Endogenous opioid actions and effects of environmental disturbance on parturition and oxytocin secretion in rats

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Summary. Blood samples were taken from conscious, chronically-catheterized rats during parturition for measurement of oxytocin by specific radioimmunoassay. After the birth of the 3rd pup, rats were allowed to remain in their nesting cage (undisturbed rats) or were transferred for 45 min to a glass bowl (disturbed rats); at the time of transfer, rats were given an i.v. injection of the opioid antagonist naloxone or saline vehicle. Subsequent parturition was prolonged in saline-treated disturbed rats, but not in naloxone-treated disturbed rats. Parturition was significantly hastened in naloxone-treated undisturbed rats. Naloxone injections were followed by a large rise in plasma oxytocin concentrations in disturbed and undisturbed rats. We conclude, from a statistical analysis of the relationship within experimental groups between plasma oxytocin concentration and speed of parturition, that the effects of disturbance and of naloxone upon parturition may be accounted for, at least in part, by their effects upon oxytocin release. However, the effects of disturbance on parturition may not be mediated entirely by activation of opioid pathways. Naloxone did not potentiate oxytocin release in non-pregnant rats, or on Day 1 *post partum*, but did potentiate oxytocin release on Day 22 of pregnancy even in rats before the onset of parturition. Endogenous opioid pathways regulating oxytocin release therefore appear to be active during late pregnancy and during parturition itself.

Keywords: opioids; oxytocin; parturition; naloxone; environmental disturbance; rat

Introduction

A pregnant rat will normally give birth to between 6 and 14 young over a period of 1–2 h on the afternoon of Day 22 or 23 of pregnancy. The progress of parturition in the rat depends upon the release of oxytocin from the maternal neurohypophysis (Higuchi *et al.*, 1985, 1986, 1987). If morphine is administered centrally or peripherally following the birth of the 3rd pup, plasma oxytocin concentrations are reduced, and the duration of parturition is greatly prolonged (Gosden *et al.*, 1985; Cutting *et al.*, 1986). We have shown previously that parturition is also disrupted in rats transferred to a novel environment following the birth of the 3rd pup, and that this disruption can be prevented by i.v. administration of the opiate receptor antagonist naloxone (Leng *et al.*, 1985). We suggested that environmental disturbance leads, in the parturient rat, to activation of endogenous opioid pathways inhibitory to the secretion of oxytocin (see Dyer, 1988).

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However, naloxone elevates oxytocin secretion during parturition in undisturbed rats as well as in disturbed rats, and on Day 22 of pregnancy even before the onset of parturition (Hartman *et al.*, 1986). Opioid pathways may therefore be actively regulating oxytocin secretion in the absence of any imposed external stress, as suggested also by an earlier study on parturition in rats implanted chronically with subcutaneous minipumps to release either naloxone or saline (Leng *et al.*, 1985).

In the present study we tested the hypothesis that the effects of naloxone and of environmental disturbance are associated with changes in plasma oxytocin concentration, and examined statistical models of interaction between the effects of disturbance and those of naloxone on parturition and on oxytocin release.

Materials and Methods

Animals. Virgin Wistar rats of 200–250 g body weight were housed overnight in groups of 4–7 with a sexually experienced male. The following morning, vaginal smears were taken and inspected for the presence of spermatozoa. The day of detection of spermatozoa was taken as Day 1 of pregnancy. Pregnancy was confirmed on Day 16 by palpation, and pregnant rats were thereafter caged singly in their 'nesting cages'. Rats that failed to become pregnant were used as non-pregnant controls for oxytocin measurements. All rats were maintained throughout on a 14 h light:10 h dark schedule (lights on at 05:00 h). On the morning of Day 22 (or on the morning of the experiment for animals sampled at other times), rats were anaesthetized with ether for implantation of a jugular catheter. The catheter was exteriorized through an incision at the back of the neck, and for its length outside the animal was enclosed in a light, wound steel sleeve.

