalamus and characterized as a potent physiological inhibitor of growth hormone (GH) secretion (1). The control of GH secretion also depends upon another hypothalamic neurohormone, GH-releasing hormone (GHRH) (2). In most species, GH secretion is pulsatile and influenced by various conditions including stress, feeding, and pharmacological manipulations that involve central neurotransmitters (3). Most studies on the neuroregulation of GH secretion have been performed in the male rat with the conclusion that GH pulses and troughs are generated by reciprocal changes of GHRH and SRIH release into the hypophysial portal blood (HPB) (4). However rats may not be the ideal model for studies on GH neuroregulation as GH secretion in the male rat is characterized by a strikingly regular ultradian rhythm, rather different to the irregular secretory bursts found in other species including humans and sheep (5). Also, the physiological regulation of GH can differ in the male rat from that in other species including humans. For example, under stress and fasting, GH levels decrease in rats and increase in humans and sheep. Furthermore, in sheep, it is possible to study the regulation of GH secretion by directly measuring the concentrations of GHRH and SRIH in HPB without stress (6). Different studies have shown that endogenous GHRH plays a major role in the control of GH secretion in sheep. In contrast, no clear inhibiting action of endogenous somatostatin on GH release could be demonstrated. Indeed, pulsatile spontaneous GH release (6, 7) as well as neostigmine- (8), clonidine- (9), and tianeptine- (10) induced GH secretion were associated with an increased release of GHRH into HPB without significant changes in SRIH levels. In this species, active immunization against SRIH did not affect GH secretion while a striking reduction in plasma GH concentrations was observed in the group of anti-GHRH animals (11). GH response to an acute GHRH stimulation was even lower in the anti-SRIH group than in the control group, suggesting that SRIH could be involved in GH regulation through the prevention of GH stores depletion and/or GHRH receptor desensitization (12, 13). Previous reports have shown that very high doses of somatostatin are required to inhibit basal and stimulated GH secretion in...
the sheep (14–17). These data suggest important interspecies differences in the physiological involvement of SRIH at the pituitary level.

In several species including humans, mice, and rats, SRIH exerts its biological effects by binding to high-affinity receptors (18). Five distinct somatostatin receptor (sst) subtype genes have recently been identified, encoding for a family of G protein-coupled receptors (19–30). Their amino acid homology ranges from 42 to 60%. Each receptor subtype is differently expressed in various tissues (31, 32) and displays distinct pharmacological properties. All five subtypes appear coupled to inhibition of adenylyl cyclase (33). Rat pituitary cells, as well as the GH3 pituitary cell line, express all five sst mRNA (31, 34). In situ hybridization combined with immunohistochemistry (35) and reverse transcription (RT)-PCR in purified somatotrophs (36) revealed that rat somatotrophs express mRNA for all five ssts. In situ hybridization analysis revealed a relatively high level of sst4 (35) and sst5 (35, 36) mRNA expression in somatotrophs as well as an important although lower expression of sst2. A heterogeneous expression of SRIH receptor subtypes has also been demonstrated in human pituitary tumours (37–39). Sst2 appears to be the most frequently expressed and sst5 is predominantly found in somatotroph adenomas (37, 38).

Because of the short half-life of SRIH in vivo, various long-acting somatostatin analogues have been developed for clinical applications (40). Recently, different groups have investigated the binding affinities of a battery of SRIH analogues for all five human and rodent sst, expressed in Chinese hamster ovary (CHO) and COS-1 cell lines. These authors have identified several relatively potent and selective analogues for sst2, sst3, and sst5. They have also reported that the potencies of various SRIH agonists in inhibiting GH release in vitro are highly correlated with their potencies in inhibiting $^{125}$I[SRIH binding to rat sst2 (41–44).

On the basis of the above-cited physiological and pharmacological studies on somatostatin and GH secretion, we investigated the pharmacological characteristics of somatostatin receptors in ovine anterior pituitary. Using the relative specificity of various SRIH analogues, we also compared the effects of SRIH and 17 analogues on GHRH-induced GH release from both male rat and ram pituitary cells in order to test if there is a species difference in the action of SRIH and its analogues.

Materials and methods

Peptides

The structure and source of SRIH 14, SRIH 28, and SRIH analogues are given in Table 1.

