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Neuroendocrinology of Prolonged Critical Illness: Effects of Exogenous Thyrotropin-Releasing Hormone and Its Combination with Growth Hormone Secretagogues*

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ABSTRACT

The catabolic state of prolonged critical illness is associated with a low activity of the thyrotropic and the somatotropic axes. The neuroendocrine component in the pathogenesis of these low activity states was assessed by investigating the effects of continuous intravenous infusions of TRH, GH-releasing peptide-2 (GHRP-2), and GHRH. Twenty adult patients, critically ill for several weeks, were studied during two consecutive nights. They had been randomly allocated to one of three combinations of peptide infusions, each administered in random order: TRH (one night) and placebo (other night), TRH + GHRP-2 (one night) and GHRP-2 (other night), or TRH + GHRH + GHRP-2 (one night) and GHRH + GHRP-2 (other night). The peptide infusions were started after a 1-μg/kg bolus and infused (1 μg/kg per h) until 0600 h. Blood sampling was performed every 20 min, and pituitary hormone secretion was quantified by deconvolution analysis. Reduced pulsatile fraction of TSH, GH, and PRL secretion and low serum concentrations of T_4, T_3, insulin growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP-3), and the acid-labile subunit (ALS) were documented in the untreated state.

Infusion of TRH alone or in combination with GH secretagogues augmented nonpulsatile TSH release 2- to 5-fold; only TRH + GHRP-2 increased pulsatile TSH secretion (4-fold). Average rises in T_4 (40–54%) and in T_3 (52–116%) were obtained with all three combinations, whereas reverse T_3 levels did not increase, except when TRH was infused alone. Pulsatile GH secretion was amplified >6- and >10-fold, respectively, by GHRP-2 and GHRH + GHRP-2 infusions, generating mean increases of serum IGF-I (66% and 106%), IGFBP-3 (50% and 56%), and ALS (65% and 97%) within 45 h. The addition of TRH did not alter the GH secretory patterns. TRH infusion increased PRL release only when combined with GH secretagogues. No effects on serum cortisol were detected.

In conclusion, the pathogenesis of the low activity state of the thyrotropic and somatotropic axes in prolonged critical illness appears to have a neuroendocrine component, because these axes are both readily activated by coinfusion of TRH and GH secretagogues. (*J Clin Endocrinol Metab* **83:** 309–319, 1998)

PROLONGED critical illness is the phase following resuscitation of an acute life-threatening disease or trauma during which vital organ functions remain dependent on intensive care support for several weeks. In this condition, feeding is unable to reverse ongoing wasting of protein, whereas fat, in contrast, is preserved or stored (1). The protein wasting results from both activated degradation and suppressed synthesis of protein. The latter determines the residual protein content, the decrease of which correlates with the duration of illness (2). Protein loss from vital organs and tissues aggravates dysfunction of the involved systems and, consequently, prolongs dependency on intensive care support, such as mechanical ventilation and artificial feeding.

In prolonged critical illness, suppression of pulsatile TSH, GH, and PRL release has been documented and related to, respectively, low serum levels of thyroid hormone and insulin growth factor-I (IGF-I), and this takes place in the presence of hypercortisolism (3–6). Circulating IGF-I has been shown to reflect protein wasting in this condition; in nonsurvivors, the tissue thyroid hormone levels are also reduced (7–9). Consequently, there is reason to believe that the above-described wasting syndrome could, at least in part, be brought about by the changes in the somatotropic and thyrotropic axes and in cortisol secretion, a hypothesis that already prompted the investigation of the anabolic properties of exogenous GH and IGF-I (10, 11).

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TRH and GHRH of hypothalamic origin are currently considered to be the major endogenous and specific secretagogues for TSH and GH, respectively (12, 13). Recently, a series of synthetic peptides (GH-releasing peptides or GHRPs) and nonpeptide agents have been shown to potently and specifically release GH (14–16) through a specific G protein-coupled receptor located in the hypothalamus and the pituitary (17). It now appears plausible that the still unknown endogenous ligand of the GHRP-receptor is one of the key factors in the physiological regulation of GH secretion.

Studying the responses to the administration of hypothalamic releasing factors in prolonged critical illness may, to a certain extent, localize the origin of the endocrine changes. In previous studies, it appeared that the GH response to a GHRH bolus is blunted, the GH response to GHRP-2 exaggerated, and the GH response to TRH paradoxical (18, 19). Similarly, the TSH response to TRH is blunted, and the TSH response to GHRH is paradoxical (18, 19). The continuous infusion of GH secretagogues is able to amplify pulsatile GH release and to generate a rise of IGF-I within 21 h (3).

To further delineate the relative importance of central (hypothalamic-pituitary) *vs.* peripheral components in the low-activity status of the thyrotropic and somatotropic axes, we investigated whether these axes could be jointly reactivated by infusing combinations of TRH, GHRP-2, and GHRH in patients with prolonged critical illness.

Methods

Patients and concomitant treatment

Because loss of protein content has been related to the time course of critical illness rather than to the underlying disease (2), only patients depending on intensive care (including mechanical ventilatory support) for at least 1 week were eligible for participation in this study. Further inclusion criteria were a stable condition without dopamine treatment for at least 48 h, because dopamine infusion has been shown to profoundly affect pituitary function in this condition (5, 20), and an expected stay in the intensive care unit (ICU) for at least another 48 h. This study was conducted between April and November 1996.

Exclusion criteria were age <18 yr; preexisting neurological, psychiatric, metabolic or endocrine disease; intracranial lesions; important liver failure (prothrombin time <20%); renal failure requiring dialysis or hemofiltration; or concomitant treatment with glucocorticoids, estrogens, somatostatin, thyroid hormones, Ca^{2+}-reentry blockers, clonidine, amiodarone, or dopamine agonists or antagonists.

A total of 20 patients (7 women, 13 men) were included (Table 1). The mean ± SEM age was 68 ± 3 yr (range, 32–87 yr). Body mass index (BMI) on ICU admission was 25 ± 1 kg/m^2 (range, 17.8–40.4 kg/m^2). Apache II score on the ICU admission day, an indicator of severity of illness with higher values reflecting a more critical condition (21), was 14 ± 1 (range 5–28).

Patients were critically ill for 25 ± 3 days (range, 12–59) at the time of inclusion. Concomitant treatment included continuous total (n = 14) or partial (n = 4) parenteral nutrition or full enteral feeding (n = 2), with normal caloric intake (a mean of 24 nonprotein Cal/kg per day, range, 12–35 Cal/kg per day) and standard composition (0.8–1.6 g/kg amino acids per day, 2.8–4.3 g/kg glucose per day, and 1–1.5 g/kg fat per day covering 25–40% of nonprotein calories) (22); inotropic support with exogenous nondopaminergic catecholamines (n = 7); antibiotics (n = 16); and analgesia and sedation with continuously infused opioids (n = 17) and/or benzodiazepines (n = 13). Plasma glucose levels were monitored; insulin was infused when plasma glucose was ≥12 mmol/L (n = 11) (23). Plasma glucose levels were elevated at the time of study start: 8.3 ± 0.5 mmol/L (range 5.7–14 mmol/L). Human albumin was continuously infused when serum levels were low (mean serum albumin concentration at inclusion was 2.8 ± 0.1 g/dL). The mean serum level of triglycerides was 188 ± 26 mg/dL (range, 66–557 mg/dL) and C-reactive protein concentration was elevated (12.5 ± 1.4 mg/dL). Continuous hemodynamic monitoring included electrocardiogram (n = 20), intra-arterial blood pressure (n = 20), central venous pressure (n = 20), and core and peripheral temperature (n = 20). During the study period of 45 h, the concomitant ICU therapy remained virtually unaltered in all patients.

