Cortistatin mimics somatostatin by inducing a dual, dose-dependent stimulatory and inhibitory effect on growth hormone secretion in somatotropes

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Abstract

Cortistatin is a recently discovered neuropeptide that is structurally related to somatostatin, the classic inhibitor of growth hormone (GH) release. Cortistatin binds with high affinity to all five somatostatin receptors (sst1–5), and, like somatostatin, cortistatin inhibits in vivo GH release in man and rats. In this report, we compared the in vitro actions of cortistatin and somatostatin using primary pig pituitary cell cultures. In this species, we have previously reported that somatostatin not only inhibits GH-releasing hormone (GHRH)-stimulated GH release at high doses, but also stimulates basal GH release at low (pM) doses, a dual response that is markedly dependent on the subpopulation of pituitary somatotropes examined. Results reported herein demonstrate that cortistatin closely mimics the dose-dependent inhibitory and stimulatory effects of somatostatin on GH secretion. As cortistatin, unlike somatostatin, binds to the human receptor for ghrelin/GH secretagogs (GHS-R), we also investigated whether cortistatin stimulates GH release through this receptor by using a synthetic, short form of cortistatin, cortistatin-8 (CST8), which lacks the sst-binding capacity of full-length cortistatin but retains its GHS-R-binding capacity. Interestingly, CST8 stimulated GH release only at low doses (10−15 M), and did not reduce GH secretion stimulated by GHRH, ghrelin, or low-dose, full-length cortistatin, yet it counteracted that induced by a nonpeptidyl GHS, L-163 255. Taken together, our results indicate that the dual, inhibitory and stimulatory effects of cortistatin on GH release closely parallel those of somatostatin and are probably mediated by the same receptor(s) and signaling pathway(s) for both peptides. Furthermore, they suggest that the pathway(s) activated by cortistatin (and somatostatin) to stimulate GH release are not initiated by GHS-R activation.

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Introduction

Cortistatin is a peptide originally cloned from neuronal tissues of man, rats and mice (de Lecea et al. 1996, 1997a, Fukusumi et al. 1997, Spier & de Lecea 2000). Cortistatin is expressed as a pre-pro-peptide in various brain regions, including cortex, hippocampus and hypothalamus (de Lecea et al. 1996, 1997b, Spier & de Lecea 2000) and in multiple peripheral tissues (de Lecea et al. 1997a, Spier & de Lecea 2000, Dalm et al. 2004), and it has been recently shown to be present in various human neuroendocrine tissues and related tumors, including the pituitary (Allia et al. 2005). Pre-pro-cortistatin is structurally homologous to pre-pro-somatostatin, with high similarity in the carboxyl terminus from which both peptides are processed (Fukusumi et al. 1997, Spier & de Lecea 2000); however, each peptide is encoded by a distinct gene (de Lecea et al. 1996, Fukusumi et al. 1997, Spier & de Lecea 2000). Human pre-pro-cortistatin may be cleaved to pro-CST, from which two mature products, CST-17 and CST-29, can be generated in man (Fukusumi et al. 1997, de Lecea et al. 1997a, Spier & de Lecea 2000). CST-17 shares 10 of the 14 amino-acid residues with somatostatin 14.

Owing to their high structural similarity, cortistatin binds all somatostatin receptors (sst1–5) with an affinity close to that of somatostatin and therefore was expected to have similar biologic activities (Fukusumi et al. 1997, Siehler et al. 1998, Spier & de Lecea 2000). However, recent studies have shown that not all of the actions of cortistatin are shared with those of somatostatin, suggesting the existence of receptors for cortistatin distinct from sst (de Lecea et al. 1996, Siehler et al. 1998, Sánchez-Alavez et al. 2000, Spier & de Lecea 2000, de Lecea 2005, Spier et al. 2005). This hypothesis has been also supported by the finding that an orphan
G-protein-coupled receptor, MrgX2, can selectively bind cortistatin, but not somatostatin (Robas et al. 2003). However, MrgX2 was found in a recent study to bind with higher affinity to proadrenomedullin N-terminal peptide 12 (PAMP12) than to cortistatin; thus, this receptor was suggested to act physiologically as a PAMP receptor in the adrenal gland rather than as a cortistatin receptor (Kamohara et al. 2005). It has also been demonstrated that in human tissues cortistatin, but not somatostatin, binds to the ghrelin/GH secretagog receptor (GHS-R), suggesting a possible functional interaction between cortistatin and the ghrelin/GHS-R system (Spier & de Lecea 2000, Deghenghi et al. 2001b, Muccioli et al. 2001).