Ovine pituitary cell cultures

Pituitary glands from lambs (6-month-old males) were removed immediately after decapitation at a local slaughterhouse and placed in phosphate-balanced salt solution (PBS) (without Ca$^{2+}$ and Mg$^{2+}$) containing fungizone (2.5 mg/l) (Gibco), gentamycin (5 mg/l) (Gibco), penicillin (10 000 U/l) (Gibco), and streptomycin (10 000 U/l) (Gibco, Cergy P). Within 10 min after lamb decapitation, pituitaries were dispersed individually at 37°C for 1 h in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) with collagenase (1 mg/ml) (Sigma Chemical Co., Saint Quentin Fallavier, France), hyaluronidase (0.5 mg/ml) (Sigma) and DNase (0.5 mg/ml) (Sigma). The dispersed cells were harvested by centrifugation for 10 min at 400 r.p.m. The non-dispersed cells were digested again at 37°C for 1 h then for an additional 5 h. Then, the cells were plated into culture dishes and incubated at 37°C in a humidified atmosphere of 95% O$_2$ and 5% CO$_2$. The next day, the cells were dispersed using trypsin solution, counted with a hemocytometer, diluted with DMEM supplemented with 10% foetal bovine serum (FBS), and randomly plated at a density of 50 000 cells/ml/well into 24-well Falcon plates. The cell cultures were incubated at 37°C in the same humidified atmosphere for 6 days and culture media were changed every 48 h.

On the seventh day in culture, the cells were washed with PBS then stimulated during 4 h with serum-free medium (DMEM+1% BSA) containing GHRH (10$^{-6}$ mol) with or without increasing concentrations of SRIH analogues (10$^{-6}$ to 10$^{-11}$ mol). Each concentration was tested in 8 wells. The medium was removed after 4 h, centrifuged, and stored at −20°C until GH RIA. Each experiment was repeated twice.

Rat pituitary cell cultures

The pituitary glands from adult male Sprague Dawley rats (3 months old), removed immediately after decapititation, were placed in PBS (without Ca$^{2+}$ and Mg$^{2+}$) with fungizone (2.5 mg/l), gentamycin (5 mg/l), penicillin (10 000 U/l), streptomycin (10 000 U/l), and 1% BSA. The anterior lobes were separated from the posterior lobes and dispersed at 37°C for 1 h in DMEM with collagenase (1 mg/ml) and hyaluronidase (0.5 mg/ml). Cell dispersion was increased by trituration of fragments through Pasteur pipettes every 15 min. Once dispersion was completed the cells were harvested by centrifugation for 2.5 min at 400 r.p.m. Then the cells were plated at a density of 50 000 cells/ml/well in 24-well plastic tissue culture dishes (Falcon) containing DMEM supplemented with 10% FBS and incubated at 37°C in a humidified atmosphere of 95% O$_2$ and 5% CO$_2$ for 6 days before the experiment. Culture media were changed every 48 h.

On the seventh day in culture, the cells were washed with PBS and stimulated during 4 h with serum-free
Table 1 Structure of SRIH 14 and its analogues.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Provenance</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRIH 14</td>
<td>Peninsula Inc.</td>
<td>H-Ala-Gly-c[Cys-Lys-Asn-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH</td>
</tr>
<tr>
<td>SRIH 28</td>
<td>Sigma</td>
<td>H-Ser-Ala-Asn Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-Phosphate-Lys-Thr-Ser-Cys]-OH</td>
</tr>
<tr>
<td>BIM 23014</td>
<td>Biomeasure Inc.</td>
<td>d-Nal-c[Cys-Tyr-o-Trp-Lys-Val-Cys]-Thr-NH₂</td>
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<td>BIM 23034</td>
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<td>o-Phe-c[Cys-Tyr-o-Trp-Lys-Val-Cys]-Nal-NH₂</td>
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<tr>
<td>SMS 201-995</td>
<td>Sandoz</td>
<td>o-Phe-c[Cys-Tyr-o-Trp-Lys-Thr-Cys]-Thr-ol</td>
</tr>
<tr>
<td>MK 678</td>
<td>Merck</td>
<td>c[N-Me-Ala-Tyr-o-Trp-Lys-Val-Phe]</td>
</tr>
<tr>
<td>BIM 23030</td>
<td>Bachem</td>
<td>c[MPA-Tyr-o-Trp-Lys-Val-Cys]-Phe-NH₂</td>
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<tr>
<td>BIM 23003</td>
<td>Biomeasure Inc.</td>
<td>c[Cys-Lys-Asn-p-Cl-Phe-o-Trp-Lys-Thr-Phe-Thr-Ser-Cys]</td>
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<td>BIM 23052</td>
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<td>o-Phe-Phe-o-Trp-Lys-Thr-Phe-Thr-NH₂</td>
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<tr>
<td>BIM 23056</td>
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<td>o-Phe-Phe-Tyr-o-Trp-Lys-Val-Phe-o-Nal-NH₂</td>
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<tr>
<td>BIM 23058</td>
<td>Biomeasure Inc.</td>
<td>o-Phe-Phe-Tyr-o-Trp-Lys-V al-Phe-Thr-NH₂</td>
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<tr>
<td>BIM 23068</td>
<td>Biomeasure Inc.</td>
<td>o-Phe-CPA-Tyr-o-Trp-Lys-Thr-Phe-Thr-NH₂</td>
</tr>
<tr>
<td>T'D8</td>
<td>D Coy</td>
<td>Tyr-Ala-Gly-c[Cys-Lys-Asn-Phe-o-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH</td>
</tr>
<tr>
<td>LDTT</td>
<td>J Rivier</td>
<td>H-Ser-Ala-Asn Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-o-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH</td>
</tr>
<tr>
<td>RC 160</td>
<td>Debiopharm</td>
<td>o-Phe-c[Cys-Tyr-o-Trp-Lys-Val-Cys]-Trp-NH₂</td>
</tr>
<tr>
<td>Choro-BIM</td>
<td>Bachem</td>
<td>c[MPA-Tyr-o-Trp-Lys-Val-Cys]-p-Cl-o-Phe-NH₂</td>
</tr>
</tbody>
</table>

