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Brief Report

A novel somatostatin receptor ligand for human ACTH – and GH –secreting pituitary adenomas

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Abstract

Objective: Somatostatin receptor ligands have come to play a pivotal role in the treatment of both ACTH- and GH-secreting pituitary adenomas. Clinical efficacy averages 30-50%, thus a considerable number of patients with Cushing's disease or acromegaly remain unresponsive to this therapeutic approach. HTL0030310 is a new somatostatin receptor ligand selective for subtype 5 over subtype 2, thus with a different receptor profile compared to clinical somatostatin receptor ligands.

Design: Assessment of the effect of HTL0030310 on hormone secretion in human ACTH- and GH-secreting pituitary adenomas *in vitro*.

Methods: Primary cultures from 3 ACTH-secreting and 5 GH-secreting pituitary adenomas were treated with 1, 10 and 100 nM HTL0030310 alone or with 10 nM CRH or GHRH, respectively. Parallel incubations with 10 nM pasireotide were also carried out. ACTH and GH secretion were assessed after 4 and 24 hour incubation; *SSTR2, SSTR3, SSTR5*, *GH* and *POMC* expression were evaluated after 24 hours.

Results: HTL0030310 reduced unchallenged ACTH and *POMC* levels up to 50% in 2 ACTH-secreting adenomas and blunted CRH-stimulated ACTH/*POMC* by 20-70% in all 3 specimens. A reduction in spontaneous GH secretion was observed in 4 GH-secreting adenomas and in 2 specimens during GHRH co-incubation. *SSTR*s expression was detected in all specimens.

Conclusions: This first study on a novel somatostatin receptor 5-preferring ligand indicates that HTL0030310 can inhibit hormonal secretion in human ACTH- and GH-secreting pituitary adenomas. These findings suggest a potential new avenue for somatostatin ligands in the treatment of Cushing's disease and acromegaly.

Keywords: somatostatin, acromegaly, Cushing's disease, pituitary adenoma, target therapy

Significance

Medical treatment plays a significant role in Cushing's disease and acromegaly, two conditions in which surgery does not always achieve remission, with somatostatin receptor ligands as the foremost target therapy. We developed a novel, somatostatin receptor 5-preferring ligand, HTL0030310, and tested its effects on a pilot series of human ACTH– and GH–secreting adenomas in vitro. Our results show that HTL0030310 inhibits ACTH synthesis and secretion; indeed, blunted basal and corticotropin-releasing hormone (CRH)–stimulated secretion was observed in all 3 specimens. The effect on GH-secreting adenomas was less homogeneous, with reduced GH secretion observed in some but not all specimens. This first study on a novel somatostatin receptor ligand offers promise for a new, potentially efficacious drug for Cushing's disease and acromegaly.

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Introduction

Somatostatin receptor ligands have come to play a pivotal role in the treatment of neurondocrine tumors, with major clinical outcomes reported for gastroenteropancreatic[1] as well as pituitary tumors.[2] Somatostatin receptor ligands are first-choice agents for medical management of GH–secreting pituitary adenomas, i.e, acromegaly,[3] and represent the only pituitary-acting drug approved for use in Cushing's disease, that is, ACTH–secreting pituitary adenoma.[4] Successful containment of excess hormone secretion is achieved in approximately 50% of patients with acromegaly[5] and slightly less in patients with Cushing's disease.[6] Thus, available somatostatin receptor ligands are ineffective in a substantial proportion of patients, and novel compounds are sorely needed. In fact, subtype selective, multireceptor ligands (ligands acting on different combinations of somatostatin receptor subtypes), somatostatin–dopamine receptor chimeras, that is, "dopastatin,"[7,8] and nonpeptidic ligands[9] are being actively pursued.

Design of viable, novel ligands is driven by knowledge of somatostatin receptor pathophysiology in neuroendocrine tissues and tumors.[5,10,11] Several studies evaluating the expression of somatostatin receptor subtypes in GH-secreting adenomas have revealed high expression of *SSTR2* and lesser amounts for *SSTR3* and *SSTR5*, albeit present to a variable extent in individual specimens.[12-15] Conversely, *SSTR5* appeared to be the prevalent receptor subtype in adenomas from patients with Cushing's disease,[16-18] with individual patients also expressing high levels of *SSTR1*.[19] Interestingly, *SSTR5* expression was linked to distinct corticotrope phenotypes.[20,21]

HTL0030310, a somatostatin receptor ligand that is selective for the SSTR5 and SSTR3 subtypes, has recently been developed through computer-aided drug design[22]; by comparison, pasireotide has similar functional and binding activity at human SSTR1, 2, 3, and 5 receptors.[23] The aim of the present study was to evaluate the effect of this novel ligand on GH- and ACTH-secreting adenomas in order to establish its potential as a medical agent for acromegaly and Cushing's disease.