The mean total ICU stay of the studied patients was 54 ± 9 days (range, 24–181). Eleven patients died on the ICU (55%), a mean 31 ± 6 days after the study. Seven patients were discharged to the ward and subsequently left the hospital (35%). One patient still remains in the ICU (5%) 5 months after study inclusion, and one patients still resides on a hospital ward (5%) 4 months after study inclusion.

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.

Study design and peptide administration

Patients were studied during a total time span of 45 h, including sampling every 20 min during two consecutive nights from 2100–0600 h and were given one study compound per night. They were randomly allocated to one of three study groups (Fig. 1, *upper*. Group 1 (n = 8) received TRH infusion (1 μg/kg bolus at 0900 h, followed by a 1 μg/kg per h continuous infusion until 0600 h) *vs.* placebo (24). Group 2 (n = 6) received TRH + GHRP-2 (1 + 1 μg/kg bolus at 0900 h, followed by a 1 + 1 μg/kg per h continuous infusion until 0600 h) *vs.* GHRP-2 infusion (1 μg/kg bolus at 0900 h, followed by a 1 μg/kg/h continuous infusion until 0600 h) (25). Group 3 (n = 6) received TRH + GHRH + GHRP-2 infusion (1 + 1 + 1 μg/kg bolus at 0900 h, followed by a 1 + 1 + 1 μg/kg per h continuous infusion until 0600 h *vs.* GHRH + GHRP-2 infusion (1 + 1 μg/kg bolus at 0900 h, followed by a 1 + 1 μg/kg per h continuous infusion until 0600 h) (26).

Within these three groups (Fig. 1, *lower*), patients were randomly assigned for the order of peptide infusion. Duration of each infusion was 21 h. This randomized, cross-over design was applied to minimize possible interference by order of peptide administration or by spontaneous recovery.

Placebo (NaCl 0.9%), TRH (200 μg/mL NaCl 0.9%; UCB, Pharma, Brussels, Belgium), GHRP-2 (Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) (50 μg/mL NaCl 0.9%), and human GHRH (50 μg/mL NaCl 0.9%; Ferring, Kiel, Germany) infusions were given through a separate lumen of a central venous catheter, inserted for clinical purposes. A PERFUSOR secura 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 in each sample, serum cortisol levels were measured every hour, and serum T_4, T_3, reverse T_3 (rT_3), IGF-I, IGFBP-3, and the acid-labile subunit (ALS) concentrations were determined before start of the infusions and in the first and the last sample of each night profile.

Blood sampling

All blood samples were collected through an arterial line inserted for clinical purposes independently of this study. The Edwards VAMP system (Baxter Healthcare Corp., Irvine, CA) was used, permitting withdrawal of undiluted blood samples from an indwelling catheter, without undue blood loss. The total amount of blood sampled per patient was 140 mL. Blood was collected into glass tubes; after clotting and centrifugation, the serum was kept frozen at −20 C until assay.

Assays

All samples of each individual patient were processed in the same assay run. Serum concentrations of TSH were measured by immunoradiometric assay using the TSH Riabead II (Abbott Labs., North Chicago, IL). The intraassay 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. Normal values range from 0.15–4.6 mIU/L.

Serum concentrations of T_4 were measured by RIA using the Tetrabead-125 Diagnostic Kit (Abbott Labs.). The intraassay coefficient of variation was 4.6% at 59 nmol/L and 4.4% at 102 nmol/L. Normal values

TABLE 1. Clinical patient data

Random	Gender	Age (yr)	BMI (kg/m^2)	Type of illness[a]	Apache II[b]	Total ICU stay (days)	Incl. day[c]	Outcome	Feeding[d]	Opioids[e]	Benzo's[e]	Insulin[e]	Catechol[e]
Placebo/TRH	M	53	23.9	Bilobectomy + pleural fistula + thoracoplasty	5	52	37	Home	TPN	Y	Y	N	N
Placebo/TRH	M	74	24.2	False aneurysm + respiratory insufficiency	19	37	25	Died	TPN	Y	Y	N	Y
Placebo/TRH	M	60	17.8	Complicated esophageal resection with colon graft	7	34	27	Home	PN+EN	N	Y	N	N
Placebo/TRH	F	32	40.4	Varicella pneumonia + MOF	28	51	29	Home	TPN	Y	N	N	N
TRH/Placebo	M	66	25.9	Lobectomy + ARDS	27	45	14	Died	TPN	Y	Y	N	Y
TRH/Placebo	M	69	24.2	Pneumonectomy + aspiration pneumonia	12	33	18	Died	PN+EN	Y	N	Y	N
TRH/Placebo	F	65	29.4	Complicated spleenectomy + MOF	21	24	12	Died	TPN	Y	N	Y	N
TRH/Placebo	M	48	24.1	Complicated pneumonectomy	11	116	59	Died	EN	Y	Y	N	N
GHRP-2/TRH+GHRP-2	M	87	22.9	False aneurysm + lung tumor + respiratory insufficiency	19	26	12	Died	TPN	Y	Y	Y	N
GHRP-2/TRH+GHRP-2	F	85	26.2	30% body surface area burns	11	69	32	Home	EN	Y	N	Y	N
GHRP-2/TRH+GHRP-2	F	76	23.9	Complicated esophageal resection + sepsis	8	108	22	ICU	TPN	Y	Y	Y	Y
TRH+GHRP-2/GHRP-2	M	66	24.2	Complicated CABG	16	36	28	Home	TPN	Y	Y	Y	N
TRH+GHRP-2/GHRP-2	M	80	26.1	Spleen and esophageal resection + respiratory insufficiency	6	42	15	Home	TPN	N	N	Y	N
TRH+GHRP-2/GHRP-2	M	76	24.0	Mitral valve replacement + respiratory insufficiency	12	32	13	Ward	TPN	Y	N	Y	Y
G+G/TRH+G+G	F	72	21.4	Postinfarction VSD + MOF	14	34	21	Died	TPN	Y	Y	N	Y
G+G/TRH+G+G	F	63	23.9	Necrotizing pancreatitis + MOF	13	181	48	Died	TPN	Y	Y	Y	Y
G+G/TRH+G+G	F	77	31.6	CABG + respiratory insufficiency	12	49	26	Died	PN+EN	N	N	Y	Y
THR+G+G/G+G	M	60	20.6	Pneumonectomy + respiratory insufficiency	13	38	24	Died	TPN	Y	Y	Y	N
TRH+G+G/G+G	M	68	22.5	Ruptured abdominal aneurysm + CABG + MOF	13	35	15	Died	TPN	Y	Y	N	N
THR+G+G/G+G	M	81	22.9	Complicated esophageal resection + sepsis	20	39	17	Home	PN+EN	Y	Y	N	N

[a] MOF, Multiple organ failure; ARDS, adult respiratory distress syndrome; CABG, complicated coronary artery bypass grafts.
[b] Apache II score after the first 24 h of intensive care.
[c] Day of stay in ICU at time of inclusion.
[d] Type of continuous feeding: TPN, total parenteral nutrition; PN+EN, parenteral and enteral nutrition; EN, full enteral nutrition.
[e] Concomitant administration of exogenous opioids, benzodiazepines, insulin, and nondopaminergic catecholamines.

