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GONADOTROPHIN RELEASING HORMONE THERAPY

C. H. MORTIMER, A. S. McNEILLY and G. M. BESSER

The Medical Professorial Unit and Department of Chemical Pathology, Saint Bartholomew's Hospital, London, EC1 7BE

SUMMARY

In view of the high proportion of patients with demonstrable hypothalamic or pituitary disease who responded to a single intravenous bolus injection of Gn-RH it was hoped that repeated administration (500 μg 8 hourly) of the decapeptide would prove to be of therapeutic value.

Treated prepubertal males (craniopharyngioma, isolated growth hormone deficiency) showed an improvement in pubic hair, a marked increase in the frequency of erections and testicular size increased.

Nine adult males with hypothalamic or pituitary disease have been treated ; in all these patients, a rapid return of potency has been observed between 2 and 17 days after starting therapy. In four of them treated for 12-18 months potency has been well maintained. In addition, spermatogenesis was induced within 7 to 26 weeks of starting therapy. FSH levels have fallen as the sperm count has risen (« inhibin ») although LH levels have been little altered. One patient's wife is now pregnant.

Female patients with anorexia nervosa (clomiphene unresponsive) and documented amenorrhea for 5-7 years, have been treated with Gn-RH resulting in ovulation and mensturation. The addition of HCG 4 500 units on day 14 in one patient also resulted in pregnancy.

Gn-RH may be of value in the treatment of male and female infertility.

INTRODUCTION

Following the work of McCann 1962, McCann and Dhariwal 1966, it was suggested that hypothalamic regulation of pituitary gonadotrophin secretion was by means of two separate releasing hormones for luteinising hormone (LH) and follicle stimulating hormone (FSH). However, Schally *et al.* (1971) were able to isolate only a single gonadotrophin releasing hormone (Gn-RH) from porcine hypothalami. Administration of this decapeptide results in both LH and FSH release and therefore has left unresolved the identity of FSH-RH, if indeed it exists at all in man.

When the synthetic material became available many centres including our own investigated its properties. It was shown that the decapeptide released both LH and

FSH in a dose related manner when 25 to 500 μg was given as an intravenous bolus (BESSER *et al.*, 1972). Subsequently a standard 100 μg test was devised sampling for LH and FSH before and 20 and 60 minutes after the injection of Gn-RH. This test was then applied to 155 patients with hypothalamic-pituitary-gonadal disease. The results showed that although 88 p. 100 were clinically hypogonadal when tested initially, only 10 p. 100 of the patients had an absent response to the releasing hormone. It was also evident that this test could not distinguish between hypothalamic and pituitary disease since identical responses could be obtained in either group, although primary gonadal failure resulted in an exaggerated response (MORTIMER *et al.*, 1973). In view of the large number of patients with demonstrable hypothalamic or pituitary disease who did respond it was hoped that repeated administration of the decapeptide would prove to be of therapeutic value.

Studies of the time course of action of 100 μg Gn-RH given by intravenous, intramuscular or subcutaneous routes showed that they were equally effective in elevating LH for 5-7 hours and FSH for 3-5 hours (MORTIMER *et al.*, 1974 *a*). Intranasal administration unfortunately was not particularly effective since at least 2 mg had to be given by this route to produce the same effect as 100 μg by the other routes (LONDON *et al*, 1973). In our studies, therefore, self administration of Gn-RH by the subcutaneous route was chosen and in view of the time course of gonadotrophin release injections were given 8 hourly. A dose of 500 μg was used since earlier studies had shown that patients with isolated gonadotrophin deficiency' might fail to respond to 100 μg, but this could be improved by increasing the dose (MARSHALL *et al.*, 1972). Patients who had previously received gonadai steroids or intramuscular gonadotrophin treatment were taken off all therapy for at least 4 months prior to Gn-RH administration.

