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Effect of naloxone on the secretion of LH in infantile and prepubertal Holstein bull calves*

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Summary. Administration of naloxone (100 mg i.v.; approximately 1·21 mg/kg body weight$^{0.75}$) to 10 intact calves (24 weeks of age) caused an acute release of LH that was similar in amplitude and duration to spontaneous discharges of LH that occur at the same age. The naloxone-induced release of LH was abolished in 9/10 calves (intact and castrated) treated with oestradiol-17β. To determine the ontogeny of opioid control of secretion of LH, 12 calves were randomly assigned to receive saline or naloxone (1·21 mg/kg body weight$^{0.75}$, i.v.) at 3, 5, 7, 9, 11, 13, 17 and 21 weeks of age. At each age, blood was collected at 10-min intervals for 4 h and saline or naloxone was administered (i.v.) after collection of the 120-min sample. Before administration of naloxone, plasma LH values increased with age ($P < 0·01$) but did not differ between the control and naloxone groups (age × treatment, $P > 0·05$). Administration of naloxone caused concentrations of plasma LH to increase at 3, 11, 13, 17 and 21 weeks of age (treatment × time, $P < 0·001$). Concentrations of LH (saline *vs* naloxone, ng/ml) reached a maximum within 20 min after treatment at Weeks 3 (0·3 *vs* 1·2), 11 (0·6 *vs* 2·6), 13 (0·6 *vs* 3·7), 17 (1·1 *vs* 2·6), and within 40 min after treatment at Week 21 (1·0 *vs* 3·5). Based on these results, it is concluded that endogenous opioids inhibit the secretion of LH during infancy and the later prepubertal periods of life in bulls but not around the time of the spontaneous increase in secretion of LH that marks the end of the infantile period.

Keywords: naloxone; LH; puberty; bulls

Introduction

Endogenous opioids have been shown to be involved in regulating secretion of luteinizing hormone (LH) in a variety of adult mammals, including rats (Bruni *et al.*, 1977), pigs (Barb *et al.*, 1985), sheep (Schillo *et al.*, 1985), cows (Whisnant *et al.*, 1984, 1985), monkeys (VanVugt *et al.*, 1984) and humans (Ropert *et al.*, 1981). In general, opiates appear to inhibit secretion of LH in those species studied (Bruni *et al.*, 1977; Parvizi & Ellendorff, 1980; Brooks *et al.*, 1986b) and administration of an opioid antagonist, such as naloxone, at various stages of the oestrous or menstrual cycle results in increased secretion of LH (Blumberg & Dayton, 1974). It has been demonstrated that gonadal steroids influence the ability of exogenous opioids and naloxone to modify the secretion of LH in rats (Gabriel *et al.*, 1983), pigs (Barb *et al.*, 1985), sheep (Trout & Malven, 1984; Brooks *et al.*, 1986a), monkeys (VanVugt *et al.*, 1983) and humans (Ropert *et al.*, 1981).

In immature rats, the ontogeny of naloxone-induced release of LH appeared to be age- and gender-dependent (Bruni *et al.*, 1977). Naloxone, when administered to immature female rats as early as 10 days of age, increased concentrations of LH in serum, but the naloxone-stimulated increase in LH was not observed in males until 30 days of age (Ieiri *et al.*, 1979; Cicero *et al.*, 1986).

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In immature rats, the presence or absence of androgens during the first week of life has been shown to affect the naloxone-stimulated release of LH (Sylvester *et al.*, 1985). For immature domestic animals, data concerning effects of opioids and naloxone on secretion of LH are limited. Administration of naloxone to ewe lambs at 23 weeks of age (Mathews & Murdoch, 1984) and to yearling ewes (Brooks *et al.*, 1986b) resulted in increased concentrations of LH in blood. Also, Rodriguez & Wise (1989) reported that naloxone induced the release of GnRH as early as 2 weeks of age in bull calves.

For bulls, puberty is defined as the age at which an ejaculate contains at least 50×10^6 spermatozoa with $\geq 10\%$ progressive motility (Wolf *et al.*, 1968) and in well-fed individuals this usually occurs between 39 and 41 weeks of age. Amann (1983) defined 2 stages of sexual development for the bull before puberty. The infantile stage, extending from birth to about 10 weeks of age, is characterized by infrequent episodes of LH release and low circulating concentrations of testosterone. The transition from the infantile to prepubertal stage is marked by a dramatic increase in LH pulse frequency and amplitude (Amann, 1983; Deaver & Peters, 1988). During the prepubertal period, the plasma concentration of testosterone increases to titres which are capable of suppressing the LH pulse generator by 20–24 weeks of age.