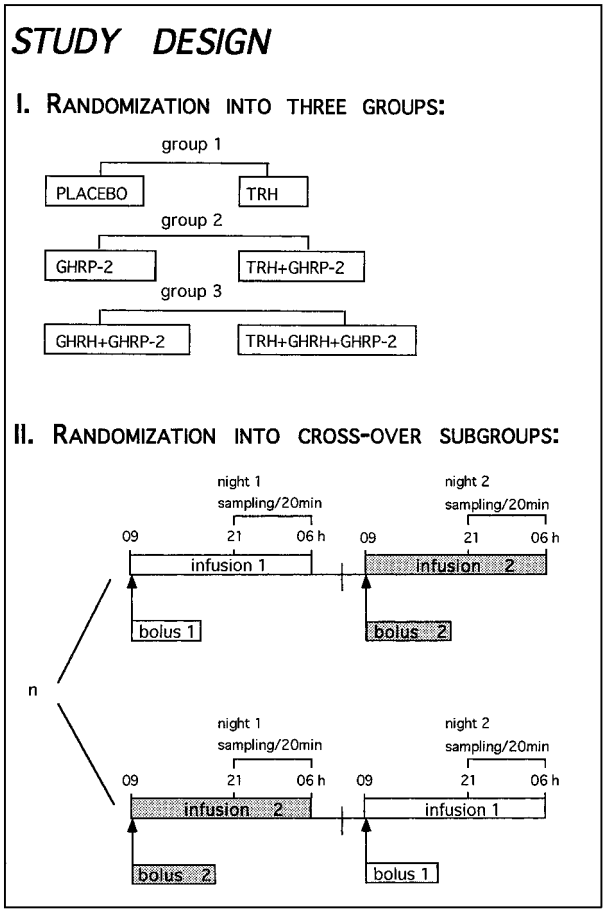

FIG. 1. *Upper*, Twenty patients were randomly allocated to one of three groups: group 1, placebo *vs*. TRH infusion (n = 8); group 2, GHRP-2 *vs*. TRH + GHRP-2 infusion (n = 6); group 3, GHRH + GHRP-2 *vs*. TRH + GHRH + GHRP-2 infusion (n = 6). *Lower*, Within these three groups, patients were randomly assigned for order of peptide infusion into two cross-over subgroups. Duration of each infusion was 21 h. Patients were studied during a total time span of 45 h, with sampling every 20 min during two consecutive nights from 2100–0600 h.

range from 71–154 nmol/L. The serum concentrations of T_3 were measured by RIA using the T_3 Riabead Kit (Abbott Labs.). The intra-assay coefficient of variation was 5.9% at 1.1 nmol/L and 4.4% at 2.8 nmol/L. Normal values range from 1.2–2.9 nmol/L. Serum concentrations of rT_3 were measured by RIA using the rT_3 Kit (Techland SA, Liege, Belgium). The intraassay coefficient of variation was 10.5% at 0.8 nmol/L and 10.3% at 4 nmol/L. Normal values range from 0.3–0.8 nmol/L.

Serum concentrations of PRL were measured by immunoradiometric assay using the PRL-IRMA Kit (Medgenix, Fleurus, Belgium). The intraassay coefficient of variation was 6.2% at 6.6 μg/L and 4.7% at 46.4 μg/L. The detection limit was 2.9 μg/L. Daytime normal levels for men are $\leq$9.9 μg/L, for females in the reproductive period $\leq$19.7 μg/L, elderly women $\leq$8 μg/L, and older men $\leq$4.9 μg/L. Sleep-associated nocturnal PRL concentrations range between 9–25 μg/L. Serum concentrations of GH in all profiles were measured by RIA using a polyclonal antibody (27). The intraassay 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.

Plasma concentrations of total IGF-I were measured by RIA, after acid-ethanol extraction. The intraassay coefficient of variation was 10.1% at 95 μg/L and 5.5% at 474 μg/L. The between-assay coefficient of variation was 14.8% at 109 μg/L and 10.1% at 389 μg/L. The detection limit was 10 μg/L. Normal range in healthy adults is 100–300 μg/L. Serum IGFBP-3 concentrations were measured by RIA as previously described (28), using antiserum R-100. The intraassay coefficient of variation was 6.2% at 2.5 mg/L and 5.5% at 5.7 mg/L, and the between-assay coefficient of variation was 11.9% at 2.9 mg/L and 14.5% at 6.3 mg/L. Normal ranges are 2.2 to 4.6 mg/L.

Serum ALS concentrations were assessed by RIA, as described elsewhere (29). The intraassay coefficient of variation was 3.4%, and the between-assay coefficient of variation was 10.5% at 5.3 mg/L and 5.4% at 24 mg/L. Normal ranges are 17–34 mg/L. Serum concentrations of cortisol were measured by RIA after extraction with dichloromethane. The intraassay coefficient of variation was 3.1% at 417 nmol/L. Normal ranges are 276–607 nmol/L at 0800 h, 0–276 nmol/L at 2000 h, and $<$50 nmol/L at 2400 h if asleep.

Data analysis

The sequential serum concentrations of TSH, PRL, and GH measured in each night profile were transformed into pituitary secretion profiles by eliminating the effect of metabolic clearance, using multiple parameter deconvolution analysis (30). This method is designed to compute hormonal half-life and the number, amplitude, and mass of underlying pituitary secretory bursts and to estimate tonic (basal or nonpulsatile) secretion (30).

Individual GH and PRL half-lives were determined in each study night and applied to the data series. For TSH, we determined a mean half-life in critical care conditions by deconvolving the distinct TRH-induced TSH peak (sampling at time 0, 20, 40, 60, and 120 min) measured in a group of subjects with similar study inclusion and exclusion criteria and using the same TSH assay (n = 10) (19). This mean TSH half-life (87 min) was applied to the data series for deconvolution analysis of the sequential serum TSH concentrations measured in each profile.

Besides mean serum concentrations, the following parameters were calculated for each hormonal profile in each subject: basal secretion rate [estimated as the amount of hormone that should be continuously released from the pituitary to achieve serum concentrations approximating the mean of the lowest 5% of all values observed, which is micrograms or milli-international units per liter of distribution volume (L_v) and per minute], amplitudes [maximal secretory rate (micrograms or milli-international units per L_v and per minute] and temporal positions of all secretory bursts, the mass of hormone secreted per burst [estimated as the area of the resolved secretion burst (micrograms or milli-international units per L_v)], and the mean pulsatile production [calculated as the product of the number of secretory bursts and the mean secretory burst mass over the time interval considered (micrograms or milli-international units per L_v over 9 h). The proportion of pulsatile secretion (percent) was calculated as the pulsatile production divided by the sum of the pulsatile and basal production, each expressed as micrograms or milli-international units per L_v over 9 h, multiplied by 100. The total GH production (μg/Lv per 9 h) was calculated as the sum of pulsatile GH production (μg/Lv over 9 h) and basal GH secretion ($\mu g/L_v \cdot min \times 560$ min).

Approximate entropy statistic (ApEn)

ApEn is a model-independent statistic for assessing regularity of time series (31, 32). ApEn measures a logarithmic likelihood that runs of patterns that are similar remain similar on next incremental comparisons. It assigns a single nonnegative number to a time series, with larger values corresponding to greater apparent process randomness. ApEn has been demonstrated to be stable to small changes in noise characteristics and to infrequent and significant artefacts. It detects variations in episodic behavior that are not reflected in changes in peak occurrences or amplitudes. Additionally, ApEn provides a direct barometer of feedback system change in many coupled systems. The calculation of ApEn was performed as previously reported (33). Because ApEn will generally increase with increasing process noise (and increasing intraassay variation), it is important to compare data sets with similar assay coefficients of variation, as performed in this study. To this end, we used a tolerance/threshold for ApEn of 0.2 times the series between sample SD, and a window/range of m = 1 consecutive samples over which to test for pattern reproducibility, as is appropriate for time series of $<$150 samples.

