

The role of endogenous opioids in neurohypophysial and hypothalamo–pituitary–adrenal axis hormone secretory responses to stress in pregnant rats

A J Douglas, H A Johnstone, A Wigger¹, R Landgraf¹, J A Russell and I D Neumann¹

Department of Physiology, University Medical School, Teviot Place, Edinburgh, EH8 9AG, UK and ¹Max Planck Institute of Psychiatry, Kraepelinstrasse 2, 80804 Munich, Germany

(Requests for offprints should be addressed to A J Douglas)

Abstract

Endogenous opioid regulation of neurohypophysial and hypothalamo–pituitary–adrenal (HPA) axis hormone secretion in response to forced swimming (90 s in deep water at 19 °C) was investigated in virgin and 21-day-pregnant rats. There was no difference in basal plasma oxytocin concentrations between pregnant and virgin rats, but the opioid antagonist, naloxone, increased basal oxytocin secretion in the pregnant rats. Forced swimming increased oxytocin secretion similarly in pregnant and virgin rats, and this response was enhanced by naloxone. In pregnant rats naloxone had a greater effect (by 3.1-fold) than in virgins, showing stronger endogenous opioid restraint of an enhanced oxytocin secretory response to stress in pregnancy. Vasopressin secretion was not increased with forced swimming in virgin or pregnant rats, and naloxone had no effect. ACTH and corticosterone secretion in response to forced swimming was attenuated in pregnant rats compared to virgin rats, measured at

5 min. Naloxone had no effect on basal plasma ACTH or corticosterone concentration, but it reduced ACTH secretion in virgin rats 5 min after forced swimming; in pregnant rats naloxone had no such effect. Naloxone removed the pregnancy-related attenuation in corticosterone secretion measured at 5 min after forced swimming. Fifteen minutes after forced swimming, plasma corticosterone concentrations were not different between groups. In the late-pregnant rats, the increases in plasma ACTH and corticosterone induced by forced swimming were significantly prolonged compared to virgins. The results show that endogenous opioid inhibition emerges in pregnancy to restrict the responses of oxytocin neurones to a stressor. In contrast, the endogenous opioid enhancement of mechanisms regulating HPA axis secretory responses in virgin rats is not evident during pregnancy.

Journal of Endocrinology (1998) **158**, 285–293

Introduction

In addition to the well-known stimulation of hypothalamo–pituitary–adrenal (HPA) axis hormone secretion by a wide variety of stressors, there are robust neurohypophysial secretory responses to stressors; for example, forced swimming, immobilisation, social defeat and intraperitoneal hypertonic saline induce increased release of oxytocin into the blood (Lang *et al.* 1983, Gibbs 1986, Wotjak *et al.* 1996), whereas ether, haemorrhage, hypoxia and noxious stimuli increase the release of vasopressin (Gibbs 1986, Yagi 1992). Oxytocin secretion in response to a stressor is restrained by endogenous opioids in female, but not in male, rats (Carter *et al.* 1986, Carter & Lightman 1987a), and opioids also influence HPA axis activity (Buckingham & Cooper 1984, Plotsky 1986, Suda *et al.* 1992, Calogero 1996). Both oxytocin and HPA axis stress responses are reduced in lactation (Carter &

Lightman 1987b, Higuchi *et al.* 1988, Walker *et al.* 1995, Windle *et al.* 1997). We have recently shown that the HPA axis response to stress is attenuated in pregnancy from day 15 onward (Neumann *et al.* 1998) as well as in lactation (e.g. Walker *et al.* 1995, Windle *et al.* 1997). Although pituitary mechanisms account for part of these inhibited secretory responses (Johnstone *et al.* 1997, Neumann *et al.* 1998), central, hypothalamic mechanisms are also likely to play a part, as parvocellular paraventricular nucleus (PVN) neurone expression of Fos in response to stress is attenuated in pregnancy (da Costa *et al.* 1996). Because stimulation of oxytocin neurone activity and secretion by the brainstem input are restrained by endogenous opioids in late pregnancy (Douglas *et al.* 1995), and secretion of another stress hormone, prolactin, is also inhibited by opioids in pregnancy (Soaje & Deis 1994), we hypothesised that there may be a common, central opioid mechanism inhibiting neuroendocrine stress responses in pregnancy.

We have now investigated whether endogenous opioids modify oxytocin secretion in response to a stressor in pregnancy. We have also compared the secretion of oxytocin with vasopressin secretion. In addition, we have sought a role for endogenous opioids in restraining the HPA axis secretory responses in pregnancy.

Material and Methods

Animals

Virgin female Sprague–Dawley rats (260–290 g body weight) were mated overnight with sexually experienced males and pregnancy was confirmed by finding a vaginal plug of semen (day 1 of pregnancy). Rats were housed singly under standard laboratory conditions (12 h light : 12 h darkness cycle, lights on at 0700 h, 22 °C, 60% humidity, food and water available *ad libitum*) for at least 7 days before surgery.

Surgery Under halothane anaesthesia and with sterile procedures, rats were implanted with a chronic jugular vein catheter (silastic tubing inside diameter 0.5 mm, outside diameter 0.75 mm, Altec, Alton, UK) 3–4 days before the experiment. The catheter was filled with heparinised (20 IU/ml, Multiparin, CP Pharmaceuticals Ltd, Wrexham, UK) sterile isotonic (0.9% w/v) saline. After surgery, the rats were housed singly and familiarised to daily handling.

Effect of naloxone on secretory responses to forced swimming in pregnant rats

Three to four days after surgery, the effect of naloxone (a general opioid antagonist) on neurohypophysial and HPA axis hormone secretory responses to forced swimming was tested. Blood samples were taken before and after treatment with naloxone or vehicle in virgin ($n=7$, body weight 282 ± 6 g; $n=8$, body weight 286 ± 9 g respectively) and 21-day-pregnant rats ($n=7$, 364 ± 9 g; $n=8$, 362 ± 9 g respectively), and subsequently after a period of forced swimming.

