Effect of transport stress on luteinizing hormone released by GnRH in dairy cows

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Abstract. Stress is thought to reduce gonadotrophin secretion in ruminants. So far, only prolonged administration of ACTH or cortisol have been shown experimentally to reduce LH release. The present experiment studied the effects of an acutely stressful situation. Fifteen min after the start of a 30 min truck journey for dairy cows plasma cortisol values exceeded 55 nmol/l, as measured by RIA. Values began to decline 15–45 min after return to the barn and reached baseline (2.5 to 20 nmol/l) 1–2 h later. Responses to 20 or 40 µg GnRH administered on day 10 of the cycle, or 48 h after prostaglandin analogue, were significantly reduced on a within-animal basis when given during the truck journey. Hence acute endogenous stimulation of pituitary-adrenal activity can interfere with pituitary LH release.

It is well known that hyperadrenal activity interferes with reproduction in cattle (Wagner & Li 1982; Moberg 1984). ACTH and cortisol individually have been shown to reduce the spontaneous preovulatory surge of LH in normally cycling cows (Stoebel & Moberg 1982; Li & Wagner 1983a) and also to reduce the amount of LH released by gonadotropin-releasing hormone (GnRH) during the oestrous cycle (Matteri & Moberg 1982; Li & Wagner 1983b). These experiments have involved pharmacological stimulation of adrenal activity; it is important to know whether similar effects may be shown in cows subjected to a stressor which causes endogenous stimulation of pituitary-adrenal activity.

Even a short truck journey (5–15 min) has a marked effect on milk and plasma cortisol value (Dobson et al. 1986). The aim of the present experiment was to assess the effect of acute stimulation of pituitary-adrenal activity (by a short truck journey) on the release of LH by low doses (20 or 40 µg) of GnRH. The earlier workers quoted above used a 100-µg dose GnRH which releases pre-ovulatory concentrations of LH (> 30 µg/l); using lower doses may stimulate the LH pulses which occur earlier in the follicular phase in cattle (Rahe et al. 1980).

Materials and Methods

Animals and experimental procedures

Four mature non-lactating Friesian cows (4–7 years; 500–600 kg body weight) were housed inside in the same tied staniings for at least 2 months prior to the experiments which took place in March and April. The cows were fed a maintenance ration of hay and concentrates and subjected to natural lighting conditions. At the beginning of the series of experiments, oestrous cycles were synchronized with 2 im injections of synthetic prostaglandin PGF₂α (cloprostenol; ICI, Macclesfield, UK) spaced 11 days apart. One day prior to each experiment, indwelling jugular catheters (outside diameter 2 mm, inside diameter 1 mm; Portex Plastics, Hythe, UK) were inserted under local anaesthesia. To maintain patency, the catheters were filled with sterile saline containing 10 U heparin/ml. Two doses of GnRH were investigated: 20 or 40 µg in 2 ml of sterile saline prepared from a synthetic natural GnRH (gonadorelin; Hoechst, Milton Keynes, UK). One day prior to each truck journey, the cows were challenged with GnRH. Blood samples (10 ml) were taken every 15 min for 30 min prior to, and 3 h after, iv injection of GnRH through the indwelling sampling catheter. The heparinized samples (10 U/ml) were centrifuged at 1000 × g as soon as possible and the plasma stored at −15°C to await hormone analysis.
Experiment 1. Ten days after an oestrus synchronized with a synthetic prostaglandin (cloprostenol, ICI, Macclesfield, UK), each cow was given 40 µg of GnRH iv and blood samples were taken as described above. The following day the cows were taken in pairs on a truck journey for a total of 30 min along tarmaced country roads. The space in the truck was 20 × 3 m for each cow, with a sloping loading ramp. Blood sampling occurred every 15 min from 1 h before the start of the journey for a total of 5.5 h. Fifteen min after the start of the journey, the truck was stopped and 40 µg of GnRH administered iv. At the end of the journey each cow returned to its original place in the shippon.

