Endocrine aspects of anovulation

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Summary

Endocrine features of anovulation are reviewed, with particular emphasis on methods of investigation. The value of, and indications for, measurements of serum gonadotrophin concentrations are discussed: it appears that FSH measurements allow better discrimination than LH measurements in diagnosing patients with primary ovarian failure. The results of stimulation tests with the synthetic form of gonadotrophin-releasing hormone are described and the relationship of tests with exogenous progestogens and clomiphene is discussed.

NINETEEN seventy-four has seen the introduction, by the Department of Health and Social Security, of a Supraregional Saturation Assay Service (SAS) which makes available to clinicians, on a nationwide basis, a variety of hormone assays hitherto restricted to those working in a few privileged centres. There seems little doubt that this new facility will greatly aid clinicians in the diagnosis and management of a wide variety of disorders. In the field of reproductive medicine eight centres are offering gonadotrophin measurements and it might be helpful to review those areas relevant to disorders in the female in which measurements of gonadotrophin concentrations are of proven clinical value. Such a review must necessarily reflect personal views and experience but recommendations will be kept within the guidelines set by the SAS gonadotrophin subgroup. This subgroup consists of all of the SAS assayists offering gonadotrophin measurements, under the chairmanship of Dr Stephen Jeffcoate.

The gonadotrophins are carbohydrate containing polypeptides which stimulate the gonads to secrete gametes and sex steroids. The gonadotrophins with which this paper is concerned are those secreted by the pituitary gland. Their synthesis and release are controlled by the action of the gonadotrophin-releasing hormone (GnRH) which is synthesized and stored in the median eminence of the hypothalamus and released into the pituitary portal circulation in response to certain neurohumoral-regulating mechanisms. This releasing hormone, which has been

isolated, analysed and synthesized all within the last 5 years, is chemically a decapeptide that interestingly has the same first two aminoacids as the tripeptide thyrotrophin releasing hormone.

The hormonal changes occurring in the ovulation cycle are depicted in Fig. 5 of the paper in this symposium by Swerdloff and Odell (page 202). During menstruation, oestrogen and progesterone levels are low and consequently the tonic centre of the hypothalamus responds by causing secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). The ovarian follicles respond by development and maturation and the active ovary secretes increasing amounts of oestrogen. When a critical amount of oestrogen has been produced the hypothalamic cyclical centre is triggered and there is then the massive midcycle preovulatory discharge of LH that causes ovulation. Subsequent formation of the corpus luteum is associated with secretion of large amounts of oestrogen and progesterone by the active ovary. The combination of these two gonadal steroids suppresses further secretion of LH and FSH. The dramatic fall of oestrogen and progesterone at the end of the luteal phase is presumably the cause of the increase of FSH and LH that restarts the next cycle (Odell *et al.*, 1971).

With this background to the basic physiology of the gonadotrophins in the female, we turn now to their measurement in clinical situations. The method with which most people are familiar is the mouse uterine weight bioassay of extracts of 24 h urine samples. This bioassay, in which the results are expressed in 'mouse units of total gonadotrophin activity', is not specific in that it responds to both LH and FSH. Furthermore, the response is altered by changing the ratio of the two hormones injected. It has now been replaced almost entirely by radio-immunoassay of the individual hormones in blood. This method has the great advantage that with it large numbers of samples can readily be processed. Furthermore, it is sufficiently sensitive to allow the determinations to be performed on small volumes of blood; however, it is not without its problems. It is important to emphasize that these assays need to be standardized in terms of widely available reference preparations. Those which are available are of

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variable purity and the particular results reported by any laboratory depend upon a number of features of the methods used (Jacobs and Lawton, 1974). However, the SAS laboratories all use the same techniques, reagents and nomenclature (Jacobs, 1974) and in this way it is hoped to provide a uniform service throughout the country.

The clinical indications for gonadotrophin measurements in the female which are of accepted clinical value can be stated. The SAS gonadotrophin subgroup recommends that the major indication for requesting gonadotrophin measurements is in the diagnosis of the cause of amenorrhoea, whether it be primary or secondary. Oligomenorrhoea and other disturbances of menstruation are not considered to be conditions where gonadotrophin measurements are of any particular diagnostic value.

