Neuropharmacology 90 (2015) 74–81

Contents lists available at ScienceDirect

Neuropharmacology

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

Oxytocin microinjected into the central amygdaloid nuclei exerts anti-aggressive effects in male rats

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ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form
29 October 2014
Accepted 20 November 2014
Available online 29 November 2014

Keywords:
Oxytocin
Intermale offensive behavior
Social exploration
Central amygdala
Dorsal raphe

ABSTRACT

We recently demonstrated that acute and chronic intracerebroventricular enhancement of brain OXT levels induces potent anti-aggressive and pro-social explorative effects during social challenges. However, the exact anatomical location in the brain where OXT exerts its action is still elusive. In the present study, we targeted two critical brain areas, i.e. the central amygdala (CeA) and the dorsal raphe (DR), both containing high levels of OXT receptors (OXTRs) and constituting important nodes of the neural circuitry related to aggression. Behavioral effects of local micro-infusion of OXT and OXTR antagonist, L368.899, (alone and combined) were evaluated in resident male rats during confrontations with an unfamiliar male intruder. Our results show that OXT microinjected into the CeA markedly reduced resident's offensive behavior and facilitated social exploration, without affecting other non-aggressive behaviors. The receptor specificity of the behavioral effects was verified when a micro-infusion of a selective OXTR antagonist nullified the changes. Pharmacological blockade of CeA OXTRs per se was without clear behavioral effects suggesting that endogenous OXT within the CeA does not play a major inhibitory role on offensiveness. Anatomical specificity was also supported by the absence of relevant behavioral effects when OXT was microinjected into more medial sub-regions of the amygdala. Likewise, within the DR neither OXT nor OXTR exerted significant effects on offensive aggression, while microinjection of the 5-HT_{1A} autoreceptor agonist in this region significantly suppressed aggression.

In conclusion, our results point at the CeA as an important brain site of action for the anti-aggressive and pro-social explorative effects induced by exogenous enhancement of brain OXT levels.

1. Introduction

Over the past decades, a number of studies have shown that the neuropeptide oxytocin (OXT) plays an essential role in regulating a variety of psychosocial functions in rodents and other mammals (Heinrichs et al., 2009; Neumann, 2008). It is crucially involved in social bonding and in-group co-operation, strengthening recognition memory for familiar conspecifics and increasing defensive behavior (Choleris et al., 2009; Hammock and Young, 2006; Winslow et al., 1993). It facilitates social approach by reducing anxiety and neuroendocrine stress/fear responses (Ebner et al., 2005; Heinrichs and Domes, 2008; Insel and Young, 2001; Knobloch et al., 2012). Moreover, it potentiates positive social interactions, increasing trust and reducing betrayal aversion in humans (Baumgartner et al., 2008; Kosfeld et al., 2005). In animals, OXT increases social exploration and decreases offensive behavior in animals (Calcagnoli et al., 2013).

Regarding social behaviors, our group recently demonstrated that both acute intracerebroventricular (icv) (Calcagnoli et al., 2013) and intranasal (Calcagnoli et al., 2014a) administration of OXT reduces intermale offensive aggression and increases social exploration displayed by a resident rat towards an unfamiliar intruder. In addition, increased offensive aggression was reported in low aggressive residents after icv administration of a selective OXT receptor (OXTR) antagonist (Calcagnoli et al., 2013).

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http://dx.doi.org/10.1016/j.neuropharm.2014.11.012
0028-3908/