Determined by double antibody RIA method using reagents provided by NIADDK Hormone Distribution Program (Bethesda, MD, USA). Specific activity of the ligand was 760±27 Ci/mmol (mean±S.E.M. of four determinations).

Hormone assays

Ovine (o) GH and rat (r) GH were determined in duplicate with a double antibody RIA method using reagents provided by NIADDK Hormone Distribution Program (Bethesda, MD, USA). oGH and rGH were labelled with ¹²⁵I using the Chloramine T method (43) and purified on an ACA 54 gel column. oGH-I-4 and rGH-I-6 were used as standards. The limit of sensitivity was 0.1 ng/ml for oGH and 0.05 ng/ml for rGH. The intra- and interassay coefficients of variation were respectively 5 and 7% for the oGH assay and 4 and 6% for the rGH assay.

[^125I]Tyr⁰-o-Trp⁸-SRIH 14 binding assay

[^125I]Tyr⁰-o-Trp⁸-SRIH 14 (T'D8) (Peninsula, Merseyside, UK) was iodinated in the presence of Chloramine T (46). Purification of labelled tracer was performed on carboxymethyl-cellulose (CM 52, Whatman Inc., Clifton, NJ, USA). The fraction retaining full immunoreactivity corresponded to 62±2% of the total radioactivity (mean of four consecutive iodinations). The specific activity of the ligand was 760±27 Ci/mmol (mean±S.E.M. of four determinations).

Male rat and ovine anterior pituitary lobes were dissected rapidly at 4°C after decapitation of animals and homogenized in a Teflon glass (Potter-Elvehjem) homogenizer in 50 mmol Tris-HCl (Merck, Rahway, NJ, USA) buffer at pH 7.5 (10%, v/w) and centrifuged for 30 min at 2000 g. The pellet was resuspended in the buffer and centrifuged at 600 g for 3 min to sediment the nuclei. The supernatant containing the membranes was then washed at 12 200 g for 20 min. The resulting pellet was gently resuspended in buffer to which had been added 0.2% BSA (Sigma) and 2×10⁻⁵ mol bacitracin (Sigma) to avoid peptide adsorption to the tube and enzymatic degradation.

Twenty-five microlitres of freshly prepared membranes, corresponding to 40–60 µg, were incubated with [¹²⁵I]Tyr⁰-o-Trp⁸-SRIH 14 at 22°C for 75 min in the presence or absence of unlabelled SRIH 14 at various concentrations (10⁻⁶ to 10⁻¹² mol). Specific binding was calculated as the difference between total and residual binding in the presence of 10⁻⁶ mol unlabelled SRIH 14. This saturable component represented between 70–80% of the total binding. The total incubation volume was 150 µl. The incubation was stopped by diluting the mixture with 2 ml ice-cold buffer. Membrane-bound [¹²⁵I]Tyr⁰-o-Trp⁸-SRIH 14 was separated from the free peptide by filtration on Whatman GF/C filters, followed by washing with 2x4 ml Tris (50 mmol; pH 7.5) buffer. Filters were recovered in
polystyrene tubes and counted in an LKB rackgamma with 72% efficiency (LKB, Rockville, MD, USA). In the absence of membrane preparations, less than 1% of the radioactive material was retained on the filter.

