Effect of short-term elevations in plasma cortisol concentration on LH secretion in prepubertal gilts

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Summary. Prepubertal gilts were fitted with jugular vein and carotid artery catheters at 148 days of age. At 160 days of age the 24 gilts were allocated to treatment in a 2×2 factorial design involving intra-carotid infusion of cortisol (10 mg in 40 ml saline) or saline alone with or without i.v. injection of 5 µg synthetic GnRH midway through the 1 h infusion. Plasma cortisol concentrations were elevated in gilts infused with cortisol ($P < 0.05$). The LH response to exogenous GnRH was reduced by cortisol infusion. Treated gilts released less LH ($P < 0.001$) and had a lower mean LH peak ($P < 0.01$) than did control gilts but the timing of the induced LH peak was not affected. In the absence of an exogenous GnRH challenge, cortisol infusion increased the endogenous secretion of LH ($P < 0.01$). These results suggest that acute elevations in plasma cortisol concentration may be involved in mediating changes in pituitary responsiveness and the secretion of LH in the peripubertal gilt.

Keywords: prepubertal gilts; elevated cortisol; LH secretion

Introduction

The most effective methods available to stimulate the attainment of precocious puberty in the gilt are contact with a mature boar and management practices such as mixing, relocation and transportation of gilts. All of these methods of puberty stimulation induce an acute stress response in the gilt, the most reliable indicator of which is a rapid elevation in plasma cortisol concentrations (Dantzer & Mormede, 1981). This response has been demonstrated in gilts during physical contact with boars (Pearce & Hughes, 1987) and during transportation (Spencer, 1980). Boar-induced precocious puberty in the gilt is delayed by adrenalectomy (Killian *et al.*, 1987) or pharmacological inhibition of adrenal function (Wodzicka-Tomaszewska *et al.*, 1985) and enhanced by the additional exposure to the stress of transport (Wodzicka-Tomaszewska *et al.*, 1985) or relocation (Eastham & Cole, 1987). The widely accepted 'gonadostat' hypothesis for the endocrine control of puberty (Ramirez & McCann, 1963) requires a change in the sensitivity of the hypothalamic–pituitary axis to steroid feedback in the peripubertal period. The acute stress response to boar exposure and/or transport may be involved in mediating these changes in feedback in the gilt.

Therefore the present study investigated the influence of acutely elevated concentrations of plasma cortisol on the endogenous and GnRH-induced release of LH from the pituitary gland of prepubertal gilts.

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Materials and Methods

Animals. Prepubertal Large White $\times$ Landrace crossbred gilts from 6 litters were housed in metabolism crates in two groups of 12 gilts at a mean age of 148 days (range 142–153 days) and a mean liveweight of 73.0 kg (range 65.0–82.0 kg). The gilts were fitted with urinary catheters (Foley 2-way) and allowed 5–9 days to acclimatize to the crates. Each gilt was then surgically implanted with indwelling jugular vein and carotid artery catheters (Silastic medical grade tubing, i.d. 1.57 mm, o.d. 3.17 mm; Dow Corning Corp., Midland MI, U.S.A.) under halothane-induced anaesthesia.

Immediately after catheterization the gilts were returned to their crates and allowed a further 4–7 days to recover from surgery before treatment began at a mean age of 160 days. Gilts were slaughtered soon after treatment and their reproductive tracts were examined to assess reproductive status.

Treatment procedures. Six gilts were allocated according to litter across each of the following treatment groups: Group 1: infusion of 40 ml saline/ethanol solution (10% ethanol); Group 2: infusion of 40 ml saline/ethanol plus injection of 5 μ g GnRH; Group 3: infusion of 40 ml saline/ethanol containing 10 mg cortisol; and Group 4: infusion of 40 ml saline/ethanol containing 10 mg cortisol plus injection of 5 μ g GnRH. Saline (8.9 g NaCl/l) or cortisol (Sigma Chemical Co., St Louis, MO, U.S.A.) was infused by connecting a previously calibrated syringe infusion pump (Unita I, B. Braun, Melsungen, A.G., West Germany) to the carotid artery catheter of the gilt. The infusion lasted 1 h from 15:17 h until 16:17 h on the day of treatment. Halfway through the infusion Groups 2 and 4 were injected with 5 μ g synthetic gonadotrophin-releasing hormone (GnRH) in 1 ml saline (Gonadorelin as Fertylgal: Intervet (Australia) Pty Ltd, Artarman, NSW, Australia) via the jugular catheter which was immediately flushed with 3 ml saline. This analogue of GnRH is approximately 10% more potent than natural GnRH.

