GH and cortisol rebound rise during and following a somatostatin infusion: studies in dogs with the use of a GH-releasing peptide

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Abstract

GH-releasing peptides (GHRPs), a class of small synthetic peptide and non-peptide compounds, act on specific receptors at both the pituitary and the hypothalamic level to stimulate GH release in both humans and other animals. GHRPs, like corticotropin-releasing hormone (CRH), also possess acute ACTH- and cortisol-releasing activity, although the mechanisms underlying the stimulatory effect of GHRPs on the hypothalamo–pituitary–adrenal (HPA) axis are still unclear. In recent years, studies in humans and other animals have provided evidence that the rebound GH rise which follows withdrawal of an infusion of somatostatin (SS) (SSIW) is due, at least in part, to the functional activation of GH-releasing hormone (GHRH) neurons of the recipient organism. Unexpectedly, in humans, SS infusion, at a dose inhibiting basal GH secretion, has been associated with an activation of the HPA axis, leading to the hypothesis that this response was mediated, at least in part, by a central nervous system ACTH-releasing mechanism activated by the SS-induced decrease in GH secretion. Interestingly, the rebound GH rise which follows SSIW was magnified by the administration of a GHRP, implying that SSIW could also be exploited to investigate the functional interaction in the process of GH and/or ACTH/cortisol secretion between endogenous GHRH (and/or other ACTH-releasing mechanisms) and GHRPs.

In the present study, six young beagle dogs were given, on different occasions, at the beginning and at the end of a 3-h i.v. infusion of SS or saline (SAL), a bolus of physiological SAL or a GHRP compound, EP51216. SSIW induced a GH rebound rise without affecting plasma cortisol concentrations, while the withdrawal of SAL infusion was ineffective on either hormone paradigm. Administration of EP51216 at the beginning of SAL infusion evoked release of both GH and cortisol, whereas EP51216 administration at the withdrawal of SAL infusion evoked somatotroph and cortisol responses which were reduced in amplitude and duration. SS infusion significantly reduced the secretion of GH elicited by EP51216 but did not affect the rise of plasma cortisol levels. Interestingly, SSIW resulted in a marked enhancement of the somatotroph and cortisol responses evoked by EP51216.

The marked rise of plasma GH levels induced by the GHRP after SSIW recalled that occurring after acute combined administration of recombinant human GHRH and EP51216, implying that exogenously delivered GHRP had synergized with the endogenous GHRH release triggered by SSIW. In contrast, acute combined administration of GHRH and the GHRP induced a cortisol response not different from that induced by GHRP alone, indicating that endogenous GHRH release was not involved in the enhanced cortisol response following EP51216 administration after SSIW. Similarly, the direct involvement of endogenous CRH could be ruled out, since i.v. administration of ovine CRH after SSIW evoked cortisol peak levels not different from those evoked by CRH at the withdrawal of SAL infusion.

In conclusion, enhancement of the GH response to EP51216 alone by SSIW, to an extent reminiscent of that following combined administration of GHRH and EP61216, reinforces the view that SSIW elicits release of endogenous GHRH. Further studies are indeed necessary for a better understanding of the mechanisms underlying the enhanced cortisol response, since from now on the involvement of endogenous GHRH or CRH can be ruled out.