An inhibitory (dose-related) action of cortistatin on basal GH secretion similar to that of somatostatin has been demonstrated in man and rats in vivo (Deghenghi et al. 2001a, Brogio et al. 2002a,b, Gottero et al. 2004). Moreover, cortistatin, like somatostatin, drastically attenuated the GH response to GHRH in healthy normal individuals (Brogio et al. 2002a,b, Gottero et al. 2004). In the pig, as in other mammalian species, somatostatin inhibits in vitro GH-releasing hormone (GHRH)-stimulated GH release at high doses (nM). However, low (pM) doses of somatostatin stimulate pig GH release from cultured somatotropes (Castaño et al. 1996, Ramírez et al. 1997a,b, Ramírez et al. 2002). The stimulatory action of somatostatin on GH release is mediated by stimulation of cAMP and nitric oxide (NO) production (Ramírez et al. 2002, Luque et al. 2005).

Given the unique action of somatostatin on GH release in pig pituitary cell cultures, we compared the effects of cortistatin with those of somatostatin on basal GH secretion. In light of the ability of cortistatin, but not somatostatin, to bind the GHS-R (Spier & de Lecea 2000, Deghenghi et al. 2001b, Muccioli et al. 2001), we also sought to determine whether cortistatin interacts with the regulation of GH release by ghrelin/GHS-R. To this end, we used a short form of cortistatin, cortistatin-8 (CST8), which lacks the sst-binding capacity of full-length cortistatin, but retains its GHS-R binding capacity, and may antagonize certain ghrelin actions (Sibilia et al. 2006).

Materials and methods

Reagents

Pig GH (pGH; USDA-B- 1, AFP-11716C) was kindly supplied by Dr A F Parlow, from the Pituitary Hormones and Antisera Center, Harbor-University of California-Los Angeles Medical Center. D-Val-modified minimum essential medium (MEM), Heps, collagenase type V, trypsin type I, soybean trypsin inhibitor I, DNaSe I, gentamicin, antibiotic–antimycotic solution, BSA and all other reagents were purchased from Sigma, unless otherwise specified. Fetal bovine serum (FBS) was obtained from Sera-Lab (Crawley Down, UK). Human cortistatin was used, as pig cortistatin has not yet been cloned, and was purchased from Peninsula (St Helens, UK). Somatostatin (1–14) was from Biogenesis (Poole, UK), GHRH (1–29) and anti-pGH were from UCB Bioproducts (Brain L’Alleud, Belgium) and human ghrelin was from Bachem (Merseyside, UK). Synthetic cortistatin-8 (CST8) was kindly provided by Dr Ezio Ghigo of the University of Turin (Turin, Italy) and the non-peptidyl GHS, L-163 255, by Merck. Tissue culture plastic ware was from Costar (Cambridge, MA, USA). Stock solutions of cortistatin, CST8, somatostatin and ghrelin were prepared with distilled deionized water, whereas GHRH was dissolved in ethanol. Aliquots of concentrated stock solutions were stored at −20 °C until use, and then they were diluted to final concentrations in MEM.

Animals and tissues

Pituitary glands from female Large-White/Landrace pigs (4–6 months old) were obtained from a local abattoir. According to European Regulations for Animal Care, after electrical stunning, animals were killed by exsanguination and immediately decapitated. Pituitaries were immediately excised and stored in sterile cold (4 °C) MEM supplemented with 0.3% BSA, 0.58% Heps, 0.22% NaHCO3 and 1% antibiotic–antimycotic solution (100 UI/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B). In the laboratory, pituitaries were washed twice with fresh medium, and the posterior lobes were discarded.

