Growth hormone-releasing peptide-2 infusion synchronizes growth hormone, thyrotrophin and prolactin release in prolonged critical illness

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Abstract

Objective: During prolonged critical illness, nocturnal pulsatile secretion of GH, TSH and prolactin (PRL) is uniformly reduced but remains responsive to the continuous infusion of GH secretagogues and TRH. Whether such (pertinent) secretagogues would synchronize pituitary secretion of GH, TSH and/or PRL is not known.

Design and methods: We explored temporal coupling among GH, TSH and PRL serum concentration profiles in 86 time series obtained from prolonged critically ill patients by nocturnal blood sampling every 20 min for 9 h during 21-h infusions of either placebo ($n = 22$), GHRH ($1 \mu\text{g/kg/h}$; $n = 10$), GH-releasing peptide-2 (GHRP-2; $1 \mu\text{g/kg/h}$; $n = 28$), TRH ($1 \mu\text{g/kg/h}$; $n = 8$) or combinations of these agonists ($n = 18$).

Results: The normal synchrony among GH, TSH and PRL was absent during placebo delivery. Infusion of GHRP-2, but not GHRH or TRH, markedly synchronized serum profiles of GH, TSH and PRL (all $P \leq 0.007$). After addition of GHRH and TRH to the infusion of GHRP-2, only the synchrony between GH and PRL was maintained ($P = 0.003$ for GHRH + GHRP-2 and $P = 0.006$ for TRH + GHRH + GHRP-2), and was more marked than with GHRP-2 infusion alone ($P = 0.0006$ by ANOVA).

Conclusions: The nocturnal GH, TSH and PRL secretory patterns during prolonged critical illness are herewith further characterized to include loss of synchrony among GH, TSH and PRL release. The synchronizing effect of an exogenous GHRP-2 drive, but not of GHRH or TRH, suggests that the presumed endogenous GHRP-like ligand may participate in the orchestration of coordinated anterior pituitary hormone release.

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Introduction

In healthy subjects, growth hormone (GH), thyrotrophin (TSH) and prolactin (PRL) are released from the anterior pituitary gland in a pulsatile fashion. GH is released nearly exclusively in pulses whereas TSH and PRL are secreted via combined pulsatile and non-pulsatile (basal) modes (1, 2). Moreover, about 30% of the TSH and PRL pulses occur concomitantly in human volunteers. The linkage between PRL and TSH secretion is lost in hypothyroidism, and restored upon treatment with thyroid hormone (3). There is also evidence for temporal coupling between GH and PRL release in humans aged between 9 and 20 years (4) and in ovine fetuses (5), but this is not evident in the human newborn (6). The pacemakers controlling the timing

of TSH, PRL and GH release are still largely unknown, and the mechanisms supervising synchrony remain undefined.

Thyrotrophin-releasing hormone (TRH) and GH-releasing hormone (GHRH) of hypothalamic origin are currently considered to be the major endogenous and specific secretagogues for TSH and GH respectively (7, 8), although neither of these releasing factors is known to control exclusively the timing of the secretory pulses. Recently, a series of synthetic peptides (GH-releasing peptides or GHRPs) have been shown to potently release GH (9), through specific receptors identified in the hypothalamus and the pituitary gland (10). Thus, it now appears plausible, albeit unproved, that the still unknown endogenous ligand of the GHRP-receptor is one of the key factors in the physiological regulation of GH secretion.

One of the hallmarks of prolonged critical illness, a condition defined by the need for intensive vital-organ function support for several weeks, is a uniformly reduced pulsatile component of GH, TSH and PRL secretion. Such blunting of amplitude (and mass) of pulsatile hormone release is reflected pathophysiologically in low circulating levels of insulin-like growth factor-I and thyroid hormones (11–13). The numbers of detectable GH, TSH and PRL secretory pulses are either normal or slightly elevated (11–13). This amplitude attenuation is reversed in significant part by relevant secretagogue infusions, which has led to the hypothesis that relative hypopituitarism in critical illness arises from a hypothalamic deficiency state, putatively that of the presumed endogenous GHRP-like ligand.

