Estradiol supplementation selectively relieves growth hormone (GH)'s autonegative feedback on GH-releasing peptide -2 (GHRP-2)-stimulated GH secretion

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Abstract:

Female gender confers resistance to GH autonegative feedback in the adult rat, thereby suggesting gonadal or estrogenic modulation of autoregulation of the somatotropic axis. Here we test the clinical hypothesis that short-term E2 replacement in ovariprival women reduces GH's repression of spontaneous, GHRH-, and GH-releasing peptide (GHRP)-stimulated GH secretion. To this end, we appraised GH autoinhibition in nine healthy postmenopausal volunteers during a prospective, randomly ordered supplementation with placebo *vs.* E [1 mg micronized 17β-E2 orally twice daily for 6–23 d]. The GH autofeedback paradigm consisted of a 6-min pulsed iv infusion of recombinant human GH (10 μg/kg square-wave injection) or saline (control) followed by iv bolus GHRH (1 μg/kg), GHRP-2 (1μ g/kg), or saline 2 h later. Blood was sampled every 10 min and serum GH concentrations were measured by chemiluminescence. Poststimulus GH release was quantitated by multiparameter deconvolution analysis using published biexponential kinetics and by the incremental peak serum GH concentration response (maximal poststimulus value minus prepeak nadir). Outcomes were analyzed on the logarithmic scale by mixed-effects ANOVA at a multiple-comparison type I error rate of 0.05. E2 supplementation increased the (mean ± SEM) serum E2 concentration from 43 ± 1.8 (control) to 121 ± 4 pg/ml (E2) (158 ± 6.6 to 440 ± 15 pmol/liter; $P < 0.001$), lowered the 0800 h (preinfusion) serum IGF-I concentration from 127 ± 7.7 to 73 ± 3.6μ g/liter ($P < 0.01$), and amplified spontaneous pulsatile GH production from 7.5 ± 1.1 to 13 ± 2.3μ g/liter per 6 h ($P = 0.020$). In the absence of exogenously imposed GH autofeedback, E2 replacement enhanced the stimulatory effect of GHRP-2 on incremental peak GH release by 1.58-fold [95% confidence interval, 1.2- to 2.1-fold] ($P = 0.0034$) but did not alter the action of GHRH (0.83-fold [0.62- to 1.1-fold]). In the E2-deficient state, bolus GH infusion significantly inhibited subsequent spontaneous, GHRH-, and GHRP-induced incremental peak GH responses by, respectively, 33%

(1–55%; $P = 0.044$ *vs.* saline), 79% (68–86%; $P < 0.0001$), and 54% (32–69%; $P = 0.0002$). E2 repletion failed to influence GH autofeedback on either spontaneous or GHRH-stimulated incremental peak GH output. In contrast, E2 replenishment augmented the GHRP-2-stimulated incremental peak GH response in the face of GH autoinhibition by 1.7-fold (1.2- to 2.5-fold; $P = 0.009$). Mechanistically, the latter effect of E2 mirrored its enhancement of GH-repressed/GHRP-2-stimulated GH secretory pulse mass, which rose by 1.5-fold (0.95- to 2.5-fold over placebo; $P = 0.078$). In summary, the present clinical investigation documents the ability of short-term oral E2 supplementation in postmenopausal women to selectively rescue GHRP-2 (but not spontaneous or GHRH)-stimulated GH secretion from autonegative feedback. The secretagogue specificity of E's relief of GH autoinhibition suggests that this sex steroid may enhance activity of the hypothalamopituitary GHRP-receptor/effector pathway.

Keywords: endocrinology | pulsatile GH secretion | GH-releasing peptide | postmenopausal women

Article:

The hormonal milieu of healthy postmenopausal women is marked by concurrent E and GH deprivation (1–4). In the latter regard, organic GH deficiency states are associated with greater risk of cardiovascular disease, dyslipidemia, intraabdominal adiposity, relative sarcopenia, osteopenic fracture, and impaired psychosocial well-being (4, 5). Thus, the prominent decline in GH secretion in E-depleted postmenopausal women may contribute to certain adverse metabolic consequences of aging (4, 6).

In the human and experimental animal, GH (and IGF-I) controls its own secretion via so-called autonegative feedback (3). For example, in the human, administration of GH for several days (an intervention, which also significantly elevates plasma IGF-I concentrations) impairs the GH secretory response to GHRH (7). In addition, acute infusion of GH can repress exercise, sleep, clonidine, and GHRH- and GH-releasing peptide (GHRP)-stimulated GH secretion rapidly in the absence of any detectable elevation of circulating IGF-I concentrations (8–14). In the rat, the mechanism of GH autofeedback involves brain GH receptors and potentiation of hypothalamic somatostatin release (3, 52). This autoregulatory pathway appears to operate via analogous mechanisms in the human because in most clinical studies, pretreatment with putative inhibitors of somatostatin release, such as L-arginine or pyridostigmine, effectually relieves negative feedback exerted by GH (3, 15, 16).

