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Ontogeny of the opioidergic regulation of LH and prolactin secretion in lactating sows II: interaction between suckling and morphine administration

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Administration of morphine to ten suckled and nine zero-weaned (piglets removed immediately after farrowing) sows was used to investigate the apparent absence of opioid regulation of LH and prolactin secretion in early lactation. Blood samples were collected at 10 min intervals at 24–30, 48–54, 72–78 h post partum, and for a 12 h period from 08:00 to 20:00 on day 10 after farrowing. Morphine (0.1 mg kg^{-1}) was administered as three i.v. bolus injections at intervals of 1 h during the last 3 h of each of the 6 h sampling periods, and at 6, 7 and 8 h after the beginning of sampling on day 10. There were significant ($P < 0.001$) group (zero-weaned versus suckled), time and morphine effects on LH secretion. Plasma LH concentrations increased ($P < 0.001$) within 48 h of farrowing in zero-weaned sows. Long-term trends of an increase in mean plasma LH in the sampling periods before treatment were attenuated in both groups by morphine treatment. Morphine also significantly inhibited ($P < 0.05$) prolactin secretion in suckled sows. In zero-weaned sows, plasma prolactin was already low at the start of sampling and did not change with time or in response to morphine treatment. Therefore, the inability to demonstrate an opioidergic involvement in the suckling-induced inhibition of LH secretion during the early post-partum period in sows is not due to a lack of opioid receptors. Furthermore, in suckled sows, morphine is stimulatory to systems that have an inhibitory effect on prolactin secretion.

Introduction

Endogenous opioid peptides have been implicated as potential modulators of gonadotrophin secretion in domestic animals (Ebling *et al.*, 1989; Haynes *et al.*, 1989; Rawlings and Churchill, 1990; Barb *et al.*, 1991; De Rensis *et al.*, 1992, 1993a,b, 1998; Britt *et al.*, 1993; Schillo, 1993; Okrasa and Kalamarz, 1996). In pigs, the opioid antagonist naloxone blocks the inhibitory effect of suckling on LH secretion in mid- and late lactation (Barb *et al.*, 1986; Mattioli *et al.*, 1986; De Rensis *et al.*, 1993a). In addition, Armstrong *et al.* (1988a) demonstrated that administration of the opioid antagonist morphine prevented the increase in LH associated with transient weaning and delayed the onset of oestrus after final weaning.

In studies on suckling-induced inhibition of LH secretion in early lactation (De Rensis *et al.*, 1993b; Sesti and Britt, 1994), suckled sows had relatively high plasma LH concentrations from parturition until an inhibitory effect of suckling on LH secretion occurred 24–48 h (Sesti and Britt, 1994) or 45–55 h (De Rensis *et al.*, 1993b) post partum. The pattern of LH secretion was typical of established lactation. However, when sows were zero weaned by removing all piglets immediately after farrowing, thereby removing the suckling-induced inhibitory input to the central nervous system, LH secretion remained high and was characterized by high frequency episodic-type release.

De Rensis *et al.* (1993a, 1998) showed that although chronic naloxone treatment was not able to reverse the suckling-induced inhibition of LH secretion in the early post-partum period, a naloxone-induced increase in LH secretion was apparent at days 10 and 11 of lactation. Similarly, a significant effect of naloxone on prolactin secretion was only established at days 10 and 11 of lactation. These data indicated temporal changes in the opioidergic regulation of LH and prolactin secretion during lactation. However, it was not possible to determine whether the absence of an effect of opioids in early lactation was due to (i) the absence of endogenous opioids; (ii) a lack of opioidergic receptors; or (iii) whether the action of endogenous opioids was masked by alternative inhibitory systems. In the present study, the second possibility was investigated.

