Hormones and Behavior 84 (2016) 136–144

Contents lists available at ScienceDirect

Hormones and Behavior

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

Brain CRF-binding protein modulates aspects of maternal behavior under stressful conditions and supports a hypo-anxious state in lactating rats

Stefanie M. Klampfl [a,1], Milena M. Schramm [a], Gwen S. Stinnett [b], Doris S. Bayerl [a], Audrey F. Seasholtz [b,c], Oliver J. Bosch [a,*]

[a] Department of Behavioural and Molecular Neurobiology, Institute of Zoology, University of Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany
[b] Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI 48109-2200, USA
[c] Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109-2200, USA

ARTICLE INFO

Article history:
Received 2 March 2016
Revised 6 June 2016
Accepted 26 June 2016
Available online 28 June 2016

Keywords:
Anxiety-related behavior
Bed nucleus of the stria terminalis
Corticotropin-releasing factor
Corticotropin-releasing factor-binding protein
Maternal aggression
Maternal care
Stress

ABSTRACT

Reduced corticotropin-releasing factor (CRF) receptor activation in the postpartum period is essential for adequate maternal behavior. One of the factors contributing to this hypo-activity might be the CRF-binding protein (CRF-BP), which likely reduces the availability of free extracellular CRF/urocortin 1. Here, we investigated behavioral effects of acute CRF-BP inhibition using 5 μg of $CRF_{(6-33)}$ administered either centrally or locally within different parts of the bed nucleus of the stria terminalis (BNST) in lactating rats. Additionally, we assessed CRF-BP expression in the BNST comparing virgin and lactating rats.

Central CRF-BP inhibition increased maternal aggression during maternal defense but did not affect maternal care or anxiety-related behavior. CRF-BP inhibition in the medial-posterior BNST had no effect on maternal care under non-stress conditions but impaired the reinstatement of maternal care following stressor exposure. Furthermore, maternal aggression, particularly threat behavior, and anxiety-related behavior were elevated by CRF-BP inhibition in the medial-posterior BNST. In the anterior–dorsal BNST, CRF-BP inhibition increased only non-maternal behaviors following stress. Finally, CRF-BP expression was higher in the anterior compared to the posterior BNST but was not different between virgin and lactating rats in either region.

Our study demonstrates a key role of the CRF-BP, particularly within the BNST, in modulating CRF's impact on maternal behavior. The CRF-BP is important for the reinstatement of maternal care after stress, for modulating threat behavior during an aggressive encounter and for maintaining a hypo-anxious state during lactation. Thus, the CRF-BP likely contributes to the postpartum-associated down-regulation of the CRF system in a brain region-dependent manner.

Introduction

The corticotropin-releasing factor-binding protein (CRF-BP) is a 37 kDa secreted glycoprotein, which binds extracellular CRF and urocortin (Ucn) 1 with similar or even greater affinity than the CRF receptors (CRF-R) (Sutton et al., 1995; Westphal and Seasholtz, 2006). Despite sharing ligands, the CRF-BP is structurally unrelated to the CRF-R (Behan et al., 1989; Orth and Mount, 1987). The CRF-BP lacks any transmembrane domains and it requires different amino acid residues in CRF for high affinity binding than the CRF-R (Behan et al., 1995a; Sutton et al., 1995). In humans, CRF-BP is found in placenta, amniotic fluid and plasma, where it is mostly implicated in parturition (Fadalti et al., 2000). In all vertebrates, CRF-BP is expressed abundantly in the central nervous system including cortical and subcortical limbic structures like the bed nucleus of the stria terminalis (BNST) (Potter et al., 1992), where the CRF-BP co-localizes with CRF and CRF-R (Potter et al., 1992). Such co-localization places the CRF-BP in an important regulatory position between the CRF-R and their ligands in these limbic brain regions (Westphal and Seasholtz, 2006).

Intriguingly, little is known about the functional roles of the CRF-BP. Deletion of the CRF-BP gene results in increased anxiety-related behavior (Karolyi et al., 1999) and impaired maternal aggression in lactating female mice (Gammie et al., 2008). CRF-BP-deficient mice also show an impaired return to homeostasis after prolonged lipopolysaccharide stress, while basal hypothalamo-pituitary-adrenal axis activity appears normal (Karolyi et al., 1999; Seasholtz et al., 2001; Stinnett et al., 2015). The actions of CRF-BP can also be reduced by pharmacological inhibition using $CRF_{(6-33)}$, a truncated version of CRF. This CRF-BP inhibitor binds with high affinity exclusively to the CRF-BP, displaces bound CRF/Ucn 1

* Corresponding author.
E-mail addresses: stefanie.klampfl@ubc.ca (S.M. Klampfl), milena.schramm@ur.de (M.M. Schramm), schaferg@umich.edu (G.S. Stinnett), doris.bayerl@ur.de (D.S. Bayerl), aseashol@umich.edu (A.F. Seasholtz), oliver.bosch@ur.de (O.J. Bosch).
[1] Current address: Department of Cellular and Physiological Sciences, Life Sciences Centre, 2350 Health Science Mall, University of British Columbia, Vancouver, B.C. V6T 1Z3, Canada.

http://dx.doi.org/10.1016/j.yhbeh.2016.06.009
0018-506X/

and, thus, increases 'free' extracellular CRF/Ucn 1 concentrations (Behan et al., 1995b). Infusion of the CRF-BP inhibitor is an elegant pharmacological tool to increase endogenous CRF/Ucn 1 concentrations. The CRF-BP inhibitor enhances learning in animal models of learning and memory (Behan et al., 1995b), blunts weight gain in obese Zucker rats (Heinrichs et al., 1996), and decreases binge drinking in rats (Albrechet-Souza et al., 2015). However, this inhibitor has not been tested for effects in maternal behavior or anxiety-related behavior postpartum, which are both known to be modulated by the CRF system.

