Inhibition of the secretion of LH in ovariectomised pigs by sustained but not repeated acute elevation of cortisol in the absence but not the presence of oestradiol

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Abstract

Prolonged stress is known to impair reproduction. It has been proposed that reproduction will also be impaired when a severe acute stress occurs during a period of elevated plasma concentrations of oestradiol, such as during the follicular phase of the oestrous cycle. In this experiment, we hypothesised that repeated acute and sustained elevation of cortisol would suppress the secretion of LH in ovariectomised pigs and that these effects would be enhanced in the presence of oestradiol negative feedback. Cortisol (or vehicle) was administered 12 hourly to ovariectomised pigs ($n=6$/treatment) for 8 days in the absence of oestradiol treatment and for a further 8 days during treatment with oestradiol. Vehicle was administered to 'control' pigs, 10 or 20 mg cortisol was administered i.v. to pigs to produce 'repeated acute' elevation of cortisol and 250 mg cortisol was administered i.m. to pigs to give a 'sustained' elevation of cortisol. Both before and during treatment with oestradiol, plasma concentrations of LH were monitored on the day before treatment, on the 4th and 8th days of treatment and following an i.v. injection of GnRH at the end of the 8th day of treatment. The repeated acute elevation of cortisol did not impair any parameters of LH secretion (i.e. mean plasma concentrations of LH, pulse amplitude or frequency, pre-LH pulse nadir or the LH response to GnRH) in the absence or in the presence of oestradiol. In contrast, when the elevation of cortisol was sustained, the mean plasma concentrations of LH and the pre-LH pulse nadir were significantly ($P<0{\cdot}05$) lower on the 8th day of treatment than on the day before treatment and on the 4th day of treatment. Nevertheless, no other parameters of LH secretion were affected and these effects only occurred in the absence (not in the presence) of oestradiol. In conclusion, cortisol needed to be elevated for more than 4 days to impair the secretion of LH, and oestradiol did not enhance the impact of cortisol on LH secretion in ovariectomised pigs.

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Introduction

Although it has been proposed that acute stress at a critical stage of the oestrous cycle, such as prior to oestrus and ovulation, can disrupt reproductive performance (Moberg 1985), experiments that we have conducted in pigs have provided no support for this (Turner *et al.* 1998*a*,*b*). For instance, when substantial activation of the hypothalamo–pituitary–adrenal axis occurred due to the imposition of a negative handling procedure every 24 h for 4–13 days prior to oestrus, there was no impairment of reproduction (Turner *et al.* 1998*b*). To impair reproduction in female pigs, stress may need to be more severe and/or more frequent than that imposed in our previous experiments. Many studies have demonstrated that administering cortisol in a manner which resulted in its sustained elevation in plasma impaired various aspects of reproduction in female pigs (Liptrap 1970, Scholten & Liptrap 1978, Barb *et al.* 1982, Fonda *et al.* 1984, Estienne *et al.* 1991) suggesting that cortisol is important in mediating the stress-induced impairment of reproduction. For instance, twice daily i.m. injection of cortisol, which resulted in a sustained elevation of plasma concentrations of cortisol, blocked the luteinizing hormone (LH) surge and ovulation in gilts (Barb *et al.* 1982). Since the secretion of LH is central to reproduction, the measurement of plasma concentrations of LH has often been used to assess the effects of stress (Blake 1975, Doney *et al.* 1976, Martin *et al.* 1981, Stoebel & Moberg 1982, Rasmussen & Malven 1983, Dobson 1988, Norman *et al.* 1994, Tilbrook *et al.* 1999) and of administering hormones of the hypothalamo–pituitary–adrenal axis (Barb *et al.* 1982, Hennessy & Williamson 1983, Paterson *et al.* 1983, Fonda *et al.* 1984, Pearce *et al.* 1988, Frautschy *et al.* 1989, Estienne *et al.* 1991) on reproduction in females. For instance, prolonged treatment of ovariectomised pigs with cortisol inhibited

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the secretion of LH (Estienne *et al.* 1991). In contrast, there have been no studies in which cortisol has been administered in a manner which resulted in the acute elevation of plasma concentrations of cortisol, as would occur in an acute stress response, and it is not known if repeated acute treatment with cortisol will also inhibit the secretion of LH. Such treatment would allow manipulation of the magnitude and frequency of elevations of plasma concentrations of cortisol to determine if substantial elevations which occur more frequently than once per day are required to impair reproduction.