The female rat shows remarkable resistance to GH autonegative feedback, compared with the male (3, 17). Although unproven, this gender difference may be because of gonadal factors and the more estrogenic milieu of the female (3, 18, 19). To test the latter postulate, the present study implements a GH autofeedback paradigm recently validated in healthy adults, wherein a single 6-min iv pulse of recombinant human GH consistently blunts spontaneous and peptidyl secretagogue-stimulated GH secretion 2 h later (10). To obviate possible confounding by nonestrogenic gonadal factors (18, 19), we examined E's impact on GH autoregulation in the postmenopausal (ovariprival) setting.

Abbreviations: CI, Confidence intervals; GHRP, GH-releasing peptide.

Materials and Methods

Subjects and study design

The study was approved by the Human Investigation Committee of the University of Virginia School of Medicine. Nine healthy postmenopausal women were studied after providing written informed consent. The mean (± SEM) age was 58 ± 3 yr, body mass index 24 ± 1.1 kg/m^2, and percentage body fat 37% ± 1.6% as assessed by hydrodensitometry (20). All subjects were nonsmokers. None was receiving systemic medications, with the exception of multivitamins, ferrous sulfate or gluconate, folate, ascorbic acid, cyanocobalamin, vitamin E, triamcinolone nasal spray (1 woman), or stable thyroid hormone replacement. Acetaminophen and ibuprofen were allowed for occasional treatment of arthritic pain or headache. Each woman had an unremarkable medical history and physical examination and normal screening laboratory tests of hepatic, renal, endocrine, metabolic, and hematologic function. Volunteers were clinically postmenopausal for at least 1 yr, as confirmed by an elevated serum FSH concentration (83 ± 12 IU/liter) and low E2 (16 ± 2 pg/ml or 66 ± 7 pmol/liter). Women previously taking E supplements were withdrawn from the hormone for at least 6 wk before entry into the study. There was no transmeridian travel (within 2 wk), night shift work, significant weight change (within 3 kg in 2 wk), acute illness, chronic disease, psychiatric illness, or substance abuse.

Volunteers were admitted to the General Clinical Research Center the evening before study to allow overnight adaptation and provision of a standardized meal. A constant eucaloric snack (turkey sandwich or vegetarian alternative) of 500 kcal containing 55% carbohydrate, 15% protein, and 30% fat was served on the evening of admission at 2000 h. To obviate food-related confounds, volunteers remained fasting after midnight until the end of sampling the next day. A regular meal was offered thereafter (at 1400 h) before discharge from the unit. Exercise activity was restricted during the admission, and the women were asked to sleep only after 2100 h and remain awake during sampling.

A prospectively randomized, patient-blinded, within-subject cross-over design was used. Volunteers were admitted to the inpatient study unit to evaluate GH secretory responses to iv bolus GHRH (1.0 μg/kg), GHRP-2 (1.0 μg/kg), or saline infusion administered 2 h after iv infusion of a single pulse of saline or recombinant human GH (10μ g/kg square-wave injection over 6 min) to impose autofeedback (10). Thus, each woman underwent a total of 12 admissions (six during placebo and six during E supplementation). Admissions were begun on day 6 of placebo or E treatment and were separated by a minimum of 48 h. Estrogen replacement consisted of 1 mg micronized 17-β E2 (Estrace) administered orally twice daily for 6–23 d. Each intervention arm was followed by a washout period of at least 6 wk to allow for physiological recovery.

The study design is illustrated in Fig. 1. Two iv catheters were inserted in forearm veins before or at 0600 h. One catheter was used for infusions and the other for blood sampling (1 cc) every 10 min from 0800 h to 1400 h. GH or saline (above) was infused at 0830 h. Secretagogues were administered 2 h later at 1030 h.