In earlier analysis of endocrine strategies to reverse the catabolic state of critical illness, we performed a series of studies on the dynamics of anterior pituitary function in prolonged critical illness (11–14). Here, we evaluate the synchrony of GH, TSH and PRL release, and whether continuously infused GHRH, GHRP-2 and TRH, as well as combinations of these agonists, can reinstate coordinated pituitary hormone secretion. We postulated that GHRP-2, but not GHRH or TRH, would confer coordinated pituitary GH, TSH and PRL release.

Subjects and methods

Patients and concomitant treatment

In 86 nocturnal blood sampling profiles, of which 66 were obtained in critically ill men and 20 in critically ill women who participated in two consecutive, prospective, randomized and open-label studies which were previously described (11–13), we determined the degree of correlation among repetitively sampled GH, TSH and PRL release over 9 h. Inclusion criteria were (i) dependency on intensive care, including mechanical ventilatory support, for at least one week, (ii) a stable condition without dopamine treatment for at least 48 h and (iii) an expected stay in the intensive care unit for at least another 48 h. Exclusion criteria for this analysis were age <18 years; pre-existing neurologic, psychiatric, metabolic or endocrine disease; intracranial lesions; significant liver failure (prothrombin time <20%); renal failure requiring replacement therapy; concomitant treatment with glucocorticoids, oestrogens, somatostatin, thyroid hormones, Ca^{2+} -re-entry blockers, clonidine, amiodarone, iodine-containing antiseptic agents, dopamine agonists or antagonists.

The mean age was 65 ± 12 (range 31–87) years and body mass index (BMI) was $26 \pm 4.8 \text{ kg/m}^2$ (range 17–40 kg/m^2). Patients were critically ill for 21 ± 10 (range 7–59) days at the time of study, and the total intensive care unit (ICU) stay was 47 ± 31 (range 16–181) days. Apache II score on the ICU admission day, an indicator of severity of illness with higher values reflecting a more critical condition (15), was 16 ± 6 (range 5–33).

Concomitant treatment included continuously administered feeding, either total parenteral feeding, combined continuous parenteral and enteral nutrition or full enteral tube feeding, with normal caloric intake (a mean of 24 non-protein cal/kg/day, range 12–35 cal/kg/day) and standard composition (0.8–1.6 g/kg amino acids per day, 2.8–4.3 g/kg glucose per day, 1–1.5 g/kg fat per day representing 25–40% of non-protein calories) (16); inotropic support with exogenous non-dopaminergic catecholamines; analgesia and sedation with continuously infused opioids and/or benzodiazepines. Plasma glucose levels were monitored; insulin was infused if necessary to keep plasma glucose $\leq 12 \text{ mmol/l}$ (17).

Ultimate survival at 6 months after study entry was 61%.

Ethical aspects

The study was approved by the Institutional Review Board of the University of Leuven School of Medicine. Informed consent from a first degree relative was obtained before patient inclusion.

Design

Patients had been randomized to receive a 21-h continuous infusion of placebo (NaCl 0.9%; $n = 22$), GHRH (50 $\mu\text{g/ml}$ NaCl 0.9%; Ferring, Kiel, Germany; 1 $\mu\text{g/kg/h}$; $n = 10$), TRH (200 $\mu\text{g/ml}$ NaCl 0.9%; Ferring; 1 $\mu\text{g/kg/h}$; $n = 8$), GHRP-2 (50 $\mu\text{g/ml}$ NaCl 0.9%; Kaken Pharmaceuticals Ltd, Tokyo, Japan; 1 $\mu\text{g/kg/h}$; $n = 28$), GHRH + GHRP-2 (1 + 1 $\mu\text{g/g/h}$, $n = 12$), or TRH + GHRH + GHRP-2 (1 + 1 + 1 $\mu\text{g/kg/h}$, $n = 6$).

The infusions were started at 0900 h and given through a separate lumen of a central venous catheter, inserted for clinical purposes. A PERFUSOR segura FT pump with a 50 ml PERFUSOR syringe (B. Braun, Melsungen, Germany) permitted precise infusions of small volumes at a constant rate. Inadvertent interruption of the infusion or unanticipated bolus injections of the peptides were thereby avoided.

Serum concentrations of TSH, PRL and GH were measured every 20 min between 2100 h and 0600 h.

Assays

All samples from each patient were processed in one assay run. All samples had detectable values using the assays described below.

