Age-specific changes in the regulation of LH-dependent testosterone secretion: assessing responsiveness to varying endogenous gonadotropin output in normal men

Peter Y. Liu, Paul Y. Takahashi, Pamela D. Roebuck, Ali Iranmanesh, and Johannes D. Veldhuis. Age-specific changes in the regulation of LH-dependent testosterone secretion: assessing responsiveness to varying endogenous gonadotropin output in normal men. Am J Physiol Regul Integr Comp Physiol 289: R721–R728, 2005. First published May 12, 2005; doi:10.1152/ajpregu.00138.2005.—Pulsatile and thus total testosterone (Te) secretion declines in older men, albeit for unknown reasons. Analytical models forecast that aging may reduce the capability of endogenous luteinizing hormone (LH) pulses to stimulate Leydig cell steroidogenesis. This notion has been difficult to test experimentally. The present study used graded doses of a selective gonadotropin releasing hormone (GnRH)-receptor antagonist to yield four distinct strata of pulsatile LH release in each of 18 healthy men ages 23–72 yr. Deconvolution analysis was applied to frequently sampled LH and Te concentration time series to quantitate pulsatile Te secretion over a 16-h interval. Log-linear regression was used to relate pulsatile LH secretion to attendant pulsatile Te secretion (LH–Te drive) across the four stepwise interventions in each subject. Linear regression of the 18 individual estimates of LH–Te feedforward dose-response slopes on age disclosed a strongly negative relationship \( r = -0.721, P < 0.001 \). Accordingly, the present data support the thesis that aging in healthy men attenuates amplitude-dependent LH drive of burst-like Te secretion. The experimental strategy of graded suppression of neuroglandular outflow may have utility in estimating dose-response adaptations in other endocrine systems.

The interconnected nature of the hypothalamo-pituitary-gonadal axis makes verifying putative deficits in the individual regulation of GnRH, LH, and Te secretion difficult in vivo (23, 24, 34, 37, 62, 65). Analytical estimation of endogenous LH-driven Te secretion illustrates a noninvasive approach to examine such issues (20, 25). Ensemble model-based predictions provide another investigative strategy (21, 22, 24). In contrast, no direct experimental assessment exists of how age modifies feedforward drive of pulsatile Te secretion by endogenous LH pulses. Appraising the actions of endogenous LH secretory bursts is significant physiologically, because the properties of LH release may be altered in aging, and the implications of such changes may not be determinable from the effects of fixed exogenous stimuli; and 2) the half-life of injected hCG is >15-fold longer and its lutropic potency multifold greater than that of LH, thereby downregulating Leydig cell steroidogenesis in the human and animal (49, 54). In principle, optimal experiments would require extraction of plasma LH to faithfully represent in vivo isoforms (4, 58), suppression of ongoing LH secretion, and reinfusion of aging pattern-specific pulses of purified LH in the donor animal.

As an alternative strategy to probe endogenous LH–Te feedforward, the present paradigm enforced four different strata of pulsatile LH output by graded inhibition of hypothalamic GnRH action (9, 26, 40). To this end, we administered saline and three escalating doses of a potent, specific, and long-acting GnRH-receptor antagonist on separate randomly ordered days and three escalating doses of a potent, specific, and long-acting GnRH-receptor antagonist on separate randomly ordered days. Diminished GnRH drive and decreased testicular steroidogenesis may contribute to relative androgen depletion in elderly men also. Indirect clinical evidence in this regard includes: 1) normal or minimally elevated LH concentrations in the face of low Te concentrations (32, 33, 59, 62, 65); 2) normal or enhanced luteinizing hormone (LH) secretion when stimulated by exogenous GnRH pulses (35, 67); 3) reduced Te concentration responses to a pharmacological human chorionic gonadotropin (hCG) stimulus (16, 30, 38, 43, 65) or to recombinant human LH (36); and 4) reduced Leydig cell number (39). In contrast, the elimination kinetics of LH and unbound (free) Te are comparable in young and older subjects (23, 25, 36). Whether altered sex-steroid negative feedback further accentuates the aging-related decrease in LH and Te secretion is not clear (34, 37, 48, 64).

