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Regulation of Growth Hormone and Prolactin Gene Expression and Secretion by Chimeric Somatostatin-Dopamine Molecules

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Dopamine (DA) regulates both prolactin (PRL) secretion and gene expression, whereas somatostatin (SRIF) inhibits GH secretion with unclear effects on GH gene expression. We therefore tested the effects of SRIF analogs and chimeric SRIF/DA compounds BIM 23A760 and BIM 23A761 on GH and PRL secretion and gene expression in primary rat pituitary cultures and pituitary tumor GH_3 and MMQ cells. Chimeric SRIF/DA molecules suppressed GH release with a similar efficacy to SRIF receptor subtype 2 agonists in rat pituitary and GH_3 cells. After 24 h, BIM 23A760 and BIM 23A761 did not exert additive effects on GH secretion, and after 48 h were less effective than the combination of respective mono-receptor agonists in GH_3 cells. Real-time PCR did not reveal changes in GH mRNA levels after treatment with SRIF analogs and SRIF/DA molecules. SRIF/DA compounds suppressed PRL and PRL mRNA in rat pituitary and MMQ cells with a similar efficacy to D_2-DA receptor agonist. In GH_3 cells, they suppressed PRL and PRL mRNA levels with a similar efficacy to SRIF receptor subtype 2 agonists. SRIF/DA molecules did not exhibit additive effects on PRL secretion and mRNA levels as compared with cotreatment with mono-receptor ligands. The results show that SRIF analogs and SRIF/DA molecules inhibit GH and PRL secretion and suppress PRL but not GH gene expression. (*Endocrinology* 148: 6107–6114, 2007)

DOPAMINE (DA) REGULATES prolactin (PRL) secretion and gene expression mediated by D_2-DA receptor (D_2D) coupling to $G\alpha_i$ proteins, resulting in inhibition of adenylyl cyclase, decreased cAMP production, and suppression of activated phosphokinase A (1, 2). GH secretion is regulated by dual hypothalamic inhibitory and stimulatory control. GHRH stimulates GH gene expression and GH secretion, whereas somatostatin (SRIF) primarily inhibits GH secretion with unclear effects on the GH gene. Pituitary SRIF action is primarily mediated by SRIF receptor subtypes 2 and 5 (SST2 and SST5) (3, 4). Most older studies (5, 6) did not show SRIF action on GH gene expression. However, more recent studies (7–10) indicate that SRIF might affect GH gene expression. SRIF also inhibits PRL secretion from human PRL-secreting pituitary adenomas *in vitro* and PRL-producing cell lines (11, 12) with unclear effects on the PRL gene.

Recently, chimeric molecules that possess potent, selective agonist activity for both SRIF and D_2D have been synthesized. These compounds have been shown to be more effective in suppressing GH and PRL secretion from cultured human GH-secreting pituitary adenomas than either octreotide or mono-receptor ligands alone (13–16).

To clarify whether SRIF analogs affect only GH and PRL secretion or also GH and PRL mRNA levels, we investigated the effects of several SRIF analogs and two chimeric SRIF/DA compounds in primary rat pituitary cells and in the GH_3 and MMQ rat pituitary tumor cell lines. To gain further insight into mechanisms for SRIF/DA chimeric molecule action, their respective efficacy was compared with that of selective SST2, SST5, SST2 plus -5, and D_2D agonists.

Materials and Methods

Compounds

SRIF receptor agonists BIM 23A760 (17), BIM 23A761 (17), BIM 23120 (18), BIM 23206 (19), and BIM 23244 (20) and D_2D agonist BIM 53097 (21) were provided by Biomeasure, Inc. (Milford, MA), and their properties are depicted in Table 1. Octreotide was purchased from Phoenix Pharmaceuticals, Inc. (Belmont, CA). Stock solutions (10^{-3} M) of these substances were prepared in 0.01 M acetic acid containing 0.1% BSA and stored at −20 C until used. Bromocriptine (2-bromo-α-ergocryptine methanesulfonate salt; Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) was first dissolved in ethanol and further diluted with cell culture medium. A separate control group for bromocriptine treatments comprised the same final maximal concentration of ethanol (0.05%) in the experimental medium.

Rat pituitary cell cultures

Normal rat pituitaries were freshly excised from euthanized 8-wk-old male Sprague Dawley rats, as approved by the Institutional Animal Use Committee. Pituitary cells were prepared as described (14), 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.

Cells were preincubated in DMEM containing 10% fetal bovine serum (Omega Scientific, Inc., Tarzana, CA) and 1% antibiotic-antimycotic (Invitrogen) for 48 h and then in serum-free medium containing 0.3% BSA for 12 h, followed by treatments with test compounds. After preliminary time-course experiments 24- and 48-h incubation time points were selected. At the end of each experiment, medium for GH and PRL assay was collected and stored at −20 C.

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Abbreviations: DA, Dopamine; D_2D, D_2-dopamine receptor; PRL, prolactin; SRIF, somatostatin; SST2, somatostatin receptor subtype 2.

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TABLE 1. Human SST and D_2D binding affinities of SRIF-14 and SRIF analogs and dopamine agonist

Compound	SST1	SST2	SST3	SST4	SST5	D_2D
SRIF-14	2.3	0.2	1.4	1.8	1.4	>1000
Octreotide	1140	0.6	34	7030	7.0	>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	>1000.0	22

Data are from radioligand binding assays to membranes from transfected CHO-K1 cells expressing human D_2D or human SST subtypes. Values are from Biomeasure, Inc. (Culler, M. D., personal communication).

GH_3 and MMQ cell lines

GH_3 cells were cultured in DMEM/F-12 and MMQ cells in RPMI 1640 (both from Invitrogen). Both media were supplemented with 15% horse serum (Omega Scientific), 2.5% fetal bovine serum, and 1% antibiotic-antimycotic. Cells were preincubated in serum-containing medium for 48 h and then in serum-free medium containing 0.3% BSA for 12 h and treated with test compounds for 24 h. To investigate whether the chimeric compounds are more effective than a combination of respective mono-receptor ligands, the action of BIM 23A760 and BIM 23A761 was compared with 24- and 48-h cotreatment with BIM 23120, BIM 23206, and BIM 53097 (SST2, SST5, and D_2D agonist, respectively).