Hormone assays. Plasma concentrations of LH were determined using a double-antibody radioimmunoassay technique previously reported and validated by Niswender *et al.* (1970). Purified pig LH (LER-2-206) was iodinated with 125 I (New England Nuclearchemical Products, Boston, MA, U.S.A.) using the chloramine-T procedure and was used for the preparation of standards in the range of 0.125 to 32 ng/ml. After dilution of anti-pig LH serum (1:12 000, GDN No. 566), 100 μ l of this serum were added to each assay tube containing 100 μ l unknown plasma and 300 μ l phosphate-buffered saline. Samples were incubated at 4°C for 24 h before 100 μ l 125 I-labelled pig LH (10 000 c.p.m.) were added and incubated for a further 72 h at 4°C. Anti-rabbit gamma globulin (200 μ l) was then added and the samples were incubated for a further 24 h at 4°C. Then 1 ml phosphate-buffered saline was added and the samples were centrifuged at 3000 *g* for 30 min before the supernatant was aspirated, 250 μ l 0.1 N-NaOH were added and the radioactivity was determined in an automatic gamma counter.

The limit of detection was 0.25 ng/ml and samples containing less than 0.25 ng/ml were assigned this value for analysis. Standard plasma samples containing high (5 ng/ml) or low (1.5 ng/ml) concentrations of LH were included in the assays and used to estimate intra- and inter-coefficients of variation, which were 9.8 and 11.6% respectively.

Plasma concentrations of cortisol were determined by a competitive binding assay previously described in detail by Barnett *et al.* (1981). The limit of detection was 0.15 ng/ml and the intra- and inter-assay coefficients of variation were 4.6 and 8.6% respectively.

Statistical analysis. Plasma patterns of cortisol and LH were analysed by measuring total areas under the hormone curves (ng.min/ml) and calculating mean levels before and after GnRH injection. This was done before, during and after cortisol infusion and over all the samples taken between 14:00 and 18:00 h. Treatment effects were assessed using factorial analysis of variance procedures and paired Student's *t* tests.

Results

The mean plasma concentrations of total cortisol and LH for the four treatment groups are shown in Fig. 1. During the 1-h infusion, gilts in Groups 3 and 4 had higher plasma cortisol concentrations than did gilts in Groups 1 and 2 ($P < 0.05$) and their plasma cortisol concentrations did not return to baseline levels until 88 min after the infusion finished. The mean total area under the cortisol curve was smaller in Group 4 than in Group 3 gilts (1.2×10^5 vs 1.5×10^5 ng.min/ml, $P < 0.01$) but this was due to individual variation among gilts in the peak level of cortisol attained and not to the GnRH injection.

The mean areas under the LH curve before and after GnRH injection in Groups 2 and 4 and equivalent times in Groups 1 and 3 are shown in Fig. 2. There was no difference in LH secretion among treatments before the GnRH injections. More LH was released in response to GnRH in gilts in Group 2 (saline) than gilts in Group 4 (cortisol) ($P < 0.001$). The mean LH peak concentration in response to GnRH was also higher in saline-infused gilts than cortisol-infused gilts

Fig. 1. The effect of treatment on (a) cortisol and (b) LH concentrations (mean $\pm$ s.e.m.) in plasma of prepubertal gilts treated with saline (Group 1), saline + GnRH (Group 2), cortisol (Group 3) or cortisol + GnRH (Group 4). Horizontal bar = period of infusion; arrow = time of GnRH injection.

(4.2 ± 0.5 vs 2.6 ± 0.2 ng/ml, $P < 0.01$) but the interval from GnRH injection to the LH peak was not affected by treatment (Fig. 1). In gilts infused with saline, injection of GnRH significantly increased the total secretion of LH ($P < 0.01$). However, among the gilts infused with cortisol there was no significant difference in LH release due to GnRH injection (Fig. 2) because the gilts which did not receive GnRH also exhibited a significant increase of LH secretion above baseline levels during the infusion. In fact the gilts infused with cortisol alone secreted more LH than did gilts infused with saline alone ($P < 0.01$, Fig. 2).