Recently, we have demonstrated that hypo-activation of CRF-R centrally (Klampfl et al., 2013) as well as in the medial–posterior BNST (mpBNST) (Klampfl et al., 2014) or the anterior–dorsal BNST (adBNST) (Klampfl et al., 2016) is essential for the adequate expression of maternal behavior and for the hypo-anxious state in lactating rats. Activation of CRF-R2 in the mpBNST and of CRF-R1 in the adBNST results in a strong reduction of maternal behavior while both receptor subtypes concomitantly mediate maternal anxiety in the mpBNST, but not the adBNST. Given that any dysregulations of the CRF system are detrimental to maternal behavior, it is important to understand the underlying mechanisms of the hypo-activation of CRF-R postpartum which is still largely unclear. The CRF-BP could represent an interesting target as it might be involved in the down-regulation of the CRF system during lactation.

Therefore, we investigated behavioral effects of CRF-BP inhibition either centrally or locally in the mpBNST or adBNST in early lactating rats. We focused on maternal care under both non-stress and stress conditions and on maternal aggression in the maternal defense test. Furthermore, given the important role of the CRF system in the expression of anxiety (Reul and Holsboer, 2002a, 2002b), we also assessed behavioral effects of CRF-BP inhibition on this emotionality. Finally, we examined CRF-BP activity patterns within the anterior and posterior BNST of lactating and virgin female rats.

Materials & methods

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 6 a.m.) with access to water and standard rat chow *ad libitum*. Females were mated and subsequently housed in groups of 3 to 4 rats until pregnancy day (PD) 18 when they underwent surgery (experiments 1–3) or were single-housed (experiment 4). For experiments 1–3, pregnant females were also single-housed after surgery to guarantee recovery and undisturbed delivery (Klampfl et al., 2013). On the day of birth, litters were culled to eight pups of mixed sexes. For experiment 4, virgin 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. All rats (experiments 1–4) were handled twice daily during the single-housing period 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 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 to international guidelines on the ethical use of animals. All efforts were made to minimize the number of rats used and their suffering.

Behavioral tests

All tests were performed between 8 a.m. and 3 p.m. in the light phase of the cycle. The delay between treatment infusion and exposure to the behavioral tests was 20 min in each test (Zorrilla et al., 2001). Each rat received the same treatment on each testing day. After infusion, dams were immediately returned to their home cage.

Maternal care

Maternal care was monitored on lactation day (LD) 1 before and after substance infusion (non-stress condition) as well as on LD 5 before and after substance infusion, which was combined with a psychosocial stressor (i.e. the maternal defense test; stress condition (Bayerl et al., 2014, 2016; Klampfl et al., 2013, 2014, 2016)). Observations were conducted for 10 s every 2nd min in 30 min blocks according to an established protocol (Bosch and Neumann, 2008). In detail, on LD 1 dams were observed under non-stress conditions from 8 to 9 a.m., infused at 9 a.m. and observation continued approximately 20 min after infusion of the last dam from 9.30–11 a.m. Additionally, dams were observed from 2 to 3 p.m. to assess potential long-lasting effects of drug treatment. On LD 5, dams were observed from 8 to 9 a.m., transported to another room, and infused at 10 a.m. Dams were tested 20 min after infusion in the maternal defense test, immediately afterwards transported back to the observation room, and maternal care was observed for another 60 min in order to assess effects of the stressor on maternal care. The main parameter for the quality of maternal care was the occurrence of arched back nursing (ABN) (Bosch, 2011), the only active nursing posture where the dam is engaged in a quiescent kyphosis (Stern and Johnson, 1990). Other behavioral parameters scored were 'hovering over the pups' and 'blanket nursing posture', which together with ABN were counted as 'total nursing', thereby indicating the quantity of maternal care (Klampfl et al., 2014). Pup retrieval/mouthing and licking/grooming were also scored. Additionally, non-maternal behaviors were scored, i.e. locomotion (including digging/burrowing and cage exploration), self-grooming, and sleeping/resting, which were summed up and are presented as 'off-nest behavior'.

Maternal aggression

To assess maternal aggression, the maternal defense test was performed on LD 5 in a separate room, to which the dams were transported 60 min prior to the test (see above). Twenty min after treatment infusion, the lactating resident was confronted with an unknown virgin female intruder in its home cage in the presence of the litter for 10 min as described previously (Bosch et al., 2005; Neumann et al., 2001). The dam's behavior was videotaped for subsequent analysis by an experienced observer blind to the treatment using JWatcher (http://www.jwatcher.ucla.edu/). 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))). The time dams spent with 'attacks', 'lateral threat', 'keep down', 'offensive upright' as well as other aggressive behaviors were summarized as 'sum aggressive'.

Anxiety-related behavior

Anxiety-related behavior was tested on the elevated plus-maze (EPM) on LD 3 as described earlier (Neumann et al., 2000; Pellow et al., 1985). Briefly, the plus shaped maze consists of two open arms (50 cm × 10 cm, 80 lx) and two closed arms (50 cm × 10 cm × 30 cm, 10 lx) surrounding a neutral square-shaped central zone (10 cm × 10 cm, 65 lx) and is elevated 82 cm over the floor. Twenty min after infusion, the rats were placed in the neutral zone of the maze and were allowed to freely explore the maze for 5 min. The percentage of time spent on the open arm versus all areas (open arm, closed arm and neutral zone) and the percentage of open arm entries versus all entries (open and closed arms) were indicators for anxiety-related behavior. The number of closed arm entries was used to measure locomotion (Neumann et al., 2000).