CLINICAL STUDY

The activation of somatostatinergic receptors by either somatostatin-14 or cortistatin-17 often inhibits ACTH hypersecretion in patients with Cushing's disease

R Giordano, A Picu, L Bonelli, F Broglio, F Prodam, S Grottoli, G Muccioli¹, E Ghigo and E Arvat

Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Turin, 10126 Turin, Italy and ¹Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, 10125 Turin, Italy

(Correspondence should be addressed to E Arvat; Email: emanuela.arvat@unito.it)

Abstract

Object: Somatostatin (SS) is known to inhibit GH and insulin, while its effect on corticotrope secretion is controversial: inhibition of ACTH secretion by agonists activating somatostatinergic receptors (sst)-2 and sst-5 was reported *in vitro*. Cortistatin (CST) not only binds all sst receptor subtypes but also possesses central actions that are not shared by SS.

Design: In nine patients with Cushing's disease (CD), ACTH, cortisol, GH, insulin, and glucose levels were studied during 120-min i.v. infusion of SS-14 (2.0 µg/kg per h), CST-17 (2.0 µg/kg per h) or saline.

Results: Both SS or CST significantly affected the hypothalamic–pituitary–adrenal axis. Cortisol was decreased to the same extent by either SS or CST ($P < 0.05$). Both SS and CST decreased ACTH, although statistical difference was reached only during CST ($P < 0.05$). Analyzing the individual responses as Δ areas under curve (Δ AUCs), a clear and consensual inhibition of ACTH and cortisol under either SS or CST was recorded in five out of nine patients. Both SS or CST inhibited ($P < 0.05$) insulin, that even showed a rebound ($P < 0.01$) at the end of infusion. GH was not modified by either peptide.

Conclusion: SS and CST often display similar inhibitory effects on the HPA axis in CD. The activation of sst receptors by both peptides is followed in almost 50% of patients by a remarkable inhibition of ACTH and cortisol hypersecretion. These findings reinforce the view that sst receptors are involved in the control of the secretory activity of tumoral corticotropic cells.

European Journal of Endocrinology 157 393–398

Introduction

The role of somatostatin (SS) in the regulation of corticotrope secretion, in both physiological and pathological conditions, is still a matter of active debate.

It has been shown that SS and SS analogs do not affect adrenocorticotropin (ACTH) and cortisol secretion in normal subjects (1–3), whereas in Cushing's disease (CD) patients ACTH secretion is, at least partially, sensitive to the inhibitory effect of native SS and SS analogs (4). No significant inhibitory effect of octreotide, a preferential somatostatinergic receptor (sst)-2 agonist, has been generally demonstrated in patients with CD (4–8); this, however, would agree with hypercortisolism-induced sst-2 down-regulation (5). In fact, some inhibitory effect of octreotide on ACTH levels in Addison's disease and Nelson's syndrome has been reported (8–10). Indeed, sst-5 seems to play an important role in the regulation of basal and corticotrope releasing hormone (CRH)-induced ACTH release by primary cultures of human corticotrope

adenomas as well as in corticotrope tumor cell lines, but not in normal corticotropes (2, 11, 12). Moreover, recent studies showed that pasireotide (SOM-230), a multi-receptor ligand SS analog displaying high affinity for sst subtypes 1–3 and 5, is able to significantly inhibit ACTH secretion in corticotropinomas *in vitro* as well as *in vivo* in some patients with CD (13, 14).

Cortistatin (CST) is a neuropeptide that represents a natural SS analog showing high structural similarity and binding with high affinity (ranging from 0.4 to 7.0 nM) to all SS receptor subtypes (15–17). In agreement with this biochemical profile, it has been demonstrated that in humans as well as in animals, CST shares the same endocrine actions of SS, i.e., inhibiting growth hormone (GH) and insulin secretion in healthy young volunteers as well as in acromegalics and hyperprolactinemic patients (18–20).