In the present study, 2 experiments were conducted to investigate the role of opioids in regulating the secretion of LH in Holstein bull calves. Two objectives of a preliminary experiment were to determine whether: (1) administration of naloxone to prepubertal bulls affected the secretion of LH and (2) blocking age-related changes in LH secretion would modify the effect of naloxone on secretion of LH. Based on the results of this study, it was considered that opioid control of secretion of LH developed after the initiation of pulsatile secretion of LH that marks the transition from the infantile to the prepubertal stage of development. Therefore, the second study was conducted to determine the ages at which naloxone would induce an increase in secretion of LH in infantile and prepubertal bull calves.

Materials and Methods

General methods. All Holstein calves used for these studies were obtained at 3–5 days of age and raised at the Dairy Breeding Research Center as previously described (Curtis & Amann, 1981). In each study, 1 day before administration of naloxone (Sigma Chemical Co., St Louis, MO, USA) calves were fitted with indwelling jugular catheters. Blood was collected in heparinized tubes and held on ice until centrifuged at 1000 **g** for 30 min. Plasma was harvested and stored until later determination of LH (Deaver & Peters, 1988) by RIA. The sensitivity of the assay for LH was 25 pg/tube and the volume of plasma assayed was 100 µl. The within- and between-assay coefficients of variation were $<12\%$ for each experiment.

Experiment 1. The calves were assigned randomly to one of the following treatments at 7·5 weeks of age: (1) intact (N = 10); (2) intact and treated with oestradiol-17β (N = 6); or (3) castrated and treated with oestradiol-17β (N = 4). A 30 mm Compudose® implant (Elanco Products Co., Indianapolis, IN, USA), placed subcutaneously in the right ear, was used to administer the oestradiol-17β. Plasma concentrations of oestradiol-17β reached a peak concentration of 24 pg/ml within 72 h, then declined and stabilized at 5–9 pg/ml within 2 weeks (Deaver *et al.*, 1988). At 24 weeks of age, samples of blood were collected every 10 min for 350 min. At the end of this period calves were given a bolus injection (i.v.) of 100 mg naloxone (1·21 mg/kg body weight$^{0.75}$) and samples were collected every 10 min for another 120 min.

Experiment 2. Bulls were assigned randomly to receive saline (N = 6; 9 g NaCl/l H_2O) or naloxone in saline (N = 6; 1·21 mg/kg body weight$^{0.75}$). In each group, the effect of saline or naloxone on secretion of LH was tested at 3, 5, 7, 9, 11, 13, 17 and 21 weeks of age. At each age blood samples were collected at 10-min intervals for 240 min and saline or naloxone was administered (i.v.) after collection of the 120-min blood sample.

Statistical analysis. Data from Exps 1 and 2 were analysed using the General Linear Model procedure for repeated measures in time (Freund & Littell, 1982). The concentration of LH in plasma was the dependent variable. In Exp. 1, treatment was the main-plot variable, and time and the interaction of time × treatment were the sub-plot variables. In Exp. 2, samples collected between 0 and 110 min (control period) and 120 and 240 min (treatment period) were analysed as separate data sets. For both periods, treatment, age and the interaction of treatment × age were main-plot variables, and time, treatment × time, age × time and treatment × age × time were the sub-plot variables. To simplify the interpretation of a significant age × treatment × time interaction during the treatment period, the statistical model for Exp. 1 was used to examine changes in LH for each age group independently.

Results

Experiment 1

As shown in Fig. 1, in 8 of 10 intact calves not receiving oestradiol-17β, there was an acute release of LH in response to naloxone; maximum concentrations occurred about 20 min after treatment. In contrast, for 6 of 6 intact and 3 of 4 castrated bulls treated with oestradiol-17β, administration of naloxone had no effect on the secretion of LH. One Group 3 bull responded to naloxone with a significant release of LH. In this bull, the concentration of LH was high at time 0 and apparently the inhibitory effect of oestradiol-17β had diminished (Fig. 1 insert).