Hormone assays

Rat GH and PRL RIAs were performed using materials provided by the National Hormone and Peptide Program (Harbor-UCLA, Torrance, CA).

Real-time PCR

Total RNA from cultured cells was extracted with Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The amount and the integrity of the RNA were assessed by measurement of OD at 260 and 280 nm. Before processing, RNA samples were treated with DNase 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 PCR were carried out in the iQ5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA). PCR were performed, recorded, and analyzed using the iQ5 Optical System Software version 1.0 (Bio-Rad). Real-time PCR amplification was carried out in a total reaction volume of 20 μl consisting of 10 μl SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 0.5 μM forward primer, 0.5 μM reverse primer, and 5 μl cDNA template (100 ng reverse-transcribed total RNA per well). β-Actin served as a reference gene used for normalization of GH and PRL mRNA levels.

Primer sequences (Invitrogen) were as follows: rat GH forward, 5′-AGGGCATCCAGGCTCTGAT-3′; rat GH reverse, 5′-GCATGTTGGCGT-CAAACTTG-3′; rat PRL forward, 5′-CATCAATGACTGCCCCACTTC-3′; rat PRL reverse, 5′-CCAAACTGAGGATCAGGTTCAAA-3′; β-actin forward, 5′-CATGTACGTTGCTATCCAGGC-3′; and β-actin reverse, 5′-CTCCTTAA-TGTCACGCACGAT-3′.

The thermal cycling profile consisted of incubation at 95 C for 4.5 min followed by 40 cycles of denaturation at 95 C for 10 sec and annealing at 55 C for 30 sec. Samples were run in triplicate. No-template control and −RT controls were run in each experiment. Melting curve analysis was performed to confirm amplification specificity of the PCR products.

Detection of SST and D_2D in MMQ cells

Total RNA from MMQ cells was extracted with Trizol reagent according to manufacturer instructions and treated with DNase I (Invitrogen) to eliminate genomic DNA contamination. Two micrograms total RNA were reverse transcribed into first-strand cDNA using SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol.

PCR mixture (50 μl) contained cDNA (2 μl), 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.5 μM forward and reverse primers, and 2.5 U *Taq* DNA polymerase (Invitrogen). PCR amplifications were performed in a Px2 Thermal Cycler (Thermo Electron Corp., Waltham, MA). After the initial denaturation step at 94 C for 2 min, 30 cycles of PCR amplification were performed as follows: denaturation at 94 C for 30 sec, annealing for 30 sec, and extension at 72 C for 30 sec. Annealing temperatures were 57.9 C for SST1, 55.5 C for SST2, 56.9 for SST3, 53 C for SST4, 56.3 C for SST5, and 52.5 C for D_2D. For each sample a −RT control was performed.

The following primers (Invitrogen) were used: SST1 forward, 5′-GTGGGTGCTGTCGCTACTGG-3′; SST1 reverse, 5-ACCGTG-GCGTCGTCTTGCTC-3′; SST2 forward, 5′-CGGGTTTCATTATCTAT-GCCTTCA-3′; SST2 reverse, 5′-GGATTTGTCCTGCTTACTGTCG-3′; SST3 forward, 5′-TCAACCAGTTCACCAGCATC-3′; SST3 reverse, 5′-CCGCACCTTCACCACAAT-3′; SST4 forward, 5′-TGGCTATCGGAT-TATGTTAC-3′; SST4 reverse, 5′-CAGCACCTCCAGTTGTTT-3′; SST5 forward, 5′-AACGCCGTCGTCTCCT-3′; SST5 reverse, 5′-GCAGCCCA-CAAACACC-3′; D_2D forward, 5′-AATGGGTCAGAAGGGAA-3′; D_2D reverse, 5′-AGTGGGCAGGAGATGG-3′. The generated PCR products were 369, 428, 329, 345, 492, and 443 bp for SST1, SST2, SST3, SST4, SST5, and D_2D, respectively. Amplification products were analyzed by agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical analysis

Statistical significance of the difference between the means was assessed with one-way ANOVA followed by Tukey's multiple comparison test (GH and PRL secretion results) or Dunnett's multiple comparison test and Tukey's test (real-time PCR data). GH and PRL levels are

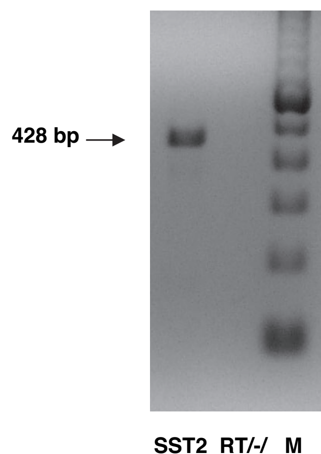

FIG. 1. SST2 mRNA expression in MMQ cells. RNA was treated with deoxyribonuclease and subjected to RT. cDNA was subjected to PCR amplification of SST2. PCR product was visualized on 2% agarose gel. Lane M, 100-bp PCR marker.

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expressed as mean ± SEM and shown as the percentage of untreated controls. EC_{50} values were determined by nonlinear regression curve analysis of dose-effect responses. GH and PRL mRNA levels were normalized to β-actin mRNA and expressed as normalized fold expression relative to the vehicle-treated control, which was defined as 1.0. Calculations were performed with statistical software GraphPad Prism (GraphPad Software, Inc., San Diego, CA). $P < 0.05$ was considered significant in all tests.

Results

SST and D_2D in MMQ cells

We confirmed expression of D_2D in MMQ cells. Of all the investigated SRIF receptor subtypes, only SST2 expression was detected, albeit weakly compared with D_2D levels (Fig. 1).