Interestingly, CST also exerts some distinct biological activities that are not shared with SS, for example some central actions (21). This evidence suggested the existence of a specific CST receptor; indeed, differently

from SS, CST is able to bind to Mas-related gene X (MrgX)-2 receptor in various central and peripheral as well as in some neoplastic tissues (22, 23). Moreover, CST but not SS is also able to bind the GH secretagogues receptor (GHS-R) that mediates endocrine and non-endocrine actions of ghrelin, its natural ligand (24, 25). However, the physiological relevance, if any, of CST binding to these receptors is still unknown.

Based on the foregoing, in order to further investigate the role of SS receptor activation in patients with pituitary ACTH-dependent hypercortisolism, the effects of the infusion of either SS-14 or CST-17, the human CST form, on ACTH, cortisol, GH, insulin, and glucose levels were studied in a group of patients with CD.

Subjects and methods

Drugs

Vials containing CST-17 [Asp-Arg-Met-Pro-c(Cys-Arg-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Ser-Ser-Cys)-Lys-NH2] were purchased from Europeptides, Argenteuil, France; vials containing SS-14 [Ala-Gly-c(Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys)-OH] were purchased from Serono, Geneva, Switzerland.

Study protocol

Nine patients with CD (5 F and 4 M; 45.1 ± 4.3 years, BMI 29.8 ± 1.6 kg/m²) were studied; these were *de novo* patients who had not been studied before, and whose clinical details are reported in Table 1. The diagnosis of CD was made by international criteria, including elevated urinary free cortisol, normal or high plasma ACTH and serum cortisol levels, absence of suppression after low-dose dexamethasone test, and adequate suppression after high-dose dexamethasone test. Based on magnetic resonance imaging findings, CD patients were diagnosed as having a) pituitary ACTH-secreting microadenoma (cases 2, 3, 5, 6, and 7) or b) pituitary ACTH-secreting macroadenoma (cases 1, 4, 8, and 9).

No patient had evidence of chiasmatic syndrome and hypopituitarism; no patient had been previously treated by surgery, radiotherapy, or medical treatment. According to the international criteria for the diagnosis of diabetes mellitus (DM) (26), three CD patients were normal glucose tolerant (N), three showed impaired glucose tolerance (IGT), and three had diabetes (DM). None of the patients underwent medical treatment influencing hormonal secretion at the time of testing. In all patients, the diagnosis of ACTH-secreting pituitary adenoma was confirmed by immunostaining after transphenoidal surgery.

All patients gave their informed consent to participate in the study that had been approved by the Ethical Committee of the University of Turin in agreement with the Declaration of Helsinki.

All the patients underwent the following testing sessions in random order (using the second randomization plan generator available on www.randomization.com) at least 3 days apart:

a) Saline (250 ml infused over 120 min); b) SS-14 (2.0 µg/kg infused over 120 min) and c) CST-17 (2.0 µg/kg infused over 120 min). These were the same doses already administered in our previous studies in both normal (19) and acromegalic (20) subjects, showing similar hormonal and metabolic effects.

After an overnight fast, testing sessions began in the morning at 0830–0900 h, 30 min after an indwelling catheter had been placed into an antecubital vein of the forearm and kept clear by slow infusion of isotonic saline. Blood samples were taken every 15 min from –15 up to +150 min after starting infusion in all sessions; all samples from an individual subject were analyzed together and during the same assay section.

ACTH, cortisol, GH, insulin, and glucose levels were assayed at each time point in every testing session.

Plasma ACTH levels (pg/ml; 1 pg/ml × 0.22 = 1 pmol/l) were measured in duplicate by IRMA (Allegro HS-ACTH: Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The sensitivity of the assay was

Table 1 Clinical and hormonal details of the patients with Cushing’s disease.

Case (n)	Age (years)	Sex	BMI (kg/m ²)	ACTH (pg/ml)	Cortisol (µg/dl)	UFC (µg/24 h)	Glycemic status	Imaging
1	47	M	25	64.3	13.0	196	N	Macroadenoma
2	27	F	22	58.8	18.9	262	N	Microadenoma
3	29	F	35	27.6	14.8	183	DM	Microadenoma
4	45	F	32	83.0	22.5	180	IGT	Macroadenoma
5	44	F	32	34.9	21.6	161	N	Microadenoma
6	41	F	35	49.8	16.9	521	IGT	Microadenoma
7	65	M	34	17.0	12.9	449	DM	Microadenoma
8	63	M	25	299.3	25.7	294	DM	Macroadenoma
9	45	M	28	68.5	29.0	175	IGT	Macroadenoma
Mean	45.1		29.8	78.1	19.5	269		
S.E.M.	4.3		1.6	28.5	1.8	43.7		

