Stimulation of release of $\beta$-endorphin and oxytocin by prostaglandin $F_{2\alpha}$ in cattle at parturition

J. E. Aurich¹, I. Dobrinski¹*, H-O. Hoppen² and E. Grunert¹

¹Clinic for Bovine Obstetrics and Gynaecology, and ²Section of Endocrinology, College of Veterinary Medicine, Hannover, Germany

Concentrations of $\beta$-endorphin and oxytocin were measured in plasma of cows before, during and after parturition. The effect of the PGF$_{2\alpha}$ analogue cloprostenol on $\beta$-endorphin and oxytocin release was investigated. During parturition, there were marked, parallel increases in $\beta$-endorphin and oxytocin concentrations. Both hormones were released in an episodic manner in conjunction with uterine and abdominal contractions. It is therefore likely that factors stimulating oxytocin release also enhance $\beta$-endorphin secretion. This suggests a role of labour or labour-associated hormones in stimulating peripheral $\beta$-endorphin release. Cloprostenol caused an immediate, pronounced increase in plasma $\beta$-endorphin and oxytocin concentrations.

Introduction

During pregnancy and parturition an increased release of $\beta$-endorphin into peripheral plasma has been shown in humans (Costatos et al., 1979; Goland et al., 1981) rats (Petraglia et al., 1985) and cattle (Aurich et al., 1990). Labour stimulates $\beta$-endorphin release in women and it has been suggested that endogenous opioids are involved in the stress response during delivery (Hoffman et al., 1984; Kofinas et al., 1985). Owing to relatively long sampling intervals in these studies, short-term changes in $\beta$-endorphin release during delivery could not be demonstrated and the exact nature of $\beta$-endorphin release during parturition remains to be clarified. Although labour is connected with increased $\beta$-endorphin concentrations in plasma, the influence of labour-associated hormones on $\beta$-endorphin secretion has not yet been examined. Interactions between opioid peptides, oxytocin and labour could be shown in laboratory animals. Opioids can inhibit oxytocin release from the neurohypophysis of parturient rats. These mechanisms are particularly activated when the animal is disturbed during delivery. In addition to central nervous mechanisms, peripheral opioids, by acting on the posterior pituitary, could have an effect on oxytocin secretion (Leng et al., 1985; Bicknell et al., 1988).

In this study we have measured short-term changes in plasma $\beta$-endorphin and oxytocin release in cattle before, during and after calving. Exogenous prostaglandin $F_{2\alpha}$ (PGF$_{2\alpha}$) has been shown to stimulate oxytocin release in postpartum cows (Ellendorff et al., 1979) and prostaglandins are involved in luteolysis and regulation of labour (Challis and Lye, 1986). We therefore investigated whether the PGF$_{2\alpha}$ analogue cloprostenol (500 µg; Estrumate; Pitman Moore, Burgwedel) after the ante-partum samples had been taken. Two cows (D and F) calved spontaneously. The mean duration of gestation at calving was 276.5 ± 3.6 days. Three animals (A, C and F) calved unassisted; for cows B, D and E, the calf was delivered by slight traction. In animal B, primary uterine inertia was diagnosed clinically. The cervix was fully dilated but the cow did not show signs of labour.

Materials and Methods

Animals

All animals in this study were German Black Pied cows from the dairy herd of the Hannover College of Veterinary Medicine. The mean duration of gestation in the herd is 283 ± 1 (±SD) days. Animals were brought to the Clinic for Bovine Obstetrics and Gynaecology at least 10 days before the expected day of calving. They were kept in individual stalls on straw and fed concentrate and hay.

