Initiation of high dose gonadotrophin-releasing hormone antagonist treatment during the late follicular phase in the macaque abolishes luteal function irrespective of effects upon the luteinizing hormone surge

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The determination of the efficacy of gonadotrophin-releasing hormone (GnRH) antagonists in blocking the luteinizing hormone (LH) surge and luteal function is important for our understanding of the control of the menstrual cycle and for clinical application. GnRH antagonists have failed to block the LH surge reliably in the non-human primate. The aim of the study was to utilize high dose GnRH antagonist treatment administered during the late follicular phase of the menstrual cycle to block the pre-ovulatory LH surge. It was postulated that the LH surge would be prevented in all animals, but if this failed subsequent luteal function would be blocked by continued suppression of LH, since the early corpus luteum is susceptible to inhibition by GnRH antagonist treatment. A group of 16 adult female stumptailed macaques (Macaca arctoides) with regular menstrual cycles were selected. The GnRH antagonist \([N{-}Ac{-}D{-}Nal(2)]\, D{-}pCl{-}Phe^2, D{-}Phe^3, D{-}(Hci)^6, Lys^4(Pr), D{-}Ala^{10}]\)GnRH (Antarelix™) (concentration 10 mg/ml) was administered as three daily s.c. injections, at a dose of 1 mg/kg on days 11, 12 and 13 of the follicular phase of the menstrual cycle. Of nine macaques in which it was judged that the treatment was commenced within 1 day of the expected LH surge (serum oestradiol >400 pmol/l), six demonstrated a decline in serum oestradiol concentrations, a total block of the LH/follicle stimulating hormone (FSH) surge and inhibition of ovulation as judged by an absence of a rise in progesterone concentrations. In the three other animals in this category, a partial LH surge occurred, but this failed to result in a functional corpus luteum. In a further three animals treatment was initiated on the day of the LH surge, and again there was absence of a subsequently functional corpus luteum. These results show that GnRH is involved at the time of the mid-cycle LH/FSH surge in the non-human primate. Initiation of high dose GnRH antagonist treatment during the periovulatory period abolishes luteal function irrespective of its effects upon the LH surge because of its long-term action and resultant withdrawal of luteal support.

Key words: corpus luteum/follicle/LH surge/macaque/ovulation

Introduction

The determination of the ability of gonadotrophin-releasing hormone (GnRH) antagonists to block the mid-cycle luteinizing hormone (LH) surge is important to elucidate the role of GnRH in controlling periovulatory events and subsequent luteal function. This understanding, in turn, has implications for the use of GnRH antagonist therapy for controlled induction of follicular development and prevention of premature LH surges, post-coital contraception and control of luteal function (Fraser and Bouchard, 1994). In previous studies, we investigated the ability of the GnRH antagonists Detirelix and Antide to block the mid-cycle LH surge in the stumptailed macaque when administered just prior to the onset of the surge. When given to animals in which the serum oestradiol concentrations had risen to the full pre-ovulatory quota, the LH surge was inhibited in only 33% of animals after Detirelix (Fraser, 1990) and in 50% after Antide (Fraser et al., 1991). The fact that there was a tendency for failure of inhibition of the surge to be associated with animals in which serum oestradiol was highest at the time of onset of treatment led us to suggest that, in the macaque, the positive feedback effect of oestradiol could overcome the inhibition of GnRH action. This was in agreement with the experiments of Knobil and co-workers in rhesus monkeys using a number of different approaches (see Hotchkiss and Knobil, 1994 for review) and by the inability of GnRH immunoneutralization to prevent the LH surge in the stumptailed macaque in contrast to the effectiveness of this approach in non-primates (Fraser et al., 1986).

Subsequently, a number of similar studies have been performed in women showing that GnRH antagonist treatment could block the LH surge or cause its delay, results which appear to differ to some extent from those in the macaque (see Fraser and Bouchard, 1994 for review).

Another reason for the failure of GnRH antagonist treatment in macaques to prevent the LH surge in all animals could be the result of inadequate inhibition of GnRH. Recently, new GnRH antagonists have been developed which have retained the potency of the previous compounds but have a decreased histaminic effect, making them more acceptable clinically, and safer to use in high doses in experimental animals. One such compound is Antarelix™, which has an additional advantage of high water solubility (Deghenghi et al., 1993). The availability of Antarelix allowed us to determine whether the administration of a high dose of this GnRH antagonist during the late follicular phase of the menstrual cycle could cause reliable blockade of the mid-cycle LH surge in the macaque. We postulated that, should the LH surge occur, the subsequent function of the corpus luteum would be blocked by the
continued presence of the antagonist, since the function of the early corpus luteum is dependent upon LH support in both macaques (Fraser et al., 1987) and women (Dubourdieu et al., 1991).

