Maternal stress and the MPOA: Activation of CRF receptor 1 impairs maternal behavior and triggers local oxytocin release in lactating rats

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A B S T R A C T
Maternal behavior and anxiety are potently modulated by the brain corticotropin-releasing factor (CRF) system postpartum. Downregulation of CRF in limbic brain regions is essential for appropriate maternal behavior and an adaptive anxiety response. Here, we focus our attention on arguably the most important brain region for maternal behavior, the hypothalamic medial preoptic area (MPOA).

Within the MPOA, mRNA for CRF receptor subtype 1 (protein: CRFR1, gene: Crhr1) was more abundantly expressed than for subtype 2 (protein: CRFR2, gene: Crhr2), however expression of Crhr1, Crhr2 and CRF-binding protein (protein: CRFBP, gene: Crhbp) mRNA was similar between virgin and lactating rats. Subtype-specific activation of CRFR, predominantly CRFR1, in the MPOA decreased arched back nursing and total nursing under non-stress conditions. Following acute stressor exposure, only CRFR1 inhibition rescued the stress-induced reduction in arched back nursing while CRFR1 activation prolonged the decline in nursing. Furthermore, inhibition of CRFR1 strongly increased maternal aggression in the maternal defense test. CRFR1 activation had anxiogenic actions and reduced locomotion on the elevated plus-maze, however neither CRFR1 nor R2 manipulation affected maternal motivation. In addition, activation of CRFR1, either centrally or locally in the MPOA, increased local oxytocin release. Finally, inhibition of CRFBP (a potent regulator of CRF activity) in the MPOA did not affect any of the maternal parameters investigated.

In conclusion, activity of CRFR in the MPOA, particularly of subtype 1, needs to be dampened during lactation to ensure appropriate maternal behavior. Furthermore, oxytocin release in the MPOA may provide a regulatory mechanism to counteract the negative impact of CRFR activation on maternal behavior.

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1. Introduction

The display of appropriate maternal behavior is the result of a variety of peripartum adaptations, including activation and inhibition of specific neurotransmitter systems in the brain. While oxytocin and vasopressin typically promote maternal behavior, and are hence up-regulated postpartum (e.g. Bosch and Neumann, 2008, 2012; Pedersen et al., 1994; van Leengoed et al., 1987), the corticotropin-releasing factor (CRF) system impedes maternal behavior and thus needs to be down-regulated (Cammin et al., 2004; Klampf et al., 2013, 2014, 2016a, b).

The CRF system consists of four ligands, CRF (protein: CRF, gene: Crh) and the urocortins 1–3, which bind with different affinities to the two CRF receptors (protein: CRFR, gene: Crhr): CRFR1 and CRFR2 (Reul and Holsboer, 2002a). In addition, activation of the CRF is regulated and, for the most part attenuated, by the...
secretory glycoprotein CRF-binding protein (protein: CRFBP, gene: Crhrbp) (Seasholtz et al., 2002; Sutton et al., 1995). CRF is the major stress neuropeptide involved in cellular, neuroendocrine, and behavioral responses to stress (Bale and Vale, 2004; Vale et al., 1981). It is released upon stressor exposure and triggers the central and peripheral stress response. Furthermore, CRF has anxiogenic and pro-depressive actions, amongst others (Reul and Holsboer, 2002a, b), which makes the CRF system one of the most promising candidate systems for treating mood disorders such as anxiety and depression.

During the postpartum period, activation of central CRFR strongly impairs maternal care and maternal aggression thereby inducing maternal neglect in lactating rats (Klampfl et al., 2013, 2014, 2016a), mice (D'Anna and Gammie, 2009; D'Anna et al., 2005; Gammie et al., 2004), and marmoset monkeys (Saltzman et al., 2011). These effects are observed not only by central activation of CRFR via intracerebroventricular (ICV) administration of CRFR agonists, but also by local stimulation of the CRFR1 and CRFR2 subtypes in the lateral septum (D'Anna and Gammie, 2009; D'Anna et al., 2005; Gammie et al., 2004), and and maintenance of maternal behavior, especially maternal care and maternal motivation (Numan and Stolzenberg, 2009). Here, the nonapeptide oxytocin has been shown to promote maternal behavior in several different species (Numan and Insel, 2003). Oxytocinergic projections from the paraventricular nucleus are thought to supply the MPOA with constantly high concentrations of oxytocin during lactation (Bosch and Neumann, 2012). In addition, oxytocin receptors are substantially up-regulated in the MPOA peripartum (Meddle et al., 2007), which facilitates finely-tuned regulation of maternal care even under constant levels of intracerebral oxytocin throughout mother–pup interactions (Bosch and Neumann, 2012). Importantly, oxytocin directly interacts with the CRF system, as oxytocin receptors are expressed on CRF neurons in the BNST and CRFR2 are expressed on oxytocin neurons in the paraventricular nucleus (Dabrowska et al., 2013), indicating a reciprocal neuromodulatory role for both peptides.

