IMMUNOREACTIVE INHIBIN CONCENTRATIONS IN SERUM THROUGHOUT THE MENSTRUAL CYCLE OF THE MACAQUE: SUPPRESSION OF INHIBIN DURING THE LUTEAL PHASE AFTER TREATMENT WITH AN LHRH ANTAGONIST

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ABSTRACT

Concentrations of immunoreactive inhibin in serum samples collected daily from six adult stumptailed female macaques during normal menstrual cycles were measured with a heterologous radioimmunoassay. Serum inhibin concentrations were low during the follicular phase of the cycle. After ovulation they began to rise, reaching a plateau between 8 and 11 days, before falling in parallel with the decline in luteal progesterone secretion. The dependence of the inhibin secretion by the corpus luteum on pituitary gonadotrophins was investigated by the administration of an LHRH antagonist [N-Ac-D-Nal(2),D-pCl-Phe²,D-Trp¹,D-hArg(Et₂),D-Ala⁶]LHRH once daily for 3 days beginning on day 8 of the luteal phase in six macaques. LHRH antagonist treatment markedly suppressed serum levels of inhibin and progesterone and these remained at the level found in the follicular phase for the remainder of the luteal phase. These results show that inhibin in the macaque is secreted into the peripheral blood almost exclusively during the luteal phase, being highest when FSH is at its nadir. Suppression of serum inhibin concentrations during the luteal phase by LHRH antagonist suggests that its secretion is integrated with the LH control of the corpus luteum.

INTRODUCTION

The structure of inhibin has recently been characterized as a glycoprotein consisting of two dissimilar subunits termed α and β (Ling, Ying, Ueno et al 1985; Miyamoto, Hasegawa, Fukuda et al 1985; Robertson, Foulds, Leversha et al, 1985). Radioimmunoassays using antibodies raised against the native molecule or synthetic peptides from the α subunit have been developed to measure circulating levels. Classically, inhibin was thought to be a product of the developing follicle and to function for the purpose of suppressing selectively the secretion of follicle stimulating hormone (FSH). It was surprising to find that in women serum concentrations of immunoreactive inhibin were highest during the luteal phase (McLachlan, Robertson, Healy et al 1987; Buckler, McLachlan, McLachlan et al 1988).

There are no reports of inhibin concentrations in peripheral blood in the non-human primate and the relationship of luteal inhibin to gonadotrophin secretion has yet to be elucidated. By using techniques of luteinizing hormone releasing hormone (LHRH) deprivation, it has recently been established that the corpus luteum of monkeys and women is dependent on pituitary luteinizing hormone (LH) secretion (Fraser, Baird, McRae et al 1985; Fraser, Abbott, Laird et al 1986a; Fraser, Nestor & Vickery 1987; Collins, Sopelak, Williams & Hodgen, 1986; Hutchison & Zeleznik, 1985; Hodges, Green, Cottingham et al 1988; Mais, Kazer, Cetel et al 1986). In the present study we measured serum concentrations of immunoreactive inhibin throughout the menstrual cycle of the stumptailed macaque and examined the consequences of administering an LHRH antagonist during the mid-luteal phase.
MATERIALS AND METHODS

Animals
Twelve adult female stumptailed macaques (Macaca arctoides) weighing 8-15 kg were used. Details of their management have been published previously (Fraser et al 1986a). The animals had demonstrated regular menstrual cycles with normal luteal phases as determined by hormonal estimations three times per week, fulfilling the criteria described previously (Fraser et al. 1986a). Blood samples (5ml) were collected daily by femoral venepuncture without anaesthesia beginning during the early follicular phase and continued for 40 days. The blood was centrifuged at 1000g for 20 min, the serum divided into two aliquots and stored at -20°C until assayed for progesterone, LH, FSH and inhibin. Aliquots for inhibin radioimmunoassay were sent to Melbourne on dry ice.