Multiple types of opioid receptor have been implicated in suppression of LH secretion, but it is generally accepted that the mu receptor subtype is principally involved (Pfeiffer *et al.*, 1983; Panerai *et al.*, 1985; Lagrance *et al.*, 1995; Walsh and Clarke, 1996). Although morphine and naloxone do not have

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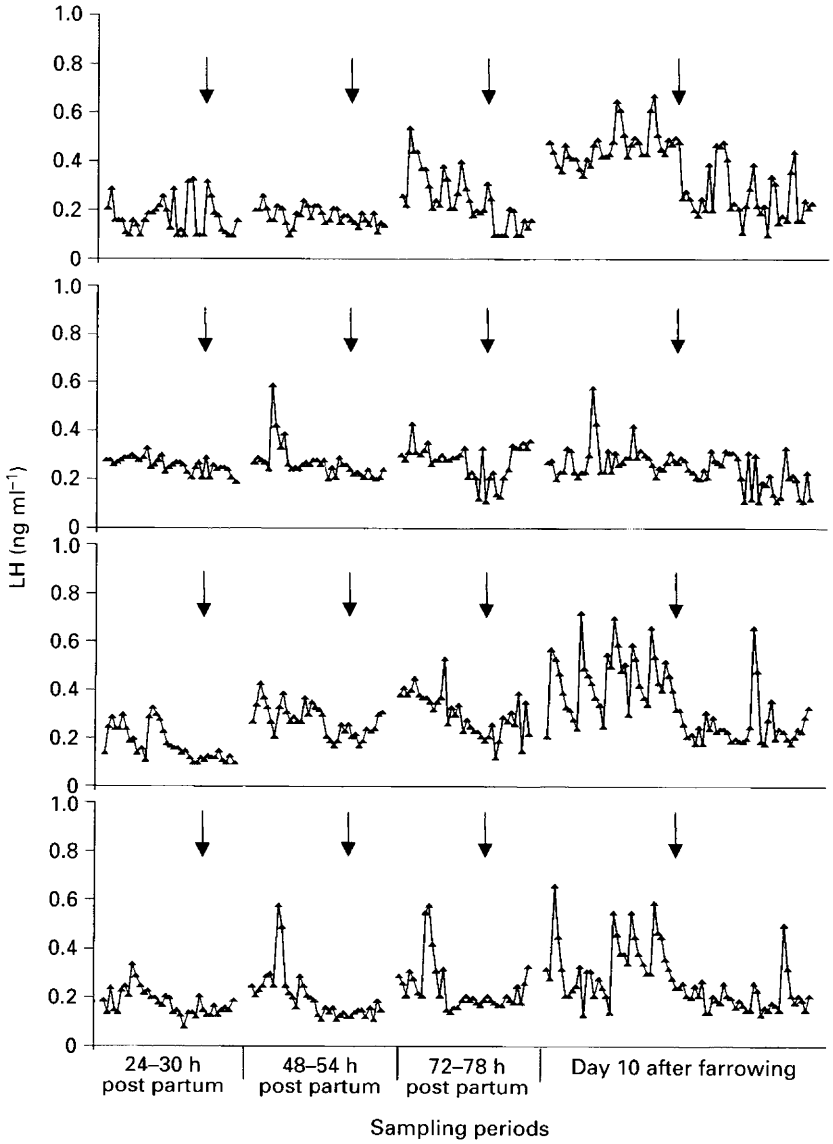

Fig. 1. Individual plasma LH profiles in four zero-weaned sows treated with morphine in early and established lactation. Arrows indicate the time of the first morphine injections.

identical affinities for all types of opioid receptor, both bind with high affinity to mu1 and mu2 receptors (Lord *et al.*, 1977; Casy and Parfitt, 1986; Pasternak, 1986; Kazmi and Mishra, 1987). Arvidsson *et al.* (1995) and Ding *et al.* (1996) identified post-synaptic mu opioid receptors in the median preoptic area and anterior hypothalamus of rats, and Lagrance *et al.* (1995) reported that GnRH neurones of guinea-pigs have functional mu receptors. In addition, morphine inhibits the firing frequency of hypothalamic neurones associated with the release of GnRH in rhesus monkeys (Kesner *et al.*, 1986). Thus, it is appropriate to use exogenous morphine treatment to determine the presence of functional opioidergic receptors in situations in which the opioid antagonist naloxone fails to elicit a response.

The aim of the present study was to determine whether administration of morphine modifies LH and prolactin secretion during early lactation in sows and to investigate the nature of the neuroendocrine mechanisms involved in the regulation of these hormones. On the basis of the results of an earlier study (De Rensis *et al.*, 1993b), agonist treatment was compared in groups of zero-weaned and suckled sows. In zero-weaned sows, it was expected that LH secretion would not be inhibited throughout the period of study, whereas prolactin would remain at basal concentrations. In contrast, in suckled sows, it was expected that LH secretion would be reduced gradually by suckling-induced inhibition. LH secretion was not antagonized with naloxone administration in the studies of De Rensis *et al.* (1993a, 1998).

