Effects of naloxone on the oestradiol-induced LH surge and cortisol release in transported cows

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Summary. Four control cows released a normal LH surge in response to 1 mg oestradiol benzoate i.m. Compared to controls, 30 min transport of 5 cows 16–18 h after oestradiol significantly delayed the onset ($P < 0.05$), suppressed the amplitude ($P < 0.01$) and reduced the total LH release ($P < 0.001$) in 4 cows and totally blocked the surge in the 5th cow. Just before the onset of transport, 5 cows were given 250 mg naloxone i.v. In 3 cows, the LH surge was delayed and reduced in amplitude and duration and was totally blocked in the other two, i.e. naloxone did not avert the detrimental effects of transport.

Transport stimulated cortisol release in cows. Cows given naloxone + transport released significantly more ($P < 0.001$) cortisol than did those subjected to transport alone. In conclusion, naloxone appeared to have further stimulated the hypothalamo-pituitary-adrenal axis of cows under stress.

Keywords: transport; LH; cortisol; naloxone; opioids; cows

Introduction

The preovulatory LH surge is a vital component of the reproductive process in animals. Defective LH surges have been implicated in various ovulatory disturbances in cattle (Nadaraja & Hansel, 1976; Kesler *et al.*, 1979; Refsal *et al.*, 1988), and events such as suckling (Radford *et al.*, 1978; Short *et al.*, 1979) and transport (Nanda *et al.*, 1988a) can delay and/or attenuate preovulatory LH surges induced in cattle by oestradiol. In response to a variety of acute stressors, cortisol is secreted (Echternkamp, 1984; Dobson, 1987) simultaneously with β -endorphin, an endogenous opioid peptide (Guillemin *et al.*, 1977). Treatment with opioid agonists blocked spontaneous or oestradiol-induced LH surges in rats (Ieiri *et al.*, 1980), sheep (Brooks *et al.*, 1986) and cattle (Nanda *et al.*, 1988b) and this could be prevented by simultaneous treatment with naloxone or other opioid antagonists. Naloxone alone on the morning of pro-oestrus advanced the LH surge in rats (Allen & Kalra, 1986). A decline in portal blood concentrations of β -endorphin occurs at the time of the preovulatory LH surge in rats (Sarkar & Yen, 1985) and sheep (Bernard *et al.*, 1988). The LH inhibition of β -endorphin manifested during the period preceding hypothalamic GnRH and plasma LH surges is removed at the time of preovulatory LH surge (Bernard *et al.*, 1988).

From the above evidence, in this study we tested the hypothesis that opioids are involved with stress-induced aberrations in the LH surge and that naloxone would avert the detrimental effects of 30 min transport at the expected onset of an oestradiol-induced LH surge in early post-partum cows (Nanda *et al.*, 1988a).

Materials and Methods

Animals. Nine lactating Holstein-Friesian dairy cows within 1 month *post partum* were housed in groups of 4 or 5 in tied stanchions and fed standard dairy concentrates, and hay and water *ad libitum*. Ovarian activity was monitored weekly by palpation *per rectum* and by measurement of plasma progesterone concentration in tail vein blood.

Treatments. Cows were given 1 mg oestradiol benzoate (Intervet Laboratories Ltd, Cambridge, UK) in 2 ml arachis oil i.m. An indwelling jugular vein catheter was inserted a day before frequent blood sampling. When applicable, the cows were transported around the time of the expected onset of the LH surge, i.e. 16–18 h after oestradiol. The cows were transported either individually or in pairs in a truck that had 3×1 metres of space for each cow. Each journey lasted for 25–30 min along metalled country roads.

