Age disrupts androgen receptor-modulated negative feedback in the gonadal axis in healthy men

Johannes D. Veldhuis,1 Paul Y. Takahashi,2 Daniel M. Keenan,3 Peter Y. Liu,1,4 Kristi L. Mielke,1
and Suanne M. Weist1

1Endocrine Research Unit, 2Department of Internal Medicine, Mayo Medical School, Mayo School of Graduate Medical Education, Center for Translational Science Activities, Mayo Clinic, Rochester, Minnesota; 3Department of Statistics, University of Virginia, Charlottesville, Virginia; and 4Department of Andrology, Concord Hospital and ANZAC Research Institute, Endocrine and Metabolic Group, Woolcock Institute of Medical Research, University of Sydney, Sydney, New South Wales, Australia

Submitted 20 May 2010; accepted in final form 29 July 2010

Veldhuis JD, Takahashi PY, Keenan DM, Liu PY, Mielke KL, Weist SM. Age disrupts androgen receptor-modulated negative feedback in the gonadal axis in healthy men. Am J Physiol Endocrinol Metab 299: E675–E682, 2010. First published August 3, 2010; doi:10.1152/ajpendo.00300.2010.—Testosterone (T) exerts negative feedback on the hypothalamo-pituitary (GnRH-LH) unit, but the relative roles of the CNS and pituitary are not established. We postulated that relatively greater LH responses to flutamide (brain-permeant antiandrogen) than bicalutamide (brain-impermeant antiandrogen) should reflect greater feedback via CNS than pituitary/periodic androgen receptor-dependent pathways. To this end, 24 healthy men ages 20–73 yr, BMI 21–32 kg/m2, participated in a prospective, placebo-controlled, randomized, double-blind crossover study of the effects of antiandrogen control of pulsatile, basal, and entropic (pattern regularity) measurements of LH secretion. Analysis of covariance showed increased LH secretion in a way of the androgen receptor (AR) remains unknown (20, 22). To examine these basic issues, one would need to (1) distinguish between hypothalamic and pituitary sites of T’s inhibition, 2) selectively block AR but not estrogen receptor, 3) quantify changes in LH secretion under both endogenous and exogenous GnRH drive, and 4) measure serum concentrations of the AR antagonist to verify subject compliance. The present investigation introduces a paradigm that attempts to satisfy these four requirements by using flutamide and bicalutamide as AR blockers that 1) act on the CNS and pituitary, respectively, and 2) do not affect estrogen receptor signaling by 3) quantifying spontaneous vs. exogenous GnRH-stimulated LH secretion and 4) measuring blood levels of both drugs to verify subject compliance. Although the paradigm focuses on central (hypothalamic-pituitary) feedback control, this emphasis does not minimize the important homeostatic role of peripheral target tissue (gonadal) responses to physiological stress (e.g., injury, exercise). Other experimental and translational models are important in exploring the corollary issue of the role of AR in the body’s repair and restoration of local tissue disruption.
SUBJECTS AND METHODS

Experimental rationale. The rationale was to antagonize AR feed-
back pathways using selective antiandrogens that either permeate
(flutamide) or do not permeate (bicalutamide) the blood-brain barrier
(8, 21). The degree to which the effect of flutamide on LH dynamics
differed from that of bicalutamide and remained distinct from the
known actions of estrogen (36) was taken as an index of AR-related
CNS feedback control. In particular, feedback differences in drug
actions (flutamide vs. bicalutamide) were quantified by specific de-
convolution measurements of LH secretion and approximate entropy.

Subjects. Each subject provided written informed consent approved
by the Mayo Institutional Review Board (IRB). Volunteers were
recruited from locally placed IRB-approved postings and received
IRB-approved reimbursement for time spent in the study.

A total of 24 healthy men participated. The age range was 20–73
yr (median 51 yr), and body mass index (BMI) was 21–32 (median
26) kg/m². Ages (no. of subjects) by 15-yr intervals were <30 (4
men), 30–45 (6 men), 45–60 (6 men), and 60–75 (8 men). Mean
mean dietary macronutrient distribution in the volunteers was 60% carbo-
hydrate, 15% protein, and 25% fat. No subject had gained or lost >2
kg weight over the preceding month or during the study. None abused
alcohol or acetaminophen. None had sexual dysfunction. Each denied
concurrent or recent major life stressors (loss of employment, death
in the family, divorce). Subjects described normal sleep habits. Medical
history and physical examination (including tests size, libido, and
potentia) were normal. There was no history of infertility, systemic
disease, hormonal therapy, or psychoactive drug use. Fasting (0800)
screening biochemical tests of endocrine, metabolic, hematological,
hepatic, and renal function were normal.

