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Gonadotrophin-releasing hormone

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After the classic animal experiments of Harris (1950), Harris and Johnson (1950), and McCann and Dhariwal (1966) it was assumed that in man too the hypothalamus controlled the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) independently by means of two releasing hormones, luteinising hormone-releasing hormone (LH-RH) and follicle stimulating hormone-releasing hormone (FSH-RH). However, Schally *et al.* (1971) were able to isolate only one gonadotrophin-releasing hormone from many thousands of porcine hypothalami. This hormone (GnRH), a decapeptide, stimulates the secretion of both LH and FSH in animals and in man and can produce differential changes in the secretion of the two gonadotrophins by interaction with the feedback effects of gonadal steroids and inhibin. The latter inhibits FSH secretion. It is probably a polypeptide and is formed in the seminiferous tubules during the final stage of spermatogenesis (which is itself stimulated by FSH).

GnRH in normal persons

MALES

In normal adults a single bolus of 25 to 500 μg of GnRH given intravenously increases LH and FSH secretion in a dose-related manner, although more LH than FSH is secreted (Besser *et al.*, 1972). The peak circulating levels occur within 10 to 30 minutes and fall gradually over the next few hours. LH remains above baseline values for 4 to 6 hours and FSH for 3 to 5 hours.

With continuous infusion plasma FSH begins to rise within 3 to 10 minutes when GnRH levels are in the range 30 to 221 ng/l. It reaches a plateau at 20 to 30 minutes. The rise in LH begins after 9 to 10 minutes when GnRH levels are 249 to 580 ng/l and reaches its plateau later than FSH. If the infusion is prolonged for several hours the early plateau is followed by a further rise. This has been taken to indicate the presence of two varieties of stored gonadotrophins, of which the more readily releasable variety accounts for the early rise. During the infusion the secretion of both gonadotrophins is pulsatile and the pulses are asynchronous. Between pulses the pituitary is presumably refractory to GnRH so far as the particular gonadotrophin is concerned.

In man feedback effects result from gonadal steroids given in physiological or higher doses and also, presumably, from inhibin in the case of FSH secretion. Men treated with oestrogen (ethinyl oestradiol 30 μg/day for three days) show suppression of LH and especially FSH secretion, whether basal or stimulated by GnRH (Mortimer *et al.*, 1973a). Pituitary responsiveness returns to the pretreatment level three days after withdrawing the oestrogen. Infusion of 17β-oestradiol to produce levels just above the normal male range suppresses the response to GnRH within 30 minutes. Evidently in men the site of negative feedback is at pituitary level. There is as yet no conclusive evidence of positive feedback of oestrogen in the male.

The effects of testosterone preparations on pituitary responsiveness to GnRH are more complex and variable. Supraphysiological doses of testosterone propionate (100 mg/day intramuscularly for 4 to 6 days) suppress basal gonadotrophin levels and the FSH response to GnRH, but there is little change in the LH responses to the releasing hormone. Suppression of the FSH response was also observed by von zur Mühlen and Köbberling (1973), who continued testosterone oenanthate administration for four weeks. After withdrawal of testosterone secretion of LH recovers more quickly than that of FSH (Lee *et al.*, 1972). The feedback effects of testosterone are complicated by the fact that it is partially converted to oestrogen. There may be actions at both pituitary and hypothalamic levels. Thus the diminishing pituitary responsiveness to GnRH over several weeks of testosterone administration could be due to the suppression of releasing-hormone synthesis in the hypothalamus with a resulting reduction in the store of pituitary gonadotrophins.

Dihydrotestosterone (DHT) has little effect on gonadotrophin secretion, and its infusion for 48 hours has been found to cause at most only very slight suppression of basal LH levels (Stewart-Bentley *et al.*, 1974).

Inhibin has clear effects on FSH secretion. Injection of sperm extracts into castrated rats

results in a fall of FSH but not LH. The evidence for such an effect in man is indirect. Thus during long-term GnRH therapy in patients with pituitary-hypothalamic disease there was a fall of FSH—but not LH—secretion as the sperm count rose (Mortimer *et al.*, 1974a) (Fig. 1). Moreover, in patients with oligo- or azoospermia receiving GnRH the response of FSH, but not of LH, is exaggerated. These results indicate that the site of inhibin feedback is at pituitary level.

