Ovarian hormones and pituitary responsiveness to gonadotrophin releasing hormone in mice

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INTRODUCTION

There is accumulating evidence which demonstrates that the ovarian steroids are involved in modulating the responsiveness of the pituitary gland to the gonadotrophin releasing hormones (Everett, 1948; Brown-Grant & Naftolin, 1972; Kalra et al., 1973; Aiyer & Fink, 1974). Aiyer & Fink (1974) indicate that with respect to the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) the magnitude and profile of responsiveness of the anterior pituitary gland to a synthetic LH releasing factor (LH-RF) at pro-oestrus are dependent on the nature and sequence of the ovarian hormones to which the hypothalamic-hypophyseal axis is exposed. Apparently oestrogen and progesterone exert both positive and negative effects on the pituitary secretion of LH, depending on the time during the cycle that the steroid is administered, the dosage employed and the length of treatment. Reports indicate that the facilitatory effect of progesterone on gonadotrophic secretion is exerted only after the hypothalamic-hypophyseal axis has been primed with oestrogen (Everett, 1948; Brown-Grant & Naftolin, 1972; Kalra et al., 1973). Arimura & Schally (1970) and Hilliard et al. (1971) suggested that progesterone may act to prevent the oestrogen induced LH surge either at the level of the hypothalamus thus affecting release of gonadotrophin releasing hormone (GN-RH) or at the level of the pituitary gland by modifying the responsiveness of the gland to GN-RH.

In the present study the role of progesterone and oestradiol 17β in modulating the responsiveness at the anterior pituitary gland to GN-RH was investigated by measuring the release of LH in ovariectomised mice which had been pretreated with these steroids.

MATERIALS AND METHODS

Animals:

Virgin female mice of the outbred Quakenbush (QS) stock, 8-10 weeks of age and weighing between 25 and 30 g were used in all experiments. The animals were maintained at 23°C-27°C and 55%-60% humidity and artificially lit from 6.00 h to 18.00 h. They were in metal stock cages and for experimental use housed in groups of 2-3 per 6×6×11 inch cage. Pellet feeding was *ad libitum*.

Hormones:

Steroid hormones were injected subcutaneously in 0.1 ml of sesame seed oil. Protein hormones were injected subcutaneously in 15% aqueous gelatine solution or in 0.9% saline. The hormones used were:
Oestradiol-17β (Sigma, USA).
Progesterone (Sigma, USA).
LH/FSH-RH (GN-RH) (National Institute of Arthritis, Metabolic and Digestive Diseases, National Institute of Health, Maryland, USA).

Collection of blood samples

Blood samples were collected from the tail vein of mice into small heparinized capillary tubes. The tubes were centrifuged and the plasma stored at -25°C until analysis.

Quantification of LH

LH was measured using a solid-phase radio immuno assay as previously described (Gidley-Baird & Bindon 1976). The assay utilizes an antiserum to ovine LH and ovine LH standards, and it measures LH levels in 20 ul of plasma with a sensitivity of less than 0.6 ng/ml.
Replicate reliability typically showed a variation of 5% or less. The mean and standard deviation for the index of precision (λ) for 10 as-

says was 0.049 ± 0.021. Ovine TSH showed about 12% cross reaction in the assay, whilst rat FSH and prolactin and ovine FSH, prolactin and GH showed no cross reaction.

Surgical procedures - ovariectomy

The technique used is that described by Emmens (1950). The animals were anaesthetized by the administration of Avertin (Winthrop Laboratories, USA) (tribromoethanol, amylene hydrate) 2.5% aqueous solution, 0.0125 ml/g body weight.

Treatment of ovariectomised mice with GN-RH

A total of 30 mice was ovariectomised and randomly allocated to 6 equal groups one week after surgery. 24 h prior to the commencement of blood sampling, Groups 1, 2 and 3 received 0.1 ml of sesame seed oil, and Groups 4, 5 and 6 received 1.0 ug oestradiol (E_2) plus 1.0 mg of progesterone (P) in 0.1 ml of sesame seed oil. After the first blood sample had been collected (zero time) the six groups received the following treatments:

Groups 1 and 4: 0.1 ml of 0.9% NaCl

Groups 2 and 5: 0.1 ug of GN-RH in 0.1 ml of 0.9% NaCl

Groups 3 and 6: 1.0 ug of GN-RH in 0.1 ml of 0.9% NaCl.

