Direct effect of prolactin, induced by TRH injection, on ovarian oestradiol secretion in the ewe

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Summary. The effect of sustained high plasma levels of prolactin, induced by repeated 2-h i.v. injections of thyrotrophin-releasing hormone (TRH; 20 µg), on ovarian oestradiol secretion and plasma levels of LH and FSH was investigated during the preovulatory period in the ewe. Plasma levels of progesterone declined at the same rate after prostaglandin-induced luteal regression in control and TRH-treated ewes. However, TRH treatment resulted in a significant increase in plasma levels of LH and FSH compared to controls from 12 h after luteal regression until 5 to 6 h before the start of the preovulatory surge of LH. In spite of this, and a similar increase in pulse frequency of LH in control and TRH-treated ewes, ovarian oestradiol secretion was significantly suppressed in TRH-treated ewes compared to that in control ewes. The preovulatory surge of LH and FSH, the second FSH peak and subsequent luteal function in terms of plasma levels of progesterone were not significantly different between control and TRH-treated ewes.

These results show that TRH treatment, presumably by maintaining elevated plasma levels of prolactin, results in suppression of oestradiol secretion by a direct effect on the ovary in the ewe.

Introduction

The role of prolactin in the control of ovarian follicle growth and development in the sheep remains unknown (see McNeilly, Glasier, Jonassen & Howie, 1982b). During the oestrous cycle plasma levels of prolactin show no significant trend until an increase coincident with oestrus and ovulation. This rise is due to both a stimulatory effect of increasing plasma levels of oestradiol in association with the first stages of follicle maturation and also a decrease in hypothalamic inhibition of prolactin secretion apparently necessary to allow the increase in luteinizing hormone releasing hormone (LH-RH) release associated with the preovulatory surge of luteinizing hormone (LH) (see McNeilly, 1980; Baird & McNeilly, 1981). During seasonal anoestrus plasma concentrations of prolactin are elevated (Walton, McNeilly, McNeilly & Cunningham, 1977). It has been suggested that this increase in prolactin may play a role in the suppression of follicle growth associated with decreased gonadotrophin secretion in anoestrus (Walton *et al.*, 1977; McNeilly *et al.*, 1982b). Some support for this hypothesis is provided by the fact that the progesterone concentration is lower during the first 15 days of pregnancy of ewes mated in March, at a time of increased prolactin levels, than in ewes mated in December (Rhind, Chesworth & Robinson, 1978).

To investigate the possible effect of high levels of prolactin in follicular steroidogenesis we have measured the changes in plasma levels of oestradiol and progesterone, LH and follicle-stimulating

hormone (FSH) during the preovulatory period in ewes in which hyperprolactinaemia was induced by repeated injections of thyrotrophin-releasing hormone (TRH).

Materials and Methods

Animals

Six Tasmanian Merino (M) and two Finnish-Landrace $\times$ Merino (F $\times$ M) ewes with ovarian transplants (Goding, McCracken & Baird, 1967) and two Finnish Landrace $\times$ Merino ewes with uterovarian transplants (Harrison, Heap & Linzell, 1968) were studied during the middle of the breeding season (January). Ewes were housed under natural lighting conditions in a covered pen at Dryden Field Station (Animal Breeding Research Organisation) Roslin, Midlothian, Scotland. The left ovary alone, or together with the oviduct, horn, and body of the uterus and cervix had been autotransplanted to the neck at least 2 years previously (Baird, Land, Scaramuzzi & Wheeler, 1976). Because of the absence of cyclic ovarian function in the ewes with ovarian transplants, premature luteal regression was induced in all animals by i.m. injection (100 μ g) of cloprostenol, a potent analogue of prostaglandin F-2 α (Estrumate: ICI, Cheshire, U.K.). Luteal regression and oestrus occurred within 72 h of this treatment (Baird & Scaramuzzi, 1975; Baird, Swanston & McNeilly, 1981).