While the impact of the hypothalamo–pituitary–adrenal axis on the reproductive axis has long been recognised (Moberg 1985), there is also evidence that gonadal steroids influence the activity of the hypothalamo–pituitary–adrenal axis (Handa *et al.* 1994, Da Silva 1995). For instance, plasma concentrations of cortisol were greatest when plasma concentrations of oestradiol were elevated in rats (Critchlow *et al.* 1963, Kitay 1963) and in rhesus macaques (Smith & Norman 1987). Consequently, it might be expected that the impact of stress on reproduction would also be greatest when circulating concentrations of oestradiol are highest. Indeed, in rhesus macaques the secretion of LH was impaired when a stressor was imposed during the follicular phase of the menstrual cycle but not when the same stressor was imposed during the luteal phase of the menstrual cycle (Norman *et al.* 1994). While oestrogen may enhance the activity of the hypothalamo–pituitary–adrenal axis and the impact of stress on reproduction, it is not known if this sex steroid influences the actions of cortisol to inhibit the secretion of LH or, conversely, if cortisol influences the feedback actions of oestradiol on LH. These issues were investigated in this experiment using ovariectomised pigs given repeated acute treatment with cortisol in the presence or absence of oestradiol negative feedback. Thus, this experiment tested the hypotheses that the repeated acute and sustained elevation of cortisol will disrupt spontaneous and gonadotrophin-releasing hormone (GnRH)-induced secretion of LH in ovariectomised pigs and that these effects will be enhanced in ovariectomised pigs treated with oestradiol.

Materials and Methods

Animals

Twenty-four pigs (17 sows and 7 gilts; Large White × Landrace) were ovariectomised 6–12 months before the experiment. The age of pigs at the commencement of the experiment was 517–700 days for sows and 396–533 days for gilts. Venous catheters were inserted in all pigs (Takken & Williams 1981) 5 or 6 days before the experiment and the pigs were housed in groups of 3 with a space allowance of 2·4 m^2 per pig. The care and experimental use of animals conformed with the requirements of the Australian Prevention of Cruelty to Animals Act 1986 and the NHMRC Australian code of practice for the care and use of animals for scientific purposes.

Procedure

A schematic representation of the treatments and protocol used in this experiment is given in Fig. 1. The pigs were allocated randomly to 4 treatments (6 pigs/treatment). Treatment consisted of i.v. injections (2 ml) and i.m. injections (2 ml) of cortisol or the appropriate vehicle for cortisol at 0900 h and 1900 h for 8 days. The vehicle for i.v. administration of cortisol was saline and that for i.m. administration of cortisol was corn oil. Thus, pigs in the 'control' treatment received i.v. injections of saline via their indwelling catheter and i.m. injections of corn oil. There were two treatments which involved repeated acute elevation of cortisol. Pigs in the '10 mg repeated acute' and '20 mg repeated acute' treatment groups received i.v. injections of either 10 mg or 20 mg cortisol (hydrocortisone sodium succinate, Solu-Cortef Sterile Powder, Upjohn Pty Ltd, Rydalmere, NSW, Australia) in saline, and i.m. injections of corn oil. The decision to use these doses was based on a study where an i.v. injection of 5 mg cortisol in pubertal gilts elevated plasma concentrations of cortisol in a similar manner to that which followed an acute stressor (Dalin *et al.* 1993). Pigs in the 'sustained' elevation of cortisol treatment group received i.v. injections of saline and i.m. injections of 250 mg cortisol (hydrocortisone 21-acetate, Sigma, St Louis, MO, USA) in corn oil. This treatment has previously been shown to result in a sustained elevation of cortisol and a disruption of the LH surge and ovulation in gilts (Barb *et al.* 1982). The plasma concentrations of cortisol which resulted from these treatments were assessed on the second day of treatment. Samples of blood (10 ml) were collected 50, 40, 30, 20, 10 and 0 min before and 2, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min after the 0900 h treatment injections. Concentrations of cortisol were measured in plasma harvested from these samples.

To determine plasma concentrations of LH, blood samples were collected every 10 min from 0900 h to 1700 h on the day before treatment commenced (day −1) and on the 4th day of treatment (day 4), and from 0800 h to 1600 h on the 8th day of treatment (day 8). At 1600 h on the 8th day of treatment, an i.v. injection of 20 μg GnRH (luteinizing hormone releasing hormone, Auspep, Parkville, Victoria, Australia) was administered to all pigs and blood samples were collected 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min thereafter. Concentrations of LH were assessed in plasma from these samples. In addition, plasma concentrations of cortisol were measured in blood samples collected 3, 4, 5, 6, 7, 8 and 9 h after the morning treatment on days -1, 4 and 8 of treatment.