At 0800 h, the catheter was attached to an extension tubing (polythene, outside diameter 1.0 mm) connected to a syringe filled with sterile heparinised saline (20 IU/ml), and the rats were left undisturbed for 90 min. Blood samples (0.65 ml), substituted immediately by sterile 0.9% saline, were taken under basal conditions at 0930 h and 1000 h. Then naloxone (5 mg/kg, 50 μ l/100 g body weight) or vehicle was injected *i.v.* and a further sample taken 15 min later. After an interval of 20–30 min, rats were exposed to forced swimming, a combined physical and emotional stressor (Abel 1994). With the extension tubing of the venous catheter still attached, rats were forced to swim for 90 s in a bucket filled with tap water

(19 °C) to a depth of about 40 cm. After the swim, the rats were gently dried, using towels, for 10 s and returned to their home cages. Further blood samples were taken 5, 15 and 60 min after forced swimming. All blood samples were collected on ice in tubes containing EDTA (5% solution, 15 μ l/100 μ l blood) supplemented with aprotinin (0.039 trypsin inhibitor units/tube; Sigma, Poole, Dorset, UK) and centrifuged. Plasma samples (200 μ l for oxytocin and vasopressin, 80 μ l for ACTH and 50 μ l for corticosterone) were stored at -20 °C until required for assay.

Hormone assays

Oxytocin and vasopressin concentrations were measured in extracted plasma samples by highly sensitive and selective radioimmunoassays (limit of detection 0.1 pg/sample; cross-reactivity of the antisera with other related peptides, including oxytocin or vasopressin, <0.7%; for a detailed description see Landgraf 1981).

Plasma ACTH was measured by radioimmunoassay using a commercially available kit (ICN, Costa Mesa, CA, USA). The intra- and interassay coefficients of variation were less than 7 and 10% respectively. Total plasma corticosterone was measured by radioimmunoassay using a scintillation proximity method. Briefly, plasma samples were denatured by incubation in borate buffer (133 mM boric acid, 68 mM NaOH; pH 7.4, 1 : 9 v/v) containing bovine serum albumin (0.5%) in a 96 well microtitre plate (Falcon) at 80 °C for 30 min. Then the samples and a range of standards were incubated with 3 H-corticosterone (Amersham Life Sciences, Little Chalfont, Bucks, UK; 11 000 c.p.m. per well) and anti-corticosterone antibody (1 : 10 000 dilution, rabbit anti-rat, a gift from the High Blood Pressure Unit, Western General Hospital, Glasgow, UK) in a total volume of 70 μ l for 1 h at room temperature. Scintillation proximity assay reagent (anti-rabbit, Amersham Life Sciences, 50 μ l, which holds antibody-bound radioactivity in close proximity to scintillant) was mixed in and incubated for a further 24 h at room temperature before counting in a β -scintillation counter. The intra-assay coefficient of variation was 6%.

Statistical analysis

Statistical analysis was performed by means of statistical software (Sigmastat, Jandel Scientific, Erkrath, Germany). Data are presented as group means \pm s.e.m. Because the data were from four groups, representing two reproductive states, and each set contained repeated measurements from the same animal, two-way analysis of variance (ANOVA, reproductive state \times time) for repeated measures followed by Newman–Keuls *post hoc* test was used to compare the interactions between all data. One-way ANOVA was also utilized to compare basal values and calculated increments in secretory responses. To analyse

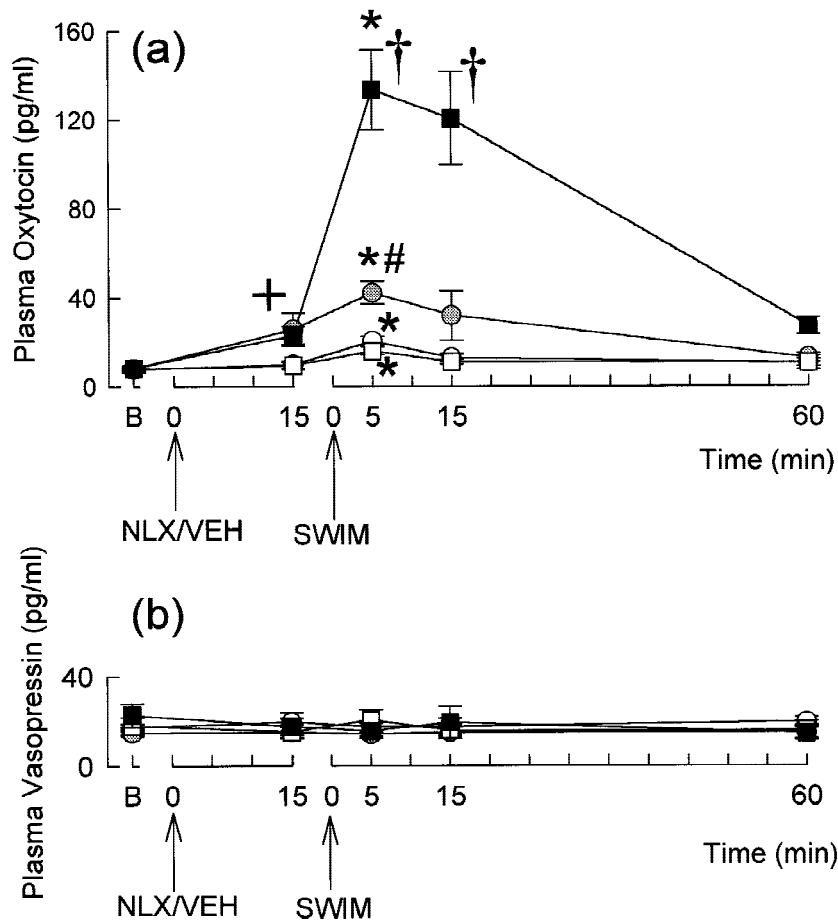


Figure 1 Effect of naloxone on neurohypophysial hormone secretory responses to forced swimming in pregnancy. Plasma oxytocin (a) and vasopressin (b) concentrations in virgin, vehicle-treated (VEH, ○, n=7) and naloxone-treated (NLX, ●, n=8) rats, and in 21-day-pregnant vehicle-treated (□, n=7) and naloxone-treated (■, n=8) rats. Newman-Keuls *post hoc* tests, * $P < 0.05$ compared with before swim in all groups; † $P < 0.05$ compared with all other groups at same time point. ‡ $P < 0.05$ pregnant naloxone-treated group compared with pregnant vehicle-treated group and compared with before injection ($P < 0.01$ one-way ANOVA for repeated measures). # $P < 0.01$, *t*-test, increment in plasma oxytocin above basal in virgin naloxone-treated group compared with virgin vehicle-treated group.

