EFFECTS OF GONADECTOMY AND OESTRADIOL TREATMENT ON PLASMA LUTEINIZING HORMONE CONCENTRATIONS IN THE MARMOSET MONKEY, CALLITHRIX JACCHUS

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SUMMARY

A heterologous double-antibody radioimmunoassay for marmoset LH is described in detail. The system uses NIAMDD rat LH-I-1 for iodination, NIAMDD rat LH-RP-1 as standard and anti-oviine rabbit LH 610V serum. The assay measures the level of marmoset LH in plasma and shows a maximum cross-reaction (B/Bo = 50%) of < 0.3% with other rat, human or bovine pituitary hormones.

The heterologous assay has been applied to the measurement of the level of LH in the marmoset after gonadectomy and after the subsequent implantation of oestradiol-17β capsules. A clear increase in the concentration of LH in the plasma was observed by day 3 after gonadectomy and in most animals the level reached a plateau by day 9. The rates of increase in the concentration of LH and the maximum levels attained after gonadectomy were similar in male and female marmosets. The secretion of LH in long-term gonadectomized marmosets was episodic. In four of the marmosets studied, the frequency of the pulsatile discharges of LH was circhoral, whereas in the remaining five animals the frequency could not be determined.

Subcutaneous implants containing oestradiol-17β were seen to have a biphasic effect on the secretion of LH in all gonadectomized marmosets. After a transient increase in the concentration of LH (positive feedback) on day 1, levels fell markedly by day 4 and were undetectable 8 days after the implants were introduced (negative feedback).

INTRODUCTION

Tonic gonadotrophin secretion in the primate is regulated by a negative feedback loop between the gonads and the hypothalamo-hypophysial axis (Knobil, 1974; Yen, Lasley, Wang, Leblanc & Siler, 1975). The interruption of this negative feedback loop by gonadectomy results in a marked increase in the levels of gonadotrophins in the circulation of man (Yen & Tsai, 1971; Monroe, Jaffe & Midgley, 1972) and the rhesus monkey (Atkinson, Bhattacharya, Monroe, Dierschke & Knobil, 1970; Knobil, 1974). The raised concentrations of gonadotrophins are the result of pulsatile discharges of these hormones by the pituitary gland (Knobil, 1974). Restoration of the feedback loop can be achieved by administration of oestrogen and in the rhesus monkey a great deal of work has been carried out using the gonadectomized animal as a model for testing the feedback properties of this hormone (Yamaji, Dierschke, Bhattacharya & Knobil, 1972; Karsch, Weick, Hotchkiss, Dierschke & Knobil, 1973; Knobil, 1974). Although the precise combination of steroids which controls tonic gonadotrophin secretion in intact animals has not yet been determined, these studies have shown that oestradiol-17β is extremely effective as an agent of negative feedback in both male and female gonadectomized rhesus monkeys.
To date, however, no similar work has been reported on any non-human primate other than the rhesus monkey.

This paper describes the development of a heterologous radioimmunoassay for marmoset luteinizing hormone (LH) and its application to the measurement of the level of LH in the plasma of the marmoset monkey, Callithrix jacchus. It also relates an initial attempt to characterize the relationship between gonadal function and the secretion of LH by the pituitary gland in this species.

**MATERIALS AND METHODS**

**Animals**

Sexually mature male and female marmoset monkeys were used. Full details of their management have been published previously (Hearn, Lunn, Burden & Pilcher, 1975). Animals were exposed to natural light with additional illumination between 05.00 and 19.00 h.

**Experimental procedure**

Nine marmosets (five male and four female) were bilaterally gonadectomized under anaesthesia (18 mg Saffan/kg; alphaxalone (0-9%, w/v) and alphadolone acetate (0-3%, w/v; Glaxo Laboratories Ltd). Animals were not sedated before venepuncture. Blood samples (0-4 ml) were taken from the femoral vein with a heparinized syringe and placed on ice immediately. The blood was centrifuged at 500 g for 20 min at 4 °C and the plasma stored at −20 °C until assayed. The schedule for bleeding is indicated in Figs 2 and 3. Implants containing oestradiol-17β were introduced at least 10 weeks after gonadectomy and were removed 8 weeks before the animals were bled at 0-5 h intervals. All 0-5 h blood samples were collected on the same day, between 09.00 and 13.00 h.