Radioautographic technique

Receptor labelling was performed as previously described (47) with minor modifications. Briefly, specimens were frozen on dry-ice and cut on a cryostat. Sixteen micrometre-thick sections were collected on gelatine-coated slides and stored at −80°C until use. Slides were then brought to room temperature and pre-incubated for 15 min in 50 mmol Tris–HCl buffer pH 7.6 containing 0.25 mmol sucrose and 0.2% BSA. The slides were then incubated for 45 min at room temperature in the above buffer supplemented with 0.1 nmol [125I]Tyr0-DTrp8-SRIH 14, bucitracin (20 mg/l), and MgCl2 (1 g/l). To determine non-specific binding, adjacent sections were incubated in the same solution supplemented with 0.1 µmol SRIH 14. Competition experiments were performed on triplicate sections. Slides were washed twice in cold buffer (2×5 min). Sections were then dried and exposed onto Hyperfilm B-max (Amersham) for three days at 4°C. After this period, films were removed, revealed with Kodak Dektol developer, and fixed in Kodak 3000 fixer. Binding was quantified by reference to iodinated standards prepared from brain paste, with the help of a computer analyser using a camera and the RAG program (Biocom, les Ulis, France) which allows for conversion of optical densities into radioactivity units.

Data analysis

Data from hormonal studies were expressed as means ± S.E.M. (n=8) and used to generate inhibition curves and obtain IC50 values. The effect of SRIH 28 and SRIH analogues were compared with SRIH 14. Data from binding studies were expressed as means ± S.E.M. of three determinations and used to generate displacement curves and obtain Kd values. Competition curves were analysed by non-linear regression using the Parker and Waud model (48) which allows for statistical evaluation of dissociation constant kinetics.

Statistical analysis was performed by two-way analysis of variance (concentration×analogues) followed by Fisher’s protected least significant difference (PLSD) test (ANOVA, Statview 512, Brain Power Inc., Calabasas, CA, USA). Four groups of analogues were classified according to their IC50s relative to SRIH 14 (IC50: 1.0) and according to their statistical analysis: group 1, highly potent peptides with IC50 significantly lower than SRIH 14 (P<0.05); group 2, peptides with a potency of action close to that of SRIH 14; group 3, weakly active SRIH analogues with IC50 significantly higher than SRIH 14 (P<0.05); group 4, inactive or mostly inactive SRIH analogues with IC50s significantly higher than group 3 analogues (P<0.05).

Correlations were calculated, with the assistance of Statview 512, by plotting the logarithm of the affinities of SRIH analogues for SST (41, 42, 49) versus the logarithm of their GH inhibition potencies. To determine if correlation coefficients were statistically different from zero a probability level (P value) was calculated by Fisher’s r to z test.

Results

Comparison between male rat and ram pituitaries’ sensitivities and binding capacities to SRIH 14

SRIH 14 was 17 times less efficient in inhibiting GH release from ovine (IC50: 5.0 nmol) than from rat (IC50: 0.3 nmol) pituitary cells. The maximum inhibition of GHRH-induced GH release was observed for SRIH at 10−7 mol in rats and 10−7 mol in sheep (Fig. 1A).

In the membrane binding assay, [125I]Tyr0-DTrp8-SRIH 14 binding sites were three times higher in rat (531±89 fmol/mg protein) than in sheep (166±27 fmol/mg protein) pituitary glands. Displacements of [125I]Tyr0-DTrp8-SRIH 14 by SRIH 14 were clearly biphasic both in rat and in sheep pituitaries (Fig. 1B). The affinity of somatostatin for the high affinity site was identical in rats and sheep. Affinity for the low affinity site, while somewhat higher in rats, was also within the same order of magnitude in both species. It appeared, however, that the ratio of high to low affinity binding sites was more important in rat than in sheep pituitary (Table 2).

By radioautography, [125I]Tyr0-DTrp8-SRIH 14 binding was evenly distributed in the anterior lobe of both rat and sheep pituitary glands while the neural lobe was not labelled (Fig. 1C). The specific binding was more important in rat than in sheep pituitaries.

Inhibitory effect of SRIH 14, SRIH 28, and their analogues on GH release from ovine pituitary cells

We have compared the potencies of SRIH 14, SRIH 28, and 17 SRIH analogues in inhibiting GHRH (10−6 mol)-induced GH release. A dose-dependent reduction of GH release was observed with all the peptides except BIM 23056 (Fig. 2).