Clinical protocol. The study was a prospective, double-blind, and
randomized crossover design. Each subject received oral placebo,
flutamide (250 mg), and bicalutamide (50 mg) three times daily for 4
days, with intensive blood sampling on the 4th day. Placebo or drug
was continued every 8 h during sampling. There was a minimum 1-mo
drug washout (8). The Mayo IRB and the FDA approved the specific
protocol for human use, contingent upon normal pretest hepatic and
renal function (transaminases and creatinine) and complete blood
counts, the absence of allergy to either drug, normal prostate-specific
antigen, and disallowance of concomitant exposure to alcohol or other
potentially hepatotoxic drugs. A data safety and monitoring board was
emanated at Mayo to verify compliance.

An indwelling intravascular (iv) catheter was placed in a forearm
vein at 0645 on the day of study, and blood samples (1.5 ml) were
withdrawn every 10 min for 8 h beginning at 0800. The first 360 min
of blood sampling served as a baseline (pre-GnRH injection). At
1400, GnRH (100 ng/kg) was given by bolus iv injection. Sampling
ceased 120 min later.

Drug assays. Serum concentrations of 2-hydroxyflutamide and
bicalutamid e were assayed on the day of blood sampling by liquid
chromatography-tandem mass spectrometry, as described (8, 34).
Sensitivity was 0.1 μg/ml for both, and interassay variabilities were
10.3 and 10.8%. Drug assays were performed on three pooled samples
from each volunteer comprising 0.1-ml aliquots of each 10-min blood
sample (n = 49) across the three separate 8-h sampling intervals. This
was done to verify subject compliance with drug ingestion.

Hormone assays. LH concentrations in the 10-min samples were
measured in duplicate on the DxI automated two-site immunoenzymatic
assay system (Beckman Instruments, Chaska, MN). Intrassay coeffi-
cients of variation (CVs) were 4.3 and 4.0% at 12 and 38.5 mIU/ml,
respectively. Interassay CVs were 9.3, 6.0, and 4.2% at 1.4, 15.6, and
48.8 mIU/ml, respectively. Procedural sensitivity was 0.2 IU/l, and the
upper analytic limit was 250 IU/l using the World Health Organization
Second International Reference Preparation 80/552 as standard. T was
measured in 10-min samples on the same robotics system. Intrassay
CVs were 6.5% at 69 ng/dl and 3.3% at 862 ng/dl. To convert ng/dl
to nmol/l, we multiplied by 0.0347. Interassay CVs were
7.4% at 1,116 ng/dl, 8.6% at 407 ng/dl, and 4.0% at 761 ng/dl. The
analytic range was 54–1,650 ng/dl. The coefficient of determination
was r² = 0.98 between robotics and liquid chromatography-tandem mass
spectrometry (LC-MS/MS; ThermoFisher Scientific, Franklin,
MA, and Applied Biosystems-MDS Sciex, Foster City, CA), as
described (31). Confirmatory LC-MS/MS was used to measure T in
0800 samples. Intraassay CVs were 3.3, 2.8, 2.2, and 2.0% at 16, 64,
184, and 927 ng/dl, respectively. Interassay CVs were 5.1, 3.8, 3.7,
and 2.8% at 17, 65, 177, and 919 ng/dl, respectively. The analytic
range is 7–2,000 ng/dl. Estradiol (E₂) concentrations were measured
in the same mass spectrometry run: sensitivity 3.5 pg/ml, interassay
CV 4.5% (24). To convert pg/ml E₂ to pmol/l, we multiplied by 3.69.

Sex hormone-binding globulin (SHBG) and albumin were mea-
sured in morning serum by Immulite 2000 [Diagnostic Products
(Siemens), Los Angeles, CA] and Roche/Hitachi 912 (Roche Diag-
nostics, Basel, Switzerland), respectively. Inter- and intra-assay CVs
were <6.0% at SHBG concentrations ranging from 5.4 to 96 mmol/l.
Inter- and intra-assay CVs were <2.0% at albumin concentrations
from 2.5 and 4.6 g/dl.