Rat pituitary cell cultures

According to the results of preliminary time-course (data not shown) and dose-dependency experiments (Fig. 2), cultures were incubated with test compounds at concentrations of 10^{-8} M (10 nM) for 24 and 48 h. The 24-h incubations with BIM 23A760 and BIM 23A761 resulted in GH suppression of 50 ± 2% ($P < 0.001$) and 42 ± 3% ($P < 0.001$) *vs.* control, respectively (Fig. 3A). The EC_{50} values were as follows: 11.4 pM (95% confidence interval, 2–82 pM) for BIM 23A760 and 79.5 pM (95% confidence interval, 28–228 pM) for BIM 23A761. After 48 h, GH levels were reduced by 38 ± 3% (BIM 23A760, $P < 0.001$) and 32 ± 6% (BIM 23A761, $P < 0.001$) *vs.* control (Fig. 3B). Suppression of GH secretion achieved with both chimeric molecules was not different from that of the selective SST2 agonist BIM 23120 (36 ± 5% after 24 h and 37 ± 4% after 48 h, $P > 0.05$ *vs.* BIM 23A760 and BIM 23A761) or octreotide (34 ± 3% after 24 h and 39 ± 3% after 48 h, $P > 0.05$ *vs.* BIM 23A760 and BIM 23A761; Fig. 3, A andB). SST5 and D_2D agonists did not significantly change GH levels. The SRIF analogs and chimeric SRIF/DA molecules did not alter GH mRNA levels (Fig. 3C).

PRL levels were reduced by 63 ± 1% (BIM 23A760, $P < 0.001$) and 58 ± 3% (BIM 23A761, $P < 0.001$) *vs.* control after 24 h and by 78 ± 1% ($P < 0.001$) and 73 ± 2% ($P < 0.001$) after 48 h, respectively (Fig. 3, D and E). EC_{50} values after 24 h incubation were as follows: 7.7 pM (95% confidence interval, 4–14 pM) for BIM 23A760 and 137 pM (95% confidence interval, 75–252 pM) for BIM 23A761. The efficacy of both chimeric molecules in suppressing PRL secretion was similar to that of the selective D_2D agonist BIM 53097 (66 ± 1% after 24 h and 61 ± 1% after 48 h, $P > 0.05$ *vs.* BIM 23A760 and BIM 23A761) and bromocriptine (66 ± 1% after 24 h and 76 ± 1% after 48 h, $P > 0.05$ *vs.* BIM 23A760 and BIM 23A761; Fig. 3, D and E).

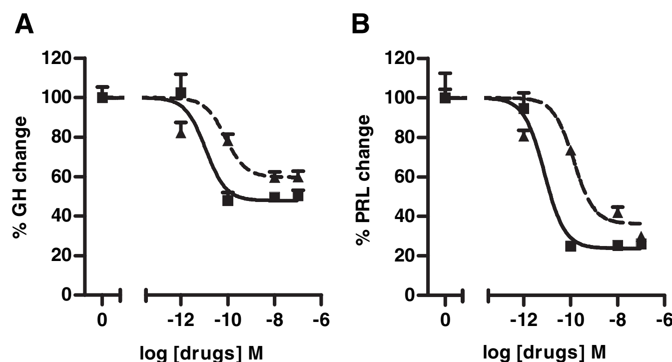

FIG. 2. Dose-dependent GH (A) and PRL (B) suppression in primary cultures of rat pituitary cells treated with SRIF/DA chimeric molecules BIM 23A760 and BIM 23A761. Results are expressed as mean ± SEM percent PRL or GH suppression *vs.* control. Each *point* represents the mean of six to eight wells. A, ■, BIM 23A760 (EC_{50} = 11.4 pM); ▲, BIM 23A761 (EC_{50} = 79.5 pM); B, ■, BIM 23A760 (EC_{50} = 7.7 pM); ▲, BIM 23A761 (EC_{50} = 137 pM).

Real-time PCR revealed suppression of PRL mRNA levels after treatment with both chimeric compounds: 2.2-fold from baseline in the BIM 23A760-treated group ($P < 0.01$) and 2-fold in the BIM 23A761-treated group ($P < 0.01$) compared with 1.8-fold decrease after incubation with BIM 53097 ($P < 0.01$) and 2.3-fold decrease in the bromocriptine-treated group ($P < 0.01$, Fig. 3F). SST2 and SST5 agonists did not affect PRL release and PRL mRNA in rat pituitary cells.

GH_3 cells

In GH_3 cells, BIM 23A760 and BIM 23A761 induced GH suppression of 25 ± 3% ($P < 0.01$ *vs.* control) and 22 ± 4% ($P < 0.01$ *vs.* control), respectively, which was similar to that obtained with the selective SST2 agonist BIM 23120 (31 ± 5%, $P > 0.05$ *vs.* SRIF/DA compounds) or SST2 plus SST5 agonist BIM 23244 (27 ± 5%, $P > 0.05$ *vs.* SRIF/DA compounds) and octreotide (35 ± 3%, $P > 0.05$ *vs.* SRIF/DA compounds; Fig. 4A). SST5 and D_2D agonists did not affect GH release from GH_3 cells.

The SRIF analogs and chimeric SRIF/DA molecules tested did not alter GH mRNA levels in GH_3 cells (Fig. 4B).

PRL levels were reduced by 31 ± 4% (BIM 23A760, $P < 0.001$) and 29 ± 5% (BIM 23A761, $P < 0.01$; Fig. 4C). PRL suppression obtained with both chimeric molecules was similar to that of selective SST2 agonist BIM 23120 (31 ± 3%, $P > 0.05$ *vs.* SRIF/DA compounds) or SST2 plus SST5 agonist BIM 23244 (29 ± 4%, $P > 0.05$ *vs.* SRIF/DA compounds) and octreotide (38 ± 4%, $P > 0.05$ *vs.* SRIF/DA compounds; Fig. 4C). SST5 and D_2D agonists did not affect PRL release from GH_3 cells.

Treatment with chimeric compounds suppressed PRL expression as evidenced by real-time PCR. Moreover, decreased PRL mRNA levels were also observed after incubation with SST2 agonist (BIM 23120), SST2 plus SST5 agonist (BIM 23244), and octreotide (Fig. 4D).

