Psychoneuroendocrinology 64 (2016) 89–98

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

CRF-R1 activation in the anterior-dorsal BNST induces maternal neglect in lactating rats via an HPA axis-independent central mechanism

Stefanie M. Klampfl[a,1], Paula J. Brunton[b], Doris S. Bayerl[a], Oliver J. Bosch[a,*]

[a] Department of Behavioural and Molecular Neurobiology, University of Regensburg, 93053 Regensburg, Germany
[b] Division of Neurobiology, The Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian EH25 9RG, UK

ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form
15 November 2015
Accepted 16 November 2015

Keywords:
Corticotropin-releasing factor
Bed nucleus of the stria terminalis
Maternal behavior
Anxiety
Hypothalamic–pituitary–adrenal axis

ABSTRACT

Adequate maternal behavior in rats requires minimal corticotropin-releasing factor receptor (CRF-R) activation in the medial-posterior bed nucleus of the stria terminalis (mpBNST). Based on the architectural heterogeneity of the BNST and its distinct inter-neural connectivity, we tested whether CRF-R manipulation in another functional part, the anterior-dorsal BNST (adBNST), differentially modulates maternal behavior.

We demonstrate that in the adBNST, activation of CRF-R1 reduced arched back nursing (ABN) and nursing, whereas activation of CRF-R2 resulted in an initial reduction in nursing but significantly increased the incidence of ABN 5 h after the treatment. Following stressor exposure, which is detrimental to maternal care, ABN tended to be protected by CRF-R1 blockade. Maternal motivation, maternal aggression, and anxiety were unaffected by any manipulation. Furthermore, under basal and stress conditions, activation of adBNST CRF-R1 increased plasma ACTH and corticosterone concentrations, whereas stimulation of adBNST CRF-R2 increased basal plasma ACTH and corticosterone concentrations, but blocked the stress-induced increase in plasma corticosterone secretion. Moreover, both the CRF-R1 and -R2 antagonists prevented the stress-induced increase in plasma corticosterone secretion. Importantly, elevated levels of circulating corticosterone induced by intra-adBNST administration of CRF-R1 or -R2 agonist did not impact maternal care. Finally, *Crf* mRNA expression in the adBNST was increased during lactation; however, *Crfr1* mRNA expression was similar between lactating and virgin rats.

In conclusion, maternal care is impaired by adBNST CRF-R1 activation, and this appears to be the result of a central action, rather than an effect of elevated circulating levels of CORT. These data provide new insights into potential causes of disturbed maternal behavior postpartum.

1. Introduction

The peripartum period is accompanied by numerous physiological and behavioral adaptations organized by the maternal brain. These changes are essential for the adequate expression of maternal behavior, thereby ensuring the proper development of the offspring, as well as for the mothers' mental health. Indeed, up to 30% of mothers that develop postpartum mood disorders show child neglect, with some committing infanticide (Brummelte and Galea, 2010b; Friedman and Resnick, 2009).

One peptidergic system that evidently contributes to such maladaptations during this highly sensitive period is the central corticotropin-releasing factor (CRF) system (Klampfl et al., 2014, 2013; Magiakou et al., 1996). The CRF system consists of CRF and its related peptides, urocortin 1, 2 and 3, which bind to CRF receptor type 1 (CRF-R1), CRF-R2, and the CRF binding protein with different affinities (Reul and Holsboer, 2002). CRF was first discovered as the main initiator of the hypothalamic–pituitary–adrenal (HPA) axis and is the major secretagogue of ACTH from the anterior pituitary into the portal blood system (Vale et al., 1981). ACTH stimulates the release of corticosterone (CORT) from the adrenal glands which plays several roles in mediating appropriate responses to stress and also exerts negative feedback control of the HPA axis at the level of the hippocampus, paraventricular nucleus (PVN), and pituitary.

* Corresponding author. Fax: +49 941 9433052.
E-mail addresses: stefanie.klampfl@ubc.ca (S.M. Klampfl), P.J.Brunton@ed.ac.uk (P.J. Brunton), doris.bayerl@ur.de (D.S. Bayerl), oliver.bosch@ur.de (O.J. Bosch).
[1] Current address: Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver BC, V6T 1Z3, Canada.

http://dx.doi.org/10.1016/j.psyneuen.2015.11.015

Moreover, CRF and its related peptides also exert central functions and influence a variety of non-social and social behaviors, e.g., increased anxiety-related behavior (Britton et al., 1986; Klampfl et al., 2014, 2013; Sahuque et al., 2006) and reduced maternal behavior (Gammie et al., 2004; Klampfl et al., 2014, 2013; Pedersen et al., 1991), even leading to pup killing in virgin rats (Pedersen et al., 1991). Intriguingly, some of these effects can be attributed to the CRF system of the bed nucleus of the stria terminalis (BNST) (Klampfl et al., 2014; Sahuque et al., 2006).

The BNST is a complex and particularly heterogeneous structure within the limbic system. It acts as a central relay site for the integration of a variety of neuronal signals, mediating behavioral as well as physiological responses. The BNST can be roughly divided into anterior and posterior divisions. The anterior part is mainly connected with hypothalamic and brainstem regions associated with autonomic activity (Dong et al., 2001a), while the posterior division is involved in controlling neuroendocrine systems and social behaviors (Dong et al., 2001a; Dong and Swanson, 2004). Such heterogeneity within a single brain region raises the question whether social (e.g., maternal behavior) and non-social behaviors (e.g., anxiety) might be differentially regulated within the BNST depending on the specific subdivision.