specifically the different responses to stress after naloxone compared with those after vehicle in the pregnant and virgin rats, the standard error of the difference between the means (vehicle- and naloxone-treated) 5 min after forced swimming was used (*t*-test, Swinscow 1983). $P < 0.05$ was considered statistically significant.

Results

Effect of naloxone on neurohypophysial hormone secretory responses to forced swimming in pregnancy

Two-way ANOVA for repeated measures of the plasma oxytocin concentrations in all groups showed a significant difference between groups ($P < 0.001$) and across time

($P < 0.001$); all other specific comparisons were derived from *post hoc* tests ($P < 0.05$), unless stated otherwise. Basal plasma oxytocin concentration was not significantly different between virgin and pregnant rats, and after forced swimming, plasma oxytocin concentration increased significantly in all groups within 5 min after the swim compared with values before the swim (Fig. 1a); there was no significant difference in the response between the vehicle-treated virgin and pregnant rats. Within 15 min after the forced swimming, the plasma oxytocin concentration had returned to pre-stress values in the virgin and vehicle-treated pregnant rats. Naloxone increased basal plasma oxytocin significantly only in pregnant rats (one-way ANOVA for repeated measures, $P < 0.05$, Fig. 1a). Naloxone also significantly enhanced the oxytocin

secretory response to forced swimming in both virgin (5.1-fold greater than basal concentration, compared with 2.6-fold greater in vehicle-treated virgin rats; $P < 0.01$, *t*-test, Fig. 1a) and pregnant rats (15.9-fold greater than basal concentration, compared with 1.9-fold greater than basal in vehicle-treated pregnant rats; $P < 0.0001$, *t*-test, Fig. 1a); the effect of naloxone in pregnancy was 3.1-fold greater than in virgins. Plasma oxytocin concentration remained increased in the naloxone-treated pregnant rats 15 min after the swim, but returned to the concentration recorded before the swim in the naloxone-treated virgin rats.

Basal plasma vasopressin concentration was not significantly different between pregnant and virgin groups, and did not alter after naloxone or with forced swimming in any group (Fig. 1b).

Effect of naloxone on HPA axis secretory responses to forced swimming in pregnant rats

ACTH Two-way ANOVA for repeated measures of the plasma ACTH concentrations in all groups showed a significant interaction between time and group ($P < 0.001$); all other specific comparisons were derived from *post hoc* tests ($P < 0.05$), unless stated otherwise. Basal plasma concentrations of ACTH did not differ significantly between virgin and day 21 pregnant rats; forced swimming significantly increased ACTH secretion in both groups compared with values before the swim and basal values, reaching a maximum at 5 min after the swim (Fig. 2a). The ACTH response to forced swimming was significantly lower in pregnant rats compared with that in virgin controls (Fig. 2a), and in the virgin rats the plasma ACTH concentration was significantly less 60 min after the forced swimming than at the peak response, whereas there was no significant decrease in the pregnant-vehicle group (Fig. 2a). Naloxone alone had no significant effect on ACTH concentrations within 15 min after administration in either virgin or pregnant rats (Fig. 2a). However, naloxone significantly attenuated the secretory response to swimming in virgins and there was a significant difference between the virgin vehicle-treated group and all other groups at 5 min after the stress stimulus; the plasma ACTH concentration in naloxone-treated pregnant rats did not differ from that in vehicle-treated pregnant rats (Fig. 2a). The increase in plasma ACTH 5 min after forced swimming, calculated as the difference from the mean basal concentration, was 245 ± 46 pg/ml in virgin rats and the increases in vehicle-treated pregnant (118 ± 20 pg/ml) and naloxone-treated virgin and pregnant rats (154 ± 30 pg/ml, 151 ± 16 pg/ml respectively) were significantly less (one-way ANOVA, $P < 0.05$). Naloxone did not significantly affect the plasma concentration of ACTH at 60 min after the swim. Further analysis showed that the effect of naloxone on the stress response was significantly different ($P < 0.05$) between virgin and pregnant rats (*t*-test

on the difference between the plasma ACTH concentration in the naloxone- and vehicle-treated groups after forced swimming and the calculated s.e.m. of the difference between the two independent means: virgins -103 ± 58 pg/ml; pregnant $+40 \pm 29$ pg/ml).

Corticosterone Two-way ANOVA for repeated measures of the plasma corticosterone concentrations in all groups showed a significant interaction between time and group ($P < 0.0001$); all other specific comparisons were derived from *post hoc* tests ($P < 0.05$), unless stated otherwise. Basal plasma concentrations of corticosterone did not differ between virgin and day 21 pregnant rats, and forced swimming significantly increased corticosterone secretion compared with values before the swim and basal values in all groups (Fig. 2b); the corticosterone secretory response to forced swimming in pregnant rats was significantly less than that in virgin rats (at 5 min after the swim only; Fig. 2b). There were no differences among the groups at 15 min after the swim, although concentrations remained similar to those at 5 min within groups. Sixty minutes after the forced swimming, in the pregnant rats the plasma corticosterone concentration had not decreased from the high concentrations at either 5 or 15 min, but that in the virgin rats had returned to basal values (Fig. 2b). Naloxone had no significant effect on basal corticosterone concentration compared with vehicle within 15 min after administration and did not significantly affect the secretory response to stress in virgins (Fig. 2b); however, the corticosterone concentration 5 min after swimming in pregnant rats treated with naloxone was significantly greater than that in the vehicle-treated pregnant rats (Fig. 2b). The increments in plasma corticosterone 5 and 15 min after forced swimming, compared with the mean basal concentration, did not show any significant differences (one-way ANOVA, $P = 0.38$, $P = 0.94$ respectively, data not shown). Naloxone did not significantly affect the plasma corticosterone concentrations at 60 min after the swim. Further statistical analysis (*t*-test on the difference between the plasma corticosterone concentration in naloxone- and vehicle-treated groups after forced swimming and the calculated s.e.m. of the difference between the two independent means) showed that the effect of naloxone on the stress response was significantly different ($P < 0.05$) between virgin and pregnant rats 5 min after stress (virgins -60 ± 66 ng/ml; pregnant $+200 \pm 62$ ng/ml).