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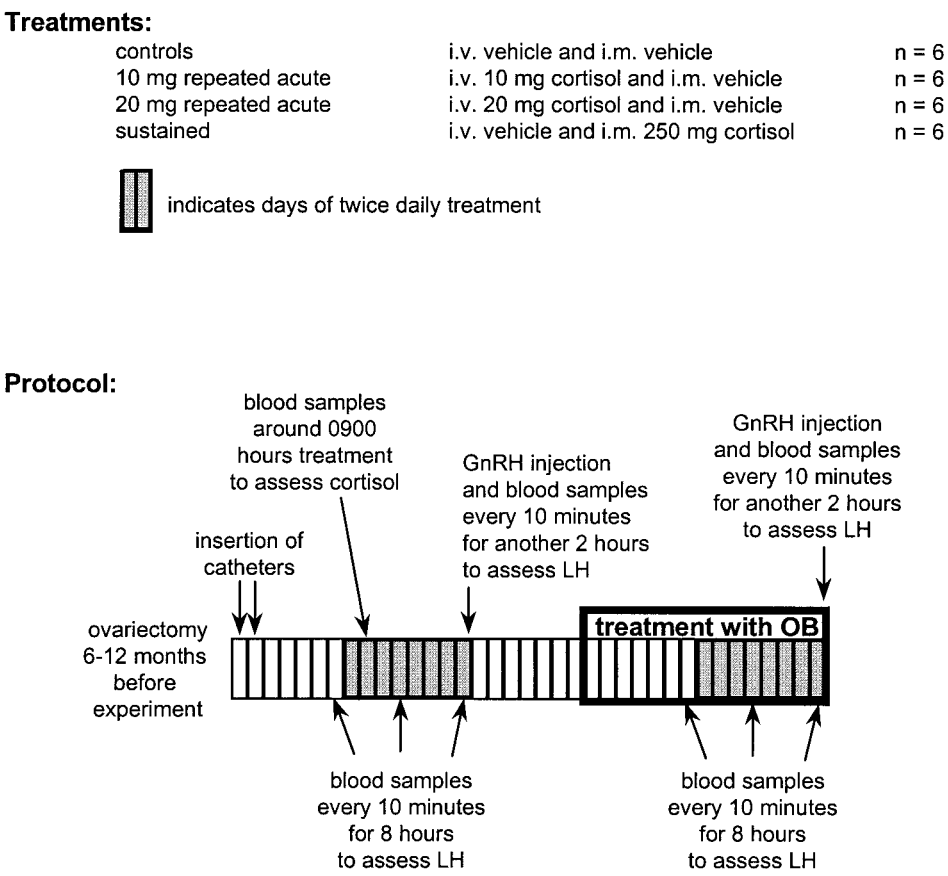

Figure 1 The treatment regimen and a schematic representation of the protocol. OB=oestradiol. Further explanation is given in the text.

The effects of sustained and repeated acute elevation of cortisol on the secretion of LH were also assessed when the pigs were undergoing treatment with oestradiol (Fig. 1). Injections (i.m.) of oestradiol (75 μg; β-oestradiol 3-benzoate, Sigma) were administered twice daily (0700 h and 1900 h) commencing one week after the end of the first treatment period. These injections were continued for the remainder of the experiment. After one week of injections of oestradiol, samples of blood were again collected at 10-min intervals from 0900 h to 1700 h (day -1). On the following day, the treatment regimen used in the first part of the experiment was resumed for 8 days. As in the first period of treatment, samples of blood were collected over 8 h on the 4th and 8th days of treatment and for a further 2 h following injection of GnRH on the 8th day. Plasma concentrations of LH and cortisol were measured in plasma harvested from these blood samples.

Radioimmunoassays

Cortisol radioimmunoassay Total plasma concentrations of cortisol were measured, following extraction of plasma using dichloromethane, by a radioimmunoassay which was developed for use in fetal sheep plasma (Bocking *et al.* 1986) and which has since been validated for use in pig plasma (Turner *et al.* 1998*a*). The mean (± S.E.M.) recovery of [^{3}H]cortisol from plasma after the extraction procedure was 92 ± 2%. There were 19 assays conducted with a mean (± S.E.M.) assay sensitivity of 0·6 ± 0·1 ng/ml. Samples that fell below the assay sensitivity were assayed again using a larger volume of sample. The intra-assay coefficients of variation were 6·3% at 33·0 ng/ml, 5·6% at 28·8 ng/ml and 7·6% at 53·1 ng/ml. The interassay coefficients of variation were 8·2% at 33·7 ng/ml, 16·1% at 29·3 ng/ml and 13·3% at 53·8 ng/ml.

LH radioimmunoassay Plasma concentrations of LH were determined using a double-antibody radioimmunoassay which was developed for analysis of porcine LH (pLH) by Niswender *et al.* (1970), modified by Peacock (1991) and Klupiec (1995) and further modified for use in our laboratory. Primary anti-body (#566 anti-porcine LH serum, Dr G D Niswender, Colorado State

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University, Fort Collins, CO, USA) was raised in rabbits (Niswender *et al.* 1970) and pLH (LER-1786–3, Dr L E Reichert Jr, The Albany Medical College, Albany, NY, USA), for use in standards and tracer, was purified according to the procedure described by Reichert (1964). Second antibody (goat anti-rabbit serum, Dr I R Young, Monash University, Clayton, Victoria, Australia) was raised in goats against rabbit immunoglobulin G. Purified pLH was labelled with radioactive iodine (^{125}I, NEN Life Science Products, Boston, MA, USA) using the Iodogen (1,3,4,6-tetrachloro-3α,6α-di-phenylglycouril, Sigma) protocol described by Salacinski *et al.* (1981). Sixteen assays were conducted with a mean (± S.E.M.) assay sensitivity of 0·17 ± 0·02 ng/ml. Samples that fell below the assay sensitivity were assayed again using a larger volume of sample. The intra-assay coefficients of variation were 7·3% at 0·4 ng/ml and 8·8% at 0·6 ng/ml. The interassay coefficients of variation were 16·4% at 0·5 ng/ml and 18·1% at 0·8 ng/ml.

