Effect of progesterone on the activation of neurones of the supraoptic nucleus during parturition

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Parturition is driven by a pulsatile pattern of oxytocin secretion, resulting from burst firing activity of supraoptic oxytocin neurones and reflected by induction of Fos expression. Rats were injected with progesterone on day 20 of pregnancy to investigate the role of the decreasing progesterone:oestrogen ratio, which precedes delivery, in the activation of supraoptic neurones. Progesterone delayed the onset of birth by 28 h compared with vehicle (control) and prolonged the duration of delivery, which was overcome by pulsatile injections of oxytocin, indicating that the slow delivery may reflect impaired oxytocin secretion. Parturient rats pretreated with progesterone had fewer Fos immunoreactive nuclei in the supraoptic nucleus than did parturient rats pretreated with vehicle. The number of Fos immunoreactive nuclei was not restored after oxytocin injection, indicating that appropriate activation of oxytocin neurones is impaired by progesterone and also that there is a lack of stimulatory afferent drive. Fos expression increased in the nucleus of the tractus solitarius during parturition in rats pretreated with either vehicle or progesterone, but not in rats that had been pretreated with progesterone and induced with oxytocin, indicating that this input was inhibited. Endogenous opioids inhibit oxytocin neurones in late pregnancy and the opioid antagonist, naloxone, increases Fos expression in supraoptic nuclei by preventing inhibition. However, progesterone attenuated naloxone-induced Fos expression in the supraoptic nucleus in late pregnancy and naloxone administered during parturition did not accelerate the duration of births delayed by progesterone administration, indicating that progesterone does not act by hyperactivation of endogenous opioid tone. RU486, a progesterone receptor antagonist, enhanced supraoptic neurone Fos expression in late pregnancy, indicating progesterone receptor-mediated actions. Thus, progesterone withdrawal is necessary for appropriate activation of supraoptic and tractus solitarius neurones during parturition.

Introduction

Parturition in rats is preceded by a decrease in plasma progesterone concentrations, resulting in a decreased ratio of plasma progesterone:oestrogen. This switch from a state of progesterone dominance during most of pregnancy to oestrogen dominance at the end is a prerequisite for myometrial changes, including the formation of gap junctions and the expression of oxytocin receptors, which are important for successful delivery (Chwalisz *et al.*, 1991; Larcher *et al.*, 1995). When the normal decrease in plasma progesterone concentrations in rats is postponed, uterine changes are prevented and gestation is prolonged (Garfield *et al.*, 1982; Bosc *et al.*, 1987). However, treatment with RU486, a progesterone receptor antagonist, enhances myometrial sensitivity to oxytocin in pregnancy (El Alj *et al.*, 1990).

In addition to their uterine effects, the sex steroid changes at term may influence the neural mechanisms involved in parturition. Intracerebroventricular administration of progesterone reduces oxytocin release in response to vaginal distension (Roberts, 1971), and progesterone and its metabolites reduce the electrical activity of supraoptic oxytocin neurones in term pregnant rats (Jiang and Wakerley, 1997). Thus, changes in oestrogen and progesterone secretion at the end of pregnancy may influence the neural pathways mediating the reflex release of oxytocin during parturition.

Administration of oestrogen or progesterone also enhances opioid synthesis and action in the hypothalamus (Bridges and Ronsheim, 1987; Genazzani *et al.*, 1990; Ingram *et al.*, 1996) and spinal cord (Dawson-Basoa and Gintzler, 1993). Endogenous opioids inhibit the activity of oxytocin neurones in late pregnancy and removal of this influence by naloxone (a general opioid antagonist) induces Fos expression in supraoptic neurones (Douglas *et al.*, 1995). During parturition, opiate drugs delay births and inhibit Fos

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expression in the supraoptic nucleus (Luckman *et al.*, 1993); opioid antagonists reverse this delay in pup births (Russell *et al.*, 1989a; Douglas *et al.*, 1993) and prevent inhibition of Fos expression (Douglas and Russell, 1993).

The aims of the present study were to investigate the importance of the decrease in progesterone secretion before term for the activation of supraoptic neurones during parturition and to determine whether sustaining progesterone concentrations delays and disrupts parturition by enhancing endogenous opioid inhibition of these neurones.

