Effect of stress-like concentrations of cortisol on follicular development and the preovulatory surge of LH in sheep

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Stress-like concentrations of cortisol increase the negative feedback potency of oestradiol in castrated male sheep. A similar cortisol-dependent response in female sheep might be expected to suppress gonadotrophin secretion and impair follicular development and ovulation. The oestrous activity of 21 female sheep was synchronized using progestogen-treated vaginal pessaries to test this hypothesis. Stress-like concentrations of cortisol (60–70 ng ml⁻¹) were established by continuous infusion of cortisol (80 μg kg⁻¹ h⁻¹; n = 13) beginning 5 days before, and continuing for 5 days after, pessary removal. Control animals (n = 8) received a comparable volume of vehicle (50% ethanol–saline) over the 10 day infusion period. Serum concentrations of oestradiol increased progressively in control sheep during the 48 h immediately after pessary removal. This increase in serum oestradiol was blocked or significantly attenuated in sheep receiving stress-like concentrations of cortisol. Preovulatory surge-like secretion of LH was apparent in control animals 58.5 ± 2.1 h after pessary removal. In contrast, surge-like secretion of LH was not observed during the 5 days after pessary removal in 54% (7 of 13) of sheep receiving cortisol. Moreover, the onset of the surge was significantly delayed in the cortisol-treated ewes that showed surge-like secretion of LH during the infusion period. The ability of episodic pulses of exogenous GnRH to override the anti-gonadal effect of cortisol was examined in a second study. Oestrous activity of 12 ewes was synchronized using progestogen-containing pessaries as described above. Ewes were randomly assigned to one of three treatment groups (n = 4 ewes per group). Animals received cortisol (100 μg kg⁻¹ h⁻¹; groups 1 and 2) or a comparable volume of vehicle (group 3) beginning 5 days before, and continuing for 2 days after, pessary removal. Pulses of GnRH (4 ng kg⁻¹ h⁻¹, i.v.; group 1) or saline (groups 2 and 3) at 1 h intervals were initiated at pessary removal and continued for 48 h. Serum concentrations of oestradiol were not significantly increased after pessary removal in sheep receiving cortisol alone. Conversely, serum concentrations of oestradiol increased progressively during the 48 h after pessary removal in control ewes and in ewes receiving cortisol and GnRH. At the end of infusion, serum concentrations of oestradiol did not differ (P > 0.05) between control (7.7 ± 0.8 pg ml⁻¹) ewes and ewes receiving cortisol and episodic GnRH (6.4 ± 1.3 pg ml⁻¹). Moreover, these values were significantly greater (P < 0.05) than the serum concentrations of oestradiol in animals receiving cortisol (1.0 ± 0.4 pg ml⁻¹) alone. Collectively, these data indicate stress-like concentrations of cortisol block or delay follicular development and the preovulatory surge of LH in sheep. In addition, episodic GnRH overrides cortisol-induced delay in follicular maturation.

Introduction

Prolonged stress, such as exposure to temperature extremes (Griffiths et al., 1970), excessive rainfall (Doney et al., 1973), transportation (Ehnert and Moberg, 1991; Smart et al., 1994) or repeated laparoscopy (Martin et al., 1981) delays or suppresses oestrous behaviour and reduces ovulation rate in sheep. Physical and psychological stressors also activate the hypothalamo–pituitary–adrenal axis and lead to marked and persistent increase in serum concentrations of glucocorticoids (Caraty et al., 1990; Guillaume et al., 1992; Komesaroff and Funder, 1994; Minton, 1994). The augmented glucocorticoid secretion induced by stress may contribute to the reduction in fertility commonly associated with stress. Exogenous glucocorticoids suppress follicular growth and development and ovulation in rodents (Smith et
al., 1971; Baldwin and Sawyer, 1974; Baldwin, 1979), primates (Cunningham et al., 1978; Hayashi and Moberg, 1990) and some domestic species (Barb et al., 1982; Stoebel and Moberg, 1982).

