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

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Concentrations of β -endorphin and oxytocin were measured in plasma of cows before, during and after parturition. The effect of the $PGF_{2\alpha}$ analogue cloprostenol on β -endorphin and oxytocin release was investigated. During parturition, there were marked, parallel increases in β -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 β -endorphin secretion. This suggests a role of labour or labour-associated hormones in stimulating peripheral β -endorphin release. Cloprostenol caused an immediate, pronounced increase in plasma β -endorphin and oxytocin concentrations.

Introduction

During pregnancy and parturition an increased release of β -endorphin into peripheral plasma has been shown in humans (Csontos *et al.*, 1979; Goland *et al.*, 1981) rats (Petraglia *et al.*, 1985) and cattle (Aurich *et al.*, 1990). Labour stimulates β -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 β -endorphin release during delivery could not be demonstrated and the exact nature of β -endorphin release during parturition remains to be clarified. Although labour is connected with increased β -endorphin concentrations in plasma, the influence of labour-associated hormones on β -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 β -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* sows (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 has an effect on plasma oxytocin and β -endorphin concentrations in late pregnant cows.

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 ($\pm$ 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 μ g; 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 β -endorphin and oxytocin release was investigated. Blood

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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-enkephalin, leu-enkephalin, dynorphin 1–8 and 1–13 from Sigma Chemie, Deisenhofen; mesotocin, arg-vasotocin, lys-vasotocin, arg-vasotocin, isotocin, 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 inter-assay 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 $0.003 \text{ mol EDTA l}^{-1}$ 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 isotocin, 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 non-specific binding, normal rabbit serum at a dilution of 1:13 300 was used instead of antiserum. ^{125}I -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; Steranti, 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 ^{125}I -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 $\pm$ 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 -

Fig. 1. β -Endorphin immunoreactivity (●) and oxytocin (○) concentrations in plasma of three late pregnant cows one day before parturition.

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

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.

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 (Dobriniski *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

Fig. 3. β-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 β-endorphin and oxytocin release during labour were influenced by the PGF_{2α} 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 κ-receptors, for which the endogenous ligands are dynorphins and not β-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 β-endorphin released at the same time as oxytocin acts on the neurohypophysis and by inhibiting

Fig. 4. Effect of cloprostenol ($n = 5$; ●) or saline ($n = 6$; ○) on (a) β-endorphin immunoreactivity and (b) oxytocin in late pregnant cows; arrow: 500 μg cloprostenol or 2 ml saline i.v.; **, ***: values for groups are significantly different ($P < 0.01$, $P < 0.001$, respectively); values are means + standard error of mean (SEM).

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 sympatho-adrenal 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 β-endorphin and oxytocin. The effect of PGF_{2α} 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 β-endorphin release has not yet been reported. It is likely that cloprostenol acts at the pituitary gland to stimulate the secretion of β-endorphin and oxytocin, but a release of β-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_{2α} might mediate an increase in β-endorphin and oxytocin release. It is not clear whether endogenous PGF_{2α} is involved in the regulation of β-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_{2 α} 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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References

