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Somatostatin analogs and chimeric somatostatin–dopamine molecules differentially regulate human growth hormone and prolactin gene expression and secretion *in vitro*

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ABSTRACT

We tested effects of selective somatostatin receptor 2 (SST2) agonist BIM-23120, SST5 agonist BIM-23206 and chimeric somatostatin–dopamine molecules (SRIF/DA) BIM-23A760 and BIM-23A761 on GH and PRL secretion and gene expression in human GH/PRL-secreting pituitary tumors *in vitro*.

In "responders" group BIM-23120 suppressed GH levels by 26 ± 4%, BIM-23206 by 31 ± 5%, BIM-23A760 by 23 ± 4%, BIM-23A761 by 39 ± 8% and D_2-dopamine agonist BIM-53097 by 31 ± 5%. Using real-time PCR we demonstrated that GH inhibition was not accompanied by decreased GH mRNA levels. PRL secretion was inhibited by BIM-23A760 (29 ± 5%), BIM-23A761 (34 ± 4%), BIM-23206 (26 ± 4%) and BIM-53097 (36 ± 2%). SRIF/DA and BIM-53097 also suppressed PRL mRNA levels.

Concluding, SST2 and SST5 agonists and SRIF/DA inhibit GH secretion, but do not suppress GH gene transcription. SRIF/DA and BIM-53097 inhibit both PRL secretion and PRL gene expression. SST5 agonist inhibits PRL secretion, but does not suppress PRL gene expression. D_2 affinity is crucial in SRIF/DA action on PRL gene expression.

1. Introduction

GH secretion from the anterior pituitary cells is controlled by complex neuroendocrine signals including hypothalamic GHRH and SRIF (for review see Giustina and Veldhuis (1998) and Luque et al. (2008)). In addition, somatotroph function is modulated by ghrelin – the endogenous ligand for the GH secretagogue receptor type Ia (GHS-RIa), acting mainly at hypothalamus to induce GH secretion in synergy with GHRH (Kojima and Kangawa, 2005; Tannenbaum et al., 2003). GHRH stimulates both GH gene transcription, synthesis and release (Giustina and Veldhuis, 1998; Melmed, 2009). GHRH may also act as a coagonist for the ghrelin receptor (Casanueva et al., 2008). SRIF inhibits GH secretion, mostly as a result of acute inhibition of hormone exocytosis, and its effect on GH biosynthesis is unclear. Inhibition of GH synthesis has not been proven conclusively. Studies in pituitary cell cultures from animals (Barinaga et al., 1985; Fukata et al., 1985) have shown that SRIF regulates GH release, but has no effect on basal levels of GH synthesis or gene transcription (for review see Luque et al. (2008)). However, other studies have shown a decrease in somatotroph GH mRNA levels (Acunzo et al., 2008; Sugihara et al., 1993; Tsukamoto et al., 1994), maintaining the controversy on this issue. Interestingly, observations in primary pituitary cell cultures from pigs and baboons (Luque et al., 2006) indicate that SRIF may exert biphasic effects on GH release (*i.e.* GH suppression at moderate to high doses and GH stimulation at low doses). The effect of SRIF and/or its analogs on human GH gene expression has not yet been thoroughly studied.

Dopamine regulates PRL secretion and gene expression acting through D_2 receptors (Ben-Jonathan et al., 2008). SRIF and its analogs inhibit PRL secretion from rat PRL-secreting pituitary tumors *in vivo* and *in vitro* (Gruszka et al., 2001, 2007; Pawlikowski et al., 1997) and human PRL-secreting pituitary adenomas *in vitro* (Hofland et al., 2004; Jaquet et al., 1999; Shimon et al., 1997). Effects of SRIF and its analogs on human PRL gene expression have not yet been reported.

Both somatostatin and dopamine receptors belong to the superfamily of G-protein-coupled receptors (GPCR). Somatostatin receptor subtypes 1, 2, 3 and 5 (SST1–SST5) predominate in normal pituitaries and pituitary adenomas (Ben-Shlomo and Melmed,

Abbreviations: D_2, D_2-dopamine receptor; GH, growth hormone; GHRH, growth hormone-releasing hormone; PRL, prolactin; SRIF, somatotropin-release inhibitory factor – somatostatin; SRIF/DA, chimeric somatostatin–dopamine molecules; SST, somatostatin receptor.

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2010; Panetta and Patel, 1995; Pawlikowski et al., 2003; Taboada et al., 2007). D_2 is the predominant dopamine receptor in normal pituitaries and pituitary adenomas (Missale et al., 1998; Neto et al., 2009; Stefaneanu et al., 2001). In most GH-secreting adenomas SST2, SST5 and D_2 are co-expressed and some of somatotroph tumors also co-express SST3 and SST1, particularly mixed GH/PRL adenomas (Neto et al., 2009; Pawlikowski et al., 2008 Taboada et al., 2007). There is also evidence that human D_2 receptors may form heterodimers with somatostatin receptors SSTR2 and SSTR5 with enhanced functional activity (Baragli et al., 2007; Rocheville et al., 2000).

Approximately 65% of patients harboring GH-secreting pituitary adenomas treated with clinically available somatostatin receptor ligands, octreotide and lanreotide (with affinity mainly to SST2), achieve control of GH secretion (random fasting GH levels <2.5 μg/l and/or normalization of IGF1 levels). Using GH cutoff of less than 1 μg/l, approximately 33% of patients can be defined as controlled (Melmed, 2009). Novel SRIF analogs with high affinity to SST2 and SST5 represent potential future treatment option in selected patients with GH-secreting pituitary tumors. Pasireotide (SOM230), SRIF analog with high affinity to SST1, SST2, SST3 and SST5, is currently being evaluated in clinical trials in patients with GH-secreting adenomas, and short-term studies show promising results (Schmid, 2008; Petersenn et al., 2010). SRIF/DA, chimeric molecules containing structural elements of both SRIF and dopamine agonists and directed against both GPCRs were shown to be effective in controlling GH and PRL secretion *in vitro* in somatotroph adenomas that were partial responders to octreotide (Jaquet et al., 2005a,b; Ren et al., 2003; Saveanu et al., 2002).