In the present study, we hypothesized that like the BNST, the MPOA CRF system is also involved in the regulation of maternal behavior. Hence, we assessed the expression profiles of both CRF and CRFBP in the MPOA and examined the effects of modulating CRF or CRFBP in the MPOA under stress and non-stress conditions. Furthermore, as one of the main mediators of maternal behavior in the MPOA is the oxytocin system (Bosch and Neumann, 2012), we hypothesized that local oxytocin release is affected by changes in CRFR signaling.

2. Materials & methods

2.1. Animals and housing

Virgin female Sprague-Dawley rats (220–250 g; Charles River Laboratories, Sulzfeld, Germany) were housed under standard laboratory conditions (change of bedding once per week, RT 22 ± 2 °C, 55% relative humidity, 12:12 h light/dark cycle, lights on at 6 a.m.) with access to water and standard rat chow ad libitum. All rats were initially housed in groups of 3–4 (for further details see below). Female rats were mated with sexually experienced male rats and pregnancy was confirmed by the presence of sperm in vaginal smears (pregnancy day (PD) 1). A separate, naïve cohort of rats was used for each experiment, and in each case rats were randomly assigned to the different treatment groups.

For experiment 1, virgin and lactating females were treated similarly; virgins were single-housed 7 days prior to brain collection, consistent with the single-housing period of the lactating rats, which were single-housed on PD 18 and killed 7 days later (lactation day (LD) 4). In experiments 2 and 5, females underwent surgery on PD 18 and were subsequently single-housed to guarantee recovery and undisturbed parturition (Klampfl et al., 2013). In experiments 3 and 4, rats were single-housed on PD 18 and underwent surgery on LD 1. On the day of birth, litters of all dams were culled to eight pups of mixed sexes. All rats were handled twice daily on PD 16–17 and during the single-housing period (except on the day of surgery and birth) to reduce non-specific stress responses during the experiments (Neumann et al., 1998).

For the maternal defense test, naïve virgin female Wistar rats (200–220 g; Charles River Laboratories) were used as intruders at random stages of their estrous cycle. Intruder rats were kept group-housed in a separate room to avoid olfactory recognition (Bosch, 2013). The experiments were approved by the Committee on Animal Health and Care of the local government and conformed with the European Directive (2010/63/EU) on the ethical use of animals. All reasonable efforts were made to minimize the number of rats used and their suffering.

2.2. Behavioral tests

All tests were performed between 8 a.m. and 3 p.m. during the light phase of the cycle. In experiments with repeated drug infusion (experiments 2 and 5), rats received the same drug throughout the experiment. After infusion, dams were immediately returned to their home cage.

2.2.1. Maternal care

Maternal care was monitored before and after drug infusion under non-stress and stress conditions (maternal defense test) (Klampfl et al., 2013, 2014, 2016a, b). Observations were made for 10 s every 2nd min in 30 min blocks according to an established protocol (Bosch and Neumann, 2008). The main parameter for the quality of maternal care was the occurrence of arched back nursing (ABN) (Bosch, 2011), an active nursing posture where the dam is strongly impairs maternal care and maternal aggression thereby inducing maternal neglect in lactating rats (Klampfl et al., 2013). In experiments 3 and 4, rats were single-housed on PD 18 and underwent surgery on LD 1. On the day of birth, litters of all dams were culled to eight pups of mixed sexes. All rats were handled twice daily on PD 16–17 and during the single-housing period (except on the day of surgery and birth) to reduce non-specific stress responses during the experiments (Neumann et al., 1998).

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2.2.2. Maternal motivation

The dams’ maternal motivation was tested in the modified pup retrieval test (PRT) as described previously (Bayerl et al., 2016). Briefly, dams were habituated to a red Perspex house for 150 min the day prior to the PRT. On the day of testing, the pups were separated from the dam for 60 min, during which the red house was re-introduced to the dam’s cage. Afterwards, the house and the dam were transferred to a plastic testing box (54 cm × 34 cm × 31 cm), which contained some bedding and the pups. Here, the number of pups retrieved into the house within the
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