Materials and methods
A total of 16 adult female stumptailed macaques (Macaca arctoides) weighing 9–14 kg was selected for study on the basis of having regular menstrual cycles as determined by examination of daily vaginal swabs and measurement of serum concentrations of progesterone and oestradiol-17β. The macaques were housed in individual cages with regular access to common exercise areas. Experiments were carried out in accordance with the Animals (Scientific Procedures) Act, UK, 1986.

Immediately prior to injection, the GnRH antagonist [N-Ac-d-Nal(2),d-pCl-Phe(3),d-Pal(4),Lys(iPr)(5),d-Ala(10)] GnRH (antarelix; europptides, argenteuil, France) was dissolved in water containing 5% mannitol to a concentration of 10 mg/ml. Macaques were given three daily injections of the GnRH antagonist administered s.c. in the thigh, at a dose of 1 mg/kg on days 11, 12 and 13 of the follicular phase (first day of menses = day 1).

Blood samples (4 ml) were collected daily throughout the study by femoral or cubital venepuncture without anaesthesia beginning on day 7 of the menstrual cycle and continuing for the next 3 weeks. Thereafter, samples were collected three times per week until the occurrence of the first post-treatment cycle. The blood was allowed to clot overnight, centrifuged at 1000 g for 20 min and the serum was stored at –20°C until required. Serum concentrations of progesterone and oestradiol-17β were measured by established radioimmunoassays (Fraser et al., 1991), detection limits for progesterone and oestradiol being 0.7 nM and 30 pM respectively. As a measure of short-term response to treatment, serum LH concentrations were determined using an in-vitro bioassay based on the production of testosterone by dispersed mouse Leydig cells as described previously (Fraser et al., 1991). Sensitivity of this assay was 6 µg LH/l of the NICHD rhesus monkey pituitary standard RP-1. Follicle stimulating hormone (FSH) was measured using a heterologous radioimmunoassay (Fraser et al., 1986) with a detection limit of 2 µg/l NICHD cyn-FSH-RP1.

Hormone profiles were compared to the normal menstrual pattern obtained from 18 control cycles in our colony collected over the previous 3 years, data being plotted around the time of the midcycle LH surge. Data for hormone concentrations were subjected to statistical analysis using one-way analysis of variance for repeated measures. The individual transformed means were further examined, where appropriate, using the Newman–Keuls test for pairwise comparisons.

Results
Although the target time for initiation of treatment was 0–24 h prior to the onset of the surge, it was inevitable that follicular development in some of the treated animals had not provided sufficient oestradiol output by the day selected for treatment while others were more advanced than intended. Since the magnitude of the late follicular phase rise in oestradiol is the most accurate predictor of the timing of the onset of the LH surge, examination of serum oestradiol concentrations during control cycles was used as an index of the accuracy of the onset of treatment. This revealed that, when oestradiol had reached a concentration of >400 pM, the LH surge would be expected to follow in 24 h.

Using this criterion it was found that four out of 16 (25%) of the s.c. treatments had been initiated when serum oestradiol was <400 pM, nine out of 16 (56%) at the correct time (as oestradiol had reached >400 pM), while three out of 16 (19%) had been started when the LH surge was already in progress. Cycles were grouped accordingly and results plotted around the day of onset of treatment and the control data plotted according to the equivalent concentration of serum oestradiol at the start of treatment.

The data for the four animals treated when serum oestradiol was <400 pM and significantly lower (P < 0.01) than in the other groups are shown in Figure 1. Antagonist administration
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Figure 2. The effect of Antarelix injection (1 mg/kg on days 11, 12 and 13 of the cycle) on serum concentrations of oestradiol, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in six macaques in whom treatment commenced when oestradiol secretion was >400 pM. Note inhibition of all hormones and absence of the LH surge (values are plotted as group means ± SEM). Shaded areas represent mean ± 1 SEM for control cycles (n = 18).

induced a fall in serum concentrations of FSH, LH and oestradiol, all of which were significantly lower (P < 0.001) than pretreatment by day 2. The LH/FSH surge and ovulation, as indicated by serum progesterone concentrations, failed to occur during that cycle.