Pituitary cell dispersion and separation of subpopulations

Anterior pituitaries were dispersed into single cells by the enzymatic and mechanical method described previously (Torronteras et al. 1993, Castaño et al. 1996, Ramírez et al. 2002). For each experiment, three to four anterior pituitary glands were pooled and dispersed together, and the cell suspension obtained (initial cell suspension (ICS)) was filtered through a nylon gauze (100 μm mesh). The cell number was assessed in a hemocytometer, and cell viability, as determined by the Trypan blue exclusion test, was consistently over 85%. Initial cell suspension (ICS, 30–40 × 106 cells) was centrifuged (3000 g, 25 min) in a hyperbolic, continuous Percoll density gradient (Amersham, Buckinghamshire, UK) at 25–80% (1033–1121 g/cm³) to separate the two subpopulations of pig somatotropes of low (LD; 1051–1064 g/cm³) and high (HD; 1076–1098 g/cm³) density, which have been characterized previously in our laboratory (Torronteras et al. 1993, Castaño et al. 1996, Ramírez et al. 2002).
Cell culture and experimental design

Monodispersed cells were plated onto 24-well culture plates at a density of $3 \times 10^5$ cells/well of ICS, LD and HD cells in 1 ml culture medium supplemented with 10% FBS and 0.1% gentamycin sulfate (50 μg/ml). After a 3-day culture period at 37°C in a humidified atmosphere (95% air: 5% CO₂), the medium was removed and cells were preincubated in 1 ml serum-free MEM for 4 h to stabilize basal GH secretion. Then, cells were incubated for 30 min with the test substance or the corresponding control vehicle. Medium was then collected, centrifuged (2000 rpm for 5 min) and stored at −20°C until GH determination.

Enzyme immunoassay

pGH concentration in the culture media was measured by a homologous enzyme immunoassay (EIA) procedure, as described in detail previously (Castaño et al. 1996), using a Rosys Anthos 2010 plate reader. Hormone employed in the EIA for both plate coating and standard was pGH, and the primary antiserum was a specific anti-pGH (developed in rabbit) at a dilution of 1:200 000. Sensitivity of the EIA was 0·65 ng of pGH/well. A minimum of four replicate wells was employed to test the effect of each agent.

RNA extraction and multiplex RT–PCR of pig pituitary receptors

Given the distinct ability of cortistatin, but not somatostatin to bind to the human GHS-R (Deghenghi et al. 2001b, Muccioli et al. 2001), we sought to determine whether the differential GH response to these peptides could be associated with a differential expression of GHS-R as well as GHRH receptor (GHRH-R). To this end, total pituitary RNA was extracted from ICS-, LD- and HD-cultured cells and reverse transcribed, and receptor mRNA levels were determined by semiquantitative multiplex PCR, as previously developed and validated in our laboratory (Luque et al. 2004a,b). Hypoxanthine phosphoribosyl-transferase (HPRT) was used as an endogenous control.

Statistical analyses

A minimum of three or four replicate wells per treatment was tested in each experiment. Samples from each experiment were analyzed in the same assay and expressed as a percentage of the corresponding control value. Results are presented as mean ± S.E.M. of at least three experiments performed on different pituitary cell preparations. Statistical analysis was carried out by one-way ANOVA, followed by a statistical test for multiple comparisons (Duncan’s multiple-range test and critical ranges) by use of the software package Statistica (StatSoft, Tulsa, OK, USA). Differences were considered significant at $P<0·05$.

Results

Effect of cortistatin and somatostatin on pGH release

Incubation of cultures of porcine pituitary cells (ICS) with increasing doses of somatostatin (SRIF) confirmed and extended our previous observations by showing that a broad range of low SRIF doses ($10^{-18}$ to $10^{-10}$ M) stimulate GH release, whereas high, micromolar doses of SRIF ($10^{-8}$ and $10^{-6}$ M) do not affect basal GH secretion (Fig. 1A).

To analyze the dose-dependent effect of cortistatin, porcine pituitary cells (ICS) were incubated with a wide range of concentrations of this peptide ($10^{-20}$-10⁻⁶ M). As illustrated in Fig. 1B, cortistatin stimulated GH secretion in a dose-dependent manner. In particular, $10^{-18}$, $10^{-16}$ and $10^{-14}$ M doses of cortistatin significantly increased GH secretion compared with vehicle-treated control.

Interaction of cortistatin with hypothalamic peptides, GHRH and SRIF

As shown in Fig. 2A, a high dose of cortistatin ($10^{-7}$ M) completely abolished GHRH-induced GH release. Conversely, a low dose of cortistatin ($10^{-15}$ M) stimulated GH secretion without modifying the stimulatory effect of GHRH.

Cortistatin and SRIF at a low dose ($10^{-15}$ M) induced comparable increases in GH release (Fig. 2B). Combined administration of low doses of SRIF and cortistatin increased GH release to the same level as that observed when each peptide was applied separately.