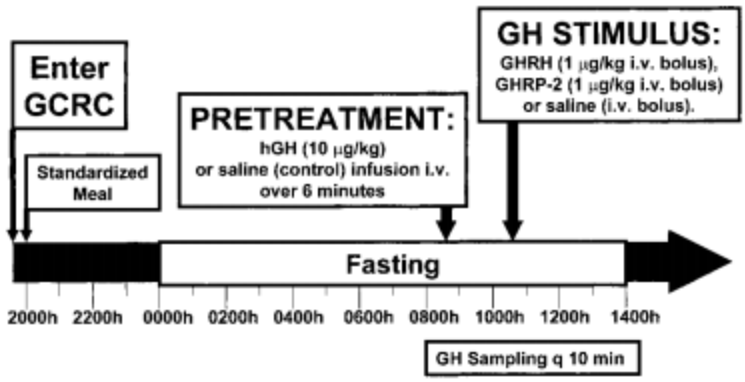

Figure 1. Prospectively randomized, placebo-controlled, within-subject cross-over experimental design to quantitate the autofeedback actions of GH on spontaneous (saline) and GHRH- and GHRP-2-stimulated GH secretion in healthy postmenopausal women supplemented with placebo or oral E2 for 6–23 d (see *Materials and Methods*).

Assays

Serum GH concentrations were measured in duplicate by automated GH chemiluminescence assay (modified Nichols Luma Tag hGH assay, Nichols Institute Diagnostics, San Juan Capistrano, CA) using 22 kDa human recombinant GH as assay standard. Sensitivity was 0.005 μg/liter with median dose-dependent inter- and intraassay coefficients of variation reported earlier (21). All 37 serum samples from each admission were assayed together. E2 and FSH concentrations were measured in morning (preinfusion) sera by automated chemiluminescence assay (ACS 180, Bayer Corp., Norwood, MA). IGF-I was measured by RIA after extraction in acid ethanol (Nichols Institute Diagnostics) (22).

Analysis of pulsatile GH release

Spontaneous pulsatile GH secretion during the 6-h saline infusion (double control day) and the summed mass of GH released after acute secretagogue stimulation were determined by multiparameter deconvolution analysis using previously validated two-component endogenous GH kinetics (23, 24). The latter comprised a rapid GH half-life of 3.5 min, a slow-phase GH half-life of 20.8 min, and a fractional (slow/total) decay amplitude of 0.63 (25).

Peak and incremental GH responses

As complementary measures, we also evaluated the simple peak (maximal) serum GH concentration and the incremental peak GH response (maximal serum GH concentration attained minus prepeak nadir value) during the 2-h poststimulus sampling period.

Statistical analysis

Data were transformed to the natural logarithmic scale before analysis to attain equal residual variation among treatment groups. Between-group comparisons are reported in terms of the fold change in the geometric mean with 95% confidence intervals (CIs) (26). Statistical computations were carried out in SAS version 6.12 with the mixed model software of PROC MIXED

(SAS/STAT 1997 changes and enhancements through release 6.12, SAS Institute, Inc., Cary, NC). Outcomes were analyzed by mixed-effects ANOVA. For each analysis, the model classification factors included the intervention type (two levels: either placebo or E), the feedback state (two levels: either saline or GH), and the secretagogue (three levels: saline, GHRH, or GHRP). When considered in the context of a split-split-plot design (27), subjects were viewed as the blocking factor, intervention as the level A factor, feedback state as the level B factor randomized within each level of factor A, and stimulus type as the level C factor randomized within each level of factor B. Parameter estimation was based on restricted maximum likelihood. The within-subject variance-covariance structure was estimated in the form of a compound symmetric matrix. Multiple comparison adjustment was based on a Fisher's least significant difference criterion with a type I error rate of 0.05. CIs are noted as 95% statistical limits.

Results

Figure 2 depicts group (mean ± SEM) serum GH concentration profiles monitored by sampling blood at 10-min intervals for 6 h in all nine women. Expanded y-axes in the insets illustrate GH responses over time to secretagogues administered 2 h after preinfusion of a single iv pulse GH or saline to quantitate GH autofeedback in the placebo and E2-supplemented milieus.

ANOVA revealed that morning (0800 h) serum E2 and IGF-I concentrations were invariant across study days 6–23 within the separate placebo and E2 replacement arms. Thus, a global mean value (n = six sessions) was calculated for these hormones in each volunteer. E2 replacement increased the latter serum E2 concentration from 43 ± 1.8 to 121 ± 4 pg/ml (158 ± 6.6 to 440 ± 15 pmol/liter; $P < 0.001$) and lowered the IGF-I concentration from 127 ± 77 to 73 ± 3.6 μg/liter ($P = 0.0096$). Deconvolution-estimated 6-h spontaneous pulsatile GH secretion during double saline control averaged 7.5 ± 1.1 (placebo) and 13 ± 2.3 μg/liter (NOREF>E2) ($P = 0.002$).