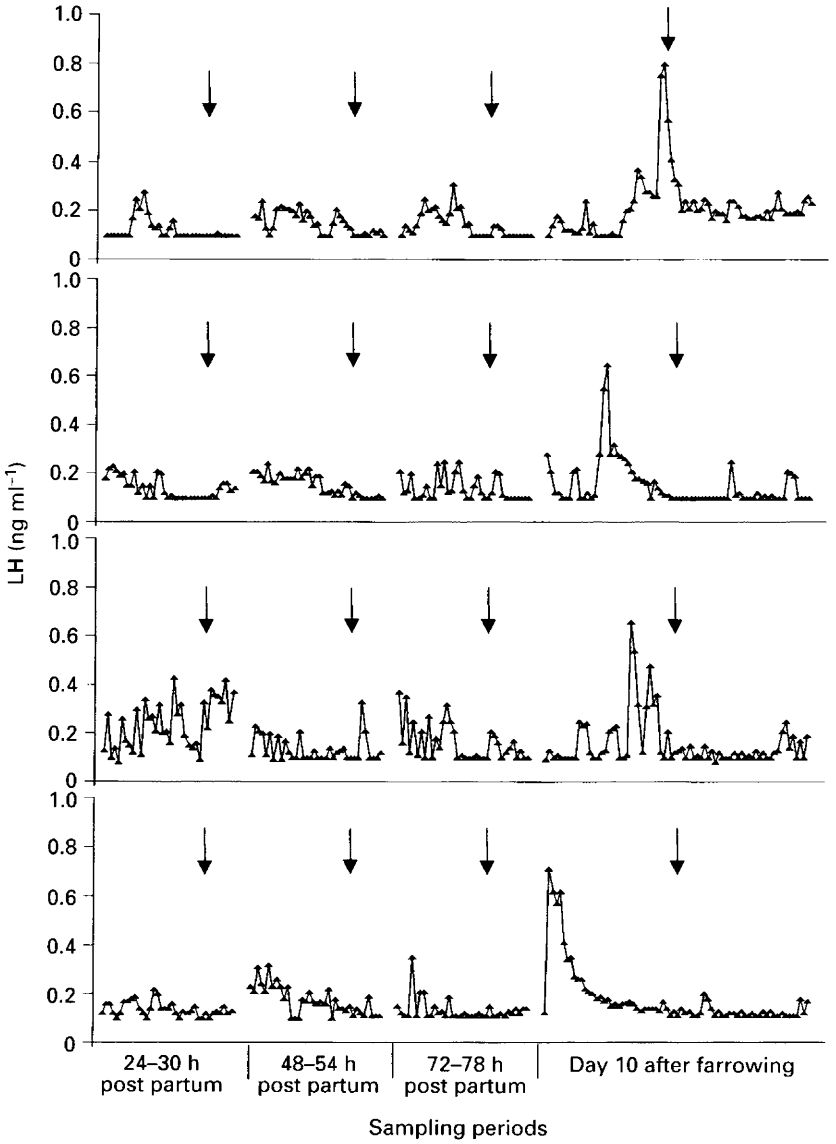

Fig. 2. Individual plasma LH profiles in four suckled sows treated with morphine in early and established lactation. Arrows indicate the time of the first morphine injections.

Materials and Methods

Animals and treatments

A total of 19 primiparous Camborough sows (Pig Improvement (Canada) Ltd) were housed in conventional farrowing crates. Water was supplied *ad libitum* and sows were fed a commercial lactation diet to appetite twice a day throughout lactation. The sows were randomly allocated to two groups. In nine sows (zero weaned), all piglets were removed 6 h after the birth of the last piglet to allow the piglets to obtain an immediate supply of colostrum from their dams before they were fostered to other sows. The remaining ten sows (suckled) suckled ten or more piglets for the duration of the study. For logistical purposes, the experiment was run as two replicates and farrowing times were grouped in 12 h blocks. Time zero was denoted as the end of each 12 h period and as far as possible sows were allocated within farrowing periods to be either zero weaned or suckled. Sows were fitted with indwelling jugular cannulae via the cephalic vein under general anaesthesia 12–18 h post partum, as described by Cosgrove *et al.* (1993), to allow subsequent stress-free frequent blood sampling. Blood samples were collected at 10 min intervals from 24–30, 48–54, 72–78 h post partum, and for a 12 h period from 08:00 to 20:00 on day 10 after farrowing. Plasma was harvested and stored at −20°C until analysis. A preliminary dose–response study established that 0.1 mg morphine kg^{-1} body weight did not produce any adverse side-effects in lactating sows, but did suppress LH secretion. Thus, 0.1 mg morphine kg^{-1} was administered as three i.v. bolus injections at intervals of 1 h during the last 3 h of each of the 6 h

