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Pulsatile Administration of Gn-RH in Hypothalamic Amenorrhea

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ABSTRACT

The physiological and pathophysiological basis of hypothalamic amenorrhoea are reviewed as well as the clinical results of chronic intermittent (pulsatile) administration of Gn-RH in the treatment of infertility. Hypothalamic amenorrhoea is considered to be the result of a deficient hypothalamic secretion of Gn-RH. By pulsatile administration of Gn-RH, which is a pre-requisite of normal pituitary gonadotrophic function, deficient endogenous Gn-RH is replaced. If an adequate dose of Gn-RH is provided, which takes into account the degree of impairment of hypothalamic function in the individual case, follicular maturation, ovulation and corpus luteum formation are achieved in nearly every treatment cycle. Although dependent also on factors other than the treated dysfunction, a high conception rate is achieved.

INTRODUCTION

Gonadotropin releasing-hormone (Gn-RH) was the second of the neurohumoral agents postulated by Harris more than three decades ago to mediate hypothalamic control of anterior pituitary function that has been isoleted, identified in its structure and synthesized. Since this was achieved by the groups of Schally and Guillemin in 1971 and the snythetic hormone became available, Gn-RH has been used extensively as tool in neuroendocrine research. Early attempts to use this decapeptide clinically for the treatment of reproductive disorders supposed to be due to an inadequate secretion of endogenous Gn-RH, however, were of only limited success. Effective therapeutic use had to await further progress in the understanding of the physiologic significance of pulsatile gonadotropin secretion and gonadal function. The demonstration that the pattern of the hypophysiotropic stimulation is of critical importance in this respect and the elucidation of the physiologic significance of pulsatile Gn-RH secretion have provided the rational basis for the efficient use of synthetic Gn-RH in the treatment of Gn-RH deficiency.These findings have also furthered the

understanding of the seemingly paradoxical antifertility effects of long acting Gn-RH analogues initially designed to compensate for the short action of the parent decapeptide and thus to simplify treatment of infertility. In this communication, following a short review on physiologic and pathophysiologic aspects of hypothalamic control of gonadotropin secretion in the human female, clinical data obtained with chronic-intermittent (pulsatile) administration of Gn-RH in hypothalamic amenorrhea (HA) will be presented.

THE PULSATILE PATTERN OF GONADOTROPIN SECRETION DURING THE NORMAL MENSTRUAL CYCLE

The pattern of gonadotropin secretion during the normal menstrual cycle is characterized by low serum levels of LH and FSH during the follicular and luteal phases of the cycle interrupted by a sharp increase of LH and FSH during midcycle which causes ovulation. It has been shown by numerous investigators that this cyclic pattern of pituitary gonadotropin secretion can be regarded as a result of negative and positive feedback effects of ovarian steroids on pituitary function (13,21).

As first demonstrated in the castrated rhesus monkey, the pituitary release of LH is pulsatile in nature reflecting a pulsatile stimulation of the pituitary gonadotrophs by hypothalamic Gn-RH (8). By measurement of immunoreactive Gn-RH in the portal stalk effluent (5) and in the cerebrospinal fluid of the third ventricle (29) of the rhesus monkey direct evidence for the secretory pattern of hypothalamic Gn-RH could be provided. The pulsatile secretion of Gn-RH is directed by the arcuate nucleus of the mediobasal hypothalamus (13). Selective destruction of this region in the brain will abolish pituitary secretion of LH and FSH. Moreover, electrophysiological studies have shown that rhythmic increases in multiunit activity in the region of the arcuate nucleus are coincident with the initiation of LH pulses in serum (14).

In the agonadal female high amplitude LH pulses are observed every 90 minutes on the average (25,31). The same studies had established that pulses with approximately this frequency, but a lower amplitude occur during the follicular phase of the cycle, while during the luteal phase low-frequency-high-amplitude pulses prevail. A more close analysis of the pulsatile pattern of the LH release revealed that from day 3 - 5 of the follicular phase until after the midcycle surge pulse frequency does not change and is maintained at one pulse every 90 minutes (21,30). During the luteal phase there is a progressive

decline in LH pulse frequency, which is lowest immediately before menstruation and increases again during the first few days of early follicular phase. There is no direct relationship between progesterone concentrations and the reduction in LH pulse frequency. The reduction, however, appears to be correlated with the duration of the progesterone elevation. The physiologic significance of the changing frequency of gonadotropin secretion during the menstrual cycle, particularly during the luteal phase, remains to be elucidated. The observation that normal menstrual cycles can be induced in women (17,19) and in rhesus monkeys (13) with essentially abolished endogenous Gn-RH secretion by the pulsatile administration of Gn-RH at an unvarying frequency, however, argues strongly against any major physiologic importance of this phenomenon for the regulation of luteal function and of follicular development.

