Effect of naloxone on plasma concentrations of prolactin and LH in lactating sows

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Summary. Six lactating sows were injected through an indwelling vena cava cannula with naloxone (2.5 mg/kg body weight) on Day 15 *post partum*. Blood samples were collected through the cannulas at 10-min intervals for 8 h before and 10 h after naloxone administration. Plasma prolactin and LH concentrations were measured by radioimmunoassay. Naloxone caused a marked suppression of plasma prolactin concentrations lasting 4–6 h. LH concentrations were also affected by naloxone: LH rose to reach maximum values 20–50 min after naloxone treatment. Pretreatment values were recorded 200–300 min after the treatment. These results indicate that endogenous opioids are involved in causing the endocrine patterns occurring during lactation, i.e. high prolactin and low LH concentrations.

Introduction

Lactation in pigs is accompanied by anoestrus and suppression of ovarian activity (Burger, 1952). From an endocrine point of view the pig's lactational anoestrus is characterized by high levels of prolactin (van Landeghem & van de Wiel, 1978; Bevers, Willemse & Kruip 1978; Stevenson, Cox & Britt, 1981; Dusza & Krzymowska, 1981) whereas LH secretion is reduced (Parvizi, Elsaesser, Smidt & Ellendorff, 1976; Stevenson & Britt, 1980; Stevenson *et al.*, 1981; Seren, Mattioli, Maffeo, Galeati & Prandi, 1984). Amongst the different causes responsible for these endocrine patterns the suckling stimulus seems to be the most important (reviewed by Edwards, 1982). However, the mechanism of action of this stimulus is still unknown.

Opioids are able to increase the secretion of prolactin in the rat (Grandison & Guidotti, 1977; Dupont, Cusan, Labrie, Coy & Li, 1977; Rivier, Vale, Ling, Brown & Guillemin, 1977; Spiegel, Kourides & Pasternack, 1982) and man (Tolis, Hickey & Guyda, 1975; Reid, Hoff, Yen & Li, 1981) and to inhibit that of LH in rats (Blank, Panerai, Friesen, 1979; Bhanot & Wilkinson, 1983; Petraglia *et al.*, 1984), men (Morley *et al.*, 1980) and sheep (Malven, Bossut & Diekman, 1984).

Since the endocrine patterns produced by opioids are similar to those occurring during lactational anoestrus, the present study was designed to investigate whether the inverse relationship between prolactin and LH occurring during lactational anoestrus in the pig is dependent upon an opiate-mediated mechanism.

Materials and Methods

Animals

Six multiparous Large White sows with an average weight of 180 kg were used. The sows were kept in farrowing crates during the entire lactational period (28 days) and were provided with food (corn-soybean meal, vitamins and minerals) and water *ad libitum*. Litters, standardized to 10 piglets per sow within 48 h of farrowing, were allowed to suck *ad libitum* throughout the experi-

mental period and the suckling frequency was recorded. A suckling event consisted of the sow lying on her side, making her teats available and of the whole litter suckling.

On the 3rd or 4th day after farrowing the sows were fitted with indwelling vena cava cannulas under metomidate–azaperone (Hypnodil–Stresnil: Janssen Pharmaceutica, Beerse, Belgium) anaesthesia. The cannulas were passed under the skin and exteriorized at the withers. At 15 days after farrowing the sows were given naloxone (Salars, Como, Italy), an opioid antagonist, at a dose of 2.5 mg/kg, dissolved in 20 ml saline (0.9% (w/v) NaCl), through the cannula as a bolus. Blood samples were taken through the cannulas at 10-min intervals for 8 h before and 10 h after naloxone administration. The blood samples were collected into heparinized tubes, centrifuged and the plasma stored at -20°C until assayed.

Hormone assays

LH. Plasma concentrations of LH were determined by a heterologous double-antibody method. This assay utilized rabbit antiserum to ovine LH (NIH-LH-S20) which is highly specific for pig LH (LER 786-3) at a working dilution of 1:40 000. Cross-reactions with pig FSH, TSH, ACTH and prolactin were $<0.01\%$. Pig LH was iodinated by using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3 α ,6 α -diphenyl glycoluril (Iodogen: Pierce Chemical Company, Rockford, U.S.A.), according to the method of Salacinski, McLean, Sykes, Clement-Jones & Lowry (1981), and purified on a column (1 $\times$ 4 cm) of dry Whatman CF-11 cellulose. The specific activity of the tracer was 35 $\mu\text{Ci}/\mu\text{g}$. The sensitivity of the assay was 0.07 ng/ml plasma. The intra-assay (10 determinations) and inter-assay (10 assays) coefficients of variation were 11.3% and 14.1% respectively. The recovery percentage of 0.5 or 2 ng pig LH added to 1 ml plasma taken from lactating sows was $106 \pm 1.4\%$ (mean $\pm$ s.e.m.; $n = 25$).