Data were analyzed using paired and unpaired Student's *t* test, and by multiple comparisons ANOVA, as appropriate. Nonnormally distributed data were logarithmically transformed before analysis. Comparison with the literature reference values for pulsatile fraction of

pituitary hormone release was performed using the two-tailed, one sample *t* test. Results are expressed as mean ± SEM unless indicated otherwise.

Results

Within each of the three study groups, the GH, TSH, PRL, and cortisol results of the cross-over subgroups were similar (Fig. 1, *lower*); accordingly, cross-over results within each group were pooled.

The releasing peptides were well tolerated. No side effects were noted during either of the peptide infusions, apart from an increased glucose intolerance during GHRP-2 and during GHRH + GHRP-2 infusions in those patients already receiving exogenous insulin infusion (Table 1); this was overcome by increasing the dose of infused insulin.

Thyroid axis

At study start, the patient population (n = 20) had a low serum T_4 (63 ± 5.2 nmol/L), a low T_3 (0.72 ± 0.08 nmol/L), and a mean rT_3 concentration just below the upper normal limit (0.78 ± 0.13 nmol/L). These variables were not different among the three groups.

TRH vs. *placebo.* The placebo profiles confirmed (4) that mean nightly serum concentrations of TSH were normal (0.92 ± 0.28 mIU/L), but that the fraction of TSH released in a pulsatile manner was reduced: 37 ± 6% *vs.* normal 65% (P = 0.002) (34, 35) (Fig. 2, *upper* and Fig. 3, *left*).

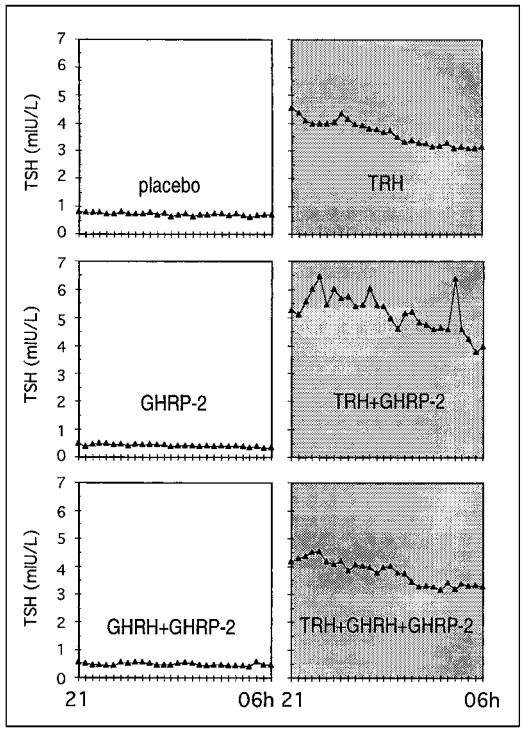

FIG. 2. Representative serum TSH concentration profiles from three critically ill men, age 69, 80, and 60 yr, respectively (two consecutive nights with sampling every 20 min between 2100–0600 h) are shown: Infusions containing TRH (1 μg/kg per h) increased nonpulsatile (basal) TSH secretion. Only when TRH was infused together with GHRP-2 (1 μg/kg per h) was pulsatile TSH secretion increased (*middle two panels*).

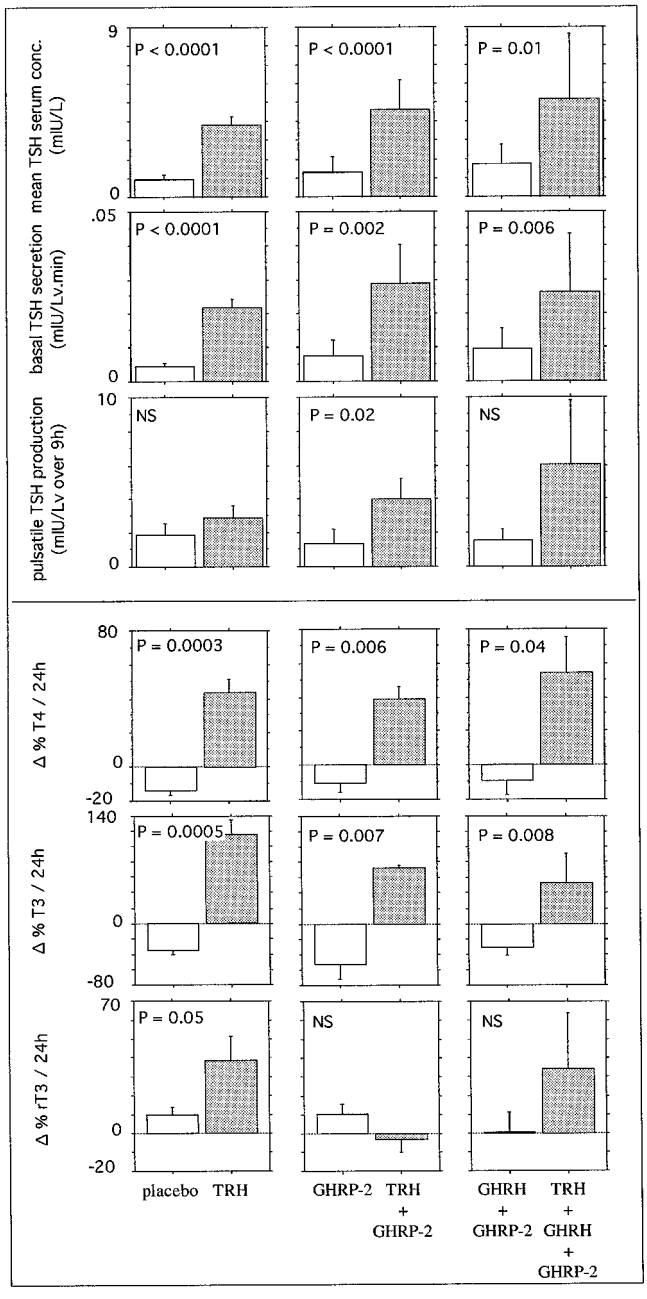

FIG. 3. *Upper*, Effects of TRH infusion (1 μg/kg per h) compared with placebo (n = 8), of addition of TRH (1 μg/kg per h) to GHRP-2 (1 μg/kg per h) infusion (n = 6), and of addition of TRH (1 μg/kg per h) to GHRH + GHRP-2 (1 + 1 μg/kg per h) infusion on mean serum TSH concentrations and basal and pulsatile TSH secretion are shown. *Lower*, Twenty four-hour changes in peripheral thryoid hormone levels in three study groups.

TRH infusion increased mean nightly TSH serum concentrations 4-fold: 3.84 ± 0.44 mIU/L *vs.* 0.92 ± 0.28 mIU/L, $P <$ 0.0001 (Fig. 2, *upper* and Fig. 3, *left*). This was mainly because of a 4-fold increase in TSH basal secretion: 0.022 ± 0.002 mIU/L_v·min *vs.* 0.005 ± 0.001 mIU/L_v·min, $P < 0.0001$. Although mean pulsatile TSH production was not significantly different, secretory burst amplitude (0.046 ± 0.015 mIU/L_v·min *vs.* 0.019 ± 0.009 mIU/L_v·min, P = 0.006) and secre-

tory burst mass (0.746 ± 0.099 mIU/L_v *vs.* 0.348 ± 0.123 mIU/L_v, $P = 0.05$) were higher during TRH infusion. The number of TSH bursts over 9 h was not significantly altered (3.9 ± 0.7 *vs.* 5.2 ± 0.4, $P = 0.1$).