- Aurich JE, Dobrinski I, Hoppen H-O and Grunert E (1990) β -Endorphin and met-enkephalin in plasma of cattle during pregnancy, parturition and the neonatal period *Journal of Reproduction and Fertility* **89** 605–612
- Bicknell RJ and Zhao B-G (1989) Secretory terminals of oxytocin neurones as a site of opioid modulation. In *Brain Opioid Systems in Reproduction* pp 289–306 Eds RG Dyer and RJ Bicknell. Oxford University Press, Oxford, New York
- Bicknell RJ, Leng G, Russell JA, Dyer RG, Mansfield S and Zhao B-G (1988) Hypothalamic opioid mechanisms controlling oxytocin neurones during parturition *Brain Research Bulletin* **20** 743–749
- Challis JRG and Lye SJ (1986) Parturition *Oxford Reviews of Reproductive Biology* **8** 61–129
- Csontos K, Rust M, Höllt V, Mahr W, Kromer W and Teschemacher HJ (1979) Elevated plasma β -endorphin levels in pregnant women and their neonates *Life Sciences* **25** 835–844
- Dobrinski I, Aurich JE, Hoppen H-O and Grunert E (1992) β -Endorphin and met-enkephalin in cattle during late gestation and parturition – release from uteroplacental tissues *Animal Reproduction Science* **27** 75–79
- Ellendorff F, Forsling M, Parvizi N, Williams H, Taverne M and Smidt D (1979) Plasma oxytocin and vasopressin concentrations in response to prostaglandin injection into the pig *Journal of Reproduction and Fertility* **56** 573–577
- Falconer J, Chan E-C, Madsen G, Thomson M, Davies J and Smith R (1988) Secretion of β -endorphin into the maternal circulation by uteroplacental tissues in response to hypoglycaemic stress *Journal of Endocrinology* **118** R5–R8
- Fuchs A-R and Fuchs F (1984) Endocrinology of human parturition: a review *British Journal of Obstetrics and Gynaecology* **91** 948–967
- Goland RS, Wardlaw SL, Stark RI and Frantz AG (1981) Human plasma β -endorphin during pregnancy, labor, and delivery *Journal of Clinical Endocrinology and Metabolism* **52** 74–78
- Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W and Bloom F (1977) β -Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland *Science* **197** 1367–1369
- Hoffman DI, Abboud TK, Haase HR, Hung TT and Goebelsmann U (1984) Plasma β -endorphin concentrations prior to and during pregnancy, in labor, and after delivery *American Journal of Obstetrics and Gynecology* **150** 492–496
- Hoffmann B, Kyrein HJ and Ender ML (1973) An efficient procedure for the determination of progesterone by radioimmunoassay applied to bovine peripheral plasma *Hormone Research* **4** 302–310
- Huszar G and Roberts JM (1982) Biochemistry and pharmacology of the myometrium at labor: regulation at the cellular and molecular levels *American Journal of Obstetrics and Gynecology* **142** 225–237
- Kofinas GD, Kofinas AD and Tavakoli FM (1985) Maternal and fetal β -endorphin response to the stress of labor and delivery *American Journal of Obstetrics and Gynecology* **152** 56–59
- Leng G, Mansfield S, Bicknell RJ, Dean ADP, Ingram CD, Marsh MIC, Yates JO and Dyer RG (1985) Central opioids: a possible role in parturition *Journal of Endocrinology* **106** 219–224
- Leng G, Mansfield S, Bicknell RJ, Chapman C, Hollingworth S, Ingram CD, Marsh MIC, Yates JO and Dyer RG (1987) Stress-induced disruption of parturition in the rat may be mediated by endogenous opioids *Journal of Endocrinology* **114** 247–252
- Leng G, Mansfield S, Bicknell RJ, Blackburn RE, Brown D, Chapman C, Dyer RG, Hollingworth S, Shibuki K, Yates JO and Way S (1988) Endogenous opioid actions and effects of environmental disturbance on parturition and oxytocin secretion in rats *Journal of Reproduction and Fertility* **84**, 345–356
- Liotta AS, Houghten R and Krieger DT (1982) Identification of a β -endorphin-like peptide in cultured human placental cells *Nature* **295** 593–595
- Norusis MJ (1986) *SPSS/PC + for the IBM PC/XT/AT*. SPSS Inc., Chicago
- Pesce G, Lang MA, Russell JT, Rodbard D and Gainer H (1987) Characterization of κ -opioid receptors in neurosecretosomes from bovine posterior pituitary *Journal of Neurochemistry* **49** 421–427
- Petraglia F, Baraldi M, Giarre G, Facchinetti F, Santi M, Volpe A and Genazzani AR (1985) Opioid peptides of the pituitary and hypothalamus: changes in pregnant and lactating rats *Journal of Endocrinology* **105** 239–245
- Schams D (1983) Oxytocin determination by radioimmunoassay. III. Improvement to subpicogram sensitivity and application to blood levels in cyclic cattle *Acta Endocrinologica* **103** 180–183
- Schams D, Schmidt-Polex B and Kruse V (1979) Oxytocin determination by radioimmunoassay in cattle *Acta Endocrinologica* **92** 258–270
- Walters DL, Schams D and Schallenger E (1984) Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during the luteal phase of the oestrous cycle in the cow *Journal of Reproduction and Fertility* **71** 479–491
- Zhao B-G, Chapman C, Brown D and Bicknell RJ (1988) Opioid–noradrenergic interactions in the neurohypophysis. II. Does noradrenaline mediate the action of endogenous opioids on oxytocin and vasopressin release? *Neuroendocrinology* **48** 25–31