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The spontaneous endogenous pulsatile release of kisspeptin is temporally coupled with luteinizing hormone in healthy women

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Objective: To evaluate the presence of a spontaneous pulsatile release of kisspeptin and whether it is temporally coupled to LH pulses.
Design: Experimental study.
Setting: Academic medical center.
Patient(s): Thirty young healthy eumenorrheic women aged 20–37 years were included in the study group. All subjects were white women admitted to the Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland.
Intervention(s): Kisspeptin, FSH, LH, E_2, PRL, and insulin were evaluated in all subjects at baseline.
Main Outcome Measure(s): All women underwent a pulsatility study measuring LH and kisspeptin plasma concentrations to assess the spontaneous episodic secretion of both hormones, sampling every 10 minutes for 2 hours from 9:00 to 11:00 a.m. for a total of 12 blood samples. Detection and specific concordance (SC) algorithms were used to detect pulses and their concordance.
Result(s): A significant endogenous secretory pattern was demonstrated for both LH and kisspeptin over the 2-hour duration of the study (2.4 ± 0.1 peaks/2 h). The computation of the SC index showed for the first time that kisspeptin and LH are cosecreted and temporally coupled at time "0," and their peaks occur at the same point in time.
Conclusion(s): The present study provides evidence supporting the hypothesis that kisspeptin is highly relevant in the regulation and modulation of reproductive functions in humans. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Kisspeptin, LH, FSH, estradiol, pulses

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Kisspeptin is the main factor controlling gonadotropins secretion. Identified for the first time in 1996 as a suppressor of the malignant melanoma metastasis process in humans (1, 2), it is considered to be a relevant factor for initiating puberty, regulating sex steroid feedback, and controlling fertility in adults. Kiss1 is the gene responsible for encoding kisspeptin.

In 2001 kisspeptin was identified as a ligand for G protein–coupled receptor 54 (GPR54), which was first described in rat brains and later in humans, Kiss-1 mRNA expression has been demonstrated in the placenta and throughout the central nervous system (CNS), including the hypothalamus. Endogenous fragments of kisspeptin 54, 14, and 13 amino acids in length were isolated from the human placenta. The common C-terminal decapeptide shared by these forms was identified as kisspeptin-10. All kisspeptin fragments, including kisspeptin-10, have a similar affinity and efficacy at the previously identified GPR54 receptor (3).

Human kisspeptin neurons are located primarily in two areas: the preoptic area and the hypothalamic arcuate nucleus (ARC), also known as the infundibular nucleus. Kisspeptin modulation of GnRH secretion has been demonstrated to be dependent on sex steroid concentrations. In fact, E_2 and P modulate kisspeptin activity both in the preoptic area and in the ARC receptors. In rodents it has been shown that preoptic kisspeptin neurons

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modulate periovulatory positive estrogen feedback to GnRH (4). A limited number of functional studies in humans suggest that both groups (preoptic and ARC) of neurons are involved in this process (5). During the periovulatory period, it is thought that GnRH neurons directly transmit a kisspeptin-controlling signal to the pituitary, understood to be the pulsatile secretion of GnRH, thus stimulating LH and FSH pulsatile release. The negative feedback of E_2 in the follicular phase and of P in the luteal phase on GnRH secretion appears to depend mainly on ARC kisspeptin neurons (5). The role of other kisspeptin neurons identified in some brain regions remains unknown. GnRH neuronal axons lead from the arcuate nucleus to the median eminence, where GnRH is released in a pulsatile manner into portal circulation (5). These findings point to the direct involvement of kisspeptin in GnRH neurosecretion (Supplemental Fig. 1, available online at www.fertstert.org). However, in humans, not all GnRH neurons receive kisspeptin contact (6–9) which may suggest that kisspeptin action/regulation of GnRH secretion involves a more complex mechanism.

Although some studies indicate that kisspeptin directly stimulates the pituitary secretion of LH and FSH based on gene expression and kisspeptin-1 receptor, kisspeptin-induced GnRH secretion appears to be the main physiologic mediator of gonadotropin secretion. Indeed, the role of kisspeptin in the regulation of GnRH secretion was demonstrated in studies using kisspeptin antagonists: Kisspeptin antagonist administration inhibited GnRH production/secretion.