Introduction

The growth hormone (GH)-releasing peptides (GHRPs) comprise a group of synthetic peptide and non-peptide analogues that can stimulate GH release through receptors separate from those related to GH-releasing hormone (GHRH) (Müller et al. 1999). The identification of a specific GHRP receptor in the pituitary, the hypothalamus and other areas of the central nervous system (CNS) (Ong et al. 1998, Yokote et al. 1998, Chen 2000) has
suggested, in the past, the existence of an endogenous ligand for these compounds and this ligand, ghrelin, has been recently identified (Bowers 2001, Kojima et al. 2001). The actions of these GHRPs, however, are not specific for GH, as they also stimulate prolactin, adrenocorticotropic (ACTH) and cortisol release in humans and other animals (Jacks et al. 1994, Chang et al. 1995, Copinschi et al. 1996, Arvat et al. 1997b). The sites of action for the release of GH by GHRPs are both in the hypothalamus and the pituitary, although the former is seemingly the privileged area (Müller et al. 1999). GHRPs act as functional antagonists of somatostatin (SS) at either hypothalamus and the pituitary, although the former is seemingly the privileged area (Müller et al. 1999). GHRPs act as functional antagonists of somatostatin (SS) at either hypothalamic sites (Müller 1997). Previous studies in humans have shown that hypothalmo–pituitary disconnection completely abolished the ACTH- and cortisol-releasing activity of GHRPs, whereas the GH-releasing activity was partially preserved (Mallo et al. 1993, Hickey et al. 1996). Thus, granted that the GH-releasing activity of GHRP is the result of a dual activation at both pituitary and hypothalamic sites (Müller et al. 1999), the stimulation of ACTH and cortisol secretion would be instead fully dependent on CNS-mediated mechanisms. In agreement with this proposition, GHRPs have been reported to stimulate GH, but not ACTH, secretion from rat pituitary (Bowers et al. 1984, Momany et al. 1984).

In humans, hexarelin (HEXA), a peptide analogue endowed with a strong GH-releasing activity, and naloxone, an antagonist of opioid receptors, allegedly acting via corticotropin-releasing hormone (CRH) (Orth 1992), have a similar stimulatory effect on ACTH and cortisol secretion, whereas their co-administration has an effect less than additive, suggesting that GHRPs also act via a CRH-mediated mechanism (Korbonits et al. 1995).

Since the ACTH and cortisol responses to HEXA as well as those to human CRH are abolished in subjects pretreated with dexamethasone (Arvat et al. 1998b) and in patients bearing a cortisol-secreting adrenal adenoma or an ectopic ACTH-secreting tumor (Ghigo et al. 1997, Arvat et al. 1998a, 1999, Grottoli et al. 1999), it would seem that the ACTH-releasing effect of GHRPs is sensitive to SS negative feedback by glucocorticoids.

In recent years, studies in animals (Miki et al. 1988, Cella et al. 1996, Rigamonti et al. 2001) and humans (Hindmarsh et al. 1991, degli Uberti et al. 1997, Cappa et al. 1999) have provided evidence that the rebound GH rise which follows withdrawal of an infusion of SS (SSIW) is due, at least in part, to the functional activation of GHRH neurons of the recipient organism.

Unexpectedly, in humans, SS infusion, at a dose that inhibited basal GH secretion, was associated with an activation of the HPA axis, and a response higher in elderly subjects than in younger adults (Ambrosio et al. 1998). Though presently unclear, this SS effect might have been mediated, at least in part, by a CNS ACTH-releasing mechanism activated by the SS-induced decrease in GH secretion (see above) (Ambrosio et al. 1998).

Interestingly, the rebound GH rise which follows SSIW can be magnified by the administration, before SS withdrawal, of a GHRP (Rigamonti et al. 2001), implying that the SSIW approach could (also) be exploited to investigate in vivo the functional interaction between endogenous GHRH (and/or other ACTH-releasing mechanisms) and GHRPs in the process of GH and/or ACTH/cortisol secretion.

Thus, the aim of the present study was that of comparing, in the dog, the GH- and cortisol-releasing activity of EP51216, a peptide of the GHRP family, administered at the beginning and at the end of an SS infusion.

Materials and Methods

Animals

Six young (4–6-year-old, two male and four female) well-trained beagle dogs, weighing between 13 and 15 kg, were used. They were exercised routinely and were fed normal dry food (Diete Standard; Charles River, Calco, Italy) once a day, at 1600 h, with water available ad libitum. They were on a 12-h light:12-h darkness regimen, with lights on at 0700 h. At the beginning of the study, the body weights of the dogs were stable and they had no observable diseases. All experiments were carried out in conscious animals. Before the experiments, animals were kept at rest in the laboratory for at least 1 h. Experiments on each dog were scheduled in a randomized order, at least 1 week apart, with continued training.

All the experiments were performed in accordance with protocols previously authorized by the Committee on Animal Care and Use of the University of Milan.