Methods

Pituitary adenoma primary cultures

Three ACTH-secreting and 5 GH-secreting adenomas (Table 1) were collected during surgery and established in culture according to our protocol.[24-27] In detail, adenomas were dispersed by enzymatic digestion and plated at 15×10^3 to 70×10^3 per well, according to specimen abundance. Cells were attached in Dulbecco's Modified Eagle Medium (DMEM), 10% fetal calf serum, and antibiotics for 2-3 days, then washed with DMEM and 0.1% bovine serum albumin (BSA) prior to challenge with 1-100 nM HTL0030310 alone or with 10 nM corticotropin-releasing hormone (CRH) or growth hormone–releasing hormone (GHRH). The range of doses used was determined according to availability of wells for testing, starting with the higher HTL0030310 concentration. Parallel experiments with 10 nM pasireotide and CRH/GHRH were also carried out. Control wells were incubated with DMEM + 0.1% BSA alone, and each treatment was performed in triplicate. DMEM and antibiotics were purchased from GIBCO (Waltham MA, United States), the remainder from Sigma-Aldrich (St. Louis, MO, United States). HTL0030310 and pasireotide were provided by Sosei Heptares, Cambridge, United Kingdom. After 4 and 24 h incubation, medium was collected for hormone measurement and cellular RNA extracted.

Hormone assays

Medium hormone concentrations were measured by ELISA assay kits produced by Biomerica, Irvine, CA, United States, for ACTH and by Invitrogen, Waltham, MA, United States, for GH, sensitivity and intraassay coefficient of variation are 0.22 pg/mL and 6.7% for ACTH and 4 pg/mL and 10% for GH. All samples from a given specimen were measured in the same run.

RNA extraction and Droplet Digital PCR

Cells were washed and RNA extracted with TRIzol reagent, Invitrogen, Waltham, MA, United States, and purified with Direct-zol RNA Microprep (Zymo, Irvine, CA, United States). RNA (500 ng) was reverse transcribed by EuroScript M-MMLV RT (Euroclone, Pero, Italy) with random primers[27] or with Sensiscript RT SuperScript (Qiagen, Hilden, Germany) if limited RNA was available.

Droplet Digital PCR was performed according to Digital MQE Guidelines[28] on QX200 Droplet Digital PCR System (Biorad, Hercules, CA, United States) for detection of *POMC* (Taqman probe Hs01596743_m1), *GH* (Evagreen chemistry: forward primer ATCCAGGCTTTTTGACAACG, reverse primer GGAGCAGCTCTAGGTTGGATT), *SSTR2* (Hs00265624_s1), *SSTR3* (Hs00265633_s1), and *SSTR5* (Hs00990407_s1) with *HMBS* as housekeeping gene (Taqman probe Hs00609296_g1). Basal expression was calculated and normalized to *HMBS* as previously described[29]; CIs were set at 95% applying Poisson's statistics (QX Manager 1.2 Standard Edition Software, Bio-Rad).

Ethical approval

The study was approved by the Ethical Committee of the University of Padua on June 20, 2020 (#4834/AO/2020, URC1782). Informed consent for secondary use of surgical tissues was obtained at referring neurosurgical centers. Study procedures adhered to the tenets of the Declaration of Helsinki. Raw data are deposited at https://doi.org/10.13130/RD_UNIMI/DXUJXX.

Analyses

Data are reported as mean ± SEM with median and interquartile range in parentheses. Gene expression is reported as fold change vs control. Nonparametric tests (Wilcoxon signed rank or Friedman, as appropriate) were used for comparisons between control and treatment(s). Significance was accepted for $P < .05$; Bonferroni's correction for multiple comparisons was applied where indicated.

Results

HTL0030310 exerted an inhibitory effect on spontaneous ACTH secretion in 2 specimens, most evident after 4 h incubation in specimen CD#2 and after 24 h incubation in specimen CD#1 (Figure 1). HTL0030310 also reduced *POMC* expression, as a reduction by 15%-40% in spontaneous expression was observed in the 3 specimens after 24 h incubation with

Table 1. Clinical features of patients with ACTH- and GH-secreting adenomas.

ACTH-secreting adenomas

Specimen	Age	Sex	Adenoma size	UFC nmol/24 h (%ULN)	ACTH ng/L	Somatostatin analog treatment
CD #1	40	F	14 mm	1326 (191%)	37	No
CD #2	17	M	7 mm	1663 (989%)	121	No
CD #3	29	F	10 mm	1702 (1013%)	136	No

GH-secreting adenomas

Specimen	Age	Sex	Adenoma size	GH ng/mL	IGF-I ng/mL (% ULN)	Somatostatin analog treatment
GH #1	35	M	30 mm	2.5	514 (187%)	Lanreotide up to 120 mg/3 weeks for 2 years
GH #2	31	M	15 mm	69.2	788 (291%)	No
GH #3	49	F	10 mm	7.87	485 (159%)	No
GH #4	38	F	15 mm	8	366 (149%)	Lanreotide 90 mg/month for 10 months; interrupted due to tumor growth
GH #5	62	F	8 mm	14	716 (424%)	No

Adenoma size refers to maximal diameter.
Abbreviations: % ULN, percentage of the upper limit of the normal range; F, female; M, male; UFC, urinary free cortisol, IGF-I, insulin-like growth factor I.