**Preparation of oestradiol-17β implants**

Silastic implants containing crystalline oestradiol-17β were prepared using a modification of the technique described by Dzuik & Cook (1966). The implants were sterilized and implanted subcutaneously through an incision made ventrolaterally in the abdominal area.

**Measurement of LH in marmosets**

Marmoset LH was measured with a heterologous double-antibody radioimmunoassay which is a modification of the assay for rat LH described by Welschen, Osman, Dullaart, de Greef, Uilenbroek & de Jong (1975). The system uses NIAMDD rat LH-I-1 for iodination, NIAMDD rat LH-RP-1 as standard and rabbit anti-ovine LH 610V (Dr J. Uilenbroek) as antiserum.

**Iodination of rat LH**

Rat LH preparation NIAMDD rat LH-I-1 was labelled with Na$^{125}$I (Radiochemical Centre, Amersham) by the chloramine-T method of Greenwood, Hunter & Glover (1963). Hormone (2 μg) was reacted with Na$^{125}$I (0-5-1-0 mCi) and chloramine T (50 μg in 0-05 M phosphate buffer, pH 7-5). The reaction was stopped after 30 s by adding 125 μg sodium metabisulphite in 0-5 ml buffer. Potassium iodide (10 mg) was then added in 0-5 ml buffer. Labelled LH was isolated from the reaction mixture by adsorption chromatography on a small column (1 × 5 cm$^2$) of Whatman CF 11 grade cellulose. The reaction mixture was applied to the column, which was then washed with 30 ml 0-12 M-barbitone buffer (pH 8-6). The $^{125}$I-labelled LH was eluted from the column by passing through 10 ml barbitone buffer containing 5% bovine serum albumin (BSA, Fraction V, nitrogen content 15-9%: Sigma) and 1 ml fractions were collected. The most immunoreactive fractions were pooled and no further purification was necessary.
Radioimmunoassay method

All incubations were carried out in duplicate. Standards, test samples and \(^{125}\text{I}\)-labelled LH were diluted in 0.01 M-phosphate buffer (pH 7.5) containing 1% BSA. A series of standards was prediluted in buffer so that the required amount of hormone could be dispensed in a volume of 200 µl. Test samples were dispensed in 50 µl portions and made up to 200 µl with buffer. Antiserum (100 µl in 0.01 M-phosphate buffer containing 0.05 M-EDTA and 0.3% normal rabbit serum) was then added and the contents of each tube were mixed and left for 3 days at 4 °C. The antiserum was used at an initial dilution of 1 : 70 000 and bound between 23 and 30% of the labelled tracer (n = 15). \(^{125}\text{I}\)-Labelled LH (approximately 10 000 c.p.m.) in 100 µl buffer was added to all tubes, which were mixed and incubated at 4 °C for a further 2 days. Separation of antibody-bound and free hormone was achieved by adding 200 µl 1 : 30 (in 0.01 M-phosphate buffer) donkey anti-rabbit gamma globulin (Burroughs Wellcome RD17) and incubation was continued at 4 °C for 12 h. Phosphate buffer (1 ml, 0.01 mol/l, pH 7.5) was added to each tube immediately before centrifugation at 500 g for 30 min at 4 °C. The supernatant fractions were discarded and the antibody-bound \(^{125}\text{I}\)-labelled LH in the precipitate was measured in an automatic gamma spectrometer.