In contrast, exogenous glucocorticoids do not suppress surge-like secretion of LH or ovulation in sheep (Moberg et al., 1981; Phillips and Clark, 1990). However, these studies used the synthetic glucocorticoid dexamethasone administered as a bolus once or twice a day. Reproductive function in sheep receiving physiological concentrations of cortisol itself has not been characterized fully. Stress-like concentrations of cortisol increase the negative feedback potency of oestradiol in castrated male sheep (Daley et al., 1999). In this research model, neither cortisol (60–80 ng ml⁻¹) nor oestradiol (2–3 pg ml⁻¹) alone significantly affected the size or pattern of LH secretion, but the combination of cortisol and oestradiol decreased LH pulse frequency and reduced basal concentrations of LH. This response is similar to the augmented feedback potency of oestradiol observed in castrated male (Sakurai et al., 1995) and female (Thomas et al., 1988; Joseph et al., 1992) sheep during the non-breeding season. The enhanced feedback potency of oestradiol during periods of increasing day length is a significant factor contributing to the interruption of follicular development and ovulation that is characteristic of female sheep during the anoestrous season (Goodman et al., 1981). It was postulated that cortisol-induced enhancement of the feedback potency of oestradiol would have a similar effect in female sheep during the normal breeding season.

In the present study, ovarian steroid production and the appearance and duration of the preovulatory surge of LH in sheep receiving stress-like concentrations of cortisol was examined. It was hypothesized that the marked and persistent increase in the serum concentration of cortisol that is characteristic of stress would impair ovarian steroidogenesis and delay the onset of surge-like secretion of LH. In addition, it was postulated that episodic administration of GnRH would reverse the anti-gonadal effect of cortisol.

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Materials and Methods

Animals

The effect of stress-like concentrations of cortisol on follicular development and ovulation was assessed using mature cyclic Rambouillet ewes (mean body weight = 53 ± 2 kg). Ewes were maintained under natural lighting in an open-sided barn with free access to water and alfalfa hay. The studies described were conducted during November and early December, a period of high reproductive activity in sheep at this latitude (38°N). All experimental procedures involving the use of animals were conducted in accordance with National Institutes of Health (NIH) Guidelines and were reviewed and approved by the Animal Use and Care Committee for the University of California.

Cannulation

Intravenous cannulae (Intramedic PE 190, Clay Adams, Parsippany, NJ) were placed in the right and left jugular veins and used for hormone infusion and blood collection, respectively. All cannulae were passed through a protective plastic tubing sheath along a halter and lead rope to the exterior of the animal holding area. Animals were freely mobile at the end of a 1 m lead. The cannulae were inserted 3 days before initiation of treatment to permit acclimation to the conditions of experimentation.

Hormone delivery

Cannulae for the delivery of cortisol or vehicle (50% ethanol–saline) were connected to syringes placed in Harvard infusion pumps (Model 2265, Harvard Bioscience, South Natick, MA). Cortisol (Sigma Chemical Co., St Louis, MO) or a comparable volume of vehicle was delivered by continuous infusion (1.0–1.2 ml h⁻¹). In Exp 2, episodic delivery of GnRH (4 ng kg⁻¹ h⁻¹; Peninsula Labs, Inc., Belmont, CA) in saline was effected by connecting the infusion pump to an automatic timer (Chronimed, Lindburg Enterprises, San Diego, CA) that was activated for 5 min each hour. A volume of 1 ml was delivered during each pulse.