Identification of LH pulses

A modification of the procedure described by Karsch *et al.* (1987) was used to define pulses of LH. Pulses were defined as abrupt increases in the concentration of LH that were greater than the assay sensitivity, exceeded the preceding value by at least three times the mean standard deviation of the LH concentrations determined by the relevant assay, and were followed by a progressive decline at a rate consistent with the half-life of LH in pigs of 28·0 min (Esbenshade *et al.* 1986). The mean plasma concentration of LH was calculated as the mean of all points in the sampling period. The amplitude of LH pulses was calculated as the difference between the peak and the preceding nadir. The number of pulses per hour was calculated as the total number of pulses in a sampling period divided by the number of hours in the sampling period (i.e. 8 h). The baseline was calculated as a mean of the pre-LH pulse nadir values. If there were no LH pulses identified in a sampling period, the mean plasma concentration of LH was used as the baseline value and the pulse amplitude and number of pulses per hour were recorded as 0.

Statistical analyses

Plasma concentrations of cortisol around the 0900 h injections on the 2nd day of treatment were compared between treatments using repeated measures analysis of variance. *Post hoc* multiple comparisons for this and subsequent analyses of variance were made using least significant differences. Comparisons were made between subjects for all analyses of variance, except where otherwise stated. Repeated measures analysis of variance was also used to compare between treatments for the plasma concentrations of cortisol from 3 to 9 h after treatment injections on each day of blood sampling. The variances of these raw data were not homogeneous so the data were subjected to log10 transformation prior to analysis. Mean plasma concentrations of LH, pulse amplitude, the number of pulses per hour and pre-LH pulse nadir were also analysed by repeated measures analysis of variance but *post hoc* multiple comparisons were made within subjects for these parameters. The LH response to GnRH was compared between treatments using repeated measures analysis of variance, and two-way analysis of variance was used to analyse the area under the LH response to GnRH curve and the peak of this curve.

Results

Plasma concentrations of cortisol

Around 0900 h treatment on the 2nd day of treatment Plasma concentrations of cortisol in pigs in the 10 mg repeated acute and the 20 mg repeated acute elevation of cortisol treatment groups were significantly ($P<0·05$) higher than those in control pigs two minutes after treatment (Fig. 2). These concentrations remained significantly ($P<0·05$) greater than those in the control pigs until 20 min after treatment in pigs in the 10 mg repeated acute elevation of cortisol treatment group, and until 110 min after treatment in pigs in the 20 mg repeated acute elevation of cortisol treatment group ($P=0·076$ 100 min after treatment). Plasma concentrations of cortisol in pigs in the sustained elevation of cortisol treatment group were significantly ($P \leq 0·05$) higher than those in control pigs 50 min after treatment and for the remainder of the sampling period (120 min; Fig. 2, inset).

From 3 to 9 h after treatment on each day of sampling Throughout the course of the experiment, plasma concentrations of cortisol from 3 to 9 h after treatment were significantly ($P<0·05$) higher in pigs in the sustained elevation of cortisol treatment group than in pigs in all other treatment groups (Fig. 3). Furthermore, plasma concentrations of cortisol were significantly ($P<0·05$) greater in pigs in the 20 mg repeated acute elevation of cortisol treatment group than in control pigs. These treatment effects were not related to the day of treatment or the presence/absence of oestradiol.

Plasma concentrations of LH

Mean concentrations Plasma concentrations of LH were significantly ($P<0·05$) higher in the absence of oestradiol than during treatment with oestradiol (Fig. 4; top panel). There was no significant change in the mean plasma concentration of LH from day -1 to days 4 or 8 of treatment in pigs in the control, 10 mg repeated acute or 20 mg repeated acute elevation of cortisol treatment

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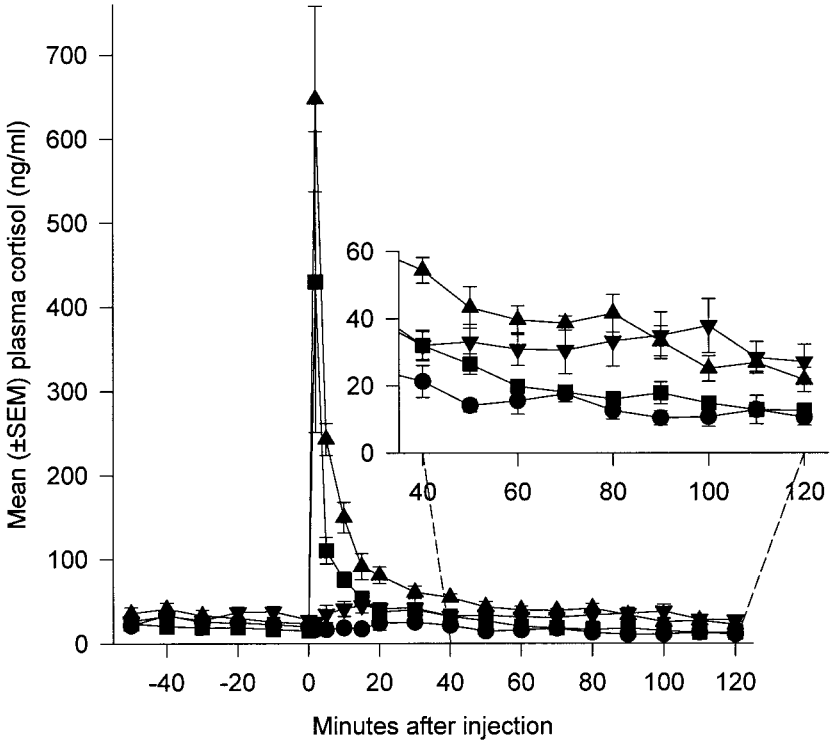