Serum concentrations of PRL were measured by IRMA using the PRL-IRMA Kit (Medgenix, Fleurus, Belgium). The intra-assay coefficient of variation was 6.2% at 6.6 $\mu\text{g/l}$ and 4.7% at 46.4 $\mu\text{g/l}$. The detection limit was 2.9 $\mu\text{g/l}$.

Serum concentrations of TSH were measured by IRMA using the TSH Riabead II assay (Abbott Laboratories, North Chicago, USA). The intra-assay coefficient

of variation was 4.3% at 1.2 mIU/l and 2.2% at 7.0 mIU/l. The detection limit was less than 0.02 mIU/l.

Serum concentrations of GH in all profiles were measured by RIA, using a polyclonal antibody (18). The intra-assay coefficient of variation was 7.3% at 6.7 µg/l and 4.6% at 14.4 µg/l. The detection limit was 0.5 µg/l. When a GH value within a GH series reached this detection limit, the complete profile of the concerned patient was re-assayed with a more sensitive IRMA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The detection limit of this IRMA was 0.02 µg/l; the intra-assay coefficient of variation was 4.2%. GH concentrations were detectable in all samples. The regression equation for comparison of both assays is: Nichols IRMA = $-0.38 + 0.83 \times \text{RIA}$, the regression coefficient being 0.83 and the correlation coefficient (R) being 0.93 ($R^2 = 0.87$; $n = 218$; $P < 0.0001$).

Analysis of synchrony of pituitary hormone release

We investigated the presence of a temporal relationship between the serial release of GH and TSH, PRL and TSH, and GH and PRL in 86 nocturnal time series.

This analysis was performed by determining the presence of significant cross-correlation between pairs of serum concentration profiles at various lags, as appropriate for time series which are relatively short as are those studied here (19–21). Within each of the six infusion groups (placebo, GHRH, TRH, GHRP-2, GHRH + GHRP-2 and TRH + GHRH + GHRP-2), correlation coefficients were calculated between the serum concentration profiles of pairs of hormones (GH/TSH; PRL/TSH; GH/PRL). The null hypothesis (cross-correlation values average no different from zero, as

expected on the basis of chance alone) was tested by R to Z transformation ($Z = R/\sqrt{(N-K)}$, where N = number of samples in each series, and K is number of lag units considered), followed by a single sample *t*-test against an expected Z score of zero. The analysis was applied at a range of time-lags (20-min intervals from -180 to $+180$ min) between the two series. The level of significance for cross-correlation was set at a protected value of $P < 0.01$.

Results (Table 1, Figs 1 and 2)

During placebo infusion, no significant correlations between paired profiles of GH and TSH, PRL and TSH, and GH and PRL were found, suggesting absence of significant synchrony between the hormonal pulse trains in these critically ill patients.

The continuous infusion of either GHRH or TRH alone did not elicit synchrony.

During continuous infusion of GHRP-2, a highly significant degree of cross-correlation emerged between GH and TSH concentrations at lag + 20 min (median $R = 0.27$ (range -0.39 to 0.74), $P = 0.002$) and at lag + 40 min (median $R = 0.18$ (range -0.58 to 0.66), $P = 0.007$); between PRL and TSH concentrations at zero lag (median $R = 0.22$ (range -0.44 to 0.81), $P = 0.0008$), at lag + 20 min (median $R = 0.31$ (range -0.44 to 0.81), $P = 0.0001$) and at lag + 40 min (median $R = 0.27$ (range -0.47 to 0.79), $P = 0.0003$), and between GH and PRL concentrations at zero lag (median $R = 0.18$ (range -0.46 to 0.80), $P = 0.005$) (Figs 1 and 2) and at lag + 20 min (median $R = 0.19$ (range -0.39 to 0.80), $P = 0.005$). Note that a plus lag denotes that values of the first-named hormone precede those of the second by that time increment.

Table 1 Cross-correlation analysis between pairs of serum concentration profiles. Significant group cross-correlation median R values (versus a null hypothesis of a cross-correlation of zero expected on the base of chance alone, $P \leq 0.007$) are depicted for lag-times from -40 minutes to $+40$ minutes.