Table 1 summarizes simple absolute peak (maximal) poststimulus serum GH concentrations in each of the 12 study conditions. ANOVA disclosed that prior GH exposure ($P = 0.012$) and secretagogue type ($P < 0.001$) both influenced the observed simple peak serum GH concentration. The effects of GH infusion and secretagogue choice were strongly interactive ($P < 0.001$). E2 supplementation also determined the maximal GH response to secretagogues ($P = 0.035$) and interacted 3-fold with secretagogue and pretreatment type ($P = 0.043$). Following a single iv pulse of GH 2 h earlier, peak serum GH concentrations during the last 4 h of saline infusion (no secretagogue given) declined comparably to 2.8± 0.68 and 2.0 ± 0.59 μg/liter in the placebo and E2 sessions, respectively. Because these values were several-fold higher than those in the non-GH-infused sessions (above), further statistical analyses evaluated incremental (postsecretagogue peak minus preinjection nadir) or deconvolution-estimated GH secretory mass responses to adjust for GH baseline (see *Materials and Methods).*

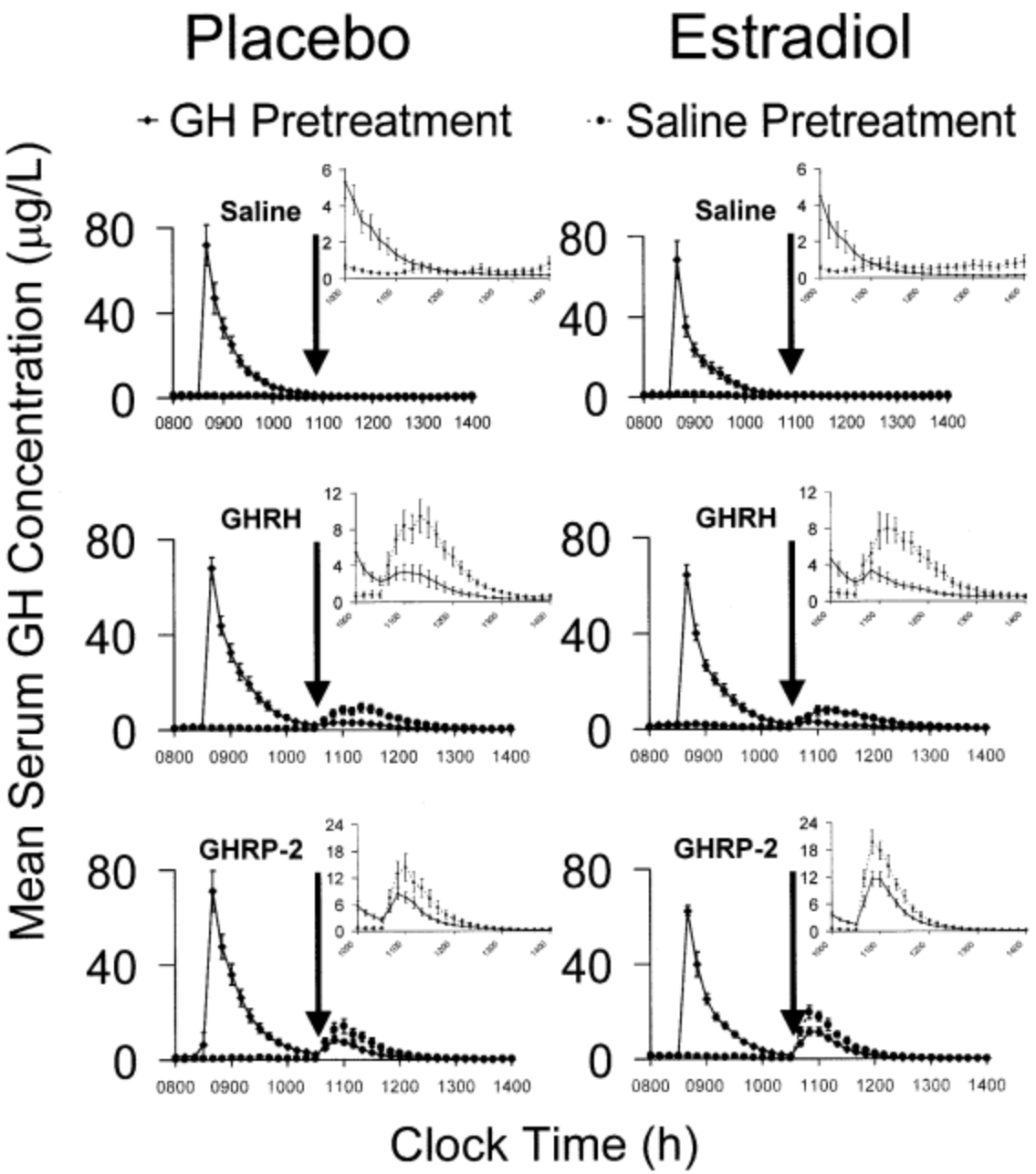

Figure 2. Group mean (± SEM) serum GH concentration profiles in nine postmenopausal women administered oral placebo *(left panels)* or E2 *(right panels)* in randomly assigned order for 6–23 d. Each study session comprised a sequential 6-min iv square-wave infusion of saline or recombinant human GH (10 μg/kg) at 0830 h to impose GH autonegative feedback followed 2 h later at 1030 h by bolus iv injection of saline, GHRH (1 μg/kg), or GHRP-2 (1 μg/kg) to stimulate GH secretion (*large arrow*) (see *Materials and Methods*). The *interrupted line* denotes the saline and the *continuous curve* the GH-predicted response. *Insets* depict the secretagogue responses.