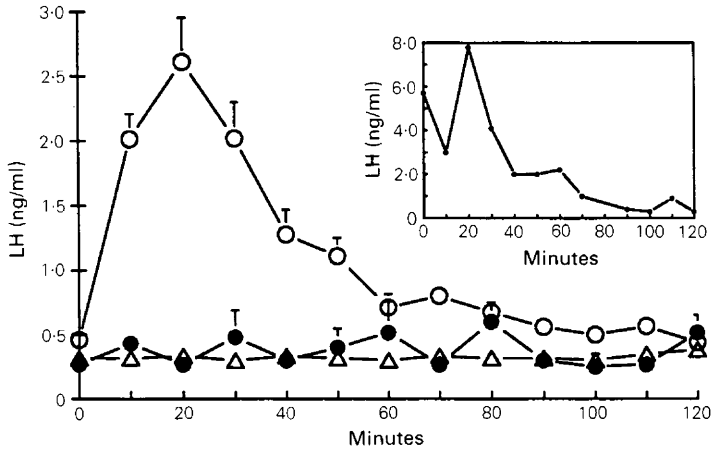

Fig. 1. Concentrations of LH in plasma after i.v. administration of 100 mg naloxone to intact bull calves (○; N = 10), intact oestradiol-treated bull calves (△; N = 6) and castrated oestradiol-treated bull calves (●; N = 3). The effects of treatment and treatment × time were significant ($P < 0{\cdot}01$). *Insert*: one castrated oestradiol-treated bull calf appeared to respond to naloxone with an increase in the release of LH.

Experiment 2

As expected, concentrations of LH during the control period increased with age ($P < 0{\cdot}05$) (Fig. 2). Injection of naloxone did not appear to influence age-related changes in LH (treatment × age and treatment × age × time, $P > 0{\cdot}1$). However, at 7 weeks of age, average concentrations of LH were slightly higher in naloxone-treated than in saline-treated calves ($0{\cdot}6 \pm 0{\cdot}07$ *vs* $0{\cdot}3 \pm 0{\cdot}02$ ng/ml).

During the treatment period there were significant interactions of treatment × age ($P < 0{\cdot}001$) and treatment × age × time ($P < 0{\cdot}001$) on concentrations of LH (Fig. 3). When these data were re-analysed within age group, there was a significant interaction of treatment × time ($P < 0{\cdot}001$) at 3, 11, 13, 17 and 21 weeks of age. Maximum concentrations of LH (saline *vs* naloxone, ng/ml) were observed at 20 min after treatment at Weeks 3 (0·3 *vs* 1·2), 11 (0·6 *vs* 2·6), 13 (0·6 *vs* 3·7) and 17 (1·1 *vs* 2·6), and at 40 min after treatment at Week 21 (1·0 *vs* 3·5).

Discussion

These experiments were conducted to determine effects of naloxone on secretion of LH in bull calves. Two major observations were made. Naloxone caused an acute release of LH in intact prepubertal bull calves but not in bull calves in which normal age-related changes in LH secretion

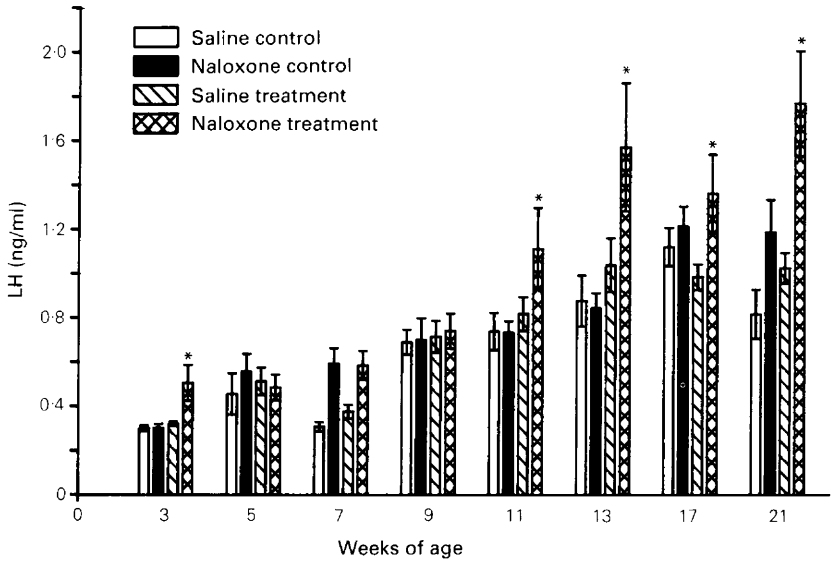

Fig. 2. Average concentrations of LH (Mean ± s.e.m.) in plasma during the control and treatment periods of Exp. 2. *Significant interaction of treatment × time ($P < 0{\cdot}001$).