Prolactin. Plasma prolactin concentrations were measured by an homologous double-antibody radioimmunoassay. The antibody used was a generous gift of Dr M. M. Bevers and details of its characteristics have been published (Bevers *et al.*, 1978). The tracer was obtained by iodination of pig prolactin (LER 2073) according to Salacinski *et al.* (1981). The tracer was purified on a column (1.4 $\times$ 80 cm) of Sephadex G100 to remove the aggregated hormones and the free iodine. The specific activity of iodinated prolactin was 37 $\mu\text{Ci}/\mu\text{g}$. The sensitivity of the assay was 1 ng/ml plasma. The intra-assay (10 determinations) and inter-assay (10 assays) coefficients of variation were 10.2 and 16.7% respectively. The recovery percentage of 25 ng prolactin added to 1 ml plasma from lactating sows was $94\% \pm 2.3$ ($n = 25$).

Statistical analysis

An LH or prolactin episode was judged to be a peak if (1) it was preceded by at least 2 values that showed a progressive decline or represented basal levels, (2) the increase in hormone concentration was at least 40% above the LH concentration immediately preceding the rise, and (3) its amplitude was greater than the sensitivity of the assay. LH peak amplitude was defined as the highest point of the peak minus the nadir concentration preceding the peak. Analysis of variance was used to examine the effect of treatment on LH and prolactin plasma concentrations; the level of significance chosen was $P < 0.01$.

Results

Typical endocrine patterns were observed during lactation. Plasma prolactin concentrations were high, showing marked individual variations of mean values (Fig. 1), e.g. 76.2 ± 3.3 ng/ml for Sow D and 29.8 ± 0.9 ng/ml for Sow F (values are mean $\pm$ s.e.m. of 48 samples). The other sows presented intermediate values. As shown in Fig. 1, suckling always produced an increase of plasma

Fig. 1. Prolactin plasma concentrations before and after naloxone administration ($\blacktriangle$, time 0) in Sows A–F. The symbols ($\circ$) represent suckling events.

prolactin with an ascending phase lasting 10–20 min and a descending phase lasting 20–30 min and followed by the rise induced by the subsequent suckling.

The levels of LH ranged between the sensitivity of the assay (0.07 ng/ml) and 1.5 ng/ml (Fig. 2). Patterns of LH secretion tended to be dissimilar among animals: in Sow E two peaks with an amplitude of 0.62 and 0.75 ng and occurring with an interval of 220 min were distinctly visible, in Sow D more frequent peaks (8 peaks/8 h) with an amplitude of 0.40 ± 0.05 ng/ml were observed, while in the remaining sows intermediate situations were recorded.

Fig. 2. LH plasma concentrations before and after naloxone administration (▼, time 0) in Sows A–F.

Naloxone administration at the dose of 2.5 mg/kg body weight resulted in a progressive decrease of plasma prolactin values (Fig. 1). The maximum inhibition of prolactin concentrations occurred 100–300 min after naloxone administration. Besides the significant reduction of mean plasma prolactin values (Table 1) prolactin peaks in response to suckling were completely suppressed (Sows E & F) or markedly reduced (Sows A, B, C & D) by naloxone. Plasma prolactin returned to pretreatment values about 6–8 h after treatment.

In 5 out of 6 sows, naloxone administration significantly increased LH plasma values (Fig. 2). The increase began immediately after the treatment to reach maximum values 20–60 min after

naloxone. Then the levels started to decline to reach pretreatment values 200–300 min after naloxone.

Sow F, which did not show any LH increase after naloxone, had unusually high LH concentrations throughout the experiment, ranging between 0.4 and 1.5 ng/ml.

The suckling frequency was not affected by naloxone treatment as shown in Table 1.