After recovery from the anaesthetic, rats were watched continuously until parturition began: red light illumination was used for observations during the dark phase of the light:dark cycle. The times of birth of each pup were noted. Up to 8 blood samples, each of 0.25–0.30 ml replaced immediately with an equal volume of heparinized saline (0.9% (w/v) NaCl), were taken from each rat for measurement of oxytocin by radioimmunoassay. Sampling was initiated following the birth of the 2nd and/or 3rd pups. After the birth of the 3rd pup, rats were given an i.v. injection of naloxone hydrochloride (2 mg/kg; Sigma, St Louis, MO, U.S.A.; 1 mg/ml solution in 0.9% NaCl) or of an equivalent volume of 0.9% NaCl alone. Thereafter, rats were allowed to remain in the nesting cage (undisturbed rats) or were transferred to an empty glass bowl, where they remained for 45 min (disturbed rats) or for 15 min, when they were returned to the nesting cage for 15 min and then again to the glass bowl for 15 min (repeatedly disturbed rats). Repeatedly disturbed rats given naloxone at the time of the first transfer were given supplementary naloxone injections of 0.5 mg/kg at the time of the second and third transfers.

Further blood samples were taken at various times during the 45 min after the birth of Pup 3. Some rats were killed at this time, and dissected to inspect the remaining contents of the uterus. In these rats, the neurointermediate lobe of the pituitary was dissected out onto solid CO₂ and stored at –70°C until assayed for total oxytocin content.

Measurement of oxytocin. Blood samples were immediately stored on ice. Plasma was separated by centrifugation, and stored at –20°C. Neurointermediate lobes were homogenized in 0.1 M-HCl. Oxytocin was measured by radioimmunoassay using the method and antiserum of Higuchi *et al.* (1985), in duplicate aliquants of each sample. Interassay and intra-assay coefficients of variation were 14.4% and 13.1% respectively (data pooled from 9 assays; the standard used for the coefficients was 260 pg/ml (13 pg/tube); 7 standards in each assay). The sensitivity of the assay varied between 2 and 4 pg/ml (0.1–0.2 pg/tube) determined from twice the s.d. about the zero binding points.

Statistical analysis of data. Comparisons of inter-birth intervals and oxytocin concentrations between the differently treated groups of animals was made by Mann-Whitney U tests. The relationship between the increment in oxytocin concentrations between the birth of Pup 3 and 8–15 min later, and the experimental treatment, was determined by maximum likelihood fit of a nonlinear model. The model assumed that the change in plasma oxytocin concentration is normally distributed with expectations specified in terms of inter-birth interval by the formulae in Table 2. The mean log oxytocin value between 8 and 15 min after the birth of Pup 3 was obtained by weighted and unweighted least squares, the differential weighting reflecting the greater variability of the naloxone treated group. All analyses were carried out using the Genstat 5 statistical programming language (Genstat 5 Reference Manual, Genstat 5 Committee, Clarendon Press, Oxford 1987) on a VAX 750 computer.

Results

Duration of parturition in undisturbed rats

The time course of parturition was similar in the uncannulated control rats, the rats cannulated but not sampled, and the blood-sampled rats: the mean interval between the birth of Pups 3 and 6

was 25.2 ± 3.8 min ($n = 9$), 28 ± 6.0 min ($n = 4$) and 24.9 ± 3.2 min ($n = 14$) in the three groups respectively. We therefore combined data from uncannulated and cannulated rats in the subsequent analysis.

In 30 control (undisturbed, saline-injected) rats at least 6 pups were either born or remained *in utero* at the end of the experiment. Pregnancies resulting in fewer than 6 pups were invariably the result of unilateral implantations: the time course of parturition was slower and more variable in such rats, and all data from rats with fewer than 6 pups were therefore excluded. Of the 30 control rats, 29 had at least 8 pups. In 19 of these 29 rats the 8th pup was born within 45 min of the 3rd, and in 6 within 30 min of the 3rd. The full time course of parturition in the control rats is shown in Fig. 1.

Fig. 1. Mean intervals ($\pm$ s.e.) between births for the first 8 pups born to 29 undisturbed rats.

Effects of disturbance on established parturition

In disturbed rats there was again no difference in the pattern of parturition between cannulated and uncannulated rats and data have been combined. In this group 23 rats had at least 6 pups, and all but one of these at least 8 pups. The 8th pup was born within 45 min of the 3rd in only 5 of these 22 rats, and within 30 min in none of the rats. The disturbance following the birth of the Pup 3 therefore resulted in a prolongation of parturition, and this effect was seen most markedly as a prolongation of the interval between the birth of Pups 3 and 4 (Fig. 2). In control rats, this interval was 9.7 ± 1.3 min ($n = 30$) compared with 19.6 ± 2.3 min ($n = 23$) in disturbed rats, whereas the intervals between the births of subsequent pups were similar in the two groups. The difference in the cumulative interval between Pups 3 and 6 (25.4 ± 2.0 min in control rats; 36.5 ± 2.0 min in disturbed rats) is therefore almost wholly attributable to the prolonged interval between the birth of Pups 3 and 4.