Study design

Following an overnight fast, two indwelling i.v. cannulae were inserted in the forelimbs at 0830 h (t0). One cannula was used for slow i.v. infusion of saline (SAL) or SS (see below), which was commenced at t0, and the other for bolus administration of SAL or compounds and for collection of blood samples. Two sets of studies were performed.

SAL or SS infusion These studies were aimed at determining the effect on GH and cortisol release of two consecutive administrations of EP51216 (GAB-NTrp...
(2-Me)-dTrp(2 Me)-Trp(2 Me)-Lys-NH₂) or SAL at the beginning and at the end of a 3-h SAL or SS infusion. The present unavailability of HEXA dictated the use of a new GHRP, EP51216, which has efficacy and potency close to those of HEXA (E_{max}=246 ng/ml and ED_{50}=223 µg/kg vs E_{max}=287 ng/ml and ED_{50}=169 µg/kg for EP51216 and HEXA respectively; A E Rigamonti, unpublished results). Reportedly, EP51216 binds to GHRP receptors (G Muccioli, personal communication). Published results). Reportedly, EP51216 and HEXA respectively; A E Rigamonti, unpublished results).

**Table 1** AUC of plasma GH and cortisol concentrations (means ± S.E.M.) in six dogs administered a bolus of SAL 0-9% (0·1 ml/kg, i.v.) or EP51216 (250 µg/kg, i.v.) at the beginning and at the withdrawal of a 3-h infusion of SAL (4 ml/h) or SS (16 µg/(kg·h))

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<td>SAL infusion</td>
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<td>SS infusion</td>
<td>1907±3 ± 62±1±5b</td>
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αP<0.01 vs the corresponding AUC value during SAL infusion and SAL bolus; βP<0.01, γP<0.05 vs the corresponding AUC during SAL infusion and EP51216 bolus.

GH and cortisol responses to a GHRP under SS infusion

(2-Me)-dTrp(2 Me)-Trp(2 Me)-Lys-NH₂) or SAL at the beginning and at the end of a 3-h SAL or SS infusion. The present unavailability of HEXA dictated the use of a new GHRP, EP51216, which has efficacy and potency close to those of HEXA (E_{max}=246 ng/ml and ED_{50}=223 µg/kg vs E_{max}=287 ng/ml and ED_{50}=169 µg/kg for EP51216 and HEXA respectively; A E Rigamonti, unpublished results). Reportedly, EP51216 binds to GHRP receptors (G Muccioli, personal communication). Published results). Reportedly, EP51216 and HEXA respectively; A E Rigamonti, unpublished results).

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αP<0.01 vs the corresponding AUC value during SAL infusion and SAL bolus; βP<0.01, γP<0.05 vs the corresponding AUC during SAL infusion and EP51216 bolus.

GHRH+GHRP challenge test In separate experiments, after a basal sample was collected at t_{<30}, dogs were given at t₀ a bolus of SAL 0-9% (0·1 ml/kg, i.v.), recombinant human GHRH (rhGHRH; 2 µg/kg, i.v.; Geref; Serono, Rome, Italy), EP51216 (250 µg/kg, i.v.) or rhGHRH+EP51216.

Blood samples for measurement of plasma GH and cortisol concentrations were collected at t_{-30}, t_{-15} and t₀, and then at 15-min intervals up to 240 min, the last blood sample being collected at t_{270}.

**GH radioimmunoassay**

Blood samples were collected in tubes containing 0·15 M EDTA and immediately chilled. Plasma was frozen until assayed for canine GH (cGH) by a double-antibody RIA. Highly purified cGH (Pituitary Hormones and Antisera Center, Torrance, CA, USA), obtained together with the specific anti-cGH antibody, through the courtesy of A F Parlow, was used for iodination and as a standard. The sensitivity of the assay was 0·39 ng/ml. The intra-assay coefficients of variation were 3·8 and 4·1% at concentrations of 12·5 and 3·1 ng/ml respectively. To avoid possible interassay variation, all samples from a given experiment were assayed in a single RIA.

Cortisol radioimmunoassay

Plasma cortisol concentrations were measured by a commercial RIA kit, provided by ICN Biomedicals (Diagnostic Division, Costa Mesa, CA, USA). The sensitivity of the assay was 0·5 µg/dl.