Discussion

We have shown that the oxytocin secretory response to forced swimming (a combined physical and emotional stressor, Abel 1994) persists into late pregnancy, with no differences in plasma concentrations between pregnant

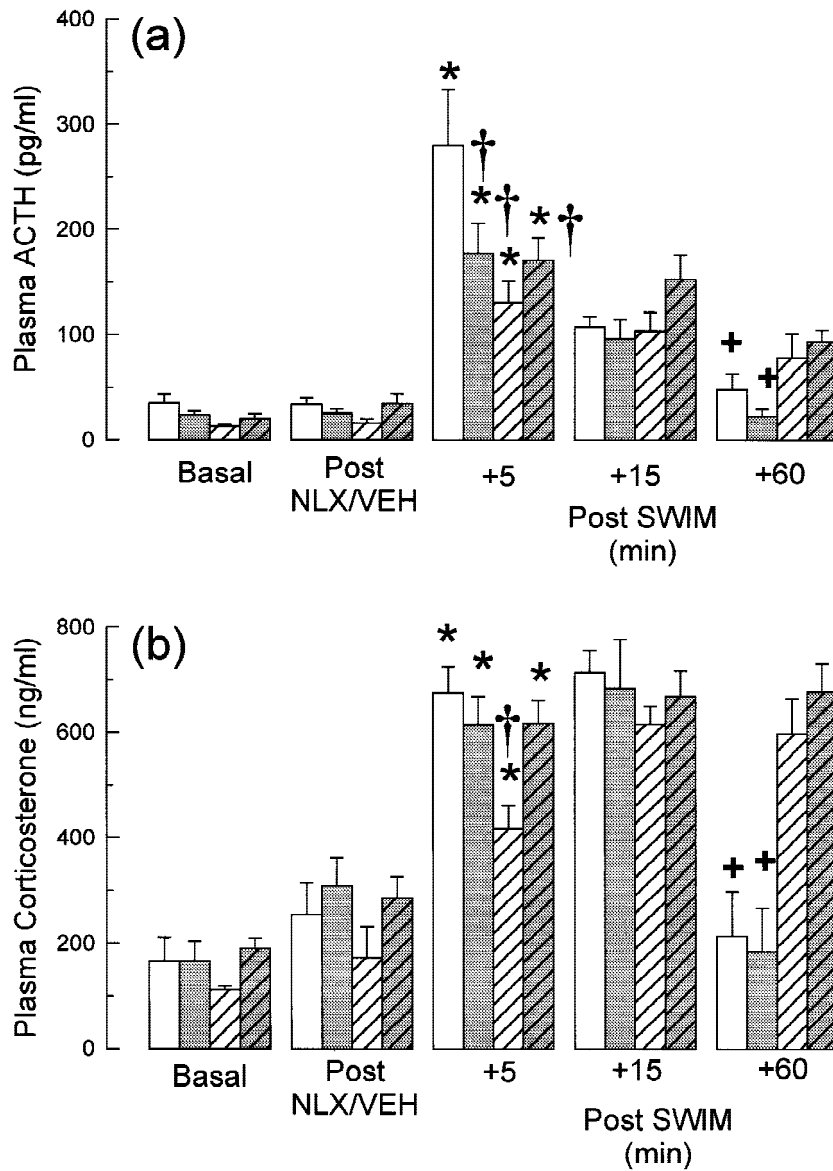


Figure 2 Effect of naloxone on HPA axis secretory responses to forced swimming in pregnancy in the same groups of rats as in Fig. 1. Data are mean \pm S.E.M. plasma ACTH (a) or corticosterone (b) concentration in vehicle-treated virgin rats (\square) and 21-day-pregnant rats (cross-hatched columns), and naloxone-treated virgin rats (stippled columns) and 21-day-pregnant rats (stippled, cross-hatched columns). Two blood samples were taken before vehicle or naloxone treatment (Basal is the mean of these), further blood samples were taken 5 and 15 min after treatment (post NLX/VEH is the mean of these) and 5, 15 and 60 min after forced swimming (Post SWIM). (a) Newman-Keuls *post hoc* tests, * $P < 0.05$ compared with before swim; † $P < 0.05$ compared with virgin vehicle-treated group at same time point; + $P < 0.05$ compared with peak secretion at 5 min after swim in same animals. (b) Newman-Keuls *post hoc* tests, * $P < 0.05$ compared with before swim; † $P < 0.05$ compared with virgin vehicle-treated group at same time point; + $P < 0.05$ compared with same groups at 5 and 15 min.