Four groups of SRIH analogues were separated according to their IC50s relative to SRIH 14 (IC50: 1) (Table 3): group a, highly potent peptides: LDTT, T0D8, SRIH 28; group b, peptides with a potency of action close to that of SRIH 14: BIM 23060, SMS 201–995, RC 160, BIM 23003, BIM 23052; group c, low active SRIH analogues: BIM 23034, BIM 23014, BIM 23059, BIM 23030, BIM 23068; group d, weakly active or inactive SRIH analogues: BIM 23042, MK 678, Chloro BIM 23030, BIM 23058, BIM 23056.
Figure 1  (A) Inhibitory effect of increasing doses of SRIH 14 on stimulated GH release in ovine or rat anterior pituitary cells in primary culture. On the seventh day of culture, cells were stimulated during 4 h with serum-free medium containing GHRH (10⁻⁶ mol in sheep and 10⁻⁸ mol in rats) and various concentrations of SRIH 14 as described in Materials and methods. Results are expressed as percentage of control (mean±S.E.M., n=8). (B) Displacement of specific [¹²⁵I]Tyr⁰-D-Trp⁸-SRIH 14 binding to ovine or rat adenohypophyseal membranes by SRIH 14. [¹²⁵I]Tyr⁰-D-Trp⁸-SRIH 14 (10⁻⁶ mol) was incubated at 22°C for 75 min with the membranes (40–60 µg) in the presence of increasing concentrations of SRIH 14. Values are the mean±S.E.M. of three determinations. Results are expressed as the percentage of displacement computed as described in Materials and methods. (C) Visualization of [¹²⁵I]Tyr⁰-D-Trp⁸-SRIH 14 binding sites on rat and sheep hypophysis. Upper micrograph: total binding (1) and non-specific binding in the presence of 0.1 µmol SRIH 14 (2) in sheep; lower micrograph: total binding (3) and non-specific binding in the presence of 0.1 µmol SRIH 14 (4) in rat. Specific binding is restricted in the anterior lobe (AL). The neural lobe (NL) is totally devoid of binding. 1E-11 stands for 10⁻¹¹ etc. Scale bar: 12 mm (1, 2); 0.6 mm (3, 4).
Table 2 Displacement of \[^{125}\text{I}]\text{Tyr}^\text{a}\text{Trp}^\text{b}-\text{SRIH 14 binding to pituitary membranes by SRIH 14. Values of } \text{K}_1 \text{ and percentage displacement for the two affinity sites.}

<table>
<thead>
<tr>
<th></th>
<th>(\text{K}_1) (10^{-12} \text{ mol})</th>
<th>%</th>
<th>(\text{K}_2) (10^{-9} \text{ mol})</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>0.5 ± 0.3</td>
<td>35 ± 5</td>
<td>3.7 ± 1.9</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>Rat</td>
<td>0.8 ± 0.1</td>
<td>71 ± 1</td>
<td>11.5 ± 2.4</td>
<td>29 ± 1</td>
</tr>
</tbody>
</table>

Competition curves were analysed by non-linear regression using the Parker and Waud model (48) which allows for statistical evaluation of dissociation constant kinetics.

**Inhibitory effect of SRIH 14, SRIH 28, and their analogues on GH release from rat pituitary cells**

The difference in IC\(_{50}\)S for SRIH 14, SRIH 28, and their analogues also allowed the separation of these peptides into four groups (Table 3): group a, highly potent peptides: BIM 23014; group b, peptides with a potency of action close to that of SRIH 14: RC 160, MK 678, BIM 23059; group c, low active SRIH analogues: SRIH 28, BIM 23003, SMS 201–995, LDTT, BIM 23034, T\(_0\)D8; group d, weakly active or inactive SRIH analogues: BIM 23052, BIM 23068, BIM 23058, BIM 23056.

**Comparison between the sensitivity of male rat and ram pituitaries to SRIH 14, SRIH 28, and their analogues**

As observed with SRIH 14, the maximum inhibition of GHRH-induced GH release was observed for most SRIH analogues at nanomolar concentrations in rats and 10^{-7} mol in sheep (Fig. 2). Furthermore, the comparison of the inhibitory action of SRIH analogues to that of SRIH 14 showed that seven peptides were much more active in ovine than in rat pituitary cells, the highest interspecies difference being observed with LDTT and T\(_0\)D8 (Table 3). Three peptides were more potent in inhibiting GH release from rat than from ovine pituitaries: MK 678, BIM 23014, and BIM 23059. One peptide (RC 160) displayed a similar potency in rat and ovine pituitary cells, higher than that of SRIH 14 in both species. Two peptides inactive in sheep, BIM 23058 and BIM 23056, displayed a weak activity in rats.

For sheep, the inhibitory potency of the analogues on GH secretion was highly correlated with rat sst5 (rsst5) affinities and also significantly correlated with mouse sst2 (mssst2). For rat, the inhibitory potency of the analogues on GH secretion was significantly correlated with human sst2 (hsst2) and mssst2 affinities and was negatively correlated with mssst3 (Table 4).

**Discussion**

Recent physiological and pharmacological studies on GH secretion have suggested that sheep provide a valuable model to further the understanding of the neuroregulation of GH secretion (5). Indeed, in sheep, the pattern of GH secretion (spontaneous pulsatility, response to stress or pharmacological agents) is very close to that observed in humans while the rat model displays some differences (5, 8–10). Furthermore, sheep pituitary cultures are easily available, human normal adult pituitary tissue being rather rare. Therefore, the purpose of our work was to study somatostatin action and receptors in sheep and to compare them to the rat, the model extensively used in that field.