Experimental design

On Day 9 of the oestrous cycle after cloprostenol injection both jugular veins in all 10 ewes were cannulated as described previously (Collett, Land & Baird, 1973; McNeilly, O'Connell & Baird, 1982a). On the side of the utero-ovarian or ovarian anastomosis, a Silastic cannula (i.d. 2.0 mm; o.d. 3.2 mm; Dow-Corning Corp., Midland, Michigan, U.S.A.) was advanced so that the tip lay near the site of the anastomosis of the utero-ovarian or ovarian and jugular vein. Animals were then kept in metabolism crates in a heated room (about 15°C) until the end of the experiment.

On the morning of Day 10, 5-ml samples of jugular venous blood were collected every hour for 6 h before to 60 h after a second i.m. injection of 100 μ g cloprostenol. From 60 h to 90 h samples were collected every 2 h. Samples (10 ml) of ovarian venous blood were collected at the same times. Samples of both jugular and ovarian venous blood were collected at 10-min intervals for two periods of 4 h during the luteal phase (−4 to 0 h) and follicular phase (24 to 28 h after cloprostenol injection). During these periods of frequent bleeding timed samples of ovarian blood were collected every hour and the plasma flow was then calculated from the haematocrit reading to allow subsequent calculation of the steroid secretion rate (Baird *et al.*, 1981). Plasma was separated by centrifugation and stored at −20°C until analysis.

From 36 h after injection of cloprostenol the ewes were tested for response to a male every 4 h to detect oestrus and continued until two successive tests were negative. The ewes were removed from the metabolism crates and observed in a pen with a vasectomized ram for 5 min.

Five of the 10 ewes (3 M and 1 F $\times$ M ovarian, 1 F $\times$ M utero-ovarian transplants) chosen at random were injected i.v. with 20 μ g TRH (Hoechst, Frankfurt, West Germany) every 2 h after cloprostenol injection for 60 h. This dose of TRH has been shown to maintain hyperprolactinaemia in ewes (Kann & Denamur, 1974).

Hormone assays

Prolactin, LH and FSH were measured in duplicate in specific double-antibody radioimmunoassays exactly as described in detail previously (McNeilly & Andrews, 1974; McNeilly, McNeilly, Walton & Cunningham, 1976; Martensz, Baird, Scaramuzzi & Van Look, 1976). The sensitivities of the assays were 0.5 ng prolactin (NIH-P-S6)/ml, 0.1 ng LH (NIH-LH-S14)/ml and 8 ng FSH

(NIH-FSH-S10)/ml. The intra- and inter-assay variations as coefficients of variation (%) were 7.7 and 10.4%, 8.9 and 11.3% and 8.4 and 12.1% for prolactin, FSH and LH respectively.

The oestradiol and progesterone concentrations in plasma were measured by specific radioimmunoassays as described previously (see Baird *et al.*, 1981). Intra- and inter-assay coefficients of variation (%) were 6.0 and 9.5% and 9.8 and 13.0% for oestradiol and progesterone respectively.

Statistical analysis

The differences in hormone levels between groups were analysed in 6-h blocks by 2-way analysis of variance with repeated measures after log transformation. A rise in LH was considered to be a pulse if the value of two consecutive samples was greater than the mean of the two previous samples (basal value) and the value of at least one of the peak samples exceeded the mean basal value by more than twice the coefficient of variation of the assay (Backström, McNeilly, Leask & Baird, 1982). Pulse frequency was analysed by χ^2 test and differences between groups by unpaired Student's *t* test.

Results

Oestrous behaviour and ovulation

All the ewes showed oestrus between 43 and 56 h after the injection of cloprostenol and TRH treatment did not affect this (mean $\pm$ s.e.m. 47.6 ± 2.5 h for controls, 50 ± 1.3 h for TRH-treated ewes). While the duration of oestrus was similar in the F $\times$ M ewes in both groups (control: 38 and 40 h; TRH-treated: 36 and 40 h) it was considerably shorter in Merino ewes after TRH treatment (control: 20, 25 and 32 h; TRH-treated: 12, 12 and 12 h). All ewes appeared to ovulate, as judged by a single estimation of plasma progesterone concentration between Days 9 and 11 after oestrus and there was no significant difference in the progesterone values between control (4.9 ± 0.4 ng/ml) and TRH-treated (4.7 ± 0.5 ng/ml) animals.