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forward relationship between pulsatile LH and pulsatile Te secretion over a wide range of endogenous LH drive.

METHODS

Sampling protocol. Eighteen men aged 23 to 72 yr (mean 45, 2–4 men/decade) participated in the study after providing written voluntary informed consent approved by the Mayo Clinic Institutional Review Board. Participants were healthy community-dwelling men within 20% of ideal body weight, who had not undertaken recent transmeridian travel (within 10 days) or consumed alcohol, caffeine, or systemic medications within eight biological half-lives. Detailed medical inventory excluded a history of infertility, systemic disease, recent weight change (exceeding 2 kg in the preceding 6 wk), hormonal therapy, or psychoactive drug use. Outpatient screening was unremarkable in relation to medical history (particularly libido and erectile function), physical examination (including testis size), and fasting morning (0800) biochemical tests of renal, hepatic, hematological, and metabolic function (plasma glucose and thyroid hormones).

Volunteers were admitted to the General Clinical Research Center for four separate overnight inpatient studies each scheduled at least 7 days apart. Blood samples (1.0 ml) were withdrawn every 10 min beginning at 1800 for a total of 18 h through a forearm intravenous catheter for later assay of serum LH and Te concentrations. Blood was allowed to clot at room temperature, and sera were frozen at −20°C. In each study session, one dose of 0 (saline), 0.1, 0.3, or 1.0 mg/m² ganirelix was administered subcutaneously at 2000 (2 h after beginning blood sampling) in a prospectively randomized double-blind fashion.

GnRH-receptor antagonist. Ganirelix is a potent, specific, and competitive GnRH-receptor antagonist used in ovulation induction protocols (1, 42). Time course studies in young men have indicated that ganirelix suppresses pulsatile LH and Te secretion by >80% in 4–8 h and maintains comparable suppression thereafter for an additional 20–24 h (52). The duration of inhibition is consistent with the plasma half-life of this peptide of 15 ± 2 h (42). Consequently, the plateau of LH and Te output was taken to be 8 h in duration beginning 8 h after ganirelix or saline injection.

Assays. LH and Te concentrations were measured in duplicate by automated immunochemiluminometry (ACS Corning, Bayer, Tarrytown, NY; see Refs. 53, 55, and 61). The LH standard was 80/552 defined against the Second International Reference Preparation. Concentration-dependent intra-assay coefficients of variation (CVs) averaged 4.7, 3.5, and 3.8% and interassay CVs 8, 3.7, and 4.7% at LH concentrations of 4.4, 18.2, and 38.8 IU/l, respectively. Procedural sensitivity was 0.05 IU/l. Te was quantitated analogously at median intra- and interassay CVs of 6.8 and 8.3% and a sensitivity of 8 ng/dl. Free Te concentrations were computed directly from measured albumin and sex hormone binding globulin concentrations, as described previously (35).
End points. Model-free end points were absolute minima and maxima of LH and Te concentration time series within the 16-h interval after saline/ganirelix administration. Minima and maxima were calculated from a five-point moving average.

Model-based outcomes were the sum of LH and Te secretory-burst mass values (pulsatile LH and Te secretion) over the 8-h interval, comprising inclusively 9–16 h after injection of saline or ganirelix. This time window encompasses stable (time-invariant) suppression of LH and Te concentrations (52). Stability was defined as a zero slope of the linear regression of LH or Te concentrations on time.