Calculation of free and bio T concentrations. Free and bio T
cent rations were calculated in each 10-min serum sample from
measured total T in each sample and pooled (session-specific) albu-
min and SHBG concentrations, as described (refer to supplemental
data in Ref. 33).

Deconvolution analysis. The 8-h LH concentration time series were
analyzed using a recently developed automated deconvolution method
described fully statistically (5). The pulse detection algorithm was
empirically validated for LH studies using hypotalamo-pituitary
sampling and simulated pulsatile time series (16). Sensitivity and
specificity are both ~93%. For preliminary pulse detection, the
Matlab-based algorithm first detrends the data and normalizes concentra-
tions to the unit interval (0, 1). Second, the program creates multiple successively decremalent potential pulse time sets, each containing one fewer burst by a smoothing process (a nonlinear adaptation of the heat diffusion equation). Third, a maximum-likeli-
hood expectation estimation method calculates all secretion and elim-
ination parameters simultaneously conditionally on each of the can-
didate pulse time sets, using the original nondetrended LH concen-
tration data. The rapid half-life of LH was represented as 18 min,
constituting 63% of the decay amplitude, and the slow half-life 90 min
(38). For T, corresponding values were 3.5 and 28 min (63% slow
decay), based upon T infusions in eight men (41). Statistical model
selection was performed using the Akaike information criterion. The
parameters (and units) are basal (nonpulsatile) and pulsatile secretion
rates (concentration units/session), mass secreted per burst (concentra-
tion units), and waveform mode (time delay to maximal secretion
after objectively estimated burst onset, min). Total LH secretion was
the sum of basal and pulsatile release. Percentage pulsatile secretion
was defined as the percentage ratio of pulsatile-to-total LH secretion.

Approximate entropy measurement of regularity. Approximate en-
tropy (ApEn; 1, 20%) was used as a scale- and model-independent
regularity statistic to quantify the orderliness (regularity) of hormone
release (25). Higher ApEn denotes greater disorderliness (irregularity)
of the secretion process. Mathematical models and clinical experi-
ments establish that greater irregularity signifies decreased feedback
control with high sensitivity and specificity (both >90%) (45). Cross-
ApEn was used to test pairwise synchrony between LH and T
(feedforward) as well as, conversely, between T and LH (feedback)
(17, 26).

Statistical comparisons. LH and T concentrations were averaged
over either 6 (before GnRH) or 2 h (after GnRH). Statistical power
was estimated as ≥90% to detect a 1.5 IU/l increase due to flutamide
in mean 6-h LH concentrations by a two-sided paired t-test if 25
men completed the study and ≥95% power to detect a 0.18 ApEn contrast
at P < 0.05. The response to GnRH was a secondary endpoint, which
was not powered. Deconvolution and ApEn measurements were
compared parametrically after log transformation and nonparametri-

cally without transformation (Kruskal-Wallis test). All primary outcomes were significant by both methods. One-way analysis of covariance (ANCOVA) was used to test the impact of the two antiandrogens on log-transformed LH secretory parameters. The covariate was the subject’s response on the placebo day. The covariate adjusts statistically for autocorrelation expected in a repeated-measurements design. Tukey’s honestly significantly different test was applied post hoc for protected comparisons among means (47). Secretory parameters are given in the supplemental tables and text as the geometric mean (95% confidence intervals) and in the figures as box-and-whisker plots. Untransformed ApEn and hormone concentrations are presented as the arithmetic means ± SE. Experiment-wise $P < 0.05$ was construed as statistically significant.

Age and BMI-dependent effects on LH secretion were analyzed via linear regression (version 9.0; Systat, Richmond, CA).

RESULTS

Baseline data. Baseline (pretreatment) physical and endocrine data in the 24 men studied here are summarized in Supplemental Table S1 (Supplemental Material for this article can be found online at the AJP-Endocrinology and Metabolism web site). All subjects completed three 8-h sampling visits (comprising a total of 3,528 LH and 3,528 T samples) without adverse events. Compliance was verified by direct assay of serum drug levels on the day of study. Regression of baseline hormone concentrations linearly on age or BMI yielded expected relationships, showing typicality of the cohort (Supplemental Table S1).