Specificity of the heterologous LH assay

To assess the specificity of the antiserum, 200 µl doubling dilutions of rat LH-I-1, human LH (hLH-Stockell–Hartree 1RC2; Hartree, 1967), human follicle-stimulating hormone (FSH, hFSH-Butt CPDS 15; Butt, Lynch & Kennedy, 1972), human thyroid-stimulating hormone (TSH, hTSH-Stockell–Hartree De 32-3; Hartree, 1966), ovine LH (oLH-S10; Reichert & Wilhelm, 1976) and bovine FSH (bFSH-CH-1-76; Cheng, 1977) were subjected to the same assay procedure as described above. Since no purified marmoset pituitary hormone preparations are available, the antiserum was tested for cross-reaction with marmoset TSH by measuring the response of plasma obtained from blood samples taken before and 0, 10, 30 and 50 min after an intramuscular injection of 20 µg thyrotrophin releasing hormone (TRH, Roche) in the LH assay. Validation of the assay was obtained by comparing the responses to an injection of luteinizing hormone releasing hormone (LH-RH) in normal and LH-RH-immunized male marmosets. (The procedure for immunization is described by Hodges & Hearn, 1977). Synthetic LH-RH (4 µg in 100 µl saline) was injected intramuscularly into normal and LH-RH-immunized male marmosets, and blood samples were collected 0 and 30 min later. The parallelism between dose–response curves for rat LH-RP-1 standard and dilutions of marmoset crude pituitary extract and plasma samples containing endogenous marmoset LH from LH-RH-treated and LH-RH-immunized marmosets was assessed to validate the assay for the measurement of LH in marmoset plasma.

Precision of the heterologous LH assay

The precision of the assay expressed as the intra- and interassay coefficients of variation (%) was assessed by repeated assay of a pool of marmoset plasma from male and female marmosets after administration of LH-RH.

Measurement of oestradiol-17β

Plasma oestradiol-17β was estimated using a specific radioimmunoassay described by Baird, Swanston & Scaramuzzi (1976). Chromatography was not required, although a recovery determination was made on each sample.

RESULTS

Specificity of LH assay

Figure 1a shows the cross-reactivity (B/Bo) of all the standard hormones investigated in the heterologous LH assay (B, radioactivity bound in the presence of unlabelled hormone;
Bo, radioactivity bound in the absence of unlabelled hormone). The slope of the dose-response curve using ovine LH-S10 standard was slightly greater than in the system which used an LH standard of rat origin (NIAMDD rat LH-RP-1). The highly purified iodination grade NIAMDD rat LH-I-1 preparation was used to assess the cross-reactivity of the various hormones tested and was given a potency value of 100%. Cross-reaction was calculated as the amount of hormone (w/w) giving 50% inhibition of binding. NIAMDD rat LH-RP-1 gave a cross-reaction of 3% while cross-reactions with bovine FSH-CH-1-76 and NIAMDD rat FSH-I-1 were 0.17 and <0.3%, respectively (cross-reaction with NIAMDD rat FSH-I-1 was measured by Welschen et al. 1975). Although all human preparations gave non-parallel inhibition curves, the antiserum clearly had a much higher

Fig. 1. (a) Cross-reactions of rat LH (●, NIAMDD rat LH-RP-1; □, NIAMDD LH-I-1), ovine LH (■, oLH-S10), bovine FSH (×, bFSH-CH-1-76) and human LH (○, hLH-Stockell-Hartree 1RC 2), TSH (△, hTSH-Stockell-Hartree De 32-3) and FSH (▲, hFSH-Butt CPDS 15) in the heterologous radioimmunoassay for LH; (b) inhibition curves for NIAMDD rat LH-RP-1 (●), pooled plasma from luteinizing hormone releasing hormone (LH-RH)-treated (△) and immunized (□) marmosets and a marmoset crude pituitary extract (▲). B, radioactivity bound in the presence of unlabelled hormone; Bo, radioactivity bound in the absence of unlabelled hormone.
affinity for human LH-IRC2 than for either human FSH-CPDS 15 or human TSH-De 32–3, both of which showed no significant inhibition of binding (<0.05%). Mean (± S.E.M.) concentrations of LH before and 10, 30 and 50 min after administration of TRH to three normal male marmosets were 30.1 ± 6.0, 29.0 ± 4.9, 31.0 ± 6.3 and 23.2 ± 3.3 ng/ml respectively. There was, therefore, no marked change in the concentration of LH after administration of TRH.