When TRH was infused for 21 h after a placebo infusion, circulating T_4 increased by 44 ± 7% over 24 h, whereas it decreased by 14 ± 2% when placebo was infused after TRH, $P = 0.0003$ (Fig. 3, *left*). Similar changes were observed for circulating T_3: +116 ± 22% *vs.* −35 ± 5%, $P = 0.0005$. Circulating rT_3 increased more during TRH infusion than during placebo: +39 ± 13% *vs.* +10 ± 4%, $P = 0.05$.

During TRH infusion, the overnight decrease of serum TSH (−1.63 ± 0.34 mIU/L) was more pronounced ($P = 0.002$) than during placebo (−0.04 ± 0.07 mIU/L) (Fig. 4). Significant TSH decreases (a decrease in TSH that was at least twice the intraassay coefficient of variation) correlated ($R^2 = 0.86$, $P = 0.003$) with the circulating T_3 levels measured at the end of the TRH infusion (0600 h) (mean 1.86 ± 0.17 nmol/L) (Fig. 4).

The infusion of TRH did not alter the regularity in the TSH secretory pattern, as indicated by similar ApEn scores during TRH and placebo infusions (0.766 ± 0.061 and 0.887 ± 0.053, respectively, $P = 0.2$).

TRH + GHRP-2 vs. *GHRP-2.* The addition of TRH to the infusion of GHRP-2 increased mean nightly serum TSH concentrations 4-fold: 4.65 ± 1.56 mIU/L *vs.* 1.32 ± 0.78 mIU/L, $P < 0.0001$ (Figs. 2 and 3, *middle*). This was because of a 5-fold increase in basal TSH secretion (0.029 ± 0.011 mIU/L_v·min *vs.* 0.005 ± 0.0004 mIU/L_v−min, $P = 0.002$) as well as to a 4-fold increase in nightly pulsatile TSH production (4.023 ± 1.213 mIU/L_v *vs.* 1.112 ± 0.78 mIU/L_v, $P = 0.02$). The latter was because of a higher secretory burst amplitude (0.024 ± 0.005 mIU/L_v−min *vs.* 0.008 ± 0.003 mIU/L_v−min, $P = 0.002$), and secretory burst mass (1.003 ± 0.3 mIU/L_v *vs.* 0.324 ± 0.198 mIU/L_v, $P = 0.02$) during TRH + GHRP-2 infusion, whereas the number of TSH bursts remained unaltered (number of bursts per 9 h: 4.3 ± 0.4 *vs.* 4.8 ± 0.4, $P = 0.4$).

When TRH and GHRP-2 were infused after GHRP-2 alone, circulating T_4 increased by 40 ± 7% over 24 h, whereas it decreased by −10 ± 6% when GHRP-2 was infused after TRH + GHRP-2, $P = 0.006$ (Figs. 2 and 3, *middle*). Similar observations were made for circulating T_3: +73 ± 3% *vs.* −52 ± 24%, $P = 0.007$. The infusion of TRH together with GHRP-2 after a GHRP-2 infusion did not alter rT_3: −3 ± 6% over 24 h *vs.* +10 ± 4% for GHRP-2 infusion alone, $P = 0.2$.

The serum T_3 concentrations, measured at the end of the TRH + GHRP-2 infusion (1.11 ± 0.092 nmol/L) were lower ($P = 0.02$) compared with those obtained after TRH infusion alone (1.86 ± 0.17 nmol/L). There was no difference in overnight changes in serum TSH during TRH + GHRP-2 as compared with GHRP-2 infusion (−0.66 ± 0.34 mIU/L *vs.* −0.27 ± 0.15 mIU/L, $P = 0.4$). The addition of TRH did not alter the regularity of TSH secretion obtained during GHRP-2 infusion, as indicated by a constant ApEn score (0.867 ± 0.071 and 0.914 ± 0.049, respectively, $P = 0.5$).

TRH + GHRH + GHRP-2 vs. *GHRH + GHRP-2.* The addition of TRH to GHRH + GHRP-2 infusion increased mean nightly serum TSH concentrations 4-fold: 5.2 ± 3.4 mIU/L *vs.* 1.76 ± 1.01 mIU/L (median TSH 2.8-fold: 1.85 mIU/L *vs.* 0.66 mIU/L), $P = 0.01$ (Figs. 2 and 3, *right*). This was essentially because of a 2.6-fold increase in basal TSH secretion: 0.026 ± 0.017 mIU/L_v−min *vs.* 0.01 ± 0.006 mIU/L_v−min, $P = 0.006$. The number of TSH bursts over 9 h remained constant (4.3 ± 0.4 *vs.* 4.3 ± 0.6).

When TRH was infused together with GHRH + GHRP-2 for 21 h after a GHRH + GHRP-2 infusion, circulating T_4 increased by 54 ± 21%, whereas it decreased by −± 9% when GHRH + GHRP-2 was infused after TRH + GHRH + GHRP-2, $P = 0.04$ (Fig. 4). Similar observations were made for circulating T_3: +52 ± 38% *vs.* −31 ± 10%, $P = 0.008$. The infusion of TRH together with GHRH + GHRP-2 after a GHRH + GHRP-2 infusion did not raise rT_3 (+35 ± 29% *vs.* +0.7 ± 10%, $P = 0.3$).

The T_3 concentrations measured at the end of the TRH + GHRH + GHRP-2 infusion (0.88 ± 0.023 nmol/L), were lower ($P = 0.004$) than those measured during TRH + GHRP-2 (1.11 ± 0.092 nmol/L), and those obtained during the infusion of TRH alone (1.86 ± 0.17 nmol/L). There was no difference in overnight changes in serum TSH during TRH + GHRH + GHRP-2 as compared with GHRH + GHRP-2 infusion (−08 ± 1.80 mIU/L *vs.* −0.61 ± 0.44 mIU/L, $P = 0.4$). The addition of TRH did not alter the regularity of TSH secretion obtained during GHRH +

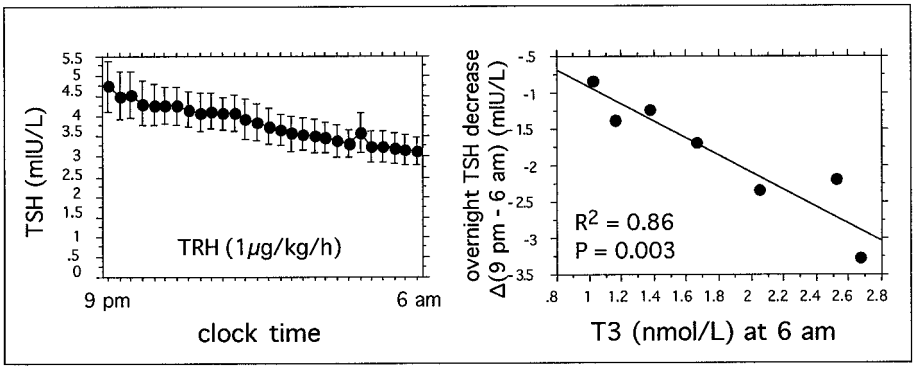

FIG. 4. Mean ± SEM serum TSH concentrations between 2100–0600 h during a TRH infusion (1 μg/kg per h) that was started at 0900 h (n = 8). In all but one patient, TSH concentration at 0600 h was significantly (at least twice variation coefficient of TSH assay) lower than at 2100 h. Overnight TSH decrease (difference between 0600 and 2100 h serum TSH concentration) correlated with serum T_3 concentration measured at end of TRH infusion (at 0600 h) ($R^2 = 0.86$ and $P = 0.003$), suggesting feedback inhibition.