However, due to the widespread expression of OXTRs within the brain (Barberis and Tribollet, 1996), and especially within structures that encompass the social behavior neural network (Goodson, 2005), it is still unclear where exactly OXT mediates these socio-behavioral effects. In the reported experiments, we tested the hypothesis that two core neural structures underlying social behavior, i.e. the central amygdala (CeA) and the dorsal raphe (DR) nucleus, are important sites where OXT regulates the behavioral response to threatening social challenges, such as during confrontation with an intruder. Several studies in humans and animals seem to support this hypothesis. From neuroimaging studies in men, intranasal OXT application has been found to dampen the amygdaloid neuronal activation and to reduce the functional connectivity between amygdala and brainstem regions involved in automatic fear reactivity, thereby attenuating the emotional and physiological response to fearful and threatening social stimuli (Coccaro et al., 2007; Gamer et al., 2010; Kirsch et al., 2005). However, contrary to these findings, in healthy women OXT appeared to increase amygdala reactivity to scenes depicting social and non-social threat (Domes et al., 2010; Lischke et al., 2012), suggesting a possible sexual dimorphism in the neuronal effects of OXT. In borderline personality disordered patients, where increased anger, aggression and impaired affective regulation have been associated with amygdala hyperactivity during confrontation with frightening situations (Donegan et al., 2003; Herpertz et al., 2001), OXT administration has been shown to normalize these abnormal behavioral and neural patterns (Bertsch et al., 2013).

In rodents, neuronal activation of the amygdala has been associated with agonistic encounters (Haller et al., 2006; Pan et al., 2010), while reduction of fear and social stress-related behaviors has been found by optogenetically stimulating endogenous OXT release, particularly in the CeA (Knobloch et al., 2012). It is in these areas that the majority of OXT immunoreactive (OXT-ir) cells (Sofroniew et al., 1983) and OXT binding sites (Freund-Mercier et al., 1987; Veinante and Freund-Mercier, 1997) have been detected.

In lactating female rats acute bilateral infusion of OXT in the CeA decreases the frequency of defensive biting and frontal attack towards a male intruder (Consiglio et al., 2005), which is in line with the observation that local infusion of OXTR antagonist 4 h prior to the resident-intruder (RI) test significantly increases the fighting behavior of lactating rats (Lubin et al., 2003). However, this is in contrast with observations in female hamsters (Ferris et al., 1992) and with the positive correlation found between OXT release in the CeA and the level of maternal defense in female Wistar rats selected for high anxiety-related behaviors (Bosch et al., 2005).

Much less is known about the OXTergic activity in the DR. This nucleus is considered to be one of the core structures of the brainstem controlling aggression and violence (Takahashi and Miczek, 2013), via its extensive, serotonin (5-HT) containing, efferent projections to various forebrain nodes within the social behavior network (Amat et al., 2005; Jacobs and Azmitia, 1992; Nakamura et al., 2008; Takahashi et al., 2010; van der Vegt et al., 2003). Hence, dysregulation of the DR 5-HTergic system has been implicated in the pathophysiology of affective disorders including anxiety, depression and suicidal (auto)aggressive behavior (Arango et al., 2002; Stockmeier, 1997). Recently, a high density of OXTRs expressing cells was reported in the DR. These receptors have been co-localized with 5-HT neurons and, at the level of the median raphe nucleus, OXT infusion was reported to increase 5-HT release (Yoshida et al., 2009). Moreover, another study has shown that the activation of OXTRs could stimulate the firing of DR 5-HT neurons (Spaethling et al., 2014). However, despite the evidence of functional OXTergic binding sites in the DR, no study has locally investigated the socio-behavioral effects induced by manipulation of OXTergic activity.

The aim of the present study, therefore, has been to pharmacologically manipulate OXT levels in the CeA and in the DR of male resident rats, and to evaluate the behavioral effects during a direct intermale confrontation.

