The effect of opioid antagonism and environmental restriction on plasma oxytocin and vasopressin concentrations in parturient gilts

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Abstract

Oxytocin plays an important role at parturition due to its involvement in uterine contractions, foetal expulsion and the onset of maternal behaviour. The role of the related neurohypophysial hormone, vasopressin, is less clear; however, there is some evidence that it is also involved in maternal behaviour and its role in osmotic regulation is well established. The aim of this study was to investigate the inhibitory effects of endogenous opioids on these hormones during the expulsive phase of parturition in the pig, and to examine how opioid restraint interacts with environmental restriction.

The subjects of this study were 31 Large White × Landrace primiparous sows (gilts). An indwelling jugular catheter was implanted under general anaesthesia at 12 days before the expected parturition day (EPD). From 5 days before the EPD 15 of the gilts were individually housed in a restrictive parturition crate without straw and 16 were individually housed in a straw-bedded pen. Blood samples were taken with increasing frequency towards and during parturition through a catheter extension to reduce disturbance. At 7.5 min after the birth of the first piglet half of the gilts in each environment received a dose of the opioid receptor antagonist naloxone (1 mg/kg, i.v.) with the remaining gilts receiving saline as a control.

Overall, there was no effect of environment on either circulating oxytocin or vasopressin. However, both oxytocin and vasopressin were inhibited by endogenous opioids during the expulsive phase. The inhibitory effects of opioids on these hormones did not appear to have any adverse effects on the progress of parturition as judged by cumulative piglet birth intervals.

The regulation of the opioid inhibition of oxytocin and vasopressin during parturition is discussed in relation to other neurotransmitters and whether opioid inhibition of these neurohypophysial hormones is part of the ‘normal’ physiological response to parturition or whether it is stress-induced.

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Introduction

The parturition process is associated with a cascade of hormonal changes as reviewed by Taverne (1992), including the release of plasma oxytocin and vasopressin from the neurohypophysis (rat: Landgraf et al. 1992, pig: Lawrence et al. 1995, cow: Landgraf et al. 1983). Pulsatile neurohypophysial oxytocin release is required for the maintenance of normal parturition (Luckman et al. 1993), and inhibition of oxytocin release by antagonists has been shown to prolong the expulsive phase in rats (Antonijevic et al. 1995) and guinea pigs (Schellenberg 1995). In the pig, prolonged parturition is associated with low basal and peak levels of oxytocin during the expulsive phase (Castren et al. 1993).

One of the major roles of oxytocin in the progress of parturition is believed to be in the co-ordination of uterine contractions (rat: Summerlee 1981, Antonijevic et al. 1995, rabbit: O’ Byrne et al. 1986, pig: Taverne et al. 1979) and foetal expulsion (Gilbert et al. 1994), with oxytocin receptors in the uterus increasing dramatically prior to parturition (Soloff et al. 1979, Alexandrova & Soloﬀ 1980).

In addition, oxytocin may play a central role in the facilitation of maternal behaviour, such as retrieval of pups and crouching in the rat (Pederson et al. 1982, Fahrbach et al. 1985) and sniffing and low-pitch bleating to the lamb in sheep (Keverne & Kendrick 1992). Another important aspect of maternal care is lactation, and it is known from work by Higuchi et al. (1985) that intramammary pressure is related to increased serum oxytocin concentrations in rats. In the pig, maximal oxytocin concentrations during a nursing bout occurs at the peak grunt rate which accompanies milk let-down (Ellendorf et al. 1982, Algers et al. 1990).
The role of neurohypophysial vasopressin is less clear; however, we know from previous work in pigs (Lawrence et al. 1995) that vasopressin does increase during the expulsive phase. One of the main roles of vasopressin is regulation of plasma osmolality, and work in cows has shown an increase in plasma osmolality at parturition which coincides with increased plasma vasopressin concentrations (Landgraf et al. 1983). Vasopressin appears to also play a role in facilitating maternal behaviour in rats. Administration of vasopressin improves the performance of maternal behaviour; however, this is somewhat delayed in comparison with oxytocin-induced improvements in maternal care (Pedersen et al. 1982).