Experiment 1

Blood samples were taken from six cows (A–F) via an indwelling jugular vein catheter on the day before calving, during calving and one day post partum. On the days before and after calving, samples were taken every 5 min for 35 min to 1 h. On the day of calving sampling began when the cow was detected to be in labour and continued at 5 min intervals until 30 min post partum. In four of the six animals (cows A, B, C and E), to facilitate a close supervision of calving, parturition was induced with a subcutaneous injection of the PGF$_{2\alpha}$ analogue cloprostenol (500 µg; Estrumate; Pitman Moore, Burgwedel) after the ante-partum samples had been taken. Two cows (D and F) calved spontaneously. The mean duration of gestation at calving was 276.5 ± 3.6 days. Three animals (A, C and F) calved unassisted; for cows B, D and E, the calf was delivered by slight traction. In animal B, primary uterine inertia was diagnosed clinically. The cervix was fully dilated but the cow did not show signs of labour.

Experiment 2

In a second group of 11 cows the effect of cloprostenol on $\beta$-endorphin and oxytocin release was investigated. Blood
samples were taken via an indwelling jugular vein catheter at 5 min intervals for 1 h. After 30 min either 500 µg cloprostenol (n = 5) or the same volume (2 ml) of saline (n = 6) was given i.v. The mean duration of gestation on the day of the experiment was 275.4 ± 2.2 days for the cloprostenol group and 276.2 ± 2.7 days for the control group.

In all samples plasma β-endorphin and oxytocin concentrations were determined and in the first sample from each day, plasma progesterone was also measured.

**Peptides and reagents**

Peptides were obtained from the following sources: human ACTH from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), Baltimore, MD; human β-lipotropin from the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Torrance, CA; met-enerkephalin, leu-enerkephalin, dynorphin 1–8 and 1–13 from Sigma Chemie, Deisenhofen; mesotocin, arg-vasotocin, lys-vasotocin, arg-vasotocin, isocotin, lys-vasopressin, somatostatin and camel (= bovine) β-endorphin from Bissendorf Biochemicals, Hannover. Acetonitrile of chromatography grade was used; all other reagents were of analytical grade.

**Hormone analysis**

β-Endorphin was extracted from plasma with C-18-cartridges (Bischoff, Leonberg) and measured by radioimmunoassay as described previously (Aurich et al., 1990). The extraction recovery for β-endorphin was 86%. The minimum detectable concentration of the β-endorphin radioimmunoassay was 19 pg; zero binding was 44%; nonspecific binding was 4%. Intra- and interassay variations were 5.9% and 9.2%, respectively, for the radioimmunoassay, and 9.6% and 11.9%, respectively, for extraction and radioimmunoassay.

Oxytocin was extracted from plasma with C-18-cartridges as described for β-endorphin by Aurich et al. (1990). The only modification was the use of 1.5% instead of 4% acetic acid for washing the cartridges. The extraction recovery was 66.3%.

Oxytocin was measured by radioimmunoassay as described by Schams (1983) with modifications. All radioimmunoassays were performed in polypropylene tubes on ice, using phosphate buffer (0.05 mol l⁻¹) with 0.003 mol EDTA l⁻¹ and 0.05% bovine serum albumin (w/v), pH 7.5 (OT buffer). The antiserum was raised in rabbits against oxytocin bound to bovine thyroglobulin. On a molar basis the antiserum crossreacted 54.3% with mesotocin, 15.6% with arg-vasotocin, 5.8% with lys-vasotocin, 1.5% with arg-vasotocin, 1.4% with isocotin, 0.4% with lys-vasopressin, 0.05% with somatostatin and less than 0.01% with camel (= bovine) β-endorphin, human β-lipotropin, leu-enkephalin, met-enkephalin, dynorphin 1–8, dynorphin 1–13 and human ACTH. The antiserum was used at a dilution of 1:13 300 in OT buffer. Standards (50 µl + 350 µl OT buffer) or resuspended sample extracts (400 µl) were incubated with 100 µl antiserum for 24 h at 4°C. For determination of nonspecific binding, normal rabbit serum at a dilution of 1:13 300 was used instead of antiserum. [125I]-labelled oxytocin (NEN, Dreieich) was used as tracer at a dilution of 10 000 c.p.m. in 100 µl in OT buffer. After addition of the tracer, incubation was continued for 48 h at 4°C. Normal horse serum, 100 µl, diluted 1:4 in phosphate buffer (0.05 mol l⁻¹) pH 7.5, was added to each tube, followed by 500 µl 4 mg dextran-T-70-coated charcoal ml⁻¹ (Separex: Sterant, St Albans) suspension for separation of free and bound ligands. After incubation for 10 min on ice, the tubes were centrifuged at 2000 g for 20 min at 4°C. Bound [125I]-labelled oxytocin in the supernatant was measured in a gamma-counter (Multigamma 1261: Pharmacia LKB, Freiburg). The minimum detectable concentration of the assay was 1.4 pg, zero binding was 49%, nonspecific binding was 5.6%. Increasing amounts of plasma (0.5–3.0 ml) displaced tracer bound to the antibody in a manner parallel to the standard curve. Intra- and interassay variations were, respectively, 7.6% and 7.8% for RIA and 7.9% and 10.8% for extraction and RIA.