sampling periods, and at 6, 7 and 8 h after the beginning of sampling on day 10.

Hormone assays

Plasma LH was quantified in all samples using the double antibody radioimmunoassay described by De Rensis *et al.* (1993b). The intra- and interassay coefficients of variation (CV) were 9.9 and 17.1%, respectively. The sensitivity of the assay, defined as 90% of total binding, was 0.13 ng ml^{-1}. Plasma prolactin was measured as described by Shaw and Foxcroft (1985) with minor modifications. Intra- and interassay CV were 9.1 and 9.7%, respectively. The overall sensitivity of the assay, defined as 85% of total binding, was 1.9 ng ml^{-1}.

Statistical analysis

Subjective assessment of the plasma LH profiles obtained in this study indicated that the adoption of computerized pulse analysis programs was inappropriate because in many cases pulsatile secretion was not evident at times when LH concentrations were clearly changing (for details see Foxcroft *et al.*, 1988). Therefore, LH profiles were analysed by the sliding windows method of Shaw and Foxcroft (1985) to provide minimum, mean and maximum characteristics of LH secretion and thus a complete and more appropriate method of analysis (Foxcroft *et al.*, 1988). For each of the first three sampling windows at 24–30, 48–54, 72–78 h post partum (periods 1, 2 and 3), the 10 min LH and prolactin samples were used to generate six 1 h means, 3 h before and 3 h after morphine injection (treatment). Hormone profiles from the 12 h frequent sampling period at day 10 (period 4) were analysed as twelve 1 h means, 6 h before and 6 h after morphine injection. The repeat measures analysis of variance within the PROC general linear model procedure of the Statistical Package and Service Solutions (SPSS) statistical package was used to test for the effects of morphine within periods. Main effects of sow group (zero weaned versus suckled), experimental replicate (first or second), and interactions between main effects and repeated measures were fitted. When significant interactions between main effects and repeated measures were established, subsequent procedures were conducted to analyse effects of repeated measures within groups. When appropriate, comparison of period means within treatment group were made using the Student–Neuman–Keul procedure (Steel and Torrie, 1980). Further evaluation of interactions between repeated measures within groups was then made using polynomial contrasts.

Results

Plasma LH

Individual patterns of LH secretion in four zero-weaned and four suckled sows are shown (Figs 1 and 2). Group mean