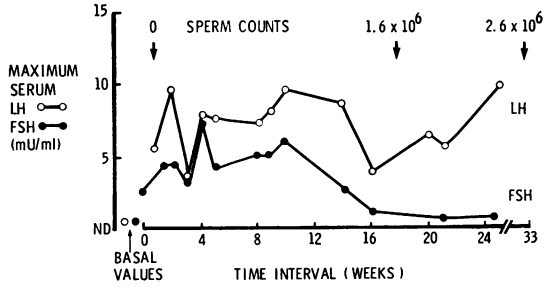

Fig. 1 *Maximum serum LH (○) and FSH (●) responses to 500 μg GnRH subcutaneously in man with isolated gonadotrophin deficiency being treated with GnRH. (Figures 1 and 2 reproduced by kind permission of the Editor and Publishers,* British Medical Journal, *1974,* **4**, *617.)*

FEMALES

The influence of gonadal steroids on pituitary responsiveness to GnRH is more complex in women than in men. Oestrogen may have both positive and negative feedback effects which are critically dependent on the time course of administration and the dose. During the normal menstrual cycle LH and FSH stimulate ovarian steroidogenesis which then modifies pituitary and hypothalamic function. During the follicular phase the gonadotrophins are thought to stimulate the development of the follicle with secretion of oestrogens. At mid-cycle the rise in circulating oestrogens induces a further surge in gonadotrophin secretion which induces ovulation. This positive feedback effect is probably activated by increasing the sensitivity of the pituitary to GnRH. This hypothesis is supported by the experiments of Nillius and Wide (1972) and Yen *et al.* (1972). They showed that serial injections of the same dose of GnRH throughout the normal menstrual cycle provoked a relatively small response in the early follicular phase and a greater response in the luteal phase. The greatest response occurred at mid-cycle coincident with the rise in plasma oestradiol.

Oestrogens may also modify hypothalamic function. At mid-cycle there is an eightfold increase in circulating GnRH, as measured by radioimmunoassay (Arimura *et al.*, 1974a; Mortimer *et al.*, 1976a). Moreover, the intramuscular administration of 1 mg 17β-oestradiol results in a rise in circulating LH, but not FSH, after 48 to 72 hours (Nillius and Wide, 1971). Other oestrogens, however, may result in the suppression of gonadotrophin levels, and the response to the releasing hormone may be suppressed in women taking oral contraceptives—that is, negative feedback. During the luteal phase pituitary responsiveness to GnRH decreases owing, at least in part, to the action of progesterone.

The effect on pituitary responsiveness to GnRH by androgens in the female is little understood since the administration of such compounds to normal women is difficult to justify. Furthermore, in view of the reduced synthesis of sex hormone-binding globulin caused by androgens and the increase caused by oestrogens (in both sexes) clearly changes in the concentration of the carrier protein may lead to pronounced differences in the ratio of bound to biologically active hormone, and therefore modify the central effect of a given concentration of total circulating hormone.

PUBERTAL CHILDREN

There is a clear pattern of gonadotrophin secretion in response to GnRH through the stages of puberty. In boys tested with 25 μg GnRH as an intravenous bolus there was an increasing LH response as puberty progressed from stage I to II and III (Tanner, 1958; Franchimont *et al.*, 1974). Although FSH also increased there was no significant difference between the responses obtained at different stages of puberty. The increase of FSH was less than that of LH in all three stages.

In girls the differences in pituitary responsiveness to GnRH during puberty are more clearly defined. The FSH response greatly increases during stage I but thereafter changes little. The LH response is slight in stage I but in stage II it increases to produce levels of LH greater than those of FSH, as in the adult. This changing pattern of pituitary responsiveness is confirmed by the similar progression of FSH and LH secretion when puberty is induced by long-term GnRH therapy.

MENOPAUSE AND 'MALE CLIMACTERIC'

At the menopause basal gonadotrophin levels rise as the normal menstrual cycle ceases and as circulating ovarian steroids diminish. Circulating endogenous immunoreactive GnRH also becomes more often detectable (Mortimer *et al.*, 1976b). There is an increase in pituitary responsiveness to injected

GnRH, especially of LH secretion, levels reaching a peak between 15 and 45 minutes after the injection. If such patients are treated with 100 μg of ethinyl oestradiol 12-hourly for five days basal serum gonadotrophin levels fall. The response of LH to GnRH is maintained but the peak is diminished and delayed to between 45 and 120 minutes. In contrast, the FSH response is increased by oestrogen treatment. These results suggest that after the menopause FSH secretion is almost maximal and is little affected by further exogenous GnRH; that oestrogens exert a negative feedback action on GnRH so that gonadotrophin levels fall; and that oestrogens sensitise the pituitary to GnRH so far as FSH, but not LH, is concerned (Franchimont *et al.*, 1974).