Experiment 2. Forty-eight hours after a cloprostenol injection, the four cows were given 40 µg of GnRH iv and bled as described above. On another occasion each cow was injected with cloprostenol and taken on a truck journey, as previously described, with GnRH (40 µg) administered 48 h after cloprostenol.

Experiment 3. This was a repeat of experiment 1 using 20 µg of GnRH throughout.

Hormone analysis
LH and cortisol were measured by radioimmunoassays verified and characterized elsewhere: LH (Alam & Dobson 1986), cortisol (Alam et al. 1986). Interassay coefficients of variation were < 14% for each assay. The LH antibody had a cross-reaction of 30% with TSH but < 1% with other pituitary hormones. The cortisol antibody had a < 10% cross-reaction with 11-substituted steroids and < 1% with other steroids. All samples from each experiment were analyzed within one assay.

Results

Behavioural response to transport
During each truck journey the cows were restrained by a rope halter only. There was some reluctance to climb the ramp into the truck after the first journey, especially by Cow 888, but during all the journeys none of the cows displayed
excessive fear except for minor stumbling when the truck went around corners. At no time did the cows pull on the halter, nor did they sit down. After each journey the cows walked quietly back to their original positions in the barn.

Cortisol response to transport

During the control sampling periods, i.e. the day prior to transport, individual cortisol values varied between 2.5 and 20 nmol/l: concentrations tended to be higher at the beginning of sampling periods than at the end. Even on the day of the third journey, values remained within this range prior to loading into the truck. Fifteen min into the journey, cortisol values exceeded 55 nmol/l in all cows on all journeys except in Cow 731 (maximum 27 nmol/l). Values began to decline 15–45 min after return to the barn and reached baseline 2–3 h later (Fig. 1).

LH response to GnRH

All animals produced maximum LH values 15–30 min after GnRH and returned to baseline values 2–3 h afterwards. Detailed results are given in Table 1: values are shown for each cow owing to variation in response between individual cows. Responses to 40 µg of GnRH in the luteal phase (Day 10: Experiment 1) were similar to those in the follicular phase (48 h after cloprostenol: Experiment 2) except for Cow 731 which responded with a pre-ovulatory type surge of LH in the follicular phase. Pre-treatment LH values were higher in this cow than in the others. The response to 20 µg of GnRH in the luteal phase (Experiment 3) was lower than to 40 µg of GnRH. In every experiment on a within-cow basis, the response to GnRH administered during transport was significantly lower than to the control GnRH injection ($P < 0.01$, Student’s paired $t$-test).

**Discussion**

The amount of LH released by the 20-µg dose of GnRH was similar to that of endogenous tonic LH pulses during the late luteal phase (Rahe et al. 1980). The higher dose of 40 µg of GnRH caused a greater release of LH similar to that during the late follicular phase, but not as great as the pre-ovulatory surge, except in cow 731 on one occasion. It would seem that these doses of GnRH may be more meaningful, physiologically, than the much higher doses used by many other authors. The main drawback for analysis was the large variation in responses between cows, so results have been considered on a within-cow basis.

This is one of the first demonstrations of stress-induced pituitary-adrenal activity causing a reduction in the LH response to GnRH. Earlier reports have depended on pharmacological means of increasing plasma cortisol concentrations (Matteri & Moberg 1982; Li & Wagner 1983).
Transport resulted in maximal secretion of cortisol 15 min after the start of the journey and declined 1–2 h after the return confirming earlier reports (Dobson et al. 1986). Although the frequency of sampling in the present experiment was only every 15 min, another work (Dobson et al. 1986) suggests that marked increases in plasma cortisol occur within 5 min. The influence of cortisol to reduce the LH response to GnRH consequently occurred after only 5–10 min of increased pituitary-adrenal activity. This is contrary to expectations from other work (Stoebel & Moberg 1982; Li & Wagner 1983b) in which prolonged cortisol hypersecretion (>3 days) was implicated: these studies used exogenous ACTH or cortisol. It is possible that stress-induced increases in endogenous ACTH and cortisol result in increased secretion of other compounds, such as the opioid β-endorphin, which may have a more immediate effect on pituitary LH release (Howlett & Rees 1986).

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References


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