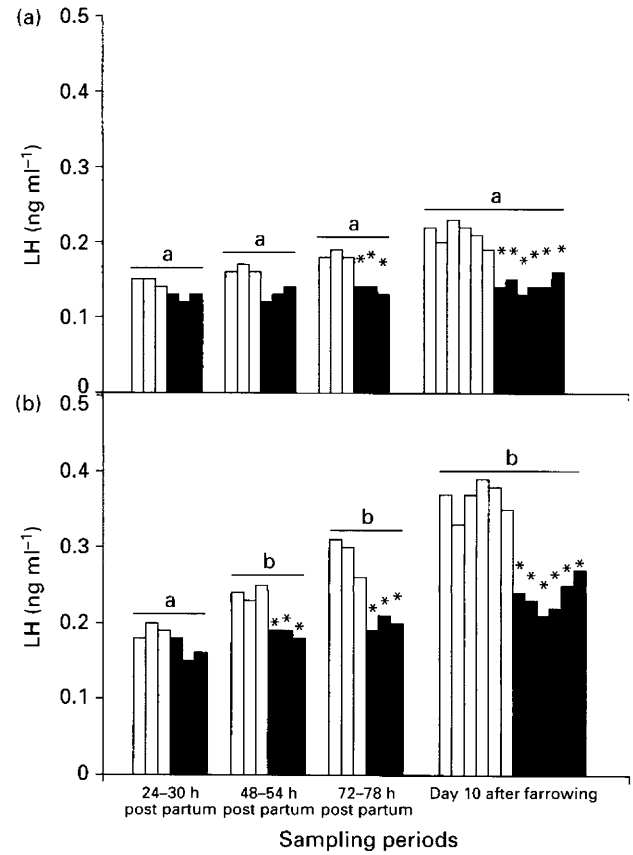

Fig. 3. Mean plasma LH in (a) suckled and (b) zero-weaned sows treated with morphine in early and established lactation. Bars indicate 1 h sampling periods; □, before and ■ after morphine treatment. Different letters within groups indicate significant differences ($P < 0.001$) in mean LH between sampling periods. Asterisks indicate differences between means within sampling periods ($P > 0.05$).

plasma concentrations of LH before and after morphine treatment during the four sampling periods (24–30, 48–54, 72–78 h and day 10 post partum) are also shown (Fig. 3). Significant ($P < 0.001$) effects of group (zero weaned versus suckled), morphine, period, and period × treatment × group interaction were established. Within group analyses revealed no effect of period of sampling on mean LH concentrations in suckled sows. However, in zero-weaned sows, in contrast to LH concentrations in the first sampling period, mean plasma LH increased ($P < 0.001$) during all subsequent periods. Morphine administration resulted in a decrease in LH secretion ($P > 0.05$) during the 72–78 h and day 10 post partum sampling periods in suckled sows, and during the 48–54, 72–78 h and day 10 periods in zero-weaned sows.

Polynomial contrasts were used to elucidate the effects of morphine within groups. In the absence of morphine treatment, a quadratic ($P < 0.001$) increase in mean LH throughout the post-partum period was established in zero-weaned sows, and a linear ($P < 0.05$) increase in suckled sows over the same period. Morphine administration resulted in attenuation of the increase in mean plasma LH across the sampling periods in both groups, as evidenced by