Materials and Methods

Animals

Female virgin Sprague–Dawley rats (body weight 225–250 g) were mated with experienced males, and after a vaginal plug was found (designated day 0 of pregnancy) the rats were kept singly with food and water available *ad libitum*, at an ambient temperature of 22°C with a 12 h light:12 h dark cycle. All procedures were performed under approved UK Home Office Licences. On day 20 of pregnancy at 09:30 h, the rats were injected i.m. with either progesterone (5 mg; Intervet Laboratories Ltd, Cambridge) or vehicle oil (containing 0.3% (v/v) cresol, 10% (v/v) benzylalcohol and 89.7% (v/v) arachis oil; Intervet Laboratories Ltd).

Experiment 1: effect of progesterone on delivery and Fos expression

From the morning of day 21 of pregnancy, rats were observed for signs of labour and delivery of pups. During the dark period, observations were continued in red light. Onset of delivery was defined as the time at which the first pup was expelled fully; the time of each subsequent pup birth was recorded.

Rats were killed in time-matched pairs, one from each of the progesterone- or vehicle-pretreated groups, either before (on days 21 and 22: prepartum), during (at 90 min after the birth of the second pup: parturient) or after delivery (6–12 h after the birth of pup 1: postpartum). The brain of each rat was removed, frozen rapidly on crushed dry ice and stored at –70°C until processed further by immunocytochemistry. The number and mass of pups were assessed post mortem.

Experiment 2: effect of oxytocin administration on delivery and Fos expression after pretreatment with progesterone

A chronic jugular vein cannula (Silastic tubing; Altec, Alton) was implanted 1 day before the experiment under brief ether anaesthesia into other rats that had been pretreated with progesterone. On the morning of the experiment, the cannulae were flushed with 0.1 ml heparinized saline (50 iu ml⁻¹) and connected to polyethylene tubing filled with either oxytocin or saline. The rats were given repeated pulse injections of oxytocin (Syntocinon; Sandoz, Hannover; 10 mU per 10 min for 2 h, and then 20 mU per 10 min for 2 h, or until 90 min after

the birth of the second pup; this dose was shown to be effective at initiating parturition by Antonijevic *et al.* (1995)) or vehicle (isotonic saline; 30 µl per 10 min for 4 h) on either day 21 or 22 of pregnancy. On day 21 of pregnancy, the rats were killed within 90 min after the injections by which time none of them had given birth. On day 22 of pregnancy, rats injected with oxytocin were killed 90 min after the birth of the second pup and were time-matched to a vehicle-injected rat (shortly after the injections but before delivery of pups). The brain of each rat was removed, frozen and stored as described earlier.

Experiment 3: effect of progesterone on plasma oxytocin concentrations and pituitary content

A chronic jugular vein cannula filled with heparinized saline was implanted into pregnant rats under brief halothane anaesthesia on the morning of day 20 of pregnancy, at the same time as the progesterone or oil injection. Blood samples (0.3 ml) were taken on the morning of the day of parturition, at the birth of pup 2 and then 10 and 20 min later. Plasma was separated by centrifugation at 13 000 r.p.m. for 5 min and the blood cells were resuspended in sterile saline and re-infused to maintain blood volume. The plasma samples were frozen at –20°C until radioimmunoassay for oxytocin was performed. Ninety minutes after pup 2 was born the rats were killed and the posterior pituitary gland of each rat was removed and homogenized in 0.5 ml of 0.2 mol HCl l⁻¹ for subsequent oxytocin analysis.

Experiment 4: effect of progesterone on naloxone-induced Fos expression in late pregnancy

The effect of progesterone on naloxone-induced Fos expression by supraoptic neurones was investigated in late pregnancy to examine whether the actions of progesterone are mediated via enhanced endogenous opioid inhibition. Pregnant rats were treated with progesterone or oil on day 20 as described earlier. After 24 h the rats pretreated with vehicle received an i.p. injection of naloxone (5 mg kg⁻¹ body weight; Sigma-Aldrich Company Ltd, Poole; a dose shown to enhance Fos expression in the supraoptic nucleus of pregnant rats by Douglas *et al.* (1995)) and the rats pretreated with progesterone were injected with either vehicle (50 µl isotonic saline per 100 g body weight) or naloxone. The rats were decapitated 90 min later and their brains were removed and frozen rapidly as described earlier.