Figure 2 Mean (± S.E.M.) plasma concentrations of cortisol (ng/ml) for pigs in the control (●), 10 mg repeated acute (■), 20 mg repeated acute (▲) and sustained (▼) elevation of cortisol treatment groups from 50 min before to 120 min after treatment (and from 40 to 120 min after treatment with an expanded scale; inset). This treatment occurred at 0900 h on the 2nd day of treatment. Significant differences are described in the Results section.

groups in the presence or absence of oestradiol or in pigs in the sustained elevation of cortisol treatment group in the presence of oestradiol. In contrast, in the absence of oestradiol, pigs in the sustained elevation of cortisol treatment group had significantly ($P<0·05$) lower mean plasma concentrations of LH on the 8th day of treatment than before treatment and on the 4th day of treatment.

Amplitude of LH pulses Overall, the amplitude of LH pulses was significantly ($P<0·05$) greater in the absence of oestradiol than during treatment with oestradiol (Fig. 4; second panel from top). Nevertheless, the amplitude of LH pulses on the 4th and 8th days of treatment was not significantly different to that recorded before treatment in pigs in any treatment group either before or during treatment with oestradiol.

Frequency of LH pulses Pulses of LH were significantly ($P<0·05$) more frequent (as measured by the number of LH pulses per hour) in the absence of oestradiol than during treatment with oestradiol (Fig. 4; third panel from top). The number of LH pulses per hour did not change from before treatment to the 4th or 8th day of treatment in pigs in any treatment group either before or during treatment with oestradiol.

Pre-LH pulse nadir Across all treatments, the baseline concentrations of LH (as measured by the pre-LH pulse nadir) were significantly ($P<0·05$) higher in the absence of oestradiol than during treatment with oestradiol (Fig. 4; bottom panel). There were no significant changes in the baseline concentrations of LH from before treatment to the 4th or 8th day of treatment in pigs in the control or 10 mg repeated acute elevation of cortisol treatment groups in the absence or presence of oestradiol or in pigs in the 20 mg repeated acute or sustained elevation of cortisol treatment groups in the presence of oestradiol. In the absence of treatment with oestradiol, pigs in the 20 mg repeated acute elevation of cortisol treatment group had significantly ($P<0·05$) higher mean pre-LH pulse nadir on the 8th day of treatment than on the day prior to treatment and on the 4th day of treatment. In contrast, pigs in the sustained elevation of cortisol treatment group had a significantly ($P<0·05$) lower mean pre-LH pulse nadir on the 8th day of treatment than on the day before treatment and on the 4th day of treatment.

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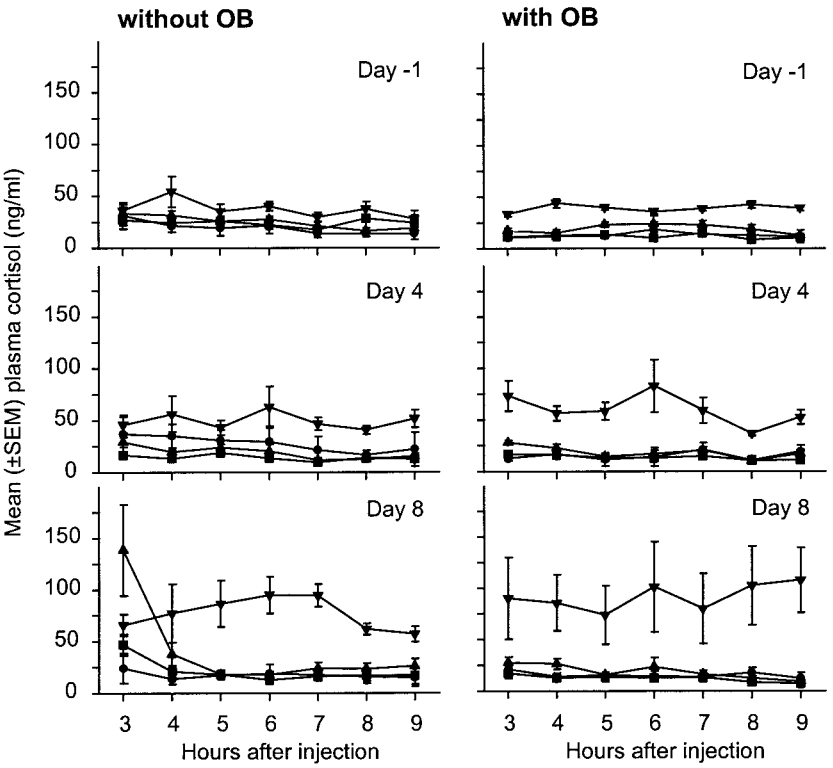

Figure 3 Mean (± S.E.M.) plasma concentrations of cortisol (ng/ml) from 3 to 9 h after treatment in pigs in the control (●), 10 mg repeated acute (■), 20 mg repeated acute (▲) and sustained (▼) elevation of cortisol treatment groups on the day prior to treatment (day -1) and on the 4th (day 4) and 8th (day 8) day of treatment both before treatment with oestradiol (without OB) and during treatment with oestradiol (with OB). Significant differences are described in the Results section.