Progesterone was determined by RIA after extraction with ether as described by Hoffmann et al. (1973). The efficiency of extraction for progesterone was 81%, the minimum detectable concentration was 10 pg and the intra- and interassay variations were 5.9% and 8.5%, respectively.

**HPLC of β-endorphin immunoreactivity**

After extraction, selected samples were subjected to reversed-phase high performance liquid chromatography (HPLC) on a C-18 column (Bischoff, Leonberg) using an LKB 2150 pump and 2151 controller (Pharmacia LKB, Freiburg). The column was eluted with a nonlinear gradient over 55 min at a flow of 0.6 ml min⁻¹. Solvent A was trifluoroacetic acid in 20% acetonitrile (v/v) and solvent B 70% acetonitrile. During 15 min, solvent B was increased from 0 to 30%, and was then raised to 40% within 20 min and 70% within 10 min. Fractions of 0.6 ml were collected, dried down in a vacuum centrifuge and stored at −20°C until radioimmunoassay.

**Statistical analysis**

For comparisons between groups a one-way analysis of variance was performed, using the SPSS statistics program (Norusis, 1986). All values given are means ± standard error of mean (SEM). The sensitivity limit of the radioimmunoassays was defined as three standard deviations from zero binding. An episode of increased β-endorphin and oxytocin release or pulse was defined according to Walters et al. (1984) as occurring when the value of the highest sample (peak) exceeded a preceding sample (basal) by at least four times the coefficient of variation of the assay for each respective peptide, and there had to be at least one more increased value before basal concentrations were reached again. The amplitude was determined by subtracting the basal from the peak value and pulse duration was defined as the time until base line values were reached again.

**Results**

**HPLC**

In the HPLC, β-endorphin immunoreactivity eluted in two adjacent but clearly separated peaks. About 70% of the β-
endorphin immunoreactivity eluted in the same position as the β-endorphin standard and 30% in the same position as β-lipotropin. The β-endorphin:β-lipotropin ratio did not differ on different days or between Expts 1 and 2.

Experiment 1

**Plasma progesterone.** Mean plasma progesterone concentrations were 2.8 ± 0.6 ng ml⁻¹ on the day before calving, 0.9 ± 0.7 ng ml⁻¹ during parturition and 0.3 ± 0.3 ng ml⁻¹ on the day post partum.