In patients with primary amenorrhoea, gonadotrophin measurements are of great value in distinguishing cases of primary ovarian failure from those due either to structural disorders in the hypothalamic pituitary region or to primary hypogonadotrophic hypogonadism. In general, it has been found that in children with ovarian dysgenesis FSH levels are raised well before LH is obviously elevated (Penny *et al.*, 1970). The diagnosis of primary amenorrhoea, therefore, requires a measurement of FSH. In addition, a karyotype analysis is obligatory to confirm Turner's Syndrome and it is also helpful in diagnosing the very rare conditions of testicular feminization and the feminizing congenital defects of steroid biosynthesis, such as deficiencies of 17 α -hydroxylase and 17 β -hydroxysteroid dehydrogenase.

FIG. 1. Serum LH concentrations in women with ovarian failure contrasted to those with other causes of secondary amenorrhoea.

FIG. 2. Serum FSH concentrations in the patients whose LH concentrations are seen in Fig. 1.

If FSH levels and the karyotype are normal, radiology of the sella and a full pituitary work-up are then required.

In secondary amenorrhoea, the first consideration is to exclude primary ovarian failure and the premature menopause. Figures 1 and 2 and the remaining figures are taken from a series of collaborative studies performed at the Central Middlesex Hospital and the Middlesex Hospital with Miss Anne Jequier and Mr Alan O'Shea. They show LH and FSH concentrations in a group of women with primary ovarian failure compared to gonadotrophin concentrations in patients with other causes of secondary amenorrhoea. Although the levels of both hormones are raised in subjects with proven primary ovarian failure, it is of interest to note the much better discrimination provided by FSH measurements (Jequier, O'Shea and Jacobs, 1975). The SAS gonadotrophin subgroup specifically recommends that FSH measurements be requested in this situation.

In the majority of patients with secondary amenorrhoea, the gonadotrophin concentrations are not elevated and in a number of these we have performed further tests using the synthetic form of the hypothalamic gonadotrophin-releasing hormone. We have routinely used a dose of 100 μ g of the synthetic form of this compound.*

* Kindly supplied by Dr W. Bogie of Hoechst Pharmaceuticals.

FIG. 3. LH response to LRH in patients with secondary amenorrhoea contrasted to those in the normal luteal phase.

Figure 3 shows the response of LH and FSH in a group of eugonadal women tested during a normal luteal phase. Both LH and FSH are released in response to this single releasing hormone and as yet a specific and distinct releasing hormone for FSH has not been described. In contrast to these results, when subjects taking an oral contraceptive containing 50 μ g of ethynyl oestradiol and 1–1.5 mg of progesterone were tested during the third week of treatment cycle, there was a marked impairment of the response of both LH and FSH (Jacobs and Jequier, 1974).

The response to the releasing hormone in two groups of patients with secondary amenorrhoea were then compared: those with post-oral contraceptive amenorrhoea and those with what Nilius and Wide (1973) have referred to as 'functional amenorrhoea', a diagnosis which excludes the amenorrhoea associated with primary ovarian failure, anorexia nervosa, drug ingestion and patients with amenorrhoea associated with structural disorders in the hypothalamic and pituitary region. The results obtained

are shown in Fig. 3. Note, firstly, the wide range of results in patients with functional secondary amenorrhoea reflecting perhaps the heterogeneity of the aetiology of this condition; secondly, the similarity of the responses in the post-oral contraceptive group to that in the bigger group of patients with secondary (functional) amenorrhoea; thirdly, the obvious overlap of the responses of these two groups of patients with secondary amenorrhoea with the responses in the normal luteal phase. This overlap has occurred in spite of the obvious differences in progesterone concentrations which may be anticipated to exist between the patients with secondary amenorrhoea and the subjects tested during the normal luteal phase. Parenthetically it might be added that the hormonal profile of post-oral contraceptive amenorrhoea appears to be quite different from that seen in the anovulation occurring during treatment with the pill.

Figure 4 shows the results of releasing hormone tests in a group of patients of both sexes with hypogonadotrophic hypogonadism. The number of men

FIG. 4. LH response to LRH in patients with hypogonadism of non-gonadal causes. (a) Pituitary hypopituitarism; (b) hypothalamic hypopituitarism; (c) isolated hypogonadotrophic hypogonadism.

and women in each group was about equal. The patients with hypopituitarism all had an endocrine deficit which affected other hormones, too. The five patients with hypothalamic disease either had diabetes insipidus or radiological or neurosurgical evidence of a disturbance in this area. Those with isolated hypogonadotrophic hypogonadism had other characteristic non-endocrine features which confirmed the diagnosis of Kallman's Syndrome. Although these results indicate that impaired responses characterize patients with hypothalamic and pituitary disorders, it is also clear that the response to exogenous gonadotrophin-releasing hormone does not distinguish patients with hypothalamic disorders from those with frank pituitary destruction. It was of course disappointing to find that in this respect the promise of thyrotrophin-releasing hormone (Hall *et al.*, 1972) was not borne out by the findings with gonadotrophin-releasing hormone, but the reasons for this failure clearly relate to the basic physiology of GnRH.

