EFFECT OF NALOXONE ON SERUM LUTEINIZING HORMONE, CORTISOL AND PROLACTIN CONCENTRATIONS IN ANESTROUS BEEF COWS

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ABSTRACT

Two experiments were conducted with the opioid antagonist naloxone to determine the effect of opioid receptor blockade on hormone secretion in postpartum beef cows. In Exp. 1, nine anestrous postpartum beef cows were used to measure the effect of naloxone on serum luteinizing hormone (LH), cortisol and prolactin concentrations. Cows received either saline (n = 4) or 200 mg naloxone in saline (n = 5) iv. Blood samples were collected at 15-min intervals for 2 h before and after naloxone administration. Serum LH concentrations increased (P<.01) in naloxone-treated cows from 1.8 ± .04 ng/ml before treatment to 3.9 ± .7 ng/ml and 4.2 ± .5 ng/ml at 15 and 30 min, respectively, after naloxone administration. In contrast, LH remained unchanged in saline-treated cows (1.6 ± .3 ng/ml). Serum cortisol and prolactin concentrations were not different between groups. In Exp. 2, 12 anestrous postpartum beef cows were used to examine the influence of days postpartum on the serum LH response to naloxone. Four cows each at 14 ± 1.2, 28 ± 3.4 and 42 ± 1.5 d postpartum received 200 mg of naloxone in saline iv. Blood samples were taken as in the previous experiment. A second dose of naloxone was administered 2 h after the first, and blood samples were collected for a further 2 h. Serum LH concentrations increased (P<.01) only in cows at 42 d postpartum. Serum LH concentrations in cows at 42 d postpartum increased from 2.1 ± .4 to 3.6 ± .6 ng/ml and from 2.2 ± .3 to 3.4 ± .5 ng/ml after the first and second administrations of naloxone, respectively. These results suggest that endogenous opioids influence LH secretion in the postpartum beef cow, and that the ability of naloxone to increase serum LH concentrations changes during the postpartum period.

(Key Words: Naloxone, LH, Cortisol, Prolactin, Beef Cows.)

Introduction

Inadequate luteinizing hormone (LH) secretion is a primary factor modulating the return to estrus in postpartum beef cows (Lamming et al., 1981). Luteinizing hormone concentrations gradually increase over time with the return of pulsatile LH secretion and ultimately the first postpartum ovulatory surge of LH (Lamming et al., 1981; Humphrey et al., 1983). Suckled cows have lower serum LH concentrations and fewer LH pulses than nonsuckled cows (Dunlap et al., 1981b), and calf removal increases serum LH concentrations and peak frequency (Walters et al., 1982). However, the mechanism by which suckling suppresses LH secretion is unknown.

A large body of evidence in other species indicates that endogenous opioid peptides are inhibitory to LH secretion (Meites et al., 1979). In addition, naloxone, an opioid antagonist, increased serum LH concentrations in the rat (Meites et al., 1979) human (Quigley and Yen, 1980) chimpanzee (Gosselin et al., 1983), sheep (Malven et al., 1984a) and pig (Barb et al., 1985). Similarly, serum cortisol concentrations increased after naloxone administration in humans (Blankstein et al., 1980) and chimpanzees (Gosselin et al., 1983). The effects of naloxone on serum prolactin concentrations are less consistent. In the human, naloxone decreased (Rubin et al., 1979), increased (Snowden et al., 1984) or had no effect (Blankstein et al., 1980) on serum prolactin concentrations, depending on the sex of the subjects and stage of the menstrual cycle. Based on these reports,
the present studies were conducted to characterize the hormonal response to naloxone in the postpartum beef cow and to determine the effect of days postpartum on the hormonal response to naloxone.

**Materials and Methods**

*Exp. 1.* Nine anestrous, postpartum (48 ± 4 d; mean ± SE) beef cows averaging 408 ± 16 kg were assigned to receive either saline (n = 4) or 200 mg naloxone\(^5\)\(^6\) dissolved in saline iv (n = 5). On the day prior to blood sampling, a cannula was inserted into a jugular vein of each cow and the ovaries were examined via rectal palpation to assess ovarian activity. This examination indicated the absence of prominent ovarian structures. Cattle were placed in stanchions on the day of bleeding and calves were removed at the beginning of blood sampling to eliminate acute effects of suckling on serum cortisol or prolactin concentrations. Blood samples (10 ml) were taken at 15-min intervals for 2 h before and 2 h after naloxone administration. Blood samples were stored overnight at 4 C, then centrifuged; serum was stored at -20 C until assayed. Serum LH, cortisol and prolactin concentrations were determined in all samples. Serum concentrations of cortisol were determined as described by Dunlap et al. (1981a), and serum prolactin concentrations were determined by radioimmunoassay as described by Whisnant et al. (1986).