ACTH, plasma concentrations at 0900 h (mean of two measurements; 1 pg/ml × 0.22 = 1 pmol/l; normal range 8–53 pg/ml); Cortisol, serum concentrations at 0900 h (mean of two measurements; 1 µg/dl × 27.59 = 1 nmol/l; normal range 8.8–26.0 µg/dl); UFC, urinary free cortisol (mean of two measurements; 1 µg/24 h × 2.76 = 1 nmol/d; normal range 30–150 µg/24 h); N, normal; IGT, impaired glucose tolerance; DM, diabetes mellitus.

1 pg/ml. The inter- and intra-assay coefficients of variation ranged from 6.9 to 8.9% and from 1.1 to 3.0% respectively.

Serum cortisol levels ($\mu\text{g/dl}$; $1 \mu\text{g/dl} \times 27.59 = 1 \text{ nmol/l}$) were measured in duplicate by RIA (CORT-CTK 125 RIA; Sorin Biomedica, Saluggia, Italy). The sensitivity of the assay was $0.04 \mu\text{g/dl}$. The inter- and intra-assay coefficients of variation ranged from 6.6 to 7.5% and from 3.8 to 6.6% respectively.

Serum GH levels ($\mu\text{g/l}$) were measured in duplicate by IRMA (hGH-CTK IRMA, Sorin Biomedica). The sensitivity of the assay was $0.15 \mu\text{g/l}$. The inter- and intra-assay coefficients of variation ranged from 2.9 to 4.5% and from 2.4 to 4.0% respectively.

Serum insulin levels (mU/l ; $1 \text{ mU/l} \times 7.17 = 1 \text{ pmol/l}$) were measured in duplicate by IRMA (INSIK-5, Sorin Biomedica). The sensitivity of the assay was 2.5 mU/l . The inter- and intra-assay coefficients of variation ranged from 6.2 to 10.8% and from 5.5 to 10.6% respectively.

Plasma glucose levels (mg/dl ; $1 \text{ mg/dl} \times 0.05 = 1 \text{ mmol/l}$) were measured by glucose-oxidase colorimetric method (GLUCOFIX, Menarini Diagnostici, Florence, Italy).

Urinary free cortisol (UFC) ($\mu\text{g/24 h}$; $1 \mu\text{g/24 h} \times 2.76 = 1 \text{ nmol/d}$) was measured in duplicate by RIA (Cortisol Bridge; Biochem Immunosystems Company, Milan, Italy). The sensitivity of the assay was $2.67 \mu\text{g/24 h}$. The inter- and intra-assay coefficients of variation ranged from 3.98 to 5.75% and from 2.64 to 8.67% respectively.

The hormonal responses are expressed as mean $\pm$ S.E.M. of absolute values, absolute, or delta ΔAUCs calculated by trapezoidal integration.

Statistical analysis

The statistical analysis was carried out using a non-parametric two-way ANOVA for repeated measures with time as a within-subject factor and treatment as a between-subject factor to analyze changes with time, and then Wilcoxon matched pairs test or Mann-Whitney *U* test as appropriate. SPSS (Statistical Package for the Social Science), version 13.0 was used for the analysis (27).

Results

Cortisol secretion was decreased to the same extent ($P < 0.05$) by either SS or CST infusion (ΔAUC : SS and CST versus saline: -403.4 ± 108.6 and -429.8 ± 182.1 vs $173.9 \pm 71.5 \mu\text{g/dl per h}$ respectively). Both SS and CST also decreased ACTH secretion (-447.8 ± 854.4 and -720.5 ± 340.0 vs $519.3 \pm 522.4 \text{ pg/ml per h}$), although statistical significance ($P < 0.05$) was reached during CST infusion only (Fig. 1).

