Effects of oestradiol on LH, FSH and prolactin in ovariectomized ewes*

T. G. Rozell and D. H. Keisler

Animal Sciences Department, University of Missouri, Columbia, Missouri 65211, USA

Summary. This study was conducted to test the hypothesis that the rate (dose/time) at which oestradiol-17 β (oestradiol) is presented to the hypothalamo-pituitary axis influences secretion of LH, FSH and prolactin. A computer-controlled infusion system was used to produce linearly increasing serum concentrations of oestradiol in ovariectomized ewes over a period of 60 h. Serum samples were collected from ewes every 2 h from 8 h before to 92 h after start of infusion, and assayed for oestradiol, LH, FSH and prolactin. Rates of oestradiol increase were categorized into high (0.61–1.78 pg/h), medium (0.13–0.60 pg/h) and low (0.01–0.12 pg/h). Ewes receiving high rates of oestradiol ($N = 11$) responded with a surge of LH 12.7 ± 2.0 h after oestradiol began to increase, whereas ewes receiving medium ($N = 15$) and low ($N = 11$) rates of oestradiol responded with a surge of LH at 19.4 ± 1.7 and 30.9 ± 2.0 h, respectively. None of the surges of LH was accompanied by a surge of FSH. Serum concentrations of FSH decreased and prolactin increased in ewes receiving high and medium rates of oestradiol, when compared to saline-infused ewes ($N = 8$; $P < 0.05$). We conclude that rate of increase in serum concentrations of oestradiol controls the time of the surge of LH and secretion of prolactin and FSH in ovariectomized ewes. We also suggest that the mechanism by which oestradiol induces a surge of LH may be different from the mechanism by which oestradiol induces a surge of FSH.

Keywords: oestradiol; LH; FSH; prolactin; ovariectomized ewes

Introduction

Serum concentrations of oestradiol increase before preovulatory surges of LH and FSH (Baird *et al.*, 1981; Goodman *et al.*, 1981; Karsch *et al.*, 1984; Keisler *et al.*, 1985). Furthermore, oestradiol is reported to stimulate secretion of prolactin in ewes (Baird *et al.*, 1981; Elsasser *et al.*, 1983). To understand how the preovulatory increase in serum concentrations of oestradiol regulates secretion of gonadotrophins in ewes, investigators have utilized ovariectomized ewes treated with injections of oestradiol (Goding *et al.*, 1969; Elsasser *et al.*, 1983; Haresign & Friman, 1983; Nett *et al.*, 1984; Clarke *et al.*, 1988), constant infusions of oestradiol (Goding *et al.*, 1969; Emons *et al.*, 1984) or implants of oestradiol (Karsch *et al.*, 1980; Foster, 1984; Keisler *et al.*, 1985). Treatment of ewes with oestradiol by these methods, however, produces patterns of serum concentrations of oestradiol which are non-linear, and therefore dissimilar to those which occur before ovulation in the ewe. By fitting regression equations to the increase in serum concentrations of oestradiol which occur during the follicular phase of cyclic ewes (Karsch *et al.*, 1984) and in lambs induced to ovulate by repeated injections of LH (Keisler *et al.*, 1985), we determined that preovulatory serum concentrations of oestradiol increase in a linear fashion at a rate of approximately 0.08 pg/h.

*Reprint requests to Dr D. H. Keisler, 160 Animal Science Research Center, University of Missouri, Columbia, MO 65211, USA.

Our objectives in the present study were to produce different rates of linearly increasing serum concentrations of oestradiol in ovariectomized ewes to: (1) determine whether the gonadotrophin surge mechanism is activated by a threshold dose of oestradiol, duration of exposure to oestradiol, or the interaction of dose and duration (i.e. rate) of oestradiol; and (2) examine the pattern of gonadotrophin secretion in response to different rates of increasing serum concentrations of oestradiol. We tested the hypothesis that the rate (dose/time) at which oestradiol is presented to the hypothalamo-pituitary axis influences the time and magnitude of the surges of LH and FSH, and the secretion of prolactin.