Therefore the importance of these two neurohypophysial hormones to the parturition process warrants concern that environmental stressors during the expulsive phase of parturition may interfere with the release of these hormones, and this has particular relevance where animals are housed under confining, putatively stress-inducing conditions. Pigs, under commercial conditions, are environmentally restricted during the parturition and lactation period, and we know from previous work (Lawrence et al. 1992) that movement into these restrictive environments (parturition crates) during the expulsive phases causes a reduction in plasma oxytocin levels and prolongs piglet expulsion, with both of these effects being naloxone-reversible. A further study by Lawrence et al. (1995) found that when pigs were given 5 days to habituate to the parturition crate before the expected day of parturition (EPD) they did not have significantly lower levels of oxytocin or vasopressin than pigs housed in a more spacious pen environment and supplied with bedding material. The aim of this study was therefore to investigate the inhibitory effects of endogenous opioids on these hormones during the expulsive phase of parturition, and to examine how opioid inhibition interacts with environmental restriction.

Materials and Methods

Animals

The subjects of the study were 32 Large White × Landrace gilts (primiparous female pigs: Cotswold Pig Development, Lincoln, Lincs, UK). The gilts were group housed in straw-bedded pens during pregnancy. For full details see Jarvis et al. (1998).

Catheterization

Approximately 15 days before the EPD all gilts had a jugular vein catheter implanted under general anaesthesia (for full details of the procedure see Lawrence et al. 1992), and they were then housed individually in straw-bedded pens. One gilt was removed from the study due to a blocked catheter.

Experimental housing

Five days before EPD the gilts were moved to either a conventional parturition crate without straw (n=15: 2·25 m in length, 0·45 m in width and 1·05 m in height) or to a pen with straw (n=16: 2·5 m × 3·0 m). Lighting was provided between 0800 h and 1600 h and the room temperature was maintained at approximately 18 °C.

Blood sampling

Blood samples (7 ml) were collected through a catheter extension which minimised the disturbance to the gilts by allowing samples to be taken from outside the crate or pen area. Samples were collected in heparinised monovette tubes (Sarstedt, Leicester, Leics, UK) at 10-min intervals in the hour before the birth of the first piglet (BFP) (baseline). This increased to 5-min intervals in the first hour following the BFP (hour 1) and then to 10-min intervals in hour 2. Samples were then taken every 15 min until 4 h post-BFP. At 7·5 min after the BFP the gilts received either naloxone (1 mg/kg body weight: Sigma Aldrich Company Ltd, Gillingham, Dorset, UK) or a control dose of saline administered via the jugular vein catheter. Saline was used to replace the volume of blood taken following each sample and the catheter and extension primed with heparinised saline (75 IU/ml). The samples were kept at 4 °C for 30 min before centrifuging. They were then spun at 3000 r.p.m. at 4 °C for 20 min. Aliquots of plasma were pipetted and stored at −20 °C for future assay.

Radioimmunoassays

Oxytocin Oxytocin was extracted using Sep-Pak C-18 cartridges and assayed in duplicate as previously described (Thornton et al. 1986) from all of the samples taken. Recoveries of standard synthetic oxytocin after extraction ranged from 85 to 90%. Inter- and intra-assay coefficients of variation were 6% and 5% respectively at 200 pg/ml and 14·8% and 13·0% at 20 pg/ml. Assay sensitivities ranged from 0·8 to 2·3 pg/ml.

Vasopressin Lysine-vasopressin was determined for all samples up to the end of the second hour following the BFP. A specific radioimmunoassay was used according to the method described by Thornton et al. (1987). The lower limit of detection of the assay is 0·05 μ units/ml and the inter- and intra-assay coefficients of variation were 12·2% and 8·3% for 1·25 μ units/ml. There was no cross-reactivity with neurophysin or corticotrophin-releasing factor.