We have shown that SRIH 14 displays a higher ratio of high affinity binding site in male rats than in rams. Binding assay and autoradiography were performed with \[^{125}\text{I}]\text{Tyr}^\text{a}\text{Trp}^\text{b}-\text{SRIH 14 (T\(_0\)D8). We chose this stable ligand because it is very general having, in humans, similar affinities for the five somatostatin receptor subtypes (IC\(_{50}\) for hsst1, 2 nmol; for hsst2, 0.2 nmol; for hsst3, 0.5 nmol; for hsst4, 3.2 nmol; for hsst5, 0.5 nmol) (D Hoyer, personal communication). Because T\(_0\)D8 is 87 times more potent in reducing GH release in rams than in male rats, and our result could be biased by this fact, we studied the displacement of labelled T\(_0\)D8 by cold T\(_0\)D8. A similar difference between rat and sheep was evident (B\(_{\text{max}}\) was 2.4-fold higher in rats than in sheep). Accordingly, SRIH 14 inhibits GH release at lower concentrations in male rat than in ram pituitary cells in vitro. This difference in inhibiting action on GH could be related to the use of two different concentrations of GHRH (10^{-6} mol for the sheep pituitary and 10^{-7} mol for the rat pituitary). In order to rule out this hypothesis, we compared, in sheep, the effect of SRIH and BIM 23014 on GH secretion after stimulation of GH by 10^{-8} mol GHRH or 10^{-6} mol GHRH. No difference was found. We chose to use 10^{-6} mol GHRH for sheep because of the lower sensitivity of the oGH RIA. Moreover, these data are in agreement with a previous in vitro report from Law et al. (50). They observed that the inhibitory potency of SRIH on GH secretion measured in rat pituitary cultures (maximum inhibition observed at 1 nmol) was much greater than that observed in experiments with the sheep pituitary cells (maximum inhibition observed at 0.1 \text{nmol}). In vivo,
Inhibition of GH secretion: species differences

A/ In sheep

B/ In rats
high doses of somatostatin must be injected to inhibit GH secretion in sheep. The i.v. infusion of somatostatin at $1 \, \text{mg/min}$ for one hour does not reduce basal plasma GH levels (14, 15). Spencer et al. have shown that a higher dose of somatostatin (5 $\text{mg/min}$) is required to lower basal secretion of GH (15). Somatotrophic responses to arginine (16) and prostaglandin E1 (PGE-1) injection (17) are blocked by i.v. injection of 500 $\text{mg}$ somatostatin, 5 and 50 $\text{mg}$ somatostatin being insufficient to reduce PGE-1-induced GH stimulation (17). Furthermore, interspecies differences in the role of SRIH in the pituitary control of GH secretion have been found; in particular, no change in spontaneous GH levels has been observed in sheep actively immunized against SRIH (11) in comparison to the rat (4).

Several SRIH analogues have been synthesized and tested in rats and humans for their ability to inhibit GH release in vitro or in vivo. So far, their action on ovine pituitary cells had not been tested. Most available studies have been devoted to the pharmacology of human subtypes transiently or stably expressed (43, 44, 49, 51, 52). In humans, most of the analogues bind to hsst2 and hsst5 with a high or relatively high affinity but no analogue binds with high affinity to hsst4 and hsst1 except a newly synthesized sst1 selective agonist: des-AA$^{1,2,7}$[oTrp$^8$,Ile$^9$]SRIH (53) which was not available in our study. In rodents, only binding affinities to msst2, msst3, and rsst5 have been reported (41, 42). None of the available analogues are strictly specific for one subtype but some analogues, such as BIM 23003, BIM 23034 and BIM 23059, have a somewhat better affinity for msst2 and rsst5 as compared with msst3.

We found that the analogues displaying a good affinity for msst2 and rsst5 also have a good inhibitory