We have recently demonstrated that reduced activation of the CRF-R within the medial-posterior BNST (mpBNST) is necessary for the optimal expression of maternal behavior postpartum (Klampfl et al., 2014). Thus, in the present study we focused on the role of the CRF system in the anterior-dorsal BNST (adBNST) containing the oval, anterodorsal, anterolateral and juxtacapsular nuclei (Fig. 1) (Dong et al., 2001b) in regulating maternal and anxiety-related behavior in lactating rats. We acutely manipulated the CRF-R1 and -R2 in the adBNST with receptor-selective agonists and antagonists and assessed the effects on maternal care, maternal motivation, maternal aggression, and anxiety-related behavior during early lactation. Furthermore, we investigated the impact of acute intra-adBNST CRF-R manipulation on HPA axis activity under both basal and stressful conditions to determine whether the physiological changes observed might account for the changes in maternal behavior. Finally, we compared mRNA expression of the *Crf* gene and its receptors in virgin and lactating rats in the adBNST and additionally the anterior-ventral BNST (avBNST; Fig. 1), which appears to be also involved in the regulation of maternal behavior (Smith et al., 2012).

2. Materials & methods

2.1. Animals

Virgin female Sprague-Dawley rats (220–250 g; Charles River Laboratories, Sulzfeld, Germany) were kept 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 0600 h) with access to water and standard rat chow ad libitum. Females were mated with experienced stud males, and housed in groups of 3–4 until pregnancy day 18. For experiment 1, females underwent surgery on pregnancy day 18 and were single-housed thereafter to guarantee recovery and undisturbed delivery, and for experiment 2, 3 and 4, females were single-housed from pregnancy day 18 to guarantee undisturbed delivery as described recently (Bayerl et al., 2014; Klampfl et al., 2013). On the day of birth, litters were culled to eight pups of mixed sexes. For experiment 4, virgin females and lactating rats were treated identically, i.e., virgins were single-housed 7 days prior to brain collection, consistent with the single-housing period of the lactating rats. During the single-housing period (except the day before and the day of delivery), all rats were handled twice a day to reduce non-specific stress responses during the experiments.

For the maternal defense test, naïve virgin female Wistar rats (200–220 g, Charles River Laboratories) selected at random stages across the estrus cycle were used as intruders. Intruder rats were housed in a separate room to avoid olfactory recognition (Bosch, 2013).

All experiments were approved by the Committee on Animal Health and Care of the local government and conformed to international guidelines on the ethical use of animals. Efforts were made to minimize the number of rats used and their suffering.

2.2. Behavioral tests

All tests were analyzed online (maternal care, pup retrieval test (PRT), elevated plus-maze (EPM)) or from video recordings (maternal defense test; http://www.jwatcher.ucla.edu) by an experienced observer blind to the treatments.

2.2.1. Maternal care

Maternal care was monitored according to an established protocol (Klampfl et al., 2014). Briefly, maternal care was observed before and after treatment, i.e., either substance infusion alone termed 'non-stress condition' or substance infusion followed by the maternal defense test, which is a psycho-social stressor (Neumann et al., 2001), termed 'stress condition' (Bayerl et al., 2014; Klampfl et al., 2014, 2013). The main parameter for the quality of maternal care was the occurrence of arched back nursing (ABN) (Bosch, 2011). Other behavioral parameters scored were hovering over the pups and blanket nursing posture, which together with ABN were counted as total nursing (quantity of maternal care). Pup retrieval/mouthing and licking/grooming were assessed as 'other maternal behaviors'. Additionally, non-maternal ('off-nest') behaviors were scored, i.e., locomotion, self-grooming, and sleeping/resting. Data is shown in 30 min blocks before and after treatment infusion with a maximal count of 15 observations per block.

2.2.2. Maternal motivation

The dams' maternal motivation was tested in the PRT (Klampfl et al., 2014). Briefly, after 60 min of pup separation, the dam was placed in a plastic box (54 cm × 34 cm × 31 cm) and the number of retrieved pups within the 15-min testing period was counted.

2.2.3. Maternal aggression

To assess maternal aggression, the maternal defense test was performed (Klampfl et al., 2014; Neumann et al., 2001). Briefly, the lactating dams (residents) were confronted with an unknown virgin female (intruder) in the dams' home cage in the presence of their litter for 10 min. The following behavioral parameters were scored: total number of attacks, latency to first attack, lateral threat, keep down, and offensive upright as well as non-aggressive behaviors (for detailed description see (Bosch, 2013)).

2.2.4. Anxiety-related behavior

Anxiety-related behavior was tested on the EPM (Klampfl et al., 2014; Pellow et al., 1985). Briefly, the dams were placed in the neutral zone of the maze and scored for 5 min. The percentage of time spent on the open arms versus all arms and of open arm entries versus all entries were taken as indicator of anxiety-related behavior. The number of closed arm entries was used to measure locomotion.