*Exp. 2.* Twelve anestrous postpartum beef cows, at 14 ± 1.2 (n = 4), 28 ± 3 (n = 4) and 42 ± 1.5 d (n = 4) postpartum, were used to examine the influence of days postpartum on the LH response to 200 mg of naloxone iv. On the day prior to blood sampling, a jugular cannula was inserted into each cow and the ovaries were examined via rectal palpation to assess ovarian activity as in Exp. 1. Calves were removed from all cows at the beginning of blood sampling. Blood samples were taken as in Exp. 1. Two hours after the first injection of naloxone all cows received a second 200-mg injection of naloxone and samples were collected for a further 2 h. Blood samples were processed as in Exp. 1 and serum LH concentrations were determined in all samples.

Data for both experiments were analyzed by a general linear model split-plot-in-time analysis of variance (Gill and Hafs, 1971) using Statistical Analysis System (SAS, 1979).

**Results**

*Exp. 1. Hormonal Responses to Naloxone.* Prior to naloxone treatment, serum LH concentrations for the two groups averaged 1.5 ± .06 ng/ml and were not different. After naloxone administration, serum LH concentrations increased (P<.01) from 1.8 ± .04 ng/ml to 3.9 ± .7 ng/ml and 4.2 ± .5 ng/ml at 15 and 30 min later (figure 1). Serum LH concentrations returned to baseline by 60 min after naloxone treatment. Saline had no effect on serum LH concentrations. One cow did not respond to naloxone with an increase in serum LH concentrations. This animal was excluded from analysis because of elevated serum cortisol concentrations.

Administration of naloxone iv resulted in an apparent elevation of mean serum cortisol concentrations from 4.5 ± 1.3 to 9.4 ± 2.8 ng/ml (figure 2), but this increase was not significant due to large variations between animals. The data from the one cow that failed to respond to naloxone with an increase in serum LH concentrations are presented in figure 3. Serum LH concentrations for this cow averaged .3 ± .08 ng/ml and were not affected by naloxone. Serum cortisol concentrations for this cow averaged 50 ± 2.6 ng/ml, which is indicative of

\(^5\)Sigma Chemical Corp., St. Louis, MO.

\(^6\)Mention of a trade name, proprietary product or specific equipment does not constitute a guarantee or warranty by the USDA or the Univ. of Georgia, and does not imply its approval to the exclusion of other products.
stress. Serum cortisol concentrations for other cows in this study averaged 8.6 ± 3.5 ng/ml.

Serum prolactin concentrations ranged from 7 to 12 ng/ml in this study, with an average of 10.8 ± 1.4 ng/ml for naloxone-treated cows and 9.3 ± 1.8 ng/ml for saline-treated cows (figure 4). Naloxone did not affect serum prolactin concentrations (P > 0.05).

**Exp. 2. Effect of Days Postpartum on the LH Response to Naloxone.** Serum LH concentrations increased after naloxone in one of four cows on d 14 and 0 of four cows on d 28 postpartum, compared with three of four cows on d 42 postpartum (figure 5). One cow at d 28 exhibited two LH increases but these occurred 45 to 60 min after treatment with naloxone. We concluded that these increases were LH pulses and not due to naloxone because of the lag time between naloxone injection and the rise in LH. In all other cases with naloxone, we have seen an immediate response. Figure 5 represents mean serum LH concentrations for all cows including those that did not respond to the two naloxone injections. Only at 42 d postpartum was the serum LH response to naloxone similar to that seen in the first experiment. Serum LH concentrations increased (P < 0.01) from 2.1 ± 0.4 to 3.6 ± 0.6 ng/ml after the first naloxone injection. In response to the second dose of naloxone two of four cows responded with increased serum LH concentrations (P < 0.01) at 42 d postpartum, while no cows responded with increased LH secretion at 14 and 28 d postpartum.