Analytical methods. Pulsatile secretion was estimated by multiparameter deconvolution analysis of each 18-h concentration profile (50). The entire 18-h concentration profile was deconvolved since, at any given time, the blood concentration reflects both current and prior secretion that is undergoing biexponential elimination. Hence, the most accurate estimates of pulsatile secretion are obtained by deconvolution of the full concentration series. However, statistical comparisons were made on values contained within the last 8 h of sampling. The deconvolution model assumed underlying Gaussian-like secretory bursts superimposed on time-invariant (nonpulsatile) basal LH and Te secretion, statistically conditioned on preestimated pulse times (57), and directly measured biexponential kinetics of LH and Te disappearance in men (17, 56). Deconvolution data are given as the mass of LH (IU) and Te (ng) secreted in bursts per unit distribution volume (liters for LH and dl for Te) in each subject over the 8-h time interval. Apparent distribution volumes of LH approximate the plasma space (51, 52), and those of Te vary among study techniques (7, 17).

Statistics. The primary postulate is that age decreases the capability of varying amplitudes of endogenously sustained LH pulses to stimulate burst-like Te secretion. To estimate feedforward drive as a function of LH output across the four experimental clamps, logarithmically transformed pulsatile Te secretion rates were regressed linearly on pulsatile LH secretion rates in each volunteer. The resultant slope estimate represents fractional stimulation of pulsatile Te production by a unit increase in pulsatile LH secretion. To test the impact of age on fractional LH-TS stimulation, the 18 slope estimates were regressed linearly on age.

Two-way ANCOVA was used to test the joint impact on pulsatile Te secretion of pulsatile LH secretion (3 fixed effects) and age (1 random effect), wherein the covariate was the response to saline. Homogeneity of slopes was tested at $P < 0.05$ by an F-statistic (27). Data are given as means ± SE or ± SD (slopes). Analyses were performed using SAS version 8.02 (SAS Institute, Cary, NC).

RESULTS

All 18 men completed all 4 visits. Potentially, 15,696 separate LH and Te measurements were available, of which ~0.5% were missing. Mean LH and total Te concentrations determined over the last 4 h of sampling on the saline day in the cohort of 18 men were $3.3 ± 0.36$ IU/l and $471 ± 39$ ng/dl (respective normal ranges are $1.2–10$ IU/l and $300–950$ ng/dl). Mean concentrations of LH and total Te did not differ with age, consistent with other reports in healthy populations (29). However, the logarithm of calculated free and bioavailable Te concentrations decreased linearly with age (both $P < 0.025$). Figure 1 illustrates Te concentration profiles in one individual aged 25 (left) and another aged 64 (right) yr, reflecting each of the four interventions studied. Data represent measurements made every 10 min beginning 2 h before (1800) and continuing for 16 h after subcutaneous injection of randomly ordered doses of ganirelix vs. saline. Increasing doses of the GnRH-receptor inhibitor progressively suppressed Te concentrations.

Figure 2 summarizes dose-dependent inhibition of maximal (peak) and minimal (nadir) LH concentrations by the GnRH-receptor antagonist in the entire study cohort ($P < 0.001$ vs. saline effect for each). Comparable inhibitory effects were observed for LH ($P < 0.001$). The time delay to reach minimal LH and Te concentrations was prolonged significantly compared with saline ($P < 0.01$) but was independent of ganirelix dose [global mean 760 ± 58 (LH) and 817 ± 46 (Te) min after ganirelix vs. 583 ± 85 (LH) and 308 ± 89 (Te) min after saline injection].

Deconvolution analysis was used to estimate pulsatile LH and Te secretion over the 8-h window before the end of sampling. This interval reflects stable suppression by ganirelix (52, 60). Table 1 gives mean pulsatile LH and Te secretion at each plateau and demonstrates that the inhibitory effect of ganirelix was dose dependent for both pulsatile Te ($P < 0.01$) and pulsatile LH ($P < 0.001$) secretion.

Figure 3 illustrates log-linear regression of the four strata of pulsatile Te secretion (dependent variable) on pulsatile LH secretion (independent variable) in the 18 individual subjects (ages shown). Each slope provides an estimate of the fractional response of pulsatile Te secretion to a unit increase in pulsatile LH secretion. The mean in the cohort was $9.4 ± 3.8$ (±SD) × $10^{-3}$ slope units. To test whether increasing age attenuates LH
feedforward on pulsatile Te secretion, the set of 18 slope estimates was regressed linearly on age. As shown in Fig. 4, slope values fell significantly with increasing age (r = −0.721, P < 0.001). The cohort slope (± SD) on age was −7.3 ± 1.8 × 10⁻⁴. Age accounted for 52% (r²) of the variance in fractional LH drive of pulsatile Te secretion.