GHRP-2 infusion, as indicated by similar ApEn scores (0.903 ± 0.059 and 0.792 ± 0.042, respectively, $P = 0.3$).

PRL

TRH vs. *placebo.* The placebo profiles confirmed (4) that mean nightly serum PRL levels were normal (19.5 ± 5.9 μg/L; median 11.4 μg/L and interquartile range 28 μg/L), and that PRL half-life was normal (63 ± 11 min), but that the fraction of PRL released in a pulsatile fashion ws reduced ($P = 0.03$): 31 ± 6% *vs*. normal 48% (36).

Surprisingly, TRH infusion did not increase serum PRL concentrations. In contrast, it suppressed pulsatile PRL production over 9 h (18.5 ± 6.8 $\mu g/L_v$ *vs*. 36.2 ± 11.6 $\mu g/L_v$, $P = 0.05$), whereas basal PRL secretion was maintained. TRH infusion did not alter the number of PRL secretory bursts over 9 h (TRH 5.0 ± 0.4 *vs*. placebo 5.7 ± 0.4, $P = 0.3$), the PRL half-life (71.8 ± 11 min *vs*. 63 ± 11 min, $P = 0.6$), or the regularity of the PRL secretory pattern, as indicated by the ApEn score (1.012 ± 0.057 *vs*. 0.911 ± 0.039, $P = 0.1$).

TRH + GHRP-2 vs. *GHRP-2.* The addition of TRH to the infusion of GHRP-2 induced a minute but consistent increase of mean nightly PRL serum concentrations: 17.3 ± 5.6 *vs*. 14.9 ± 5 μg/L, $P = 0.02$. This was exclusively because of an increase in basal PRL secretion: 0.210 ± 0.081 $\mu g/L_v \cdot min$ *vs*. 0.166 ± 0.072 $\mu g/L_v \cdot min$, $P = 0.03$.

Again, the number of PRL secretory bursts over 9 h (5.5 ± 0.2 *vs*. 5.8 ± 0.3, $P = 0.4$) as well as PRL half-life (60 ± 10 min *vs*. 58 ± 12 min, $P = 0.6$) remained unaltered. Moreover, the addition of TRH to GHRP-2 infusion significantly increased irregularity of the PRL secretory pattern, as indicated by a higher ApEn score ($P = 0.01$) during TRH + GHRP-2 infusion (0.97 ± 0.028) compared with that obtained during the infusion of GHRP-2 alone (0.856 ± 0.041).

TRH + GHRH + GHRP-2 vs. *GHRH + GHRP-2.* The addition of TRH to the infusion of GHRH + GHRP-2 also induced minute but consistent increase in mean nightly PRL serum concentrations: 23.5 ± 5.1 μg/L *vs*. 21.1 ± 5.6 μg/L, $P = 0.04$. This occurred without significant alterations of the different deconvolution-derived parameters including PRL half-life (67.9 ± 14.3 min *vs*. 68.1 ± 8.3 min, $P = 0.99$), and ApEn score (0.969 ± 0.021 *vs*. 0.892 ± 0.033, $P = 0.2$).

Somatotropic axis

At inclusion, the study population (n = 20) had low serum concentrations of IGF-I (86 ± 6 μg/L), IGFBP-3 (1.88 ± 0.12 mg/L), and ALS (7.21 ± 0.61 mg/L). These variables were not different among the three groups.

The placebo profiles (n = 8) confirmed that mean nightly GH concentrations were normal (2.3 ± 0.4 μg/L), and that GH half-life was normal (17.3 ± 1.6 min), but that the fraction of GH released in a pulsatile fashion was reduced ($P < 0.0001$): 48.1 ± 5.2% *vs*. normal 99% (3, 36). Moreover, the GH secretory pattern appeared to be relatively irregular, as indicated by a high ApEn score (0.868 ± 0.076) (37).

The addition of TRH to the infusions of either placebo, GHRP-2, or GHRH + GHRP-2 did not alter within 24 h any of the deconvolution-derived parameters of the GH secretory patterns (Fig. 5); the ApEn score; or serum IGF-I, IGFBP-3, or ALS (data not shown).

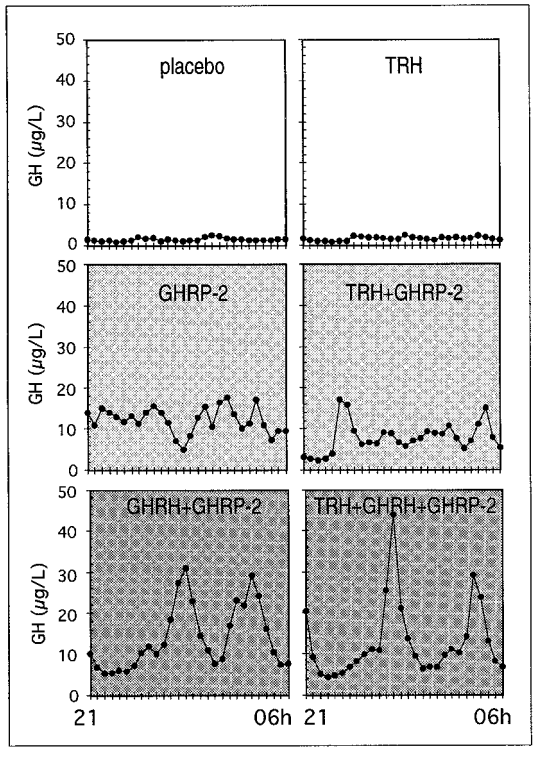

FIG. 5. Representative overnight serum GH concentration profiles obtained in three patients (one man age 69 yr and two women age 85 and 63 yr, respectively) during two consecutive nights, with sampling every 20 min between 2100–0600h. Infusion of TRH alone or in combination with GHRP-2 or GHRH + GHRP-2 did not detectably alter GH secretory patterns.

Consequently, GH secretion is reported over two study nights, and the generation of IGF-I, IGFBP-3, and ALS are described over 45 h during either placebo, GHRP-2, or GHRH + GHRP-2 infusion (Figs. 5 and 6). Nightly mean serum GH concentration was different in the three groups ($P = 0.005$): 2.8 ± 0.5 μg/L during placebo (±TRH), 17.3 ± 6.8 μg/L during GHRP-2 (±TRH), and 24 ± 4.1 μg/L during GHRH + GHRP-2 (±TRH).

Nightly pulsatile GH production was different in the three groups ($P = 0.008$): 29 ± 7 $\mu g/L_v$ over 9 h during placebo (±TRH), >6-fold higher during GHRP-2 (±TRH) infusion (180 ± 66 $\mu g/L_v$ over 9 h), and >10-fold higher during GHRH + GHRP-2 (±TRH) infusion (307 ± 85 $\mu g/L_v$ over 9 h).

Nightly basal GH secretion was different in the three groups ($P = 0.009$): 0.052 ± 0.007 $\mu g/L_v \cdot min$ during placebo (±TRH), >5-fold higher during GHRP-2 (±TRH) infusion (0.309 ± 0.131 $\mu g/L_v \cdot min$), and >7-fold higher during GHRH + GHRP-2 (±TRH) infusion (0.328 ± 0.074 $\mu g/L_v \cdot min$).

IGF-I change was different in the three groups ($P = 0.0004$): placebo (±TRH) infusion did not significantly alter the low serum IGF-I levels over 45 h: 91 ± 14 μg/L at study start and 98 ± 17 at the end of the second study night ($P = 0.6$).

