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Aromatase Inhibition Causes Increased Amplitude, but not Frequency, of Hypothalamic-Pituitary Output in Normal Women

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Abstract

Objective—To better understand the site and mode of action of aromatase inhibitors.

Setting—Academic research environment.

Patients and Design—5 eumenorrheic (non-PCOS), early follicular phase women of normal BMI (mean = 20.47 $\pm$ 0.68 kg/m²) and 12 normal weight, mid-reproductive aged, early follicular phase, normal BMI (mean = 20.8 $\pm$ 1.7 kg/m²) historical controls.

Interventions—2.5mg letrozole daily for 7 days. Women were sampled with daily, first morning voided urine collections, thrice weekly blood sampling, and 4 hours of q 10 minute frequent blood sampling

Main outcome measures—Serum LH measured using a well-characterized immunofluorometric assay. LH pulse characteristics were compared between treated and control groups using t tests.

Results—Mean LH and LH pulse amplitude more than doubled in women who had taken letrozole compared to controls, but the LH pulse frequency did not differ between women taking letrozole and controls.

Conclusions—These results indicate that the release of negative feedback inhibition of estradiol on the hypothalamic-pituitary axis in normal women by aromatase inhibitors creates an amplitude-related increase in endogenous hypothalamic-pituitary drive. The finding that mean LH and LH

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pulse amplitude, but not frequency, increased after letrozole suggests a possible pituitary site of action.

Keywords

Hypothalamic-Pituitary-Gonadal Axis; Luteinizing Hormone; Aromatase Inhibitor; Letrozole; Pituitary; Ovary

Introduction

Clomiphene citrate, a mixed estrogen receptor agonist, has been a first-line agent for ovulation induction for over 45 years (1). Its mechanism of action involves opening the negative feedback loop of estradiol on the hypothalamic-pituitary axis, resulting in a greater than 50% increase in endogenous follicle stimulating hormone (FSH) and luteinizing hormone (LH) (2). The increased gonadotropin stimulation, in turn, leads to ovulatory rates of 60–85% and cumulative pregnancy rates of 30–40% over 3–6 cycles (1,2).

Recent studies indicate a potential role for aromatase inhibitors in ovulation induction (3). Similar to clomiphene, aromatase inhibitors are taken orally for a relatively short period of time, are relatively inexpensive, and have few side effects (3,4). The overall efficacy of letrozole appears comparable to or better than clomiphene and tamoxifen, and it appears to be associated with reduced multiple follicular development (1,3,5), and a significantly reduced rate of multiple gestation compared to clomiphene citrate (5).

Despite the increased use and clinical potential of letrozole for ovulation induction, its precise mechanism of action remains unconfirmed. Because it is not a mixed agonist, the response of the hypothalamic-pituitary axis to letrozole cannot be assumed to be identical to that of clomiphene. The current research is focused on whether letrozole's mechanism of action is primarily driven by a hypothalamic site of action (which would favor an increase in the *frequency* of GnRH-LH pulses) or a pituitary site of action, (which would favor an increase in LH pulse *amplitude* without a change in pulse frequency). We examined the pulsatile LH response of normally cycling adult women to administration of an aromatase inhibitor to localize its site of action.

Materials and Methods

Participants

This protocol was approved by the Albert Einstein College of Medicine Clinical Research Center Protocol Review Committee, and Committee on Clinical Investigations. All participants provided their informed consent before participation. Five women who met the following inclusion criteria were recruited between July 2009 through May 2010: a. aged 18 – 40, b. body mass index (BMI) 18 – 25 kg/m², c. no history of chronic disease affecting hormone production, metabolism, or clearance, d. normal Thyroid Stimulating Hormone (TSH) at screening, e. baseline hemoglobin > 11 g/dL, and f. regular menstrual cycles every 25–35 days. Exclusion criteria included use of medications known to alter or interact with reproductive hormones (e.g., thiazolidinediones, metformin), and excessive exercise (> 4 hours per week). Participants underwent transvaginal ultrasound examination at screening in order to exclude those with ovarian pathology.

Measurements

Participants took 2.5mg letrozole daily for 7 days starting on menstrual cycle days 1 – 4. On day 6 of letrozole administration, women underwent an 8-hour, q10 minute blood sampling

session, with an intravenous bolus of 75 ng/kg of GnRH given at 4 hours. GnRH stimulation portion of the experimental design is a part of a larger study that is currently ongoing. Each participant therefore had a four-hour window in which to assess LH pulse dynamics prior to the administration of exogenous GnRH. Participant characteristics analyzed included age, BMI, LH pulse frequency, LH pulse amplitude, and mean serum LH.