Measurement of LH in plasma

Parallelism

Serial dilutions of marmoset plasma and a crude marmoset pituitary extract were parallel to the inhibition curve obtained with NIAMDD rat LH-RP-1 standard (Fig. 1b) over the range 31–87% B/Bo (no significant departure from parallelism: P>0.05, ANOVA). Although parallelism was not found at the extremities of the curves, all plasma samples measured fell within the limits of parallelism quoted. There was no significant departure from parallelism (P>0.2, ANOVA) between serial dilutions of marmoset plasma and the marmoset pituitary extract, over the complete range of dilutions. The non-parallelism above 87% B/Bo and below 31% B/Bo seen when comparing marmoset plasma with NIAMDD rat LH-RP-1 standard is therefore a feature of the hormone and is not due to a plasma effect. A volume of 50 µl marmoset plasma (as used in the assay) showed no non-specific interference. The slight inhibition of binding produced by 200 µl plasma was probably caused by low levels of residual LH which can occur in LH-RH-immunized animals.

Response to LH-RH

Mean (± S.E.M.) concentrations of LH before and 30 min after administration of LH-RH to normal male marmosets were 29.1 ± 2.5 and 102.1 ± 6.4 ng/ml respectively (n= 15). The change in the concentration of LH in the plasma (ΔLH) during this period was therefore 73.0 ng/ml. Immunization against LH-RH inhibited the release of LH in response to injection of LH-RH. Pretreatment levels of LH in immunized male marmosets were no longer detectable (<20 ng/ml) and mean (± S.E.M.) concentrations 30 min after the LH-RH

![Graph](image-url)

Fig. 2. Mean (± S.E.M.) concentrations of LH in the plasma of male (●, n = 5) and female (×, n = 4) marmosets before and after gonadectomy.
Oestradiol-17\(\beta\) implanted (10–20 µg/day)

Fig. 3. Mean (± s.e.m.) concentrations of LH in the plasma of male (●, n = 5) and female (×, n = 4) gonadectomized marmosets before and after the introduction of oestradiol-17\(\beta\) implants. Mean (± s.e.m.) concentrations of oestradiol in the plasma of male and female gonadectomized marmosets before and after the introduction of oestradiol-17\(\beta\) implants are shown in the lower part of the figure (n = 9).

Injection were 25.7 ± 1.7 ng/ml (n = 3). As pre-injection levels of LH were undetectable in immunized animals, it was not possible to obtain a value for ΔLH. Nevertheless, it is clear that immunization against LH-RH both depresses the level of LH in the circulation and inhibits the pituitary response to stimulation by exogenous LH-RH.

**Sensitivity**

The limit of detection of the assay (\(B/Bo = 90\%\)) ranged from 0.7 to 0.9 ng/tube. Since the dilutions of marmoset plasma and rat standard become non-parallel above 87%, \(B/Bo\),
LH levels in gonadectomized marmosets

a working sensitivity of the assay of 1 ng/tube was adopted. With a 50 μl plasma sample, the detection limit was therefore 20 ng LH-RP-1 equivalent/ml.

**Precision**

Intra- and interassay variations (expressed as the coefficient of variation) of results on replicate samples were 3-6% (n = 12) and 7-4% (n = 15), respectively.

**Effect of gonadectomy on LH secretion**

The concentrations of LH (±S.E.M.) before and after gonadectomy in five male and four female marmosets are shown in Fig. 2. The rate of increase in the concentration of LH and the maximum level attained after gonadectomy were similar in all animals, regardless of their sex. In each case, a clear increase (P<0.01, paired t-test) in the level of LH in the plasma was observed by day 3 after gonadectomy and in most animals the level reached a plateau by day 9. Mean (±S.E.M.) concentrations of LH in the plasma 10 weeks after gonadectomy (measured in samples taken just before the insertion of implants) were not significantly higher than those observed after 2 weeks (P>0.2, paired t-test).