Analyzing the individual responses in terms of ΔAUC , clear-cut and consensual inhibition of both ACTH and

cortisol under either SS or CST was recorded in five out of nine patients (cases 1–5). Corticotrope refractoriness to either SS or CST was recorded in two out of nine patients (cases 8 and 9), both with a pituitary macroadenoma; one case showed some inhibition under SS but not under CST (case 6), while another showed some inhibition under CST but not under SS (case 7). When the individual responses were evaluated in terms of absolute AUC, variable but consensual inhibition of both ACTH and cortisol levels under either SS or CST was recorded in four out of nine patients (cases 2, 3, 4, and 5); refractoriness to either SS or CST was recorded in three out of nine patients (cases 1, 8, and 9); discordant responses to SS or CST persisted in the same two cases (Table 2).

Insulin secretion was inhibited ($P < 0.05$) by SS and CST (-1155.6 ± 197.3 and -962.2 ± 249.9 vs $-7.2 \pm 77.3 \text{ mU/l per h}$ saline); insulin levels showed a significant rebound ($P < 0.01$) at the end of the administration of the two peptides (Fig. 2).

Glucose levels were unchanged under administration of both SS and CST (-913.9 ± 292.4 and -401.8 ± 261.9 vs $-607.5 \pm 241.5 \text{ mg/dl per h}$; data not shown).

Lower basal GH secretion was not significantly modified by SS and CST (-7.8 ± 7.9 and -3.7 ± 5.9 vs $-3.4 \pm 2.0 \text{ mg/dl per h}$; data not shown).

Side effects

No significant side effect was recorded in any subject. No medication was required and none had to stop any testing session.

Discussion

The results of the present study demonstrate that SS and CST often display similar inhibitory effects on the HPA axis in patients with CD: in fact, in almost 50% of patients a clear reduction of ACTH and cortisol levels was observed.

Data regarding the effects of SS receptors activation in patients with CD are controversial. No change in ACTH secretion has been observed in some studies where either native SS or octreotide, a preferential sst-2 agonist, was administered acutely (4–6). Instead, octreotide was able to exert some inhibitory effect on ACTH levels in other ACTH hypersecretory states, such as in Addison's disease and Nelson's syndrome (8–10). The lack of any effect of octreotide in CD would have an explanation. In fact, glucocorticoids exert negative modulation of sst-2 expression in corticotrophic adenoma cells (5). Moreover, glucocorticoid receptor antagonists induce sensitivity to the inhibitory effect of SS on ACTH secretion from normal rat corticotrophic cells (28). Thus, it is possible that the lack of ACTH inhibition under exposure to octreotide in CD simply

Figure 1 Mean ($\pm$ S.E.M.) Adrenocorticotropin (ACTH) (pg/ml; $1 \text{ pg/ml} \times 0.22 = 1 \text{ pmol/l}$) and cortisol ($\mu\text{g/dl}$; $1 \text{ }\mu\text{g/dl} \times 27.59 = 1 \text{ nmol/l}$) levels and ΔAUCs in Cushing's disease patients under infusion of somatostatin (SS), cortistatin (CST), or saline. * $P < 0.05$ versus saline.

reflects sst-2 down-regulation induced by hypercortisolism. Differently from sst-2, sst-5 have been found refractory to the negative influence of glucocorticoid; moreover, this receptor subtype has been supposed to play a more important role in the regulation of basal and stimulated ACTH release, at least in corticotrope tumors (12). In agreement with this assumption, another synthetic SS analog displaying high affinity for sst subtypes 1–3 and 5, pasireotide (SOM-230), has been found able to inhibit ACTH release from human corticotropinoma cells *in vitro* (13) and even *in vivo* in some patients with CD (14).

By studying the effects of native SS and CST, another natural ligand of all sst subtypes (16, 17), on the HPA axis we intended to further clarify whether play any significant role on ACTH and cortisol secretion in pituitary ACTH-dependent Cushing's syndrome. The exposure of sst to either SS or CST also offered the possibility to test the reproducibility of the corticotropic response, if any, to these peptides. In fact, in patients with acromegaly as well as in normal subjects, the exposure to CST-17 and native SS led to reproducible inhibition of GH and insulin secretion and sometimes even of prolactin secretion (18–20).