LH response to GnRH

While there was a significant ($P<0·05$) elevation of plasma concentrations of LH in pigs in all treatments following i.v. injection of 20 mg GnRH both before and during treatment with oestradiol (Fig. 5), the LH response to GnRH was significantly ($P<0·05$) greater in the absence of oestradiol than when oestradiol was present. Repeated measures analysis of variance found no significant differences between treatments in the plasma concentrations of LH following injection of GnRH either in the presence or absence of oestradiol. Furthermore, the area under the LH response to GnRH curve (ng/ml/120 min) and the peak height of this response (ng/ml) were significantly ($P<0·05$) greater in the absence of oestradiol (120 ± 16, $2·3 \pm 0·3$ respectively) than in the presence of oestradiol (54 ± 4, $1·2 \pm 0·1$ respectively) but there were no significant differences between treatments for these parameters.

Discussion

In this experiment, the repeated acute elevation of cortisol did not impair the secretion of LH in ovariectomised pigs in the absence or in the presence of oestradiol negative feedback, indicating that this aspect of reproduction is insensitive to such treatment. Moreover, the GnRH-induced release of LH was not affected by the repeated acute elevation of cortisol in ovariectomised pigs, suggesting that this treatment did not impair the ability of the pituitary gland to respond to GnRH, and this result was not influenced by treatment with oestradiol. These results are consistent with those of previous experiments in our laboratory (Turner *et al.* 1998*a*,*b*), which showed that the imposition of acute stressors that resulted in the repeated acute elevation of plasma concentrations of cortisol did not impair reproduction. Also in keeping with previous studies (Barb *et al.* 1982, Fonda *et al.* 1984, Estienne *et al.* 1991) is the finding that the sustained elevation of cortisol reduced plasma concentrations of LH. Nevertheless, this did not occur until the 8th day of treatment, and did not occur in ovariectomised pigs treated with oestradiol. This result suggests that, in animals where the secretion of LH is not restrained by negative feedback of ovarian hormones, plasma concentrations of cortisol need to be elevated for a considerable period before plasma concentrations of LH are reduced. It would appear that, even

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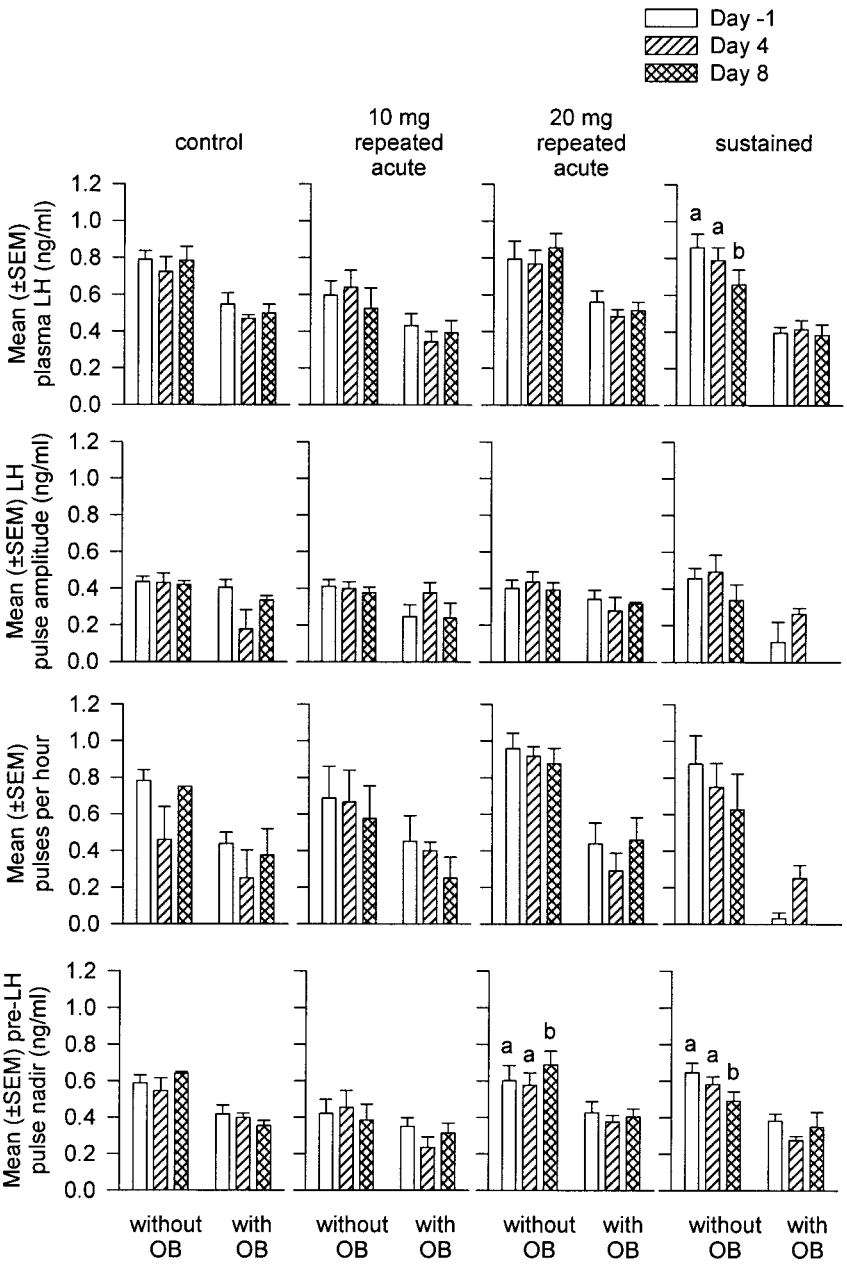