Blood samples were collected at 0.25, 0.5, 1.0 and 2.0 h after this injection.

Statistics

Analyses of variance and F-tests were performed using the program *Facanova* designed for use on the *Cyber 7200* at the University of Sydney.

RESULTS

The effect of injection of GN-RH in saline on LH release in ovariectomised mice

The effect of treatment with GN-RH on plasma LH levels is shown in Figure 1.

The data were subjected to analysis of variance to examine the effect of steroid pretreatment and GN-RH treatment. The analysis showed that there was a significant ($P < 0.001$) increase in plasma levels of LH with treatment on this response.

There was a significant ($P < 0.01$) effect of steroid treatment and a significant effect ($P < 0.05$) of dose of GN-RH on the response of LH. There was a significant ($P < 0.001$) difference in LH levels between animals receiving no GN-RH and those receiving 0.1 or 1.0 ug of GN-RH. This was not seen for control animals.

DISCUSSION

The essential findings of the present study is that GN-RH causes a rapid, transient and dose dependent increase in plasma LH in ovariectomised mice. Peak levels of LH were reached 0.25 to 0.5 h after injection of GN-RH in both oil and steroid pretreated animals. A large rise and fall in LH levels is followed by a small rise around 2 hours after GN-RH injection. Arimura et al (1972) and Bremner et al. (1976) reported that a biphasic release of LH also occurred in the ewe and ram following either a single intracarotid injection of GN-RH or prolonged infusion of GN-RH over 4 hours.

Steroid pretreatment of ovariectomised mice was included as pituitary LH and FSH is high in ovariectomised animals (Gay and Sheth, 1972), in which there is no negative feedback of ovarian steroids on pituitary gonadotrophin release. Debeljuk et al. (1972) showed that treatment of dioestrous rats with oestrogen and progesterone lowered basal serum LH concentration. In the present study, pretreatment of mice with oestrogen and progesterone resulted in a depression of LH levels in control animals and a significantly larger release of LH in GH-RH treated animals in comparison with animals not pretreated with steroids. Debeljuk et al. (1972) reported that the release of LH following GN-RH injection into dioestrous rats pretreated with oestrogen and progesterone was smaller than in the control group. This difference in results may be attributable to a difference in sensitivity of diostrous rats or to pretreatment of animals with a different dose ratio of oestrogen to progesterone than used in this study.

The fact that pretreatment with steroids resulted in lower levels of LH and a more pronounced stimulating effect of GN-RH agrees well