Our present findings indicate that either SS or CST infusion is followed by significant decrease of both mean ACTH and cortisol secretion in patients with CD, suggesting the activation of sst by both peptides. Unfortunately, we did not perform an *in vitro* study aimed at evaluating direct effects of both SS and CST on pituitary extracts of our patients, which, indeed, would throw light on our suggestion.

Actually, mean hormonal response reflected clear inhibition exerted by both peptides in five out of nine patients, some inhibition exerted by SS or CST in two cases, and no effect of any peptide in the remaining two cases, at least when ΔAUC were considered.

Analyzing the individual responses, SS and CST generally displayed the same concordant influence on ACTH and cortisol secretion. Thus, once again, it seems clear that SS and CST share the same endocrine actions, reflecting the activation of sst that are bound with almost equal affinity by both peptides (16, 17). This also implies that the inhibitory effect of sst activation on corticotropic function in patients with CD is reproducible. On the other hand, as CST has also been demonstrated to bind other specific receptors, e.g. MrgX2 and GH secretagogues-receptor type 1a (GHS-

Table 2 Adrenocorticotropin (ACTH) and cortisol area under curves (AUCs) and Δ AUCs in patients with Cushing's disease (CD) under infusion of somatostatin (SS), cortistatin (CST), or saline.

	Case (n)	SS		CST		Saline	
		AUCs	Δ AUCs	AUCs	Δ AUCs	AUCs	Δ AUCs
ACTH (pg/ml per h)	1	10 075.5	-172.5	11 423.3	95.2	7755.8	4203.8
	2	3546.0	-1014.0	2645.3	665.3	4191.8	1407.8
	3	2946.8	-1973.3	2837.3	-1002.8	4386.0	138.0
	4	4635.8	-2672.3	4188.0	-2412.0	6759.8	-1064.3
	5	3469.5	-3202.5	2979.0	-1857.0	4734.8	258.8
	6	3084.0	-1884.0	4140.8	264.8	6210.0	270.0
	7	2080.5	604.5	1482.8	-149.3	2664.8	636.8
	8	47 366.3	5186.3	45 408.8	-1043.3	35 467.5	-712.5
	9	13 098.0	1098.0	10 394.5	-645.5	11 415.0	-465.0
Cortisol (μ g/dl per h)	1	1619.9	-502.8	1663.9	34.3	1442.4	196.8
	2	776.0	-245.1	1455.6	-1324.8	1863.1	484.3
	3	689.2	-532.3	661.5	-242.0	1832.0	45.2
	4	1005.0	-755.3	1385.2	-765.1	1645.2	61.2
	5	1266.0	-398.4	1878.9	-1199.0	1926.7	33.1
	6	1146.6	-825.0	1475.3	-129.0	1709.3	-115.8
	7	1572.7	-542.8	1096.8	-417.6	1923.0	531.0
	8	3688.3	88.3	3460.8	32.4	3497.1	239.1
	9	2482.5	82.5	2242.5	142.5	2190.7	90.7

ACTH (pg/ml; 1 pg/ml $\times$ 0.22 = 1 pmol/l); cortisol (μ g/dl; 1 μ g/dl $\times$ 27.59 = 1 nmol/l).

R1a) (22–24), whether the activation of sst receptors by the peptide overrides any peculiar endocrine actions possibly mediated by the activation of these receptors remains, at present, unknown.

In terms of extent of inhibition, neither SS nor CST was able to normalize ACTH and cortisol hypersecretion in any patient; this could, however, simply reflect the short-term exposure to SS receptor agonists. In fact, preliminary data from Arnaldi *et al.* reported normalization of the HPA axis in some patients with CD under chronic treatment with pasireotide (14). Therefore, the possibility that treatment with SS analogs that also activate sst-5 and display more prolonged action (such as the new universal ligand pasireotide) could be effective for treatment of CD, at least in some cases, is open.

In our study, patients with CD exposed to SS or CST did not show any inhibition of GH secretion, but this would simply reflect the low somatotrophic secretory function that connotes patients with hypercortisolism (29); on the other hand, GH measurement by an ultra-sensitive assay would likely be needed to record a significant inhibitory effect (30).