Effect of cortistatin on pig somatotrope subpopulations

Similar to the stimulatory effect observed with low-dose cortistatin in whole cultures of pig pituitary cells (ICS), a low dose ($10^{-15}$ M) of cortistatin stimulated GH release in both LD (Fig. 3A) and HD (Fig. 3B) subpopulations of porcine somatotropes. However, a high dose ($10^{-7}$ M) of cortistatin stimulated GH release only in HD somatotropes (Fig. 3B). In contrast, a high dose ($10^{-7}$ M) of cortistatin blocked the GH stimulatory effect of GHRH in LD somatotropes (Fig. 3A), but this effect was not observed in HD somatotropes (Fig. 3B).

Quantification of mRNA levels of GHRH-R and GHS-R

Examination of the relative expression levels of GHRH-R and GHS-R in somatotrope subpopulations
revealed that both receptors were more abundantly expressed in HD cells than in LD or ICS cells (Fig. 4).

Effect of CST8 on GH release

To compare the effects of CST8 with those of SRIF and cortistatin, total pituitary cell cultures (ICS) were treated with a wide range of CST8 doses (Fig. 5). In these assays, only a single low dose (10^{-15} M) of this peptide significantly stimulated GH release to 162% of control levels. In two additional experiments, CST8 at 10^{-16} and 10^{-14} M also stimulated GH release significantly, albeit moderately, to 132% and 142% of vehicle-treated control respectively (data not shown).

Interaction of CST8 with GHRH, full-length cortistatin, ghrelin and the nonpeptidyl GHS agonist, L-163 255

As shown in Fig. 6A, a high dose (10^{-7} M) of CST8 did not inhibit GHRH-stimulated GH release. Interestingly, a low dose (10^{-15} M) of CST8 not only increased GH release, but also acted additively with GHRH to further stimulate GH secretion. On the other hand, the stimulatory effect of a low dose (10^{-15} M) of CST8 was blocked by a high dose (10^{-7} M) of cortistatin (Fig. 6B).
Conversely, a high dose (10^{-7} M) of CST8 did not inhibit GH release induced by low-dose (10^{-15} M) cortistatin.

We next investigated the possible interaction of CST8 with ghrelin and a nonpeptidyl GHS agonist, L-163 255. As shown in Fig. 7A, high-dose (10^{-7} M) CST8 did not inhibit ghrelin-induced GH release. Interestingly, combined administration of both high and low doses of CST8 (10^{-7} and 10^{-15} M respectively) with ghrelin induced an additive stimulation of GH release. In contrast, high-dose (10^{-7} M) CST8 blocked the stimulatory effect of L-163 255 on GH release (Fig. 7B) while low-dose (10^{-15} M) CST8 did not alter L-163 255-stimulated GH secretion.

**Discussion**

Ramírez et al. (1997a), can stimulate or enhance GH secretion in vivo in pigs (Anderson et al. 1991, Farmer et al. 1992), and can also enhance GH secretion in vivo in other species (Turner & Tannenbaum 1995, Leal-Cerro et al. 2002). In this report, we demonstrate that somatostatin at a wide range of low doses (10⁻¹⁸–10⁻¹⁰ M) directly stimulates GH release in pig pituitary cells, in a dose-dependent manner. At higher doses (10⁻⁸ M or greater), somatostatin had no effect on basal GH secretion. Interestingly, cortistatin, like somatostatin, also stimulated GH release from cultured porcine somatotropes when applied at low doses, albeit the magnitude and dose-dependency of its effects were less patent.

We also investigated the possible interaction of cortistatin and GHRH in somatotrope cells. Our group has previously shown that a high dose of somatostatin inhibited GHRH-induced GH release (via inhibition of adenylate cyclase (AC)), whereas a low dose did not alter the effect of GHRH (Castaño et al. 1996, Ramírez et al. 1997a). When pig pituitary cells were treated with a combination of cortistatin and GHRH, high doses of cortistatin (10⁻⁷ M) fully abolished GHRH-induced GH release, whereas a low dose (10⁻¹⁵ M) of cortistatin

Figure 6 Secretory response of cultured pig pituitary cells to treatment with two doses of CST8 (10⁻⁷ and 10⁻¹⁵ M)±10⁻⁸ M GHRH (A) or two doses (10⁻⁷ and 10⁻¹⁵ M) of full-length cortistatin (B). Data are expressed as percentage of basal values (100%) in control experiments, and are the mean±S.E.M. of five separate experiments. P<0·05: a, vs control; b, vs 10⁻¹⁵ M CST8; c, vs GHRH.