Materials and Methods

Twelve mature, chronically (≥ 3 months) ovariectomized and hysterectomized ewes of mixed breeding were selected for study. Ewes were randomly assigned before each trial to receive either an intravenous infusion of saline (0.9% (w/v) NaCl in sterile distilled water; pH 7.0) or a saline solution containing oestradiol. Five trials were conducted between June and December 1988, with 3 or more weeks between each trial. Two days before each trial, ewes were relocated from pasture to indoor 2 m $\times$ 2 m pens and housed for 7 days. While indoors, ewes were fed 4 kg of a mixed grain ration 3 times per day and fresh water was available at all times. Ewes were tied to stanchions and fitted with indwelling jugular cannulae 24 h before infusion. Cannulae were placed caudally in each jugular vein, one for infusion and the other for blood sampling. During each trial, ewes were infused with saline or oestradiol delivered in 100 μ l drops by a computer-controlled, gravity-flow infusion system programmed to produce linearly increasing serum concentrations of oestradiol in each ewe over a period of 60 h. This was accomplished by increasing mass (volume) of oestradiol delivered over time.

Solutions of oestradiol to be infused were made by diluting a concentrated solution of oestradiol (150 μ g/ml in ethanol) in 3 litres of saline. Solutions of oestradiol (Sigma Chemical Co., St Louis, MO, USA) varied in concentration from a maximum of 720 ng/ml to a minimum of 10 ng/ml. A total of 2 litres of saline or oestradiol in saline were delivered to each animal during the 60-h infusion period.

Blood samples (10 ml) were collected at 2-h intervals beginning 8 h before infusion and ending 32 h after infusion. Samples were allowed to clot at room temperature for 4 h before centrifugation. Serum was decanted and stored at -20°C until assayed. Serum concentrations of oestradiol, LH, FSH and prolactin were determined by radioimmunoassays.

Assays. Serum concentrations of oestradiol were determined in duplicate 500 μ l samples. Sample and standard curve tubes were twice extracted with anhydrous diethyl ether (4.0 ml/tube). Efficiency of extraction of oestradiol from samples averaged 92%. A standard solution of oestradiol in methanol was pipetted in triplicate to create a standard curve with the following points: 0.25, 0.50, 1.0, 2.5, 5.0, 10.0 and 20.0 pg/tube. The methanol containing the oestradiol standard was evaporated and 100 μ l of a 1% BSA solution in PBS were then added to each standard curve and sample tube. Oestradiol antiserum (100 μ l of a 1:85 500 dilution of antiserum; Butcher *et al.*, 1974) was added to each tube except total and non-specific binding tubes. Tubes were incubated at 37°C for 5 min, then at 4°C for 1 h, after which 100 μ l 3-iodo-oestradiol-17 β (4.0 pg/tube; ICN Biomedicals, Inc., Carson, CA, USA) were added to each tube. Tubes were then incubated for an additional 15 h at 4°C , and then 800 μ l of a charcoal-dextran suspension in double-distilled water were added to each tube except total tubes. Tubes were vortexed and incubated for 10 min at 4°C , then centrifuged at 4°C and 3000 g for 10 min. The supernatant was decanted into a separate set of tubes and each was counted for 1 min in a gamma counter. The intra- and inter-assay coefficients of variation for the oestradiol assay were 9.71% (N = 8) and 12.8% (N = 8), respectively. Minimum detectable concentrations of oestradiol were 0.37 pg/tube.

Serum concentrations of LH were assayed in duplicate 100 μ l samples by the methods described by Fogwell *et al.* (1977), utilizing RAoLH TEA #35 ovine LH antiserum (Adams *et al.*, 1975). The inter- and intra-assay coefficients of variation were 3.2% (N = 5) and 12.6% (N = 9), respectively, with a minimum detectable concentration of 50 pg/tube.

Serum concentrations of FSH were determined in duplicate 25- μ l samples as described by Keisler *et al.* (1983) utilizing materials and methods provided by NIDDKD. Inter- and intra-assay coefficients of variation were 12.0% (N = 9) and 19.0% (N = 9), respectively, and minimum detectable concentrations of FSH were 60 pg/tube.

Serum concentrations of prolactin were determined in duplicate 10- μ l samples as described by Keisler *et al.* (1983), utilizing materials and methods provided by NIDDKD. Inter- and intra-assay coefficients of variation were 15.2% (N = 9) and 17.4% (N = 9), respectively, and minimum detectable concentrations of prolactin were 80 pg/tube.