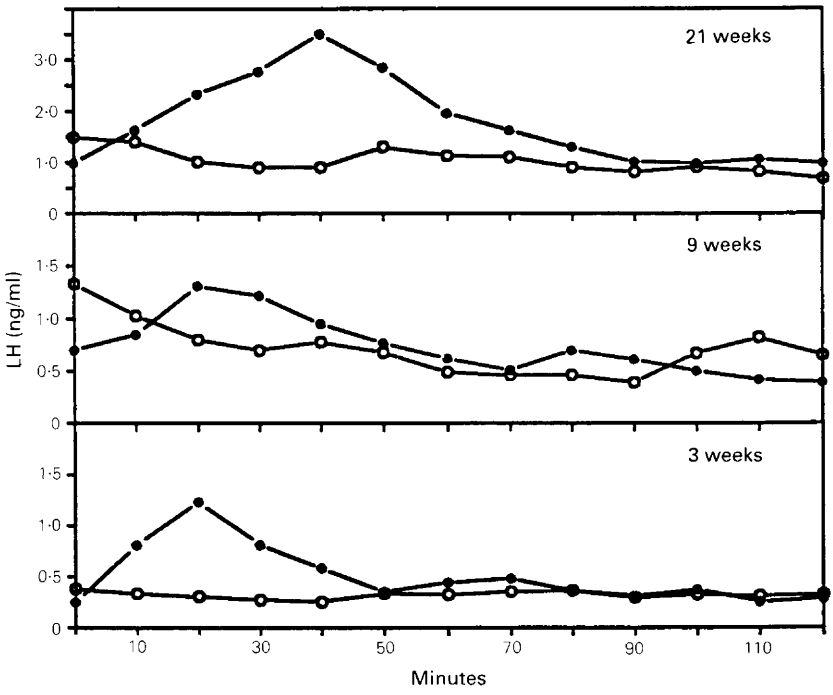

Fig. 3. Examples of changes in concentrations of LH in plasma over time following the i.v. administration of saline (○) or naloxone (1·21 mg/kg body weight$^{0.75}$; ●) at 0 min. Each point represents the average value of 6 calves. The interaction of treatment × time was significant ($P < 0{\cdot}001$) at 3 and 21 weeks of age but not at 9 weeks of age ($P > 0{\cdot}1$).

were prevented by administration of oestradiol-17β. Secondly, the effect of naloxone on the release of LH was age-dependent, since the release of LH was induced during the early infantile and mid-prepubertal periods but not around the time of the spontaneous increase in pulsatile secretion of LH that marks the end of the infantile period (Amann, 1983).

With the 'gonadostat' hypothesis, Ramirez & McCann (1963) suggested that gonadal oestrogens inhibited the hypothalamic centres controlling the secretion of LH, and puberty followed a decrease in sensitivity of the hypothalamus to the inhibitory effects of oestrogen. In sheep and cattle, castration of infantile animals results in a precocious onset of pulsatile secretion of LH and the ability of oestradiol-17β to inhibit LH secretion decreases with age (Foster & Ryan, 1979; Day *et al.*, 1984). Therefore, the basic tenets of the 'gonadostat' hypothesis appear to apply to ruminants. The neurochemical mechanisms which modulate hypothalamic sensitivity to oestradiol are unclear. Several groups of investigators have suggested that the ability of endogenous opioids to inhibit the secretion of LH decreases before the onset of puberty. For example, Bhanot & Wilkinson (1983) demonstrated that the ability of an opioid agonist to inhibit the release of LH decreased with age in male gonadectomized rats. Also, while the release of LH after administration of naloxone was initially observed on Days 15 and 35 of life in females and males, respectively (Ieiri *et al.*, 1979; Sylvester *et al.*, 1985; Cicero *et al.*, 1986), neonatal castration of males decreased and neonatal administration of oestradiol increased the age at which naloxone was first capable of inducing the release of LH. Based on these studies it was suggested that early exposure to oestradiol-17β influenced the activity of hypothalamic opioid neurones during sexual maturation (Sylvester *et al.*, 1985; Watson *et al.*, 1986).