Design of Experiment 1

The oestrous activity of 21 ewes was synchronized using vaginal pessaries (Chrono-gest, Intervet International, Boxmeer) impregnated with a synthetic progestogen (40 mg flugestone acetate). Synchronization of follicular development and ovulation was effected by removal of the first pessary 14 days after insertion. Ten days after removal of the first pessary, ewes received a second pessary that remained in place for an additional 10 days. Use of the two pessary synchronization regimen ensured that the luteal phase preceding the period of experimentation was of uniform duration in all ewes. Ewes were randomly assigned to one of two treatments groups. Animals in groups 1 and 2 received cortisol (80 µg kg⁻¹ h⁻¹; n = 13) or vehicle (n = 8), respectively, beginning 5 days before, and continuing for 5 days after, removal of the second vaginal pessary (pessary removal). Blood samples were collected once a day before pessary removal. Samples were collected at 3 h intervals beginning at pessary removal and continuing to the end of infusion. Blood was allowed to clot on ice. Serum was removed within 24 h of sample collection, rapidly frozen, and stored at −20°C for later endocrine analysis.

Design of Experiment 2

The oestrous activity of 12 ewes was synchronized using progestogen-containing pessaries as described above. Ewes were randomly assigned to one of three treatments groups (n = 4 ewes per group). Animals received cortisol (100 µg kg⁻¹ h⁻¹; groups 1 and 2) or a comparable volume of vehicle

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Fig. 1. Serum concentrations of oestradiol during the 60 h immediately after pessary removal in sheep receiving cortisol (80 μg kg⁻¹ h⁻¹; ●, ▲) or a comparable volume of vehicle (n = 8; ▼). Sheep receiving cortisol were divided into those that did (n = 6; ▲) and those that did not (n = 7; ●) display preovulatory surge-like secretion of LH during the 5 days after pessary removal. At each time point, treatment means with different superscripts are significantly different (P < 0.05). Mean values not marked with a superscript are not significantly different (P > 0.05).

Fig. 2. Serum concentrations of LH after pessary removal in sheep receiving cortisol (80 μg kg⁻¹ h⁻¹; ●, ▲) or a comparable volume of vehicle (n = 8; ▼). Sheep receiving cortisol were divided into those that did (n = 6; ▲) and those that did not (n = 7; ●) display preovulatory surge-like secretion of LH during the 5 days after pessary removal. The LH profile for those sheep displaying surge-like release of LH has been adjusted so that the LH peak for individual sheep is aligned with the average time interval from pessary removal to surge peak that is characteristic of each specific treatment group (58.5 and 74.3 h after pessary removal for sheep receiving vehicle and cortisol, respectively).

(group 3) beginning 5 days before, and continuing for 2 days after pessary removal. Animals received pulses of GnRH (4 ng kg⁻¹ h⁻¹; group 1) or saline (groups 2 and 3) at 1 h intervals beginning at pessary removal and continuing for 2 days. Blood samples were collected at 6 h intervals beginning at pessary removal and continuing to the end of infusion. Blood was collected to clot on ice. Serum was collected and stored as described earlier.

Hormone analysis

Serum concentrations of LH, oestradiol and cortisol were determined using validated procedures (Adams et al., 1975; Sakurai et al., 1992; Daley et al., 1999). The LH (NIAMDDolLH-26) reference standard was a gift from the National Hormone and Pituitary Program (Baltimore, MD). In all cases, intra- and interassay coefficients of variation were < 10%. The minimum sensitivity of the LH, oestradiol and cortisol assays was 0.2 ng ml⁻¹, 0.6 pg ml⁻¹ and 1 ng ml⁻¹, respectively.

Statistical analyses

Statistical significance of treatments was assessed by ANOVA. When significant treatment effects were observed, mean comparisons were made using Duncan’s multiple-range test. Data are presented as mean ± SEM. Chi-squared analysis was used to determine the significance of differences among treatments in the number of ewes displaying surge-like secretion of LH (Gill, 1978).

Results

Experiment 1

Serum concentrations of cortisol in ewes receiving vehicle alone did not differ from the pretreatment concentration (18 ± 4 ng ml⁻¹). Conversely, the serum concentration of cortisol was increased to 64 ± 9 ng ml⁻¹ within 24 h of initiation of infusion in ewes receiving exogenous cortisol at 80 μg kg⁻¹ h⁻¹. This serum concentration of cortisol was maintained for the remainder of the infusion period. Final serum concentrations of cortisol were 67 ± 10 and 18 ± 3 ng ml⁻¹ in ewes receiving cortisol and vehicle, respectively.