**Discussion**

These results indicate that naloxone clearly elevated serum LH concentrations in postpartum anestrous beef cows, suggesting a role for
opioid peptides in the suppression of LH secretion during the postpartum anestrous period. These data are in agreement with research in cycling rats (Meites et al., 1979), women (Quigley and Yen, 1980), chimpanzees (Gosselin et al., 1983), ewes (Malven et al., 1984a) and gilts (Barb et al., 1985).

Suckling depresses serum LH concentrations in the cow (Dunlap et al., 1981b) but the mechanism of the suckling-induced inhibition of LH secretion is unknown. In the postpartum rat, suckling increased plasma β-endorphin concentrations (Riskind et al., 1984), but whether β-endorphin in the peripheral circulation can influence LH secretion is unclear. Most evidence points to the hypothalamus as the site of action for naloxone and opioids. Naloxone had no effect on LH release from rat pituitary explants (Cicero et al., 1979). In contrast, naloxone increased gonadotropin releasing hormone (GnRH) release from the hypothalamus of rats (Wilkes and Yen, 1981) and humans (Rasmussen et al., 1983) in vitro. Suckling may affect brain opioid activity in the cow; Malven et al. (1984b) reported that hypothalamic levels of dynorphin changed following calf removal. The ability of naloxone to increase serum LH concentrations is evidence for a physiological role for the endogenous opioids in inhibiting LH secretion in the postpartum cow.

In the second experiment naloxone treatment had no effect on serum LH concentrations at 14 and 28 d postpartum. These results may be interpreted as indicating that endogenous opioid tone changes with time postpartum. In rats, brain concentrations of β-endorphin decreased after parturition from concentrations seen during late gestation (Wardlaw and Frantz, 1983). In the cow endogenous opioid concentrations may be higher during the early postpartum period, and a larger dose of naloxone may be needed to increase serum LH concentrations.

Other factors could also explain the failure of naloxone to induce LH release at d 14 and 28 postpartum. Webb et al. (1977) reported that pituitary responsiveness to GnRH did not fully recover until 20 to 30 d postpartum. Still some response would be expected if naloxone caused release of GnRH at d 14 and 28. Hypothalamic content of GnRH does not differ over time postpartum (Moss et al., 1985), but releasable pools of GnRH could be different. Finally, an inhibitory neuronal system, other than the opioids, could be active on d 14 and 28.

Serum cortisol concentrations were increased by naloxone treatment in humans (Blankstein et al., 1980) and chimpanzees (Gosselin et al., 1983). In contrast, naloxone administration did not increase serum cortisol concentrations in the present study. However the dose of naloxone or the physiological state of the animal may influence these results. The cow represented in figure 3 did not exhibit increased serum LH concentrations after naloxone. This animal had elevated serum cortisol concentrations indicative of stress and could explain the failure of this animal to respond to naloxone. High serum levels of cortisol inhibit LH secretion (Li and Wagner, 1983). Also, Guillemiin et al. (1977) demonstrated that stress results in the release of endogenous opioids as well as adrenocorticotropic hormone and glucocorticoids. This cow may have had elevated levels of endogenous opioids, and therefore the dose of naloxone may have been insufficient to stimulate LH release in the face of elevated opioid tone.

Administration of naloxone decreased serum prolactin concentrations in rats (Meites et al., 1979) and men (Rubin et al., 1979). In women, naloxone treatment had no effect on serum prolactin concentrations in the follicular phase (Blankstein et al., 1980) but serum prolactin...
concentrations were increased after naloxone in the luteal phase (Snowden et al., 1984; Cetel et al., 1985). Recently, Piva et al. (1985) found no effect of naloxone on serum prolactin concentrations in the rat. In this study, no change in serum prolactin concentrations occurred after naloxone administration. However, based on the results in women, the prolactin response might be different in cycling cows and might vary according to the stage of the cycle.

The results of these studies provide evidence that naloxone increases serum LH concentration in the anestrous postpartum beef cow after approximately 40 postpartum. Furthermore, it is suggested that endogenous opioid peptides may inhibit LH secretion during at least the latter part of the postpartum period. The lack of effect of naloxone during the early postpartum period may indicate changing brain opioid tone over time postpartum. Serum cortisol and prolactin concentrations were not affected by naloxone administration. Further research is needed to clarify the role of the endogenous opioid peptides in regulating LH secretion in the anestrous postpartum beef cow.

Literature Cited