In a further experiment, a progesterone receptor antagonist was used to determine whether the actions of progesterone on inhibition by endogenous opioids were mediated via a cytoplasmic progesterone receptor. On day 20 of pregnancy, pregnant rats were treated with progesterone as described earlier. After 24 h, either RU486 (10 mg mifepristone kg⁻¹ body weight; NIMH Chemical Synthesis Program, Research Biochemicals International, Natick, MA) or oil were administered to the rats i.m. After 6 h the rats received an i.p. injection of either naloxone (5 mg kg⁻¹ body weight) or vehicle. The three treatment groups were: progesterone + oil + naloxone; progesterone + RU486 +

vehicle; and progesterone + RU486 + naloxone. After 90 min the rats were killed by decapitation and the brain of each rat was removed and frozen.

Experiment 5: effect of naloxone on progesterone-delayed parturition

In a separate experiment, progesterone or oil was administered to rats as described earlier and a chronic jugular vein cannula was implanted on day 20 of pregnancy. On the morning of expected parturition catheter lines were attached to the jugular cannulae and at the birth of pup 2, either naloxone (5 mg kg⁻¹ body weight) or vehicle (50 µl (100 g)⁻¹ body weight) was injected i.v.; the subsequent time of birth of each pup was recorded for each rat.

Immunocytochemistry

Coronal brain sections (15 µm thickness) were cut on a cryostat and mounted on gelatinized slides. Sections containing the hypothalamic supraoptic nucleus (Expts 1 and 2: throughout supraoptic nucleus with similar rostro-caudal sections for each rat; Expt 3: at 6280–6060 µm anterior to the interaural line in all rats) or brainstem nucleus of the tractus solitarius (A1/C1 region, also containing area postrema for all rats) were selected and processed for Fos immunocytochemistry. Sections were incubated with a rabbit polyclonal anti-Fos antiserum (G. I. Evan and D. Hancock; Imperial Cancer Research Fund, London) at a dilution of 1:10000 at 4°C for 24 h. A second antibody (anti-rabbit IgG; Vector Laboratories, Peterborough) coupled to peroxidase was used at 1:500 for 24 h (Antonić et al., 1995; Luckman, 1995). Brains from the naloxone experiments were processed as described by Douglas *et al.* (1995), with the rabbit anti-rat Fos polyclonal antibody (Oncogene Sciences, Cambridge) at a dilution of 1:1000, and the secondary antibody at a dilution of 1:500. Immunolabelling was visualized using the nickel intensified (glucose-amino-oxidase) 3',3'-diaminobenzidine method, which results in dark purple cell nuclei. Immunoreactive cells were counted with the identity of the sections coded, using a microscope with a × 10 objective and a brightfield condenser. Between six and ten sections of supraoptic nucleus or nucleus of the tractus solitarius were evaluated from each rat.

Oxytocin radioimmunoassay

Plasma oxytocin concentrations were measured by the modified (Douglas *et al.*, 1995) radioimmunoassay of Higuchi *et al.* (1986). Assay sensitivity was 2.4 pg ml⁻¹ and the intra-assay coefficient of variation was 6%. The oxytocin content of the pituitary gland was measured in the supernatant of homogenized posterior pituitary glands using the radioimmunoassay of Bicknell *et al.* (1984).

Statistical analysis

The data were analysed mainly using non-parametric

statistical tests. Comparisons between two groups were made with the Mann-Whitney *U*-test for independent groups or the Signed rank test if paired. For comparisons between more than two groups a Kruskal-Wallis test, followed by Dunn's post hoc test, was used. Two-way ANOVA for repeated measures was used for comparing birth times in Expt 5; the post hoc test was Student-Newman-Keuls. A value of $P < 0.05$ was considered significant. All data are expressed as mean ± SEM.

Results

Experiment 1: effect of progesterone on delivery and Fos expression

Rats pretreated with vehicle ($n = 11$) gave birth between 11:00 and 20:00 h on day 21 of pregnancy. By the time the last rat pretreated with vehicle started to deliver, none of the rats pretreated with progesterone ($n = 10$) had given birth. All the rats pretreated with progesterone gave birth between 09:00 h on day 22 and 10:00 h on day 23 of pregnancy, a mean 28 h later than control rats ($P < 0.001$, Fig. 1a). Parturition was also prolonged in rats pretreated with progesterone compared with rats pretreated with vehicle ($P < 0.05$, Fig. 1b). Rats pretreated with progesterone had a mean 3 ± 1 pups remaining in the uterus when they were killed 90 min after the birth of pup 2 compared with no pups remaining in the uterus of the rats pretreated with vehicle. In the postpartum group, neonatal pup survival was decreased significantly ($86 \pm 6\%$) compared with rats pretreated with vehicle ($99 \pm 1\%$) ($P < 0.05$), although the pups were larger ($P < 0.05$), and maternal behaviour was lacking in 9 of 13 rats pretreated with progesterone, but was present in all controls (assessed subjectively).