Plasma levels of prolactin, gonadotrophins and steroids

For presentation and analysis of the changes in plasma levels of these hormones results have been centred around both the injection of cloprostenol and the peak plasma level of LH at the preovulatory surge.

Prolactin. The injection of 20 μ g TRH at the time of cloprostenol injection caused an immediate highly significant ($P < 0.001$) increase in plasma levels of prolactin compared to those in control animals (Text-fig. 1). The repeated TRH injections resulted in a sustained (2- to 10-fold) increase throughout the treatment period, with the prolactin increasing to a similar extent after each TRH injection (Text-fig. 2). In control animals prolactin levels increased at the time of the LH surge and thereafter decreased to values similar to those before cloprostenol injection.

Progesterone. Plasma levels of progesterone declined rapidly within 6 h of the injection of cloprostenol and plasma levels fell to < 1 ng/ml within 24 h in all animals. There was no significant difference in response between control and TRH-treated ewes (Text-fig. 3).

LH. By 6 h after cloprostenol injection plasma levels of LH increased significantly ($P < 0.01$) in the control (0.7 ± 0.4 to 1.6 ± 0.4 ng/ml) and TRH-treated (1.4 ± 0.4 to 3.2 ± 1.2 ng/ml) animals. While LH concentrations before cloprostenol injection were not significantly different, the values in TRH-treated ewes from 6 to 24 h after injection were greater than in controls ($P < 0.01$, Text-fig. 1a). Plasma levels of LH remained significantly ($P < 0.01$) higher in TRH-treated than control ewes between 36 and 6 h before the LH peak (Text-fig. 1a). The peak preovulatory surge occurred at similar times after cloprostenol injection in both groups (control: 61 ± 4 h, range 52–68

Text-fig. 2. Concentration of prolactin in jugular venous plasma of ewes 24–28 h after induction of luteal regression by injection of 100 µg cloprostenol on Day 10 of the cycle and treated with 20 µg TRH every 2-h (●—●). ○, Controls. Each point represents the mean $\pm$ s.e.m. of 5 observations.

h; TRH-treated: 55 ± 4 h, range 41–65 h). Peak levels (controls: 258 ± 79 ; TRH-treated: 206 ± 49 ng/ml) and cumulative release of LH during the surge (controls: 551 ± 62 ; TRH-treated: 462 ± 83 ng/ml, –6 to +6 h around peak) were not significantly different.

FSH. After the injection of cloprostenol, plasma levels of FSH increased slightly but then declined in the control and TRH-treated ewes (Text-fig. 1b). By 24 h after cloprostenol, levels were significantly ($P < 0.05$; paired t test) lower than preinjection levels in control ewes (104 ± 2.3 and 68 ± 13 ng/ml) but not in TRH-treated ewes (110 ± 17 and 98 ± 15 ng/ml).

Between 36 and 6 h before the LH surge, FSH levels remained suppressed in control ewes. However, during this time the FSH values in TRH-treated ewes were significantly ($P < 0.001$) higher than controls although a significant ($P < 0.05$) decline did occur before the start of the LH surge.

Coincidental to the LH surge, there was a 4-fold increase in FSH in both groups, giving similar peak levels (control: 248 ± 42 ng/ml; TRH-treated: 306 ± 37 ng/ml; Text-fig. 1c). FSH levels then returned to basal values (control: 56 ± 18 ng/ml; TRH-treated: 82 ± 17 ng/ml) between 8 and 10-h and subsequently rose again to peak values (control: 218 ± 70 ng/ml; TRH-treated: 237 ± 31 ng/ml) 16 to 33 h after the LH peak. There was no significant difference between control and TRH-treated ewes in these changes in FSH around the LH surge.

Oestradiol. Between 6 and 12 h after cloprostenol injection there was a rise in the secretion of oestradiol which was similar in control and TRH-treated ewes (Text-fig. 1c). This increase continued until levels reached a peak between 44 and 66 h after cloprostenol injection. However, oestradiol values were significantly ($P < 0.05$) lower between –24 and +12 h around the LH surge

Text-fig. 1. Concentration of FSH, LH and prolactin in jugular venous plasma and oestradiol in ovarian venous plasma of ewes after injection of 100 µg cloprostenol on Day 10 of the cycle and treated with 20 µg TRH every 2 h. The results have been grouped around the time of the cloprostenol injection (PG) or the LH peak. Each point represents the mean $\pm$ s.e.m. of 5 observations (4 for oestradiol).