Infusion of GHRP-2 (±TRH) increased serum IGF-I by 66% from 86 ± 8.1 μg/L to 143 ± 20 μg/L ($P = 0.01$). Infusion

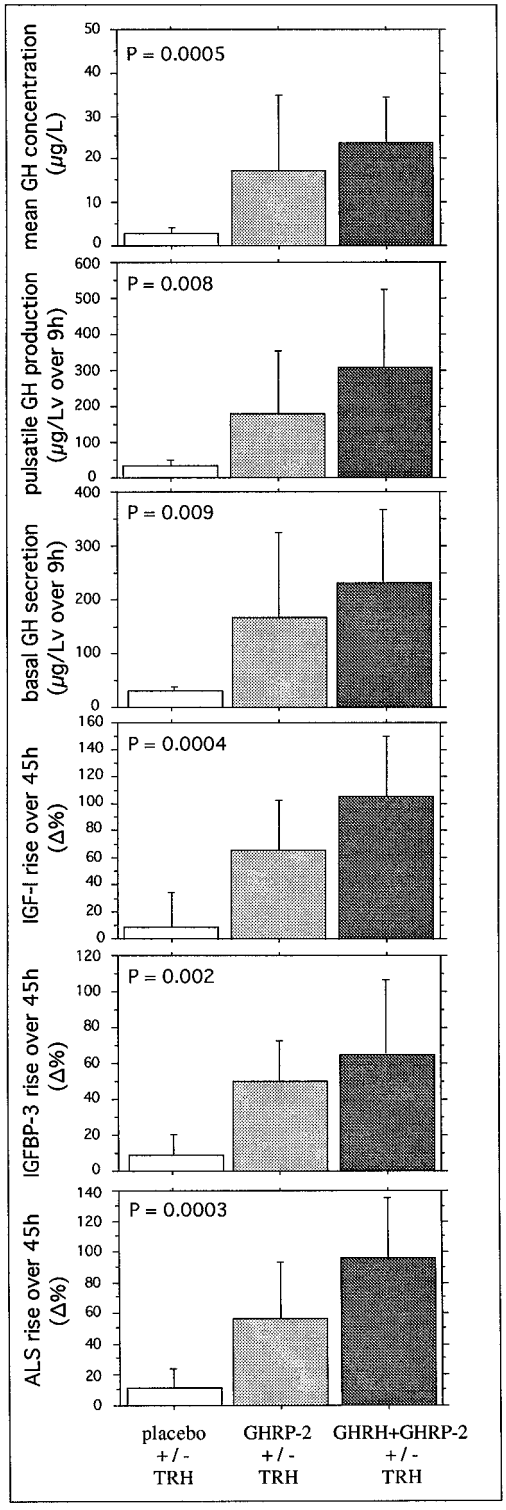

FIG. 6. Effect on mean serum GH concentration, pulsatile GH production, basal GH secretion, and increments over 45 h in IGF-I, IGFBP-3, and ALS, obtained with a 45-h infusion of placebo ± TRH (n = 8), a 45-h infusion of GHRP-2 ± TRH (n = 6), and a 45-h infusion of GHRH + GHRP-2 ± TRH (n = 6) were distinct as calculated with ANOVA. *Bars*, Represent means + 95% confidence interval.

of GHRH + GHRP-2 (±TRH) increased serum IGF-I by 106% from 78 ± 6.7 μg/L to 161 ± 17 μg/L ($P = 0.001$).

IGFBP-3 change was different in the three groups ($P = 0.002$): placebo (±TRH) infusion did not alter the low IGFBP-3 levels over 45 h: 2.11 ± 0.22 mg/L at study start and 2.32 ± 0.27 mg/L at the end of the second study night ($P = 0.1$). Infusion of GHRP-2 (±TRH) increased IGFBP-3 levels by 50% from 1.73 ± 0.08 mg/L to 2.61 ± 0.22 mg/L ($P = 0.004$). Infusion of GHRH + GHRP-2 (±TRH) increased IGFBP-3 levels by 65% from 1.73 ± 0.27 to 2.66 ± 0.20 mg/L ($P = 0.0003$).

ALS change was different in the three groups ($P = 0.0003$): placebo (±TRH) infusion did not alter the low ALS levels over 45 h: 8.53 ± 0.97 mg/L at study start and 9.61 ± 1.21 mg/L at the end of the second study night ($P = 0.12$). Infusion of GHRP-2 (±TRH) increased ALS levels by 56% from 6.97 ± 0.87 mg/L to 10.63 ± 1.33 mg/L ($P = 0.01$). Infusion of GHRH + GHRP-2 (±TRH) increased ALS levels by 96% from 5.68 ± 1.08 to 10.77 ± 1.83 mg/L ($P = 0.003$).

Increments over 45 h of IGFBP-3 and ALS correlated positively with serum IGF-I rise ($R^2 = 0.49$, $P = 0.0006$ and $R^2 = 0.42$, respectively, $P = 0.002$), as well as with each other ($R^2 = 0.64$, $P < 0.0001$).

An exponential regression line universally fitted the relationship between GH secretion and the 45-h increments in IGF-I, IGFBP-3, or ALS, as shown in Fig. 7.

Cortisol

The addition of TRH did not change mean nightly cortisol concentrations obtained during placebo (449 ± 27 nmol/L and 407 ± 23 nmol/L, respectively, $P = 0.1$), GHRP-2 (417 ± 76 nmol/L and 407 ± 74 nmol/L, respectively, $P = 0.4$), or GHRH + GHRP-2 infusion (484 ± 22 nmol/L and 477 ± 30 nmol/L, respectively, $P = 0.8$).

Discussion

The finding of a uniformly reduced pulsatile GH, TSH, and PRL secretion in a context of low IGF-I and thyroid hormone levels, and the observation of a spontaneous rise of serum TSH preceding recovery led to the hypothesis of the presence of a neuroendocrine component in the pathogenesis of the low activity status of the somatotropic and thyrotropic axes in the chronic, catabolic phase of critical illness (3, 4, 38). Continuous infusion of GHRP-2 or GHRH + GHRP-2 has previously been shown to amplify pulsatile GH secretion and to raise serum IGF-I in this condition (3). The present study reveals that adding TRH to the continuous infusion of GH secretagogues concomitantly activates the thyrotropic axis and PRL secretion, without attenuating the GH-releasing properties of the GH secretagogues. The substantial peripheral responsiveness to amplified GH and TSH secretion points towards a predominantly central, rather than peripheral, origin of the low circulating IGF-I and thyroid hormone levels in the chronic phase of intensive care dependency.