Many neuronal nuclei in the supraoptic nucleus expressed Fos during spontaneous parturition in rats pretreated with vehicle but not in those pretreated with progesterone (Fig. 2a,b). Rats pretreated with vehicle showed a significant increase in the number of Fos immunoreactive cell nuclei in the supraoptic nucleus during parturition on day 21 of pregnancy compared with either before, or 6–12 h after, delivery of pups (Kruskal-Wallis all groups $P < 0.02$, $P < 0.05$ post hoc test; Fig. 2c). No increase in Fos expression in the supraoptic nucleus was observed in rats pretreated with progesterone when parturition occurred on day 22 or 23 and there was significantly less Fos expression than in rats pretreated with vehicle. With progesterone treatment there was no change in Fos expression before or after delivery compared with either day 21 of pregnancy or with vehicle controls (Fig. 2c). Fos expression in the nucleus of the tractus solitarius increased during parturition in rats pretreated with either vehicle or progesterone (Fig. 2e) compared with both prepartum and postpartum rats.

Experiment 2: effect of oxytocin administration on delivery and Fos expression after pretreatment with progesterone

Of the five rats pretreated with progesterone and injected

Fig. 1. Effects of progesterone on the onset and progress of delivery in rats. (a) Each circle represents the time of birth of the first pup for individual rats injected with progesterone (●, $n = 10$) or vehicle (○, $n = 11$) on day 20 of pregnancy (09:30 h). The x-axis indicates the time of day, bars represent the dark phase. Rats pretreated with progesterone delivered significantly later than vehicle-pretreated controls ($***P < 0.001$, Mann–Whitney U test). (b) Time between delivery of pups 2 and 6 in rats pretreated with progesterone. The data show the cumulative time for delivery of pups 2–6 in rats pretreated with either vehicle (□, $n = 11$) or progesterone (Prog) (■, $n = 10$); the delivery time was significantly shorter in rats pretreated with progesterone induced with oxytocin injections (OT) (▨, $n = 6$) compared with rats treated with progesterone alone ($*P < 0.05$, Kruskal–Wallis).

with oxytocin pulses on day 21 of pregnancy, and the three rats injected with vehicle pulses, none gave birth during the 4 h injection period. In contrast, six of eight rats pretreated with progesterone that were given oxytocin injections on day 22 of pregnancy started to deliver during the injection period, which was significantly earlier than in progesterone-treated rats that were not given oxytocin ($P < 0.05$). None of the rats pretreated with progesterone that were given saline on day 22 of pregnancy gave birth during the injection period.

All rats that had been pretreated with progesterone and in which delivery had been induced with oxytocin on day 22 of pregnancy showed behaviour typical of normal delivery. The time between delivery of pups 2–6 was not significantly different from spontaneous deliveries in rats pretreated with vehicle and was significantly shorter than spontaneous deliveries in rats pretreated with progesterone only (Fig. 1b, $P < 0.05$). The saline-injected rats that had been pretreated with progesterone were not observed during parturition but were killed as time-matched prepartum controls for measurement of Fos expression (see Methods).

Although injections of oxytocin on day 22 of pregnancy

induced delivery, the number of Fos immunoreactive nuclei in the supraoptic nucleus was not significantly higher than in rats injected with saline that did not deliver (Fig. 2d), and was similar to spontaneous parturition in rats pretreated with progesterone (Fig. 2c). In the nucleus of the tractus solitarius, Fos expression was also not significantly changed during oxytocin-induced parturition compared with prepartum (Fig. 2f), in contrast to spontaneous parturition in rats pretreated with progesterone (Fig. 2e).