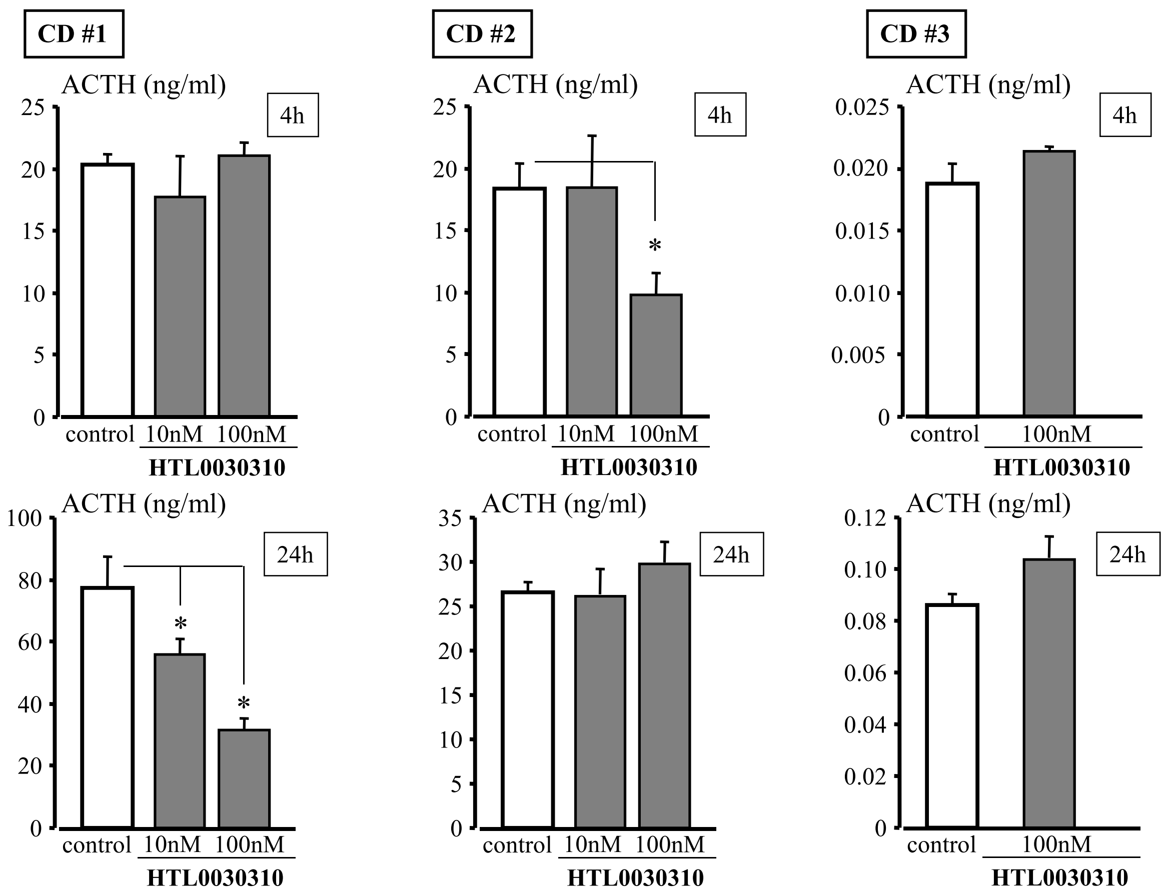

Figure 1. Effect of HTL0030310 on spontaneous ACTH secretion in ACTH-secreting adenomas. Concentrations at 4 and 24 h in each specimen are shown. White bar: control wells; gray bar: wells incubated with 10 or 100 nM HTL0030310. Adenomatous specimens are identified by a sign number, * indicates $P < 0.05$ vs control.

100 nM HTL0030310 (Figure 3A); on average, *POMC* expression was ~80% of control (Table 2, $P < .05$).

The effect of HTL0030310 on CRH-stimulated ACTH secretion was more evident than on spontaneous secretion, as blunted responses were observed in all 3 adenomatous specimens (Figure 2). The inhibitory effect of pasireotide—albeit tested a single concentration—appeared less pronounced (Figure 2 and Table 2). Of note, the effect of HTL0030310 appeared dose dependent (Table 2 and Figure 2). Further, HTL0030310 markedly blunted CRH-stimulated *POMC* expression with decreases by 40%-60% compared to CRH-incubated wells (Figure 3B and Table 2). All specimens expressed *SSTR2*, *SSTR3*, and *SSTR5* (Table S1); no clear association between receptor expression and the inhibitory

Table 2. Average effect of HTL003010 on ACTH secretion and *POMC* expression in ACTH-secreting adenomas.