In repeatedly disturbed rats, the effects of the disturbance on parturition were less easy to analyse. The interval between the birth of Pups 3 and 4 (18.8 ± 2.3 min; $n = 18$) was similar to that in the rats disturbed only once—not surprisingly since for the first 15 min after the birth of Pup 3 these two groups were treated identically. To examine the effect of the subsequent return to the nesting cage, and then 15 min later of a second transfer to the glass bowl, we compared the distribution of births in these three 15-min intervals for Pups 3 to 8 (for rats with at least 8 pups). Of the 18 repeatedly disturbed rats, 3 gave birth to Pup 8 within 30 min of Pup 3, but only 6 of the remaining 15 rats gave birth to any pups in the 15 min following return to the glass bowl (Table 1). By contrast, in the corresponding time interval (31–45 min after the birth of Pup 3) 21 out of the 23 disturbed rats gave birth to 1 or more pups. It therefore appeared that the disruption of parturition was effected mainly by the environment of the glass bowl itself rather than by the act of physically

Fig. 2. Effects of disturbance and of naloxone hydrochloride injection (i.v.; 2 mg/kg body wt) on the progress of parturition in rats. Means ($\pm$ s.e.) of inter-birth intervals for the birth of Pups 4, 5 and 6. Hatched bars are data from naloxone-treated rats; open bars are from saline-treated rats. The number of intervals contributing to each bar is given in parentheses above each bar. * $P < 0.05$; ** $P < 0.01$ (Mann-Whitney U test).

disturbing the rat by moving it from one place to another since in the repeatedly transferred rats, transfer from cage to bowl resulted in a disruption of parturition whereas the reverse transfer did not. Moreover, the data indicate that rats remaining in the bowl for 45 min had, after 30 min, become tolerant to the disruptive effects of this environment upon parturition since, comparing parturition in the period 30–45 min after the birth of Pup 3, fewer of the repeatedly disturbed rats gave birth than did the disturbed rats.

Effects of naloxone on parturition

The time course of parturition in the disturbed rats given naloxone was significantly faster than in disturbed rats given saline, and was similar to that in undisturbed control rats (Fig. 2). In repeatedly disturbed rats also, naloxone treatment reversed the disruption of parturition completely. However, undisturbed, naloxone-treated rats gave birth significantly more quickly than did disturbed, naloxone-treated rats or undisturbed control rats (Fig. 2). In 15 undisturbed, naloxone-treated rats the mean interval between the birth of Pups 3 and 6 was 17.6 ± 1.6 min compared with 25.4 ± 2.0 min in 30 undisturbed, saline-treated rats ($P < 0.01$).

Table 1. Numbers of rats giving birth (Pups 4–8 only) per 15-min period following transfer from their nesting cage to a glass bowl after the birth of Pup 3

	Time after birth of Pup 3		
	0–15 min	16–30 min	31–45 min†
	Birth:no birth	Birth:no birth	Birth:no birth
Disturbed rats*	10:13 (bowl)	20:3 (bowl)	21:2 (bowl)
Repeatedly disturbed rats	9:9 (bowl)	15:3 (cage)	6:9 (bowl)*
Significance‡	ns	ns	$P < 0.001$

*Remained in the glass bowl for 45 min.

†Three animals which gave birth to Pup 8 within 30 min of Pup 3 are excluded from the analysis of birth distributions in the period 31–45 min.

‡The significance values are for a test of equality of proportions obtained by exact contingency table analysis.