Experiment 3: effect of progesterone on plasma oxytocin concentration and pituitary content

Basal plasma oxytocin concentrations were not different between rats pretreated with progesterone ($n = 6$; 10.9 ± 2.3 pg ml⁻¹) and vehicle ($n = 11$; 9.8 ± 1.4 pg ml⁻¹) on the morning of parturition. After the birth of pup 2, plasma oxytocin concentrations increased to 13.5 ± 2.3 and 11.7 ± 1.4 pg ml⁻¹ (10 min after birth of pup 2), and then to 20.7 ± 5.1 and 16.7 ± 6.5 pg ml⁻¹ (20 min after birth of pup 2), in rats pretreated with progesterone and vehicle, respectively. There were no significant differences between the two groups. Posterior pituitary oxytocin content was 0.8 ± 0.1 µg and 0.9 ± 0.2 µg in rats pretreated with progesterone and vehicle, respectively. These values are not significantly different.

Experiment 4: effect of progesterone on naloxone-induced Fos expression in late pregnancy

High Fos expression was observed in the supraoptic nucleus of late pregnant rats pretreated with vehicle and given naloxone, thus confirming the results of Douglas *et al.* (1995). Fos expression was significantly lower in rats pretreated with progesterone that were given naloxone than in rats pretreated with vehicle (Fig. 3a; Kruskal–Wallis, $P < 0.05$), and was not significantly different from rats pretreated with progesterone that were given saline. Fos expression in the nucleus of the tractus solitarius of the same rats was not affected by naloxone or progesterone treatments (Fig. 3b; Kruskal–Wallis). Thus, the inhibitory effect of progesterone pretreatment on Fos expression was not reversed by naloxone and, therefore, is not attributable to activation of central opioid mechanisms by progesterone.

RU486 was given in another experiment to antagonize progesterone action to determine whether the central effects of progesterone were mediated by a cytoplasmic progesterone receptor. Fos expression in the supraoptic nucleus of rats pretreated with progesterone and then given naloxone was within the same low range as that in rats treated in a similar manner earlier (compare Fig. 4a with Fig. 3a). Rats pretreated with progesterone to which RU486 was administered additionally on the morning of the experiment had significantly enhanced Fos expression in the supraoptic nucleus compared with those that did not receive RU486 treatment, whether or not naloxone was administered (Fig. 4a; Kruskal–Wallis, $P < 0.05$). Fos expression in the nucleus of the tractus solitarius was similar among groups (Fig. 4b,

Fig. 2. Fos expression in the supraoptic nucleus and nucleus of the tractus solitarius during parturition in progesterone pretreated rats. The photomicrographs show representative sections of supraoptic nucleus immunolabelled for Fos, taken from rats during parturition after pretreatment with (a) vehicle or (b) progesterone. Scale bar represents 100 μ m. Arrows indicate Fos immunopositive neuronal nuclei. OC: optic chiasma. The number of Fos immunoreactive nuclei per supraoptic nucleus (c,d) or nucleus of the tractus solitarius (e,f) profile were counted. (c) Mean $\pm$ SEM counts in the supraoptic nucleus from rats pretreated with vehicle ($\square$) or progesterone ($\blacksquare$), Kruskal–Wallis with all vehicle and progesterone groups, $P < 0.02$; $*P < 0.05$, Dunn's test versus prepartum and postpartum values, and to parturient (part.) in progesterone pretreated rats. (d) Mean $\pm$ SEM counts in the supraoptic nucleus from rats pretreated with progesterone and given either oxytocin ($\blacksquare$) or saline ($\square$) pulses on day 21 or day 22 of pregnancy. Oxytocin-injected rats on day 22 of pregnancy went into parturition ($\boxtimes$). (e) Mean $\pm$ SEM counts in the nucleus of the tractus solitarius from the same rats as in (c), Kruskal–Wallis, $P < 0.01$; $*P < 0.05$, Dunn's test compared with prepartum and postpartum groups in both vehicle and progesterone pretreated groups. (f) Mean $\pm$ SEM counts in the nucleus of the tractus solitarius from the same rats as in (d). There was no increase in Fos expression in the supraoptic nucleus during parturition after progesterone pretreatment, but there was an increase in Fos in the nucleus of the tractus solitarius. In oxytocin-induced parturition there was no increase in Fos expression in either the supraoptic nucleus or nucleus of the tractus solitarius. The numbers of rats per group are indicated in parentheses.