**Statistical analysis**

GH and cortisol values are expressed either as absolute values (means ± S.E.M.; ng/ml and µg/dl respectively) (see Figures) or as mean ± S.E.M. area under the plasma concentration vs time curve (AUC_{0–180}; SAL and AUC_{180–270}; SAL for SAL-infusion studies; AUC_{0–180}; SSWiW and AUC_{180–270}; SSWiW for SSiWiW studies; AUC_{0–150}; GHRH+GHRP, AUC_{0–150}; GHRH+GHRP and AUC_{0–150}; saline for GHRH+GHRP studies; ng/ml per min and µg/dl per min respectively), calculated by the trapezoidal integration (see Results and Table 1).

Since no differences in hormone levels between male and female dogs were detected in the different experiments, as in a previous study (Cella et al. 1995), the data were pooled.

Statistical evaluation of differences in absolute GH concentrations and mean values of AUCs among the different experimental conditions was performed by the Student–Newman–Keuls test, preceded by one-way ANOVA. P<0.05 was taken to be statistically significant.

**Results**

Profiles of mean plasma GH and cortisol concentrations during the i.v. infusion of SAL or SS and bolus...
Administration of SAL, EP51216 or oCRH, and after a challenge test with SAL, GHRH, EP51216 or GHRH+EP51216 are shown in Figs 1–5 and Table 1.

Administration of a SAL bolus at the beginning and at the end of SAL infusion did not affect baseline plasma GH levels (Table 1). SS infusion elicited a progressive decrease in plasma GH concentrations which did not, however, reach statistical significance vs GH values of SAL-infused dogs (AUC₀–¹₈₀ cGH: 824.5 ± 132.5 ng/ml per min vs 553.1 ± 118.4 ng/ml per min, P = not significant (NS)) (Table 1). SS infusion significantly reduced EP51216-stimulated GH release (AUC₀–¹₈₀ cGH: 1907.3 ± 621.5 ng/ml per min vs during SAL, P < 0.01) (Fig. 1 and Table 1), without affecting significantly the cortisol response (AUC₀–¹₈₀ cortisol: 487.7 ± 136.8 µg/dl per min vs during SAL, P = NS) (Fig. 2 and Table 1). Interestingly, a marked enhancement of the somatotroph and cortisol responses to EP51216 was observed following SSIW (AUC₁₈₀–²₇₀ cortisol: 4433.6 ± 845.5 ng/ml per min vs during SAL, P < 0.05; AUC₁₈₀–²₇₀ cortisol: 319.6 ± 632.7 µg/dl per min vs during SAL, P < 0.01) (Figs 3 and 4 and Table 1).

The striking increase in somatotroph response elicited by GHRP after SSIW mimicked the pattern present after SS infusion significantly reduced EP51216-stimulated GH release (AUC₀–¹₈₀ cGH: 1907.3 ± 621.5 ng/ml per min vs during SAL, P < 0.01) (Fig. 1 and Table 1), without affecting significantly the cortisol response (AUC₀–¹₈₀ cortisol: 487.7 ± 136.8 µg/dl per min vs during SAL, P = NS) (Fig. 2 and Table 1). Interestingly, a marked enhancement of the somatotroph and cortisol responses to EP51216 was observed following SSIW (AUC₁₈₀–²₇₀ cortisol: 4433.6 ± 845.5 ng/ml per min vs during SAL, P < 0.05; AUC₁₈₀–²₇₀ cortisol: 319.6 ± 632.7 µg/dl per min vs during SAL, P < 0.01) (Figs 3 and 4 and Table 1). The striking increase in somatotroph response elicited by GHRP after SSIW mimicked the pattern present after SS infusion significantly reduced EP51216-stimulated GH release (AUC₀–¹₈₀ cGH: 1907.3 ± 621.5 ng/ml per min vs during SAL, P < 0.01) (Fig. 1 and Table 1), without affecting significantly the cortisol response (AUC₀–¹₈₀ cortisol: 487.7 ± 136.8 µg/dl per min vs during SAL, P = NS) (Fig. 2 and Table 1). Interestingly, a marked enhancement of the somatotroph and cortisol responses to EP51216 was observed following SSIW (AUC₁₈₀–²₇₀ cortisol: 4433.6 ± 845.5 ng/ml per min vs during SAL, P < 0.05; AUC₁₈₀–²₇₀ cortisol: 319.6 ± 632.7 µg/dl per min vs during SAL, P < 0.01) (Figs 3 and 4 and Table 1). The striking increase in somatotroph response elicited by GHRP after SSIW mimicked the pattern present after
acute combined administration of GHRH and EP51216 (AUC\(_{0-150}\) GH: 19 704·3 ± 4480·1 ng/ml per min vs GH response to GHRH alone, 2394·7 ± 846·1 ng/ml per min, \(P<0·01\); vs GH response to EP51216 alone, 7949·5 ± 941·6 ng/ml per min, \(P<0·01\)) (Fig. 3). By contrast, administration of GHRH failed to modify the cortisol secretion elicited by EP51216 (AUC\(_{0-150}\) cortisol: 151·7 ± 34·2 µg/dl per min vs cortisol response to EP51216, 219·4 ± 62·4 µg/dl per min, \(P=\text{NS}\)) (Fig. 4).