As expected, SS and CST displayed clear inhibitory effect on insulin secretion, while no significant change in glucose levels was recorded.

In conclusion, this study shows that SS and CST often display similar inhibitory effects on the HPA axis in patients with CD. In fact, in almost 50% of patients, the activation of SS receptors by both peptides is followed by a remarkable inhibition of ACTH and, in turn, cortisol hypersecretion. These findings reinforce the view that SS receptors are involved in the control of the secretory activity of tumoral corticotrophic cells.

Figure 2 Mean ($\pm$ S.E.M.) insulin levels (mU/l; 1 mU/l $\times$ 7.17 = 1 pmol/l) and Δ AUCs in Cushing's disease patients under infusion of somatostatin (SS), cortistatin (CST), or saline. * P < 0.05 versus saline.

Acknowledgements

The present study was supported by MURST (Grant 2004-062974), University of Turin and SMEM

Foundation. The authors wish to thank Dr A Bertagna and Mrs A Barberis for their skillful technical assistance.

References

- Brown MR, Rivier C & Vale W. Central nervous system regulation of adrenocorticotropin secretion: role of somatostatins. *Endocrinology* 1984 **114** 1546–1549.
- Stafford PJ, Kopelman PG, Davidson K, McLoughlin L, White A, Ress LH, Besser GM, Coy DH & Grossman A. The pituitary–adrenal response to CRF-41 is unaltered by intravenous somatostatin in normal subjects. *Clinical Endocrinology* 1989 **30** 661–666.
- Volpi R, Chiodera P, Capretti L, Caiazza A, Caffari G, Magotti MG, Boni S & Coiro V. Inhibition by somatostatin of the growth hormone, but not corticotropin response to angiotensin II in normal men. *Hormone Research* 1996 **45** 269–272.
- Lamberts SWJ, Krenning EP & Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocrine Reviews* 1991 **12** 450–481.
- Stalla GK, Brockmeier SJ, Renner U, Newton C, Buchfelder M, Stalla J & Muller OA. Octreotide exerts different effects *in vivo* and *in vitro* in Cushing's disease. *European Journal of Endocrinology* 1994 **130** 125–131.
- Ambrosi B, Bochicchio D, Fadin C, Colombo P & Faglia G. Failure of somatostatin and octreotide to acutely affect the hypothalamic–pituitary–adrenal function in patients with corticotropin hypersecretion. *Journal of Endocrinological Investigation* 1990 **13** 257–261.
- Lamberts SW, van der Lely AJ & Hofland LJ. New somatostatin analogs: will they fulfil old promises? *European Journal of Endocrinology* 2002 **146** 701–705.
- Lamberts SW, Uitterlinden P & Klijn JM. The effect of long-acting somatostatin analogue SMS 201-995 on ACTH secretion in Nelson's syndrome and Cushing's disease. *Acta Endocrinologica* 1989 **120** 760–766.
- Fehm HL, Voigt KH, Lang R, Beinert KE, Raptis S & Pfeiffer EF. Somatostatin: a potent inhibitor of ACTH-hypersecretion in adrenal insufficiency. *Klinische Wochenschrift* 1976 **54** 173–175.
- Tyrrell JB, Lorenzi M, Gerich JE & Forsham PH. Inhibition by somatostatin of ACTH secretion in Nelson's syndrome. *Journal of Clinical Endocrinology and Metabolism* 1975 **40** 1125–1127.
- Spada A, Reza-Elahi F, Lania A, Bassetti M & Atti E. Inhibition of basal and corticotropin-releasing hormone-stimulated adenylate cyclase activity and cytosolic Ca^{2+} levels by somatostatin in human corticotropin-secreting pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 1262–1268.
- van der Hoek J, Waaijers M, van Koetsveld PM, Sprij-Mooij D, Feelders RA, Schmid HA, Schoeffter P, Hoyer D, Cervia D, Taylor JE, Culler MD, Lamberts SW & Hofland LJ. Distinct functional properties of native somatostatin receptor subtype 5 compared with subtype 2 in the regulation of ACTH release by corticotroph tumor cells. *American Journal of Physiology. Endocrinology and Metabolism* 2005 **289** E278–E287.