and virgin rats (see also Neumann *et al.* 1988); this contrasts with lactation, when the oxytocin secretory response to a stressor is greatly reduced (Carter & Lightman 1987*b*, Higuchi *et al.* 1991, Neumann *et al.* 1995). Oxytocin secretion in response to forced swimming in pregnancy was strongly enhanced by naloxone, which thus indicates that endogenous opioids actually mask an exaggerated response after exposure to this stressor. The lack of a vasopressin response to the forced swim stress used in our studies is consistent with previous reports of findings in male and female rats (Lang *et al.* 1983, Kasting 1988, Wotjak *et al.* 1996), and shows a highly selective activation of the neurohypophysial oxytocin system by the swim stressor. Oxytocin secretory responses to stress, therefore, are restrained by endogenous opioids during pregnancy. Endogenous opioids have previously been demonstrated to inhibit oxytocin, but not vasopressin, secretory responses to immobilisation stress in male (Samson *et al.* 1985) and female virgin rats (Carter *et al.* 1986), and we now show similar endogenous opioid inhibition of oxytocin responses to forced swimming in female rats. Both μ - and κ -opioid systems may be responsible (Carter & Lightman 1987*a*). Endogenous opioids are co-localised and co-secreted with oxytocin and vasopressin (Watson *et al.* 1982, Meister *et al.* 1990) and there is substantial evidence that endogenous κ -opioids restrain stimulated oxytocin secretion at the level of the neurosecretory terminals in the neurohypophysis (Bicknell & Leng 1982). However, previous studies have indicated that, at the level of the neurohypophysis, endogenous κ -opioid inhibitory mechanisms are down-regulated at the end of pregnancy (Sumner *et al.* 1992, Douglas *et al.* 1993), suggesting a greater role for μ -opioids, which act centrally and not at the neurohypophysis (Russell *et al.* 1993).

It is clear that endogenous μ -opioids strongly inhibit oxytocin neurone activity and secretion in late pregnancy (Douglas *et al.* 1995), and that this is manifest mainly on oxytocin neurone cell bodies and their inputs, rather than on the nerve terminals in the neurohypophysis (Douglas *et al.* 1993). The present study has demonstrated that an endogenous opioid mechanism not only restrains oxytocin secretion from an expanded neurohypophysis store in pregnancy (Douglas *et al.* 1993), but also strongly restricts the oxytocin neurone response to forced swimming in pregnancy, as with responses to other stimuli, such as peripheral administration of cholecystokinin during gestation (Douglas *et al.* 1995), and to birth (Hartman *et al.* 1986, Leng *et al.* 1987, 1988, Lawrence *et al.* 1992). This action could be on oxytocin cell bodies themselves, as they have opioid receptors (Inenaga *et al.* 1994, Sumner *et al.* 1992), or on the input pathways to these neurones mediating the stress stimulus, perhaps from the brainstem (Onaka *et al.* 1995). However, endogenous opioids are not responsible for the reduced responsiveness of oxytocin neurones to osmotic stimulation in pregnancy (Bull & Russell 1992), or to electrical stimulation of lamina

terminalis (Bull *et al.* 1994) and thus there is likely to be a selective action of opioids on inputs to oxytocin neurones. No consistent changes have been described in magnocellular or parvocellular neurone prodynorphin or proenkephalin A mRNA expression, which are indicators of opioid synthesis, in pregnancy (Schriefer 1991, Douglas & Russell 1994, Douglas *et al.* 1993). However, an increased hypothalamic content of β -endorphin (Wardlaw & Frantz 1983, Dondi *et al.* 1991, Broad *et al.* 1993) and pro-opiomelanocortin mRNA in the arcuate nucleus (Redmond *et al.* 1996) have been described before parturition, which may account for the central endogenous opioid influence on oxytocin neurones in pregnancy.

In parturition, the increased secretion of oxytocin is reduced by the stress of environmental disturbance and the intervals between pup births increase (Leng *et al.* 1987, 1988). The evident dichotomy between these reports and the stimulation of oxytocin secretion by stressors in virgin female and male rats in other previous studies (Lang *et al.* 1983, Gibbs 1986, Carter & Lightman 1987*b*, Wotjak *et al.* 1996) and in the present study on pregnant rats, may be apparent rather than real. Thus the effects of a stressor on oxytocin secretion in parturition could be secondary to the slowing of parturition through another mechanism, with consequent reduced positive feedback stimulation of oxytocin secretion. The effect of naloxone to increase oxytocin secretion in these environmentally disturbed rats (Leng *et al.* 1987) may simply reveal the underlying stimulatory effect of environmental stress on oxytocin secretion as in pregnancy.

This study confirms that secretion of ACTH and corticosterone in response to a stressor are reduced during late pregnancy (Neumann *et al.* 1998) and this is comparable to the reduced HPA axis response to stressors previously reported in lactation (e.g. Walker *et al.* 1995). Naloxone attenuated the increase in ACTH concentration in virgins in response to forced swimming, revealing that endogenous opioids enhance ACTH secretory responses. Naloxone did not attenuate ACTH secretion in response to forced swimming in pregnant rats, indicating loss of the endogenous opioid-enhancing effects on the ACTH secretory response seen in virgin rats. Corticosterone responses showed a trend similar to those of the ACTH responses: there was a pregnancy-related attenuation in corticosterone concentration 5 min after forced swimming and naloxone reversed this; also, high ACTH and corticosterone concentrations after the swim were prolonged in pregnant rats compared with those in virgin rats. However, naloxone did not cause a decrease in the corticosterone response to forced swimming in virgin or pregnant rats at either 5 or 15 min after the swim, although the corticosterone response in virgin rats was not significantly greater than that in virgin or pregnant rats given naloxone. This is in contrast with the response of ACTH, perhaps because the maximal response of the adrenal cortex to ACTH is limiting (Keller-Wood *et al.* 1984). In addition, adrenal

sensitivity to ACTH is increased in pregnancy (Carr *et al.* 1981, Dupouy *et al.* 1975, Waddell & Atkinson 1994), and therefore changes in corticosterone concentration will not necessarily parallel those of ACTH.