Plasma oxytocin concentrations during parturition

After birth of Pups 2 and 3, oxytocin concentrations were 21.4 ± 2.1 ($n = 19$ rats) and 21.3 ± 1.5 pg/ml ($n = 63$ rats; both significantly higher than Day 22 pre-parturition samples; $P < 0.001$). In all groups of saline-treated rats, whether disturbed or undisturbed, oxytocin concentrations rose progressively in the 40 min following the birth of the 3rd pup, but the increase was greater in the undisturbed rats (Fig. 3). Naloxone injection in disturbed and undisturbed rats resulted in a prompt and large rise in plasma oxytocin (Table 2) that was sustained for at least 40 min. The mean plasma concentration 40 min after the birth of the Pup 3 was 48.2 ± 8.0 pg/ml in 12 naloxone-treated disturbed rats; 72.1 ± 11.4 pg/ml in 10 naloxone-treated undisturbed rats; and 31.8 ± 2.7 pg/ml in 10 control, undisturbed rats.

Fig. 3. Mean plasma oxytocin concentrations ($\pm$ s.e.) before and during parturition, measured by radioimmunoassay in jugular blood samples from conscious, chronically catheterized rats. The number of rats is given in parentheses for samples from non-pregnant rats, from rats on Day 22 of pregnancy before the onset of parturition, and from rats at the time of birth of Pup 2. The data shown for samples at and following the birth of Pup 3 all come from 11 rats; n is between 7 and 11 for each of these points.

Effects of disturbance upon plasma oxytocin concentrations

For each rat, we calculated the increment in plasma oxytocin (Δ) measured between the birth of Pup 3 and the mean oxytocin concentration measured in samples taken between 8 and 15 min later (Table 2). A model was fitted with expectations $E(\Delta)$ as given in Table 2. Initially an unweighted fit was made, but as the variability of increments in the naloxone-treated animals was much greater than in the saline-treated animals it seemed more appropriate to use a weighted fit of the model giving greater weight to the less variable data (Table 3). Weighting inversely proportional to the variances suggested a relative weight for the data from saline-treated animals of about 25 as compared to 1 for the naloxone-treated animals. For a test that there was no influence of disturbance on plasma oxytocin concentration (i.e. that the true value of ρ , the proportionate influence of disturbance as defined in Table 2, is 1), the t values were 6 and 4.3 for the two fits respectively on at worst 8 degrees of freedom. These results are significant at $P < 0.001$ and $P < 0.01$ for unweighted and weighted fits respectively. Therefore, considering the results as a whole, disturbance is associated with a significant depression of plasma oxytocin concentration.

Table 2. Parameters of the statistical model used to test the hypothesis that the influences of naloxone and of disturbance upon the progress of parturition in rats can be accounted for by their effects upon plasma oxytocin concentration

Treatment group			Plasma oxytocin increments		
Injection	Disturbance	$E(\Delta)^*$	Mean Δ	s.e.m.	No. of rats
Saline	Undisturbed	δ_s	6.6	4.8	9
Saline	Disturbed	$\rho \cdot \delta_s$	2.4	1.8	20
Naloxone	Undisturbed	δ_n	89.9	20.5	11
Naloxone	Disturbed	$\rho \cdot \delta_n$	36.3	9.6	19

*Measured between birth of Pup 3 and 8–15 min later.

Table 3. Estimates of the parameters of the statistical model defined in Table 2 following an unweighted fit of the data, and a fit giving a weighting to the data from saline-treated animals which reflected the lower variance in these animals

$E(\Delta)$	Unweighted fit		Weighted fit	
	Estimate	s.e.	Estimate	s.e.
δ_s	6.4	10.9	6.5	3.0
δ_n	89.9	11.4	90.1	15.4
ρ	0.40	0.11	0.40	0.14

Relation between plasma oxytocin concentrations and birth intervals

We analysed the relationship between the birth interval 3–4, and the mean oxytocin concentration (log) measured in samples taken between 8 and 15 min after the birth of Pup 3. Fitting

regression lines relating these two variables for each of the 6 experimental groups gave the regression coefficients shown in Table 4. Within each group there was a downward trend, although this reached significance only for naloxone-treated disturbed rats. However, carrying out a pooled regression, allowing different intercepts for each group but a common slope, the regression is significant ($P < 0.001$) and the difference between the intercepts is not significant ($P > 0.05$). Therefore, although this analysis was only approximate, it indicated that the effects both of naloxone and of disturbance on the speed of parturition may be accounted for entirely by the relationship between the speed of parturition and the plasma oxytocin concentration.