In later life some men experience symptoms similar to those of some menopausal women. The so-called 'male climacteric' presents with flushing attacks and lack of energy and of motivation. There is usually no evidence of endocrine disease and plasma testosterone levels may be normal or at the lower limit of normal for adult men. The FSH level is normal unless the total sperm count is low but LH levels may be raised and the LH response to GnRH is exaggerated. It may be that as testicular function begins to fail normal levels are maintained by the drive from increased LH levels, but as testicular failure progresses testosterone levels can no longer be maintained and symptoms of testosterone deficiency appear. However, intramuscular testosterone often produces improvement despite normal plasma testosterone levels. This syndrome is not uncommon, but the mechanisms involved are unclear.

GnRH in pathological states

In order to study the hypothalamic-pituitary-gonadal axis tests have been devised based on measurements of serum LH and FSH after an intravenous injection of GnRH, the preferred dose being 100 μg. Blood is withdrawn before and 20 minutes (around the time of the peak response) and 60 minutes after the injection of GnRH. The response is compared with the results from normal controls.

An absent response is defined as one in which the rise in gonadotrophin is less than three times the within-assay coefficient of variation of the basal levels of gonadotrophin (a change of about 15% for LH and 18% for FSH, depending on the laboratory). If the rise exceeds this but is less than the normal range at 20 and 60 minutes it is regarded as an impaired response. If the 60-minute value is the same as or exceeds that at 20 minutes the response is defined as delayed. Values which rise above the normal range at 20 and 60 minutes are considered to be exaggerated. Absent, impaired, or exaggerated responses are pathological and imply endocrine dysfunction. Delayed responses occur in normal subjects and if the gonadotrophin levels reached remain within the normal range the response is not necessarily pathological (Mortimer *et al.*, 1973b). In women with amenorrhoea the response is compared with that obtained during the follicular phase of normal women, since this would seem to be the best functional equivalent.

GnRH TEST IN HYPOTHALAMIC AND PITUITARY DISEASE

The term 'isolated gonadotrophin deficiency' is applied to patients with partial or absent puberty in whom basal serum gonadotrophin levels are low and do not respond to clomiphene and in whom there is no evidence of deficiency of other pituitary hormones. An impaired growth hormone response to insulin may be seen in some such patients but they are not stunted. Indeed, being eunuchoid they tend to be tall. Clinically it may be confused with delayed puberty, but in that basal gonadotrophin levels and clomiphene responsiveness are normal and there are no other features typical of isolated gonadotrophin deficiency—for example, anosmia or incurving of the little finger.

Patients with isolated gonadotrophin deficiency usually respond to 100 μg GnRH with normal or impaired LH or FSH responses. However, those who do not respond to 100 μg always respond to 500 μg, especially if repeated subcutaneously every eight hours. The basic defect is presumably a deficiency of endogenous hypothalamic GnRH, although increased destruction of GnRH and diminished pituitary sensitivity have also been suggested. But in two such patients given an intravenous injection of synthetic GnRH we found a normal disappearance curve and a small but significant increase of the serum levels of LH and FSH from low basal levels.

In patients with a craniopharyngioma or other suprasellar tumours the response to GnRH is extremely variable, whether or not treatment by surgery or irradiation, or both, has been given. Some such patients respond to GnRH when they do not respond to clomiphene. A normal response to GnRH may be seen even though the secretion of growth hormone, thyrotrophin, or ACTH is impaired. A similarly variable response to GnRH is also found in patients with non-functioning tumours of the pituitary, even when they appear clinically to have hypogonadism, and also in idiopathic hypopituitarism and in acromegaly or Cushing's syndrome with hypogonadism. In acro-

megaly GnRH may also release growth hormone, which indicates the non-specificity of pituitary cell receptors in this condition (Faglia *et al.*, 1973).