Fig. 3. Effect of progesterone on naloxone-induced Fos expression in late pregnant rats. Data are number of Fos immunoreactive nuclei per supraoptic nucleus (a) or nucleus of the tractus solitarius (b) in day 21 pregnant rats pretreated with progesterone (Prog) or vehicle (Oil) and given naloxone (NLX) or vehicle saline (Veh) 90 min before collection of the brain. Fos expression in the supraoptic nucleus was attenuated in rats pretreated with progesterone; Fos expression in the nucleus of the tractus solitarius did not alter with treatment. Kruskal–Wallis, $P < 0.05$, * $P < 0.05$ versus Oil + NLX group (post hoc test). The numbers of rats per group are indicated in parentheses.

Kruskal–Wallis). Therefore, it appears that progesterone prevents activation of supraoptic neurones in late pregnancy by a mechanism that at least partly involves progesterone receptors.

Experiment 5: effect of naloxone on progesterone-delayed parturition

In this experiment, progesterone administration on day 20 of pregnancy caused a mean delay of 19 h 25 min ($n = 11$) in the onset of parturition compared with rats pretreated with vehicle ($n = 11$; $P < 0.001$, Mann–Whitney U test). The progress of parturition was also prolonged after progesterone treatment, but naloxone administered on the birth of pup 2 did not alter the inter-pup birth intervals in rats pretreated with either vehicle or progesterone compared

Fig. 4. Effect of RU486 on Fos expression in progesterone- and naloxone-treated late pregnant rats. Data are number of Fos immunoreactive nuclei per supraoptic nucleus (a) or nucleus of the tractus solitarius (b) in day 21 pregnant rats pretreated with progesterone (Prog), given either RU486 or vehicle (Oil) 24 h later and given naloxone (NLX) or vehicle saline (Veh) 90 min before collection of the brain. In rats given RU486, Fos expression increased in the supraoptic nucleus, but not in the nucleus of the tractus solitarius. Kruskal–Wallis, $P < 0.05$, * $P < 0.05$ versus Prog + Oil + NLX group (post hoc test). The numbers of rats per group are indicated in parentheses.

with saline controls (Fig. 5). The mean cumulative time between the birth of pups 2–6 in the rats pretreated with progesterone ($n = 11$; 74.1 ± 6.9 min) was significantly longer than the mean for rats pretreated with vehicle ($n = 11$; 48.5 ± 6.5 min; two-way ANOVA for repeated measures: across groups: $P < 0.05$; across pups: $P < 0.0001$; interaction between group and sample: $P < 0.05$). Thus, the slow progress of parturition in rats pretreated with progesterone was not reversed by naloxone, which is consistent with the conclusion that the central mechanisms of action of progesterone do not include enhancement of central opioid tone.

Fig. 5. Effect of naloxone on progress of delivery in rats pretreated with progesterone. The data points show the cumulative times for delivery of pups after injection at pup 2 in vehicle pretreated rats (treated with vehicle (○, $n=5$) or naloxone (□, $n=6$)) or progesterone pretreated rats (treated with vehicle (●, $n=5$) or naloxone (■, $n=6$)). Naloxone had no effect on pup delivery times and did not reverse the progesterone-induced prolongation of delivery. * $P < 0.05$ combined progesterone groups versus vehicle groups, two-way ANOVA for repeated measures, interaction between group and pup number, $P < 0.05$. NLX: naloxone; Veh: vehicle.

Discussion

The results of the present study show that progesterone administered on the day before expected parturition in pregnant rats delays the onset of delivery by about 1 day and prolongs delivery. Normally, uterine sensitivity to oxytocin remains low until a few hours before birth, at which time the expression of uterine oxytocin receptors increases and the uterus becomes highly sensitive to oxytocin (Fuchs *et al.*, 1983). In humans, rats and sheep, concentrations of uterine oxytocin receptors increase after oestrogen treatment, whereas administration of progesterone inhibits the effects of oestrogen (Maggi *et al.*, 1991; Burgess *et al.*, 1992). Thus, the ineffectiveness of oxytocin on delivery on the expected day of parturition in rats pretreated with progesterone probably reflects the low numbers of functional oxytocin receptors in the uterus after progesterone treatment. Progesterone might also act non-genomically on the oxytocin receptors in the uterus to limit oxytocin activation (Grazzini *et al.*, 1998).