Table 4. Regression of the interval between births of Pups 3 and 4 and the plasma oxytocin concentration (log) at 8–15 min after birth of Pup 3

Treatment group		Constant (s.e.)	Slope (s.e.)	<i>t</i>	No. of rats
Injection	Disturbance				
Naloxone	Undisturbed	24.4 (10.1)	–8.99 (4.99)	–1.80	10
Naloxone	Disturbed	54.5 (13.3)	–24.40 (7.52)	–3.35	11
Naloxone	Repeatedly disturbed	27.1 (15.8)	–11.18 (9.25)	–1.21	5
Saline	Undisturbed	42.4 (15.3)	–23.4 (10.7)	–2.17	8
Saline	Disturbed	28.9 (14.0)	–6.1 (11.1)	–0.55	12
Saline	Repeatedly disturbed	62.3 (18.2)	–33.8 (13.8)	–2.44	5

Pooling all groups, the regression is highly significant ($P < 0.001$).

Looking more closely for evidence of independent effects of disturbance and of naloxone upon the progress of parturition we broke the treatment term into a 2×2 factorial (combining data from disturbed and repeatedly disturbed groups since there was no difference between the treatment of these groups in the relevant time period). We obtained a significant difference in the intercepts of the regression lines fitted to disturbed and undisturbed animals ($P < 0.05$). The improvement in the model achieved by allowing different slopes and intercepts for these two groups is significant ($P < 0.05$; F on 2 and 59 d.f. = 3.79). Therefore, while there is virtually no effect of naloxone on the relationship between speed of parturition and plasma oxytocin concentration, the disturbed animals may have a larger intercept and a steeper slope for the regression of speed of parturition against plasma oxytocin concentration (Fig. 4).

Effects of naloxone on plasma oxytocin concentration before and after parturition

Plasma oxytocin concentrations in non-pregnant rats were 6.8 ± 1.1 pg/ml ($n = 25$), and did not change significantly following injection of naloxone or saline (Fig. 5). On Day 22 of pregnancy, oxytocin concentrations in rats before the onset of parturition were 13.1 ± 1.3 pg/ml ($n = 17$), significantly higher than in non-pregnant rats ($P < 0.01$; Fig. 3), and in these rats oxytocin concentrations were not significantly affected by saline injection but were significantly elevated after naloxone (Fig. 5). On the day after parturition plasma oxytocin concentrations averaged 11.7 ± 1.7 pg/ml ($n = 19$; significantly higher than in non-pregnant rats). Oxytocin concentrations in these rats were unaffected by saline or naloxone injection (Fig. 5). We therefore found evidence for endogenous opioid inhibition of oxytocin release on Day 22 of pregnancy but not in non-pregnant rats or in rats on Day 1 *post partum*. The rise following naloxone in parturient rats was significantly greater than in Day 22 pre-parturition samples ($P < 0.01$), hence endogenous opioid inhibition of oxytocin release appeared to be greatest during parturition itself.

Fig. 4. Effects of naloxone and of disturbance during labour on plasma oxytocin concentrations, measured by radioimmunoassay from jugular blood samples. Rats were removed from their nesting cage into a glass chamber following the birth of Pup 3 (disturbed rats), and at this time were given an i.v. injection of naloxone hydrochloride (2 mg/kg body wt) or 0.9% saline. The lines shown are regressions of the interval between the births of Pups 3 and 4, and the mean log plasma oxytocin concentration measured between 8 and 15 min after the birth of Pup 3. The group means are shown for naloxone-treated disturbed rats (▲); naloxone-treated undisturbed rats (■); saline-treated disturbed rats (●), and saline-treated undisturbed rats (*).

Neurointermediate lobe content of oxytocin

The neurointermediate lobe content of oxytocin was elevated on Day 22 of pregnancy in glands removed before the onset of parturition ($3.64 \pm 0.13 \mu\text{g/gland}$, $n = 9$, vs $2.79 \pm 0.12 \mu\text{g/gland}$, $n = 11$, non-pregnant rats). Content was significantly lower in glands removed 45 min after the birth of Pup 3, but no differences were found between disturbed and undisturbed rats or between naloxone-treated and saline-treated rats ($2.83 \pm 0.13 \mu\text{g/gland}$ ($n = 7$) in undisturbed, control rats; $2.55 \pm 0.14 \mu\text{g/gland}$ ($n = 12$) in repeatedly disturbed, saline-treated rats; $2.55 \pm 0.11 \mu\text{g/gland}$ ($n = 11$) in repeatedly disturbed, naloxone-treated rats; no significant differences between these groups; value for each group significantly lower than that for glands removed before the onset of parturition).