Postmenopausal women with pituitary or hypothalamic disease may have basal gonadotrophin levels and a response to GnRH that are within the normal range for premenopausal women, but that should not be taken as indicating normal pituitary function since high basal levels and an exaggerated response to GnRH are to be expected. Similarly, in men with oligo- or azoospermia, together with low testosterone levels and clinical hypogonadism, the absence of raised gonadotrophin levels and of an exaggerated response to GnRH indicates hypothalamic or pituitary disease.

In men isolated FSH deficiency is recognised and is characterised by oligo- or azoospermia with normal plasma testosterone levels. The response to GnRH is reduced or absent, indicating a failure of pituitary synthesis.

OTHER DISORDERS

Patients with primary gonadal failure from any cause—for example, Turner's syndrome or Klinefelter's syndrome—have raised basal gonadotrophin levels with an exaggerated response to the releasing hormone if hypothalamic-pituitary function is normal. Patients with precocious puberty show normal gonadotrophin responses to GnRH in the absence of space-occupying cerebral lesions.

Anorexia nervosa commonly presents with primary or secondary amenorrhoea. The response to 100 μg GnRH is very variable. With a dose of 50 μg, however, there is usually a reduced response when weight loss is extreme, and it may be of the prepubertal type with FSH secretion exceeding that of LH. With recovery of body weight the response returns to the normal adult pattern (Palmer *et al.*, 1975; Nillius and Wide, 1975). These changes are also found in the rare cases of anorexia nervosa in men. Since there is a close correlation between body weight and the age of onset of puberty (Frisch and Revelle, 1970) and since pituitary responsiveness depends in part on continued exposure to GnRH (Mortimer *et al.*, 1974a) the amenorrhoea which accompanies anorexia nervosa may possibly represent the occurrence of acquired GnRH deficiency.

Infertility with hyperprolactinaemia is common and found in both sexes. It may be associated with galactorrhoea. It occurs in patients with pituitary tumours, acromegaly, or the polycystic ovary syndrome; on cessation of oral contraception with no evidence of localised hypothalamic or pituitary disease; and in patients on drugs which raise prolactin levels. In women it is accompanied by oligo- or amenorrhoea. In patients with this syndrome the gonadotrophin response to GnRH is usually normal or even exaggerated but on rare occasions it is absent. There is no correlation between the maximal gonadotrophin levels achieved and basal or TRH-stimulated prolactin levels. This indicates that in women the amenorrhoea is not normally due to the inability of the pituitary to synthesise or release gonadotrophins when stimulated, though there is probably a disturbance of their cyclical release. Probably also prolactin blocks the action of gonadotrophins on the gonads. Patients can usually be treated effectively with bromocriptine but a poor result may be expected if there is an impaired or absent gonadotrophin response to GnRH.

The polycystic ovary syndrome, diagnosed by laparotomy, laparoscopy, or pelvic pneumography, is another cause of infertility, and although some cases are associated with hyperprolactinaemia most are of uncertain aetiology. Katz and Carr (1976) investigated 15 such patients and in some found that basal LH levels were raised, as shown previously by Yen *et al.* (1970), and that an exaggerated response occurred after GnRH. Basal FSH levels were always within the normal range. These patients tended to have high oestradiol levels and secondary amenorrhoea and were classified as Group 1. The other patients had normal basal LH values and a normal response to GnRH and tended to have anovulatory cycles; they were classified as Group 2. It remains to be seen whether this classification on the basis of gonadotrophin responses to GnRH is valid or whether it is an artificial distinction based on only one particular aspect of endocrine dysfunction. Whatever the underlying pathology clearly synthesis of pituitary gonadotrophins and the mechanisms for their release are intact and responsive to GnRH stimulation.

The testicular feminisation syndrome is characterised by the resistance of the target organs to the action of testosterone. This leads to the development of individuals who are phenotypically female although genetically male with functioning male gonads. These patients have normal male testosterone levels but high basal gonadotrophin levels, which may not fall with testosterone administration. This suggests that the pituitary (and possibly hypothalamus) is also resistant to its action. As a result the gonadotrophin responses to GnRH may be exaggerated.

Primary dysfunction of the seminiferous tubules gives rise to azoospermia or oligospermia, usually defined as a sperm count of less than $5 \times 10^6/mm^3$, plasma testosterone levels are normal. As would be expected, LH levels are normal but FSH levels are

raised, owing to lack of inhibin, and show an exaggerated response to GnRH.