Table 1. Absolute peak serum GH concentrations (baseline uncorrected) attained during placebo and E2 treatment stimulated by the injection of GHRH, GHRP-2, and saline 2 h after GH infusion to induce GH autofeedback

Stimulus	Saline preinfusion	GH preinfusion	*P* value[a]
Placebo			
Saline	0.88 ± 0.24	2.8 ± 0.68	<0.001
GHRH	11 ± 1.7	4.1 ± 1.0	0.012
GHRP-2	16 ± 3.1	9.0 ± 1.3	<0.001
E2			
Saline	1.2 ± 0.35	2.0 ± 0.59	0.042
GHRH	9.3 ± 1.96	3.6 ± 0.94	<0.001
GHRP-2	20 ± 2.4	12 ± 1.7	0.029

Values are the mean ± SEM (n = 9) peak serum GH concentrations (μg/liter) attained after the indicated stimulus.
[a] Multiple comparison test *P* values of the null hypothesis of no GH autonegative feedback effect on the indicated stimulus in the placebo *vs.* E2-replaced milieus. Analyses were based on a least significant difference criterion applied to the logarithms of the ratios of absolute peak serum GH concentration responses over 2 h. The indicated secretagogues were infused 2 h following a 6-min iv pulse of GH *vs.* saline (see *Materials and Methods*).

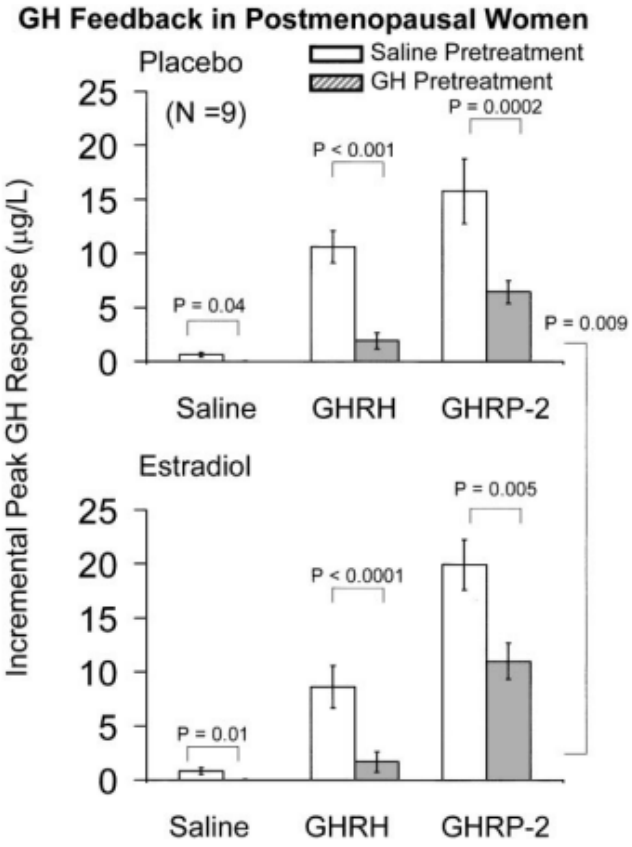

Figure 3. Impact of placebo (top) *vs.* E2 (bottom) administration on incremental peak serum GH concentration responses micrograms/liter) to saline, GHRH, and GHRP-2 injected by iv bolus 2 h following the 6-min pulsed infusion of recombinant human GH (*vs.* saline) to induce autonegative feedback. Inset *P* values denote contrasts between saline and GH pretreatment, except for *P* = 0.009. The latter compares the GHRP-2 effect in the face of GH autofeedback for E *vs.* placebo ingestion. Data are the mean ± SEM (n = 9).