PATTERN OF GONADOTROPIN SECRETION IN PATIENTS WITH HYPOTHALAMIC AMENORRHEA

Complete absence or severe reduction of pulsatile gonadotropin release results in impairment of follicular maturation, anovulation and amenorrhea (16, 19). While this obtains physiologically before puberty or during pregnancy and lactation, it is pathological in other periods of reproductive life. Since there is substantial indirect evidence that cause of this kind of amenorrhea is a reduced stimulation of the anterior pituitary gland by Gn-RH and since Gn-RH is secreted from the hypothalamus, it is referred to as hypothalamic amenorrhea (16,22).

The term "hypothalamic amenorrhea" was coined by Klinefelter and associates in 1943 (12) to describe amenorrhea of suprapituitary origin. Due to some cases described in the original publication, however, it was later on confined to psychogenic amenorrhea. In this communication, hypothalamic amenorrhea is used in its broader original sense and consequently applies for patients with lesions of the pituitary stalk or hypothalamus, anorexia nervosa, Kallmann's syndrome as well as for idiopathic or psychogenic amenorrhea.

Since endogenous Gn-RH cannot be measured reliably in peripheral blood direct evaluation of hypothalamic function is presently not possible. Therefore, the diagnosis of hypothalamic amenorrhea is essentially based on the exclusion of other causes of amenorrhea, such as hyperprolactinemia, hyperandrogenemia, primary ovarian failure, genital tract defects as well as internal and neurological diseases. Primary pituitary failure is excluded by the ability to stimulate pituitary gonadotropic function by pulsatile administration of Gn-RH.

Based on studies in amenorrhoic patients, prepubertal subject and experimental animals the view has been advanced that hypothalamic amenorrhea forms a pathophysiological continuum, reflecting a gliding scale of impairment of hypothalamic Gn-RH secretion and consequently gonadotropin production and follicular development and it was furthermore proposed that the extent of this impairment can be assessed by the response to Gn-RH-, gestagen-, and clomiphene-administration (16,22). The reactions in those simple tests have therefore been used a criteria for grading of amenorrhoic patients according to the severity of hypothalamic impairment and for selection of the appropriate therapy (table 1).

Table 1. Grading of hypothalamic amenorrhea on the basis of clomiphene-, gestagen- and Gn-RH-tests, respectively

Grade	Result of test
1	Clomiphene positive (bleeding)
2	Gestagen positive (bleeding) Clomiphene negative (no bleeding)
3	Gestagen negative (no bleeding) with pituitary response to 100 µg of Gn-RH i.v.
3a	"adult" response
3b	"prepubertal" response
3c	no response

Recent studies on the pulsatile pattern of LH in serum, the frequency of LH pulses, overall LH and FSH levels during a 24 hour period as well as on ultrasonographic visualization of ovarian follicles in 20 patients suffering from hypothalamic amenorrhea supported this view (figure 1) (30). The number of LH pulses was lowest in grade 3c patients and increased gradually until a value comparable of that of the normal menstrual cycle was reached in grade 2 patients. Only in grade 3b patients an increase in pulse frequency during sleep became apparent, while in all other grades pulses were found to be evenly distributed between sleep and awake periods. Amplitude of some LH pulses, however, was considerably larger during sleep than during awake periods in grade 3b, 3a and grade 2 subjects. Overall LH and FSH levels increased parallel to the number of LH pulses up to grade 3a and 2, respectively, but failed to reach values typical for the early follicular phase of the cycle. In clomiphene