PREPUBERTAL MALES : PUBERTY AND POTENCY

Initially five prepubertal patients were treated aged 14 to 22 years. Of these, four had craniopharyngiomas and one isolated growth hormone deficiency. All were clomiphene negative except one (craniopharyngioma). Treatment resulted in progressive increases in both gonadotrophins with the FSH response being greater initially than that of LH. This prepubertal pattern of response (FRANCHIMONT *et al.*, 1974) was later reversed becoming adult in type. (fig. 1). Of the five patients, two were still growing with intramuscular growth hormone therapy and so Gn-RH treatment was discontinued after 4 weeks since it was considered undesirable to induce puberty while they still had growth potential. However, therapy was continued in two of them (craniopharyngioma, isolated growth hormone deficiency). Before treatment one had pre-adolescent genitalia at Stage 1 puberty (TANNER, 1958). After 8 weeks of treatment this patient showed an increase in spontaneous erections with Stage 3 pubic hair. The other was Stage 3 before therapy but after nine weeks showed an improvement in pubic hair with a marked increase in the frequency of erections. Testicular size increased from 4-6 ml before treatment to 6-12 ml after 20 weeks in these patients.

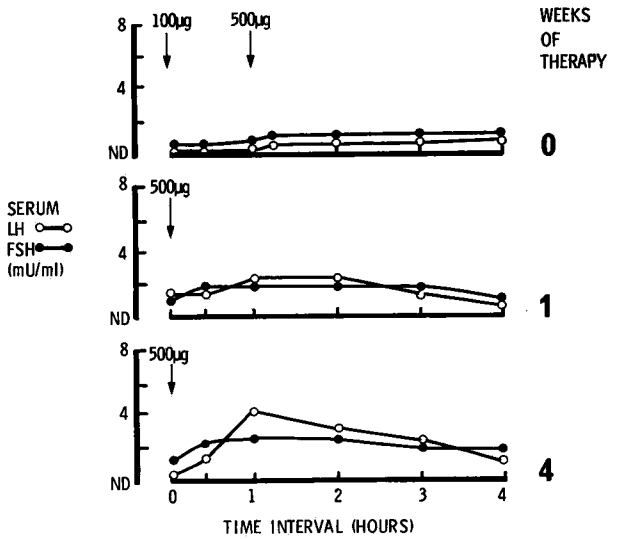

FIG. 1. — *Serum LH ○ and FSH ● responses to Gn-RH in a prepubertal male aged 14 years with a craniopharyngioma*

ADULT MALES : POTENCY AND SPERMATOGENESIS

Nine adult males with hypothalamic or pituitary disease have been treated. Of these, six had isolated gonadotrophin deficiency ; one craniopharyngioma ; one acromegaly treated with external pituitary irradiation and one a diffuse hypothalamic tumour of unknown origin. In all patients there was an increase in potency between 2 and 17 days after starting therapy, despite testosterone levels below the normal adult male range in all but two. The rapid return of potency in these patients despite levels of testosterone normally associated with impotence suggests that these behavioural effects may not be entirely androgen mediated. It is of interest that MOSS and MCCANN (1973), PFAFF (1973) have reported an increase in the number of lordotic responses in ovariectomised and hypophysectomised rats following subcutaneous administration of the releasing hormone. The role of Gn-RH in psychogenic impotence remains to be defined and we await the results of a double blind crossover trial being carried out in conjunction with Professor R. HALL and co-workers in Newcastle.

Four adult males have been treated for 12-18 months and potency has been well maintained. In addition, spermatogenesis was induced within 7 to 26 weeks of starting therapy. Two with « isolated » gonadotrophin deficiency and the craniopharyngioma patient had low or undetectable basal gonadotrophins, and all were clomiphene unresponsive. During initial therapy the prepubertal type of gonadotrophin response to Gn-RH was seen although later as in the prepubertal boys this pattern became adult in type. During therapy the maximum LH levels following a therapeutic 500 µg dose of Gn-RH showed no definite pattern. However, in all three patients the maximum FSH response began to decline before spermatazoa were seen

on seminal analysis and showed a progressive fall into the normal basal male range throughout therapy (fig. 2).