Statistical analysis

Progress of parturition To determine differences between environment and injection groups in the length of
parturition (time from birth of first to last piglet), piglet interval and cumulative piglet interval a two-way analysis of variance was carried out (ANOVA; Genstat version 5).

Hormonal analysis The data were non-normal and therefore were log10 transformed. An analysis of variance (Genstat version 5) was used to determine if there were any differences between the treatment groups in the hour before the BFP. As no differences between the environments were found in terms of plasma oxytocin or vasopressin, no baseline level was removed from subsequent data. The data were analysed using a repeated measures analysis of variance (ANOVA; Genstat version 5), with a blocked structure for pig and time. Factors were environment (two levels; crate (C) and pen (P)), injection (two levels; naloxone (N) or saline (S)) and treatment (four levels; P/N, P/S, C/N, C/S). To assess whether a change in plasma concentrations of vasopressin and oxytocin had occurred at the onset of parturition, a Wilcoxon Rank Sign test was carried out between concentrations at −10 and 0 min in relation to the BFP.

In addition, to describe the pattern of response following injection, a linear regression model (Minitab, version 7·2) was fitted over time from the injection of either naloxone or saline until the end of sampling for oxytocin and vasopressin.

Results

Progress of parturition and piglet information

There was no difference in the inter-piglet birth intervals or the length of the expulsive phase of parturition between the treatments (mean parturition length (h)=3·82 (C/N), 4·59 (C/S), 3·10 (P/N), 2·67 (P/S), standard error of the difference (s.e.d.)=0·74). The cumulative piglet interval appeared to be longer in the C/S gilts, particularly as parturition progressed; however, this did not reach significance (Fig. 1: F 1,29=0·38, not significant (n.s.)). Full details on piglet mortality are described in Jarvis et al. (1998).

Hormonal analysis

Oxytocin There were no significant differences between the treatment groups during the baseline period (Fig. 2) and therefore no baseline was removed from subsequent values (F3,20=1·57, n.s.). Plasma oxytocin concentrations did increase slightly during the baseline period towards the onset of the expulsive phase of parturition (time: F4,57=3·27, P<0·05) irrespective of environment. There was a rapid increase in plasma oxytocin between the sample taken at 10 min before the onset of parturition and the delivery of the first piglet (Wilcoxon statistic (W)=189·0, P<0·001, Fig. 2); however, this again was not affected by environment (F1,23=0·45, n.s.). Plasma oxytocin concentrations increased rapidly in response to administration of naloxone (effect of injection type: F1,23=16·05, P<0·001) and remained elevated during hour 1 of parturition in comparison with the saline controls (Fig. 2). This elevation of plasma oxytocin following naloxone administration was similar for gilts in both environments (effect of environment: F1,23=1·16, n.s.). During hour 1 plasma oxytocin concentrations increased gradually in the saline-treated animals and by the start of

- Figure 1 Mean cumulative inter-birth interval between piglets. Cumulative birth intervals (min) between piglets 1 and 2 (interval 1) up to piglet 1 to 11 (interval 10) for the four treatment groups: C+N (solid line, n=8), C+S (tightly dashed line, n=7), P+N (dashed and dotted line, n=8), P+S (widely dashed line, n=8). S.E.D. is indicated.
- Figure 2 Mean plasma oxytocin around the BFP is indicated by the solid vertical line at 0 min. Injection of naloxone (solid line, n=16) or saline (dashed line, n=15) is indicated by the solid vertical line at 7·5 min post-BFP. Due to the lack of environmental effects the data are presented by injection type only. S.E.D. is indicated.

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hour 2 levels were similar between the two injection groups. There was no effect of injection type \((F_{1,26} = 0.01, \text{n.s.})\) or environment \((F_{1,26} = 0.24, \text{n.s.})\) during hour 2 or hours 3–4 (effect of injection type: \(F_{1,27} = 0.20, \text{n.s.}\); effect of environment: \(F_{1,27} = 2.06, \text{n.s.}\)). There was a decrease in plasma oxytocin during the last 30 min of this time-period (time: \(F_{7,122} = 3.20, P < 0.01\)).