Figure 3 highlights the effects of specific secretagogues on incremental peak GH responses assessed during placebo and E2 supplementation. In the absence of GH autofeedback, E2 administration potentiated the stimulatory effect of GHRP-2 on the incremental peak GH response by 1.58-fold (CI 1.2- to 2.1-fold) that observed during placebo ingestion ($P = 0.009$). In contrast, E2 replacement did not alter the incremental peak GH response to GHRH, which was 0.83-fold (CI 0.62- to 1.1-fold) the placebo value.

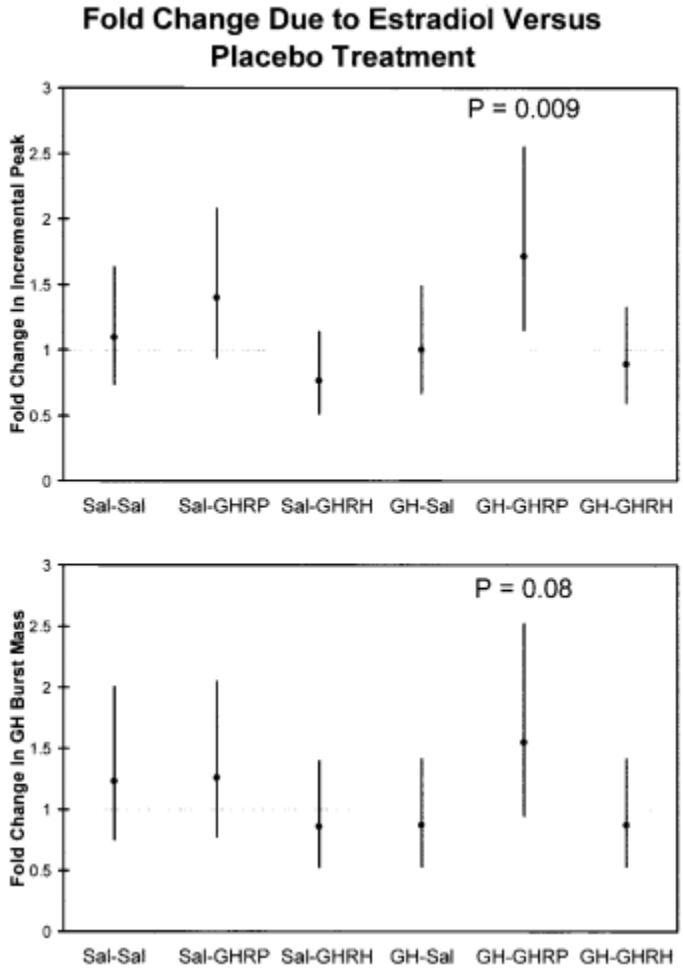

Figure 4. Fold changes (±95% CI) in incremental peak serum GH concentrations (*top panel*) and the summed mass of GH secreted per 2 h (*bottom panel*) in response to bolus saline, GHRH, or GHRP-2 injection for E *vs.* placebo pretreatment in nine women (see *Materials and Methods*). *P* values denote probability of falsely rejecting the null hypothesis of no effect of E for the indicated intervention.

During placebo ingestion, prior infusion of GH suppressed spontaneous (saline-infused), GHRH, and GHRP-2-induced incremental peak GH responses by 33% (CI 1–55%; $P = 0.044$ *vs.* saline preinfusion), 79% (CI 68–86%; $P < 0.0001$), and 54% (CI 32–69%; $P = 0.0002$), respectively. Thus, autoinhibition of saline was less than that of GHRH. During E2 replacement, pretreatment with GH repressed secretagogue-stimulated incremental peak GH responses by 39% (10–59%) (spontaneous GH release, $P = 0.014$ *vs.* saline pretreatment), 75% (63–83%; GHRH

stimulus, $P < 0.0001$), and 44% (17–62%; GHRP-2 stimulus, $P = 0.0046$) (Fig. 4, A). Thus, in the E2-enriched milieu, GHRP-2 was less inhibited than GHRH. E2 supplementation augmented the GHRP-2-stimulated incremental peak GH response in the face of GH autofeedback by 1.7-fold (CI 1.2- to 2.5-fold), compared with placebo administration ($P = 0.009$) without altering spontaneous GH release or that stimulated by GHRH.