Treatment	ACTH (% control)		*POMC* (% control)
	4 h	24 h	24 h
10 nM HTL003010	93.6 ± 6.8 (93.6; 90.8-96.9)	85.8 ± 13.2 (85.8; 79.2-115)	
100 nM HTL003010	90.4 ± 18.3 (103; 78.5-108)	90.6 ± 13.3 (112; 76-116)	78.5 ± 8.0[a] (82.4; 72.8-86.2)
10 nM CRH	229.7 ± 25.6[a] (204; 201-242)	197.1 ± 31.1[a] (206; 172-226)	131 ± 6.6[a] (124.9; 124-134)
Treatment	**ACTH (% CRH)**		***POMC* (% CRH)**
	4 h	**24 h**	**24 h**
10 nM CRH + 1 nM HTL003010	86.5 ± 1.78 (86.5; 85.6-87.3)	93.9 ± 1.7 (93.9; 93,1-94.8)	
10 nM CRH + 10 nM HTL003010	74.7 ± 16 (67.4; 59.3-86.4)	90.4 ± 12.1 (100.3; 83.4-102)	
10 nM CRH + 100 nM HTL003010	59.9 ± 19.2[b] (61.4; 43.6-76.9)	86.2 ± 4.8[b] (85.2; 81.8-90.1)	49.2 ± 6.4[b] (49. 1;43.5-54.8)
10 nM CRH + 10 nM pasireotide	83.9 ± 19.3[b] (92.9; 69.8-102.4)	94.5 ± 2.6[b] (96.2; 92.8-97.0)	69.2 ± 6.7[b] (71.4;63.8-75.5)

Data are provided as means ± SEM with median and interquartile range in parentheses.
[a]$P < .05$ vs control wells.
[b]$P < .05$ vs CRH-treated wells.

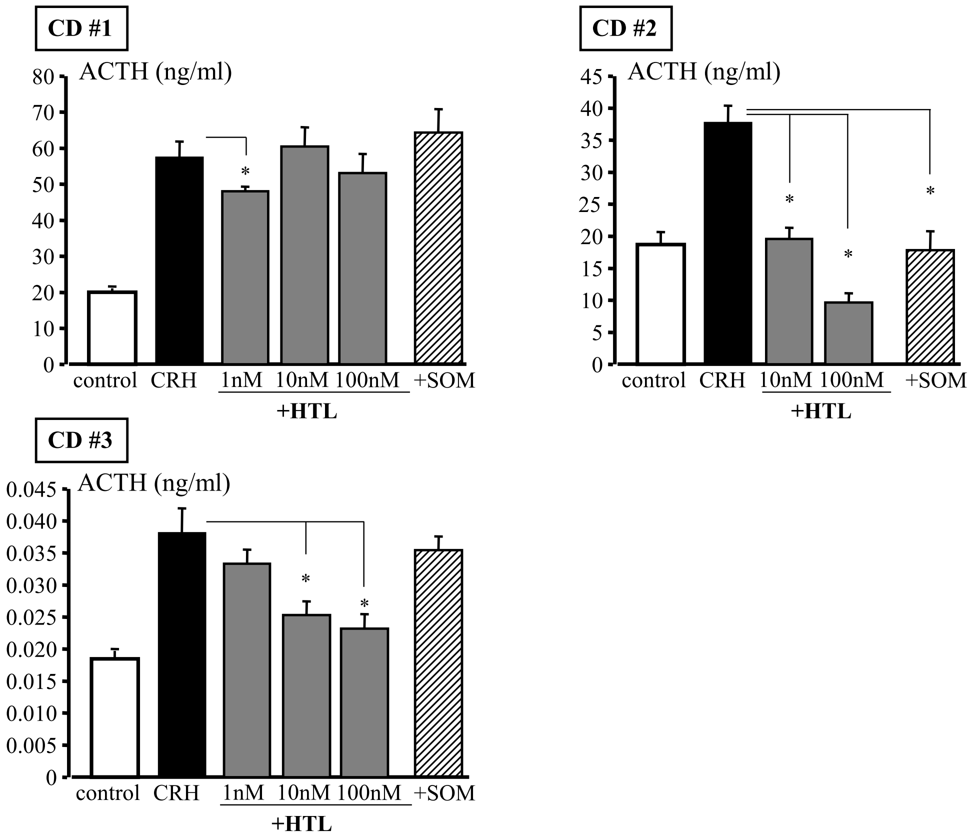

Figure 2. Effect of HTL0030310 on CRH-stimulated ACTH secretion in ACTH-secreting adenomas. Concentrations at 4 h in each specimen are shown. White bar: control wells; black bar: wells incubated with 10 nM CRH; gray bar: wells incubated with 10 nM CRH and 1-100 nM HTL0030310 (HTL); striped bar: wells incubated with 10 nM CRH and 10 nM pasireotide (SOM). Adenomatous specimens are identified by a sign number, * indicates $P < 0.05$ vs control.

effect of HTL0030310 on basal and CRH-induced response was apparent.

The effect of HTL0030310 was tested in 5 GH-secreting adenomas and revealed variable results across specimens. A clear reduction in spontaneous GH secretion at both 4 and 24 h was observed in 2 specimens (GH #1 and GH #2); in 2 further specimens (GH #3 and GH #4), the reduction was apparent either at 4 or at 24 h incubation, indicating a different time–