Overall, the pattern of oxytocin over the period following injection of either naloxone or saline was quite different (Fig. 2). Control gilts receiving saline showed a fairly steady rise in oxytocin over the 4 h, resulting in a fairly low constant and positive gradient \((\text{oxytocin} = 146 + 0.7 \text{ time})\). Naloxone-treated gilts showed an elevation in plasma oxytocin immediately following injection resulting in a high constant, with levels declining over the following 4 h leading to a negative gradient \((\text{oxytocin} = 327 - 0.4 \text{ time})\).

**Vasopressin** Plasma vasopressin concentrations were unaffected by treatment group during the baseline period (Fig. 3: \(F_{3,21} = 0.68, \text{n.s.}\)); however, an increase in plasma vasopressin was seen between 50 and 30 min (time: \(F_{4,65} = 3.17, P < 0.05\)). Plasma vasopressin increased from the BFP to the samples 5 min later in the gilts about to receive naloxone; however, this was not significantly higher than the saline-treated group (effect of injection: \(F_{1,25} = 0.25, \text{n.s.}\), Fig. 3). Once naloxone had been administered, plasma vasopressin concentrations were elevated in these animals (Fig. 3) compared with the control animals during hour 1 \((F_{1,25} = 8.98, P < 0.01)\) and hour 2 \((F_{1,23} = 5.86, P < 0.05)\). Plasma vasopressin concentrations were not affected by environmental restriction during either of these time-periods.

Overall, the pattern of vasopressin following injection was fairly similar across the naloxone- and saline-treated groups which led to the lines of best fit having similar gradients (Fig. 3). However, the naloxone line, due to an increase immediately following injection, had a higher constant than that of the line of best fit for the saline group \((\text{naloxone: vasopressin} = 1.46 - 0.02 \text{ time}; \text{saline: vasopressin} = 1.39 - 0.001 \text{ time})\).

**Discussion**

Consistent with Lawrence et al. (1995), this study found no effects of environmental restriction on oxytocin or vasopressin release during parturition. This is also consistent with measurements of the hypothalamic–pituitary–adrenal (HPA) axis, in which gilts housed in crates and pens had concentrations of plasma cortisol which were not significantly different during parturition (Jarvis et al. 1998). The lack of effect of environmental restriction on oxytocin release is reinforced by a similar lack of effect on duration of parturition and piglet birth intervals.

In addition, although an opioid inhibition of oxytocin occurred, this opioid restraint did not interact with the parturition environment. It is known that oxytocin is inhibited by opioids in response to acute stress during parturition in the rat (Leng et al. 1988) and the pig (Lawrence et al. 1992). This suggests that either both of the environments used in this study are sub-optimal and stress-inducing to a parturient pig or, secondly, that opioid restraint is a part of ‘normal’ parturition in the pig.

Opioid inhibition of oxytocin may play a role in the initiation of the expulsive phase of parturition of ‘unstressed’ rats (Douglas & Bicknell 1993) in that the release of oxytocin that occurs at the onset of parturition results from a desensitisation of oxytocin nerve terminals to the inhibitory effects of κ opioids. Once parturition is in progress it has also been suggested that opioids may be involved in the pulsatile release of oxytocin: the magnitude of pulsatility is unaffected by naloxone administration; however, the frequency of pulses of oxytocin is increased in response to naloxone (Gilbert et al. 1997). As the pig is a litter-bearing animal then it is crucial that oxytocin is released in a way that maintains optimal uterine function–

There may be advantages in restraining oxytocin release in the pig to either conserve pituitary content of oxytocin or prevent desensitisation of uterine oxytocin receptors. Therefore release of the majority of available oxytocin at the onset of parturition may be disadvantageous in relation to the birth of later piglets. Indeed, there is now evidence that the likelihood of a piglet being born dead is higher during the later stages of farrowing, and particularly following a long birth interval (Fraser et al. 1997); however, this has not been shown to be through attenuation of uterine contractility.