Deconvolution-estimated (postsecretagogue summed) GH secretory burst mass was used as a complementary analytical technique to correct for baseline (presecretagogue infusion) serum GH concentrations following iv GH *vs.* saline infusions (above). Table 2 shows the percentage reduction of this measure owing to GH autoinfusion in the E2-deficient and E2-sufficient states. During placebo administration, GH infusion inhibited subsequent GH secretory-burst mass responses to all three secretagogues in the rank order of GHRH (mean 80% inhibition), GHRP-2 (51%), and saline (44%), wherein inhibition of GHRH significantly exceeded that of saline. During E2 replacement, the rank order was GHRH (79%), saline (60%), and GHRP-2 (39%), wherein autofeedback on GHRH was significantly greater than that on GHRP-2. Figure 4, B illustrates corresponding fold changes in the poststimulus summed GH secretory burst mass during E2 *vs.* placebo supplementation. During GH autofeedback, supplementation with E2, compared with placebo, increased the mass of GH secreted after the GHRP-2 stimulus by 1.5-fold (CI 0.95- to 2.5-fold; $P = 0.078$). In contrast, the corresponding effects of E2, compared with placebo, on the saline and GHRH stimuli were 0.87-fold (0.53- to 1.4-fold) and 0.87-fold (0.53- to 1.4-fold; $P = 0.57$ each).

Table 2. Impact of E2 supplementation on autofeedback inhibition of summed GH secretory burst mass

Stimulus	**Prior intervention**			
	Control	***P* value**[a]	**E2**	***P* value**[a]
Saline	44 (11–64)[b]	0.014	60 (37–75)	<0.0001
GHRH	80 (68–87)[b]	<0.0001	79 (67–87)[b]	<0.0001
GHRP-2	51 (22–69)	0.0026	39 (5–61)[b]	0.031

Data are percentage suppression with 95% statistical confidence intervals.

[a] *P* values test the null hypothesis of no detectable GH autofeedback.

[b] $P < 0.05$ for the comparison between the indicated paired values within a column.

Figure 5 highlights the dispersion among the nine volunteers of individual incremental peak serum GH concentration responses to E in the GHRP-2-stimulated GH-autofeedback setting ($P = 0.009$).

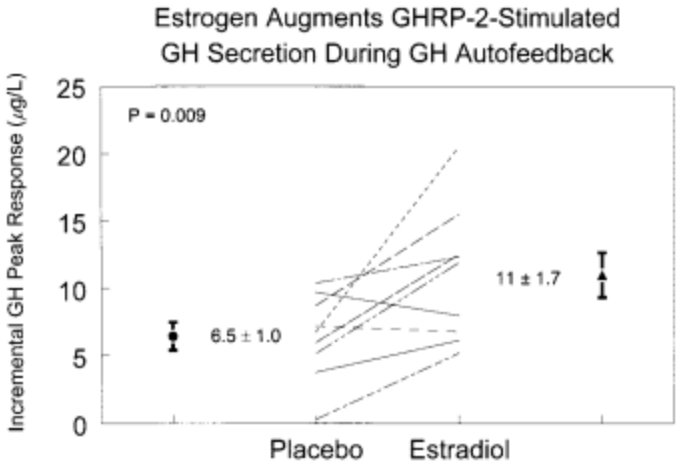

Figure 5. Dispersal of individual incremental peak serum GH concentration responses owing to E *vs.* placebo intervention in nine postmenopausal women. The incremental peak GH response was defined as the within-subject algebraic difference between the GHRP-2-stimulated maximal (peak) and the prestimulus nadir serum GH concentration in each woman. Volunteers were monitored for 2 h following GH infusion to impose autofeedback (Fig. 1, see *Materials and Methods*). Data values are the mean ± SEM ($P = 0.009$).

Discussion

The present clinical investigation of GH autonegative feedback demonstrates that a single iv pulse of recombinant human GH rapidly and significantly suppresses exogenous GHRH- and GHRP-2-stimulated and spontaneous pulsatile GH secretion in postmenopausal women. The degree of autoinhibition of GHRH-stimulated GH secretion averaged 79% (68–88%), compared with 33% (1–55%) for spontaneous GH release and 54% (32–69%) for GHRP-2-induced GH release. Relative sparing of GH's autorepression of the GHRP stimulus was also observed in healthy young men and children (3, 11, 12). The lesser susceptibility of the GHRP (than GHRH) stimulus to GH autonegative feedback could indicate that GH autoregulation in the human axis does not block GHRP-stimulated endogenous GHRH release because the latter typically synergizes with and is released by GHRPs (3, 28). In sheep, GHRP infusion triggers hypothalamic GHRH secretion into hypophyseal portal blood in the absence of GH autofeedback (29, 30). Alternatively, the preferential escape of GHRP-2 from autoinhibition by GH could reflect partial antagonism of endogenous somatostatin action by GHRP-2 (3, 16, 31–35).