Previous studies on the efficacy of SRIF analogs and SRIF/DA in pituitary tumors from acromegaly patients were limited to the evaluation of GH and PRL secretion. The effect of SRIF and its analogs on human GH gene transcription is unclear, and the effects of SRIF/DA on human GH and PRL mRNA have not yet been reported. We have previously shown that inhibition of GH release is not accompanied by decreased GH mRNA levels in primary and tumorous rat pituitary cells (Gruszka et al., 2007). The aim of the present study was to simultaneously investigate the effects of selective SST2 (BIM-23120) and SST5 (BIM-23206) agonists and SRIF/DA (BIM-23A760 and BIM-23A761) on human GH and PRL secretion and gene expression in mixed GH/PRL-secreting pituitary tumors *in vitro*.

2. Materials and methods

2.1. Compounds

Somatostatin receptor agonists: BIM-23120, BIM-23206, BIM-23244, chimeric somatostatin–dopamine molecules BIM-23A760 and BIM-23A761, and D_2 receptor agonist BIM-53097 were provided by Biomeasure, Inc. (Milford, MA), and their properties are shown in Table 1. Stock solutions (10^{-3} mol/l) of these substances were prepared in 10^{-2} mol/l acetic acid containing 0.1% BSA (Sigma–Aldrich) and stored at −20 °C until used.

Table 1
Human SST and D_2 binding affinities of SRIF-14, SRIF analogs and D_2 agonist BIM 53097 (IC_{50} (nmol/l)). Data are from radioligand binding assays to membranes from transfected CHO-K1 cells expressing human D_2 or human SST subtypes. Values are from IPSEN, Biomeasure, Inc. as previously published (Gruszka et al., 2007).

	SST1	SST2	SST3	SST4	SST5	D2
SRIF-14	2.3	0.2	1.4	1.8	1.4	>1000
BIM-23A760	622	0.03	160	>1000	42.0	15
BIM-23A761	462	0.06	52	>1000	3.7	27
BIM-23120	1000	0.34	412	1000	213.5	>1000
BIM-23206	1152	166	1000	1618	2.4	>1000
BIM-23244	>1000	0.3	133	>1000	0.7	>1000
BIM-53097	>1000	>1000	>1000	>1000	>1000	22

2.2. Human pituitary adenomas

Use of human tissue was approved by the Institutional Review Board, and patients gave written consent for anonymous tissue collection. Clinical characterization of the patients is summarized in Table 2. A part of each surgically removed tumor from acromegaly patients was mechanically dispersed and enzymatically digested in DMEM (Invitrogen Corp., Grand Island, NY) containing 0.35% collagenase type IA, 0.15% hyaluronidase and 0.3% BSA (Sigma–Aldrich) at 37 °C for 40 min according to the protocol previously described (Gruszka et al., 2006). Cells were cultured in DMEM supplemented with 10% FBS for 48 h, then serum-starved for 12 h and treated with test substances. According to the results of preliminary time-course and dose-dependency experiments (data not shown), cultures were incubated with test compounds at concentrations of 10^{-8} mol/l for 24 h. Medium for hormonal assays and RNA for real-time PCR was collected.

2.3. Hormone assays

Human GH in culture medium was measured using a radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 0.9 μg/l. Human PRL was measured by immunoradiometric assay (Coat-A-Count Prolactin IRMA, Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 0.1 μg/l.

2.4. Real-time PCR

Total RNA from cultured cells was extracted with Trizol Reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. RNA samples were treated with DNase I (Deoxyribonuclease I, Amplification Grade, Invitrogen) to eliminate genomic DNA contamination. Total RNA was reverse transcribed into first-strand cDNA using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol. For each new batch of cDNA a control sample containing no reverse transcriptase was performed (/−/RT control).

Real-time PCRs were carried out in the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA) according to the protocol previously described (Gruszka et al., 2007), recorded and analysed using the iQ5 Optical System Software ver. 1.0 (Bio-Rad Laboratories, Inc.). Briefly, real-time PCR amplifications were carried out with 10 μl SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 5×10^{-7} mol/l forward primer, 5×10^{-7} mol/l reverse primer and 5 μl cDNA template (100 ng reverse-transcribed total RNA per well). β-Actin served as an internal reference for normalization of GH and PRL mRNA levels.

Primer sequences (Invitrogen) were as follows: human GH forward: 5′-CAGGAGTGTCTTCGCCAACA-3′, human GH reverse: 5′-TCCCCATCAGCGTTTGGAT-3′, human PRL forward: 5′-TCATCTGGTCACGGAAGTACGT-3′, human PRL reverse: 5′-TGCCCTCTAGAAGCCGTTTG-3′, β-actin forward: 5′-CATGTACGTTGCTATCCAGGC-3′, β-actin reverse: 5′-CTCCTTAATGTCACGCACGAT-3′.

Thermal cycling profile consisted of incubation at 95 °C for 4.5 min followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s. Samples were run in triplicate. Controls comprising either no template, or /−/RT were run in each experiment.

2.5. Statistical analysis

Statistical significance of the difference between means was assessed with one-way analysis of variance (ANOVA) followed by Tu-