All gilts had small undeveloped uteri and ovaries containing only small follicles (< 3 mm in diameter) at slaughter.

Fig. 2. The effects on mean $\pm$ s.e.m. area under the LH curve of treatment of prepubertal gilts with saline (Group 1), saline + GnRH (Group 2), cortisol (Group 3) or cortisol + GnRH (Group 4). *Or equivalent time in non-GnRH-injected gilts. a vs b: values are significantly different ($P < 0.001$); c vs d: values are significantly different ($P < 0.01$); e vs f: values are significantly different ($P < 0.05$).

Discussion

Acute elevations of plasma cortisol achieved by intra-carotid infusion of cortisol in prepubertal gilts significantly reduced the responsiveness of the pituitary gland to a single injection of synthetic GnRH. Although Fonda *et al.* (1984) observed no significant effect of exogenous cortisol or ACTH on GnRH-induced secretion of LH in intact prepubertal gilts, they used a very large dose of 400 μ g GnRH which may have over-ridden any changes in pituitary responsiveness. The dose of GnRH used in the present study induced an LH release within the normal physiological range for gilts reaching puberty (Esbenshade *et al.*, 1982). The concentrations of plasma cortisol obtained as a result of the infusion were also within the range of levels reported for gilts approaching puberty (Diekman & Trout, 1984) although they were higher than levels observed in gilts exposed to boars (Pearce & Hughes, 1987). The effects of treatments observed in the present study therefore probably reflect physiological rather than pharmacological responses.

Work with female rats (Baldwin & Sawyer, 1974) and male sheep (Matteri *et al.*, 1984) has shown that elevated plasma corticosteroid concentrations can reduce pituitary responsiveness to exogenous GnRH. The present study shows that this effect also operates in the female pig and may explain why elevations in plasma cortisol can depress the preovulatory surge of LH in mature gilts (Barb *et al.*, 1982) and why ACTH suppresses the increase in LH in prepubertal gilts after ovariectomy only when the adrenal glands are present (Fonda *et al.*, 1984).

Liptrap & Raeside (1983) reported that short-term cortisol infusions enhanced the pituitary responsiveness of mature boars to exogenous GnRH. However, high levels of plasma corticosteroids induced by exogenous ACTH have been reported to depress LH secretion in boars (Liptrap & Raeside, 1975; Pitzel *et al.*, 1984). It therefore appears that the effect of corticosteroids on pituitary responsiveness to GnRH may vary with the method used to increase plasma

corticosteroids, the duration of the increase and also with the sex and physiological status of the animals.

In the absence of exogenous GnRH, LH secretion was greater in cortisol-infused than in saline-infused gilts. This may simply be due to random occurrence of LH pulses in individual animals or it may represent a real increase in the endogenous release of LH induced by cortisol. Evidence to support the latter suggestion has been obtained by Li (1987) who found that incubation of pig pituitary cells with cortisol increased their basal secretion of LH but decreased their LH release in response to exogenous GnRH. This finding is in complete agreement with our results *in vivo* and suggests that corticosteroids may play a complex role in regulating LH secretion in female pigs.

Lutz *et al.* (1984) reported that endogenous serum LH increased immediately before puberty in gilts and this was suggested to be a consequence of a decrease in the sensitivity to negative oestrogen feedback on gonadotrophin secretion which is consistent with the 'gonadostat' hypothesis of puberty attainment (Ramirez & McCann, 1963). However, Lutz *et al.* (1984) also reported that initial changes in serum LH secretion in the peripubertal gilt occurred while serum oestrogen concentrations were constant and work with women (Conte *et al.*, 1981) and ewes (Foster & Ryan, 1979) has shown that the peripubertal rise in LH is also independent of gonadal steroids. Furthermore, it has been proposed from work with rats that the change in the sensitivity of the gonadostat occurs after, not before, the pubertal rise in LH (Andrews *et al.*, 1981). These studies suggest that other factors may also be involved in controlling prepubertal gonadotrophin secretion. Our observations that acute elevations of plasma cortisol altered endogenous LH secretion suggests that plasma cortisol concentrations are such a factor in the prepubertal gilt. The physiological significance of the acute elevation of plasma cortisol known to occur in the gilt during exposure to mature boars (Pearce & Hughes, 1987) may therefore be to increase basal LH secretion at the time of puberty onset.

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