SRIF/DA compounds vs. combination of mono-receptor ligands in GH_3 cells

The 24-h treatment with BIM 23A760 did not exert additive effects on GH secretion compared with cotreatment with the SST2 agonist BIM 23120 and D_2D agonist BIM 53097 (GH suppression of 27 ± 4% and 16 ± 4%, respectively, $P > 0.05$ *vs.* BIM 23A760; Fig. 5A). After 48 h, combination of BIM 23120 and BIM 53097 resulted in greater GH suppression (33 ± 4%) than treatment with BIM 23A760 alone (17 ± 3%, $P < 0.01$; Fig. 5B). Similarly, BIM 23A761 was less effective in suppressing GH after 48 h (16 ± 2%) than a combination of BIM 23120, BIM 23206, and BIM 53097 (SST2, SST5, and D_2D agonist, respectively, 32 ± 3%, $P < 0.01$ *vs.* BIM 23A761; Fig. 5B). GH mRNA levels remained unchanged in all the

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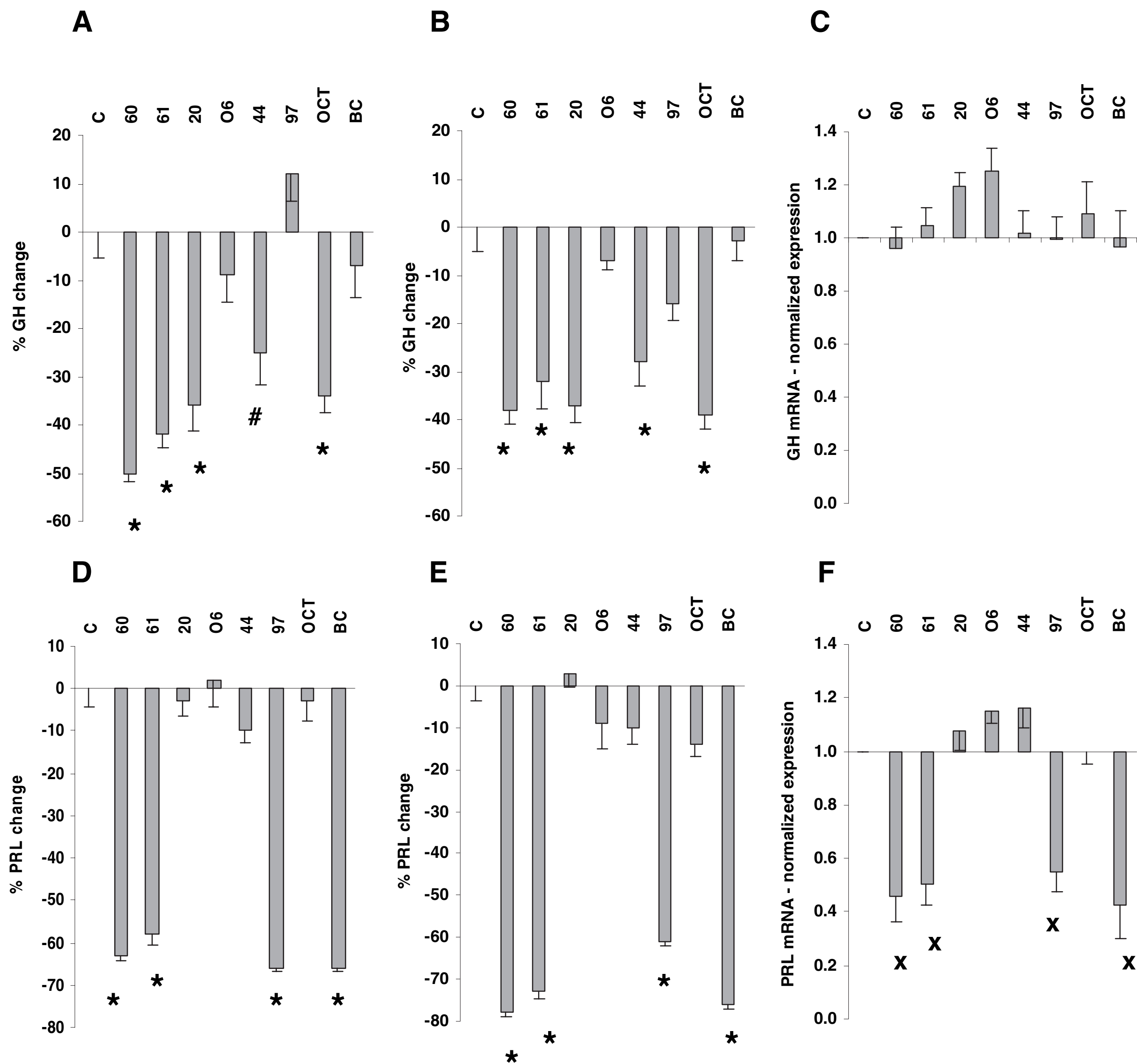

FIG. 3. Effects of SRIF/DA chimeric molecules BIM 23A760 (60) and BIM 23A761 (61), D_2D agonists BIM 53097 (97) and bromocriptine (BC), and SRIF receptor agonists BIM 23120 (20), BIM 23206 (06), BIM 23244 (44), and octreotide (OCT) on GH and PRL secretion and mRNA expression in primary culture of rat pituitary cells after 24 h (A, C, D, and F) and 48 h (B and E) incubation (all test substances at concentrations of 10^{-8} M). Results are expressed as mean ± SEM percent GH or PRL secretion change *vs.* vehicle-treated control cells. Each *point* represents the mean of seven to eight wells. GH and PRL mRNA levels were analyzed by real-time PCR. Values (mean ± SEM) of three different experiments, with three replicates per group, are expressed as fold change from vehicle-treated control, which was assigned a value of 1.0. GH and PRL mRNA levels were normalized to β-actin mRNA. *, $P < 0.001$; ×, $P < 0.01$; #, $P < 0.05$ *vs.* control.

experimental groups, both after 24 and 48 h of treatment (Fig. 5, C and D).

The chimeric compounds were similarly effective in suppressing PRL secretion from GH_3 cells after 24 h as the respective mono-receptor agonists combined together (PRL suppression of 27 ± 2% with BIM 23A760 *vs.* 36 ± 2% with BIM 23120 plus BIM 53097, $P > 0.05$, and 28 ± 2% with BIM 23A761 *vs.* 38 ± 4% with BIM 23120 plus BIM 23206 plus BIM 53097, $P > 0.05$; Fig. 6A). After 48 h, BIM 23A761 was less effective than the combination of the respective mono-receptor agonists (36 ± 1% *vs.* 47 ± 2%, $P < 0.05$; Fig. 6B). A similar decrease in PRL mRNA levels was obtained after incubation with the chimeric compounds and the combination of mono-receptor ligands, after both 24 and 48 h (Fig. 6, C and D).