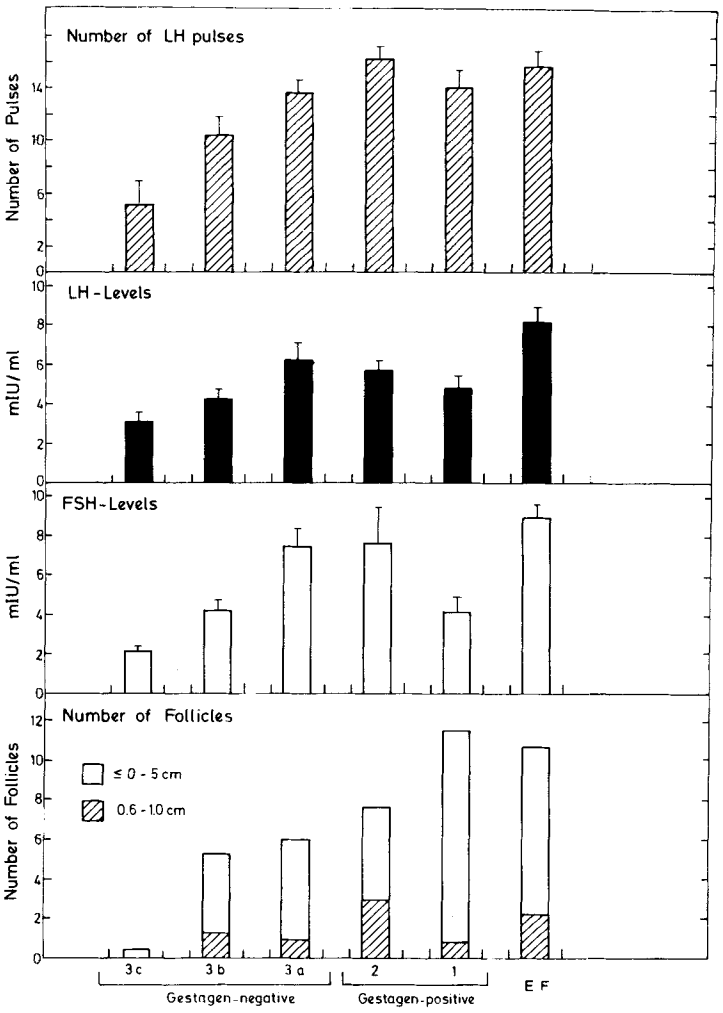

Fig. 1. Compositive showing number of LH pulses in 24 hours, mean LH and FSH levels of the 24-hours sampling period and number of class I and class II follicles in patients suffering from hypothalamic amenorrhea graded according to response to Gn-RH-, gestagen- and clomiphene-administration. Bars indicate mean ± SEM. The corresponding values for the early follicular phase (day 3-7) of 13 normal menstrual cycles (EF) are given for comparison and represent values of 8-hours sampling periods. The number of follicles given under EF represents the maximum number observed for each class of follicles. From Wildt et al. (1983), with permission (30).

positive patients, LH and particularly FSH levels declined again and this may be attributed to negative feedback inhibition by the elevated levels of estradiol found in those patients.

Considerable follicular development up to large antral stage has been found in ovarian biopsies of amenorrhoic patients (24). This was reflected by the number of follicles identified by ultrasound, which increased parallel to the number of LH pulses from essentially undetectable in grade 3c patients to a hight comparable to that found during the phase of maximal follicular development during the early follicular phase of the cycle in clomiphene positive patients. Thus, hypothalamic amenorrhea is characterized by a reduced frequency and amplitude of gonadotropin secretion which is reflected by the concomitant reduction of ovarian follicular growth. In this context, a reduction of frequency of pulsatile gonadotropin secretion is distinctive for the most severe grades of hypothalamic amenorrhea, while a reduction in amplitude characterizes less pronounced grades of this disorder.

One is tempted to speculate that the reduction of frequency and amplitude of pulsatile gonadotropin secretion closely reflects a corresponding reduction of frequency and amplitude of hypothalamic Gn-RH secretion, butthis has still to await experimental proof. In any event, the findings provided by these investigations stronly support the earlier view that hypothalamic amenorrhea forms a pathophysiological continuum on the basis of a reduced Gn-RH secretion (16,21, 22). They furthermore demonstrate, by showing a close correlation between the secretory pattern of gonadotropins and the results of the Gn-RH, gestagen- and clomiphene tests the validity of the grading system based on these test and therefore support its use for the assessment of residual hypothalamic function in patients suffering from hypothalamic amenorrhea.

The pattern of gonadotropin secretion in patients suffering from different grades of hypothalamic amenorrhea closely resembles that observed during the developmental process of puberty (3,32). At least from a descriptive point of view, hypothalamic amenorrhea may therefore be viewed as a regression into puberty or prepuberty in patients suffering from secondary amenorrhea, or as an arrest of the developmental process in those presenting with the primary form of this disorder. Such a mechanism has already been proposed for development of amenorrhea in anorexia nervosa (4,9) but seems to apply for other forms of hypothalamic amenorrhea also.