Mean concentrations. Six-hour pre-GnRH (baseline) and 2-h post-GnRH (stimulated) LH concentration vs. time profiles were obtained by 10-min sampling during each intervention (Fig. 1). Prior to GnRH injection, LH and T concentration curves appeared to separate in the descending rank order of flutamide > bicalutamide > placebo. ANCOVA confirmed this inference (flutamide $P < 0.001$ vs. placebo, bicalutamide $P < 0.01$ vs. placebo, overall $P < 0.001$, covariate $P < 0.001$; Fig. 2). By post hoc analysis, LH and T responses to flutamide exceeded those responses to bicalutamide by $P < 0.002$ and $P < 0.017$, respectively (Fig. 2, left). Changes in baseline total T concentrations were corroborated by mass spectrometry, viz. 454 ± 32 (placebo), 644 ± 34 (flutamide, $P < 0.001$ vs. placebo) and 563 ± 37 ng/dl (bicalutamide, $P < 0.005$ vs. placebo) ($P < 0.015$ for antiandrogen comparison). Concentrations of E$_2$ also rose during exposure to antiandrogens ($P < 0.001$ for both vs. placebo, $P < 0.015$ for drug comparisons; Supplemental Table S2). Estrone levels did not vary among the three sessions.

Fig. 1. Time profiles of LH (top) and testosterone (T; bottom) concentrations observed in 24 men by sampling blood every 10 min for 8 h after administration of placebo, flutamide, or bicalutamide for 4 days. Sampling began at 0800. Gonadotropin-releasing hormone (GnRH; 100 ng/kg) was injected by intravenous bolus after the 1st 6 h. Data are the arithmetic means ± SE.

Fig. 2. Antiandrogens [flutamide (Fl) or bicalutamide (Bi)] augment baseline LH and T concentrations and peak GnRH-stimulated T concentrations compared with placebo (Pl). Data are the arithmetic means ± SE of 6-h values before and 2-h values after GnRH injection. *, ø, and † P values were determined by analysis of covariance and by post hoc Tukey’s test. Differing (unshared) letters denote P < 0.05 contrast between the corresponding means.
GnRH stimulation. A submaximally stimulatory dose of GnRH (100 ng/kg) elicited similar absolute peak LH concentrations in all three treatment conditions (P = 0.31). Each response was significantly greater than the 6-h baseline mean (P < 0.001; Fig. 2, right). GnRH injection per se did not alter 2-h mean or peak T levels, which remained higher in the presence of flutamide than bicalutamide (P = 0.013) and both P < 0.001 over placebo.

Deconvolution analysis of LH. Pulsatile and basal (norpulsatile) LH secretion rates were compared by ANCOVA. Flutamide and bicalutamide each stimulated basal LH secretion (P < 0.001 vs. placebo), with a nonsignificant trend toward a larger effect by flutamide (P = 0.065; Fig. 3, left). Only flutamide augmented pulsatile LH secretion and concomitantly inhibited GnRH-evoked LH release. Flutamide elevated only basal T secretion, and the magnitude of this effect exceeded that of bicalutamide (P = 0.032). Both antiandrogens increased LH pulse frequency (P < 0.001 vs. placebo), but flutamide induced a twofold larger incremental change (P = 0.013; Supplemental Table S3). Only flutamide stimulated 6-h pulsatile LH secretion (P = 0.003 vs. placebo; Fig. 3, middle). Neither antiandrogen significantly affected the size of LH secretory bursts, although there was a trend toward a decrease (P = 0.082; Fig. 4). Flutamide compared with bicalutamide abbreviated LH secretory bursts (decreased the mode, P = 0.035 overall, P = 0.032 for flutamide vs. bicalutamide; Supplemental Table S3). Although both flutamide and bicalutamide stimulated total (basal plus pulsatile) LH secretion (P < 0.001), flutamide had the greater effect (P = 0.002). Incremental (stimulus minus baseline) GnRH-induced pulsatile LH secretion was less after flutamide than placebo (overall P = 0.015 with a covariate effect of P < 0.001; Fig. 3, right). This was not true for bicalutamide.