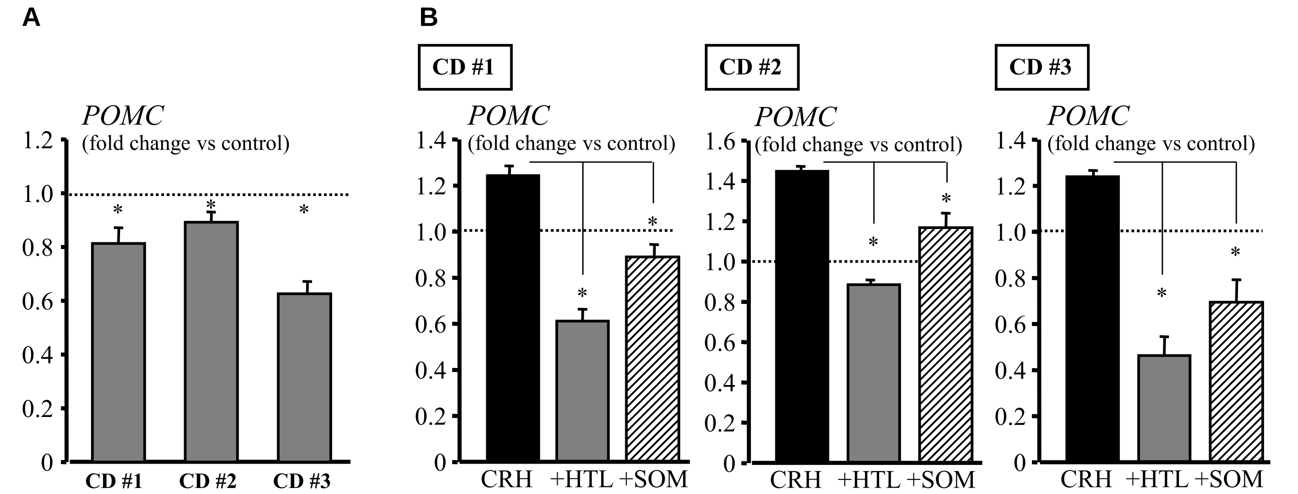

Figure 3. Effect of HTL0030310 on *POMC* expression in ACTH-secreting adenomas. (A) *POMC* expression in wells incubated with 100 nM HTL0030310. (B) *POMC* expression in wells incubated with 10 nM CRH (black bar); 10 nM CRH and 100 nM HTL0030310 (gray bar; HTL); and 10 nM CRH and 10 nM pasireotide (striped bar; SOM). Data are shown relative to expression in control wells (dashed line). Adenomatous specimens are identified by a sign number, * indicates $P < 0.05$ vs control.