Four cows served as controls and were given oestradiol alone. Another group of 5 cows was transported 16–18 h after oestradiol. On another occasion, the same 5 cows received 250 mg naloxone hydrochloride (Sigma, St Louis, MO, USA) in 10 ml 0.9% (w/v) sodium chloride solution injected through the jugular venous catheter immediately before the journey. The transport and the transport + naloxone treatments were given in a crossover design with an interval of 1 week. Heparinized blood samples were collected at the time of oestradiol administration, every 2 h from 16 to 32 h after oestradiol to characterize the LH surge, and every 15 min from 1 h before to 2.5 h after each journey, to assess changes in cortisol concentrations. The blood samples were centrifuged immediately for 10 min at 1000 g and plasma stored at -15°C until analysis.

Hormone analyses. Plasma progesterone, LH and cortisol were analysed by radioimmunoassays described elsewhere (Kanchev *et al.*, 1976; Alam & Dobson, 1986; Alam *et al.*, 1986; respectively). Intra- and inter-assay coefficients of variation were $<10\%$ except for 14.7% inter-assay variation for cortisol. Assay sensitivity, defined as twice the standard deviation above the buffer control, was 0.015 ng/ml for progesterone, 0.5 ng/ml for LH and 0.1 ng/ml for cortisol.

Statistical analysis. An increase in LH values to ≥ 10 ng/ml in 2 consecutive samples collected 2 h apart was considered as an LH surge. The first rise to ≥ 10 ng/ml was considered as the onset of the surge. The time of onset, the duration, the peak value and the area under the curve measured with a planimeter (arbitrary units) in treated cows were compared by Student's *t* tests with corresponding values in control cows. The comparison between 2 treatment groups was made by a paired Student's *t* test. The maximum rise above the pre-treatment cortisol concentration and the areas under the curve of cortisol released after transport and naloxone + transport treatments were also compared by paired Student's *t* test.

Results

All the cows remained acyclic during the period of investigation with plasma progesterone concentration <0.5 ng/ml at each oestradiol treatment.

The characteristics of the oestradiol-induced LH surge in the control, the transported and the naloxone + transported cows are given in Table 1. All the control cows had a normal LH surge. In the transported cows, the surge was significantly delayed, shortened, of reduced amplitude and had a reduced area under the curve ($P < 0.05$ – $P < 0.01$) in 4 cows, and was totally blocked in the fifth. The LH surge in the naloxone + transported cows was also delayed and reduced in 3 cows and was totally blocked in the other 2. The characteristics of the LH surges in the transport and naloxone + transport groups were not different ($P > 0.05$).

Table 1. Characteristics of the LH surge in response to oestradiol in control, transported (30 min), and naloxone (250 mg) + transport-treated cows in the early post-partum period

Treatment	Total no. of cows	Onset (h) after oestradiol	Duration (h)	Peak conc. (ng/ml)	Area under the curve (Arbitrary Units)
Control	4	18.0 ± 2.8	7.0 ± 1.2	64.7 ± 17.1	52.2 ± 8.1
Transport	5†	$23.5 \pm 3.4^*$	$3.5 \pm 1.0^{**}$	$35.0 \pm 10.9^*$	$24.4 \pm 11.3^{**}$
Naloxone + transport	5‡	23.3 ± 5.8	$3.3 \pm 1.2^{**}$	$26.7 \pm 4.9^*$	$15.3 \pm 4.7^{***}$

Values are mean $\pm$ s.d.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with the control values.

†One cow did not produce an LH surge and values are not included.

‡Two cows did not produce an LH surge and values are not included.

All cows released cortisol in response to transport (Fig. 1). The peak cortisol concentration occurred 30–45 min after the start of the journey. The mean peak concentration was 4.4 ± 1.8 times higher than the pretreatment cortisol concentration in the transported cows and 5.4 ± 2.0

times higher in the naloxone + transported cows. The area under the cortisol curve was significantly higher ($P < 0.001$) after naloxone + transport (58.0 ± 14.0 units) than after transport alone (32.0 ± 5.3 units).

Fig. 1. Plasma cortisol concentrations (mean $\pm$ s.e.m.) in 5 cows in response to 30 min transport alone (●---●) or simultaneously with 250 mg naloxone i.v. (●—●). Naloxone was injected and transport began at time 0.