Text-fig. 3. The concentration of progesterone in jugular venous plasma of ewes after injection of 100 µg cloprostenol on Day 10 of the cycle. Each point represents the mean $\pm$ s.e.m. of 5 observations.

in TRH-treated than control ewes (Text-fig. 1c). Peak values of oestradiol occurred significantly ($P < 0.001$) earlier relative to the LH surge in TRH-treated than control ewes (TRH-treated: -32 , -20 , -20 and -12 h; control: -18 , -10 , -8 and -4 h). By 12 h after the LH peak in both groups, oestradiol declined to values lower than at any stage of the preovulatory phase (Text-fig. 1c).

Pulsatile release of LH and oestradiol

The change in pulsatile secretion of LH and oestradiol and the associated changes in FSH during the luteal phase and 24–28 h after cloprostenol injection were assessed from results of 4-h periods of intensive bleeding at each stage. The number of LH pulses in the 20 h of observation rose from 8 in the luteal phase to 18 and 20 in the control and TRH-treated ewes. Pulse amplitude did not alter during this time (luteal phase: 0.8 ± 0.3 ; follicular phase, control: 0.9 ± 0.3 ng/ml; TRH-treated 0.9 ± 0.2 ng/ml). However, basal levels of LH were significantly ($P < 0.05$) higher in the TRH-treated ewes (Text-fig. 4). All LH pulses were accompanied by an increase in oestradiol secretion. However, while in control animals there was a marked increase in both basal and peak secretion of oestradiol in the follicular than in the luteal phase, this was drastically reduced in the TRH-treated ewes (Text-fig. 4). FSH levels in the control and TRH-treated ewes were significantly lower ($P < 0.01$) in the follicular than luteal phase, but those in the follicular phase were significantly ($P < 0.001$) lower in control than TRH-treated ewes.

Text-fig. 4. Pulsatile secretion of LH, FSH and oestradiol in ewes on Day 10 (luteal phase) and 24–28 h after injection of cloprostenol (follicular phase). The results have been grouped round the LH peak. Each point represents the mean $\pm$ s.e.m. of 5 observations.

Discussion

The present study demonstrated that a sustained increase in plasma levels of prolactin in the preovulatory phase of the oestrous cycle in the ewe is associated with a significant reduction in ovarian secretion of oestradiol. Since plasma levels of both LH and FSH were higher than in control ewes, the results imply that the high levels of prolactin reduced oestradiol secretion by a direct action at the ovarian level.

We have confirmed that repeated injections of TRH will maintain an increase in prolactin concentrations over control values (Kann, Martinet & Schirar, 1977) and responsiveness is maintained through the 60-h injection period. However, in spite of this increase in prolactin concentration, injection of prostaglandin resulted in a marked decline in plasma levels of progesterone and functional luteolysis at the same rate as in untreated ewes. This contrasts with results for the rat in which prolactin appears to protect the corpus luteum from prostaglandin-induced luteolysis (Behrman, Greenwich, Hichens & MacDonald, 1978). However, this difference may only be due to the use of a more active analogue of prostaglandin in the present experiment rather than the PGF-2 α used in the rat studies.

Throughout most of the preovulatory period, basal levels of LH were higher in TRH-treated than control ewes. It was also clear that the increase in pulse frequency of LH associated with this period was unaffected. Nevertheless, the greater increase in basal level of LH in the TRH-treated ewes only resulted in inducing oestradiol secretion at one third that of normal throughout the preovulatory phase. Indeed, since the peak values of oestradiol occurred significantly earlier in TRH-treated than control ewes it was also clear that the follicle(s) producing the oestradiol could not respond with the normal large increase in oestradiol secretion that occurs in response to the first increase in basal LH at the start of the preovulatory surge of LH (Baird *et al.*, 1981). These results

clearly imply a reduction in responsiveness of the ovary to LH. The fact that plasma levels of FSH were also higher in TRH-treated than control ewes throughout the preovulatory phase was presumably a result of the reduction in negative feedback due to the reduced levels of oestradiol, and perhaps other ovarian factor(s) such as inhibin, during the preovulatory phase (see Baird & McNeilly, 1981).