The macaques were injected with LHRH antagonist [N-Ac-D-Nal(2), D-pCl-Phe3, D-Trp3, D-hArg(Et2)4, D-Ala10]LHRH (detirelix: Syntex, Palo Alto, CA, USA) dissolved in 0.9% saline: propylene glycol (1:1, v/v) and administered subcutaneously at a dose of 300 μg/kg for 3 consecutive days beginning on day 8 after the mid-cycle LH surge. This time was determined by use of a rapid progesterone radioimmunoassay together with an LH radioimmunoassay as described previously (Fraser et al 1985). Serum concentrations of FSH were also measured by a radioimmunoassay described previously (Fraser, McNeilly, Abbott & Steiner, 1986b).

Inhibin concentrations were determined by a heterologous radioimmunoassay utilizing a rabbit antiserum (no.1988) raised against purified bovine 31kDa inhibin and iodinated bovine 31kDa inhibin as tracer. As macaque inhibin is not available we employed samples of a stock of serum (MR-1) from gonadotrophin-treated women as a source of non-radioactive inhibin standard in the radioimmunoassay. The potency of this material was assessed by comparison with a partially purified human follicular fluid inhibin preparation (Buckler et al 1988). The bio and immunoactivity of the standard and details of the radioimmunoassay specificity have been reported elsewhere (Robertson, Tsonis, McLachlan et al 1988b). In the radioimmunoassay the human serum stock gave a dose-response curve parallel to that given by serum from female stumptailed macaques, as judged by the lack of significant differences in the slopes of the logit-log dose response curves. The volume of serum in each tube was equalised to 200μl by addition of serum from post-menopausal women which contained undetectable concentrations of inhibin. All samples were assayed in three assays with a between-assay variation of 4%. The within-assay variation, as assessed from the average index of precision, was 0.035. The detection limits of the assays were between 0.3 and 0.55 U/ml. A pool of serum from a macaque ovariectomized 2 weeks previously read 0.54 U/ml which was at the detection limit of the radioimmunoassay. The assay showed < 0.5% cross-reaction with transforming growth factor-β, activin A, Mullerian inhibitory substance and the subunits of inhibin produced by reduction and alkylation.

Statistical analysis
Changes in hormone concentrations within the two groups were statistically evaluated using two-way analysis of variance for repeated measures. Where a significant change with time was indicated, different time points were compared using Newman-Keuls t-test.

RESULTS

Serum concentrations of progesterone, immunoreactive inhibin and FSH, centred around the day of the mid-cycle LH surge (day 0), are plotted in Fig. 1. Serum concentrations of FSH and progesterone followed the course described previously; progesterone concentrations reached a plateau between days 8-11 and then declined (Fraser et al 1986a). Inhibin concentrations were low during the follicular phase in all animals though a small but significant (P< 0.05) rise occurred on the day before and the day of the LH surge. On day 1 of the luteal phase inhibin concentrations returned to the mid-follicular phase range prior to the onset of a sustained rise from day 2. Levels of inhibin reached maximal on days 9-12 before falling as the luteal phase came to an end. As the serum concentrations of progesterone and inhibin declined, serum concentrations of FSH, which are at a nadir during days 8-12 of the luteal phase, began to increase.

Prior to treatment, hormone concentrations in the LHRH antagonist-treated macaques were similar to controls. LHRH antagonist administration was followed by a marked fall in serum concentrations of progesterone and immunoreactive inhibin. The levels of these hormones
DISCUSSION

These results demonstrate that in the macaque the secretion of immunoreactive inhibin into the peripheral blood occurs predominately during the luteal phase and follows a pattern of secretion similar to that of progesterone. The very small rise in inhibin, evident during the late follicular phase and on the day of the LH surge, may be less marked than the preovulatory rise seen in women (McLachlan et al. 1987). This may represent a species variation or may be related to the use of a heterologous radioimmunoassay. The predominant secretion of serum inhibin during the luteal phase is similar to the situation in women (McLachlan et al. 1987). The close correlation of serum immunoreactive inhibin and progesterone concentrations suggests that the corpus luteum is the source of inhibin during the luteal phase, a concept supported by the demonstration of the expression of the inhibin gene by the human corpus luteum (Davis, Kroowski, McLachlan & Burger 1987).