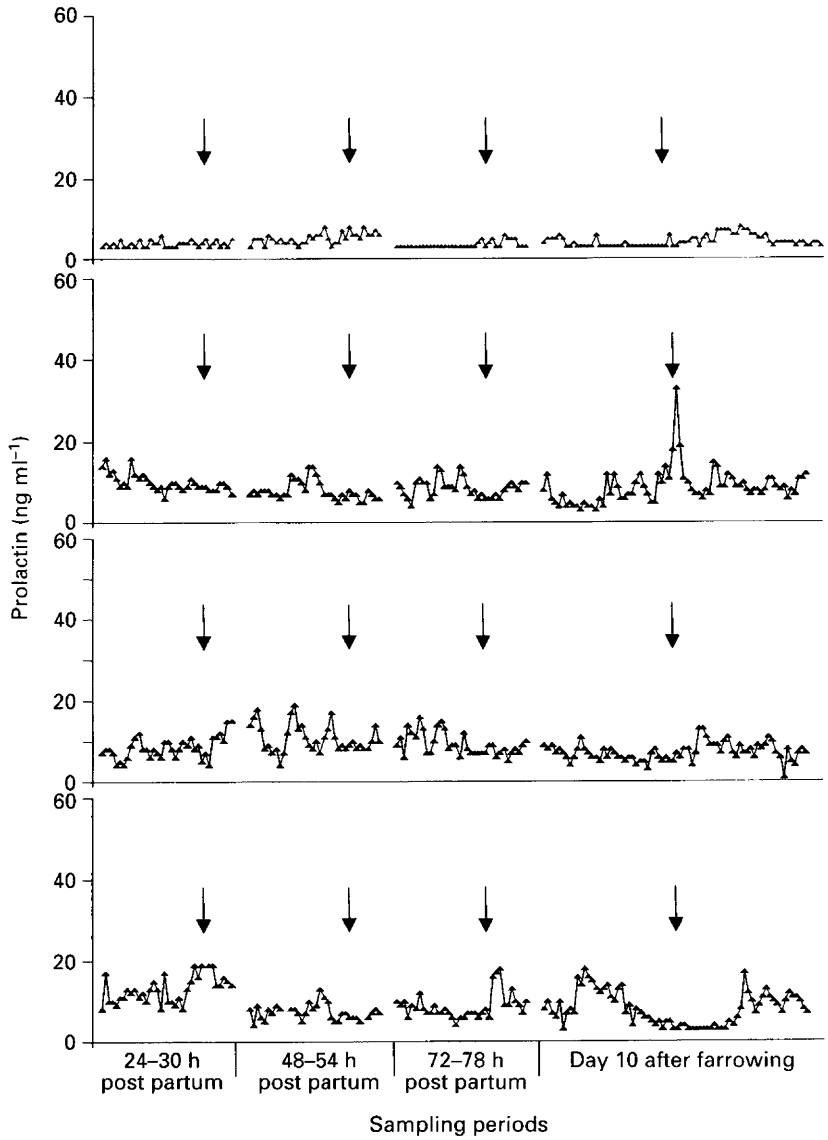

Fig. 4. Individual plasma prolactin profiles in four zero-weaned sows treated with morphine in early and established lactation. Arrows indicate the time of first morphine injections.

a linear relationship between mean plasma LH and time post partum ($P < 0.05$) in zero-weaned sows, and no relationship in suckled sows.

Plasma prolactin

Individual patterns of prolactin secretion in four zero-weaned and four suckled sows are shown (Figs 4 and 5). Mean plasma concentrations of prolactin during the four sampling periods are also shown (Fig. 6). There were significant ($P < 0.001$) group, period and morphine effects on plasma prolactin. Within group comparisons established that in suckled sows, plasma prolactin was already high at the beginning of sampling and did not change with time. In suckled sows, morphine administration resulted in a decrease ($P < 0.05$) in mean plasma prolactin across all periods. In zero-weaned sows, plasma prolactin was already low at the beginning of sampling, did not change with time, and was not affected by morphine treatment.

Discussion

As in previous studies, the pattern of LH secretion did not allow LH pulse characteristics to be described precisely due to the high frequency, low and variable amplitude and episodic-like release of LH. However, a significant increase in mean plasma LH occurred in the zero-weaned sows, beginning 48 h post partum. These observations are in