	GH/TSH Lag time (min)					PRL/TSH Lag time (min)					GH/PRL Lag time (min)				
	-40	-20	0	20	40	-40	-20	0	20	40	-40	-20	0	20	40
Without GHRP-2															
Placebo ($n = 22$)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GHRH ($n = 10$)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
TRH ($n = 8$)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
With GHRP-2															
GHRP-2 ($n = 28$)	—	—	—	0.27	0.18	—	—	0.22	0.31	0.27	—	—	0.18***	0.19	—
GHRH+GHRP-2 ($n = 12$)	—	—	—	—	—	—	—	—	—	—	—	—	0.36***	0.35	—
TRH+GHRH+GHRP-2 ($n = 6$)	—	—	—	—	—	—	—	—	—	—	—	0.30	0.48***	—	—

Median R values were obtained by calculation of cross-correlation between pairs of 86 GH, TSH and PRL time series of serum concentrations, composed by sampling every 20 min for 9 h during random infusion of placebo, GHRH (1 µg/kg/h), TRH (1 µg/kg/h), GHRP-2 (1 µg/kg/h), GHRH+GHRP-2 (1+1 µg/kg/h) or TRH+GHRH+GHRP-2 (1+1+1 µg/kg/h). At positive lags, the first hormone in the pair leads the second hormone value by the indicated time lag (min).

*** $P = 0.0006$ via factorial ANOVA for each of the following infusion paradigms: placebo, GHRP-2, GHRH+GHRP-2, TRH+GHRH+GHRP-2.

— No significant cross-correlations were detected.

Figure 1 Box and whisker plots of GH and PRL cross-correlation R values at zero lag (displaying for each group the 10th, 25th, 50th, 75th and 90th percentile of R values as well as all R values above the 90th and below the 10th percentile as separate dots (○)) depicting absent cross-correlation during infusion of placebo ($n = 22$), and indicating that the significant cross-correlation calculated during continuous infusion of GHRP-2 ($1 \mu\text{g/kg/h}$; $n = 28$) is increased by combining this secretagogue with GHRH ($1 \mu\text{g/kg/h}$; $n = 12$) and with TRH+GHRH ($1 + 1 \mu\text{g/kg/h}$; $n = 6$). $P = 0.0006$ via ANOVA.

When GHRH was added to the infusion of GHRP-2, there was no longer a significant cross-correlation between GH and TSH and between PRL and TSH, but the degree of cross-correlation between GH and PRL profiles was increased at zero lag (median $R = 0.36$ (range -0.07 to 0.55), $P = 0.003$) (Figs 1 and 2) and at lag $+20$ min (median $R = 0.35$ (range -0.24 to 0.49), $P = 0.0002$).

When TRH was added to the combination of GHRP-2 and GHRH, there was a further increase in the correlation between GH and PRL, as compared with GHRP-2 alone and compared with GHRH + GHRP-2 ($P = 0.0006$ with factorial ANOVA for placebo/GHRP-2/GHRH + GHRP-2/TRH + GHRH + GHRP-2) at lag zero (median 0.48 (range 0.07 to 0.74), $P = 0.006$) (Figs 1 and 2). At lag -20 min, GH was correlated with PRL (median $R = 0.30$ (range 0.12 to 0.67), $P = 0.004$), whereas the asynchrony between TSH and PRL and between GH and TSH profiles remained.

Discussion

During prolonged critical illness, spontaneous pulsatile secretion of GH, TSH and PRL is known to be uniformly reduced, through impairment of pulse amplitude rather than pulse frequency, which can be reamplified by continuous infusion of GH secretagogues and TRH (11–14). The current study revealed an additional characteristic of the nocturnal secretory pattern of anterior pituitary hormones during prolonged critical illness: normal synchrony among GH, TSH and PRL release

Figure 2 Nocturnal serum concentration profiles of GH (thick line) and PRL (thin line), representative for the group finding of absent synchrony between GH and PRL release during continuous infusion of placebo, and significant increase in synchrony by addition of GHRP-2 ($1 \mu\text{g/kg/h}$), GHRH ($1 \mu\text{g/kg/h}$) and TRH ($1 \mu\text{g/kg/h}$) to the infusion. The illustrative profiles were obtained in 4 men aged 69, 66, 68 and 60 years, with a BMI of respectively 24.2, 24.2, 22.4 and 20.6 kg/m^2 , who were critically ill for 18, 28, 15 and 24 days respectively. The time series are composed by sampling every 20 min for 9 h (2100 h–0600 h). The cross-correlation R values for GH and PRL release at zero lag for these 4 patients were 0.117, 0.212, 0.490 and 0.555 respectively.