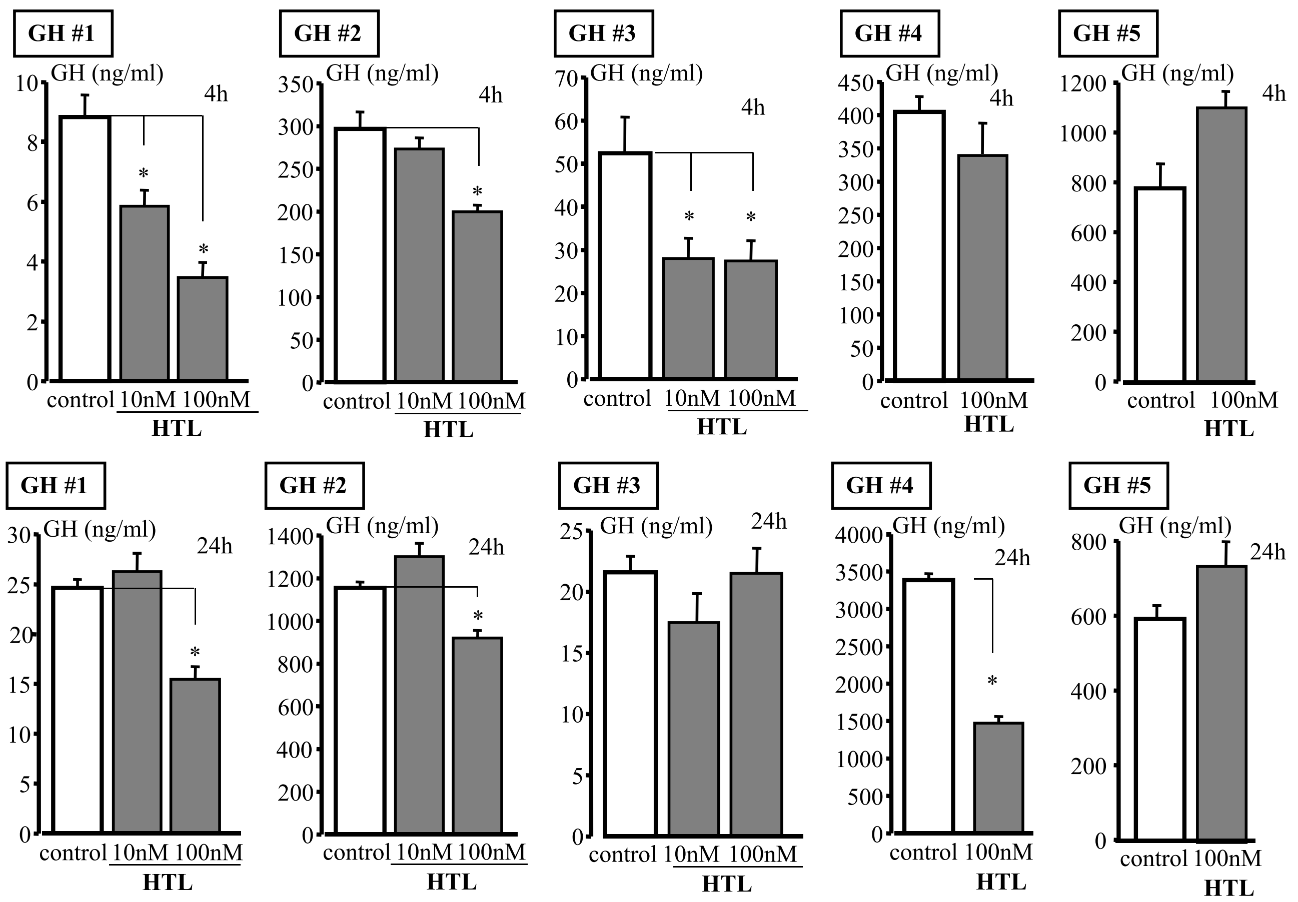

Figure 4. Effect of HTL0030310 on spontaneous GH secretion in GH-secreting adenomas. Concentrations at 4 and 24 h in each specimen are shown. White bar: control wells; gray bar: wells incubated with 10 or 100 nM HTL0030310 (HTL). Adenomatous specimens are identified by a sign number, * indicates $P < 0.05$ vs control.

response range to the receptor ligand. HTL0030310 did not exert a noticeable effect in the remaining adenoma specimens (Figure 4). All specimens expressed *SSTR2*, *SSTR3*, and *SSTR5* (Table S1); no clear relation between *SSTR* expression and the response to HTL0030310 was apparent. On average, GH synthesis and secretion during 10 and 100 nM HTL0030310 incubation did not differ significantly from control wells (Table 3).

Table 3. Average effect of HTL003010 on GH synthesis and secretion in GH-secreting adenomas.

Treatment	GH (% control)		*GH* (% control)
	4 h	24 h	24 h
10 nM HTL003010	90.6 ± 21.4 (91.0; 89.2-91.7)	94.9 ± 7.71 (93.8; 80.0-108)	
100 nM HTL003010	88.7 ± 14.9 (80.0; 72.0-84.1)	90.5 ± 14.2 (82.0; 80.1-99.4)	99.5 ± 2.2 (98.6; 97-102)
10 nM GHRH	116.4 ± 9.63 (107; 103-115)	140.8 ± 14.6* (137; 131-141)	86.6 ± 12.6 (86.0; 80-89)
Treatment	**GH (% GHRH)**		***GH* (% GHRH)**
	4 h	**24 h**	**24 h**
10 nM GHRH + 100 nM HTL003010	104.6 ± 20.0 (86.0; 83.5-107)	86.8 ± 22.0 (64.7; 60.2-113)	104.8 ± 24.0 (100; 89.4-117)
10 nM GHRH + 10 nM pasireotide	142.9 ± 67.1 (91.4; 74.3-160)	84.3 ± 16.3 (79.8; 60.9-103)	101.1 ± 14.7 (103; 80.8-119)

Data are provided as means ± SEM with median and interquartile range in parentheses.
GHRH, growth hormone–releasing hormone.
[a]*P* < .05 vs control wells.