THE FUNCTIONAL ROLE OF THE HYPOTHALAMUS IN THE REGULATION OF GONADOTROPIN SECRETION

The physiological significance of the pulsatile pattern of Gn-RH secretion did not become apparent until recently, when it was shown that only pulsatile and not continuous administration of Gn-RH was able to maintain pituitary gonadotropic function in ovariectomized rhesus monkeys, in which endogenous Gn-RH secretion had been abolished by lesions in the medio-basal hypothalamus (1). The requirement of a pulsatile stimulation with Gn-RH by the pituitary gonadotrophs may explain, why administration of long acting analogues of the decapeptide was essentially unsuccessful in the treatment of secondary amenorrhea (10), and did even deterioriate pituitary gonadotropic function in normal women (2,7). Moreover, it could be demonstrated with the model of the hypothalamus lesioned rhesus monkey that the site of action of estradiol in exhibiting negative and positive feedback effects on the pituitary secretion of LH and FSH is localized on the level of the pituitary rather than on the level of the brain (23). In hypothalamus lesioned but otherwiese intact female rhesus monkeys the pulsatile administration of an unvarying amount of Gn-RH at a physiologic frequency induced menstrual cycles which were not different from spontaneous ones (15). Thus, the endocrine regulation of the menstrual cycle of primates appears to be fundamentally different from that of the estrous cycle of the rat. While in the rat the rostral part of the hypothalamus seems to be essential in the mediation of chrono-biological signals and positive feedback reactions. the assumption of such a "cyclic center" appears no longer to be justified for the primate. In the primate the function of the hypothalamus in the regulation of the menstrual cycle is only a "permissive" one (15).

In women with severe hypothalamic amenorrhea, a condition functionally comparable with that of the hypothalamus lesioned female rhesus monkey, chronic intermittent (pulsatile) administration of Gn-RH with an unvarying dose and at an unchanged frequency of one pulse every 90 minutes resulted in follicular maturation, ovulation and corpus luteum formation (16,17) the endocrine pattern of the normal menstrual cycle could be completely replicated.

Thus, it could be shown that the concept of the permissive function of the hypothalamus developed in the rhesus monkey could be extended to the human female. These results have been confirmed by other investigators (6,11,26,27) and with the development of chronic-intermittend (pulsatile) administration of Gn-RH by means of a small computerized pump ("Zyklomat", Ferring GmbH, Kiel,

FRG) as a new and practical mode of treatment of infertility in hypothalamic amenorrhea clinical advantage has been taken of these new insights into the physiology of the human menstrual cycle (18,19,22).

CLINICAL RESULTS OF PULSATILE ADMINISTRATION OF GN-RH IN HYPOTHALAMIC AMENORRHEA

Since the first introduction of pulsatile administration of Gn-RH to women with hypothalamic amenorrhea treatment cycles have so far been completed in our institution. The patients were selected for pulsatile treatment on the basis of the criteria described above. Only patients with hypothalamic amenorrhea of grades 2 - 3c were considered suitable for Gn-RH substitution.

Dose of Gn-RH. Follicular maturation and ovulation could be induced by intravenous application of 2.5 - 20 µg of Gn-RH per pulse in patients suffering from hypothalamic amenorrhea grades 2 - 3b, respectively. Some patients suffering from grade 3c of hypothalamic amenorrhea may require a dose of 15 - 20 µg of Gn-RH per pulse, while others with the same degree of severity of hypothalamic impairment ovulate with a dose of 5 µg per pulse intravenously. The different dose requirements among patients of the same grade is not clear.

There is a dose response relationship between the dose of Gn-RH administered per pulse and the ovarial response, as indicated by studies performed in patients with hypothalamic amenorrhea grade 3b (22). The mean estradiol and progesterone levels of the cycles induces with 15 - 20 µg/pulse were all above those obtained in cycles with 2.5 - 5 µg/pulse.

Substitution during the Luteal Phase. The normal luteotrophic hormone in the human is pituitary LH (28). In severe hypothalamic amenorrhea corpus luteum function immediately caeses following termination of pulsatile Gn-RH substitution a few days after ovulation (20). Continuation of pulsatile administration of Gn-RH during the whole luteal phase resulted in normal luteal function as indicated by the length of the luteal phase, the progesterone levels in serum and conceptions. Previously, it was suggested to support the luteal function by one to three injections of 2500 IU of HCG once ovulation had been obtained by Gn-RH (18). There is, however, no indication on the basis of our data (22) that one method of luteal substitution is superior over the other in terms of pregnancy rate obtained.