### Table 3

<table>
<thead>
<tr>
<th>Peptides$^1$</th>
<th>Sheep $^{(1)}$ IC$_{50}^2$ (1 = $5 \times 10^{-9}$ mol)</th>
<th>Rat $^{(2)}$ IC$_{50}^2$ (1 = $0.3 \times 10^{-9}$ mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDTT</td>
<td>0.0089</td>
<td>2.29</td>
</tr>
<tr>
<td>SRIH 28</td>
<td>0.014</td>
<td>1.23</td>
</tr>
<tr>
<td>T'D8</td>
<td>0.068</td>
<td>4.36</td>
</tr>
<tr>
<td>BIM 23060</td>
<td>0.1</td>
<td>ND</td>
</tr>
<tr>
<td>SMS 201-995</td>
<td>0.14</td>
<td>1.86</td>
</tr>
<tr>
<td>RC 160</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>BIM 23003</td>
<td>0.4</td>
<td>1.23</td>
</tr>
<tr>
<td>BIM 23052</td>
<td>0.57</td>
<td>20.83</td>
</tr>
<tr>
<td>SRIH 14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>BIM 23034</td>
<td>1.12</td>
<td>4.35</td>
</tr>
<tr>
<td>BIM 23014</td>
<td>1.62</td>
<td>0.074</td>
</tr>
<tr>
<td>BIM 23059</td>
<td>2.95</td>
<td>0.375</td>
</tr>
<tr>
<td>BIM 23030</td>
<td>4.31</td>
<td>ND</td>
</tr>
<tr>
<td>BIM 23036</td>
<td>5.38</td>
<td>26.23</td>
</tr>
<tr>
<td>BIM 23042</td>
<td>14.45</td>
<td>ND</td>
</tr>
<tr>
<td>MK 678</td>
<td>89.08</td>
<td>0.26</td>
</tr>
<tr>
<td>Chloro BIM 23030</td>
<td>$&gt;100$</td>
<td>ND</td>
</tr>
<tr>
<td>BIM 23058</td>
<td>$&gt;100$</td>
<td>99.61</td>
</tr>
<tr>
<td>BIM 23056</td>
<td>$&gt;1000$</td>
<td>$&gt;1000$</td>
</tr>
</tbody>
</table>

$^1$SRIH analogues are listed by their efficacy on ovine pituitaries.

Values are given relative to that for SRIH 14 ($IC_{50} = 1.0$), with the IC$_{50}$ values for SRIH 14 given in parentheses.

Four groups of SRIH analogues have been separated according to the statistical analysis of two-way analysis of variance (concentration × analogues): a, highly active peptides; b, active peptides; c, low active peptides; d, weakly or inactive peptides.

ND, not determined.

### Table 4

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Sheep/GH Correlation ($r$)</th>
<th>P values</th>
<th>Rat/GH Correlation ($r$)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRIH 14</td>
<td>1</td>
<td>—</td>
<td>0.493</td>
<td>0.0731</td>
</tr>
<tr>
<td>msst2</td>
<td>0.545</td>
<td>0.0342</td>
<td>0.665</td>
<td>0.0162*</td>
</tr>
<tr>
<td>rsst5</td>
<td>0.641</td>
<td>0.0085**</td>
<td>0.343</td>
<td>0.2834</td>
</tr>
<tr>
<td>msst3</td>
<td>0.290</td>
<td>0.3012</td>
<td>-0.785</td>
<td>0.0015**</td>
</tr>
<tr>
<td>hsst2</td>
<td>0.506</td>
<td>0.0536</td>
<td>0.698</td>
<td>0.0065**</td>
</tr>
<tr>
<td>hsst5</td>
<td>0.472</td>
<td>0.0755</td>
<td>0.294</td>
<td>0.3382</td>
</tr>
<tr>
<td>hsst3</td>
<td>0.261</td>
<td>0.3982</td>
<td>-0.382</td>
<td>0.2273</td>
</tr>
<tr>
<td>hsst1</td>
<td>0.486</td>
<td>0.0659</td>
<td>-0.023</td>
<td>0.9412</td>
</tr>
<tr>
<td>hsst4</td>
<td>0.288</td>
<td>0.3040</td>
<td>-0.156</td>
<td>0.6197</td>
</tr>
</tbody>
</table>

$^*P < 0.05, **P < 0.01$ compared with zero.
effect on GH secretion in rats as well as in sheep. Furthermore BIM 23056, a peptide with no affinity for both hsst2 (IC50>1000 according to Patel & Srikanth (49) and IC50=250 according to Hoyer et al. (54)) and msst2 (IC50>1000 according to Raynor et al. (42)), is only weakly effective on GH secretion in rat and totally inactive in sheep. These results confirm that sst2 is the main receptor involved in the inhibition of GH secretion in rats (compare correlation coefficients in Table 3) as already suggested (41, 42, 55). Sst2 and sst5 (compare correlation coefficients in Table 3) are also likely to be the main receptors involved in GH inhibition in sheep though only the cloning of ovine sst receptor and pharmacological studies on ovine clones will give the final answer.

Large differences between rats and sheep have been observed in pituitary sensitivity to several SRIH analogues as compared with SRIH 14, some of them being far more active in sheep, others more active in rats. For example, LDTT and T17D8 are respectively 257- and 87-fold more active in reducing GH release in sheep than in rat pituitary cells. BIM 23052 has a good inhibitory activity in sheep but is nearly inactive in rats. In contrast, MK 678 is much more potent in inhibiting GH secretion in rats (IC50=0.26 relative to SRIH 14) than in sheep (IC50=89.1). The differences in procedures between rat and sheep cultures due to the compositional differences between rat and sheep pituitaries do not contribute to the observed species differences. Using the rat cell dispersion technique for sheep increases debris and consequently increases data variability, but no differences in sensitivity to SRIH and SRIH analogues could be discerned. Differences in SRIH levels in hypophysial portal blood could explain the differences between male rat and ram observed for the SRIH inhibitory effect on GH. Though it is difficult to compare somatostatin levels in rat and sheep as the two species are studied in different conditions (under anaesthesia and stress for rat, under physiological conditions for sheep), no difference could be found between SRIH levels in sheep and in rats (6, 7, 56).