Oxytocin pulses on day 22 of pregnancy advanced the onset of birth by about 6 h and restored the progress of delivery fully, indicating that insufficient endogenous oxytocin release may have contributed to the slow deliveries observed. Although there is controversy about whether expression of the oxytocin gene in the magnocellular neurones increases in pregnancy, expression of the oxytocin gene does increase near term and during parturition (Douglas *et al.*, 1998), requiring withdrawal of progesterone (Crowley *et al.*, 1995; Thomas *et al.*, 1999). In rats, oxytocin is also synthesized in the uterus and mRNA content increases during late pregnancy. Progesterone administration as

described above does not change the expression of uterine oxytocin mRNA (Douglas *et al.*, 1997), indicating that availability of uterine oxytocin is not a factor.

During parturition in rats, oxytocin secretion occurs in a pulsatile pattern that is superimposed upon an increased background secretion (Higuchi *et al.*, 1986). The delay in onset and progress of parturition after progesterone administration does not appear to be due to a complete lack of oxytocin secretion, as no significant differences in plasma oxytocin concentration were observed before or during birth, or in pituitary oxytocin content between rats pretreated with progesterone or vehicle. However, it is clear from many studies (Luckman *et al.*, 1993; Antonijevic *et al.*, 1995) that the pulsatile pattern of oxytocin secretion is critical to the normal progress of parturition; hence, it seems most likely that the slower birth profile in rats pretreated with progesterone was a result of a lack of pulsatile secretion of oxytocin. Importantly, and consistent with this interpretation, when rats pretreated with progesterone were given i.v. bolus oxytocin treatment, parturition proceeded at a normal rate.

Pulsatile oxytocin secretion is a consequence of burst-like increases in the firing rate of magnocellular oxytocin neurones, triggered as a reflex by distension of the birth canal before delivery of each pup (Summerlee, 1981; Higuchi *et al.*, 1986). Increased activity in parturition is reflected by expression of Fos in the magnocellular neurones in the supraoptic nucleus of the hypothalamus (Luckman *et al.*, 1993, 1996; Antonijevic *et al.*, 1995; Lin *et al.*, 1995, 1998a,b), and is found in both oxytocin and vasopressin neurones at this time (Antonijevic *et al.*, 1995; Lin *et al.*, 1995). Fos expression in these neurones is a marker of their response or activation and is thought to be a result of synaptic activation of the neurones rather than simply increased electrical activity (Luckman *et al.*, 1994). The lack of Fos expression in the supraoptic nucleus in parturient rats pretreated with progesterone is consistent with a lack of appropriate activation of oxytocin neurones. Furthermore, although oxytocin injections restored delivery, they did not significantly enhance expression of Fos in the supraoptic nucleus during parturition, indicating that any positive feedback stimuli from the contracting uterus or birth canal during parturition fail to activate supraoptic neurones in progesterone-treated rats. In the present study, both vasopressin and oxytocin neurones may have expressed Fos: vasopressin secretion increases during parturition (Kumaresan *et al.*, 1979) and vasopressin receptors are present on the uterus (Maggi *et al.*, 1991). As administration of vasopressin antibodies does not affect parturition, the role of vasopressin may be to prevent haemorrhage.

As Fos expression is normally enhanced in the supraoptic nucleus and the nucleus of the tractus solitarius during both spontaneous and oxytocin-induced parturition (Luckman *et al.*, 1993; Antonijevic *et al.*, 1995), progesterone could be acting at one or more sites in the feedback from the uterus. This pathway has been shown, by retrograde labelling and double immunolabelling, to include a monosynaptic noradrenaline-mediated link from the nucleus of the tractus solitarius to the supraoptic nucleus (Meddle *et al.*, in press), and noradrenaline release increases in the supraoptic nucleus during parturition (Herbison *et al.*, 1997).

Progesterone receptors are located in several brainstem regions (Haywood *et al.*, 1999), including the gracile nucleus and the noradrenergic neurones of the nucleus of the tractus solitarius. Progesterone is also reported to reduce the noradrenaline content of the hypothalamus and the medulla (Chaudhuri *et al.*, 1994). Although oxytocin injections advanced the onset of parturition in rats pretreated with progesterone, they did not increase Fos expression in the supraoptic nucleus and nucleus of the tractus solitarius as occurs during normal parturition. As no activation of the supraoptic and tractus solitarius neurones, in terms of neuronal Fos expression, was observed in the oxytocin-stimulated rats pretreated with progesterone, it appears that progesterone continued to restrain neural pathways. Hence, it is possible that there is a progressive removal of the inhibitory actions of progesterone at the end of pregnancy, firstly at the uterus, then at the brainstem, and lastly at the supraoptic neurones. Therefore, a sequential change in progesterone effects on neurones before parturition may be a prerequisite for initiation of spontaneous labour.