Statistical analysis. The rate at which serum concentrations of oestradiol increased in each ewe was determined by fitting a regression line to assayed serum concentrations of oestradiol determined for each ewe during the 60-h infusion period. For purposes of analysis, the rates of oestradiol increase were categorized into 3 groups: 0.01–0.12 pg/h, 0.13–0.60 pg/h or 0.61–1.78 pg/h. Differences in time of onset and magnitude of the surge of LH among the 3 groups were tested using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS, 1985) and, when appropriate, by Duncan's New Multiple Range Test (Steel & Torrie, 1980). The relationship between

dose and duration of oestradiol exposure at which each ewe responded with a surge of LH was determined by fitting a non-linear, non-polynomial regression line (NLIN procedure; SAS, 1985) to values obtained from each ewe. Differences in the profile of serum concentrations of FSH and prolactin among the 3 groups of rates were determined by analysis of variance using a split-plot design, with time and the interaction of time and rate of oestradiol increase in the subplot (Gill & Hafs, 1971). The correlation between the pattern of serum concentrations of oestradiol and the pattern of FSH and prolactin in each ewe was determined by using the regression (REGRESSION) procedure of SAS (1985).

Results

Oestradiol infusion

Linearly increasing serum concentrations of oestradiol were established and maintained over a 60-h infusion period in 37 of 52 attempts. Samples collected during the 15 attempts in which linear infusions failed to be maintained for 60 h were not assayed for gonadotrophins. The rate at which oestradiol increased during successful oestradiol infusions ranged from 0.01 to 1.78 pg/h and all patterns of serum concentrations of oestradiol were different from those in saline-treated ewes ($N = 8$; $P < 0.05$). No differences were found in serum concentrations of oestradiol as the result of infusions of oestradiol at different times during the year (June to December).

Effects on the surge of LH

Ewes which received oestradiol at high rates ($N = 11$) responded with a surge of LH 12.7 ± 2.0 h after oestradiol began to increase (Fig. 1). In contrast, when serum concentrations of oestradiol increased at medium ($N = 15$) and low ($N = 11$) rates, the interval to the onset of the surge of LH increased to 19.5 ± 1.7 and 30.9 ± 2.0 h, respectively (Fig. 1). The dose and duration of oestradiol at which each of the 37 ewes responded with a surge of LH are illustrated in Fig. 2. A non-linear, non-polynomial regression line represented by the equation: dose increase in oestradiol in pg/ml = $29.45[1/(\text{hour of infusion at which the surge of LH occurred} - 7)^{0.506}]$ (solid line; Fig. 2) illustrates the relationship between dose and duration of oestradiol at which each ewe responded with a surge of LH. Magnitude of the surge of LH was 22.6 ± 4.1 , 33.1 ± 3.5 and 33.5 ± 4.1 ng/ml for ewes receiving high, medium and low rates of increasing concentrations of oestradiol, respectively, and were not different ($P > 0.05$). Ewes receiving saline ($N = 8$) did not respond with a surge of LH. Patterns of serum concentrations of LH produced during the negative feedback effects of oestradiol, and initial serum concentrations of LH did not differ ($P > 0.05$) among the 5 trials.

Effects on serum concentrations of FSH

The patterns of serum concentrations of FSH and oestradiol in each ewe were negatively correlated ($R = -0.58$; $P < 0.05$). Serum concentrations of FSH decreased over time ($P < 0.05$; Fig. 3) in ewes receiving high and medium, but not low, rates of oestradiol, compared to saline-infused ewes. Serum concentrations of FSH were not different ($P > 0.05$) in ewes receiving high and medium rates of oestradiol. The time at which concentrations of FSH were maximally decreased was not different ($P > 0.05$) between ewes receiving high and medium rates of oestradiol. Initial serum concentrations of FSH throughout the infusion period did not differ ($P > 0.05$) among the 5 trials.