The administration of the LHRH antagonist caused a complete suppression of luteal function with progesterone and inhibin levels falling to follicular phase values. The fall in inhibin was slower than for progesterone, due perhaps to the longer half-life of inhibin in the circulation (Robertson, Hayward, Irby et al. 1988a). The effects of this antagonist on serum concentrations of FSH and bioactive LH during the luteal phase in separate experiments in the stumptailed macaque have been the subject of detailed investigations (Fraser et al. 1985, 1986a, 1987). These results demonstrated a rapid decline in LH pulses after administration of the LHRH antagonist. Studies in cynomolagus monkeys have also shown that it is the decline in LH, but not FSH, which is responsible for the fall in serum concentrations of progesterone (Collins et al. 1986). By the mid-luteal phase serum concentrations of FSH are at a nadir; these are only marginally reduced by the LHRH antagonist. From the present results, we suggest that any remaining FSH is derived from the corpus luteum, which is the principal source of FSH following ovulation.

Figure 1. Serum concentrations of progesterone, inhibin and FSH in control macaques (○) and in animals receiving 300 μg/kg LHRH antagonist (ANT) s.c. at days 8, 9 and 10 after the mid-cycle LH surge (●). n=6 per group, values are plotted as the mean (± SEM).

were significantly lower (P<0.001) than controls by the first day after starting treatment. Serum progesterone concentrations had fallen to 22% of pretreatment values within 24h after the first injection of antagonist, and had reached follicular-phase values by 48h. This suppression was sustained for the remainder of the luteal phase and premature menstruation occurred in all animals. Inhibin concentrations fell more slowly than progesterone, being 71% of immediate pretreatment values by 24h after the first injection of antagonist and 36% by 48h. Thereafter inhibin concentrations remained at low levels for the duration of the "luteal phase". Serum FSH concentrations were at a nadir at the time of LHRH antagonist administration and the resulting fall in mean FSH during the 4 days after starting treatment was not significant.
that luteal phase inhibin secretion is driven, like progesterone (Fraser et al 1986a), by LH. In addition, human luteinized granulosa cells in long-term culture synthesize inhibin in the presence of LH but not FSH (Tsonis, Hillier & Baird 1987).

The feedback role of inhibin during the menstrual cycle has yet to be elucidated. During the luteal phase, serum inhibin is likely to be responsible for the low levels of FSH. FSH is lowest when serum inhibin is maximal and its decline, during the late luteal phase, is followed by a rise in serum FSH. The fact that serum FSH concentrations do not rise in response to the premature decline in inhibin induced by the LHRH antagonist shows that FSH release is predominantly under the control of LHRH release and that the rise in response to withdrawal of negative feedback requires the presence of LHRH. However, the fact that FSH secretion falls more slowly than LH after interference with LHRH action may, in part, be a consequence of withdrawal of the negative feedback specifically on pituitary FSH release (Mais et al 1986). These observations have been made generally during the follicular phase when the levels of FSH are elevated and levels of inhibin are low. Thus, it is tempting to conclude that the other major component of the developing follicle, oestradiol, is primarily involved in the negative feedback control of FSH secretion during the follicular phase. During the luteal phase the low level of follicular development, caused by the low pituitary output of FSH, is likely to be due, in part, to the high serum concentrations of inhibin. Oestradiol produced by the corpus luteum also serves to suppress FSH at this time (Zeleznik, Hutchison & Schuler, 1987). Further studies are required to elucidate the relative roles of these two hormones in control of FSH secretion.

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REFERENCES