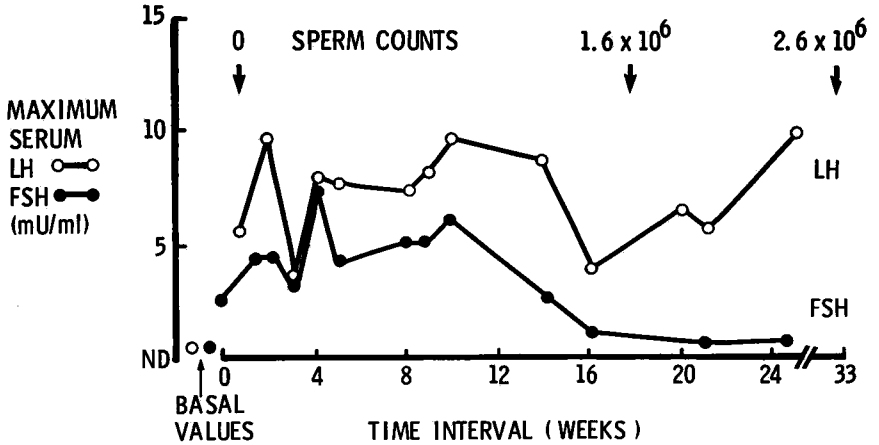

FIG. 2. — *Maximum serum LH ○ and FSH ● responses to 500 μg Gn-RH subcutaneously in a man with isolated gonadotrophin deficiency*

The gonadotrophin results in the patient with acromegaly were different since he had an elevated basal FSH level of 17.3 mU/ml (normal range less than 0.2 to 5.9 mU/ml, MRC standard 68/39) although the basal LH was normal at 3.8 mU/ml (normal range less than 0.4 to 6 mU/ml, MRC standard 68/40). He had much reduced potency, low plasma testosterone and was azoospermic. When tested with 100 μg Gn-RH he had a normal LH response but an exggerated FSH rise. The maximum FSH response at the beginning of treatment showed levels greater than 50 mU/ml but fell progressively before and following the appearance of spermatozoa (fig. 3). During treatment in these four patients total sperm counts have risen from zero (or 600.000 dead spermatozoa in one patient who had received human chorionic gonadotrophin 4 months prior to the study) to maximum levels of 7.8 and 60.8 million (isolated gonadotrophin deficient patients), 146 million (acromegalic) and 432 million (craniopharyngioma) with a motility of 40-70 p. 100. This was accompanied by an increase in testicular volume from 1-3 mls before to between 8-15 mls after 52 weeks of treatment. In all patients in whom spermatogenesis has occurred FSH levels have fallen as the sperm count has risen although LH levels have been little altered. This suggests that a factor (inhibin) has been released which selectively impairs FSH secretion. Also, since circulating Gn-RH levels were maintained by repeated subcutaneous injection it would seem that the site of action of inhibin is primarily at the pituitary level. It is also clear that pituitary responsiveness in male subjects may be modified by circulating substances other than testosterone or oestrogen (plasma 17β-oestradiol levels were within the normal male range throughout therapy except on three isolated occasions).

Following one year of therapy the acromegalic patient was treated with bromocriptine in order to suppress growth hormone secretion (THORNER *et al.*, 1974). Gn-RH therapy was continued and the total sperm maintained around 130 million. This patient's wife is now 3 months pregnant.

Gn-RH therapy has been used also to treat patients with oligo-or azoospermia. (ZARATE *et al.*, 1973). Treatment with 500 μg subcutaneously, twice daily for 6 months resulted in an increase from a total count of 1-5 million basally to 10 million. The count then fell despite continued therapy. Three of their patients showed no significant improvment although all had an increase in motility. It remains to be seen if Gn-RH therapy is of benefit in patients with primary gonadal dysfunction resulting in infertility.

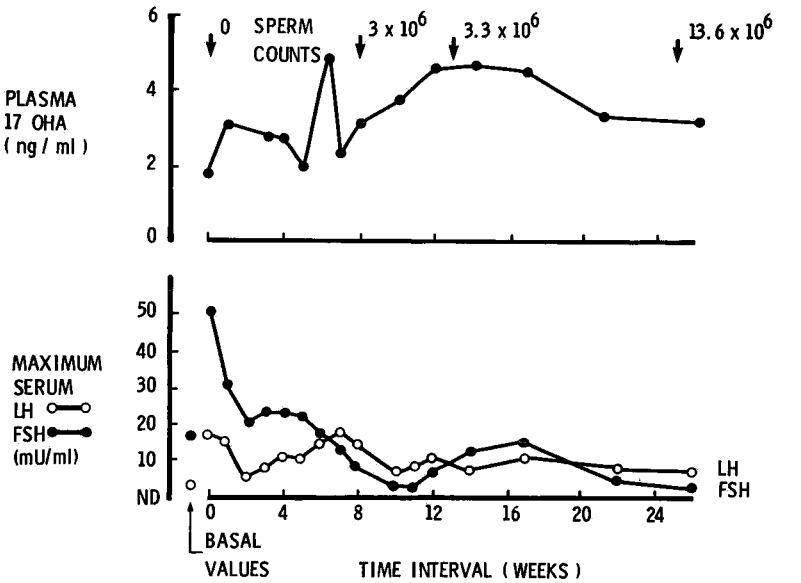

FIG. 3

Top : *Plasma 17 hydroxyandrogen levels* (normal range 5-22.5 ng/ml) *and increasing total sperm counts in a man with acromegaly.*