The serum concentration of oestradiol was 0.7 ± 0.2 pg ml⁻¹ at pessary removal and did not differ (P > 0.05) between ewes receiving cortisol or vehicle alone. Mean serum concentrations of oestradiol in ewes receiving vehicle increased progressively during the period immediately after pessary removal, reaching a maximum concentration of 10.4 ± 5.7 pg ml⁻¹ at 48 h after pessary removal (Fig. 1). In contrast, continuous infusion of cortisol suppressed (P < 0.05) serum concentrations of oestradiol during the period after pessary removal. Serum oestradiol was maintained at basal concentrations (< 0.7 pg ml⁻¹) throughout the 5 days after pessary removal in 7 of 13 animals receiving stress-like concentrations of cortisol. Serum concentrations of oestradiol were increased progressively during the 24 h immediately after pessary removal in the remaining cortisol-treated animals (6 of 13). However, serum concentrations of oestradiol 24-48 h after pessary removal were significantly (P < 0.05) lower than concentrations in control animals.
Table 1. Effect of continuous infusion of cortisol (80 μg kg⁻¹ h⁻¹) or a comparable volume of vehicle (50% ethanol–saline) on the onset and size of the preovulatory LH surge in sheep

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Sheep displaying surge-like release of LH</th>
<th>Interval to LH surge (h)</th>
<th>Duration of LH surge (h)</th>
<th>Size of LH surge (ng ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>8</td>
<td>58.5 ± 2.1*</td>
<td>11.9 ± 0.9</td>
<td>24.8 ± 2.4</td>
</tr>
<tr>
<td>Cortisol</td>
<td>13</td>
<td>6</td>
<td>74.3 ± 6.6†</td>
<td>10.5 ± 0.6</td>
<td>24.8 ± 6.1</td>
</tr>
</tbody>
</table>

*Number of ewes displaying a preovulatory surge-like secretion of LH during the 5 days after pessary removal.
†Interval from pessary removal to the peak of the LH surge.
‡Interval during the surge when serum concentrations of LH exceeded 2 ng ml⁻¹.
§Serum concentration of LH at the peak of the surge.
‖Values within a column that do not share a common superscript are significantly different (P < 0.05).

Preovulatory surge-like secretion of LH was evident in all control ewes (8 of 8) receiving vehicle alone, and the peak of the surge was apparent 58.5 ± 2.1 h after pessary removal (Fig. 2). In contrast, continuous infusion of cortisol blocked or delayed the appearance of the surge-like secretion of LH. Preovulatory surge-like secretion of LH was only apparent in cortisol-treated sheep (6 of 13) in which serum oestradiol was significantly increased during the period after pessary removal. Among cortisol-treated sheep displaying an ovulatory surge, the duration and size of the surge did not differ from the surge in control sheep. However, the interval from pessary removal to the peak of the surge was significantly (P < 0.05) extended (74.3 ± 6.6 h after pessary removal) relative to the size and timing of the surge in control animals (Table 1). Similar to serum concentrations of oestradiol, serum concentrations of LH in the remaining cortisol-treated sheep (7 of 13) did not increase above basal concentrations (< 1 ng ml⁻¹) during the period after pessary removal.

Experiment 2

Continuous infusion of cortisol at a rate of 100 μg kg⁻¹ h⁻¹ increased serum concentrations of cortisol to 83.0 ± 6.0 ng ml⁻¹ at 24 h after the start of infusion. Serum cortisol was maintained at this concentration for the remainder of the infusion period. In contrast, serum concentrations of cortisol in control animals receiving vehicle alone were 15.2 ± 3.4 ng ml⁻¹.