Intravenous Versus Subcutaneous Application of Gn-RH. The same catheter used for the i.v. application of Gn-RH was also used for the subcutaneous route, however without the addition of heparin to the hormone containing solution. The catheter was placed into the fat tissue of the lower adominal wall. Ovulations could be induced with doses of 5 - 20 µg/pulse in patients with hypothalamic amenorrhea of grades 2 - 3b and with 20 µg/pulse in a patient with hypothalamic amenorrhea grade 3c following the removal of a craniopharyngeoma (20). Four pregnancies were obtained with the s.c. route. However, in contrast to the i.v. application with a 100% ovulation rate, the adequate dose per pulse provided, there was an incidence of only 13 ovulatory cycles in 21 s.c. applications of Gn-RH. However, all these patients who did not ovulate during s.c. application, had ovulatory cycles when Gn-RH was intravenously applied at the same dose level. Delayed resorption of Gn-RH from the subcutaneous fat tissue might result in insufficient serum levels of Gn-RH for adequate stimulation of the pituitary gonadotrophs.

Ovulation- and Pregnancy-Rate. The adequate dose of Gn-RH provided ovulation and normal luteal function can be expected in every i.v. treatment cycle. The ovulation rate is reduced, when the s.c. route is chosen. Definitive treatment failure (no ovulation) was only observed when the diagnosis of hypothalamic amenorrhea was not correct

The pregnancy rate is remarkably high. Of 30 patients 26 became pregnant. One patient had two successful pregnancies two years apart. Twenty four pregnancies are completed with 29 children born, among them 3 sets of heterozygous twins and one set of triplets. Five patients aborted of whom one patient had two sequential abortions probably due to active cytomegaly. Four of these patients conceived thereafter again and had uneventful pregnancies so far. Totally, 32 conceptions were obtained in 30 patients.

The pregnancy rate, however, is critically dependent upon whether or not additional factors causing infertility of the couple are present (i.e. tubal or andrological factors). 64 treatment cycles were applied in 27 favourable couples, in whom the hypothalamic amenorrhea constitutes the only cause of infertility of the couple and 29 pragnancies were obtained (2.2 cycle per pregnancy). Totally, the pregnancy rate is comparable to the normal population. In 97 ovulatory treatment cycles 32 conceptivum occurred (3.0 cycles per pregnancy).

Ovarian Overstimulation and Multiple Pregnancies. The feedback mechanisms of ovarian steroids on the pituitary secretion of the gonadotropic hormones

are operative during pulsatile administration of Gn-RH. Clinical signs of ovarian overstimulation have therefore not been observed during 130 treatment cycles. There is, however, a dose response relationship between the dose of Gn-RH and the ovarian response, which is mediated by a dose related pituitary secretion of gonadotropins. If it is taken into consideration that the recruitment of follicles, the selection of the dominant follicle and the suppression of the other accompanying follicles is dependent to a certain degree upon the gonadotropic stimulation, it has to be expected that a gonadotropic stimulation of the ovaries resulting in discrete chemical overstimulation must cause an increased incidence of multiple pregnancies as compared to the normal population. In our study 4 multiple pregnancies were obtained out of 30 conseptions. One of these multiple pregnancies was obtained by too high a dose for the respective grade (20 µg/pulse in grade 3b of hypothalamic amenorrhea).

CONCLUSIONS

Pulsatile administration of Gn-RH by means of a portable pump ("Zyklomat") has proven to be an efficient and practical method for the induction of ovulation as a treatment of infertility in hypothalamic amenorrhea. The results obtained with this method of treatment are critically dependent upon the correct selection of patients as far as the diagnosis of hypothalamic amenorrhea is concerned. Patients with hypothalamic amenorrhea, previously treated with human gonadotropins are suitable for this mode of treatment. Further intensive studies have to demonstrate whether other anovulatory conditions, such as polycystic ovarian disease and hyperprolactinemia (17), are also suitable for pulsatile Gn-RH administration.

In our study 32 conceptions were obtained in 30 patients. These favourable results are obtained due to a rather physiological stimulation of the ovaries during chronic intermittent (pulsatile) administration of Gn-RH. On the basis of operating negative and positive feedback mechanisms of the ovarian steroids on the pituitary secretion of the gonadotropins during treatment, the follicle itself regulates the required amount of gonadotropin stimulation. However, since there is a relationship between the Gn-RH dose per pulse applied and the reaction of the pituitary-ovarian axis, the lowest efficient dose of Gn-RH in reliably inducing ovulatory cycles should be chosen.

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