**Plasma β-endorphin and oxytocin.** On the day before calving plasma oxytocin concentrations were relatively low; no major changes in β-endorphin and oxytocin could be detected during 1 h of sampling at intervals of 5 min (Fig. 1). During calving there was a marked increase in β-endorphin and oxytocin release in all six animals (Fig. 2). Both hormones were released in an episodic manner. Highest concentrations were seen in conjunction with rupture of the fetal membranes and the expulsion or extraction of the calf. Oxytocin release increased in all animals and β-endorphin secretion was increased in five out of the six cows (A, B, C, D, F) during the expulsive phase of parturition. In addition, during the hour preceding expulsion of the calf, episodes of marked β-endorphin or oxytocin release or release of both compounds occurred in three of the cows (A, C, D) and, in conjunction with milking 20 min after delivery of the calf, β-endorphin and oxytocin release was stimulated in one animal (E). The release of β-endorphin and oxytocin occurred more or less in parallel; nine out of 12 β-endorphin pulses coincided with episodes of increased oxytocin release and only one oxytocin pulse was not accompanied by increased β-endorphin secretion. Pulse amplitude was 140.1 ± 24.4 pg ml⁻¹ for β-endorphin and 136.0 ± 37.8 pg ml⁻¹ for oxytocin. The number of pulses per h was 1.2 ± 0.4 and 1.0 ± 0.3 for β-endorphin and oxytocin, respectively; the mean duration of episodes of β-endorphin secretion was 22 ± 2 min and the average duration of increased oxytocin release 29 ± 4 min. In cow B during the first 70 min of sampling, concentrations of oxytocin in plasma were below the detection limit of the radioimmunoassay and no fluctuations in β-endorphin concentrations were seen. When the fetal membranes were ruptured manually and the calf was pulled into the cervix, the release of β-endorphin and oxytocin into the peripheral blood increased but remained below values measured in the other five animals. Plasma concentrations of β-endorphin and oxytocin were low at one day post partum and no episodic release could be detected (Fig. 3).

**Experiment 2**

**Plasma progesterone.** Progesterone concentrations before injection of cloprostenol or placebo were 3.9 ± 0.9 ng ml⁻¹ for the cloprostenol group and 3.5 ± 1.1 ng ml⁻¹ for control animals.

**Plasma β-endorphin and oxytocin.** Before application of cloprostenol or saline, β-endorphin and oxytocin concentrations were low; no significant differences were found between groups. Cloprostenol caused an immediate, pronounced increase in plasma concentrations of both peptides (Fig. 4). For 15 min after cloprostenol injection, β-endorphin concentrations were significantly higher (P < 0.001 and P < 0.01) than in corresponding control animals. Oxytocin release was significantly increased (P < 0.001 and P < 0.01) for 20 min after application of cloprostenol compared with the placebo group.

**Discussion**

In this study, we found an increased release of β-endorphin in parturient cows which is in agreement with previous results from different species (Csontos et al., 1979; Goland et al., 1981; Petraglia et al., 1985; Aurich et al., 1990). In addition, our results show that, during delivery, β-endorphin can be released in an episodic manner. Highest plasma β-endorphin concentrations were found in conjunction with uterine and abdominal contractions and distension of the cervix during rupture of the fetal
membranes and expulsion of the calf. No distinct episodes of increased β-endorphin release could be detected on the day before calving or one day post partum. This result suggests a role of labour or labour-associated hormones in stimulating β-endorphin release into peripheral plasma. In all animals, most episodes of β-endorphin release during calving coincided with peak plasma oxytocin concentrations. In cows with primary uterine inertia, no plasma oxytocin could be detected and β-endorphin concentrations were low before the obstetrical intervention and extraction of the calf. For oxytocin, our results are in agreement with those of Schams et al. (1979), who also found maximum oxytocin concentrations in cows during the expulsive phase of labour. It is likely that, during calving, factors stimulating oxytocin release also cause, at least in part, an increased secretion of β-endorphin.

Our results do not allow conclusions about the source of β-endorphin in peripheral plasma. Although β-endorphin is released primarily from the anterior pituitary (Guillemin et al., 1977), in pregnant sheep submitted to hypoglycaemia, the placenta has been identified as an additional source of β-endorphin (Falconer et al., 1988). β-Endorphin can also be synthesized by human placental tissue (Liotta et al., 1982). In contrast, in cattle submitted to a Caesarean section, β-endorphin concentrations in blood taken from a branch of the uterine vein were not higher than in samples taken simultaneously from the jugular vein (Dobrinski et al., 1992), hence no β-endorphin secretion from uteroplacental tissues into the maternal circulation could be demonstrated. However, β-endorphin release in Caesarean section patients could be different from cows with normal parturition. Although β-endorphin in plasma of parturient cows is likely to come from the pituitary, a uteroplacental contribution to β-endorphin concentrations in peripheral plasma is also possible.