Effects on serum concentrations of prolactin

The patterns of serum concentrations of prolactin and oestradiol were positively correlated ($R = 0.77$; $P < 0.05$; Fig. 4). Serum concentrations of prolactin increased ($P < 0.05$) in ewes

Fig. 1. Relationship between serum concentrations of oestradiol and onset of the surge of LH in ewes receiving (a) high ($N = 11$), (b) medium ($N = 15$) and (c) low ($N = 11$) rates of increasing concentrations of oestradiol. Hour 0 represents the point at which infusions of oestradiol were initiated. Standard error bars represent least squares means standard error in each of the three treatment groups, for oestradiol (E; vertical bars) and for time of the surge of LH (horizontal bars).

receiving high and medium rates of oestradiol, but concentrations of prolactin in ewes which received low rates of oestradiol were not different ($P > 0.05$) from those in saline-infused ewes. Maximum serum concentrations of prolactin and the time at which maximum serum concentrations of prolactin was obtained did not differ ($P > 0.05$) among ewes receiving high and medium rates of oestradiol. Initial serum concentrations of prolactin did not differ ($P > 0.05$) among the 5 trials.

Fig. 2. Relationship between the dose and duration of exposure to increasing concentrations of oestradiol at which each ewe responded with a surge of LH (open circles) as represented by the non-linear, non-polynomial regression line described by the equation: dose increase in oestradiol in pg/ml = $29.45[1/(\text{hour of infusion at which the surge of LH occurred} - 7)^{0.506}]$.

Discussion

The relationship between dose of oestradiol and duration of oestradiol exposure (the interaction of which is rate) at which a surge of LH occurred in ovariectomized ewes was studied. Ewes receiving high rates of oestradiol had a surge of LH an average of 12 ± 3.0 h after oestradiol began to increase, and this 12-h period was not dependent on the dose of oestradiol at the time of the surge of LH. Other investigators have found an interval of about 12 h between oestradiol treatment and the surge of LH in response to injection (Goding *et al.*, 1969; Elsasser *et al.*, 1983; Haresign & Friman, 1983; Nett *et al.*, 1984), constant infusion (Goding *et al.*, 1969; Emons *et al.*, 1984) or implantation of oestradiol (Foster, 1984; Keisler *et al.*, 1985). This minimum amount of time is most probably required for protein synthetic events which are stimulated by oestradiol, such as synthesis of GnRH (Zoeller & Young, 1988; Ronnekleiv *et al.*, 1989) and LH (Landefeld & Kepa, 1984; Leung *et al.*, 1988; Marshall *et al.*, 1989), and synthesis and up-regulation of GnRH receptors in the pituitary (Moss *et al.*, 1981; Crowder *et al.*, 1982; Nett *et al.*, 1984; Clarke *et al.*, 1988; Gregg & Nett, 1989). Nett *et al.* (1984), however, reported that a surge of LH occurred after 4 h if oestradiol was injected in the presence of a constant infusion of GnRH. Therefore, much of the 12-h period observed in the present and other studies may be necessary to increase synthesis and/or secretion of GnRH. Regardless of the mechanisms involved, a delay of 12 h after oestradiol exposure may enable the surge of LH to be of sufficient amplitude and duration to cause ovulation and formation of the corpus luteum.

Ewes given low rates of oestradiol in the present study responded with a surge of LH which occurred at a lower dose, but required a longer duration of oestradiol exposure, than did ewes receiving high rates of oestradiol. The dose of oestradiol at which the surge occurred in ewes receiving low rates of oestradiol was 3.25 ± 0.2 pg, which may be the minimum increase in serum concentrations of oestradiol necessary to stimulate a surge of LH. A minimum dose is perhaps necessary to ensure that sufficient oestradiol receptors are bound to initiate a response.

We suggest that our findings support the hypothesis that the minimum dose and minimum duration of oestradiol exposure necessary to induce a surge of LH are influenced primarily by the rate at which oestradiol is presented to the hypothalamic LH surge centre. Although the mechanism by which the rate of oestradiol increase interacts with the surge centre is unknown, its functional significance may be to provide the ewe with a way of compensating for rates of oestradiol increase which are above or below normal.

Fig. 3. Response of FSH to (a) high, (b) medium and (c) low rates of increasing serum concentrations of oestradiol. Hour 0 represents the point at which infusions of oestradiol were initiated. Standard error bars represent least squares means standard error in each of the 3 groups, for oestradiol (E; solid) and for FSH (broken).