Bottom : *Maximum serum LH* ○ *and FSH* ● *responses to 500* μg *Gn-RH subcutaneously.*

FEMALES : OVARIAN STEROIDOGENESIS, OVULATION AND MENSTRUATION

In 1972, AKANDE *et al.*, reported a series of studies in 8 patients with secondary amenorrhoea although the diagnosis was not recorded in all of them. However, although a rise in gonadotrophins was observed ovulation occurred in only one who had ovulated spontaneously while basal sampling was being carried out prior to the study. Other studies were subsequently reported (KELLER 1973 ; BRECKWOLDT *et al.*, 1974) but the value of Gn-RH therapy for ovulation induction remained undecided. More recent studies have produced ovulation in 2 of 16 women with secondary amenorrhoea following depot-medroxyprogesterone acetate or chlormadinone therapy for contraceptive purposes (ZANARTU *et al.*, 1974). However, the low success rate achieved suggests that the optimal regimen for reliable ovulation induction remains to be found.

In our own study, we have treated four patients aged between 19 and 29 years with anorexia nervosa and documented amenorrhoea for 5-7 years. These patients were either at their ideal body weight or between 3.5 and 5.8 kgs below this, and each was clomiphene unresponsive. Basal total urinary oestrogens were between 4 and 17 μg/24 hours measured fluorimetrically (BROWN *et al.*, 1968). Initial therapy with 500 μg Gn-RH was given by subcutaneous injection 8 hourly for 7 days, then stopped and restarted on days 12-14. During this study samples for serum gonadotrophins were collected 10 hours after the last dose of releasing hormone given the previous night. Basal serum gonadotrophins were low or absent in all these patients although during therapy these was a rise with more LH than FSH secreted. During the first seven days urinary oestrogens rose to a maximum between 62 and 135 μg/24 hrs (normal mid-cycle range between 30 and 120 μg/24 hrs). When therapy was discontinued after 7 days gonadotrophins became low or undetectable with a fall in urinary oestrogens. There was then a further rise when treatment was restarted between days 12-14. In all subjects there was a rise in 24 hours total urinary oestrogens between days 18 and 28 despite being off therapy preceeded by a rise in basal serum gonadotrophins. These results therefore indicate that the patients had adequate pituitary LH

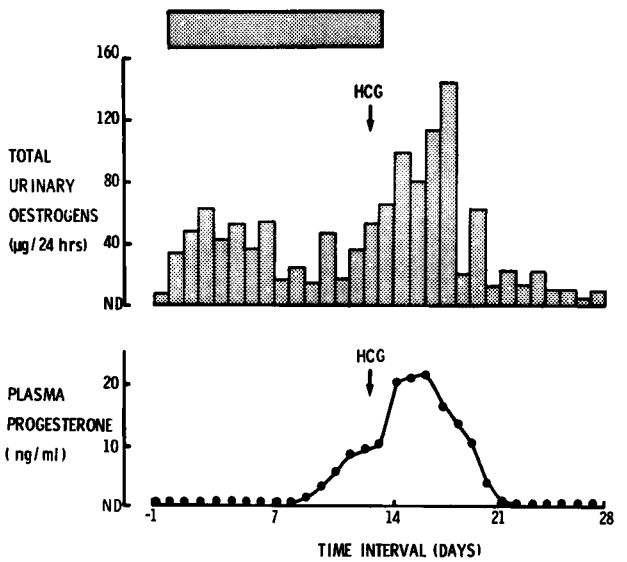

FIG. 4. — *Total 24 hrs urinary oestrogen and plasma progesterone responses to Gn-RH 500 μg 8 hourly subcutaneously in a woman with anorexia nervosa*