Deconvolution analysis of T. Flutamide elevated basal (norpulsatile) T secretion significantly compared with both placebo

---

**Fig. 3.** *Left and middle:* basal (norpulsatile) and pulsatile LH (top) and T (bottom) secretion in 24 men studied during administration of Pl, Fl, or Bi. *Right:* pulsatile LH and T secretion after GnRH injection. Data are box-and-whisker plots (median, interquartile range). Individual dots are outside the 80% confidence interval. Open bars, Pl; gray bars, Fl; black bars, Bi.

**Fig. 4.** Primary LH and T pulse characteristics assessed by deconvolution analysis: secretory burst frequency (left) and secretory burst mass (right). Data are presented as in Fig. 3.
Baseline LH ApEn increased the most during flutamide administration ($P < 0.001$ vs. placebo, $P = 0.044$ vs. bicalutamide, covariate $P < 0.001$), albeit significantly during bicalutamide exposure ($P = 0.006$ vs. placebo; Fig. 5, top left). Only flutamide increased LH ApEn after GnRH injection ($P = 0.012$ overall, $P < 0.001$ covariate effect, $P = 0.009$ for flutamide vs placebo, $P = 0.27$ for bicalutamide vs. flutamide). Antiandrogens did not affect T ApEn values before ($P = 0.15$) (Fig. 5, top right) or after ($P = 0.11$) GnRH injection. Cross-ApEn, which is a measurement of joint-series irregularity (viz., paired-series asynchrony), revealed that LH $\rightarrow$ T feedforward asynchrony was higher (pairwise synchrony was lower) during flutamide than bicalutamide administration ($P = 0.034$ overall, $P = 0.045$ for flutamide $>$ bicalutamide). This may reflect less effective LH pulsations in driving T in that T $\rightarrow$ LH feedback asynchrony did not change ($P = 0.14$; Fig. 5, bottom).

**Linear regression analysis.** There was a consistently negative effect of age on the mode (time delay to maximal LH secretion within a burst) determined over 8 h under all three study conditions ($P = 0.005$ placebo, $P < 0.001$ flutamide, $P = 0.004$ bicalutamide, $r^2 = 0.30$ to 0.47; Fig. 6). The slope on age was more negative during flutamide than placebo or bicalutamide administration ($P \leq 0.025$).

During flutamide administration, BMI was a positive, and age a negative, joint correlate of percentage pulsatile LH secretion (overall $P = 0.0004$, $r^2 = 0.52$, BMI $P = 0.028$, age $P = 0.0011$; Supplemental Fig. S1). The negative effect of age, but not the positive effect of BMI, was evident during placebo ($P = 0.012$, $r^2 = 0.25$) and bicalutamide ($P = 0.016$, $r^2 = 0.25$) treatment. Conversely, during flutamide exposure, BMI was a negative determinant ($P = 0.0061$) and age a positive determinant ($P = 0.0083$) of basal LH secretion (overall $P = 0.0009$, $r^2 = 0.59$; Supplemental Fig. S2).

Serum concentrations of 2-hydroxyflutamide averaged $1.1 \pm 0.09$ $\mu$g/ml (median 0.93, range 0.45–2.3) and bicalutamide $7.9 \pm 0.26$ $\mu$g/ml (median 7.5, range 5.8–10.9). Neither correlated with age ($P > 0.70$). However, bicalutamide levels correlated negatively with BMI ($r = 0.18, P = 0.041$). 2-Hydroxyflutamide levels behaved similarly but at a nonsignificant trend level ($r^2 = 0.14, P = 0.073$; Supplemental Fig. S3).

**DISCUSSION**

The present studies introduce a novel, short-term, physiological homeostasis-perturbation paradigm to investigate brain and pituitary/peripheral sites of AR-related feedback regulation of basal, pulsatile, and entropic (orderly) LH secretion. The

![Fig. 5. Top: impact of PI and antiandrogens on approximate entropy (ApEn) of LH (left) and T (right) release over 6 h in 24 men. Bottom: feedforward (LH $\rightarrow$ T) and feedback (T $\rightarrow$ LH) paired synchrony within male gonadal axis as estimated by cross-ApEn. Data are depicted as described in Fig. 2. Higher ApEn or cross-ApEn denotes greater irregularity (less orderliness or less joint synchrony).](http://ajpendo.physiology.org/)