- Hofland LJ, van der Hoek J, Feelders R, van Aken MO, van Koetsveld PM, Waaijers M, Sprij-Mooij D, Bruns C, Weckbecker G, de Herder WW, Beckers A & Lamberts SW. The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5. *European Journal of Endocrinology* 2005 **152** 645–654.
- Arnaldi G, Polenta M, Cardinaletti M & Boscaro M. Potential indications for somatostatin analogs in Cushing's syndrome. *Journal of Endocrinological Investigation* 2005 **28** 106–110.
- de Lecea L, Criado JR, Prospero-Garcia O, Gautvik KM, Schweitzer P, Danielson PE, Dunlop CL, Siggins GR, Henriksen SJ & Sutcliffe JG. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. *Nature* 1996 **381** 242–245.
- de Lecea L. Cortistatin: a natural somatostatin analog. *Journal of Endocrinological Investigation* 2005 **28** (Supp) 10–14.
- Fukusumi S, Kitada C, Takekawa S, Kizawa H, Sakamoto J, Miyamoto M, Hinuma S, Kitano K & Fujino M. Identification and characterization of a novel human cortistatin-like peptide. *Biochemical and Biophysical Research Communications* 1997 **232** 157–163.
- Broglio F, Arvat E, Benso A, Gottero C, Prodham F, Grottoli S, Papotti M, Muccioli G, van der Lely AJ, Deghenghi R & Ghigo E. Endocrine activities of cortistatin-14 and its interaction with GHRH and ghrelin in humans. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3783–3790.
- Gottero C, Prodham F, Destefanis S, Benso A, Gauna C, Me E, Filtri L, Riganti F, Van Der Lely AJ, Ghigo E & Broglio F. Cortistatin-17 and -14 exert the same endocrine activities as somatostatin in humans. *Growth Hormone and IGF Research* 2004 **14** 382–387.
- Grottoli S, Gasco V, Broglio F, Baldelli R, Ragazzoni F, Gallenca F, Mainolfi A, Prodham F, Muccioli G & Ghigo E. Cortistatin-17 and somatostatin-14 display the same effects on GH, PRL and insulin secretion in patients with acromegaly or prolactinoma. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1595–1599.
- Spier AD & de Lecea L. Cortistatin: a member of the somatostatin neuropeptide family with distinct physiological functions. *Brain Research Reviews* 2000 **33** 228–241.
- Robas N, Mead E & Fidock M. MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *Journal of Biological Chemistry* 2003 **278** 44400–44404.
- Allia E, Taraba E, Volante M, Cerrato M, Ghigo E, Muccioli G & Papotti M. Expression of cortistatin and MrgX2, a specific cortistatin receptor, in human neuro-endocrine tissues and related tumours. *Journal of Pathology* 2005 **207** 336–345.
- Deghenghi R, Papotti M, Ghigo E & Muccioli G. Cortistatin, but not somatostatin, binds to growth hormone secretagogue (GHS) receptors of human pituitary gland. *Journal of Endocrinological Investigation* 2001 **24** RC1–RC3.
- van der Lely AJ, Tschop M, Heiman ML & Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews* 2004 **25** 426–457.
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2004 **27** S5–S10.
- Nelder JA. A user's guide to the evaluation of statistical packages and systems. *International Statistical Review* 1974 **42** 291–298.
- Lamberts SW, Zuyderwijk J, Den Holder F, Van Koetsveld P & Hofland L. Studies on the conditions determining the inhibitory effect of somatostatin on adrenocorticotropin, prolactin and thyrotropin release by cultured rat pituitary cells. *Neuroendocrinology* 1989 **50** 44–50.
- Magiakou MA, Mastorakos G, Gomez MT, Rose SR & Chrousos GP. Suppressed spontaneous and stimulated growth hormone secretion in patients with Cushing's disease before and after surgical cure. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 131–137.
- Chapman IM, Hartman ML, Straume M, Johnson ML, Veldhuis JD & Thorner MO. Enhanced sensitivity growth hormone (GH) chemiluminescence assay reveals lower postglucose nadir GH concentrations in men than women. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 1312–1319.

Received 9 March 2007

Accepted 2 July 2007