In six of the nine macaques treated when oestradiol had reached >400 pM, similar suppressive effects were observed. Results are shown in Figure 2. Oestradiol declined markedly by day 1 (P < 0.001) and remained significantly suppressed for the duration of the treatment cycle. The LH/FSH surge and ovulation were prevented. FSH concentrations were basal at the start of treatment, because of negative feedback as a result of high serum concentrations of oestradiol; they therefore did not fall significantly, but remained at basal levels for the duration of the cycle. LH concentrations fell significantly (P < 0.01) by day 2 of treatment and remained suppressed for the remainder of the 5 day period of analysis.

In the remaining three animals in this category, serum oestradiol concentrations did not decline in response to treatment. On day 2 there was a clear rise in LH secretion. The resultant LH surge was 50% of that in control cycles but was not accompanied by a rise in FSH. A small increase in serum progesterone which lasted a few days was detected after the LH surge. Thereafter all hormones were suppressed for the remainder of the cycle, there being no indication of a functional corpus luteum (Figure 3).

In the three macaques in which treatment coincided with the LH surge, a small progesterone rise occurred during the first few days after treatment, but otherwise pituitary–ovarian function, based on serum concentrations of FSH and oestradiol, was suppressed for the remainder of the cycle (Figure 4). Mean time to return to ovulation was 52 ± 4 days (mean ± SEM) from end of treatment.

Antarelix was well tolerated, there being no indication of local or systemic histamine reactions.

Discussion

To establish whether GnRH is required during the mid-cycle LH surge, inhibition of GnRH must be delayed until as near as possible to the onset of this anticipated rise. Administration of antagonist at the mid-follicular phase is inappropriate because the hypothalamus is deprived of concentrations of oestradiol required to induce positive feedback and the pituitary is deprived of GnRH priming. In the present study, treatment was initiated in nine macaques at the optimal time during the menstrual cycle. The LH surge was totally abolished in 66% of macaques and attenuated in the remaining animals. This compares with an inhibition in 33% of animals after Detirelix and 50% following Antide treatment (Fraser, 1990; Fraser et al., 1991). The high dose 3 day treatment with Antarelix has provided our most convincing evidence to date that the pre-ovulatory surge of LH/FSH in the macaque requires the presence of GnRH.

It is still not clear why Antarelix treatment was not 100% effective. In previous studies we have taken the view that the most likely explanation for failure to prevent the surge in all animals was because, in treatment failures, the serum oestradiol concentrations had reached the positive feedback threshold by day 2. The LH/FSH surge and ovulation, as indicated by serum progesterone concentrations, failed to occur during that cycle.
to block the rising oestradiol concentrations. On the other hand, in the majority of animals in which oestradiol was also in the pre-ovulatory range, the dominant follicle was suppressed, presumably because they were in a less advanced stage of maturity. Serum oestradiol concentrations had declined markedly in these animals 1 day after starting GnRH antagonist treatment. At that time serum concentrations of LH and FSH were not significantly suppressed. It is likely that LH and FSH did decline after treatment but had recovered to basal values when the blood sample was taken at 24 h, as we observed when more detailed analysis was performed (Fraser, 1990), and that the follicle was susceptible to this deprivation in gonadotrophin.

Figure 3. The effect of Antarelix injection (1 mg/kg on days 11, 12 and 13 of the cycle) on serum concentrations of oestradiol, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in three macaques in whom treatment commenced on the day of the LH surge. Note absent luteal function (values are plotted as group means ± SEM). Shaded areas represent mean ± 1 SEM for control cycles (n = 18).

Figure 4. The effect of Antarelix injection (1 mg/kg on days 11, 12 and 13 of the cycle) on serum concentrations of oestradiol, progesterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) in three macaques in whom treatment commenced on the day of the LH surge. Note absence of luteal function (values are plotted as group means ± SEM). Shaded areas represent mean ± 1 SEM for control cycles (n = 18).

Our studies, using regimens likely to result in greater exposure to antagonist, have been associated with a higher number of successful inhibitions, suggesting that the efficacy of treatment is also related to the ability of the antagonist to block the effects of GnRH. We now know that in macaques, as in non-primate species, a GnRH surge accompanies the LH surge (Xia et al., 1992; Pau et al., 1993). It is likely that in macaques the achievement of the optimal concentration of oestradiol leads to both positive feedback effects at the level of the pituitary and an increase in GnRH release, making complete inhibition of the LH surge extremely difficult.