GnRH therapy

MALES

A number of males with delayed puberty or infertility have been treated with GnRH for months or years. Some had proved lesions of the pituitary or hypothalamus, some isolated Gn deficiency, and some were presumed to have isolated FSH deficiency. FSH deficiency was diagnosed on the finding of normal LH and testosterone levels but an FSH response to GnRH which was low or normal despite testicular biopsy evidence of reduced spermatogenesis with maturation arrest.

In many cases there was a rapid increase of potency followed by an increase of sperm count and motility, often well into the normal range. The best results were obtained with 500 μg given eight-hourly by subcutaneous injection. When GnRH was withdrawn there was a reversal of both. With treatment a prepubertal response to GnRH was usually converted to the adult type (Fig. 2) (Zarate *et al.*, 1973; Mortimer *et al.*, 1976b; Mortimer and Besser, 1976).

Since the improvement in potency is so rapid, often preceding a return of plasma testosterone levels to normal, the GnRH may have some direct action. This is suggested by the experiments of Moss and McCann (1973) and Pfaff (1973), who found that GnRH increased the sexual responsiveness of female rats even after their ovaries and pituitary had been removed.

PREPUBERTAL FEMALES

GnRH therapy for delayed puberty in females has not been explored to the same extent as in males. However, in two patients with isolated gonadotrophin deficiency such treatment established a cyclical rhythm of oestrogen excretion (Fig. 3) but without ovulation or menstruation during the first year of treatment. One of them menstruated four weeks after stopping treatment and the other began regular periods with evidence of ovulation when treatment was continued beyond one year.

ADULT FEMALES: INDUCTION OF OVULATION

Initial attempts to induce ovulation with GnRH were disappointing, probably owing to the inadequacy of diagnosis and the use of too small a dose. The introduction of bromocriptine for hyperprolactinaemia and the use of clomiphene and hCG therapy have reduced the indications for therapy with either gonadotrophin or GnRH. However, the latter has been tried in patients with secondary amenorrhoea, especially those due to anorexia nervosa or occurring on withdrawal of depot contraceptive therapy. In one series of eight patients with secondary amenorrhoea from a variety of causes (Akande *et al.*, 1972) GnRH produced a rise in gonadotrophins but ovulation occurred in only one patient, and this began before GnRH was given. The value of GnRH therapy therefore remained in doubt, as also in the case of patients in whom ovulation and pregnancy occurred when they were treated with GnRH and clomiphene simultaneously (Keller, 1973). Similar limited success was obtained by Zanartu *et al.* (1974), who induced ovulation in only 2 out of 16 women with secondary amenorrhoea after the withdrawal of contraceptive drugs. Brechwoldt *et al.* (1974), who used a synthetic analogue, concluded that it was unsuitable for inducing ovulation since its value was limited by pituitary responsiveness. All these studies indicate that the optimal regimen for reliably inducing ovulation remains to be found.

In view of our success in inducing spermatogenesis with 500 mg eight-hourly we used this dose in patients with anorexia nervosa who had had amenorrhoea for five to seven years and were clomiphene unresponsive (Mortimer *et al.*, 1975). The patients were either at their ideal body weight or slightly below it. Basal urinary oestrogens were within the normal range for the early follicular phase. Initially, basal serum gonadotrophins were low or absent but increased during seven days of therapy as did the urinary oestrogens, which rose

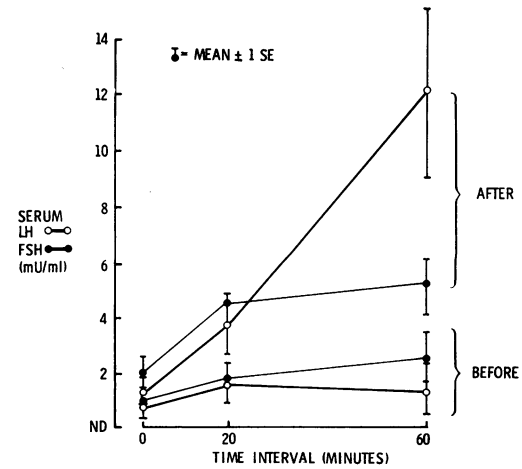

Fig. 2 *Serum LH* (○) *and FSH* (●) *responses to a standard test dose of 100 μg intravenous GnRH in five patients before and after completing 4 to 6 weeks GnRH therapy.*