Table 1. Suckling frequency (expressed as sucks $\times$ h⁻¹) and mean plasma prolactin values ($\pm$ s.e.m.) determined in sows during a period of 8 h before and 6 h after naloxone

Sow	Before treatment		After treatment	
	Suckling frequency	Prolactin (ng/ml)*	Suckling frequency	Prolactin (ng/ml)*
A	1.50	32.1 $\pm$ 0.9	1.50	10.0 $\pm$ 0.7
B	1.37	41.8 $\pm$ 1.0	1.50	17.8 $\pm$ 2.6
C	1.25	63.2 $\pm$ 2.5	1.33	29.4 $\pm$ 2.6
D	1.50	76.2 $\pm$ 3.3	1.33	33.2 $\pm$ 3.2
E	1.37	45.0 $\pm$ 1.9	1.33	10.9 $\pm$ 2.2
F	1.00	29.8 $\pm$ 0.9	1.33	5.6 $\pm$ 0.4

* Means of 48 samples taken before and 36 samples after naloxone.

Discussion

The high levels of prolactin observed in lactating sows in this study support previous findings (van Landeghem & van de Wiel, 1978; Bevers *et al.*, 1978; Stevenson *et al.*, 1981; Dusza & Kryzmowska, 1981). In particular the discharge of prolactin in response to the suckling stimulus is in agreement with the result of van Landeghem & van de Wiel (1978). On the other hand, we found low values of LH, except for Sow F, in agreement with the findings of Parvizi *et al.* (1976), Stevenson & Britt (1980), Stevenson *et al.* (1981) and Seren *et al.* (1984).

The administration of naloxone produced a reduction of prolactin values. This effect cannot be accounted for by a reduced suckling frequency after the treatment. These results indicate that in the sow an opioid-mediated mechanism seems to be responsible for the hyperprolactinaemia occurring during lactation. An antiprolactinaemic effect of naloxone has already been described for lactating rats (Miki, Sonnetag, Forman & Meites, 1981; Sirinathsinghji & Martini, 1984).

Naloxone increased the concentrations of LH in 5 out of 6 sows. Endogenous opioids are known to inhibit LH secretion in cyclic rats (Blank *et al.*, 1979; Bhanot & Wilkinson, 1983; Petraglia *et al.*, 1984), sheep (Malven *et al.*, 1984) and men (Morley *et al.*, 1980). Sirinathsinghji & Martini (1984) have demonstrated that, in the rat, endogenous opioids are involved also in the suppression of LH levels occurring during lactation. Our data extend these results to the sow.

The high levels of LH recorded in Sow F throughout the experimental regimen and the failure to influence these levels with naloxone seem to indicate that in this animal the suppressive mechanisms, possibly involving opioids, which normally inhibit LH during lactation, were not effective.

Hyperprolactinaemia has been described as able to affect LH secretion in the rat (Lu *et al.*, 1976), man (Roland, Lequin, Schellerens & de Jong, 1975) and sheep (Kann, Martinet & Schirar, 1977), and so one possible hypothesis to explain the inverse relationship occurring between LH and prolactin during lactation is that suckling stimulates the secretion of prolactin which in turn suppresses LH secretion. However, the present results seem to indicate that both high prolactin and low LH concentrations are the results of the same factor, acting through an opioid-mediated

mechanism. This is supported by the results of previous experiments in which bromocriptine suppressed prolactin concentrations in lactating sows without influencing LH values (Seren *et al.*, 1984; Mattioli & Seren, 1985). Similar results were obtained by Sirinathsinghi & Martini (1984) who did not find any effect of bromocriptine-suppressed prolactin levels on LH secretion in lactating rats whereas naloxone could prevent the inverse relationship between LH and prolactin seen after suckling. However, the role of prolactin *per se* in the suppression of LH secretion cannot be completely disregarded because the results of Bevers *et al.* (1983) indicate that prolactin contributes to the suppression of LH values.

Since stressful stimuli can increase the amounts of endogenous opiate in the central nervous system (Akil, Richardson, Barchas & Li, 1978; Akil & Watson, 1980), it might be supposed that a stimulus as potent as suckling would increase the production of opiates. Anatomical bases for such an hypothesis have been shown by Martin & Vogit (1981) and Vanderhaeghen, Lostra, Liston & Rossier (1983) who reported that oxytocin and opioids are co-localized in the same hypothalamic neurones. A suckling stimulus capable of releasing oxytocin might therefore cause opioid secretion as well.

Ellendorff, Elsaesser, Parvizi, Schakel & Smidt (1985) reported that oxytocin administration in sows after weaning maintains the anoestrus. These data, taken together with the finding that oxytocin can directly affect ACTH secretion through an opioid-mediated mechanism (Coiro *et al.*, 1985), indicate that oxytocin secreted in response to suckling may be the factor, acting through an opioid-mediated mechanism, responsible for the endocrine patterns of lactating pigs.

The difference in the duration of the effect of naloxone on prolactin and LH is of interest and warrants further studies.

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