Serum concentrations of oestradiol increased progressively during the period after pessary removal in ewes receiving vehicle alone (Fig. 3). In contrast, serum oestradiol was maintained at basal concentrations in ewes receiving stress-like concentrations of cortisol. Concurrent episodic administration of GnRH reactivated follicular development and oestradiol secretion in cortisol-treated sheep. Serum concentrations of oestradiol at 48 h after pessary removal did not differ (P > 0.05) between control ewes (7.7 ± 0.8 pg ml⁻¹) and ewes receiving cortisol and episodic GnRH (6.4 ± 1.3 pg ml⁻¹). These values were significantly greater (P < 0.05) than the serum concentrations of oestradiol observed in animals receiving cortisol alone (1.0 ± 0.4 pg ml⁻¹).

Fig. 3. Serum concentrations of oestradiol during the 48 h after pessary removal in sheep (n = 4 ewes per group) receiving cortisol (100 μg kg⁻¹ h⁻¹; ▲, △) or a comparable volume of vehicle (●) beginning 5 days before, and continuing for 48 h after pessary removal. Sheep received pulses of GnRH (4 ng kg⁻¹ h⁻¹, i.v.; ◆) or saline (▲, ●) at 1 h intervals during the 48 h after pessary removal. At each time point, treatment means denoted with an asterisk are significantly different (P < 0.05) from the other mean values at that time point.

Discussion

These results demonstrate that continuous administration of stress-like concentrations of cortisol to sheep during the late luteal and early follicular phases of the reproductive cycle suppresses oestradiol secretion and, presumably, follicular growth and development. Similarly, preovulatory surge-like secretion of LH is also blocked in more than 50% of cortisol-treated sheep. Moreover, the onset of the surge is significantly delayed in cortisol-treated sheep that show a surge of LH. Collectively, these observations indicate that stress-like concentrations of cortisol suppress ovarian function in sheep. This anti-ovulatory effect of cortisol is consistent with the response to exogenous glucocorticoid reported in women (Cunningham et al., 1978), rodents (Baldwin and Sawyer, 1974), and other domestic species (Barb et al., 1982; Stoebel and Moberg, 1982). However, these
results differ from the findings of Phillips and Clarke (1990) which indicated that administration of a synthetic glucocorticoid once a day did not compromise follicular development or ovulation in sheep. Although the physiological basis for this discrepancy is not clear, differences in the type (dexamethasone versus cortisol), method of administration (bolus once a day versus continuous infusion) or dose (2 mg DEX day\(^{-1}\) versus 2 mg cortisol kg\(^{-1}\) day\(^{-1}\)) of glucocorticoid may be contributory factors.

In the present study, attempts were made to approximate the marked and persistent increase in the serum concentration of cortisol that is observed in sheep during exposure to repetitive or persistent stress by infusion of the natural glucocorticoid. Continuous infusion of cortisol at rates of 80–100 µg kg\(^{-1}\) h\(^{-1}\) established serum concentrations of cortisol comparable with those in sheep during exposure to moderate stressors, such as transportation, repeated laparoscopy, or isolation and restraint (Martin et al., 1981; Ehnert and Moberg, 1991; Minton, 1994). Use of the natural glucocorticoid at physiological concentrations strengthens the conclusion that stress-like concentrations of cortisol impair fertility in sheep.

As observed in control animals, serum oestradiol concentrations increase progressively during the normal follicular phase and reach a peak just before the ovulatory surge of LH (Webb and England, 1982; Sakurai et al., 1992; Scaramuzzi et al., 1993). Large preovulatory follicles are the primary source of the steroid and little oestradiol is produced by small or atretic follicles (Carson et al., 1981). Therefore, serum concentrations of oestradiol can provide an approximate measure of follicular maturation. In contrast to the process of follicular development and ovulation in control animals, ewes receiving stress-like concentrations of cortisol showed two alternate patterns of oestradiol secretion during the period after pessary removal. One group of animals failed to display a preovulatory LH surge and serum oestradiol was maintained at basal concentrations throughout the period of cortisol delivery. This is likely to reflect the absence of significant follicular growth during this period. Huet et al. (1997) reported that only ovine follicles larger than 3.5 mm in diameter acquired the capacity to synthesize oestradiol. The observations of the present study indicate that cortisol may arrest follicular development before that stage of maturation.