**Effect of oestradiol-17β implants on LH secretion**

Subcutaneous implants containing oestradiol-17β inhibited the secretion of LH in gonadectomized male and female marmosets (Fig. 3). The concentration of LH in the plasma fell significantly (P<0.001, paired t-test) 8 days after the implants were introduced, and remained undetectable for the rest of the study. With the sampling frequency used, it was not possible to detect any difference between the response of male and female marmosets to oestrogen (the difference between levels of LH in the plasma of male and female marmosets on day 4 is not significant; P>0.05, Student's t-test). This is particularly interesting with respect to the concentration of LH 24 h after implantation. A clear surge of LH was observed at this time in all nine animals, indicating that LH is released in response to an oestrogen challenge in castrated as well as ovariectomized marmosets. The levels of oestradiol-17β in the blood produced by the implants are shown in Fig. 3. There was an initial peak in the concentration of oestradiol-17β in the plasma on day 1, with a subsequent decline to fairly constant levels with mean (±S.E.M.) values ranging from 0.53 ± 0.05 to 0.61 ± 0.05 ng/ml.

![Fig. 4. Concentrations of LH in the plasma of individual male (a) and female (b) marmosets gonadectomized for approximately 20 weeks.](image-url)
Episodic secretion of LH in gonadectomized marmosets

The concentrations of LH in the plasma of long-term gonadectomized marmosets bled at 0-5 h intervals for 4 h are shown in Fig. 4. In each animal, secretion of LH was episodic with as many as four peaks occurring within the 4 h period. The frequency of the pulsatile discharges of LH is not clear from these results. Although it appears to be circadian in four of the animals, the periodicity of LH release in the remainder cannot be determined from the sampling frequency used.

DISCUSSION

The heterologous radioimmunoassay for LH described in this paper has been applied to the measurement of the concentration of LH in the marmoset monkey after gonadectomy and after the subsequent implantation of oestradiol-17β capsules.

Although absolute validation of the assay must await the purification of marmoset pituitary hormones, the data obtained here indicate that the assay is capable of measuring marmoset LH. Comparison of dilutions of marmoset plasma and crude marmoset pituitary extract with NIAMDD rat LH-RP-1 shows that the assay measures marmoset LH in plasma. The slight inhibition of binding obtained with human TSH-De 32-3 is probably due to contamination of this preparation with LH. Although it is not anticipated that marmoset TSH and human TSH-De 32-3 would show immunochemical similarity, the data relating human TSH and LH are relevant in showing that the LH assay described here clearly has a much higher affinity for human LH than for human TSH. Since there was no apparent increase in the concentration of radioimmunoassayable LH in the plasma of marmosets after administration of TRH, it is assumed that the LH assay is not measuring significant amounts of marmoset TSH (providing TRH does release TSH in the marmoset). The data on human TSH-De 32-3 and the administration of TRH therefore provide indirect evidence that significant cross-reaction with TSH in the heterologous LH assay is unlikely.

Physiological validation of the assay (responsé to administration of LH-RH and gonadectomy) shows that the heterologous assay effectively measures marmoset gonadotrophins, but it does not give any indication of how much FSH is being measured. Cross-reaction studies with FSH preparations of human, rat and bovine origin showed a maximum cross-reaction of <0.3% with NIAMDD rat FSH-I-1. Since this assay uses a rat LH tracer, it is unlikely that the cross-reactivity of FSH from any other species will be greater than that of rat FSH. Thus, although a precise determination of the degree of cross-reaction with marmoset FSH cannot be made at present, it is reasonable to assume that it will not exceed 0.3%. These results indicate that this heterologous LH assay provides a reliable method for measuring LH in the plasma of the marmoset monkey, although until purified marmoset LH becomes available, the accuracy of the assay cannot be determined.