MMQ cells

In MMQ cells, BIM 23A760 and BIM 23A761 induced PRL suppression of 36 ± 6% ($P < 0.001$) and 38 ± 3% ($P < 0.001$ *vs.* control), respectively, acting with similar efficacy to BIM 53097 (34 ± 4%, $P > 0.05$ *vs.* SRIF/DA compounds; Fig. 7A). PRL levels were also decreased by octreotide (30 ± 3%, $P < 0.01$ *vs.* control) but not by the SST5 agonist BIM 23206. BIM 23120 and BIM 23244 suppressed PRL secretion by 22 ± 5%

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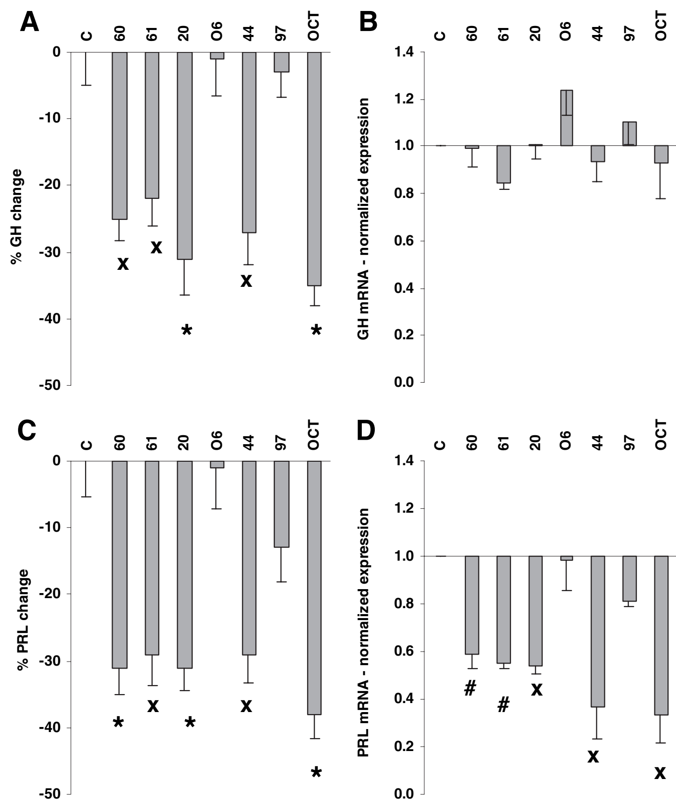

FIG. 4. Effects of SRIF/DA chimeric molecules BIM 23A760 (60) and BIM 23A761 (61), D_2D agonist BIM 53097 (97) and SRIF receptor agonists BIM 23120 (20), BIM 23206 (06), BIM 23244 (44), and octreotide (OCT) on GH and PRL secretion and mRNA expression in GH_3 cells after 24 h incubation (all test substances at concentrations of 10^{-8} M). Results are expressed as mean ± SEM percent GH or PRL secretion change *vs.* vehicle-treated control cells. Each point represents the mean of eight wells. GH and PRL mRNA levels were analyzed by real-time PCR. Values (mean ± SEM) of three different experiments, with three replicates per group, are expressed as fold change from vehicle-treated control, which was assigned a value of 1.0. GH and PRL mRNA levels were normalized to β-actin mRNA. *, $P < 0.001$; ×, $P < 0.01$; #, $P < 0.05$ *vs.* control.

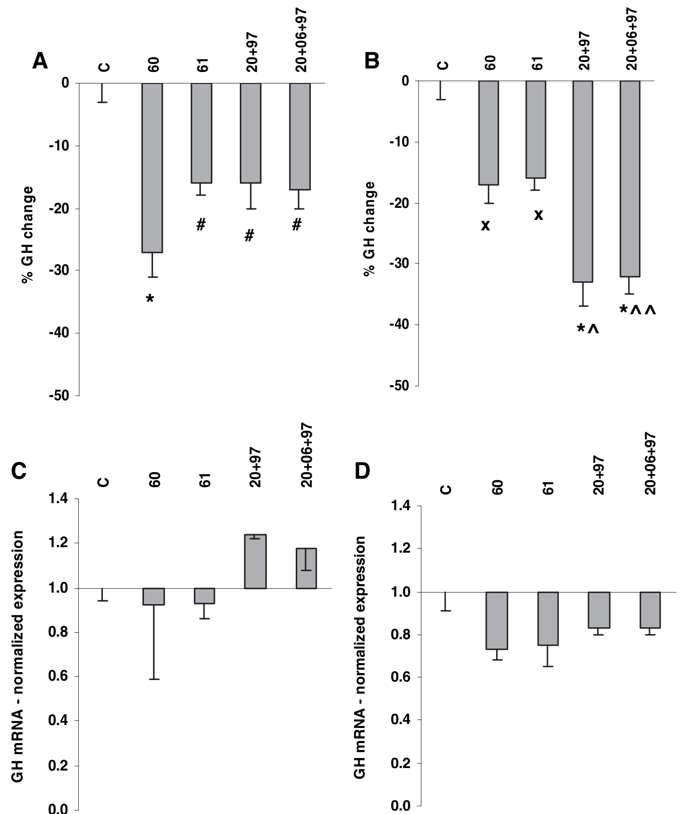

FIG. 5. Effects of SRIF/DA chimeric molecules BIM 23A760 (60) and BIM 23A761 (61) and the combination of respective mono-receptor ligands BIM 23120 (20), BIM 23206 (06), and BIM 53097 (97) on GH secretion and GH mRNA expression in GH_3 cells after 24 and 48 h incubation (all test substances at concentrations of 10^{-8} M). Results are expressed as mean ± SEM percent GH secretion change *vs.* vehicle-treated control cells. Each point represents the mean of five to 10 wells. GH mRNA levels were analyzed by real-time PCR. Values (mean ± SEM) are expressed as fold change from vehicle-treated control, which was assigned a value of 1.0. GH mRNA levels were normalized to β-actin mRNA. *, $P < 0.001$; ×, $P < 0.01$; #, $P < 0.05$ *vs.* control; ^, $P < 0.01$ *vs.* BIM 23A760; ^^, $P < 0.01$ *vs.* BIM 23A761.