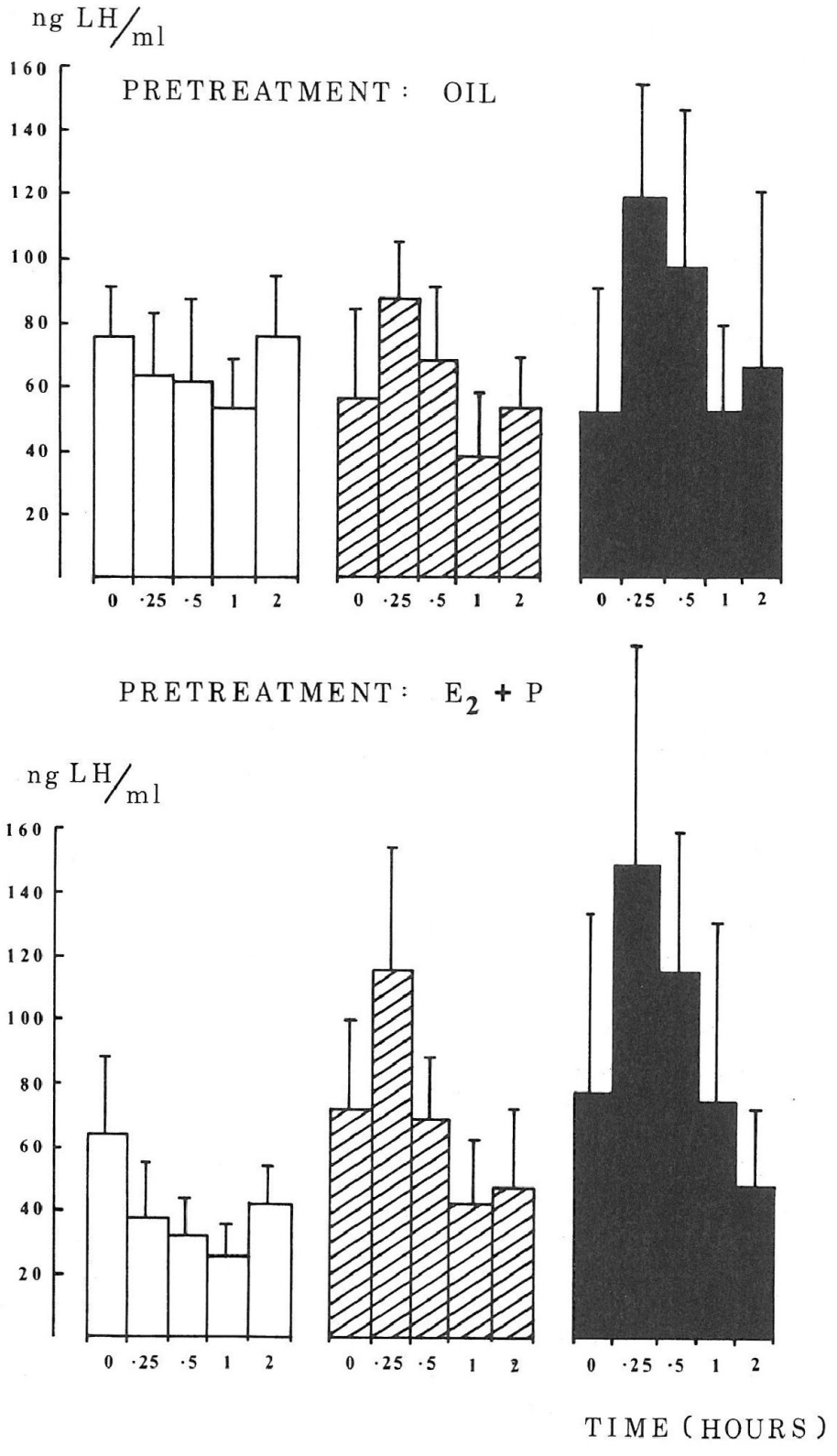

Fig. 1. Comparison of plasma LH levels in ovariectomised mice following treatment with GN-RH in saline.

□ 0 ug GN-RH
▨ 0.1 ug GN-RH
■ 1.0 ug GN-RH

The vertical bars represent +1 standard deviation.

with findings in other species that ovarian steroids cause a heightened responsiveness of the pituitary gland to GN-RH.

Summary
In the present study the role of progesterone and oestradiol in modulating the responsiveness at the anterior pituitary gland to gonadotrophin releasing hormone (GN-RH) was investigated by measuring the release of luteinizing hormone (LH) in ovariectomised mice which had been pretreated with these steroids.
A significant release of LH was seen in the animals receiving GN-RH. Pretreatment with oestrogen and progesterone depressed LH levels in the animals which did not receive GN-RH, and resulted in a larger release of LH in the animals receiving GN-RH in comparison with the control group pretreated with oil.

Yhteenveto / K. Pelkonen
Työssä selvitettiin sitä, miten progesteroni ja estradioli vaikuttavat aivolisäkkeen etulohkon vasteeseen gonadotropiinia vapauttavalle hormonille (GN-RH). Hiiristä, joilta oli poistettu munarauhaset ja joita oli käsitelty etukäteen näillä steroideilla, mitattiin LH:n vapautumista.
GN-RH-käsitellyissä eläimissä vapautui merkittävsti LH:ta. Esikäsittely estrogeenilla ja progesteronilla laski LH-tasoja eläimissä, jotka eivät saaneet GN-RH: ta, ja sai aikaan lisääntyneen LH-vapautumisen GN-RH: ta saaneissä eläimissä verrattuna öljyä saaneeseen kontrolliryhmään.

Sammendrag
I denne undersøgelse undersøgtes progesterons og østradiols evne til at modulere hypofysens følsomhed over for gonadotrophin releasing hormone (GN-RH) ved at måle ændringer af plasmaniveauet af luteinizing hormone (LH) i ovariectomerede mus forbehandlet med steroidhormonerne.
GN-RH behandlingen resulterede i forhøjet LH niveau. Forbehandling med de gonadale hormoner førte til en sænkning af LH niveauet hos de dyr, som ikke fik GN-RH, og til en stigning af LH niveauet hos de dyr, som fik GN-RH.

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