The alternate pattern of oestradiol secretion in cortisol-treated sheep was characterized by a gradual and progressive increase in the serum concentration of oestradiol. This may indicate that follicular development is retarded, but is not blocked completely. This contention is supported by the observation that an ovulatory surge of LH is apparent in this group of animals. However, both the progression of oestradiol secretion and the onset of the surge were significantly delayed relative to the temporal pattern observed in control animals.

The two patterns of oestradiol secretion in cortisol-treated sheep in the present study are similar to the two alternate responses to stress during the follicular phase of monkeys (Xiao et al., 1998). The physiological basis for these two patterns of follicular response, arrested or delayed development, is unclear and is currently the subject of investigation. Two alternate hypotheses may account for this variation between animals in the pattern of response. One hypothesis is that the stage of the follicular wave at which stress or stress-like concentrations of cortisol is introduced may influence the follicular response. Alternatively, the threshold of cortisol required to arrest follicular development fully may vary among sheep. According to this proposal, increasing the duration or extent of cortisol stimulation may increase the proportion of sheep showing full arrest of follicular development. In this regard, it is interesting to note that oestradiol secretion was suppressed in all ewes that received cortisol at 100 µg kg\(^{-1}\) h\(^{-1}\) (Expt 2), but in only 50% of ewes that received cortisol at 80 µg kg\(^{-1}\) h\(^{-1}\) (Expt 1).

Stress-like concentrations of cortisol may arrest or retard folliculogenesis by acting directly at ovarian loci. In vitro studies demonstrated that glucocorticoids compromise several key steps in follicular development and steroidogenesis in rodents (Schoonmaker and Erickson, 1983). Alternatively, cortisol may act at hypothalamic or hypophyseal sites to decrease gonadotrophin secretion and, thereby, limit the amount of LH and FSH available to support follicular development and ovulation.

This proposal is consistent with the findings of Daley et al. (1999) that the negative feedback potency of oestradiol is enhanced in orchidectomized sheep during concurrent exposure to high concentrations of cortisol. If stress-like concentrations of cortisol have a similar effect in female sheep, the effect is likely to be most prominent during the early follicular phase when a circhoral pattern of GnRH and gonadotrophin secretion supports follicular maturation. The critical role that circhoral secretion of GnRH plays in ovarian function is indicated by the arrest of follicular development after administration of a GnRH antagonist (Campbell et al., 1990) or immunoneutralization of endogenous GnRH (McNeilly et al., 1984; Sakurai et al., 1992). Similarly, follicular development and ovulation are reinstated during seasonal anoestrus by episodic delivery of GnRH (McLeod et al., 1982). The present study demonstrates that episodic administration of GnRH also reactivates follicular development and ovulation in sheep receiving stress-like concentrations of cortisol. This indicates that stress-like concentrations of cortisol decrease the activity of the GnRH pulse generating system. Similar actions of glucocorticoids at hypothalamic or hypophyseal loci have been observed in rodents (Baldwin and Sawyer, 1974) and women (Sakotos et al., 1993). Similarly, the activity of the GnRH secretory apparatus is apparently decreased in sheep and primates during stress (Chen et al., 1992; Battaglia et al., 1998). It is postulated that the infertility induced by stress or stress-like concentrations of cortisol is due, at least in part, to a cortisol-dependent increase in the negative feedback potency of oestradiol. During the follicular phase of the oestrous cycle the augmented negative feedback potency of oestradiol would be expected to suppress the frequency or amount of episodic release of GnRH and decrease gonadotrophin secretion below the threshold required to sustain the normal progression of follicular development.

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