Administration of oCRH at the beginning and at the withdrawal of SAL significantly increased plasma cortisol concentrations in comparison with the control experiment (SAL bolus at the beginning and at the withdrawal of SAL infusion) (AUC\(_{0-180}\) cortisol: 411·7 ± 68·4 µg/dl per min vs 223·4 ± 46·5 µg/dl per min, \(P<0·01\); AUC\(_{180-270}\) cortisol: 271·5 ± 58·7 µg/dl per min vs 109·1 ± 23·0 µg/dl per min, \(P<0·01\)) (Fig. 5). SS infusion did not affect these responses, making unlikely the involvement of endogenous CRH in the cortisol response to GHRP (AUC\(_{0-180}\) cortisol: 382·7 ± 73·5 µg/dl per min vs during SAL infusion, \(P=\text{NS}\); AUC\(_{180-270}\) cortisol: 280·5 ± 74·6 µg/dl per min vs SAL infusion, \(P=\text{NS}\)) (Fig. 5).

Under the same experimental conditions (either SAL or SS infusion), CRH induced no changes in plasma GH concentrations (data not shown).

Discussion

Reportedly, SSIW in both animals (Cella et al. 1996, Rigamonti et al. 2001) and humans (degli Uberti et al. 1997, Cappa et al. 1999) elicits a rebound GH rise, which has been attributed to a hypothalamic component, i.e. disinhibition of GHRH neuronal function (Plotsky & Vale 1985, Robinson et al. 1990, Cella et al. 1996).

The presence of the clear-cut enhancement of the GH response to EP51216, a synthetic GHRP hexapeptide, after SSIW confirmed the results of a previous study (Rigamonti et al. 2001) and, in view of the known functional interactions between GHRPs and GHRH,
reinforced the proposition that SSIW acts by disinhibiting hypothalamic GHRH neurons, allowing synergy of the exogenously administered GHRP with endogenously released GHRH (Massoud et al. 1997, Rigamonti et al. 2001).

Although the dose of SS we used was supraphysiologically, the GH-releasing activity of EP51216 was only partially reduced by SS infusion, a finding likely due to the ability of GHRPs to counteract the action of SS (Fairhall et al. 1995, Rigamonti et al. 1998).

During SS infusion, plasma cortisol levels progressively decreased, though the decline did not reach statistical significance vs baseline cortisol titers. These data are in essence consistent with a wealth of human and other animal results, pointing to an inhibitory effect of SS on the HPA system (Fehm et al. 1976, Richardson & Shonbrunn 1981, Brown et al. 1984, Petraglia et al. 1986, Volpi et al. 1996). In clear contradiction of these findings, Ambrosio et al. (1998) have recently reported that in young and elderly healthy subjects infusion of a pharmacological dose of SS, while inhibiting basal GH secretion, increased plasma ACTH and cortisol concentrations. The discrepancy between their findings and the results of other studies is difficult to interpret. In our study, SS infusion failed to reduce plasma GH levels consistently, whereas in the work by Ambrosio et al. (1998), the increase in ACTH and cortisol levels during SS infusion was related to SS-induced suppression of GH release, and, hence, decrease in the GH negative feedback on the CNS. This would have allowed the release of an ACTH secretagogue (endogenous ligand of GHRPs or ghrelin?).