Figure 4 Mean (± S.E.M.) plasma concentrations of LH (ng/ml; top panel), LH pulse amplitude (ng/ml; second panel from top), pulses per hour (third panel from top) and pre-LH pulse nadir (ng/ml; bottom panel) for pigs in the control, 10 mg repeated acute, 20 mg repeated acute and sustained elevation of cortisol treatment groups on the day prior to treatment (day − 1) and on the 4th (day 4) and 8th (day 8) day of treatment both before administration of oestradiol (without OB) and during administration of oestradiol (with OB). The absence of a bar represents a value of 0. Different superscripts on bars within a panel indicate a significant (P<0·05) difference.

though cortisol is capable of reducing plasma concentrations of LH when its elevation in the plasma is sustained, the repeated acute elevation of cortisol in this experiment was not sufficient to achieve this.

The sustained elevation of cortisol achieved in this experiment may have been ineffective in reducing plasma concentrations of LH in ovariectomised pigs treated with oestradiol because the LH parameters were reduced by the

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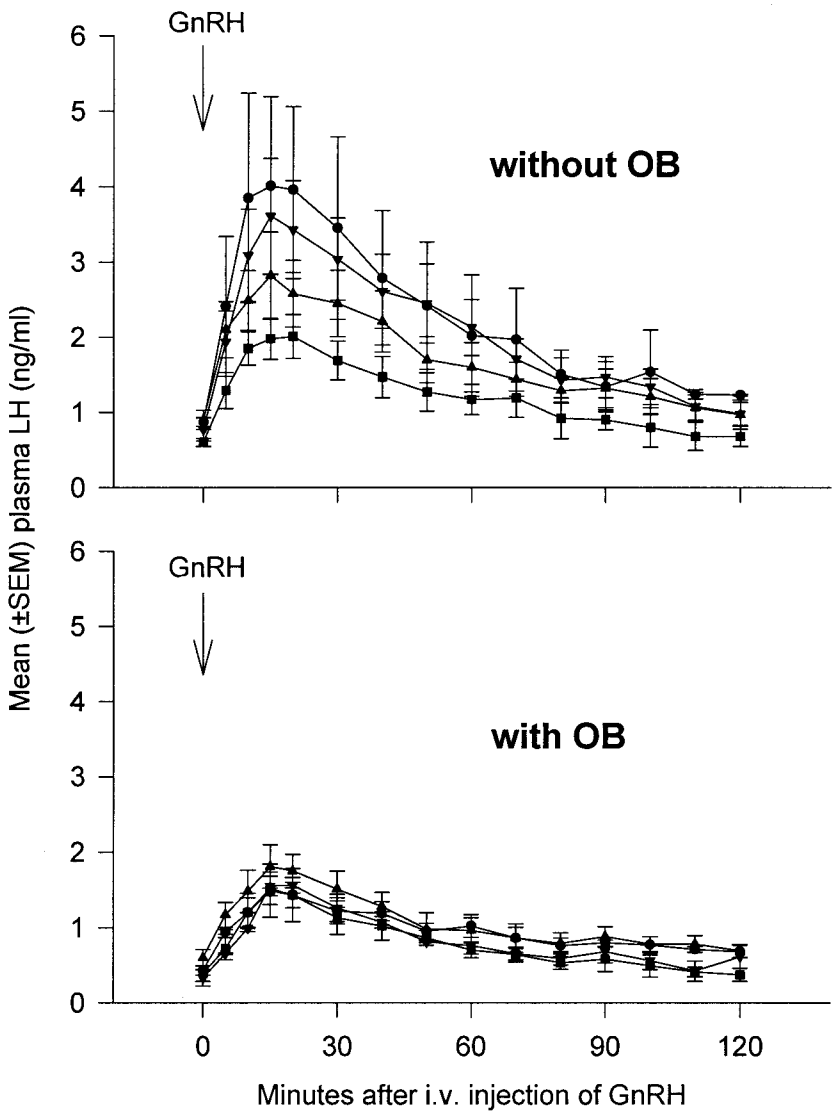