In four of the six cows in Expt 1, parturition had been induced with cloprostenol. It can be assumed that, as far as the

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**Fig. 2.** Concentrations of β-endorphin immunoreactivity (●) and oxytocin (○) in plasma of six cows during parturition: (a) rupture of fetal membranes; (b) feet or nose of calf visible; (c) strong abdominal contractions; (d) expulsion or extraction of the calf; calf born; (e) milking; (f) manual exploration of the genital tract. Note different scales on y-axis for individual animals.
Fig. 3. \(\beta\)-Endorphin immunoreactivity (●) and oxytocin (○) concentrations in plasma of three cows one day after parturition.

regulation of labour and oxytocin release during calving are concerned, cloprostenol-induced parturition is comparable to spontaneous calvings. Because calving occurred at least 24 h after injection of cloprostenol, it is unlikely that \(\beta\)-endorphin and oxytocin release during labour were influenced by the PGF\(_{2\alpha}\) analogue in Expt 1.

In rats, opioids, by inhibiting oxytocin release, participate in the regulation of labour and timing of the interval between the birth of different pups (Leng et al., 1985). However, the direct opioid effect on oxytocin neurosecretory terminals is mediated primarily via \(\kappa\)-receptors, for which the endogenous ligands are dynorphins and not \(\beta\)-endorphin (Pesce et al., 1987; Bicknell and Zhao, 1989), but opioids also suppress the release of noradrenaline from neurohypophyseal noradrenergic terminals by acting on an as yet unidentified opioid receptor. Noradrenaline facilitates oxytocin release; a decrease in noradrenergic stimulation therefore leads to an indirect inhibition of oxytocin secretion from the neurohypophysis (Zhao et al., 1988). It is possible that anterior pituitary \(\beta\)-endorphin released at the same time as oxytocin acts on the neurohypophysis and by inhibiting

noradrenergic stimulation of oxytocin secretion participates in the regulation of oxytocin pulses during parturition.

Three out of six cows in Expt 1 required obstetrical assistance. This could be explained by the fact that during calving, the cows were irritated by the presence of the investigator. It is well known that parturition can be interrupted when the animal is disturbed. This inhibition of labour is mediated via the sympathoadrenal system (Huszar and Roberts, 1982; Fuchs and Fuchs, 1984) and, at least in the rat, also by opioidergic systems (Leng et al., 1987, 1988). Without the interference of sampling, all cows might have calved unassisted. This interpretation is supported by the fact that there was no cause for dystocia such as fetomaternal disproportions or postural abnormalities and the calves were delivered uneventfully by slight traction.

Exogenous cloprostenol stimulated a prompt increase in secretion of \(\beta\)-endorphin and oxytocin. The effect of PGF\(_{2\alpha}\) on oxytocin release in late pregnant cows is in agreement with results obtained from postpartum sows (Ellendorff et al., 1979). A comparable effect of prostaglandins on \(\beta\)-endorphin release has not yet been reported. It is likely that cloprostenol acts at the pituitary gland to stimulate the secretion of \(\beta\)-endorphin and oxytocin, but a release of \(\beta\)-endorphin from the placenta cannot be excluded. We have also not distinguished luteal oxytocin release, as reported for cycling cows (Walters et al., 1984), from neurohypophyseal secretion, nor shown how endogenous PGF\(_{2\alpha}\) might mediate an increase in \(\beta\)-endorphin and oxytocin release. It is not clear whether endogenous PGF\(_{2\alpha}\) is involved in the regulation of \(\beta\)-endorphin and oxytocin release during labour, or whether the experimentally induced relationship between
β-endorphin, oxytocin and prostaglandins is of physiological significance in parturient animals or is due to pharmacological effects caused by a luteolytic dose of cloprostenol.

It is concluded that β-endorphin, like oxytocin, is released in an episodic manner during parturition in cows and that both hormones are released concomitantly in conjunction with uterine and abdominal contractions and distension of the uterine cervix. The PGF$_{2α}$ analogue cloprostenol appears to stimulate a prompt increase in secretion of β-endorphin as well as oxytocin when administered to cows in late pregnancy.

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