Discussion

The present results confirm that environmental disturbance prolongs parturition, as first shown in the mouse (Newton *et al.*, 1966, 1968). Notwithstanding large variability between animals, the results also provide clear evidence both that the interval between deliveries is related to plasma oxytocin concentration, and that the effects of disturbance upon the speed of parturition may, at

Fig. 5. Effects of injection of naloxone hydrochloride (i.v.; 2 mg/kg body wt; closed symbols) or 0.9% saline (open symbols) on plasma oxytocin concentrations, measured by radioimmunoassay from jugular blood samples in conscious rats. Injections of saline or naloxone were given immediately after the second sample (arrow). Values are mean $\pm$ s.e.m. for the number of rats given in parentheses.

least in part, be accounted for by depression of plasma oxytocin concentrations. As in a previous study (Leng *et al.*, 1987), naloxone administration prevented the disruption of parturition, apparently by elevating oxytocin concentrations in the circulation. In our previous studies, rats were anaesthetized with tribromoethanol for cannulation, and such anaesthesia late in pregnancy significantly delays the onset of parturition. This was not true of the present study in which ether anaesthesia was used: all rats gave birth, as expected, on Day 22 or Day 23 of pregnancy. The effects of disturbance, and their reversibility by naloxone, are therefore not features only of retarded parturition (Leng *et al.*, 1987).

Our results differed in one important respect from those previously obtained using tribromoethanol anaesthesia: naloxone markedly hastened parturition in undisturbed animals in the present, but not in the previous, study. Therefore, comparing disturbed and undisturbed animals, it is clear that naloxone does not eliminate the effects of disturbance. The difference between the two studies may therefore reflect additional stress in the tribromoethanol-treated animals associated with the retardation of the onset of parturition, although within each study we found no correlation between the time of onset of parturition and the duration of parturition.

The present study demonstrated that naloxone elevates oxytocin secretion in disturbed and undisturbed parturient rats. Electrophysiological recordings from oxytocin cells during parturition in conscious rats suggest that, on top of an elevated background release, oxytocin is also released in pulses, and that the occurrence of pulses is correlated with the births of individual pups (Summerlee, 1981). The half-life of oxytocin in rat plasma is about 1.5 min (Higuchi *et al.*, 1985),

but the disappearance time of a bolus of oxytocin introduced into the circulation by a brief, synchronized burst of activity from oxytocin cells is likely to be much less, since the disappearance rate will be dominated by the speed of dilution of the bolus into the whole volume of circulating blood. An analogous pulsatile release of oxytocin occurs during suckling in lactating rats, and to detect such release by RIA requires very frequent blood sampling (Higuchi *et al.*, 1985, 1986). In the present experiments samples were taken at 5- or 10-min intervals, and plasma concentrations tended to be consistent within animals (disregarding the effects of naloxone). Thus our sampling provided no direct information on the rate or amplitude of pulsatile hormone release, and this qualification requires us to be circumspect about some conclusions.

The relationship of oxytocin concentration to inter-birth interval is not simple. In control rats, the intervals between births were fairly constant between the birth of Pups 2 and 7, although oxytocin concentrations rose progressively over this period. Naloxone injections produced a prolonged elevation of plasma oxytocin, but only a transient effect on the speed of parturition. The naloxone-treated disturbed rats had higher plasma oxytocin concentrations than did the saline-treated undisturbed rats, but the progress of parturition was very similar in these two groups. Nevertheless, within each experiment group there was a positive correlation between plasma oxytocin concentration and the speed of parturition, and overall this correlation was highly significant. It is likely that during parturition oxytocin is released in pulses superimposed upon an elevated background secretion, and that a pulse of oxytocin triggers the delivery of each pup (Summerlee, 1981). The background secretion of oxytocin, measured in the present experiments, may contribute to the sensitivity of the uterus to pulses of oxytocin but probably does not fully determine the timing of births.