To test the assumption that age does not determine the relative potency of ganirelix in inhibiting pulsatile Te secretion, we regressed the log of the Te/LH secretion ratio on ganirelix dose in each subject (Fig. 5A). The resultant set of inhibitory slopes (n = 18) was then regressed linearly on age (Fig. 5B). Age did not alter the effect of ganirelix on the LH-to-Te secretion ratio. Statistical power was >95% to detect a 30% contribution of age to the variance in the pulsatile LH-to-Te secretion ratio at P < 0.05. In addition, we examined the slopes obtained in Fig. 5A to test whether the mean slope significantly differed from zero. The mean of these slopes was 1.54 ± 0.21 (median 1.46; P < 0.01), indicating that greater ganirelix blockade enhanced the pulsatile Te-to-LH secretion ratio. This outcome is also consistent with the greater percentage fall in LH (85%) than Te (35%) secretion in response to GnRH-receptor antagonism (Table 1), thus suggesting that some Te secretion is not acutely LH dependent.

**DISCUSSION**

The present data support the postulate that age significantly attenuates pulsatile LH-dependent drive of episodic Te secretion in normal men. This inference depends on deconvolution and regression analysis of four experimentally controlled graded decrements in burst-like LH and Te secretion in each of 18 subjects. Varying plateaus of pulsatile LH output were achieved by selective antagonism of hypothalamic GnRH-dependent secretion of LH and thereby gonadal Te. Statistical contrasts demonstrated that the negative impact of age on endogenous LH-driven burst-like Te secretion accounts for 52% of the interindividual variability in pulsatile Te production in this cohort of volunteers aged 23–72 yr.

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**Table 1. Dose-dependent impact of ganirelix on pulsatile LH and Te secretion in men**

<table>
<thead>
<tr>
<th>Ganirelix Dose, mg/m²</th>
<th>LH, IU/L/1/8 h⁻¹</th>
<th>Te, 10³ × (ng/dl⁻¹/6 h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.4 ± 4.2⁺</td>
<td>18.1 ± 2.3⁺</td>
</tr>
<tr>
<td>0.1</td>
<td>18.5 ± 2.5⁺</td>
<td>13.2 ± 1.8⁺</td>
</tr>
<tr>
<td>0.3</td>
<td>13.9 ± 2.3⁺</td>
<td>13.2 ± 1.8⁺</td>
</tr>
<tr>
<td>1.0</td>
<td>7.9 ± 1.9⁺⁺</td>
<td>11.8 ± 1.7⁺⁺</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 18 men. LH, luteinizing hormone; Te, testosterone. Ganirelix dose-response effects were *P < 0.001 for pulsatile LH and †P < 0.01 for pulsatile Te. Within a column, unshared (unique) alphabetic superscripts denote significantly different means based on Tukey’s test.
Multiple loci of hypothalamo-pituitary-testicular impairment appear to underlie the gradual fall in free and bioavailable Te concentrations in aging men (29, 54). The present analyses consider particularly the LH-Te interface in healthy individuals. LH and Te are both secreted in pulses (12, 25, 33, 50, 66). LH secretory bursts represent a relevant, but not necessarily exclusive, feedforward signal to the testis, inasmuch as discrete pulses of infused LH or hCG stimulate Te secretion in the rat, dog, sheep, horse, and human (10, 20, 31, 52, 63). On the basis of this physiological background, we used escalating doses of a potent and long-acting selective GnRH-receptor antagonist to enforce stepwise reductions in pulsatile LH output. The GnRH blocker suppressed both pulsatile Te secretion rates (model based) and peak Te concentrations (model free) in a dose-dependent fashion. Analyses of the paired LH and Te time series indicated that any given amplitude of LH pulses is less effective in driving burst-like Te secretion in the aging than young male. This inference extends earlier model-based mathematical predictions using cross-correlation (linear) and logistic (nonlinear) analyses of concomitant LH and Te concentration profiles in cohorts of men in the age extrema >60 vs. <30 yr (25, 34). Analogously, in one study, administration of pulses of GnRH (100 ng/kg) intravenously every 90 min for 14 days elevated mean LH concentrations comparably in five older and five young men but failed to increase bioavailable and free Te concentrations maximally in older individuals (35). The current data differ by way of demonstrating that 1) the decline in pulsatile Te secretion in aging men occurs continuously over six decades of life, and 2) impaired testicular steroidogenic responsiveness operates over a 5.6-fold range of pulsatile LH secretion in healthy individuals.

