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The Importance of Signal Pattern in the Transmission of Endocrine Information: Pituitary Gonadotropin Responses to Continuous and Pulsatile Gonadotropin-Releasing Hormone*

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ABSTRACT. We tested the hypothesis that pulsatile GnRH stimulation of the pituitary is required for normal gonadotropin secretion in humans. We administered GnRH in pulsatile and continuous regimens in varying order to each of five women with hypothalamic amenorrhea and presumed endogenous GnRH deficiency. Mean serum levels of GnRH were similar during the pulsatile and continuous regimens. All women ovulated during the pulsatile regimen (progesterone, >31.8 nmol/L (10 ng/mL); none ovulated during the continuous regimen. Compared to pretreatment levels, FSH and estradiol, as measured by RIA, and LH, as measured by bioassay, increased significantly during the pulsatile GnRH regimen, but not during the continuous regimen. However, LH and α-subunit, as measured by RIA, increased significantly during both continuous and pulsatile GnRH administration. We conclude that a pulsatile pattern of GnRH is essential to normal functioning of the human female reproductive axis. Continuous administration of GnRH, producing mean serum levels of the peptide indistinguishable from those found during pulsatile administration, stimulates some rise in a nonbioactive form of radioimmunoassayable LH-like material and α-subunit, but does not stimulate bioactive LH, FSH, estradiol, or progesterone and does not lead to ovulation. (*J Clin Endocrinol Metab* **72:** 1286–1289, 1991)

TWO ASPECTS of hormonal signals are classically recognized as critical in regulating the function of target tissues: the molecular structure of the hormone and the hormone level that circulates in blood. More recently, it has become clear that nearly all hormones are secreted episodically, at intervals from a few minutes to hours (1). It is unclear whether the pattern of hormonal secretion is of general importance in endocrine signalling.

In ovariectomized, arcuate nucleus-lesioned monkeys, pulsatile GnRH infusions stimulate and maintain gonadotropin secretion (2), while continuous infusions do not. Pulsatile GnRH infusions are also effective in stimulating the pituitary-gonadal axis in men and women with GnRH deficiency resulting in hypogonadism (3–6). It has been assumed from animal studies (2) that continuous GnRH administration would be ineffective in humans. However, it is not known whether women with GnRH deficiency respond to continuous GnRH infusion in a dosage range that is known to be effective for inducing gonadotropin secretion and ovulation when administered in a pulsatile fashion. This is of practical as well as theoretical interest, since the continuous delivery of GnRH using a low dose of a long-acting analog or a sc implant would be much simpler than pulsatile administration, which requires an automated portable pump. In this study, we assessed the physiological and clinical importance of the pattern of GnRH in the regulation of gonadotropin secretion and ovulation.

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Materials and Methods

Subjects

Five women with the syndrome of idiopathic hypothalamic amenorrhea were studied. All had undergone normal puberty, but had experienced secondary amenorrhea for at least 1 yr at the time of study. They were otherwise in excellent health and on no medications. None was a marathon runner or elite athlete, and none had anorexia nervosa or bulimia. All patients had hormonal profiles characterized by low estradiol (E_2) and

progesterone levels and low gonadotropin levels (Table 1). Serum testosterone and PRL levels and thyroid function tests were normal.

Protocol

All patients were treated with both pulsatile and continuous GnRH regimens in varying orders. GnRH was delivered as either a single sc bolus every 90 min or a continuous sc infusion using portable infusion pumps, Auto Syringe (Travenol) or Pump Pal I (BioMedical Devices), worn 24 h/day, 7 days/week. The starting dosage of GnRH was 6 μg every 90 min, except for patient 3 in whom the starting dosage was 12 μg every 90 min.