and FSH reserve although the release of the gonadotrophins was impaired while off therapy due to a persistent deficiency of endogenous Gn-RH. Therapy resulted in rises in total 24 hour urinary oestrogens (and plasma oestradiol) to levels comparable to those seen in normal cycles. The occurrence of spontaneous release of gonadotrophins between days 18-28 despite being off treatment indicates that there had been positive feedback of the rise in circulating oestrogens at the hypothalamic-pituitary level. Following this treatment one patient menstruated for the first time in 6 years, although there was no biochemical evidence of ovulation in any of the 4

patients. Two patients were retested after 12-14 weeks off therapy with clomiphene and both now showed a rise in urinary oestrogens followed by a rise in urinary pregnanediol indicative of ovulation. Both patients also menstruated. The other two instead of clomiphene received a further course of Gn-RH 500 μg 8 hourly except this was continued for 14 days, at which time an injection of 4.500 units of HCG was given intramuscularly (fig. 4). In both patients there was a rise in urinary oestrogens together with a rise in plasma progesterone from less than 1 ng/ml to 6.4 and 9.9 ng/ml on day 14 *before* HCG was given. Both patients later menstruated.

Further studies involving the administration of Gn-RH 500 μg 8 hourly for 28 days has resulted in ovulation (as determined by a rise in urinary pregnanediol from undetectable to 6.5 mg/24 hrs) and menstruation. The addition of HCG 4.500 units on day 14 in one patient resulted in ovulation and pregnancy. Continuous therapy initially for 1 month and then for a further 4 months in a patient with isolated gonadotrophin deficiency has resulted in a cyclical increase in 24 hour total urinary oestrogens from 1 to 30 μg/24 hrs and increase in breast size although ovulation and menstruation have not yet occurred.

It would appear therefore that Gn-RH therapy will result in ovarian steroidogenesis and ovulation. This may be followed by menstruation although no female has reported the increase in libido noted by the men. Since Gn-RH therapy for ovulation induction results in the production of oestrogen and progesterone levels normally seen during the menstrual cycle, it is hoped that hyperstimulation of the ovary and multiple births will be avoided.

CONCLUSION

Studies with Gn-RH indicate that the single decapeptide is capable of promoting synthesis as well as the differential release of LH and FSH by the modulation of pituitary responsiveness by gonadal steroids and inhibin. It may be of value in the treatment of male and female infertility and suggests that although a distinct FSH-RH may yet exist in man, it is not essential for these events to occur. Future studies involving depot preparations and analogues more resistant to enzymatic degradation may further widen the scope of Gn-RH therapy.

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RÉSUMÉ

TRAITEMENT PAR L'HORMONE DE LIBÉRATION DES GONADOTROPINES ((Gn-RH)

Puisqu'une proportion élevée (88 p. 100) de patients avec des troubles hypothalamiques ou hypophysaires répondent au Gn-RH, il était possible d'espérer que des administrations répétées (500 µg toutes les 8 heures) du décapeptide auraient une certaine valeur thérapeutique.

Quelques adolescents mâles prépubères traités avec le Gn-RH ont effectivement montré une augmentation importante de la fréquence d'érection et de la taille testiculaire.

De même, 9 patients mâles adultes souffrant de troubles hypothalamiques ou hypophysaires ont tous montré un retour rapide de la puissance entre 12 et 17 jours après le début du traitement au Gn-RH. Celle-ci s'est maintenue chez quatre d'entre eux traités pendant 12 à 18 mois. En outre, la spermatogenèse a été induite 7 à 26 semaines après le début du traitement. Les niveaux plasmatiques de FSH ont diminué lorsque le nombre de spermatozoïdes s'est élevé dans l'éjaculat (« inhibine ») bien que les niveaux de LH n'aient pas été modifiés. La femme de l'un des patients est actuellement enceinte.

Des femmes avec anorexie nerveuse et aménorrhée depuis 5-7 ans et ne répondant pas à un traitement au clomiphène ont reçu des injections de Gn-RH pendant des durées variables jusqu'à 28 jours. L'ovulation et la menstruation ont été obtenues. L'injection supplémentaire de 4 500 unités de HCG le 14e jour chez une patiente a permis l'installation d'une grossesse.

Ainsi, le Gn-RH peut être utile dans le traitement de la stérilité mâle ou femelle.

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