Neuropharmacology 121 (2017) 130–139

Contents lists available at ScienceDirect

Neuropharmacology

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

Oxytocin receptor neurotransmission in the dorsolateral bed nucleus of the stria terminalis facilitates the acquisition of cued fear in the fear-potentiated startle paradigm in rats

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A R T I C L E I N F O

Article history:
Received 9 February 2017
Received in revised form
4 April 2017
Accepted 25 April 2017
Available online 26 April 2017

Keywords:
Oxytocin
Fear
Anxiety
Startle
Learning
BNST

A B S T R A C T

Oxytocin (OT) is a hypothalamic neuropeptide that modulates fear and anxiety-like behaviors. Dorsolateral bed nucleus of the stria terminalis ($BNST_{dl}$) plays a critical role in the regulation of fear and anxiety, and expresses high levels of OT receptor (OTR). However, the role of OTR neurotransmission within the $BNST_{dl}$ in mediating these behaviors is unknown. Here, we used adult male Sprague-Dawley rats to investigate the role of OTR neurotransmission in the $BNST_{dl}$ in the modulation of the acoustic startle response, as well as in the acquisition and consolidation of conditioned fear using fear potentiated startle (FPS) paradigm. Bilateral intra-$BNST_{dl}$ administration of OT (100 ng) did not affect the acquisition of conditioned fear response. However, intra-$BNST_{dl}$ administration of specific OTR antagonist (OTA), ($d(CH_2)_5^1$, $Tyr(Me)^2$, Thr^4, Orn^8, des-Gly-NH_2^9)-vasotocin, (200 ng), prior to the fear conditioning session, impaired the acquisition of cued fear, without affecting a non-cued fear component of FPS. Neither OTA, nor OT affected baseline startle or shock reactivity during fear conditioning. Therefore, the observed impairment of cued fear after OTA infusion resulted from the specific effect on the formation of cued fear. In contrast to the acquisition, neither OTA nor OT affected the consolidation of FPS, when administered after the completion of fear conditioning session. Taken together, these results reveal the important role of OTR neurotransmission in the $BNST_{dl}$ in the formation of conditioned fear to a discrete cue. This study also highlights the role of the $BNST_{dl}$ in learning to discriminate between threatening and safe stimuli.

1. Introduction

Oxytocin (OT) is a hypothalamic neuropeptide that modulates a wide range of social behaviors, as well as fear and anxiety-like behaviors, for review see (Neumann and Slattery, 2016). Although substantial evidence suggests that OT has anxiolytic properties (Bale et al., 2001; Ellenbogen et al., 2014; Ring et al., 2006), the role of OT neurotransmission in the regulation of conditioned fear appears to be more complex and brain site-specific. When applied intracerebroventricularly (ICV) prior to the fear conditioning, OT reduces cued fear expression, but OT impairs cued fear extinction when delivered prior to the extinction training (Toth et al., 2012). In addition, OT in the central amygdala (CeA) (Knobloch et al., 2012) and medial prefrontal cortex (Lahoud and Maroun, 2013) reduces contextual fear expression, but the opposite effect is observed when OT is delivered into the basolateral amygdala (Lahoud and Maroun, 2013), or when OT receptors (OTR) are overexpressed in the lateral septum (Guzman et al., 2013). In the fear potentiated startle (FPS) experiments systemic OT decreases background anxiety, but it has no effect on cued or contextual fear, when administered systemically or ICV (Ayers et al., 2011; Missig et al., 2010). In these experiments, cued fear was measured as a potentiation of the startle amplitude that occurred in a presence of conditioned stimulus (CS^+). Background anxiety was expressed as a potentiation of the startle amplitude during noise-alone trials (CS^-) that occurred after the first presentation of CS^+ (measured in a novel context). Contextual fear was measured as a startle potentiation to noise-only trials (no CS presentation) in the training context.

Dorso-lateral bed nucleus of the stria terminalis ($BNST_{dl}$) is a key

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http://dx.doi.org/10.1016/j.neuropharm.2017.04.039

brain area translating stress into sustained anxiety (Dabrowska et al., 2013; Daniel and Rainnie, 2016; Davis et al., 2010; Sparta et al., 2013). While involvement of the BNST in the light- (Walker and Davis, 1997) or corticotropin-releasing factor (CRF) potentiated startle (Lee and Davis, 1997) is well documented, the role of the BNST in the modulation of cued fear is less apparent. BNST lesions disrupt expression of contextual fear (Sullivan et al., 2004), as well as conditioned fear response to long-lasting cues (Davis et al., 2010), but not short, discrete cues (Gewirtz et al., 1998; Hitchcock and Davis, 1991; LeDoux et al., 1988). However, most of the initial reports stem from lesion studies, whereas accumulating evidence from cell-type specific manipulations supports the notion that the BNST might also be involved in the conditioned fear response to discrete cues, for review see (Gungor and Pare, 2016). The BNST has one of the highest expression levels of OTR (Dabrowska et al., 2011; Dumais et al., 2013; Tribollet et al., 1988; Veinante and Freund-Mercier, 1997) and receives dense OT inputs from the paraventricular nucleus of the hypothalamus (Dabrowska et al., 2011; Knobloch et al., 2012), yet the role of OTR neurotransmission in the BNST in the regulation of fear and anxiety is unknown.