Secretion of LH by the pituitary gland increases markedly after gonadectomy in male and female marmosets; the greatest increment in the concentration of LH occurred during days 1-3. The initial increase in the level of LH in the plasma almost certainly occurred earlier than day 3, but since the first sample was not collected until this time there are no data to show this. However, Reeves, O'Donnell & Denorscia (1972) observed that the level of LH in the plasma increases within 12 h of castration in the ram and Goldman & Porter (1970) reported that female hamsters ovariectomized during dioestrus have markedly increased levels of LH in the serum within 3-5 h of gonadectomy. In male and female rhesus monkeys (Atkinson et al. 1970) and in women (Yen & Tsai, 1971), increases in the level of LH in the plasma occur within 1 or 2 days of gonadectomy. In the rat, the increase in the level of LH after gonadectomy is much more rapid in the male (within 8 h) than in the female (within 1 or 2 days; Gay & Midgley, 1969) of the species. In contrast to this and in agreement with work on the rhesus monkey (Atkinson et al. 1970), there does not appear to be a sex difference in the marmoset.
Blood samples collected at 0.5 h intervals reveal an episodic release of LH in the long-term gonadectomized marmoset. Similar fluctuations in the levels of LH in the plasma have been observed in a number of other species such as the rat (Gay, 1970), the rhesus monkey (Dierschke, Bhattacharya, Atkinson & Knobil, 1970), the sheep (Reeves et al. 1972) and man (Yen, Tsai, Vandenberg & Rebar, 1972) and it is now generally accepted that these variations in the concentration of LH are the result of pulsatile discharges of LH by the pituitary gland. The frequency of the discharges appears to vary between species. In the present study the rhythm of LH discharge was circoral in four animals but appeared to be irregular in the remainder. Reeves et al. (1972) observed an average cycle length in ovariec-tomized ewes of 52 min, Dierschke et al. (1970) reported a mean period of oscillation in the level of LH in the plasma of gonadectomized rhesus monkeys of 75 min (with a mode of 60 min) and Gay (1970) described a periodic increase in the level of LH in gonadectomized rats occurring at 15 min intervals. Reeves et al. (1972) and Dierschke et al. (1970) showed in the ewe and rhesus monkey respectively, that regular periods of LH discharge could only be detected when the sampling frequency was of the order of every 10–20 min. Initially, blood samples were taken every hour in these species (Atkinson et al. 1970; Reeves et al. 1972) and in both cases the discharges of LH appeared completely random. As the size of the marmoset precludes the withdrawal of large quantities of blood it was not possible to increase the sampling frequency in the present study. If a greater sampling frequency had been used, a regular (circal) period of LH discharge might have been observed in more of the animals.

Implantation of Silastic capsules containing crystalline oestradiol-17β inhibited secretion of LH in male and female marmosets. This demonstration of a negative feedback effect of oestradiol confirms earlier reports (Yamaji et al. 1972; Karsch et al. 1973; Legan, Gay & Midgley, 1973) that this steroid is capable, on its own, of restoring the negative feedback loop between the gonads and the hypothalamo-hypophysial axis which is interrupted by gonadectomy. The rate of release of oestradiol-17β by the implants, estimated to be between 10 and 20 μg/day, produced concentrations of oestradiol-17β in the plasma (after the peak on day 1) similar to those found in the late follicular stage of the ovarian cycle in the intact female marmoset (Hearn & Lunn, 1975). With this dose, no significant difference was observed between the responses of male and female marmosets to oestradiol-17β. Coincident with the peak in the level of oestradiol on day 1 was a marked increase in the secretion of LH in all animals, indicating that oestradiol-17β is capable of exerting both positive and negative feedback actions. A biphasic effect of oestradiol on the secretion of LH has been demonstrated in intact and ovariec-tomized female rats (Legan et al. 1973), rhesus monkeys (Karsch et al. 1973) and women (Yen & Tsai, 1972). The results obtained here confirm a previous observation (Hodges & Hearn, 1978) that castrated marmosets are also sensitive to a positive feedback action of oestradiol-17β and will release LH when stimulated with this hormone. Hodges and Hearn (1978) demonstrated that a single injection of oestradiol benzoate initially induces negative feedback (4–12 h), with a subsequent positive feedback effect after 24 h. In the present study, the first blood sample was not taken until 24 h after implantation of the capsules and so any initial negative action which may have occurred has not been detected.

Although absolute validation of this heterologous LH assay is still required, the system described in this paper will enable investigation into the control of LH secretion in the marmoset monkey to proceed. A greater understanding of the reproductive endocrinology of this interesting New World primate may in turn provide an insight into certain aspects of reproduction in man.

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