From our data as a whole, disturbance in mid-parturition was accompanied by depressed secretion of oxytocin. Naloxone resulted in a marked potentiation of oxytocin release during parturition, and it seems that this potentiated release was sufficient to overcome the disruption of parturition caused by environmental disturbance. Since naloxone increased the speed of parturition in undisturbed rats also, the simplest explanation is that environmental disturbance slows parturition by suppressing oxytocin release, possibly by mechanisms independent of endogenous opioid pathways, and that naloxone speeds parturition by elevating oxytocin secretion but similarly acts independently of whether the animal has been disturbed. Thus the data support our original hypothesis that endogenous opioids regulate oxytocin release during normal parturition, and may be involved in 'spacing' the deliveries of successive pups (Leng *et al.*, 1985).

Another role for opioid pathways may be in determining the onset of parturition. Jones & Summerlee (1986a, b) have suggested that secretion of the ovarian hormone relaxin is involved in the co-ordination of central and peripheral events necessary for parturition to proceed, and that one role of relaxin in the period before the onset of parturition is to inhibit oxytocin release via the activation of brain opioid pathways. In late pregnancy, the concentration of relaxin in the maternal circulation of the rat is high and falls precipitously shortly before the onset of parturition (Sherwood *et al.*, 1980). Recent evidence suggests that the elevated relaxin concentrations of late pregnancy inhibit oxytocin secretion via opioid pathways (Summerlee *et al.*, 1984; O'Byrne & Summerlee, 1985; O'Byrne *et al.*, 1986). Chronic infusions of exogenous relaxin delay the onset of parturition and suppress oxytocin release during parturition (Jones & Summerlee, 1986a, b), and these effects of relaxin are reversed by naloxone.

Inhibition of oxytocin release by relaxin would conserve neurohypophysial oxytocin stores for parturition, might result in a further sensitization of the uterus already sensitized by the proliferation of oxytocin receptors (Fuchs & Poblete, 1970), and would prevent premature uterine activation by oxytocin until the relaxin-induced softening of the cervix was complete (Harkness & Harkness, 1959). Withdrawal from prolonged exposure to opioids results in hypersecretion of oxytocin (Bicknell *et al.*, 1988), and the rapidly falling plasma concentration of relaxin on Day 22 of pregnancy (Sherwood *et al.*, 1980) may similarly provoke an opioid-withdrawal hypersecretion of oxytocin to initiate or help maintain parturition.

This hypothesis is supported by some of our data. We demonstrated that there is tonic opioid inhibition of oxytocin release on Day 22 of pregnancy before the onset of parturition, whereas little opioid influence was discernible in non-pregnant rats or on Day 1 *post partum*. On the other hand, naloxone effects on oxytocin release were greater during parturition, whereas it might be expected that, if opioid withdrawal was already underway, then naloxone should be less effective at this time. However, oxytocin release is not linearly related to the discharge rate of oxytocin neurones: there is a marked frequency-facilitation of oxytocin release, which means that a given increase in firing rate will produce a larger oxytocin release if the basal concentration of firing is high (Leng & Bicknell, 1984). The high concentrations of oxytocin release sustained throughout parturition suggest that oxytocin cells indeed have a high degree of basal activity throughout parturition (see Summerlee, 1981).

Which opioid system is involved in oxytocin regulation during parturition remains uncertain. Oxytocin release is under inhibitory opioid control at the level of the neurohypophysis, probably by dynorphin co-released with vasopressin (Bicknell *et al.*, 1985; Summy-Long *et al.*, 1986; Zhao *et al.*, 1988). If this opioid system is active during parturition, then elevated vasopressin concentrations might also be found. Vasopressin release is elevated throughout pregnancy in the rat (Lindheimer *et al.*, 1985). However, in both rabbits (Haldar, 1970) and rats (Hartman *et al.*, 1986) little vasopressin is released during parturition as compared with oxytocin.

Oxytocin secretion is also under opioid control at the concentration of the neurosecretory cell bodies (Bicknell *et al.*, 1988), which appear to be innervated by a β -endorphinergic projection from the hypothalamic arcuate nucleus (Sawchenko *et al.*, 1982). Brain β -endorphin concentrations are elevated throughout pregnancy in the rat (Wardlaw & Frantz, 1983), and remain elevated until 1–2 days *post partum*. Relaxin administration depresses the electrical activity of oxytocin neurones (Summerlee *et al.*, 1984), hence the endogenous opioid control of oxytocin release during parturition is likely to be exerted, at least in part, centrally rather than at the neurohypophysis.

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