Increasing doses of the GnRH-receptor antagonist elevated the ratio of pulsatile Te to pulsatile LH secretion by ~50% independently of age. This finding excludes a direct inhibitory effect...
effect of ganirelix on Leydig cells, which inference mirrors that for other agents in this class (40). Greater suppression of LH than Te secretion could indicate that a basal-like component of Te production is sparingly dependent on LH stimulation (8, 20, 25, 28, 33, 44, 66).

By way of qualifications, first, no comprehensive studies are available that directly quantitate the dose-responsive actions of purified or biosynthetic LH on pulsatile Te secretion over a span of ages in the intact experimental animal or human. When available, such data will ultimately be important to compare with the accompanying estimates based on endogenous LH pulses. Second, our use of saline and three nonzero doses of the GnRH-receptor antagonist (72 study sessions) allowed linear approximation of testicular steroidogenic sensitivity to pulsatile LH drive. Comprehensive estimation of nonlinear dose-response properties (potency, sensitivity, and efficacy) would require achieving supraphysiological LH pulses to verify attainment of maximal Leydig cell steroidogenesis. Third, aging attenuates the mass of LH contained in secretory bursts (and hence blunts the incremental amplitude of LH peaks) by 35–50, and disrupts the orderliness of the LH release process (41, 59, 61, 65). The precise impact of chronically reduced amplitudes and irregular patterns of LH secretion on gonadal steroidogenesis is not known. Thus further studies will be required to assess whether prolonged normalization of LH pulse amplitude and regularity will enhance Te secretion in the older male. Recent experimental data suggest that reduced Te production in the aged male rat is not reversible by in vivo delivery of fixed LH pulses for 5 days (14). On the other hand, sustained withdrawal of endogenous LH in the young adult rat potentiates LH-dependent Te secretion in the older animal (5). Such data suggest that prolonged exposure to LH in adulthood may induce deleterious intragonadal factors, such as oxidative stress-related products of steroidogenesis, transforming growth factor-β, or inflammatory cytokines (5, 14, 44).

In summary, an experimental paradigm comprising randomly ordered, separate-day administration of saline and varying doses of a selective GnRH-receptor antagonist delineates a prominent age-related decline in the apparent sensitivity of burst-like Te secretion to endogenous LH pulses in normal men. Longitudinal studies and exogenous LH dose-response analyses will be required to corroborate the inferred decline in Leydig cell responsiveness with age in men, and basic laboratory investigations will be needed to establish the precise biochemical bases of reduced LH-stimulated Te biosynthesis in the older male.

ACKNOWLEDGMENTS

We thank Kris Nunez for manuscript support and Jason Kerkvliet for performing the ganirelix assays.

GRANTS

Studies were supported in part by National Institutes of Health Grant M01 RR-00585 to the General Clinical Research Center of the Mayo Clinic and Foundation and Grants RO1 AG-023133 and DK-006717. P. Y. Liu was supported by a Neil Hamilton Fairley Research Fellowship from the National Health and Medical Research Council of Australia (Grant ID 262025).

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