Discussion

All the transported cows had an abnormal LH surge in response to oestradiol, confirming earlier results (Nanda *et al.*, 1988a). Administration of naloxone at the onset of transport did not avert the detrimental effects of transport. In fact, more cows failed to release an LH surge and even the responding cows released a reduced amount of LH after naloxone + transport than after transport alone. Although it could not be proved statistically because of the low number of animals, naloxone appeared to have added to the stressful effects of transport on LH release. Unlike unstressed rats (Allen & Kalra, 1986), naloxone failed to advance the onset of the LH surge in the transported cows.

Transport alone increased the release of cortisol in cows, as reported by Dobson (1987) and Mitchell *et al.* (1988). Incorporation of naloxone with transport further stimulated the release of cortisol beyond that of transport alone. Naloxone alone elevated cortisol concentrations in cows, to an extent directly proportional to the pre-treatment cortisol concentrations (Nanda *et al.*, 1989) suggesting that naloxone augments stress-induced cortisol release. Similar studies on farm animals are not available for comparison, but ACTH and corticosterone were greater after photic or audiogenic stress in naloxone-injected rats compared to saline-treated controls (Siegel *et al.*, 1982). On the other hand, both morphine and naloxone prevented elevation of plasma corticosteroids in response to ether stress in rats (Gibson *et al.*, 1979). The difference in the effects of naloxone may be due to the different types of opioid receptors involved. Moreover, opioid regulation of cortisol in

rats is unlike that of cows; cortisol concentrations are suppressed by opioid agonists in cows (Nanda *et al.*, 1989), but elevated in rats (Grossman & Rees, 1983).

An opioidergic system is actively involved in the regulation of LH release in early post-partum cows as revealed by increased LH responses to naloxone (Whisnant *et al.*, 1986; Cross *et al.*, 1987). There are also high opioid receptor concentrations in parts of the brain known to release GnRH (Trout & Malven, 1988). If stress were opioid-mediated, naloxone would be expected to counteract the suppressive effects of stress on the LH surge. The inability of naloxone to do so may therefore disprove our hypothesis. However, this conclusion may not be true. Apart from the effects of naloxone on the GnRH-related opioids, naloxone also affects the release of cortisol. Since ACTH is co-secreted with β -endorphin (Guillemin *et al.*, 1977) from a common precursor, pro-opiomelanocortin (Imura *et al.*, 1983), an increase in cortisol may reflect an exaggerated increase in the β -endorphin concentration after treatment with naloxone as well as transportation. This may explain the tendency to further suppression of oestradiol-induced LH surges by naloxone in the transported cows.

Plasma concentrations of β -endorphin increase after acute physical stress to cows (Shutt & Fell, 1988). A temporal relationship has been reported between a rise in plasma β -endorphin and suppression in the tonic LH release in sheep in response to isolation and confinement (Malven, 1987), *E. coli* bacteraemia (Leshin & Malven, 1984) or acupuncture (Malven *et al.*, 1984). Treatment with naloxone failed to relieve the LH suppression in these situations. Naloxone also failed to induce LH release in cows with increased plasma cortisol concentrations (Gregg *et al.*, 1986; Whisnant *et al.*, 1986).

The release of β -endorphin is in part regulated by its own negative feedback mechanism (Carr *et al.*, 1982), which may be interrupted by the receptor antagonist, naloxone. A continuing stressor (transport) and a lack of negative feedback may explain the (suspected) increase in β -endorphin (as reflected by cortisol release). The further stimulation of the hypothalamo-pituitary-adrenal axis, which may have implications for LH release, rendered this model unfit to study our hypothesis. A lack of improvement in LH release after naloxone in stressful situations may not, however, be interpreted as an exclusion of opioid involvement.

In conclusion, naloxone failed to avert the detrimental effects of transport stress on oestradiol-induced LH surge. However, this may not disprove our hypothesis that stress suppresses LH through opioids, because naloxone given to transported cows appeared to aggravate further the release of opioids as reflected by the increased cortisol release.

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