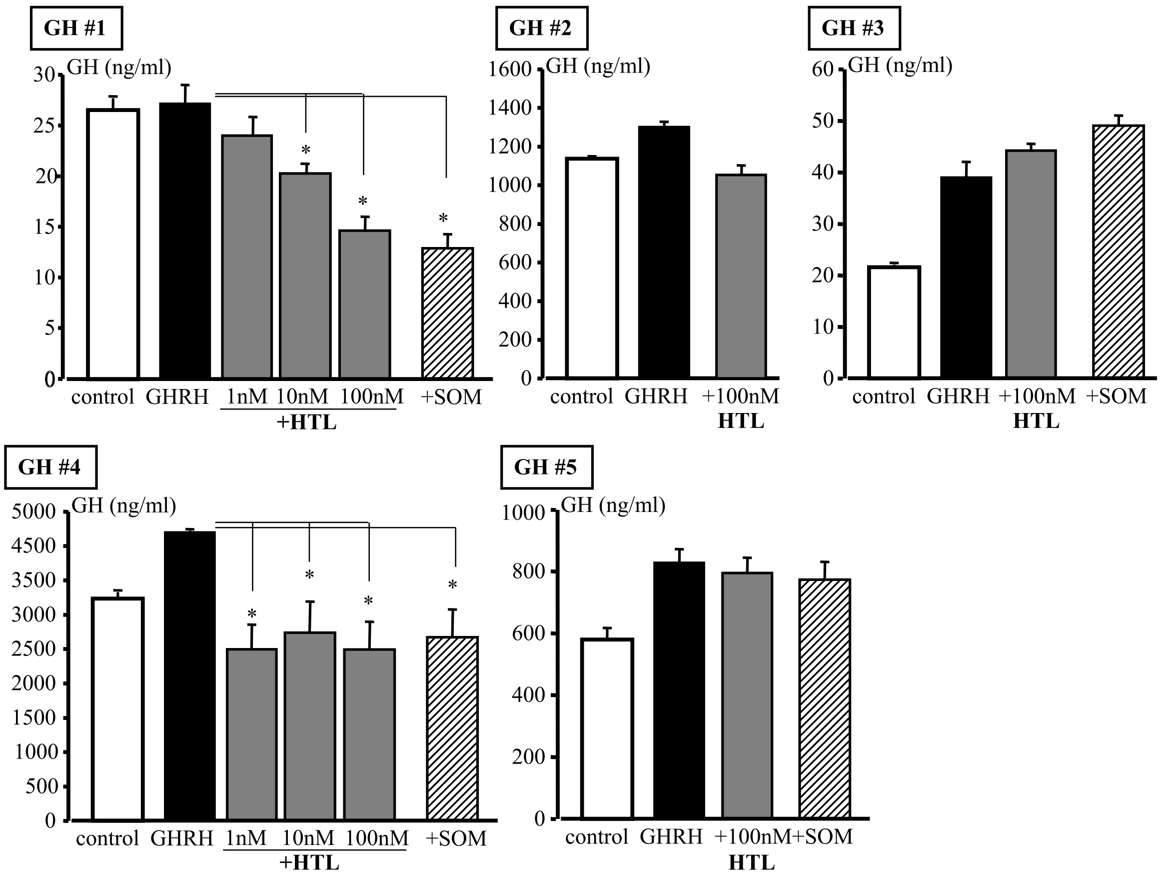

Figure 5. Effect of HTL0030310 on GHRH-stimulated GH secretion in GH-secreting adenomas. Concentrations at 24 h in each specimen are shown. White bar: control wells; black bar: wells incubated with 10 nM GHRH; gray bar: wells incubated with 10 nM GHRH and 1-100 nM HTL0030310 (HTL); striped bar: wells incubated with 10 nM GHRH and 10 nM pasireotide (SOM). Adenomatous specimens are identified by sign number, * indicates *P* < 0.05 vs control.

Our series included both GHRH-responsive and GHRH-unresponsive adenomas. During coincubation, HTL0030310 blunted the GH response to GHRH in one specimen (GH #4) and decreased GH secretion in specimen GH #1, albeit unresponsive to GHRH. Both adenomas proved sensitive to the inhibitory effect of both HTL0030310 and pasireotide (Figure 5). HTL0030310 did not exert an appreciable effect in the remaining samples. On average, no significant changes in the GH response to GHRH were observed during coincubation with HTL0030310 or pasireotide (Table 3). As an ancillary comment, both GH #1 and GH #4 were collected from patients treated with somatostatin receptor ligands prior to removal of the adenoma, although escape from control led to surgery.

Discussion

Somatostatin receptor ligands are the mainstay of medical therapy for neuroendocrine tumors and novel formulations are continuously being developed for patients unresponsive to currently available drugs.

HTL0030310, a potent SSTR5 receptor ligand with high selectivity over SSTR2 (>100-fold), was developed by computer-aided drug design together with optimized homology models of SSTR5 and SSTR2.[22] This is the first report on the effect of HTL0030310 on pituitary adenomas. We tested specimens from patients with acromegaly as well as with Cushing's disease as these patients are most likely to benefit from somatostatin receptor ligand treatment.

The effect of HTL0030310 proved of particular interest in specimens from patients with Cushing's disease. We observed a reduction in spontaneous and CRH-stimulated ACTH secretion as well as suppression of *POMC* expression. Not unexpectedly, sensitivity to HTL0030310, as well as to pasireotide, varied among the 3 specimens, in keeping with the known variability in tumor corticotrope phenotypes.[20,26,30] Of note, no study had previously reported on ACTH synthesis in human corticotroph adenomas incubated with somatostatin receptor ligands.

Somatostatin receptors involved in corticotrope regulation are mainly subtypes 2, 3, and 5[31,32] acting through adenylate cyclase and MAPK pathways, as shown in AtT-20 cells.[32] Human corticotrope adenomas express several somatostatin receptors with subtype 5 the most abundant isoform and other subtypes present to a variable extent.[16-18]

The path to elucidate the effect of somatostatin on ACTH secretion in Cushing's disease proved complex. Initial studies with somatostatin itself or its first-generation receptor ligand, that is, octreotide, failed to observe consistent inhibition of ACTH secretion.[33] Further studies revealed that corticosteroids themselves interfered with inhibition by somatostatin,[33] as dexamethasone reduced somatostatin subtype 2 expression.[34] Indeed, *SSTR2* expression was lower in adenomatous specimens from patients with Cushing's disease with high urinary free cortisol levels prior to surgery, compared to patients in whom presurgical normalization of cortisol had been achieved with adrenal-acting drugs.[35] Conversely, expression of *SSTR5* was unaffected by dexamethasone *in vitro*[34] and hypercortisolism *in vivo*,[35] paving the way to subtype 5–targeting ligands in Cushing's disease. Several studies, albeit in small series of corticotrope adenomas, revealed that pasireotide inhibits ACTH secretion in roughly half of tested specimens[16-18,35] and appeared mostly superior to octreotide both in terms of potency and efficacy.[36] These findings were confirmed in clinical trials, with octreotide proving by and large inefficacious[33,37] whereas pasireotide contained excess hormone secretion in some 30% of patients with Cushing's disease.[6] It follows that a considerable proportion of corticotrope adenomas is not sensitive to the multireceptor ligand.