The factors which determine the response to this releasing hormone are: (1) the dose of releasing hormone; (2) the pituitary content of gonadotrophins; (3) the prevailing steroid milieu.

The pituitary content of gonadotrophin depends upon the presence of endogenous GnRH, and when the production of this is low, as for instance occurs in hypothalamic disorders, the response to exogenous GnRH is small. Some of the poorest responses have occurred in patients with severe anorexia nervosa: as shown in Fig. 5, when patients are tested in the phase of maximum weight loss a severely impaired response of LH occurs. When tested after the regain of weight but before the resumption of menstruation, on occasion, exaggerated responses have been found,

a result which has been interpreted as indicating a recovery of hypothalamic pituitary function before ovarian activity has resumed sufficiently to contribute to the regulation of gonadotrophin secretion (Jequier *et al.*, 1974). Of course, the response returns to normal, as indeed may full reproductive function, when the patient is in full remission—indicating the full evolution of a functional hypothalamic hypopituitarism.

In essence, the management of patients with secondary amenorrhoea embraces two problems.

(1) Does the patient have evidence of ovarian or hypothalamic-pituitary disease?

(2) What is the patient's prognosis for fertility?

In answer to the first question, gonadotrophin measurements are of inestimable value in the diagnosis of ovarian failure. Indeed, the value of ovarian biopsy in the diagnosis of patients with ovarian failure is questionable now that these measurements are readily available. Exclusion of hypothalamic or pituitary disease requires careful radiology of the sella and evaluation of other pituitary hormones (Jacobs and Nabarro, 1969).

The prognosis for fertility depends essentially upon the outlook for induction of ovulation. Once ovarian and hypothalamic or pituitary pathology has been excluded, the problem is whether the patient will respond to clomiphene or whether the much more onerous therapy with exogenous gonadotrophins will be required. One of the ways this has been evaluated has been to challenge the patient with a large dose of progesterone and to see whether post progesterone bleeding occurs. Recently the endocrine background of this test has been investigated and Goldenberg *et al.* (1973) have shown a striking increase of LH levels after the injection of progesterone in a group of

FIG. 5. LH and FSH response in patients with anorexia nervosa.

patients who bled and who were shown subsequently to ovulate after a course of clomiphene. It is generally accepted that bleeding following exogenous progesterone indicates good oestrogen production by the ovary and it implies therefore, that, at the level of the tonic control of gonadotrophin secretion, there is an intact hypothalamic-pituitary-ovarian axis. The peak of LH produced in this test in those who respond to exogenous progesterone is analogous to the simulated preovulatory peak that can be produced in the post-menopausal woman (Odell and Swerdloff, 1968) and the ovariectomized rat (Swerdloff, Jacobs and Odell, 1972) by adding progestogens to therapy with oestrogens. This positive response of LH presumably indicates that the cyclical centre, too, is intact. It is this physiological relevance which gives the progesterone test its clinical value in reproductive medicine, since a response to clomiphene requires both hypothalamic centres to be intact (Odell *et al.*, 1971).

Clinical experience with the progesterone test is as yet limited but so far patients responding to this test subsequently respond to clomiphene, as judged by measurements of basal body temperature, by measurements of plasma progesterone concentrations and by the subsequent development of bleeding. Its great advantage is that a positive response should encourage one to pursue clomiphene therapy, even if the response in the initial courses is disappointing. We are currently evaluating the progesterone test in conjunction with tests with the releasing hormone and with clomiphene, the hope being to develop a

shorter test which carries both diagnostic and prognostic information.

In conclusion, although measurements of circulating gonadotrophins have proved of immense value in physiological research, in reproductive medicine there are few conditions in the female in which measurements of LH and FSH are of clearly established clinical value. On the whole, one gets little information from LH concentrations not available from measurements of FSH. The SAS gonadotrophin subgroup recommends that requests for gonadotrophin assays (preferably FSH) be made in the following conditions. Firstly, amenorrhoea, whether primary or secondary. High levels indicate ovarian failure, low levels suggest hypothalamic-pituitary dysfunction and may indicate the need for a subsequent stimulation test. Finally, in postmenopausal patients with pituitary tumours, a low level of LH and FSH provides easily acquired evidence of an endocrine deficit.

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