LH pulsatility results were compared to non-contemporaneous, previously published data from 12 women who had LH pulsatility assessments using q15-min blood sampling in an identical LH assay. Prior examination of the role of sampling interval on LH pulse detection indicates that 10 and 15 minute sampling intervals for LH yield essentially identical results (6). The control women were aged 20–33 years, had a normal BMI, were ovulatory by luteal phase progesterone, had no systemic disease, and had no history of excessive exercise (7). For both groups, serum LH was measured using a solid-phase, two-site specific immunofluorometric assay (DELFI; Perkin Elmer, Turku, Finland) (8). The inter-assay and intra-assay coefficients of variation for serum LH were 5.5 and 2.3%, respectively. Serum estradiol and testosterone were also measured using DELFIA reagents. The assay limit of detection for testosterone was 3.28 nmol/L (11 ng/dl) and the intra-assay CV was 12.9% at the level of the lowest standard.

Statistical Analysis

In each group, LH pulse frequency, amplitude, and mean were the outcomes of interest. LH pulse frequency was determined by two methods (9,10), both of which yielded similar results; the data are shown using the modified Santen and Bardin method, which defines LH pulses as a 20% increment over the preceding nadir. Amplitude was calculated as the peak value of the LH pulse minus the preceding nadir. Mean LH was calculated as the average level across the first four hours of sampling, prior to the administration of exogenous GnRH. In the controls, LH pulse frequency was determined by dividing the mean pulse frequency for the 12 hour study by 3. Group means were compared using Student's t-test with a two-tailed alpha of 0.05. Analyses were performed using STATA 9.2 (StataCorp LP, College Station, TX).

Results

Participant Characteristics (Table 1)

The mean age ($\pm$ SD) of the women who took letrozole was years, not different from controls (24.9 ± 4.8 years; $p = 0.07$). The mean BMI ($\pm$ SD) of the letrozole group was, which was nearly identical to the controls (20.8 ± 1.7 ; $p = 0.82$). Menstrual cycle length ($\pm$ SD) of the participants in this study was within normal limits at days.

LH Pulsatility Parameters (Table 2; Figures 1 and 2)

Letrozole treated women had 2.0 ± 0.6 LH pulses per 4 hours, compared to 2.4 ± 1.7 per 4 hours ($p = 0.55$) in controls. The mean LH pulse amplitude for the letrozole treated group was more than twice that of controls; 5.1 ± 1.2 IU/L vs. 1.6 ± 0.7 IU/L ($p < 0.01$). Mean LH for the letrozole treated women was also substantially increased compared to controls, 9.4 ± 1.8 IU/L vs. 3.4 ± 0.7 IU/L ($p < 0.01$). GnRH bolus did not change the LH pulse frequency (pre, 2.0 ± 0.5 vs. post, 1.8 ± 0.4 , $p = 0.74$), while the mean LH after stimulation increased by approximately 50% (data not shown).

Sex Steroids

Serum estradiol levels have decreased, as expected, to the assay limit of detection during letrozole administration and remained there for the course of the cycle. Testosterone increased transiently during letrozole administration, from a mean pre-letrozole minimum of

0.86 ± 0.27 nmol/L to a maximum of 1.64 ± 0.33 nmol/L (p=0.02) in the 4 women who had available serum for testosterone measurement.

Discussion

Aromatase constitutes the rate limiting step in the conversion of C19 steroids (testosterone and androstenedione) into C18 steroids (estradiol and estrone). Non-steroidal aromatase inhibitors target the active site of the aromatase cytochrome P450 and bind in a competitive manner to prevent estrogen synthesis (11). Aromatase mRNA has been localized in the pituitary gland, as well as several areas of the hypothalamus, in zebrafish (12) and rainbow trout (13). Rats exhibit moderate levels of aromatase within the periventricular preoptic nucleus and medial preoptic nucleus, with several other areas of the hypothalamus demonstrating low but detectable aromatase activity (14). Non-human primates demonstrate aromatase mRNA in both the hypothalamus and pituitary gland. Within the hypothalamus, male old-world primates demonstrate activity in the preoptic and ventromedial nuclei (15). In humans, brain aromatase expression includes the preoptic nucleus of the hypothalamus (16). Thus, there is reason to believe that aromatase inhibition could cause effects at both hypothalamic and pituitary levels.