Serum E_2 levels were measured by the clinical laboratory using a rapid RIA throughout the period of GnRH administration. If serum E_2 levels did not increase to greater than 75 pmol/L by days 10–14 of the initial regimen, the GnRH dosage was increased by an increment of 6 μg every 90 min, and E_2 monitoring was continued in the same fashion for the next 10–14 days. This sequence was repeated until either a sustained E_2 response was noted or for a total of 7–9 weeks. If a persistent E_2 elevation was noted, the GnRH dosage level was not altered, and monitoring was continued until menses occurred. Menses occurred in all women with pulsatile GnRH at a dosage of 6–12 μg every 90 min. Continuous GnRH dosages were increased further to 18 μg (in four patients) and 24 μg (in two patients) every 90 min. During the period of study each patient received one cycle of continuous and one cycle of pulsatile GnRH administration. In addition to rapid E_2 determinations by the clinical laboratory, blood was drawn every 1–3 days for subsequent determination of serum LH, FSH, E_2, and progesterone levels by RIA. Ovulation was defined by a progesterone level greater than 31.8 nmol/L (10 ng/mL). In each patient, serum α-subunit and LH bioactivity were measured in blood samples obtained after at least 1 week of each GnRH dosage regimen.

During each GnRH regimen, subjects were admitted to the University of Washington Clinical Research Center for frequent blood-sampling studies. Blood sampling was performed every 20 min for 6 h through an indwelling catheter immediately before initiation of GnRH infusion and between 7–15 days on each GnRH regimen. Serum was obtained, stored at −20 C and subsequently assayed for LH and FSH by RIA, allowing assessment of the patterns of gonadotropin secretion before and during GnRH regimens. When GnRH was being administered, blood samples were also obtained every 10 min for 2 h. These samples were immediately centrifuged and flash-frozen in dry ice-methanol, serum was stored at −70 C, and pools comprised of equal aliquots of serum obtained over 90 min were assayed for GnRH. The RIAs for LH (7), FSH (7), E_2 (8), progesterone (9), GnRH (10), and α-subunit (11) and the *in vitro* bioassay for LH (7) were performed as previously described. All samples from an individual patient were run in the same assay. Data were analyzed using repeated measures analysis of variance with Fisher PLSD multiple comparison procedures.

The study protocol was approved by the University of Washington Human Subjects Review Committee, and informed consent was obtained from each patient.

Results

All women ovulated during pulsatile GnRH administration, as confirmed by a rise in serum progesterone levels to greater than 31.8 nmol/L (10 ng/mL). In all patients, serum E_2 levels increased, and subsequent ovulation occurred with pulsatile GnRH infusions at dosages of 12 μg every 90 min or less. In contrast, progesterone and E_2 elevations were not achieved, and no patients ovulated during any of the continuous GnRH infusions despite increasing GnRH dosages up to 18–24 μg every 90 min.

Mean GnRH levels were comparable during the pulsatile and continuous GnRH regimens (39 ± 5 *vs.* 44 ± 7 ng/L, respectively; $P > 0.6$). Higher serum levels of GnRH were eventually reached during the continuous GnRH infusions at higher dosages.

Results of the frequent blood sampling to assess LH patterns are shown in Fig. 1 (data from patient 5). Before initiating GnRH treatment, LH (RIA) was consistently very low and without significant pulsatility. During continuous GnRH infusion (6 μg/90 min), LH (RIA) levels were higher and nearly constant, ranging from 35–40 μg/L. During pulsatile GnRH (6-μg bolus every 90 min), LH (RIA) levels were higher than during continuous infusion, and clear pulses of LH were demonstrable after each GnRH bolus.

A characteristic hormonal profile throughout the entire study period from patient 5 is shown in Fig. 2. LH RIA levels were very low at the onset of study, but rose rapidly and were maintained at nearly constant levels above baseline throughout continuous GnRH infusions in escalating doses ranging from 6–24 μg/90 min. FSH RIA levels initially increased, but were not maintained during continuous GnRH infusion. E_2 and progesterone

TABLE 1. Patient characteristics before study

Patient no.	Age (yr)	Ht	Wt (lb)	E_2 (pmol/L)	LH (μg/L)	FSH (μg/L)	Progesterone (nmol/L)
1	27	5′6″	128	44	6	35	1.6
2	33	5′8″	132	44	15	112	1.6
3	31	5′5″	122	55	12	128	1.6
4	27	5′8″	134	117	12	112	1.6
5	27	5′3″	132	44	8	68	2.2