![Fig. 6. Linear regression of the mode of LH secretory bursts on age in 24 men sampled every 10 min for 8 h during exposure to placebo, flutamide, and bicalutamide. Unshared letters A and B denote $P \leq 0.025$ difference in the indicated slopes and SD.](http://ajpendo.physiology.org/)
4-day protocol utilized flutamide to block hypothalamic, pituitary, and peripheral AR pathways and bicalutamide to inhibit principally pituitary and peripheral AR pathways (21). Independent data indicate that radioactive flutamide but not bicalutamide permeates the brain readily in animals (8). Both antiandrogens bind potently to rat, dog, and human prostate AR and inhibit T- and dihydrotestosterone-induced cell growth and gene expression. Moreover, flutamide is able to block AR action on human neurons (11). Neither AR antagonist associates significantly with estrogen, progesterone, glucocorticoid, or mineralocorticoid receptors (2, 14). The rationale was that, to the degree that LH secretion and regularity responses to flutamide are distinct from those to estrogen and those to bicalutamide, they reflect CNS more than pituitary/peripheral AR pathways. According to this concept, the accompanying analyses indicate that 1) CNS mechanisms of AR-mediated negative feedback especially modulate GnRH/LH secretory-burst frequency and duration, pulsatile LH secretion, the incremental LH response to GnRH, total (basal plus pulsatile) LH secretion, and orderliness (regularity) of the LH secretory process; 2) pituitary/peripheral mechanisms of AR action determine basal (nonpulsatile) LH secretion; and 3) age and BMI reciprocally control basal LH secretion and percentage pulsatile LH secretion in healthy men. Several peripheral measurements, such as hepatic IGF-I, SHBG, and albumin concentrations (not shown), did not change over the short-term study. However, longer-term androgen deprivation is associated with increases in insulin and triglyceride levels, abdominal visceral fat, and possibly blood pressure in clinical studies (1, 3, 4). These alterations are mimicked by AR knockout in mice (46). The short-term paradigm used here avoids confounding by these metabolic changes.

The main effects of CNS-permeable flutamide compared with placebo were to stimulate LH secretory burst frequency, augment both basal and pulsatile and thereby total LH secretion, and increase LH ApEn (irregularity). Except for enhanced basal LH secretion, bicalutamide was unable to reproduce the direction and the magnitude of flutamide’s effects. A minimal increase in LH pulse frequency by bicalutamide was significantly less than that induced by flutamide ($P = 0.013$). The small increase might reflect a decrease in false-negative or an increase in false-positive errors associated with elevated basal LH secretion, some CNS penetration of bicalutamide, and/or AR-dependent feedback via the median eminence (43). Although $E_2$ concentrations rose more during flutamide than bicalutamide exposure, $E_2$ actually suppresses rather than augments basal, pulsatile, and entropic LH secretion (20). Thus, the regulatory mechanisms underlying the distinctive effects of flutamide compared with bicalutamide probably involve greater disinhibition of CNS AR-repressed GnRH secretion, since more frequent pulses of GnRH can augment LH pulse frequency, pulsatile LH secretion, and LH irregularity (7, 23, 45). The flutamide-induced phenotype of gonadotropin release also resembles that observed recently in older men (18, 19). Accordingly, a possible mechanism underlying aging effects would be diminished restraint by CNS AR. In this context, several studies report fewer sex steroid receptors in the brain, pituitary, and other tissues of aged than of young animals and humans (10, 12, 27, 29, 35).

Increased age was associated with depressed, and increased BMI with elevated, percentage pulsatile LH secretion (see SUB-ECTS AND METHODS) and reciprocal changes in basal (nonpulsatile) LH secretion (Supplemental Figs. S1 and S2). The joint relationships were evident only during flutamide exposure and accounted statistically for 52 and 59% of intersubject variability. The associations indicate that aging and adiposity alter the ratio of basal/pulsatile GnRH secretion in opposite directions when the activity of CNS AR pathways is muted. Both effects are putatively central, since neither age nor BMI altered peak LH responses to exogenous GnRH. In contradistinction, the reduced incremental LH response to GnRH during flutamide exposure may reflect the recognized negative effect of $E_2$ at the pituitary level (36, 43).