One mechanism of progesterone action might be directly on supraoptic neurones or their immediate inputs by enhancing inhibitory opioid tone (Onaka *et al.*, 1995; Ingram *et al.*, 1996). Endogenous opioids strongly inhibit oxytocin neurone responses during parturition (Bicknell *et al.*, 1988). During parturition, the opioid agonist morphine delays birth and inhibits Fos expression in oxytocin neurones (Russell *et al.*, 1989a; Luckman *et al.*, 1993). Normal birth profiles can be restored by administration of pulsatile exogenous oxytocin, but this does not prevent the attenuated Fos expression by the supraoptic nucleus (Luckman *et al.*, 1993). However, enhanced endogenous opioid inhibition does not explain progesterone inhibition of the neurones, as progesterone prevented naloxone-induced Fos expression in the supraoptic nucleus in late pregnancy and instead may have attenuated the endogenous opioid inhibition of supraoptic neurones.

Another inhibitory action of progesterone mediated by progesterone receptors was indicated, as administration of RU486 to rats pretreated with progesterone enhanced Fos expression by supraoptic neurones compared with control rats pretreated with progesterone, whether or not naloxone was administered. RU486 could act in the hypothalamus, but progesterone receptors have not been found in magnocellular neurones of the supraoptic nucleus in rats (Fox *et al.*, 1990), although weak expression has been reported in the paraventricular nucleus (Thomas *et al.*, 1999). If not acting directly on supraoptic neurones, RU486 could cause their disinhibition by acting on inputs. Progesterone receptors are expressed in several cell groups that project to the magnocellular nuclei. In particular, they are located in perinuclear GABA neurones (Thind and Goldsmith 1997), zona incerta and the ventromedial nucleus (Fox *et al.*, 1990). Neurones in the medial and periventricular preoptic areas and circumventricular areas (Fox *et al.*, 1990; Brown *et al.*, 1996) also express progesterone receptors, although lesions of this region have no effect on the progress of parturition (Russell *et al.*, 1989b). The arcuate nucleus also densely expresses progesterone receptors, including in opioid-containing neurones (Bethea and Widmann, 1996), and there is a non-opioid inhibitory pathway from the arcuate nucleus

to the supraoptic nucleus (Leng *et al.*, 1988; Ludwig and Leng, 2000). RU486 could additionally, or alternatively, act on brainstem progesterone receptors to antagonise inhibition there, but the lack of RU486 on Fos expression in the nucleus of the tractus solitarius indicates that no stimulatory signals were being received from inputs (for example, the contracting uterus) (Antonijevic *et al.*, 1995). Removal of the effect of progesterone only, in the absence of an excitatory input or disinhibition, would not be expected to induce Fos expression. Thus, the action of RU486 is likely to be central, rather than on the uterus. Supporting this contention, neither labour nor parturition was induced in the RU486-treated rats by 6 h after treatment, although at 24–48 h after treatment it is reported to enhance uterine sensitivity to oxytocin *in vitro* (El Alj *et al.*, 1990) and to induce parturition (Bosc *et al.*, 1987).

As progesterone did not act via endogenous opioids to inhibit the neurones of the supraoptic nucleus during pregnancy and parturition, the evidence indicates that other central mechanisms are involved. It is now established that progesterone acts via non-genomic cell membrane-mediated mechanisms in the brain, including in the supraoptic nucleus. Neuroactive progesterone metabolites inhibit oxytocin neurones during late pregnancy (Jiang and Wakerley, 1997), at least partly via inhibitory GABAA receptors (Brussaard *et al.*, 1999; Thomas *et al.*, 1999) and this affect normally decreases around parturition when GABAA receptor subunit expression changes in parallel with a decreasing progesterone:oestrogen ratio (Fenelon and Herbison, 1996; Brussaard *et al.*, 1997). Thus, the progesterone treatment may have acted directly on oxytocin neurones to enhance or prolong GABAA receptor activation and, therefore, inhibition of the neurones. Thus, progesterone appears to act either directly to inhibit supraoptic neurones or on one or more of their non-opioid inputs from within the hypothalamus, and possibly also on the brainstem feedback pathway.

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