In GH-secreting adenomas, incubation with HTL0030310 reduced GH secretion to a variable extent. Further, the inhibitory effect of HTL0030310 proved more evident on spontaneous rather than GHRH-stimulated GH secretion, but this may reflect the proportion of GHRH-unresponsive adenomas[38,39] in the present series. Interestingly, the inhibitory effect was observed at both 4 and 24 h incubation in 2 specimens and only at one time point in the remaining 2 specimens. Most studies on GH-secreting pituitary adenoma primary cultures report on findings at one time point only[40-42]; thus, our findings provide information also on the time-dependent response to the somatostatin receptor ligand. The study by Hofland et al.[43] on long-term primary cultures in GH-secreting adenomas is worth mentioning in this context as the inhibitory effect of octreotide appeared to change over time. Overall, although the average effect of HTL0030310 on GH secretion did not reach statistical significance, a clear-cut reduction was observed in individual specimens. These findings both uphold the importance of developing novel somatostatin receptor ligands and support the concept of precision medicine targeted to individual responsiveness.[5]

Somatostatin receptor ligands are the mainstay of treatment for patients with acromegaly,[3,5] although resistance to both first (i.e., octreotide and lanreotide) and second (i.e., pasireotide) generation somatostatin receptor ligands represents an enduring problem for long-term disease control. Prediction of the response to somatostatin receptor ligand therapy has been extensively investigated, and current knowledge points to somatostatin receptor expression in tumoral somatotropes as the main marker.[44-46] *In vitro*, somatotrope tumor sensitivity to octreotide and SSTR2-selective ligands is correlated with somatostatin receptor subtype 2 expression.[46,47] The association between sensitivity to pasireotide *in vitro* and *SSTR2* expression was less clear-cut, suggesting a role for other somatostatin receptors,[40] in keeping with its multireceptor profile. In terms of inhibition of GH secretion *in vitro*, octreotide and pasireotide were comparable,[16,40] although sensitivity to the two ligands varied considerably across specimens.[40] In the present series, 2 patients had been treated with lanreotide for several months and surgery performed upon escape from control. Expression of *SSTR2*, *SSTR3*, and *SSTR5* was detected in all our samples, with variable levels across adenomas; specimens from the 2 patients treated with ligands prior to surgery did not appear markedly different from the remaining samples, although, obviously, the small size does not allow clear comparisons to be drawn. Whether somatostatin receptor expression is affected by treatment or is intrinsic to tumor biology remains to be established.

In conclusion, our study, the first to evaluate the effect of a novel somatostatin receptor 5-preferring ligand, shows that HTL0030310 can restrain hormone synthesis and secretion in human pituitary adenomas. Further studies on modulation of somatostatin receptor gene and protein expression as well as comparisons with additional somatostatin receptor ligands, for example, octreotide, will shed additional light on the efficacy of this ligand and pave the way to its use in the clinic. Of note, these findings support the development of novel somatostatin receptor ligands to identify new, potentially efficacious drugs for Cushing's disease and acromegaly.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Conflict of interest: The following authors are employees for Sosei Heptares: C.P.M., E.S., D.H., A.G., K.A.B., A.J.H.B., and M.B. D.R., S.A., G.O., M.B., D.F., M.P.T., M.L., G.L., C.S., and F.P.G. have no conflicts of interest to declare.

Author contributions

Daniela Regazzo (Conceptualization [supporting], Data curation [supporting], Methodology [lead], Writing—review & editing [supporting]), Serena Avallone (Data curation [equal], Methodology [equal], Writing—review & editing [equal]), Cliona MacSweeney (Conceptualization [supporting], Formal analysis [supporting], Funding acquisition [supporting], Project administration [supporting], Writing—original draft [supporting], Writing—review & editing [supporting]), Eugenia Sergeev (Methodology [equal], Writing—review & editing [equal]), David Howe (Conceptualization [equal], Writing—review & editing [equal]), Alex Godwood (Conceptualization [equal], Writing—review & editing [equal]), Kirstie Bennett (Conceptualization [equal], Writing—review & editing [equal]), Alastair Brown (Conceptualization [equal], Writing—review & editing [equal]), Matt Barnes (Conceptualization [equal], Writing—review & editing [equal]), Gianluca Occhi (Data curation [equal], Methodology [equal], Writing—review & editing [equal]), Mattia Barbot (Data curation [equal], Writing—review & editing [equal]), Diego Faggian (Data curation [equal], Writing—review & editing [equal]), Maria Pia Tropeano (Data curation [equal], Writing—review & editing [equal]), Marco Losa (Data curation [equal], Formal analysis [equal], Writing—review & editing [equal]), Giovanni Lasio (Data curation [equal], Formal analysis [equal], Writing—review & editing [equal]), Carla Scaroni (Conceptualization [equal], Formal analysis [equal], Writing—review & editing [equal]), and Francesca Pecori Giraldi (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Funding acquisition [lead], Methodology [lead], Project administration [lead], Writing—original draft [lead], Writing—review & editing [lead]).

Data availability

Raw data are available at https://doi.org/10.13130/RD_UNIMI/DXUJXX.

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