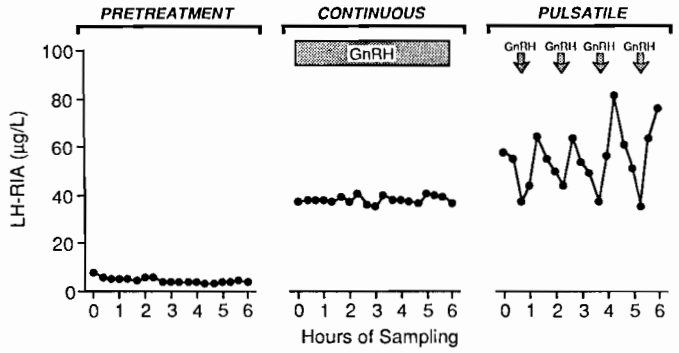

FIG. 1. LH (determined by RIA) measured every 20 min for 6 h in patient 5 during the pretreatment period and during the continuous and pulsatile GnRH regimens.

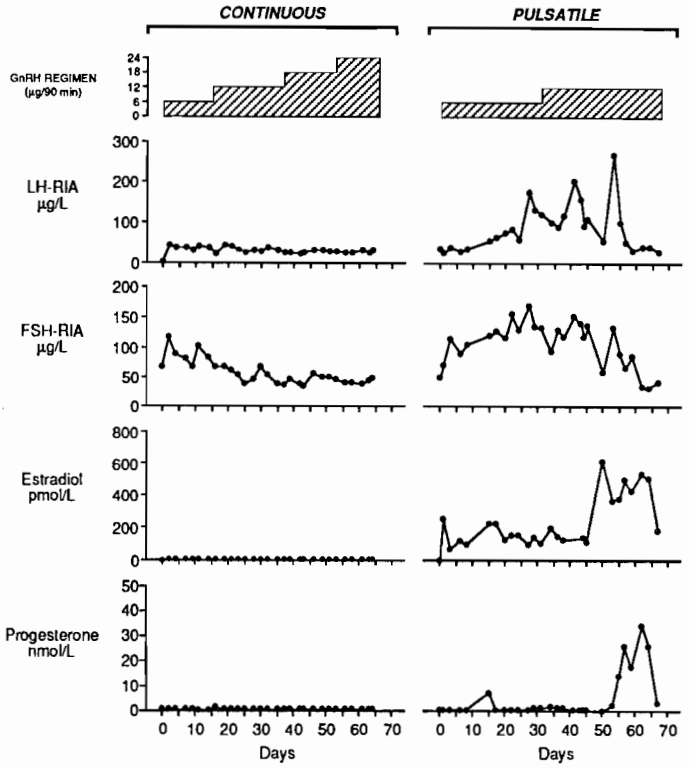

FIG. 2. Hormone data obtained every 1–3 days throughout the continuous and pulsatile infusions of GnRH in patient 5. GnRH dosages are indicated in the *cross-hatched areas*. Menses occurred on day 68 of the pulsatile regimen and never during the continuous regimen.

levels remained low and unchanged, despite nearly normal gonadotropin levels by RIA. In contrast, pulsatile GnRH infusion at a dose of 6 μg/90 min resulted in a gradual and sustained rise in both LH and FSH (RIA) levels with an associated, but poorly sustained, rise of E_2 and progesterone levels. The subsequent increase in pulsatile GnRH dosage to 12 μg/90 min resulted in a further rise in LH (RIA) levels and ovulation, characterized by progesterone levels greater than 31.8 nmol/L and a sustained E_2 elevation. The individual hormonal profiles during GnRH infusions were similar in the remaining patients.

Mean hormone levels in all five women, measured in frequent blood samples during the pretreatment and initial GnRH dosage regimens, are shown in Fig. 3. During the continuous GnRH infusions, LH (RIA) increased from 12 ± 2 during baseline to 33 ± 3 μg/L ($P < 0.05$). In contrast, serum LH bioactivity was unchanged during continuous GnRH infusion (180 ± 39 compared to 131 ± 4 μg/L during baseline; $P > 0.05$). Serum FSH (RIA) and E_2 levels during continuous GnRH treatment (90 ± 13 μg/L and 106 ± 51 pmol/L, respectively) were unchanged compared to baseline (91 ± 17 μg/L and 62 ± 19 pmol/L, respectively; $P > 0.05$). α-Subunit levels increased during continuous GnRH infusions from 0.45 ± 0.20 μg/L during baseline to 1.48 ± 0.27 μg/L ($P < 0.05$).