Figure 7 Secretory response of cultured pig pituitary cells to treatment with two doses of CST8 (10⁻⁷ and 10⁻¹⁵ M) in combination with 10⁻⁸ M ghrelin (A) or 10⁻⁸ M L-163 255 (B). Data are expressed as percentage of basal values (100%) in control experiments, and are the mean±S.E.M. of five (ghrelin) and four (L-163 255) separate experiments. P<0·05: a, vs control; b, vs ghrelin or L-163 255 alone; c, vs 10⁻⁸ M CST8; d, vs 10⁻¹⁵ M CST8.
stimulated GH secretion without altering the effect of GHRH. Therefore, these data demonstrate that the effects of cortistatin in pig are identical to those of somatostatin, suggesting that the actions of these peptides are exerted through the same receptors and/or intracellular signaling mechanism (Ramírez et al. 2002, Luque et al. 2005, 2006). This hypothesis is supported by the observation that combined administration of a low, stimulatory dose of both somatostatin and cortistatin did not augment GH secretion over that achieved by each peptide alone, while a high dose of either peptide suppressed the stimulation caused by a low dose of the other peptide (data not shown).

We have previously reported that the population of pituitary somatotropes is in fact composed of two morphologically and functionally distinct cell subpopulations (LD and HD) that can be isolated by Percoll density gradient (Castaño et al. 1996, Ramirez et al. 1997b, 2002). In LD cells, the inhibitory effect of high-dose somatostatin on GHRH-stimulated GH release and the stimulatory effect of low-dose somatostatin on basal GH release can be observed. However, in the HD subpopulation, somatostatin stimulates GH release irrespective of dose and has no effect on GHRH-stimulated GH release (Ramirez et al. 1997b). Therefore, we studied the effect of cortistatin on GH release in LD and HD subpopulations, and our results demonstrate that cortistatin evokes the same GH response as that shown previously for somatostatin. These results underscore the functional similarities between cortistatin and somatostatin in the control of GH release, and strongly support the view that their actions in somatotropes are exerted through the same receptors. Recent studies from our group using nonpeptidyl somatostatin agonists, selective for each of the five sst, suggest that sst1 and sst2 mediate the inhibitory effects of somatostatin on GH release, whereas sst5 activation exerts stimulatory effects on GH release (Luque et al. 2006). In line with this, recent results indicate that sst5 is expressed at higher levels in HD subpopulations, where somatostatin and cortistatin at both high and low doses have a stimulatory effect (Delgado et al. 2002, Luque et al. 2006).

We have demonstrated that the actions of cortistatin are virtually identical to those observed with somatostatin on pig somatotropes and therefore their actions might be exerted via the same sst receptors. However, given the ability of cortistatin, but not somatostatin, to bind the human GHS-R (Deghenghi et al. 2001b,c, 2003, Muccioli et al. 2001), it has been suggested that some actions of cortistatin could be conveyed through GHS-R signaling (Spier & de Lecca 2000, Muccioli et al. 2001, Deghenghi et al. 2001a,b,c, 2003). In line with this, examination of relative expression levels of GHS-R in the somatotrope cell subtypes reveals that HD cells, which are responsive only to the stimulatory actions of somatostatin and cortistatin, express more GHS-R than the LD subpopulation. These observations would suggest that the stimulatory actions on pig GH release of cortistatin (and perhaps somatostatin) could be mediated, at least in part, via GHS-R. To explore this possibility, pig pituitary cells were treated with a truncated cortistatin analog, CST8, which is able to bind selectively only the GHS-R, and not the sst. In fact, CST8 has recently been shown to behave as an antagonist of GHS-R by counteracting the response of ghrelin on gastric acid secretion (Sibilia et al. 2006). In our model, however, CST8 stimulated GH release, although, unlike intact cortistatin or somatostatin, it elicited a more restricted effect, in that it acted only at doses from 10⁻¹⁶ to 10⁻¹⁴ M. Consistent with the inability of CST8 to bind to sst, a high dose of CST8 did not inhibit GHRH-stimulated GH release. Interestingly, a low dose of CST8 (10⁻¹³ M) additively augmented GHRH-induced GH release, thus showing that CST8 exerts its effects, at least in part, through different receptor(s) and signaling mechanisms than those used by full-length cortistatin. Consistent with this idea, the stimulatory effect of low doses (10⁻¹⁵ M) of CST8 was blocked by 10⁻⁷ M full-length cortistatin, whereas a high dose (10⁻⁷ M) of CST8 did not inhibit GH release induced by low-dose cortistatin.