We have previously shown that, in pregnancy, down-regulated anterior pituitary mechanisms contribute to the attenuated HPA axis responses, as there is a reduced pituitary ACTH secretory response to exogenous corticotrophin-releasing hormone (CRH) *in vivo* (Neumann *et al.* 1998), attenuated cAMP production in response to CRH *in vitro* and decreased CRH receptor binding (Johnstone *et al.* 1997). We have now shown that, in pregnancy, the enhancing action of endogenous opioid on ACTH release in response to forced swimming, which is normally seen in virgins, is removed. The reduction in ACTH secretion in response to the stressor after naloxone in virgins is consistent with the findings of previous studies showing that opioid antagonists reduce ACTH and corticosterone secretion in response to a stressor in male rats (degli Uberti *et al.* 1995). Naloxone is likely to be exerting its effects on the HPA axis via the hypothalamus (Wang *et al.* 1996), and thus may affect hypothalamic-pituitary mechanisms through CRH release. μ -Opioids appear to mediate the naloxone-induced reduction in stress responses (Cover & Buckingham 1989), whereas κ - and δ -opioids may modulate HPA axis hormone secretion under basal conditions (Iyengar *et al.* 1986, Plotsky 1986). The κ -opioid effects probably occur within both the hypothalamus (Nikolarakis *et al.* 1987) and the anterior pituitary (Calogero 1996). We are not aware of any direct action of naloxone on the adrenal cortex.

The removal of endogenous opioid enhancement on central mechanisms regulating HPA axis responses to stressors in pregnancy may partly underlie the reduced activation of parvocellular PVN neurones by immobilisation (da Costa *et al.* 1996) and decreased CRH mRNA expression in the PVN (Douglas & Russell 1994). Together, these changes constitute evidence for reduced feed-forward activity in the hypothalamo-pituitary component of the HPA axis in pregnancy. The prolonged increase in ACTH and corticosteroid secretion after stress in the pregnant rats is evidently not consistent with enhanced fast negative feedback in pregnancy, but is consistent with either a prolonged adrenocortical secretory response or reduced metabolic clearance, perhaps as a result of increased circulating corticosteroid binding globulin in pregnancy (Seal & Doe 1967). Maternal plasma corticosterone concentrations may be additionally contributed to by the fetus (Dupouy *et al.* 1975). In addition, increased oxytocin secretion after the swimming stress may enhance the secretion of ACTH or corticosterone, or both, by actions on the corticotrophs or adrenal cortex (Samson & Schell 1995, Stachowiak *et al.* 1995, Link *et al.* 1993).

The placenta limits the exposure of the fetus to high concentrations of maternal HPA axis hormones, as ACTH does not cross from the maternal to the fetal circulation

(Dupouy *et al.* 1980) and the transfer of glucocorticoid is regulated by several placental enzymes, of which 11β hydroxysteroid dehydrogenase Type II (Seckl *et al.* 1995) predominates in the last few days of pregnancy, inactivating corticosterone (Burton & Waddell 1994). Thus the reduced immediate peak ACTH secretory response to the stressor in pregnancy may act in concert with placental mechanisms to protect the fetus from excessive concentrations of corticosteroid toward the end of pregnancy which could have potentially lifelong deleterious effects (Weinstock 1997). Possible changes in feedback mechanisms in pregnancy are currently under further investigation.

In conclusion, oxytocin neurone secretory responses to exposure to forced swimming are not reduced, but are instead strongly restrained from responding in an exaggerated fashion, by endogenous opioids in late pregnancy. This opioid restraint will contribute to conservation of the neurohypophysial store of oxytocin for primary use in promoting uterine contractions in parturition, when about a third of the oxytocin content is depleted within about 2 h (Fuchs & Saito 1971). HPA axis secretion in pregnancy in response to forced swimming is no longer stimulated by endogenous opioids, which may contribute to protecting the fetus from exposure to excessive concentrations of corticosteroid in the mother during initial responses to stress.

Acknowledgements

We thank Dr Philip Bull and Gabriele Kohl for excellent technical assistance including quantitative analysis of plasma hormones. Supported by DAAD/ARC (British Council), and Deutsche Forschungsgemeinschaft. H A J is a BBSRC Research Student. I D N is a recipient of the Heisenberg stipend of the DFG.

References

- Abel EL 1994 A further analysis of physiological changes in rats in the forced swim test. *Physiology and Behaviour* **56** 795–800.
- Bicknell RJ & Leng G 1982 Endogenous opiates regulate oxytocin but not vasopressin secretion from the neurohypophysis. *Nature* **298** 161–162.
- Broad KD, Kendrick KM, Sirinathsinghji DJ & Keverne EB 1993 Changes in proopiomelanocortin and pre-proenkephalin messenger-RNA levels in the ovine brain during pregnancy, parturition and lactation and in response to estrogen and progesterone. *Journal of Neuroendocrinology* **5** 711–719.
- Buckingham J & Cooper TA 1984 Differences in hypothalamo-pituitary-adrenocortical activity in the rat after acute and prolonged treatment with morphine. *Neuroendocrinology* **38** 411–417.
- Bull PM & Russell JA 1992 Oxytocin secretory responses to hypernatraemia and inhibition by morphine in pregnant urethane-anesthetised rats. *Journal of Physiology* **452** 210P.