($P > 0.05$ *vs.* control) and 23 ± 4% ($P > 0.05$ *vs.* control), respectively. Real-time PCR revealed suppressed PRL mRNA expression after treatment with the chimeric compounds or with the selective D_2D agonist BIM 53097 but not with any of the SST2 and SST5 agonists (Fig. 7B).

SRIF/DA compounds vs. combination of mono-receptor ligands in MMQ cells

Neither BIM 23A760 nor BIM 23A761 produced additive effects on PRL secretion compared with the combination of respective mono-receptor ligands, both after 24 and 48 h. After 24 h, PRL levels were suppressed by 37 ± 2% with both BIM 23A760 and BIM 23A761, 38 ± 2% with BIM 23120 plus BIM 53097, and 36 ± 3% with BIM 23120 plus BIM 23206 plus BIM 53097 ($P < 0.001$ *vs.* control; Fig. 8A). After 48 h, PRL suppression obtained with the respective compounds was 46 ± 1, 34 ± 5, 40 ± 4, and 49 ± 1% ($P < 0.001$ *vs.* control; Fig. 8B). Similarly, additive effects on PRL mRNA expression were not observed after treatment with SRIF/DA compounds (Fig. 8, C and D).

Discussion

GH secretion and GH mRNA expression

BIM 23A760 and BIM 23A761 suppressed GH secretion from cultured rat pituitary cells with a similar efficacy to that observed for SST2 agonists, whereas the SST5 agonist BIM 23206 was ineffective. Both SST2 and SST5 regulate GH secretion in human somatotroph adenoma cells (3) and in primary human fetal pituitary cells (4). Rat somatotrophs express all five SRIF receptor subtypes (22); however, our results provide additional evidence that SST2 is the major receptor subtype responsible for rat GH inhibition and that participation of the SST5 isoform in rat GH inhibition is of lesser importance (23). In GH_3 cells, both SRIF/DA chimeric molecules suppressed GH similarly to the selective SST2 agonist BIM 23120 or SST2 plus SST5 agonist BIM 23244 and octreotide. SST5 and D_2D agonists did not alter GH release from GH_3 cells, which was not surprising considering that these cells do not express SST5 or D_2D (24).

The 24- and 48-h treatment with SRIF/DA chimeric compounds did not result in additive effects on GH secretion

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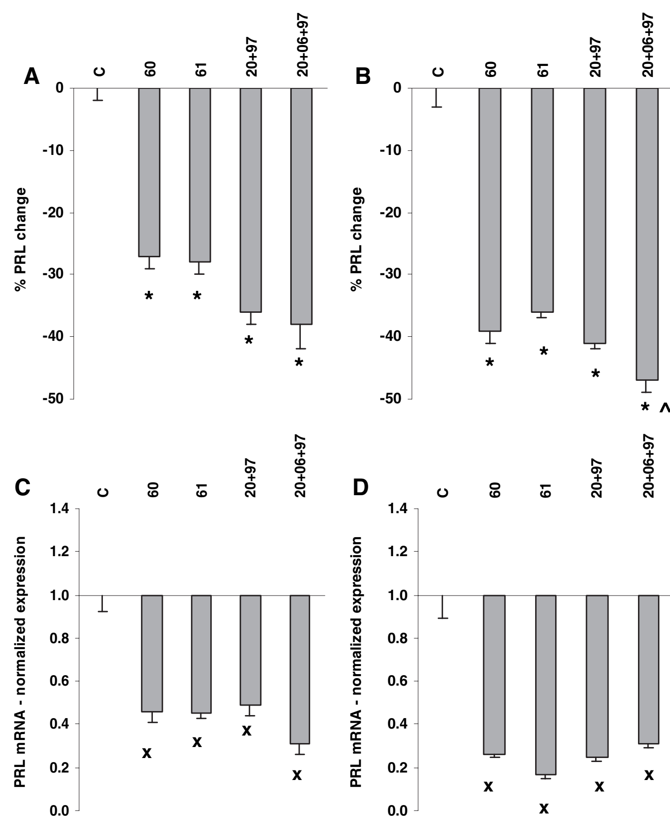

FIG. 6. Effects of SRIF/DA chimeric molecules BIM 23A760 (60) and BIM 23A761 (61) and the combination of respective mono-receptor ligands BIM 23120 (20), BIM 23206 (06), and BIM 53097 (97) on PRL secretion and PRL mRNA expression in GH_3 cells after 24 and 48 h incubation (all test substances at concentrations of 10^{-8} M). Results are expressed as mean ± SEM percent PRL secretion change *vs.* vehicle-treated control cells. Each *point* represents the mean of five wells. PRL mRNA levels were analyzed by real-time PCR. Values (mean ± SEM) are expressed as fold change from vehicle-treated control, which was assigned a value of 1.0. PRL mRNA levels were normalized to β-actin mRNA. *, $P < 0.001$; ×, $P < 0.01$ *vs.* control; ˆ, $P < 0.05$ *vs.* BIM 23A761.