Direct kisspeptin action on GnRH neurons is supported by rodent and primate studies. Humans who develop "inactivating" mutations of the gene encoding kisspeptin and/or its receptor show the occurrence of hypogonadotropic hypogonadism (10). However, in patients in whom "activating" mutations were found, precocious puberty is observed, suggesting that kisspeptin is an important modulator of pubertal maturation and of the pulsatile GnRH secretion (11). Interestingly, in humans, although kisspeptin release stimulates the release of both gonadotropins, the activity for LH is more pronounced.

GnRH secretion models, followed by secretion of LH in different phases of the menstrual cycle, are modulated by a feedback of gonadal steroid hormones. During the follicular phase of the menstrual cycle, GnRH activity and LH secretion are limited by negative feedback effects determined by E_2. The reversal of negative to positive feedback regarding the midcycle LH surge is not yet well understood. Because GnRH neurons do not express estrogen receptors (ERs), the transmission of the ovulation trigger signal, forwarded from the gonads to the hypothalamic GnRH neurons, is likely mediated by a different, distinct neuronal population. Evidence obtained in recent studies indicate that kisspeptin neurons are the putative "missing link," mediating both negative and positive feedback of sex steroids (5).

On such basis, we aimed to evaluate whether a temporal coupling might be measured in plasma concentrations of kisspeptin and LH in a group of eumenorrheic young women, with the use of a simple pulsatility study.

MATERIALS AND METHODS

Study Group

Thirty young healthy women were included in the study group. All of the women were white and admitted to the Department of Gynecological Endocrinology, Poznan University of Medical Sciences, Poznan, Poland.

Inclusion criteria were age 20–35 years (mean 25.6 ± 6.1), regular ovulatory menstrual cycle (28 ± 5 days) during past year, and two recent ovulatory cycles documented with the use of hormonal evaluation (P level in luteal phase >9 ng/mL).

Before her enrollment, each subject gave written informed consent. The protocol was approved by the local Ethics Committee of the Poznan University of Medical Sciences. This study was not registered as a clinical trial, because it was an observational study.

Clinical Evaluation

Each subject was interviewed by a physician, who obtained data on past and current diseases, allergies, family history of endocrinologic, psychiatric, or neurologic illnesses, weight changes, and complete menstrual and medication histories.

No subjects were under hormone treatment during the 6 months before the study, and none were receiving hormone therapy at the time of the study. No subjects had chronic diseases requiring continuous therapy, and all subjects denied recent weight loss, eating disorders, or excessive physical activity. No mood or behavior disturbances were reported at the time of the enrollment; psychiatric disorders were excluded with the use of DSM V criteria. No signs of hyperandrogenism (i.e., hirsutism, acne, or balding/alopecia, or a score ≥ 8 on the modified Ferriman-Gallwey scale) were observed at the onset of the study. Patients with current or past infertility and miscarriage diagnoses were excluded. Eleven patients reported at least one pregnancy ending with live term birth, and the other 19 patients were nulliparous with no desire for pregnancy at the time of the evaluation.

The following clinical characteristics were recorded in all subjects: age, age at menarche, history of menstrual cycles, intermenstrual interval length, weight, height, body mass index.

Study Protocol

All subjects underwent a pulsatility study to determine kisspeptin and LH plasma concentrations. The pulsatility study was performed during the middle follicular phase of the menstrual cycle, days 8–10 from the reported onset of last menstrual period. The midfollicular state was confirmed with the use of transvaginal ultrasound, showing the presence of a dominant follicle measuring 10–17 mm, the lack of a luteal body, and serum E_2 concentrations in the range of 100–200 pg/mL. Only patients who ovulated during the studied cycle and whose P concentrations measured >9 ng/mL from day 16–20 of the cycle were included in the study. A heparin-containing intravenous line was placed in the antecubital vein 45–60 minutes before commencing venous sampling, and blood was withdrawn every 10 minutes for 2 hours