Debate as to the role of GnRH during the LH/FSH surge has continued for many years. Certainly in the macaque, experimental manipulations support a role for oestrogen acting
upon the GnRH primed pituitary to induce an LH surge (see Fraser and Bouchard, 1994; Hotchkiss and Knobil, 1994, for reviews). During the normal cycle in women there is little indication for an increase in GnRH pulse frequency (Adams et al., 1994). It is also clear that an LH surge leading to ovulation can be induced in anovular women and monkeys by an unchanging frequency and dose of exogenous GnRH (Hotchkiss and Knobil, 1994). Using different doses of GnRH antagonist during the early, mid and late follicular phases and during the time of the mid-cycle LH surge, Hall et al. (1994) found that LH could be most consistently suppressed at the time of the LH surge. This led them to suggest that in women there is a decreased release of GnRH during the preovulatory LH surge and that the surge was brought about by other positive influences.

Despite these observations, an essential role for GnRH during the LH/FSH surge in women now seems certain from a series of recent studies using GnRH antagonists. These have demonstrated that the LH surge can be abolished or delayed during the period of effective GnRH antagonist administration (Ditkoff et al., 1991; Frydman et al., 1992; Dubourdieu et al., 1994; Leroy et al., 1994). In some cycles in women this short-term GnRH antagonist treatment can cause the dominant follicle to undergo atresia and the LH surge of that cycle is eliminated. In others, the LH surge is delayed until after the antagonist treatment has stopped and oestradiol secretion has resumed. It is not known whether treatment outcome is related to individual differences in clearance of the antagonist, or to the status of the pituitary and dominant follicle, or which factors influence how long the pituitary–ovarian axis can be put ‘on hold’.

Additional experiments provide further support for a positive role for GnRH throughout the LH surge in women. When GnRH antagonist is administered during the late follicular phase, concomitant administration of oestradiol does not result in an LH surge (Dubourdieu et al., 1994). It is assumed that under these circumstances the oestrogen stimulates an endogenous GnRH surge but pituitary LH is not released because of antagonist blockade of the GnRH receptor. The GnRH antagonist fails to block the LH surge when administered together with pulsatile GnRH in a dose which is sufficiently high to override receptor blockade (Dubourdieu et al., 1994). These results show that the inhibition of the LH surge by GnRH antagonist is the result of inhibition of GnRH action at the receptor level.

Thus, it seems that, although evidence for a rise in GnRH secretion during the LH surge in women has not been forthcoming, GnRH is nevertheless essential for the surge to occur. In the macaque, it is known that a GnRH surge occurs but inhibition of the consequent LH surge is more difficult to achieve than in women. This may be because of greater difficulty in suppressing both development of the dominant follicle and the increasing concentrations of GnRH. A partial LH surge may therefore occur as a result of enhanced pituitary sensitivity.

Irrespective of whether or not the LH surge was inhibited or whether GnRH antagonist treatment was not initiated until after the surge had commenced, there was no functional corpus luteum during the treatment cycle. This strongly suggests that the LH surge is more difficult to prevent than the GnRH mediated drive to the early corpus luteum in the macaque.

Although detailed measurements of serum LH concentrations throughout the luteal phase of the treatment cycle were not performed in the present study, we have previously demonstrated the susceptibility of the early corpus luteum to LH withdrawal by GnRH antagonist treatment during all stages of the luteal phase in the stumptailed macaque (Fraser et al., 1986), findings which have been confirmed in the human (Dubourdieu et al., 1991). In our previous study (Fraser et al., 1991), Antide was detectable in the blood for many weeks, even though pituitary–ovarian function was again becoming active. This suggests that the best indicator of the biological effectiveness of the compound is the suppression of FSH and oestradiol, which we have demonstrated clearly in the present study. From our current demonstration of suppression of serum concentrations of FSH and oestradiol, it is reasonable to assume that corpus luteum function was abolished as a result of long action of the antagonist via suppression of pituitary gonadotrophin secretion.

Reduction in luteal function could also be a direct consequence of an attenuated LH surge (Chandrasekher et al., 1994), although our findings of near normal luteal function after attenuation of the LH surge following Antide administration do not support this (Fraser et al., 1991). We cannot exclude the possibility of a direct action of the GnRH antagonist on the primate ovary, although we doubt the significance of any such effect (Fraser et al., 1996). Clinically, our results indicate that a long-acting or orally active GnRH antagonist preparation may function as a method of post-coital fertility control. On the other hand, the use of long-acting preparations should be avoided when antagonists are employed for controlling the timing of the LH surge in infertility treatment to prevent deleterious effects upon luteal function.

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References


Fraser, H.M. (1990) Inhibitory effects of treatment with an LHRH antagonist on the ovulatory cycle are reduced when administered during the late follicular phase. *Contraception*, 41, 73–83.


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