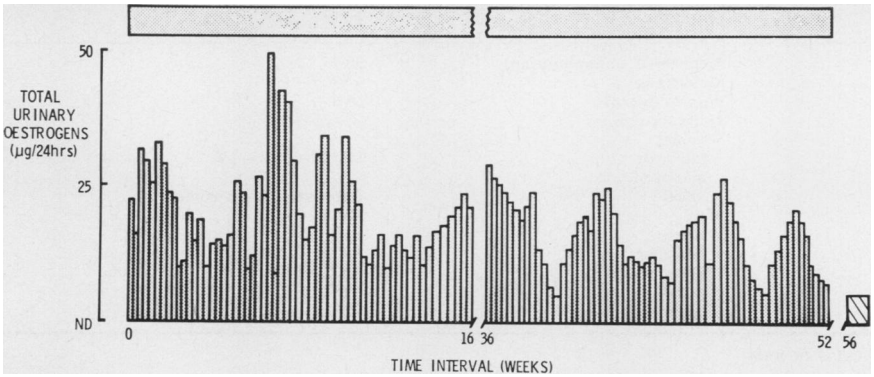

Fig. 3 *Cyclical 24-hour total urinary oestrogen excretion in a female patient with 'isolated gonadotrophin deficiency' throughout one year of treatment with GnRH 500 μg eight-hourly subcutaneously. Hatched square represents occurrence of patient's first period.*

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to the maximum of the range encountered at mid-cycle. Therapy was then discontinued and the gonadotrophin levels became low or undetectable with a fall in urinary oestrogens (Fig. 4). More treatment was given on days 12-14 and this was followed between days 18 and 28 by a small rise in gonadotrophins and a further rise in oestrogen excretion. These results indicated that the patients had adequate pituitary LH and FSH reserve although the release of the gonadotrophins was impaired in the absence of therapy due to a persistent deficiency of endogenous GnRH. The spontaneous release of gonadotrophins between days 18 and 28 in the absence of treatment indicated that there had been positive feedback by the increased levels of circulating oestrogens at the hypothalamic-pituitary level. After treatment one patient menstruated for the first time in six years although there was no biochemical evidence of ovulation in any of the four patients. Two patients subsequently became responsive to clomiphene with a return of ovulation, and others ovulated after a more prolonged course of GnRH (14-28 days). No patient reported an increase in libido.

Nillius and Wide (1975) also succeeded in inducing ovulation with GnRH alone although they noted that hCG may be a useful addition during the second half of the expected cycle to facilitate pregnancy if there is evidence of luteal phase insufficiency. Since the oestrogen and progesterone responses in these studies resemble those seen in the normal menstrual cycle it would seem that GnRH 500 μg subcutaneously eight-hourly preserves the normal feedback relationship of the hypothalamic-pituitary-ovarian axis. It is hoped that this will prevent the occurrence of the hyperstimulation syndrome and the multiple births which are far too commonly encountered with human menopausal gonadotrophin therapy.

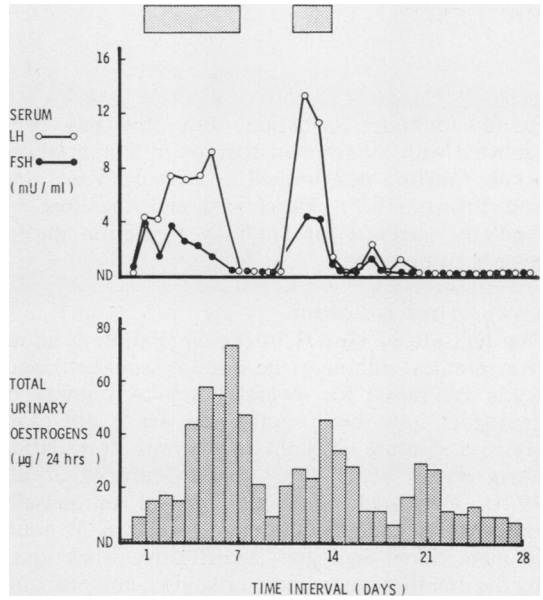

Fig. 4 *Anorexia nervosa. Top: Serum LH (○) and FSH (●) levels measured 10 hours after last dose of GnRH 500 μg subcutaneously the previous night. Intermittent therapy shown by stippled bars. Bottom: 24-hour total urinary oestrogen excretion. (Reproduced by kind permission of the Editor and Publishers,* Clinics in Endocrinology and Metabolism, *1977,* **6**, *1.*)