- Bull PM, Douglas AJ & Russell JA 1994 Opioids and coupling of the anterior peri-third ventricular input to oxytocin neurones in anaesthetized pregnant rats. *Journal of Neuroendocrinology* **6** 267–274.
- Burton PJ & Waddell BJ 1994 11 beta-Hydroxysteroid dehydrogenase in the rat placenta: developmental changes and the effects of altered glucocorticoid exposure. *Journal of Endocrinology* **143** 505–513.
- Calogero AE 1996 The kappa-opioid receptor agonist MR-2034 stimulates the rat hypothalamic–pituitary–adrenal axis: studies *in vivo* and *in vitro*. *Journal of Neuroendocrinology* **8** 579–585.
- Carr RR, Parker CR, Madden JD, MacDonald PC & Porter JC 1981 Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. *American Journal of Obstetrics and Gynecology* **139** 416–421.
- Carter DA & Lightman SL 1987a Opioid control of oxytocin secretion: evidence of distinct regulatory actions of two opiate receptor types. *Life Sciences* **40** 2289–2296.
- Carter DA & Lightman SL 1987b Oxytocin responses to stress in lactating and hyperprolactinaemic rats. *Neuroendocrinology* **46** 532–537.
- Carter DA, Williams TD & Lightman SL 1986 A sex difference in endogenous opioid regulation of the posterior pituitary response to stress in the rat. *Journal of Endocrinology* **111** 239–244.
- da Costa APC, Wood S, Ingram CD & Lightman SL 1996 Region-specific reduction in stress-induced *c-fos* mRNA expression during pregnancy and lactation. *Brain Research* **742** 177–184.
- Cover PO & Buckingham J 1989 Effects of selective opioid-receptor blockade on the hypothalamo–pituitary–adrenocortical responses to surgical trauma in the rat. *Journal of Endocrinology* **121** 213–220.
- Dondi D, Maggi R, Panerai AE, Piva F & Limonta P 1991 Hypothalamic opiate tone during pregnancy, parturition and lactation in the rat. *Neuroendocrinology* **53** 460–466.
- Douglas AJ & Russell JA 1994 Corticotrophin-releasing hormone, proenkephalin A and oxytocin mRNAs in the paraventricular nucleus during pregnancy and parturition in the rat. *Gene Therapy* **1** (suppl 1) S85.
- Douglas AJ, Dye S, Leng G, Russell JA & Bicknell RJ 1993 Endogenous opioid regulation of oxytocin secretion through pregnancy in the rat. *Journal of Neuroendocrinology* **5** 307–314.
- Douglas AJ, Neumann I, Meeren HKM, Leng G, Johnstone LE, Munro G & Russell JA 1995 Central endogenous opioid inhibition of supraoptic oxytocin neurones in pregnant rats. *Journal of Neuroscience* **15** 5049–5057.
- Dupouy JP, Coffigny H & Magre S 1975 Maternal and foetal corticosterone levels during late pregnancy in rats. *Journal of Endocrinology* **65** 347–352.
- Dupouy JP, Chatelain A & Allaupe P 1980 Absence of transplacental passage of ACTH in the rat: direct experimental proof. *Biology of the Neonate* **37** 96–102.
- Fuchs AR & Saito S 1971 Pituitary oxytocin and vasopressin content of pregnant rats before, during and after parturition. *Endocrinology* **88** 574–578.
- Gibbs DM 1986 Vasopressin and oxytocin: hypothalamic modulators of the stress response: a review. *Psychoneuroendocrinology* **11** 131–140.
- Hartman RD, Rosella-Dampman LM, Emmert SE & Summy-Long JY 1986 Inhibition of release of neurohypophysial hormones by endogenous opioid peptides in pregnant and parturient rats. *Brain Research* **382** 352–359.
- Higuchi T, Honda K, Takano S & Negoro H 1988 Reduced oxytocin response to osmotic stimulus and immobilization stress in lactating rats. *Journal of Endocrinology* **116** 225–230.
- Higuchi T, Bicknell RJ & Leng G 1991 Reduced oxytocin release from the neural lobe of lactating rats is associated with reduced pituitary content and does not reflect reduced excitability of oxytocin neurons. *Journal of Neuroendocrinology* **3** 297–302.
- Inenaga K, Nagatomo T, Nakao K, Yanihara N & Yamashita H 1994 Kappa selective agonists decrease postsynaptic potentials and calcium components of action potentials in the supraoptic nucleus of rat hypothalamus *in vitro*. *Neuroscience* **58** 331–340.
- Iyengar S, Kim HS & Wood PL 1986 Kappa opiate agonists modulate the hypothalamic–pituitary–adrenocortical axis in the rat. *Journal of Pharmacology and Experimental Therapeutics* **238** 429–436.
- Johnstone HA, Douglas AJ, Seckl JR & Russell JA 1997 Changes in 11 β -hydroxysteroid dehydrogenase type I (11 β -HSD1) activity and corticosterone receptor mRNA in the rat hippocampus and paraventricular nucleus (PVN). *Society for Neuroscience Abstracts* **23** 1241.
- Kasting NW 1988 Simultaneous and independent release of vasopressin and oxytocin in the rat. *Canadian Journal of Physiology and Pharmacology* **66** 22–26.
- Keller-Wood M, Shinsako J & Dallman MF 1984 Interaction between stimulus intensity and corticosteroid feedback control of ACTH. *American Journal of Physiology* **247** E489–E494.
- Landgraf R 1981 Simultaneous measurement of arginine vasopressin and oxytocin in plasma and neurohypophysis by radioimmunoassay. *Endocrinologie* **78** 191–204.
- Lang RE, Heil JWE, Ganten D, Hermann K, Unger T & Rascher W 1983 Oxytocin unlike vasopressin is a stress hormone in the rat. *Neuroendocrinology* **37** 314–316.
- Lawrence AB, Petherick JC, McLean K, Gilbert CL, Chapman C & Russell JA 1992 Naloxone prevents interruption of parturition and increases plasma oxytocin following environmental disturbance in parturient sows. *Physiology and Behavior* **52** 917–923.
- Leng G, Mansfield S, Bicknell RJ, Brown D, Chapman C, Hollingsworth S, Ingram CD, Marsh MI, Yates JO & 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, Hollingsworth S, Shibuki K, Yates JO & Way SA 1988 Endogenous opioid actions and effects of environmental disturbance on parturition and oxytocin secretion in rats. *Journal of Reproduction and Fertility* **84** 345–356.
- Link H, Dayanithi G & Gratzl M 1993 Glucocorticoids rapidly inhibit oxytocin-stimulated adrenocorticotrophin release from rat anterior pituitary cells, without modifying intracellular calcium transients. *Endocrinology* **132** 873–878.
- Meister B, Villar MJ, Ceccatelli S & Hokfelt T 1990 Localisation of chemical messengers in magnocellular neurones of the hypothalamic supraoptic and paraventricular nuclei: an immunocytochemical study using experimental manipulations. *Neuroscience* **37** 603–633.
- Neumann I, Pittman QJ & Landgraf R 1995 Release of oxytocin within the supraoptic nucleus: mechanisms, physiological significance and antisense targeting. *Advances in Experimental Medicine and Biology* **395** 173–183.
- Neumann ID, Johnstone H, Liebsch G, Shipston M, Landgraf R, Russell JA & Douglas AJ 1998 Attenuated responses of the hypothalamo–pituitary–adrenal axis to stress in pregnant rats involve adenohipophysial changes. *Journal of Physiology* **508** 289–300.
- Nikolarakis K, Pfeiffer A, Stalla GK & Herz A 1987 The role of CRF in the release of ACTH by opiate agonists and antagonists in rats. *Brain Research* **421** 373–376.
- Onaka T, Luckman SM, Antonijevic I, Palmer JR & Leng G 1995 Involvement of the noradrenergic afferents from the nucleus tractus solitarius to the supraoptic nucleus in oxytocin release after peripheral cholecystokinin octapeptide in the rat. *Neuroscience* **66** 403–412.
- Plotsky PM 1986 Opioid inhibition of immunoreactive corticotrophin-releasing factor secretion into the hypophysial–portal circulation of rats. *Regulatory Peptides* **16** 235–242.
- Redmond K, Douglas AJ, Bicknell RJ & Russell JA 1996 Evidence for plasticity in the β -endorphin innervation of the supraoptic nucleus during pregnancy in the rat. *Journal of Physiology* **495P** 13P.
- Russell JA, Coombes JE, Leng G & Bicknell RJ 1993 Morphine tolerance and inhibition of oxytocin secretion by κ -opioids acting on the rat neurohypophysis. *Journal of Physiology* **469** 365–386.