The mode (time delay from the onset to the maximum) of LH secretory bursts was lower during flutamide than bicalutamide administration. Whether this is due to higher GnRH/LH pulse frequency is not known. However, LH secretory burst mode also declined significantly with age, explaining 30–47% of intersubject variability. Blockade of CNS (flutamide) but not peripheral (bicalutamide) AR pathways heightened the negative effect of age twofold. Mechanistically, either impaired central AR action or higher $E_2$ concentrations might explain this outcome. The latter seems unlikely, inasmuch as LH secretory burst modes are comparable in the early and late follicular phases of menstruating women despite markedly different $E_2$ concentrations (13). Abbreviation of the secretory burst mode would be consistent with more rapid onset of the exocytotic process and/or a shorter duration of fusion of pituitary secretory vesicles at the gonadotrope membrane (6).

Flutamide and bicalutamide are selective nonsteroidal AR antagonists approved for use in patients with prostate cancer (21, 28). Neither antiandrogen exhibits significant intrinsic androgenicity (15, 30, 32). The nominal half-life of the active metabolite of flutamide (a 2-hydroxy derivative) is 8 h and of bicalutamide is 5.8 days (8). Therefore, prestudy pharmacokinetic predictions were that loading doses of flutamide and bicalutamide should yield therapeutic serum concentrations by day 4. Mass spectrometric drug assays confirmed this, as well as subject compliance, since 2-hydroxyflutamide and bicalutamide concentrations on the day of sampling averaged 1.1 and 7.9 μg/ml, respectively (8, 21). Whereas age did not influence drug levels, BMI was a negative predictor of bicalutamide (and nonsignificant flutamide) concentrations. This finding, if confirmed, would have relevance to drug-dosing schedules in overweight and obese patients.

By way of caveats, flutamide and bicalutamide elevated total serum $E_2$ concentrations by 25–43%. Thus, antiandrogen-associated changes in LH secretion might arise in principle from either blunted AR action or heightened $E_2$ availability. The latter is excluded in the case of the four main effects of flutamide, since estrogen deprivation rather than excess in men is required to augment GnRH/LH pulse frequency, basal and pulsatile LH secretion, and the irregularity of LH release (20, 37, 43, 44). Direct measurements of brain interstitial fluid drug concentrations in humans would ultimately be required to verify animal data regarding differential CNS uptake of these antiandrogens. However, the nine (9) separate analytically documented differences in LH and $T$ responses to the two drugs strongly support the notion of nonidentical effects. Moreover, although the $K_i$ of flutamide and bicalutamide

AJP-Endocrinol Metab • VOL 299 • OCTOBER 2010 • www.ajpendo.org
differ, the present work directly documents therapeutic androgen levels for both drugs based upon antineoplastic activity. The two AR antagonists might act differently on the pituitary, but there is no evidence for this given the existence of a single AR. Larger prospective studies would be needed to verify inferred relationships between basal LH secretion and age or BMI. More prolonged sampling duration could also be used to corroborate the pulsatility and entropy distinctions observed here. Longer-term studies with emphasis on possible body compositional changes would be required to test the impact of altered peripheral AR function on muscle, bone, and fat metabolism. At present, long-term studies have been done only in patients with prostatic cancer (1, 3, 4, 9).

In conclusion, a CNS-permeant antiandrogen markedly accelerates GnRH/LH pulse frequency, elevates pulsatile LH secretion, increases the irregularity of the LH secretory process, and abbreviates LH secretory bursts compared with a placebo and non-CNS-permeant AR blocker. Both antiandrogens augment basal LH secretion. Age and BMI influenced basal (nonpulsatile) LH secretion. These outcomes point to distinct AR-related pituitary/peripheral and CNS feedback dependencies of regulated LH secretion in healthy men studied during short-term disruption of physiological homeostasis. The approach illustrated here does not minimize the corollary importance of other experimental translational models that explore the role of AR in the repair of the effects of local tissue stress (e.g., injury, exercise).

GRANTS

Studies were supported in part by National Center for Research Resources (Rockville, MD) Grant M01-RR-00585 to the Mayo Clinic Center for Translational Science Activities and by National Institute on Aging (Bethesda, MD) Grants RO1-AG-31763 and R21-AG-23777.

DISCLOSURES

The authors have nothing to declare.

REFERENCES