SIDE EFFECTS OF GNRH

When GnRH is given intravenously or intramuscularly no side effects occur apart from discomfort at the site of intramuscular injection. Subcutaneous injections, however, result in minor but transient discomfort and erythema. Even after repeated subcutaneous injections of GnRH 1·5 mg for up to 132 weeks Mortimer *et al.* (1976b) have not

Authority	*Subjects*	*Level (pg/ml)*
Keye *et al.* (1973)*	Prepubertal children (mean)	30·7
	Men (mean)	67·9
	Women (mean)	69·6
Jeffcoate and Holland (1974)†	Normal subjects	<0·25–3·5
Arimura *et al.* (1974a)‡	Women	
	follicular	Undetectable – 1·8
	mid-cycle‖	2-17
	luteal	Detectable in 2/5 women
Mortimer *et al.* (1976a)§	Men	<0·2
	Women	
	follicular	<0·2
	mid-cycle	<0.2-1·5
	luteal	<0·2
	postmenopausal	<0·2-2·5

Table *Circulating GnRH levels in man*

*Unextracted assay.
†Methanol extraction.
‡Ethanol extraction.
‖Lower level estimated from published illustration.
§Vycor extraction.

found antibody formation. But this has been reported with intermittent therapy in a man, whose sexual function deteriorated as a result (Van Loon and Brown, 1975). Patients should therefore be regularly screened for antibody formation during long-term treatment.

ANALOGUES OF GnRH

The half-life of GnRH in the circulation is about five minutes, although the ensuing gonadotrophin levels are raised for several hours. A number of analogues have been synthesised which are more active and more resistant to enzymic degradation (Arimura *et al.*, 1974b; Vilchez-Martinez *et al.*, 1974). One such analogue instilled intranasally raises the gonadotrophin levels for 12 to 24 hours in man. Some analogues are effective when given by the mouth, nose, rectum, or vagina, and probably oral or nasal administration will become the standard method.

Derivatives with predominantly inhibitory effects on gonadotrophin secretion are being developed and may provide a means of non-steroidal contraception.

Methods of assay

RADIOIMMUNOASSAY

Hitherto radioimmunoassays for GnRH have been too insensitive for measuring basal plasma levels. This problem has now been overcome (Mortimer *et al.*, 1976a) with the use of Vycor (a leached silica glass) for the extraction, but some of the GnRH is eluted before the major peak. A similar fractionation occurs with methanol extraction, but this does not necessarily indicate that plasma GnRH includes a 'big' or protein-bound form. The results obtained with our new method are much lower than those of other authors (Table) and further work is required to determine whether these results represent intact endogenous GnRH or biologically inactive fragments.

BIOASSAY

There have been few attempts to measure GnRH in the peripheral circulation by *in-vivo* bioassay, which is unlikely to achieve the sensitivity of the radioimmunoassay. In the method of Reichlin (Malacara *et al.*, 1972), the gonadotrophins are extracted from 40 ml of plasma with methanol and, after transfer into saline, injected into an ovariectomised rat. GnRH activity is assessed by the increase of plasma LH values 10 minutes after injection. The latest assay of these authors is based on direct injection of 1 ml of human plasma (Seyler and Reichlin, 1974). However, this gives GnRH concentrations of 10-50 ng/l in normal men compared with 0·2 ng/l found in our immunoassay, and up to 400 ng/l in normal women compared with 9 ng/l by our method.

We have developed an *in-vitro* bioassay based on the perfused pituitary cell column technique described by Lowry (1974). Mechanically dispersed rat anterior pituitary cells are mixed with an inert matrix and then perfused in a small plastic column. LH is released in response to synthetic GnRH in a dose-related manner and the preparation is sensitive to 50 ng/l GnRH. This method allows detection of biologically active endogenous GnRH if large volumes of plasma are extracted with Vycor in a similar manner to that developed for the radio-

immunoassay (Mortimer *et al.*, 1976a). Results given by this bioassay are consistent with those obtained by radioimmunoassay (Yeo *et al.*, 1977).

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