Here, we examined the effects of OT and OTR antagonist administration into the $BNST_{dl}$ on the acoustic startle response (ASR), as well as on the acquisition and consolidation of cued and non-cued fear using FPS paradigm. Using *in vivo* pharmacological approach we demonstrate for the first time that OTR neurotransmission in the $BNST_{dl}$ facilitates the acquisition, but not consolidation, of conditioned fear to a discrete cue.

2. Material and methods

2.1. Animals

Adult male Sprague-Dawley rats aged 44–48 days old and weighing 175–199 g were purchased from ENVIGO (IL). The rats were housed in groups of three on a 12 h light/dark cycle (light 7 a.m. to 7 p.m.) with free access to water and food. The rats were allowed to adapt to this environment for one week before the experiments began. A total of 97 animals were used in these experiments. All experimental procedures were approved by the Institutional Animal Care and Use Committees at Rosalind Franklin University of Medicine and Science, and were performed in accordance with the US National Institutes of Health guidelines.

2.2. Drugs

OT (H-2510, Bachem Inc., CA) and the selective OTR antagonist (OTA, H-2908, Bachem Inc., CA), $(d(CH_2)_5^1, Tyr(Me)^2, Thr^4, Orn^8, des\text{-}Gly\text{-}NH_2^9)$-vasotocin (Manning et al., 2012) were stored in −80 Celsius degrees freezer and diluted in artificial cerebrospinal fluid (ACSF, pH = 7.4) before an experiment.

2.3. Surgery

Rats weighing between 230 and 270 g were deeply anesthetized with mix of isoflurane and oxygen and placed in a stereotaxic frame (Model 900; Kopf, CA). Ketoprofen was used as an analgesic (5 mg kg^{-1}, subcutaneous; Zoetis Inc., MI). Rats were bilaterally implanted with guide cannula (22-gauge, 7 mm length; Plastics One, Roanoke, VA) aimed at the $BNST_{dl}$ using the following stereotaxic coordinates (15° coronal angle, from bregma, AP: +0.1 mm; ML: ±3.4 mm; DV: −5.25 mm). Stereotaxic coordinates were based on the rat brain atlas (Paxinos and Watson, 2007) and adapted from the $BNST_{dl}$ coordinates we have published before (Dabrowska et al., 2016). Guide cannulae were positioned 2 mm above the $BNST_{dl}$ and fixed with dental cement bonded to stainless steel screws inserted into the surface of the skull. A dummy cap (7 mm length; Plastics One, Roanoke, VA) was inserted into the guide cannula to maintain it free of obstructions. Following surgery, the rats were allowed to recover for 4–7 days, during which they were handled and their cannulas checked daily to habituate them to the injection procedure.

2.4. Drug administration

OT (100 ng), OTA (200 ng), or ACSF (all in volume of 0.5 μl per side) were injected bilaterally into the $BNST_{dl}$ through microinjector (28-gauge, 7 mm length; Plastics One, Roanoke, VA). Injections were made at a rate of 0.25 μl min^{-1} using microinjection pump (Harvard Apparatus, MA), connected via polyethylene tubing (PE-20) to Hamilton syringes. After the injection, the microinjector was left in place for additional 5 min for adequate diffusion. The doses of OT and OTA were chosen based on previous studies on fear and anxiety in rats (Bale et al., 2001; Lahoud and Maroun, 2013; Neumann and Slattery, 2016; Neumann et al., 2000; Toth et al., 2012).

2.5. Acoustic startle apparatus

All experiments were conducted in eight, identical SR-LAB startle chambers with cylindrical animal enclosures (San Diego Instruments, San Diego, CA). A high-frequency loudspeaker, mounted 24 cm above the enclosures, provides background noise as well as the startle eliciting white-noise bursts (WNB). During the FPS, a single LED bulb positioned on the ceiling inside the startle chamber was used as the visual conditioned stimulus (CS). In addition, a grid floor made of stainless steel bars placed inside the enclosures was used to deliver foot shocks as the unconditioned stimulus (US). The presentation and sequence of all stimuli as well as recording of the responses were automatically controlled by the SR-LAB software (San Diego Instruments).

2.6. Acoustic startle response (ASR)

On day 1 (habituation), rats were placed in the cylindrical enclosures inside the chambers for 20 min. On day 2 (pre-test), rats were placed in the same enclosures, where after 5 min acclimation they were presented with 30 startle eliciting WNB (95 dB, 50 ms, inter-trial-interval 30 s). A background white-noise (70 dB) was continuously played throughout the whole session. On day 3 (test), rats received 30-startle eliciting WNB (same as above) 10 min after bilateral administration of OT (n = 7), OTA (n = 7) or ACSF (n = 7) into the $BNST_{dl}$ (Fig. 1).

In all experiments, animals were assigned to the experimental groups based on their baseline ASR (pre-test) in order to generate experimental groups with balanced average ASR and to reduce variability in stress-reactivity. All chambers were cleaned with a 70% ethanol solution between sessions.

2.7. Fear potentiated startle (FPS)

The FPS procedures were modified based on previous studies (Ayers et al., 2011; Missig et al., 2010; Sink et al., 2013; Walker et al., 2009a). On days 1 and 2, separate cohort of rats underwent habituation and pre-test sessions, respectively (as above). On the following day (fear conditioning), animals were placed in the cylindrical enclosures containing a grid floor conveying foot shocks. After 5 min acclimation, animals received 10 presentations of a 3.7 s cue light (CS), each co-terminating with a 0.5 s foot shock (US; 0.5 mA, inter-trial-interval 60–180 s). A background noise was absent during the conditioning session. Twenty-four hours later,