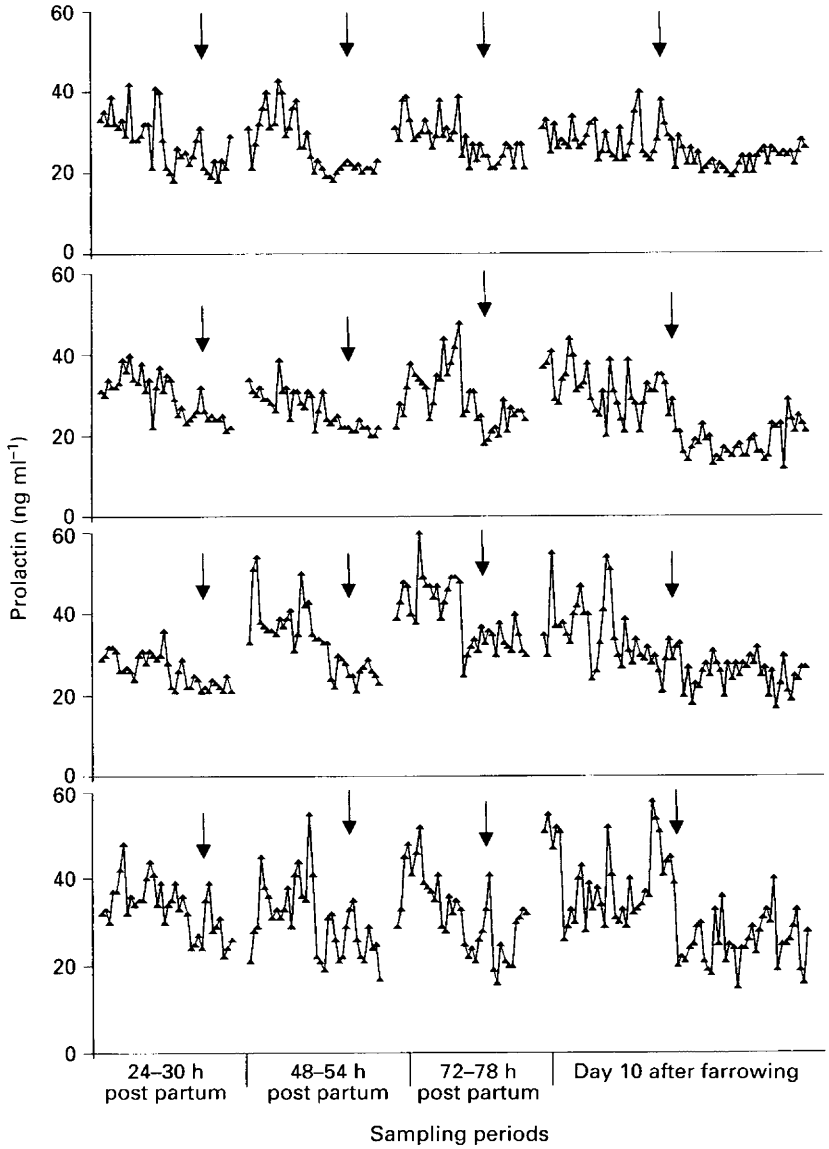

Fig. 5. Individual plasma prolactin profiles in four suckled sows treated with morphine in early and established lactation. Arrows indicate the time of first morphine injections.

agreement with other studies (De Rensis *et al.*, 1993b). The absence of any comparable increase in LH secretion over time in suckled sows confirms that the suckling stimulus is the main factor that blocks LH secretion during early lactation in sows.

In contrast to other studies in suckled sows (Tokach *et al.*, 1992; De Rensis *et al.*, 1993b; Sesti and Britt, 1994) and rats (Dondi *et al.*, 1991), time-dependent suckling-induced inhibition of LH was not observed in the period immediately after birth. This may be due, in part, to the experimental protocol used in the present study, which did not include sampling immediately after farrowing, during which period LH secretion was consistently high in previous studies (De Rensis *et al.*, 1993b).

With regard to the neuroendocrine control of gonadotrophin secretion, treatment with opioid antagonists consistently increases LH secretion during lactation in sows, indicating that an inhibitory opioidergic mechanism can be an important mediator of the inhibitory effects of suckling (Armstrong *et al.*, 1988b; Barb *et al.*, 1991; Britt *et al.*, 1993; De Rensis *et al.*, 1998). The observed effect of morphine on LH secretion is consistent with this proposal and with the study of Armstrong *et al.* (1988a), in which a similar response was observed in a number of sows at day 25 of lactation.

The effect of morphine on LH secretion in suckled sows in mid-lactation, when episodic LH secretion is at a very low frequency, is particularly interesting. Several studies have reported that opioidergic effects on LH involve mu and

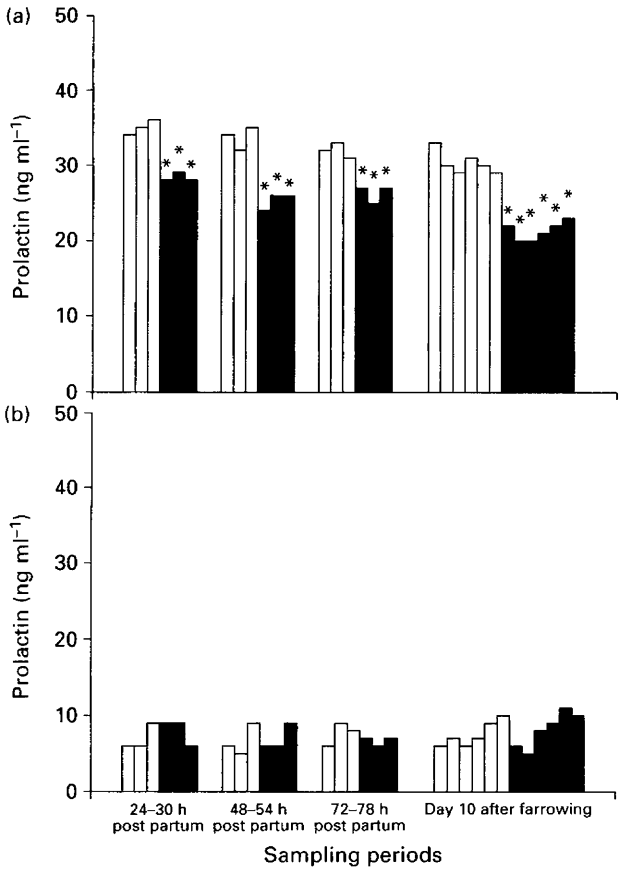

Fig. 6. Mean plasma prolactin in (a) suckled and (b) zero-weaned sows treated with morphine in early and established lactation. Bars indicate 1 h sampling periods; □, before and ■ after morphine treatment. Asterisks indicate differences between means within sampling periods ($P > 0.05$). In suckled sows, morphine treatment resulted in a decrease in mean plasma prolactin, but no effects of morphine were observed in zero-weaned sows.