In an attempt to clarify whether CST8 exerts its actions on pig cells by binding to the GHS-R, as previously reported in rats (Sibilia et al. 2006), we tested the effects of CST8 in combination with ghrelin. Surprisingly, a high dose of CST8 (10⁻⁷ M) did not inhibit GH release induced by ghrelin. In fact, high and low doses of CST8 (10⁻⁷ and 10⁻¹⁵ M) combined with 10⁻⁸ M ghrelin induced an additive stimulation of GH release. These results differ from the recently described antagonistic action of CST8 on ghrelin inhibition of gastric acid secretion when administered centrally in rats in vivo, which was attributed to CST8 binding to GHS-R1a (Sibilia et al. 2006). This apparent discrepancy in CST8 action can relate to the different species, doses and biologic model studied (ghrelin i.c.v. action on rat neurons vs in vitro ghrelin action on pig somatotropes). However, our present data strongly indicate that CST8 activates a receptor and/or signaling pathway distinct from that used by ghrelin in pig somatotropes, or, as recently proposed for ghrelin and synthetic GHSs (Holst et al. 2005), CST8 may be acting via the same GHS-R as ghrelin, but by promoting additional, distinct receptor–receptor interactions (dimers) that would thereby enhance the GH-releasing action of ghrelin. In agreement with this latter idea, we found that the combined administration of a low dose of CST8 with 10⁻⁸ M of the nonpeptidyl GHS agonist, L-163 255, did not cause an additive stimulatory effect comparable to that found for ghrelin. Furthermore, 10⁻⁸ M CST8 inhibited L-163 255-stimulated GH release, thereby
suggesting that CST8 antagonizes a receptor subtype or receptor conformation specifically activated by this synthetic, nonpeptidyl GHS. Interestingly, we have previously reported that, while ghrelin stimulates GH release in pig somatotropes by activating both AC and phospholipase C/protein kinase C (PLC/PKC) second messenger routes (Malagón et al. 2003), L-163 255 activates only PLC/PKC (Gracia-Navarro et al. 2002). In this context, our results suggest that CST8 modulates PLC/PKC signaling via a receptor distinct from that used by ghrelin or by enabling conformations of the known GHS-R (dimers) distinct from those affected by ghrelin (Holst et al. 2005).

To summarize our present results in the context of previous findings, we propose a working model (Fig. 8) wherein somatostatin and full-length cortistatin bind to sst1 and sst2 to exert their inhibitory actions on GH release in pig somatotropes by inhibition of AC pathway, whereas sst5 mediate their stimulatory actions (Luque et al. 2006) probably via AC activation (Ramírez et al. 2002). Moreover, ghrelin stimulates GH release by activating both AC and PLC pathways (Malagón et al. 2003), either by binding to distinct binding pockets of the GHS-R (Feighner et al. 1998) and/or enabling dimeric GHS-R forms (Holst et al. 2005), or by binding and activating an alternate, different receptor (Chen 2000). In this working model, we also propose that L-163 255 can bind only to the specific GHS-R-binding pocket or receptor combination that allows coupling to the PLC/PKC route to stimulate pig GH release (Gracia-Navarro et al. 2002), and that CST8 can use a similar binding mechanism to activate or impair these signals, depending on the presence of other GHS-R agonists. Studies in our laboratory are continuing to clarify these complex signaling pathways.

In conclusion, our results demonstrate that cortistatin strongly mimics the dual in vitro actions of somatostatin on GH pig release, suggesting that both peptides mediate their endocrine effects on somatotropes through the same receptors and signaling mechanisms. When the present findings in vitro are viewed together with previous reports in vivo on the endocrine actions of cortistatin (Deghenghi et al. 2001a, Broglio et al. 2002a,b, Gottero et al. 2004) and with the recent demonstration of immunoreactive cortistatin in normal and tumoral human neuroendocrine tissues, which express high levels of sst (Allia et al. 2005), it becomes apparent that the possible local, auto/paracrine actions of cortistatin in pituitary and other endocrine tissues merit further investigation.

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