was absent (3, 4). Exogenous GHRP-2 synchronized in part GH, TSH and PRL secretion, as evidenced by significant cross-correlation among their serum concentration profiles. When infused alone, neither GHRH nor TRH altered GH, TSH or PRL co-release, but when GHRH and TRH were infused together with GHRP-2, this triple secretagogue combination increased the

synchrony between GH and PRL release patterns, as indicated by an increased degree of cross-correlation. However, despite statistical significance, the absolute values of the median cross-correlation coefficients were relatively small, which may indicate substantial variability and/or procedure 'noise' as expected in a relatively short time series as those studied here. Nevertheless, this pilot observation throws new light upon the hitherto still obscure systems orchestrating human anterior pituitary hormone release.

The mechanisms underlying the absence of synchrony among pituitary GH, TSH and PRL release during protracted critical illness and the synchronizing effects apparently exerted by GHRP-2 are only speculative. One possibility is that the normal hypothalamic control mechanisms orchestrating the secretory activity of somatotrophs, lactotrophs and thyrotrophs are altered. The synchrony induced by continuous GHRP-2 infusion points to the capacity of GHRP-2 to partially coordinate (directly or indirectly) anterior pituitary hormone release and, accordingly, suggests that the presumed endogenous GHRP-like ligand may participate in the physiological regulation of co-pulsatility. GHRH, TRH, somatostatin and dopamine are among the other candidate pacemakers for coupled hormone release. Continuously infused GHRH or TRH (separately) did not relieve the asynchrony. In addition, when GHRH and TRH were infused continuously together with GHRP-2, the synchrony between TSH and PRL and between GH and TSH induced by GHRP-2 alone disappeared. Together, these findings could be interpreted to suggest a simplified model in which pulsatile release of GHRH and TRH from the hypothalamus might participate in supervising the simultaneous release of TSH and PRL and of GH and TSH. This reasoning follows, if one assumes that continuously infused GHRH and/or TRH supplants or impedes pulsatile endogenous GHRH and/or TRH actions. On the other hand, the finding of an increased degree of correlation between GH and PRL profiles by addition of GHRH and TRH to the infusion of GHRP-2 suggests that pulsatile GHRH and TRH release by the hypothalamus may contribute, if anything, to desynchronizing GH and PRL release rather than synchronizing it. Consequently, in our view, somatostatin and/or dopamine as episodic inhibitors are more plausible candidate pacemakers for the concomitant secretion of GH and PRL, allowing for possible actions of GHRP on these regulators. Episodic hypothalamic input in the face of a constant GHRP drive could account for pulsatile GH and PRL release despite an unvarying GHRP stimulus.

Alternatively, synchrony may be based on local paracrine and/or autocrine control of joint hormone release by anterior pituitary cells, such as mammosomatotrophs or other (unclassified) plurihormonal cells (22). In this interpretation, the synchronizing effect of continuous GHRP-2 infusion may be related to increased secretory activity of these cell populations or

even to induction of functional differentiation or transdifferentiation within pituitary cell types.

Finally, GHRP-2 might have induced significant cross-correlation by co-altering hormonal half-lives. This mechanism appears less plausible, as GHRP-2 has been shown not to modify the GH or TSH half-life, although it appeared to reduce PRL half-life by 27% and/or increase its basal release (11, 12).

Previously described studies have led to the hypothesis that prolonged critical illness may be a condition of relative deficiency of the presumed endogenous GHRP-like ligand (11–14). The present findings of absent synchrony among GH, TSH and PRL release and of synchrony restoration by GHRP-2 administration further supports this novel hypothesis.

In conclusion, concomitant release of GH, TSH and PRL appears to be absent in prolonged critical illness, and an exogenous GHRP-2 drive seems to exert a synchronizing effect. This pilot observation needs to be confirmed in future studies involving a larger number of patients and longer sampling intervals. It also remains to be elucidated whether GHRP-2 exerts this synchronizing effect at the level of hypothalamic control or at the level of uni- or plurihormonal pituitary cell types.

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