kappa receptors (Goodman *et al.*, 1980; Cicero *et al.*, 1983; Pfeiffer *et al.*, 1983; Panerai *et al.*, 1985; Laedem and Karla, 1985), whereas Wiesner *et al.* (1985) reported that delta, but not mu, receptors are implicated in the opioidergic inhibition of LH. Whatever the types of receptor involved, the results of the present study indicate that receptors that are sensitive to morphine are not occupied fully during established lactation in sows, and that treatment with an exogenous opioid agonist is able to effect an even greater inhibition of LH secretion.

The results of the present study also indicate that opioid receptors are present during early lactation in sows, and that when they are activated by morphine administration, there is a decrease in LH secretion. This observation, viewed in the context of the lack of a naloxone (opioid antagonist) effect on LH secretion during early lactation (De Rensis *et al.*, 1993a, 1998), indicates that endogenous opioid secretion may be absent during the early post-partum period in sows. Alternatively, it is possible that even if endogenous opioids are released in early lactation and are antagonized effectively by naloxone treatment, a more dominant inhibitory mechanism is present at this time. The results of the polynomial contrasts indicated that the inhibitory effect of morphine increased as lactation progressed. This change appears to parallel the trend for naloxone reported by De Rensis *et al.* (1993a, 1998), which becomes more effective in increasing LH secretion over time from farrowing. Collectively, these data support the concept of the gradual emergence of a functionally important inhibitory opioidergic mechanism in the regulation of gonadotrophin release during the post-partum period in sows.

Similar to the findings of other studies (Stevenson and Britt, 1981; De Rensis *et al.*, 1993b), prolactin concentrations in suckled sows were high soon after farrowing, whereas plasma prolactin was low during the first period of sampling in zero-weaned sows and did not change during the later part of the experiment. These data confirm that the suckling stimulus is an essential part of the mechanism that regulates prolactin secretion after farrowing.

The observation that morphine inhibited prolactin secretion in suckled sows is in contrast to studies in other species in which exogenous opiates had either a stimulatory effect or no effect on prolactin secretion (Bruni *et al.*, 1977; Shaar and Clemens, 1980; Grossman *et al.*, 1982; Schillo *et al.*, 1985; Callahan *et al.*, 1988; Peck *et al.*, 1988a,b; Estienne *et al.*, 1990). The data from the present study are also inconsistent with the observation that administration of naloxone, an opioid antagonist, resulted in a decrease in prolactin in lactating sows (De Rensis *et al.*, 1993a). However, Armstrong *et al.* (1988a) also reported that morphine suppressed rather than increased prolactin concentration in lactating sows, and suggested that this effect may be due to the dose used, the duration of treatment, or to previous exposure to high concentrations of endogenous opioids during parturition. With regard to LH, the inhibitory effect of morphine may be explained by the presence of separate opiate receptor subtypes. It is generally accepted that opioidergic receptors involved in prolactin secretion belong to the mu (Rossi *et al.*, 1983; Akil *et al.*, 1984) and kappa (Zukin and Zukin, 1981) classes. However, Koening *et al.* (1984) reported that the beta endorphin effect on prolactin is not blocked by a mu1 antagonist, indicating that the mu1 receptor is not involved. Therefore, in contrast to a general stimulatory effect of opioids on prolactin secretion (Ben-Jonathan *et al.*, 1989), administration of morphine to lactating sows appears to stimulate neuroendocrine mechanisms that inhibit prolactin secretion.

In conclusion, this study confirms that in the period immediately after birth, the suckling stimulus is associated with suppression of LH secretion and increased plasma prolactin concentrations. Secondly, although studies using the opioid antagonist naloxone indicated that the inhibition of LH secretion at this time does not involve an opioidergic mechanism, this appears to relate to a lack of endogenous opioids rather than to a lack of opioid receptors.

The inhibitory effect of morphine on prolactin secretion in suckled sows during lactation indicates that opiate receptors that are sensitive to morphine are not involved in the regulation of prolactin secretion during lactation, or that morphine stimulates the activity of systems that have an inhibitory effect on prolactin secretion.

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