In healthy subjects, TRH infusion increases both basal and pulsatile TSH secretion (24). In prolonged critical illness, TRH infusion alone increased only basal TSH release. The mechanisms underlying the lack of effect on the pulsatile component are unclear, but may include an illness-induced

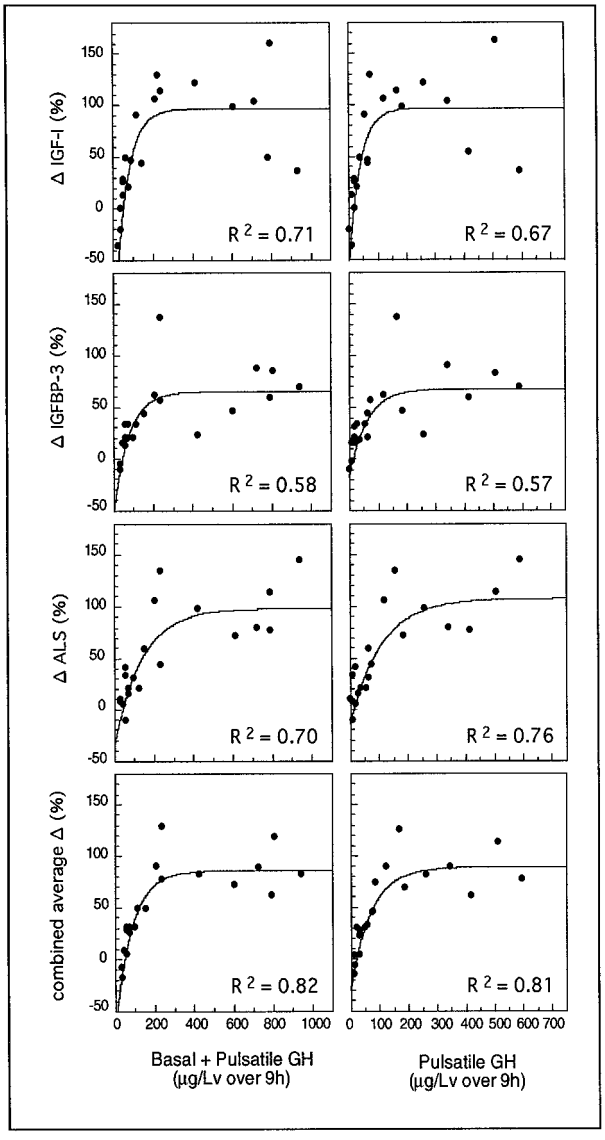

FIG. 7. Exponential regression of relationship between GH secretion and 45-h increments in IGF-I, IGFBP-3, or ALS. Δ IGF-I (percent) correlated with total (basal + pulsatile) GH production ($R^2 = 0.71$) and with pulsatile GH production ($R^2 = 0.67$). Δ IGFBP-3 (percent) correlated with total (basal + pulsatile) GH production ($R^2 = 0.58$) and with pulsatile GH production ($R^2 = 0.57$). Δ ALS (percent) correlated with total (basal + pulsatile) GH production ($R^2 = 0.70$) and with pulsatile GH production ($R^2 = 0.76$). Combined average Δ (percent) of IGF-I, IGFBP-3, and ALS (an index of responsiveness to GH) correlated with total (basal + pulsatile) GH production ($R^2 = 0.82$) and with pulsatile GH production ($R^2 = 0.81$). The regression lines indicate that severely impaired (pulsatile) GH secretion during prolonged critical illness allows circulating IGF-I to decrease progressively, whereas amplifying GH secretion with GH secretagogues substantially increases IGF-I levels, up to a certain level, above which further increase in GH secretion has little or no additional effect.

TRH resistance (39), or an increased suppressive tone on the thyrotropes, *e.g.* exerted by endogenous dopamine (5, 18, 40), somatostatin (41), or cortisol (42), all of which are known to suppress TSH pulse amplitude.

When TRH was infused together with GHRP-2, it also increased pulsatile TSH secretion, thereby normalizing the TSH response (24). This finding is consistent with the previously formulated hypothesis of a deficiency of the putative endogenous GHRP-like ligand in the catabolic condition of prolonged critical illness (3, 19). Further support for this hypothesis is provided by the PRL response to TRH infusion. When TRH is administered alone, PRL secretion is paradoxically suppressed, whereas coinfusion of GHRP-2 tends to normalize the PRL response to TRH. None of the peptide infusions altered TSH or PRL pulse frequency, indicating that neither TRH, GHRH, nor GHRP-2 are involved in the pacing of pulsatile TSH or PRL secretion.

Continuous TRH infusion results in a striking increase of serum T_4 and T_3. When TRH was infused alone, there was also a rise in circulating rT_3, indicating incomplete peripheral conversion of increased T_4 into T_3. When TRH was infused together with GHRP-2 or with GHRH + GHRP-2, the increase of serum T_3 concentrations was less pronounced but circulating rT_3 was not altered. This finding is in line with *in vitro* data indicating that GHRPs in large doses have a direct inhibitory effect on T_3 synthesis in human thyroid follicles (43). The observation that circulating rT_3 did not rise when TRH was infused together with GH secretagogues, suggests that the concomitant increase of GH secretion amplifies the efficacy of the peripheral conversion of increased T_4 into T_3, as has been defined in earlier independent studies of GH action (44).

The effect of TRH infusion in critically ill patients appears to be self-limiting; once serum T_3 approaches the upper-normal range, the TSH response to TRH decreases (Fig. 4). Thus, TRH infusion does not appear to overcome the negative feedback exerted by thyroid hormones at the pituitary level, which serves as a safety mechanism preventing overstimulation of the thyroid gland (45). Consequently, the continuous infusion of TRH, as compared with T_4 or T_3 administration, appears to be a theoretically safer strategy to drive the thyrotropic axis in prolonged critical illness. Continuous TRH infusion did not detectably alter any of GHs secretory characteristics during placebo, GHRP-2, or GHRH + GHRP-2 infusion, suggesting that the paradoxical GH response, previously observed after a bolus injection of TRH in critically ill patients, is a transient phenomenon (18).

GHRP-2 and GHRH + GHRP-2 infusions were found to elicit >6-fold and >10-fold increases of GH secretion, respectively, and to be associated with striking rises of serum IGF-I, IGFBP-3, and ALS, thus confirming previous findings over 21 h (3) and extending them over 45 h. Hence, the somatotropes of critically ill patients appear to maintain responsiveness to GH secretagogues over at least 45 h, despite increasing serum IGF-I, which has been shown to suppress GH release in the fasting human (46).

Close correlations were observed between total or pulsatile GH secretion on one hand and separate or combined serum markers (IGF-I, IGFBP-3 and ALS) of GH responsiveness on the other hand (47, 48). In addition to their regulation by GH, both IGF-I and ALS are regulated by nutritional status (49), although nutritional factors do not appear to have compromised the GH responsiveness in this study. The correlation between GH secretion and serum IGFBP-3 was somewhat weaker, suggesting that IGFBP-3 may be less directly GH dependent than IGF-I or ALS. Nutritional factors

and IGF-I itself may also affect IGFBP-3 (49, 50). Furthermore, IGFBP-3 may be subject to limited proteolysis in critically ill patients, which may affect its ability to complex with ALS and thus its stability in the circulation (51).

The aforementioned correlations between variables of GH secretion and indices of responsiveness to GH were obtained along exponential regression lines (Fig. 7). This relationship indicates that circulating IGF-I, IGFBP-3, and particularly ALS further increase in proportion to GH secretion up to a certain point, beyond which further increase of GH secretion has apparently little or no additional effect. It is noteworthy that the latter point corresponds to a pulsatile GH secretion of approximately 200 $\mu g/L_v$ over 9 h or less, a value that can be evoked by the infusion of GHRP-2 alone.

Together, these findings corroborate the retention of considerable responsiveness to GH, thus further delineating the distinct pathophysiological paradigm of the chronic, nonthriving phase of critical illness, as opposed to that of the acute phase, which is thought to be primarily a condition of GH resistance (52). In conclusion, the present investigations indicate that low serum T_4, T_3, IGF-I, IGFBP-3, and ALS levels in the chronic phase of critical illness may have at least in part a hypothalamic origin. The alterations occurring in the thyroid and somatotropic axes are reversed to a significant degree by the combined infusion of TRH and GH secretagogues. The extent to which this finding should be considered a pharmacological effect is at present unclear. It also remains to be determined whether this endocrine reactivation by intervening at the hypothalamic-pituitary level can be maintained over a longer interval, and whether it will enhance clinical indices of recovery from critical illness.

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