Acute administration of EP51216, at the beginning and at the end of SAL infusion, induced an increase in plasma cortisol levels. This effect might be due to ACTH release following the activation of central mechanisms, since it is known that GHRP administration does not induce any release of ACTH/cortisol in animals with hypothalamic–pituitary disconnection (Hickey et al. 1996). Despite the alleged CNS mechanisms in the GHRP-induced ACTH/cortisol secretion (Orth 1992, Arvat et al. 1997), the role of CRH and arginine-vasopressin in this context is not known, although ghrelin, the endogenous GHRP ligand, has recently been reported to increase hypothalamic levels of CRH mRNA when injected intracerebroventricularly into stressed mice (Asakawa et al. 2001).

Alternatively, to explain our findings, it cannot be ruled out that EP51216 was acting directly at the pituitary level, since ‘paradoxical’ responses of freshly dispersed or cultured anterior pituitary cells to hypothalamic-releasing hormones have been reported recently (Villalobos et al. 1997, Roudbaraki et al. 1999). Multi-responsiveness to hypothalamic releasing hormones would thus be a genuine property of normal anterior pituitary cells (Villalobos et al. 1997).

It would seem from our study that, at least under physiological conditions, the stimulatory action of GHRPs is sensitive to the negative feedback of adrenal steroids. The cortisol response to the second GHRP administration after withdrawal of SAL was, in fact, significantly reduced when compared with the AUC of plasma cortisol levels following the first bolus of the peptide. In this context, it is noteworthy that, in normal volunteers, a dexamethasone pre-treatment abolished the ACTH-releasing effect of HEXA (Arvat et al. 1998).

Finally, in contrast to the decrease in GHRP-stimulated GH secretion under SS infusion (see above), the cortisol response was not affected in the same experimental conditions. The apparent unresponsiveness to SS of the HPA axis under stimulation by GHRP would reinforce the proposition that GHRPs act through different pathways in the regulation of ACTH and GH secretion (Arvat et al. 1997, 1998b, Grottoli et al. 2000).

It is known that the somatotroph response to GHRH may be modulated differently, positively or negatively by ACTH or CRH administration respectively (Raza et al. 1998). To assess the possible contribution of endogenous GHRH after SSIW to the GHRP-stimulated plasma cortisol secretion, a challenge test with combined administration of GHRH and GHRP was performed. Despite the striking synergism present in the somatotroph response, the rise in plasma cortisol levels following combined administration of the two peptides was not different from that elicited by EP51216 alone.

Similarly, it is unlikely that the enhancement of circulating cortisol levels induced by EP51216 after SSIW may be related to a release of endogenous CRH (Katakami et al. 1988, Volpi et al. 1996). In fact, under the same experimental conditions, CRH administration after SSIW did not modify the cortisol response.

At present, we have no evidence to exclude the possibility that SSIW, in addition to GHRH, may also induce the release of the endogenous ligand of GHRP (or ghrelin) produced by hypothalamic neurons and/or by oxyintic glands, and that this event might be responsible for cortisol secretion (Ambrosio et al. 1998). Even though this would detract from the specificity of the SSIW test (combined endogenous GHRH and ghrelin release?), perhaps a better understanding would be achieved of the central GH regulatory mechanisms (Tannenbaum & Bowers 2001) and of the differential diagnosis of growth disorders (Cappa et al. 1999, Rigamonti et al. 2001).

In conclusion, the enhancement of the somatotroph response to GHRPs after SSIW supports the view that SSIW increases the endogenous GHRH tone and might be used in humans in the diagnosis of GH disorders (Cappa et al. 1999). Further studies are necessary for a better understanding of the mechanisms underlying the enhancement of the cortisol response, since from now on the involvement of endogenous GHRH or CRH can be ruled out.
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References


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