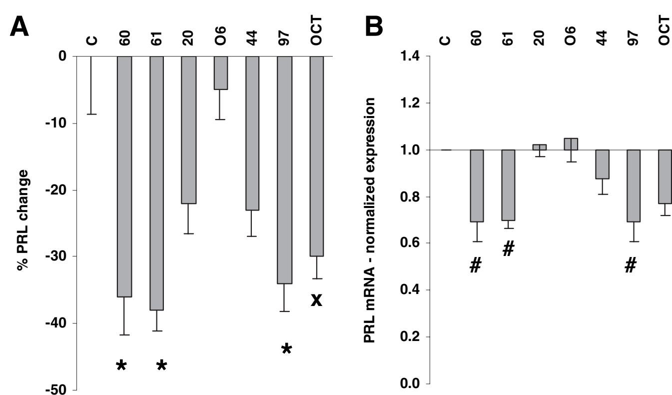

FIG. 7. Effects of SRIF/DA chimeric molecules BIM 23A760 (60) and BIM 23A761 (61), D_2D agonist BIM 53097 (97) and SRIF receptor agonists BIM 23120 (20), BIM 23206 (06), BIM 23244 (44), and octreotide (OCT) on PRL secretion and PRL mRNA expression in MMQ cells after 24 h incubation (all test substances at concentrations of 10^{-8} M). Results are expressed as mean ± SEM percent PRL secretion change *vs.* vehicle-treated control cells. Each *point* represents the mean of eight wells. PRL mRNA levels were analyzed by real-time PCR. Values (mean ± SEM) of three different experiments, with three replicates per group, are expressed as fold change from vehicle-treated control, which was assigned a value of 1.0. PRL mRNA levels were normalized to β-actin mRNA. *, $P < 0.001$; ×, $P < 0.01$; #, $P < 0.05$ *vs.* control.

from GH_3 cells compared with cotreatment with respective mono-receptor agonists. Conversely, after 48 h, both SRIF/DA molecules were less effective than combined treatment with mono-receptor ligands. However, considering that GH_3 cells lack SST5 and D_2D, these results can be interpreted as a lower efficacy of the SST2 component of the SRIF/DA chimeric molecules in suppressing GH secretion compared with the SST2 agonist BIM 23120 at concentration of 10^{-8} M.

Previously, Saveanu *et al.* (13) demonstrated that BIM 23A387, a chimeric molecule with affinity for SST2 and D_2D, was more potent than either BIM 23023 (SST2 agonist) or BIM 53097 (D_2D agonist), either alone or in combination, in suppressing both GH and PRL secretion from human GH adenomas. The mean EC_{50} for GH suppression by BIM 23A387 (0.2 pM) was 50 times lower than that of the individual SST2 and D_2D agonists. However, at nanomolar concentrations, mean maximal inhibition of GH release induced by BIM 23A387, BIM 23023, and BIM 53097 was similar. In another study (14), BIM 23A387 (0.4 nM) exhibited greater GH suppression in human GH-producing tumor cells compared with combined cotreatment with individual SST2 (BIM 23023) and D_2D (BIM 53097) agonists, but the effectiveness of the SRIF/DA chimeric molecule decreased with increasing doses from 0.4 to 40 nM. Jaquet *et al.* (16) described two individual patterns of dose-related inhibition of GH by BIM 23A387, BIM 23244 (SST2 plus -5 agonist), and octreotide. In the first group of tumors, enhanced GH-suppressive effects with BIM 23A387 were observed (mean EC_{50}, 1 *vs.* 10 pM for BIM 23244 and 150 pM for octreotide), whereas in the second group, no differences between EC_{50} values were observed. In six of 13 tumors, BIM 23A387, BIM 23A760, and BIM 23A761 achieved greater maximal suppression of GH secretion than octreotide. Mean EC_{50} values were 10, 2, and 4 pM for respective chimeric molecules *vs.* 150 pM for octreotide.

Clearly, optimal results of treatment with SRIF/DA chimeric compounds are likely achieved in cells coexpressing both SRIF receptors (SST2 and/or SST5) and D_2D. Previous studies have shown that SST2, SST5, and D_2D, in variable amounts, were coexpressed in all (13, 15, 16) human GH-secreting adenomas tested. In another study (25), SST2 was expressed in 25, SST5 in 16, and D_2D in 16 of 25 adenomas tested.

Our study did not reveal significant effects of the SRIF analogs and chimeric SRIF/DA molecules on GH gene expression in GH_3 cells and in normal rat pituitary cells. Most studies support the hypothesis that although SRIF is a potent inhibitor of *in vivo* and *in vitro* GH release, it does not suppress GH mRNA levels in pituitary cells (26). Some reports, however, show discordant results (7, 8). Morishita *et al.* (10) investigated effects of GHRH and SRIF on GH gene 5′-promoter activity in MtT/S cells and found that SRIF modestly but significantly suppressed GHRH-induced GH gene transcription, although SRIF alone did not influence basal promoter activity. There are few reports of GH mRNA levels in

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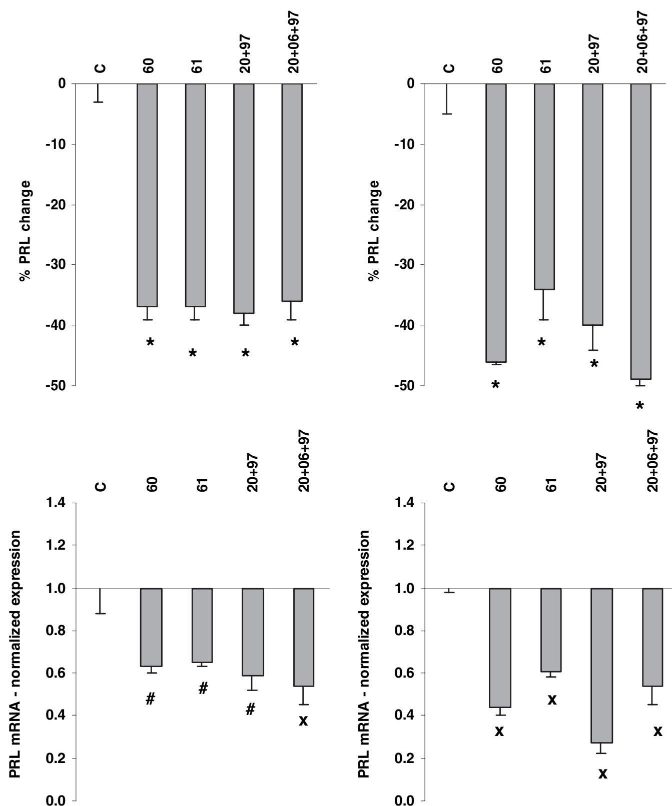