An alternative, although not mutually exclusive, possibility is that opioid inhibition of oxytocin occurs as a
response to parturition ‘stress’ (e.g. Lawrence et al. 1992). It is known that HPA activity is elevated during farrowing (Lawrence et al. 1994, Jarvis et al. 1998) indicating that the progress of parturition is associated with increased physiological stress. Opioids are released in response to stress and pain (Dalayeuon et al. 1993), and it is known that an opioid-mediated analgesic system occurs at parturition in the pig (Jarvis et al. 1997b). There are a number of intrinsic aspects of the delivery process which appear as potentially stress-inducing, including the discomfort, pain and novelty associated with the experience of uterine contractions and actual expulsion. It is also likely that the initial response of the primiparous sow to her first-born piglet will be neophobic as this is most likely the first time she will have encountered a neonate.

The suggestion that opioid inhibition of oxytocin is a result of stressors associated with the parturition process is reinforced by the pattern of release over the expulsive phase in saline-treated gilts. Oxytocin is very much inhibited by opioids at the beginning of the expulsive phase; however, there appears to be some relaxation of this inhibition as parturition progresses. This may be due to the gilt experiencing and therefore adapting to the intrinsic stress-inducing aspects of parturition.

The present study has found that the release of vasopressin in the pig, consistent with other studies in rats (Wells & Forsling 1991, Van de Heijning et al. 1991) is under inhibition by opioids. Previous work in rats (Van de Heijning et al. 1991) provided evidence that opioid receptor subtypes µ and κ were involved in the control of oxytocin and vasopressin. The antagonist used in the present study, naloxone, preferentially binds to µ receptors, suggesting the possible involvement of this opioid receptor subtype in the inhibition of vasopressin in the pig.

Plasma vasopressin levels in the pigs receiving saline were, although elevated, fairly constant over the 2 h following the BFP. This would suggest a more constant opioid inhibition of vasopressin over hours 1 and 2. This is reinforced by vasopressin levels in the naloxone-treated pigs beginning to fall back down to the level of the saline-treated pigs.

The role of vasopressin at parturition is unclear; although neurohypophysial release of vasopressin is elevated during parturition, which is known to be associated with increased opioid inhibition of oxytocin (Van de Heijning et al. 1991), there remains an inhibitory effect of opioids. The rise in vasopressin in cows at parturition is known to coincide with increased plasma osmolality (Landgraf et al. 1983), suggesting its role in osmolality regulation.

Evidence from sheep work suggests that the opioid inhibition of vasopressin is modulated by glucocorticoids (Currie et al. 1994), and it is known that cortisol is elevated in the pig during the pre–parturient (Lawrence et al. 1994, Jarvis et al. 1997a) and the parturient periods (Lawrence et al. 1994, Jarvis et al. 1998). In addition, there is also evidence in men that γ-aminobutyric acid (GABA) inhibits vasopressin with opioids playing a mediatory role (Otake et al. 1991, Chiodera et al. 1993). However, the prevalence of GABA receptor subtypes is known to change around parturition and lactation in relation to the different magnocellular neurons (Fenelon & Herbison 1996) and therefore may come to play an inhibitory role on vasopressin and oxytocin at parturition.

Another important factor to consider in relation to inhibition of neurohypophysial hormones is the free radical nitric oxide (NO). It is known that NO inhibits vasopressin and oxytocin release in rats (Kadekaro et al. 1997), and it has been shown that NO prolongs parturition and disrupts maternal behaviour (Okere et al. 1996a), and also interferes with milk transfer to pups (Okere et al. 1996b). In addition, there appears to be a modulatory effect of GABA on NO inhibition of oxytocin and vasopressin (Choinedera et al. 1996).

In conclusion, release of vasopressin and oxytocin in the pig is under opioid inhibition during parturition. It is unclear whether this opioid restraint serves a physiological function, such as maintaining optimal uterine functioning over the entire expulsive phase, or whether it reflects stress–inducing aspects of the parturition process. However, work in rats and humans would suggest that the role of opioids is a mediatory one and therefore it will be important in the future to also consider other factors such as GABA and NO.

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