2. Material and method

2.1. Animals and housing conditions

As in our previous etho-pharmacological studies (Calcagnoli et al., 2013, 2014a, 2014b), male wild-type Groningen rats (*Rattus norvegicus*) were used as experimental subjects (4.5 months old and average weight 400 ± 50 g). This strain of rats descended from pairs of wild-trapped specimens that were outbred under conventionalized conditions for over 35 generations in our laboratory (de Boer et al., 2003). Adult male specimens of this rat strain are very suitable for intermale aggression research as substantially higher levels of offensive behavior are displayed compared to virtually all other commercially available laboratory strains (de Boer et al., 2003). After 120 days of age, each resident was housed in large observation cages (80 × 55 × 50 cm) together with an oviduct-ligated but gonadally-intact female to prevent social isolation and allow normal sexual activity, required to stimulate territorial behavior. Throughout the experimental period, the animals were maintained under standard housing conditions (12: 12 h light/dark photoperiod, lights off at 13:00 h; ambient temperature 21 ± 2 °C; humidity 50 ± 5%) with *ad lib*. access of food (Hope Farms, RMH-B) and water. All experimental and behavioral procedures (Fig. 1) were approved by the Animal Ethics Committee on Care and Use of Laboratory Animals (DEC 5824) of Groningen University and were conducted in agreement with Dutch laws (Wet op de Dierproeven 1996) and European regulations (Guideline 86/609/EEC).

2.2. Surgical procedures

All stereotaxic surgical procedures were performed under isoflurane/oxygen anesthesia, using sterile conditions. To minimize discomfort directly linked to the surgery, analgesic (0.1 ml Finadyne) and antibiotic (0.25 ml Penicillin) compounds were subcutaneously injected. Stainless steel guide cannulas (CeA: custom-made 15 mm, 23-gauge cannula; DR: 22-gauge, C313, Plastic One, Roanoke, VA) were placed stereotactically, according to the brain map of Paxinos and Watson (6th edition, 2007) with the cannula tip 2 mm above the target area (coordinates for the bilateral cannulation into the CeA: 2.2 mm posterior to bregma, ±4.3 mm lateral to midline and 6.3 ventral to the skull, with a tooth bar at −3.3 mm. Coordinates for the cannulation into the DR: 7.6 mm posterior to bregma, 2.0 mm lateral to midline, under a 12° lateral angle and 6.8 ventral to the dura mater, with a tooth bar at −3.3 mm). The cannulas were affixed to the skull with anchoring screws and dental cement. Stylets were inserted into the cannulas to maintain patency. For the first 24 h post-operation, rats were singly housed in their home cage, and then again housed together with the same tubal-ligated female companion of the pre-surgery period. During the 10-day recovery period, the weight and the well-being of the animals were daily checked. Moreover, animals were habituated to the infusion procedure in order to avoid non-specific stress responses during the experimental sessions.

2.3. Behavioral testing

After full recovery, a baseline ethogram was assessed for each animal using a RI test, according to the standard protocol described by Koolhaas and colleagues (Koolhaas et al., 2013, 1980; Olivier et al., 1995). Unfamiliar naïve male Wistar rats (Harlan, The Netherlands, 4 months old, average body weight 300 ± 50 g) socially housed in groups of five in transparent macrolon type IV cages (60 × 60 × 20 cm) were used as intruder animals. The lower weight/size of the intruder facilitated the establishment of the resident's dominance. The female partner of the experimental rat was removed from the observation cage approximately 30 min prior to the start of the test.

As previously described (Calcagnoli et al., 2013), the baseline behavioral testing was performed across four consecutive days. During the first three days, an attack latency test was performed by introducing an intruder into the home cage of the experimental resident animal. The test was terminated shortly after the occurrence of the first full attack. When the resident failed to attack within the first 10 min of testing, the attack latency time was scored as 600 s and the test was terminated. On the fourth day, the interaction between the resident and the intruder was videotaped for the 10 min following either the first attack or the introduction of the intruder, in case of no attack (complete RI test). Videos were analyzed and all behaviors expressed within the 10-min observation were evaluated. Specific behavioral elements were grouped into the following broad behavioral categories in order to promote a clear representation of the data: (1) *offensive behavior* (lateral threat, clinch, keep down, chase, upright posture); (2) *social explorative behavior* (moving towards, investigation and ano-genital sniffing of the intruder, crawl over, social grooming); (3) *non-social exploration* (ambulation, rearing, sniffing, scanning, digging); (4) *inactivity* (sitting, lying, freezing) and (5) *self-grooming* (washing,