These results indicate that the reduction in secretion of oestradiol is not a consequence of failure of normal gonadotrophin secretion but imply a suppression of responsiveness to gonadotrophin stimulation at the ovarian level in the TRH ewes. Clearly, the raised plasma levels of prolactin could be responsible for this suppression.

Normal secretion of oestradiol requires both the stimulation by LH of androgen production, principally androstenedione and testosterone, from the theca and its conversion to oestradiol by an active aromatase system induced by FSH and possibly LH in the granulosa cell layer of the follicle (Moor, 1977; Armstrong, Weiss, Selstam & Seamark, 1981; Baird & McNeilly, 1981; McNatty, Gibb, Dobson, Thurley & Findlay, 1981). Injection *in vivo* of large amounts of prolactin during pro-oestrus in the rat inhibits the production of oestrogen by follicles incubated *in vitro* although the site of this inhibition is not known (Uilenbroek, van der Schoot, den Besten & Lankhorst, 1982). However, high levels of prolactin can block both the induction by FSH of aromatase in rat granulosa cells *in vitro* (Dorrington & Gore-Langton, 1981) and LH-induced androgen production from ovarian thecal and interstitial cells *in vitro* (Magoffin & Erickson, 1982). It is not clear from our present results which, if either, of these two possible points of steroid inhibition are responsible for the suppression of oestradiol secretion, although preliminary observations suggest that thecal androgen production may be reduced (McNeilly *et al.*, 1982b). It is also possible that prolactin may interfere with the induction and maintenance of LH receptors on the theca and LH and FSH receptors on granulosa cells of the developing preovulatory follicle. These receptors appear and increase in number during the final stages of follicle maturation (Carson, Findlay, Burger & Trounson, 1979; Webb & England, 1982). However, this seems unlikely as most studies of the rat indicate that prolactin is part of the hormonal complex necessary for the induction and maintenance of LH receptors in the follicle and corpus luteum (Richards & Williams, 1976; Holt, Richards, Midgley & Reichert, 1976).

In spite of the reduced levels of oestradiol, the preovulatory surges of LH and FSH and the second peak of FSH occurred normally in the TRH-treated ewes. Clearly, therefore, sufficient oestradiol had been produced to elicit the normal positive feedback response. However, the duration of oestrus, another biological indicator of preovulatory oestrogen secretion, was substantially shorter in the TRH-treated than in the control Merino ewes. Such an effect was not seen in the Finn-Merino ewes, perhaps because of a different sensitivity to oestrogen (Land, Thompson & Baird, 1972). Nevertheless, luteal function, in terms of plasma levels of progesterone, were similar in the control and TRH-treated ewes, suggesting that sufficient oestradiol was secreted in the preovulatory phase in TRH-treated ewes to allow apparently normal follicular maturation. However, such alterations in the steroid milieu of the follicle might affect the maturation of the oocyte. It has been suggested that the intrafollicular hormonal environment is important (McNatty *et al.*, 1981) and a role for prolactin has been suggested in oocyte maturation (Baker & Hunter, 1978; Nolin, 1980) and in the production by the follicle of oocyte maturation inhibitory factor (Channing & Evans, 1982). High levels of prolactin have been implicated in reduced progesterone levels and fertility in sheep mated towards the end of the breeding season (Rhind *et al.*, 1978).

The present study provides direct evidence that treatment of sheep with TRH results in a suppression of LH and FSH-induced follicular oestradiol secretion in the preovulatory phase by a direct action on the ovary. This action is presumed to be through the induction of sustained high levels of prolactin but repeated injections of TRH may also result in an increase in thyroid-stimulating hormone (TSH) and the thyroid hormones triiodothyronine and thyroxine (Fraser & McNeilly, 1982) and this alteration in thyroid status might also be implicated.

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