- Samson WK & Schell DA 1995 Oxytocin and the anterior pituitary gland. *Advances in Experimental Medicine and Biology* **395** 355–364.
- Samson WK, McDonald JK & Lumpkin MD 1985 Naloxone-induced dissociation of oxytocin and prolactin releases. *Neuroendocrinology* **40** 68–71.
- Schriever JA 1991 Diethylstilbesterol- and pregnancy-induced changes in rat neurointermediate lobe oxytocin, arginine vasopressin, methionine enkephalin and dynorphin. *Neuroendocrinology* **54** 185–191.
- Seal US & Doe RP 1967 The role of corticosteroid binding globulin in mammalian pregnancy. *Experimental Medicine International Congress Series* **132** 697–706.
- Seckl JR, Benediktsson R, Lindsay RS & Brown RW 1995 Placental 11 beta-hydroxysteroid dehydrogenase and the programming of hypertension. *Journal of Steroid Biochemistry and Molecular Biology* **55** 447–455.
- Soaje M & Deis RP 1994 A modulatory role of endogenous opioids on prolactin secretion at the end of pregnancy in the rat. *Journal of Endocrinology* **140** 97–102.
- Stachowiak A, Macchi C, Nussdorfer GG & Malendowicz LK 1995 Effects of oxytocin on the function and morphology of the rat adrenal cortex: *in vitro* and *in vivo* investigations. *Research in Experimental Medicine* **195** 265–274.
- Suda T, Sato Y, Sumitomo T, Nakano Y, Tozawa F, Iwai I, Yamada M & Demura H 1992 β -Endorphin inhibits hypoglycemia-induced gene expression of corticotrophin-releasing factor in the rat hypothalamus. *Endocrinology* **130** 1325–1330.
- Sumner BEH, Douglas AJ & Russell JA 1992 Pregnancy alters the density of opioid binding sites in the supraoptic nucleus and posterior pituitary gland of rats. *Neuroscience Letters* **137** 216–220.
- Swinscow TDV 1983 *Statistics at Square One*. London: British Medical Association.
- degli Uberti EC, Petraglia F, Bondanelli M, Guo AL, Valentini A, Salvadori S, Crisculo M, Nappi RE & Genazzani AR 1995 Involvement of mu-opioid receptors in the modulation of pituitary-adrenal axis in normal and stressed rats. *Journal of Endocrinological Investigation* **18** 1–7.
- Waddell BJ & Atkinson HC 1994 Production rate, metabolic clearance rate and uterine extraction of corticosterone during rat pregnancy. *Journal of Endocrinology* **143** 183–190.
- Walker C-D, Trotter G, Rochford J & Lavellee D 1995 Dissociation between behavioural and hormonal responses to the forced swim stress in lactating rats. *Journal of Neuroendocrinology* **7** 615–622.
- Wang XQ, Imaki T, Shibasaki T, Yamauchi N & Demura H 1996 Intracerebroventricular administration of beta-endorphin increases the expression of c-fos and of corticotropin-releasing factor messenger ribonucleic acid in the paraventricular nucleus of the rat. *Brain Research* **707** 189–195.
- Wardlaw SL & Frantz AG 1983 Brain beta-endorphin during pregnancy, parturition, and the postpartum period. *Endocrinology* **113** 1664–1668.
- Watson SJ, Akil H, Fischli W, Goldstein A, Zimmerman E, Nilaver G & van Wimersma Greidanus TB 1982 Dynorphin and vasopressin: common localisation in magnocellular neurones. *Science* **216** 85–87.
- Weinstock M 1997 Does prenatal stress impair coping and regulation of hypothalamic-pituitary-adrenal axis? *Neuroscience and Behavior Reviews* **21** 1–10.
- Windle RJ, Wood S, Shanks N, Perks P, Conde GL, da Costa APC, Ingram CD & Lightman SL 1997 Endocrine and behavioural responses to noise stress: comparison of virgin and lactating female rats during non-disrupted maternal activity. *Journal of Neuroendocrinology* **9** 407–414.
- Wojtak CT, Kubota M, Liebsch G, Montkowski A, Holsboer F, Neumann I & Landgraf R 1996 Release of vasopressin within the rat paraventricular nucleus in response to emotional stress: a novel mechanism of regulating adrenocorticotrophic hormone secretion. *Journal of Neuroscience* **16** 7725–7732.
- Yagi K 1992 Suppressive vasopressin response to emotional stress. *Japanese Journal of Physiology* **42** 681–703.

Received 26 September 1997

Revised manuscript received 29 January 1998

Accepted 24 March 1998