During the pulsatile GnRH infusions, both serum LH (RIA; 82 ± 9 μg/L) and LH bioactivity (634 ± 75 μg/L) increased significantly above pretreatment levels (both $P < 0.05$). Serum FSH RIA and E_2 levels also increased significantly to 133 ± 12 μg/L and 414 ± 161 pmol/L, respectively (both $P < 0.05$). Compared to baseline, α-subunit levels also rose (1.48 ± 0.28 μg/L; compared to

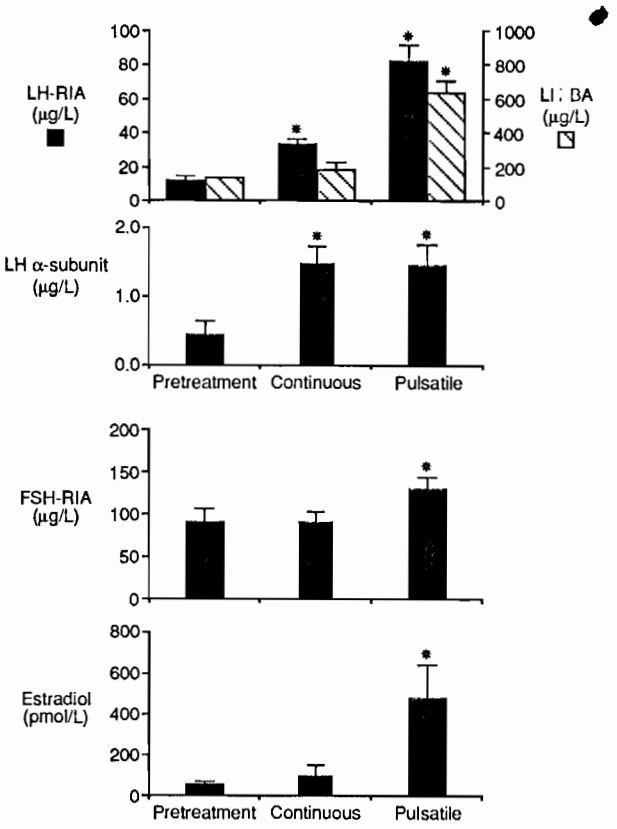

FIG. 3. Hormone data (mean ± SEM) for all five patients before treatment and during the initial continuous and pulsatile regimens of GnRH. Samples during treatment were obtained after at least 1 week on each regimen. *Asterisks* refer to significant ($P < 0.05$) differences from pretreatment control values. BA, Bioassay.

baseline, $P < 0.05$), but not higher than during continuous GnRH infusions ($P > 0.05$).

Discussion

In women with hypothalamic amenorrhea, ovulation was consistently induced by the pulsatile sc infusion of physiological dosages of GnRH, but not by continuous GnRH infusions at the same or higher doses. Comparable mean serum GnRH levels were achieved initially with the pulsatile and continuous infusion styles, and the pituitary responded with the sustained output of pulsatile and continuous patterns of LH secretion, as measured by RIA. Serum LH (RIA) levels increased significantly during both pulsatile and continuous GnRH infusions, while LH bioactivity, FSH (RIA), and E_2 levels increased only during the pulsatile GnRH infusions. These findings suggest that a pulsatile pattern of GnRH stimulation is required for normal pituitary gonadotropin and ovarian function and is, therefore, essential to the normal functioning of the human female reproductive axis.

Evidence is accumulating that the pulsatile pattern of hormone secretion is an important determinant of end-organ response in various endocrine systems. In ovariectomized hypothalamus-lesioned monkeys, pulsatile GnRH infusion stimulates normal pituitary LH and FSH secretion, while continuous GnRH infusion suppresses gonadotropin secretion (2). Insulin (12), GH (13), and glucagon (14) have also been reported to be more effective when administered in a pulsatile compared to a continuous fashion.