Recent cloning of a specific GHRP receptor and cognate endogenous ligand, ghrelin, in the rat and human and expression mapping of this pathway have distinguished GHRP from GHRH-dependent signaling systems (3, 16, 36–38). In the present study, E2 potentiated the actions of GHRP-2 but not GHRH. This secretagogue specificity would be consistent with E's minimal (or in some cases inhibitory) effects on GHRH gene and/or GHRH receptor expression in the rodent (2, 3, 16, 39–42). In contrast, E may modulate GHRP-receptor/effector activity in the postnatal and pubertal rat and the GH-transgenic mouse (3, 16). In the human, administration of (ethinyl) E2 for 3–10 d amplifies acute GHRP-stimulated GH release by nearly 2-fold in prepubertal girls and postmenopausal women (43, 44). In addition, the stimulatory effects of GHRPs are maximal in the sex-steroid enriched milieu of mid-to-late puberty (44, 45). Although hypothalamic (but not pituitary) binding of GHRP analogs declines in aging humans (46), the promoter for the

GHRP-receptor gene contains a putative hemi-E-responsive element (47). Thus, GHRP receptor expression might be upregulated by E2. On the other hand, GH secretion evoked by a single iv bolus of a GHRP-receptor agonist did not vary across the menstrual cycle (48), differ in men and women (48), or increase following prolonged low-dose (0.05 mg/d) transdermal E2 delivery in postmenopausal women (49). Thus, further clinical investigations will be important to clarify whether and how E regulates GHRP-receptor/effector activity.

An alternative notion that E acts to restrict hypothalamic somatostatin release and thereby augment GH secretion is not supported by the majority of experimental data in the rodent (2, 3, 16, 39, 40). Fewer data are available to address this issue directly in the human (2, 3). Although short-term E2 supplementation can reduce the submaximal inhibitory effects of infused somatostatin in postmenopausal women (50), the failure of E to amplify GHRH-stimulated or spontaneous GH secretion speaks against substantial withdrawal of somatostatinergic repression during GH autofeedback. This reasoning assumes that GH autonegative feedback is mediated via somatostatinergic restraint (above). Indeed, putative inhibitors of somatostatin outflow, such as the cholinergic agonist, pyridostigmine, and the amino acid, L-arginine, mute GH autonegative feedback in the human (15, 51). The present results also argue against (but do not exclude) E-induced release of an endogenous GHRP, like ghrelin, which would be expected to synergize with exogenous GHRH (28, 53, 54). Whether E might facilitate hypothalamic secretion of GHRH cannot be ascertained from the present data.

Brain GH receptor density declines with aging in humans (55). Estrogen downregulates hepatic GH receptors in the rat and rabbit but upregulates central nervous system GH receptor expression (2, 3, 56). Because increased GH receptor expression would be expected to enhance GH-receptor- dependent autonegative feedback, the former would not explicate E2's rescue of GHRP-2-induced GH secretion.

The ability of E to impede GH autonegative feedback is consistent with its stimulation of irregular patterns of GH release, as monitored by higher approximate entropy in the female (18, 19, 22, 57, 58). In particular, attenuated negative feedback *per se* evokes more disorderly GH secretion (57, 59–61). Conversely, heightened negative feedback imposed by the infusion of somatostatin or IGF-I promotes more orderly GH output (50, 60). Accordingly, E's stimulation of more irregular GH release may reflect its ability to mute GH autonegative feedback and/or reduce IGF-I negative feedback (below).

Oral E2 administration lowered plasma IGF-I concentrations by approximately 40%, as observed elsewhere (3, 22, 57, 59, 62, 63). This effect of E may be important mechanistically here because IGF-I can exert autoinhibitory effects on both the hypothalamus and pituitary gland (31–35, 64–67). Thus, withdrawal of IGF-I negative feedback during E exposure may contribute to the amplification of GHRP-2-stimulated GH secretion (43). Of note, a single sc injection of recombinant human IGF-I suppresses exogenous hexarelin, more than GHRH-stimulated GH secretion in healthy adults (68). This differential susceptibility of GHRP (over GHRH) actions to inhibition by IGF-I negative feedback could account for the preferential relief of GH autofeedback on GHRP-2 observed here.

In summary, short-term oral E2 replacement in postmenopausal women augments pulsatile GH secretion and selectively mutes GH autonegative feedback on GHRP-2-stimulated GH release. Spontaneous and GHRH-driven GH secretion are not affected by pretreatment with a dose of this sex steroid. E2-dependent amplification of GH secretion could thus reflect facilitation of endogenous GHRP-receptor/effector signaling. This mechanism may also explicate GHRP's more pronounced stimulation of GH secretion in the E-enriched milieu of normal puberty in girls.

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