In contrast to the positive feedback effects, negative feedback actions of oestradiol on secretion of LH were not responsive to the rate of oestradiol increase. The negative feedback actions of oestradiol are therefore not merely the inverse of its stimulatory actions on mechanisms which initiate a surge of LH. A possible explanation for inhibitory effects of oestradiol on secretion of LH is that oestradiol may directly activate a sequence of events in the pituitary which include decreases in: (1) adenylate cyclase activity (Harden *et al.*, 1980), (2) releasable pituitary stores of LH (Koninckx *et al.*, 1976), (3) number of cells responsive to GnRH (Lloyd & Childs, 1988), and/or (4) plasma membrane potentials. Alternatively, the inhibitory effects of oestradiol may be mediated by an oestradiol-induced release of hypothalamic proteins which may inhibit the secretion of LH (Hwan & Freeman, 1987).

In cyclic ewes, surges of FSH occur concomitantly with surges of LH (Baird *et al.*, 1981). In the present study, however, no surges of FSH occurred in the 37 successful infusions of oestradiol and secretion of FSH was inhibited in a dose-dependent manner by infusion of oestradiol at different rates. Our results provide evidence that oestradiol regulates the secretion of FSH differently from that of LH, and that the preovulatory surge of FSH may be mediated by ovarian factors other than

Fig. 4. Response of prolactin to (a) high, (b) medium and (c) low rates of linearly increasing serum concentrations of oestradiol. Hour 0 represents the point at which infusions of oestradiol were initiated. Standard error bars represent least squares means standard error in each of the 3 groups for oestradiol (E; solid) and for prolactin (broken).

oestradiol. Other investigators have found suppression of FSH concurrent with stimulation of LH in long-term ovariectomized ewes given an implant (Clarke *et al.*, 1989) or injection (Elsasser *et al.*, 1983) of oestradiol. Oestradiol was also found to cause a dose-dependent suppression of FSH in ewes both *in vitro* and *in vivo* (Henderson *et al.*, 1989). However, injection or implantation of oestradiol results in patterns of serum concentrations of oestradiol in ewes which are different from those found in intact ewes before ovulation, and therefore patterns of oestradiol caused by injection or implantation in these studies may have been insufficient to cause the surge of FSH. Serum concentrations of FSH were also observed to decrease as oestradiol increased in cyclic ewes (Baird *et al.*, 1981). These investigators, however, observed that a surge of FSH occurred after oestradiol concentrations had decreased following ovulation. Our findings provide evidence that the pre-ovulatory increase and subsequent decline in oestradiol may not be responsible for the surge of FSH.

The mechanism by which oestradiol inhibits secretion of FSH is unknown, but may be different from the mechanism by which oestradiol inhibits secretion of LH. Secretion of FSH was inhibited in a dose-dependent manner, while inhibition of LH was not dose-dependent in the present study.

Shupnik *et al.* (1988) reported that oestradiol regulates synthesis of FSH differently from that of LH. This may account in part for continued inhibition of FSH by oestradiol, even during a surge of LH which is presumed to be caused by increasing concentrations of GnRH (Clarke & Cummins, 1982; Levine & Duffy, 1988).

In the present study, serum concentrations of prolactin reached maximum levels while concentrations of oestradiol continued to increase in ewes receiving high rates of oestradiol, indicating that prolactin production was maximally stimulated before infusion of oestradiol was stopped. Therefore, secretion of prolactin may be stimulated by increasing concentrations of oestradiol until a maximum level is achieved; then prolactin concentrations decrease regardless of further increases in oestradiol. However, until maximum values of prolactin are reached, increases in oestradiol are needed to stimulate further increases in prolactin. Other investigators have found that increasing concentrations of oestradiol stimulate secretion of prolactin in intact ewes (Baird *et al.*, 1981) and ovariectomized ewes (Elsasser *et al.*, 1983). Conversely, serum concentrations of prolactin did not increase in ewes receiving implants of oestradiol (Deaver & Dailey, 1983), further suggesting that concentrations of oestradiol must increase to stimulate secretion of prolactin in ewes.

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