FIG. 8. Effects of SRIF/DA chimeric molecules BIM 23A760 (60) and BIM 23A761 (61) and the combination of respective mono-receptor ligands BIM 23120 (20), BIM 23206 (06), and BIM 53097 (97) on PRL secretion and PRL mRNA expression in MMQ cells after 24 and 48 h incubation (all test substances at concentrations of 10^{-8} M). Results are expressed as mean ± SEM percent PRL secretion change *vs.* vehicle-treated control cells. Each *point* represents the mean of five to six wells. PRL mRNA levels were analyzed by real-time PCR. Values (mean ± SEM) are expressed as fold change from vehicle-treated control, which was assigned a value of 1.0. PRL mRNA levels were normalized to β-actin mRNA. *, $P < 0.001$; ×, $P < 0.01$; #, $P < 0.05$ *vs.* control.

human GH-secreting pituitary adenomas. In these studies (9, 27), GH mRNA levels were determined by automatic quantification of grain numbers in individual adenoma cells. There was no effect of 10 nM octreotide on GH mRNA levels in 24-h incubations in three adenomas tested (27). In a 96-h incubation, GH mRNA levels increased in two and slightly decreased in one of three adenomas. In a second study (9), in GH-secreting pituitary adenoma tissue obtained from seven patients treated preoperatively with octreotide, GH mRNA levels were significantly lower than in adenomas derived from 18 untreated patients.

Our study provides evidence that SRIF-dopaminergic ligands and SST2 agonists inhibit GH secretion with a similar efficacy but do not suppress GH gene expression in normal rat pituitary and GH_3 cells.

PRL secretion and PRL mRNA expression

We report that the SRIF/DA chimeric molecules BIM 23A760 and BIM 23A761 suppress PRL secretion from normal rat pituitary cells and MMQ and GH_3 cell lines and also negatively regulate PRL gene expression in these experimental models.

The efficacy of both chimeric molecules in suppressing PRL secretion in normal rat pituitary cells was similar to that achieved with the selective D_2D agonist BIM 53097 and bromocriptine. PRL release from rat pituitary cells was not affected by SST2 or SST5 agonists, in accordance with previous findings that SRIF agonists do not inhibit PRL release in normal, but only in estradiol-treated, rat anterior pituitary cells (23). PRL secretion results are consistent with real-time PCR results showing decreased PRL mRNA levels in groups treated with both chimeric molecules, BIM 53097, and bromocriptine. Thus, the effect of BIM 23A760 and BIM 23A761 on PRL gene expression as well as on PRL release from normal rat pituitary cells appears to depend selectively on their D_2D affinity.

In GH_3 cells, PRL secretion was suppressed in groups treated with both chimeric molecules, selective SST2 agonist BIM 23120, SST2 and -5 agonist BIM 23244, and octreotide but not in those treated with the SST5 or D_2D agonist. Because GH_3 cells express SST1 and SST2 receptors and lack functional DA receptors (24), the effect of SRIF/DA chimeras and other tested SRIF analogs on PRL secretion from these cells depends on their respective affinity for SST2. We also report that PRL suppression by SST2 agonists in GH_3 cells is accompanied by suppressed PRL mRNA expression. The effect of BIM 23A760 and BIM 23A761 on PRL gene expression in GH_3 cells cannot be attributed to the affinity of these compounds for D_2D, because these are not expressed in this cell line, and seems to depend solely on their SST2 affinity.

SRIF/DA chimeric compounds did not exert additive effects on PRL secretion or mRNA expression in GH_3 cells compared with cotreatment with respective mono-receptor agonists. After 48 h, BIM 23A761 was even less effective in reducing PRL levels than combined incubations with mono-receptor ligands. However, considering the absence of SST5 and D_2D in GH_3 cells, these results can be interpreted as a lower efficacy of the SST2 component of SRIF/DA chimeric molecules in suppressing PRL secretion and PRL mRNA levels compared with the SST2 agonist BIM 23120 at a concentration of 10^{-8} M.

In MMQ cells, BIM 23A760 and BIM 23A761 suppressed PRL gene transcription and inhibited PRL release with a similar efficacy to that of the D_2D agonist BIM 53097. Of the SRIF receptor agonists tested, only octreotide decreased PRL secretion but not PRL mRNA level in MMQ cells. MMQ cells express D_2D (28), and expression of SRIF receptors has not previously been reported in these cells. We show that MMQ cells weakly express SST2, compared with D_2D. This observation explains the lack of response to most tested SRIF analogs in MMQ cells. Weak SST2 expression in MMQ cells is also likely the reason why no additive effects on PRL secretion and PRL mRNA expression were obtained with SRIF/DA compounds.

We conclude that chimeric SRIF/DA molecules suppress PRL gene expression and inhibit PRL release from cultured rat pituitary and MMQ cells with a similar efficacy to the D_2D agonist. In GH_3 cells, they suppress PRL gene expression and inhibit PRL secretion with a similar efficacy to SST2 agonists.

In summary, we have investigated effects of several SRIF

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analogs and two chimeric SRIF/DA compounds in cultured primary rat pituitary cells and GH_3 and MMQ cell lines. Substances with affinity to SST2, including both SRIF/DA molecules, suppressed GH secretion but did not affect GH mRNA expression in rat pituitary and GH_3 cells. SRIF/DA molecules suppressed PRL secretion and also PRL mRNA expression in rat pituitary and MMQ cells with a similar efficacy to the D_2D agonist. In GH_3 cells, they suppressed both PRL and PRL mRNA levels with a similar efficacy to SST2 agonists. SRIF/DA molecules did not exert additive effects on GH and PRL secretion and PRL mRNA levels in GH_3 and MMQ cells as compared with mono-receptor ligand cotreatment.

SRIF/DA chimeric compounds are new therapeutic molecules shown to suppress GH and PRL secretion from human pituitary adenomas. Results of our *in vitro* studies provide additional evidence supporting the clinical application of SRIF/DA chimeric molecules in patients with GH-secreting or mixed (GH/PRL) pituitary adenomas with coexpression of SST2 and/or SST5, as well as D_2D. Studies of SRIF and D_2D expression are required in a large population of somatotroph adenomas to assess the dimensions of the patient population likely to benefit from treatment with SRIF/DA chimeric compounds.

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