These species differences observed in pituitary sensitivity to several SRIH analogues may be explained by differences in the structure of SRIH receptor or in the ratio of sst2 and sst5 subtypes in somatotrophs. The latter hypothesis warrants the elucidation of ovine sst subtypes.

Differences in binding capacities of some analogues to human and rat sst have already been observed. Raynor et al. (41, 42) obtained strikingly different results in rodents from those obtained by other authors in humans (49, 54). They have also demonstrated major differences between hsst5 and rsst5 pharmacological properties although there is 80.5% sequence homology between the two receptors (51). Whereas rsst5 recognized several SRIH analogues, octapeptides, hexapeptides, and linear peptides with high to moderate affinities, hsst5 bound to most of the SRIH analogues with a much lower affinity, except for SRIH 14 and SRIH 28. For example, BIM 23052 binding is 1000 times more potent for rsst5 than for hsst5. Although a great similitude of sequence has been found between homologous receptor subtypes, it is well known that minute sequence differences can cause large pharmacological variations (57). 5-Hydroxytryptamine1B (5HT1B) receptor also displays species differences in pharmacological properties. Indeed, human 5HT1B receptor is identical to rodent 5HT1B receptor in binding to 5HT but it differs profoundly in binding to many drugs. Oksenberg et al. (57) have shown that replacement of a single amino acid in the human receptor (threonine at residue 355) with a corresponding asparagine found in rodent 5HT1B receptors makes the pharmacology of the receptors essentially identical.

Alternatively, differences in SRIH-mediated signal transduction pathways have been observed between rats and sheep and could be the source of interspecies differences in somatotroph sensitivity to several SRIH analogues. The signal transduction mechanisms for the SRIH receptor subtypes are not yet completely elucidated. Each sst subtype is linked to multiple transmembrane effectors (58) but it remains to be determined whether these different signalling mechanisms are cell- or sst subtype-selective, if they function in parallel or in interdependent pathways, and what their relative role is with respect to the overall biological response. However, a reduction of cyclic AMP production is likely to be involved after activation of each receptor subtype, although the importance of its participation in the inhibition of GH release is not established. Interestingly, Law et al. (50) have shown that SRIH 14 inhibits GHRH-stimulated cyclic AMP levels in rat, but not in sheep pituitary cell cultures. They have suggested that the low inhibitory action of SRIH analogues on ovine pituitary cells is explained by this lack of action on adenyl cyclase. This transduction system seems generally less involved in sheep pituitary activities since, unlike in rats, sheep pituitary adenyl cyclase is not stimulated by neurohormones such as corticotrophin-releasing hormone (59).

The regulation of ion channels appears to be a major mechanism by which SRIH induces its physiological responses. The threshold for activation of voltage-gated Ca2+ (60) and voltage-dependent K+ (61) currents differs between sheep and rat somatotrophs and may also explain these species differences in SRIH sensitivity.

In conclusion, our data confirm and extend the quantitative differences between rats and sheep with regard to the inhibitory action of SRIH on GH secretion. The differences observed between humans, rats, and now sheep in the binding capacities of SRIH analogues to each sst indicate that ligand-binding properties of a given receptor subtype cannot be extrapolated across species. Therefore, cloning of sheep sst subtypes appears
necessary to understand the mechanisms of action of SRIH in the inhibition of GH secretion in sheep.

Acknowledgements

Reagents for ovine and rat GH assay were provided by the NIADDK Hormone Distribution Program. The authors wish to thank J P Moreau (Biomeasure Inc., Milford, MA, USA), S F Brady (Merck & Co. Inc., West Point, PA, USA), J Rivier (The Salk Institute, La Jolla, CA, USA), and Ph. Leroux (GREM, University of Rouen, France) for their kind gifts of SRIH analogues. They are also grateful to L’H Ouatifli, F. Boudouresque (INSERM U297, Marseille, France), and C B Srikant (McGill University, Montreal, QUE, Canada) for helpful discussions. The scientific interest and continuous support of Dr Tissier (Ipsen, Signes, France) are gratefully acknowledged. N Briard is supported by a fellowship from IPSEN France and Regional Council Provence Alpes Côte d’Azur.

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Received 18 February 1997
Accepted 16 June 1997