We have attempted to answer a central question regarding the site of action of aromatase inhibitors in the hopes of gaining insight into the mode of estradiol action within the brain. Our data favor a pituitary site of action for aromatase inhibition. The increase in amplitude and mean LH that we observed was highly significant and resulted in a more than doubling of LH pulse amplitude and mean LH. An approach similar to ours has been used to study the localization of the site action of clomiphene citrate in 11 women by Kerin et al (17). In that study, LH pulse frequency doubled with no change in LH pulse amplitude, whereas we observed the opposite changes. Taken together, these findings are complimentary and suggest hypothalamic site of action for clomiphene and pituitary site of action for letrozole. Prior studies in men with idiopathic hypogonadotropic hypogonadism, using testolactone to block aromatase activity, observed a lack of testosterone mediated suppression of LH secretion in men who were receiving exogenous pulsatile GnRH (18). In this model, hypothalamic function is essentially 'clamped' and thus the lack of LH suppression in the face of aromatase inhibition is in agreement with our findings implicating a pituitary site of action for letrozole. However, in another study, administration of testolactone to women with polycystic ovary syndrome resulted in an increase in both amplitude of LH and frequency of LH pulses, implying both hypothalamic and pituitary sites of action (19). In these latter studies, the use of testolactone, and not a more specific aromatase inhibitor, may have been partially responsible for the findings. Moreover, neither study involved aromatase inhibition in normal women, who may have feedback circuits that differ from men or from women with polycystic ovary syndrome. Nonetheless, the prior literature favors at least a partially pituitary-based site of action for aromatase inhibition.

Aromatase inhibitors are currently under investigation for the treatment of endometriosis, as they have been shown to significantly reduce pelvic pain in patients with endometriosis (20). A chief clinical limitation to the use of aromatase inhibition in normally cycling women is the side effect of ovarian cyst formation. Oral contraceptives have been used as an adjuvant treatment, to suppress pituitary gonadotropin output and thereby prevent recurrent ovarian cysts (21). Our study confirms the potential of aromatase inhibition to activate the hypothalamic-pituitary axis and points up the need to suppress pituitary output when treating women whose reproductive axis is intact.

This study is limited by several factors. The small sample size of five women in our experimental group may have been inadequate to detect a significant change in pulse

frequency. However, we found no evidence of a trend towards a faster LH frequency, as would be expected if removal of estradiol negative feedback at the hypothalamus was operative. If anything the trend is towards a lower LH pulse frequency in the letrozole treated women compared to controls. Mean LH levels were also approximately doubled after letrozole administration. A second limitation is the use of historical controls. The LH data used in calculations for controls was originally recorded over a 12 hour period. The recalculation of LH pulse frequency in this group and the brief interval of sampling in the women who took letrozole likely made these estimates less stable. However, our findings of a mean follicular phase pulse frequency of approximately 1 per hour, is in agreement with the literature (22). While the relatively short duration of the frequent blood sampling session may represent a limitation to our ability to calculate reliable pulse frequency estimation, the 4 hour sampling of LH pulsatility has been used by several groups in the past with robust results (23–25). Short duration of a session may influence power of the study to detect a difference in the desired outcome and, therefore, produce a Type II error. Our study observed an unambiguous and significant difference in LH pulse amplitude and mean LH thus demonstrating that we had adequate power in this subject sample to detect an effect of this size.

Additionally, the effects of aromatase inhibition on kisspeptin, an amplifier of hypothalamic GnRH (26), are not well understood and may be contributing to our results as it plausible that kisspeptin might be modulated by letrozole. A final limitation is the lack of a study group in which both an aromatase inhibitor and exogenous GnRH are administered; a reduction in LH amplitude in such a study would further support a primarily pituitary site of action of aromatase inhibitors.

In summary, we herein extend the existing body of literature on aromatase inhibitor use by our observation of the effects of letrozole on the hypothalamic-pituitary-ovarian axis in normal women. We have determined that the release of negative feedback inhibition of estradiol on the hypothalamic-pituitary axis in normal women by aromatase inhibitors creates an amplitude-related increase in endogenous hypothalamic-pituitary drive. The finding that mean LH and LH pulse amplitude, but not frequency, increased after early follicular phase letrozole administration suggests a possible pituitary, not hypothalamic, site of action.

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Figure 1. LH Pulse frequency (A), amplitude (B) and mean LH in letrozole treated women (solid bars) compared to untreated controls (unfilled bars). Asterisk signifies statistical significance.

Figure 2.

LH pulses over representative four-hour frequent blood sampling studies. Objectively determined LH pulses are depicted by arrows in a letrozole-treated woman (closed squares) and an untreated woman in the early follicular phase (open squares).

TABLE 1

Participant Characteristics and Outcomes

	Letrozole, n=5	Control, n=12 [REF. 6]	P Value
Age, years	30.8±5.9	24.9 ±4.8	0.09
BMI, kg/m	21.0±1.0	20.8±1.7	0.81
Menstrual Cycle Length	29.2±1.2	Data N/A	

TABLE 2

Participant Characteristics and Outcomes

	Letrozole, n=5	Control, n=12 [REF. 6]	P Value
LH Pulse Frequency/4 hours	2±0.5	2.4±0.5	0.55
LH Pulse Amplitude	5.1±1.1	1.6±0.2	<0.01
Mean LH, IU/Liter	9.4±1.6	3.4±0.2	<0.01