Figure 5 Mean (± S.E.M.) plasma concentrations of LH (ng/ml) following an i.v. injection of 20 mg GnRH in pigs in the control (●), 10 mg repeated acute (■), 20 mg repeated acute (▲) and sustained (▼) elevation of cortisol treatment groups on the 8th day of treatment, both before administration of oestradiol (without OB; upper panel) and during administration of oestradiol (with OB; lower panel). There were no significant differences between treatments.

negative feedback actions of oestradiol to an extent where differences between treatments were masked. Alternatively, the detrimental actions of cortisol on the secretion of LH may have been directly attenuated by oestradiol. This latter explanation does not support the finding that in rhesus macaques, 6 h of chair restraint inhibited the secretion of LH during the follicular phase of the menstrual cycle, when oestrogens are elevated, but not during the luteal phase (Norman *et al.* 1994), or the finding, in ovariectomised sheep, that 4 h of isolation/restraint stress inhibited the secretion of LH in the presence and absence of oestradiol (Tilbrook *et al.* 1999). Nevertheless, there might be species differences in the ability of oestrogens to influence the impact of stress on reproduction and/or the discrepancies between the results of these studies might be due to differences in the experimental paradigms. For instance, while stressors were imposed in the studies on rhesus macaques (Norman *et al.* 1994) and sheep (Tilbrook *et al.* 1999), cortisol was injected in the current experiment.

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The mechanism by which the sustained elevation of cortisol reduced the mean plasma concentrations of LH in the absence of oestradiol is not clear. This reduction in mean LH was not associated with a decrease in the frequency or amplitude of LH pulses. This is surprising as it would be expected that cortisol would act at the hypothalamus (Estienne *et al.* 1991) and/or at the pituitary (Pearce *et al.* 1988) to disrupt the secretion of LH and that this would be manifest in an impairment of the frequency and/or amplitude of LH pulses. Instead, the reduction in mean plasma concentrations of LH was associated with a reduction in mean pre-LH pulse nadir suggesting that basal secretion of LH was impaired. The site at which cortisol acted to produce this result is not clear and could include the hypothalamus and/or pituitary. The finding that the responsiveness of the pituitary to exogenous GnRH was not altered by treatment with cortisol supports studies by Fonda *et al.* (1984) and by Estienne *et al.* (1991) but not that by Pearce *et al.* (1988) which found that, in pre-pubertal pigs, the LH response to GnRH was lower when GnRH was administered mid-way through a 1-h infusion of cortisol than during an equivalent infusion of saline. Nevertheless, our results indicate that the response of the pituitary to GnRH was not affected by cortisol. The approach used in the current experiment assessed the ability of the pituitary to release stores of LH from gonadotrophs in response to a large dose of exogenous GnRH and not the ability of the pituitary to respond to the continuous endogenous pulses of GnRH that occur in ovariectomised animals. The decrease in the pre-LH pulse nadir may, therefore, be due to a decrease in the ability of the pituitary to respond to endogenous pulses of GnRH and/or a decrease in the GnRH stimulus from the hypothalamus, and/or direct effects at the gonadotroph to alter the intracellular mechanisms which regulate synthesis and secretion of LH. Since the secretion of LH was not inhibited by the administration of cortisol during treatment with oestradiol, there is no evidence in this experiment to suggest that cortisol increased the negative feedback actions of oestrogens.

Although repeated acute elevation of cortisol did not impair LH secretion, an increase in the pre-LH pulse nadir occurred on the 8th day of twice daily i.v. treatment with 20 mg cortisol, in the absence of oestradiol. This apparent increase in basal secretion of LH did not occur in pigs in the 10 mg repeated acute elevation of cortisol treatment group, nor in either of the repeated acute elevation of cortisol treatment groups in the presence of oestradiol. Furthermore, despite the increase in basal plasma concentrations of LH in these pigs, there were no changes in the mean plasma concentrations of LH or the frequency or amplitude of LH pulses. The mechanism and the physiological significance of this increase in basal plasma concentrations of LH are unknown.

In conclusion, the results of this experiment indicate that the repeated acute elevation of cortisol, similar to that which would follow severe acute stress, did not inhibit the secretion of LH in ovariectomised pigs, in the absence or in the presence of oestradiol negative feedback. These results provide further support for the contention that reproduction in female pigs appears to be resistant to repeated acute stress. Indeed, it appears that, in ovariectomised pigs, the elevation of plasma concentrations of cortisol needs to be sustained for more than 4 days to achieve suppression of LH. Furthermore, this effect may be influenced by oestradiol because, in this experiment, 8 days of sustained elevation of plasma concentrations of cortisol reduced plasma concentrations of LH in ovariectomised pigs in the absence but not in the presence of oestradiol. The decrease in plasma concentrations of LH was associated with a decrease in basal concentrations of LH but there were no changes in the frequency or amplitude of LH pulses or the LH response to an injection of GnRH.

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