Oestrogens have been reported to influence the morphology and function of neurones in neonatal (Gorski, 1985) and mature (Brawer *et al.*, 1980, 1983) mammals. Deaver *et al.* (1988) observed that implanting immature bulls with oestradiol-17β resulted in an increased density of staining of GnRH fibres in the stalk median eminence (SME). This observation was interpreted as an indication that exogenous oestradiol-17β decreased the release of GnRH from the SME. In our Exp. 1, treatment of bulls with oestradiol-17β-releasing implants from 7·5 to 24 weeks of age prevented a naloxone-induced release of LH. Based on this observation we initially proposed that opioid inhibition of LH secretion developed after a decline in hypothalamic sensitivity to oestradiol-17β and the spontaneous increase in pulsatile secretion of LH, and that oestradiol-17β blocked the maturation of this system in prepubertal bull calves. However, in Exp. 2, calves clearly responded to an injection of naloxone with a discharge of LH at 3 weeks of age. We therefore reject our initial hypothesis and conclude that the opioid pathways are functional before or soon after birth in bull calves.

The idea that opioid pathways are functioning to control the release of LH during the infantile period is consistent with the earlier hypothesis of Bhanot & Wilkinson (1983). Also, this conclusion is in agreement with the recent report by Rodriguez & Wise (1989) who found that naloxone induced the release of GnRH in Holstein bull calves at 2 weeks of age. However, they also observed an increased release of GnRH in calves injected with naloxone at 5 and 8 weeks of age, but a concomitant increase in LH only at 8 weeks of age. These findings contrast with our observations and there are several differences in the experimental paradigms that might account for the differences between these two studies. Firstly, the dose of naloxone used in this study was lower than that used by Rodriguez & Wise (1989). Secondly, in calves used for this study (see Deaver *et al.*, 1988) and previous reports (Curtis & Amann, 1981; Amann, 1983; Deaver & Peters, 1988) the incidence of spontaneous discharges of LH increased between 5 and 9 weeks of age; this increase in LH pulse frequency appeared to be delayed due to the hypophysial portal cannulation procedure used by Rodriguez & Wise (1989). Also, previous investigators have reported that some discharges of GnRH are not accompanied by a release of LH in ewes (Thiery & Pellitier, 1981; Levine *et al.*, 1982) and calves (Rodriquez & Wise, 1989). Therefore, we cannot rule out the possibility that, at 5, 7 and 9 weeks, naloxone induced a release of GnRH that was of insufficient amplitude to elicit a detectable increase in peripheral LH or that gonadotrophs might have been refractory to further stimulation by GnRH.

It is difficult to explain why naloxone failed to induce the release of LH in Exp. 1. Possible explanations to account for a lack of response would include: (1) the dose of naloxone was too low to overcome a high degree of opioid inhibition; and (2) the releasable pool of LH was not large enough to allow for a discharge of LH of sufficient magnitude to be detected in peripheral blood. We suggest that the inability of naloxone to induce the release of LH in oestradiol-treated calves reflects a level of activity of opioid neurones that could not be effectively antagonized by the dose of naloxone used.

Finally, age-related differences in the response of LH to naloxone cannot be explained solely by differences in plasma concentrations of endogenous steroids. In Holstein bulls during the infantile stage, plasma concentrations of testosterone remain low, while concentrations of oestradiol-17β decrease slightly (Amann *et al.*, 1986; Deaver *et al.*, 1988). Therefore, in Exp. 2, it is difficult to account for differences in the LH response to naloxone in terms of changing concentrations of testosterone and/or oestradiol-17β.

We conclude from these studies that administration of naloxone results in an age-dependent effect on the secretion of LH; this effect can be blocked by exogeneous oestradiol. Endogenous opioids modulate some of the negative feedback effects of steroids on the secretion of LH, and, in bull calves, the initiation of pulsatile release of LH is a consequence of reduced opioid neuronal activity and/or a decrease in tissue sensitivity to these peptides.

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