Mean GnRH levels in our patients were comparable during the continuous and pulsatile infusions of GnRH when administered at the same initial dosage. Therefore, differences in mean serum GnRH levels cannot explain the greater pituitary responsiveness observed with pulsatile GnRH infusions. Much higher GnRH levels were eventually reached during higher dosage continuous GnRH infusions, but normal gonadotropin secretion and ovulation were still not achieved. Serum LH bioactivity did not significantly rise above baseline during continuous GnRH administration. Serum FSH, E_2, and progesterone levels also remained unchanged during the continuous GnRH treatment. α-Subunit levels increased similarly during pulsatile and continuous GnRH infusion and may have accounted for some of the measured increase in LH (RIA) during the continuous GnRH infusions. We hypothesize that continuous physiological levels of GnRH may stimulate pituitary output of LH α-subunit and other biologically inactive or less bioactive forms of LH, and that GnRH pulses are instrumental in promoting the output of the biologically active forms of LH and FSH and normal ovarian activity. Consistent with this concept, excessively rapid or continuous GnRH is ineffective in stimulating LH β-subunit mRNA in the rat (15).

In summary, we found that in the woman with hypothalamic amenorrhea, pulsatile administration of GnRH is more effective than a comparable dose of GnRH given continuously in stimulating secretion of biologically active LH and FSH and inducing normal ovarian function. We conclude that in addition to the molecular structure and mean level of a hormone, the signal pattern is an important component in the transmission of endocrine information.

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References

1. Krieger DT, ed., Endocrine rhythms. New York: Raven Press; 1979.
2. Belchetz PE, Plant TM, Kakai Y, Keogh EG, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science. 1978;202:631–3.
3. Hurley DM, Brian R, Outch K, et al. Induction of ovulation and fertility in amenorrheic women by pulsatile low-dose gonadotropin-releasing hormone. N Engl J Med. 1984;310:1069–74.
4. Crowley Jr WF, McArthur JW. Stimulation of the normal menstrual cycle in Kallmann's syndrome by pulsatile administration of GnRH. J Clin Endocrinol Metab. 1980;51:173–5.
5. Skarin G, Nillius SJ, Wide L. Pulsatile low dose luteinizing hormone-releasing hormone treatment for induction of follicular maturation and ovulation in women with amenorrhea. Acta Endocrinol (Copenh). 1982;101:78–86.
6. Sheckter CB, McLachlan RI, Tenover JS, et al. Stimulation of serum inhibin concentrations by gonadotropin releasing hormone in men with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab. 1988;67:1221–4.
7. Bremner WJ, Matsumoto AM, Sussman A, Paulsen CA. Follicle stimulating hormone and spermatogenesis. J Clin Invest. 1981;68:1044–52.
8. Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WJ. Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors, and sperm production despite high estradiol levels. J Clin Endocrinol Metab. 1983;56:720–8.
9. Soules MR, Steiner RA, Clifton DK, Bremner WJ. The effects of inducing a follicular phase gonadotropin secretory pattern in normal women during the luteal phase. Fertil Steril. 1987;47:45–53.
10. Matsumoto AM, Karpas AE, Southworth MB, Dorsa DM, Bremner WJ. Evidence for activation of the central nervous system-pituitary mechanism for gonadotropin secretion at the time of puberty in the male rat. Endocrinology. 1986;119:362–9.
11. Tenover JS, Matsumoto AM, Plymate SR, Bremner WJ. The effects of aging in normal men on bioavailable testosterone and luteinizing hormone secretion: response to clomiphene citrate. J Clin Endocrinol Metab. 1987;65:1118–26.
12. Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC. Pulsatile insulin has greater hypoglycemic effect than continuous delivery. Diabetes. 1983;32:617–21.
13. Clark RG, Jansson JO, Isaksson O, Robinson IC. Intravenous growth hormone: growth responses to patterned infusions in hypophysectomized rats. J Endocrinol. 1985;104:53–61.
14. Weigle DS, Koerker DJ, Goodner CJ. Pulsatile glucagon delivery enhances glucose production by perifused rat hepatocytes. Am J Physiol. 1984;64–8.
15. Dalkin AC, Haisenleder DJ, Ortolano GA, Ellis TR, Marshall JC. The frequency of gonadotropin-releasing hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. Endocrinology. 1989;125:917–24.