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Oxytocin in the prelimbic medial prefrontal cortex reduces anxiety-like behavior in female and male rats

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Summary The neuropeptide oxytocin (OT) is anxiolytic in rodents and humans. However, the specific brain regions where OT acts to regulate anxiety requires further investigation. The medial prefrontal cortex (mPFC) has been shown to play a role in the modulation of anxiety-related behavior. In addition, the mPFC contains OT-sensitive neurons, expresses OT receptors, and receives long range axonal projections from OT-producing neurons in the hypothalamus, suggesting that the mPFC may be a target where OT acts to diminish anxiety. To investigate this possibility, female rats were administered OT bilaterally into the prelimbic (PL) region of the mPFC and anxiety-like behavior assessed. In addition, to determine if the effects of OT on anxiety-like behavior are sex dependent and to evaluate the specificity of OT, male and female anxiety-like behavior was tested following delivery of either OT or the closely related neuropeptide arginine vasopressin (AVP) into the PL mPFC. Finally, the importance of endogenous OT in the regulation of anxiety-like behavior was examined in male and female rats that received PL infusions of an OT receptor antagonist (OTR-A). Overall, even though males and females showed some differences in their baseline levels of anxiety-like behavior, OT in the PL region of the mPFC decreased anxiety regardless of sex. In contrast, neither AVP nor an OTR-A affected anxiety-like behavior in males or females. Together, these findings suggest that although endogenous OT in the PL region of the mPFC does not influence anxiety, the PL mPFC is a site where exogenous OT may act to attenuate anxiety-related behavior independent of sex.

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1. Introduction

Oxytocin (OT) is a nonapeptide synthesized within the hypothalamic paraventricular (PVN) and supraoptic nuclei. OT neurons of the hypothalamus project to the posterior pituitary and secrete OT into the bloodstream, where its peripheral actions are critical to the processes of lactation and parturition (Gimpl and Fahrenholz, 2001). Besides peripheral release, OT also reaches many regions of the forebrain either through diffusion following dendritic release (Ludwig and Leng, 2006) or via axonal projections from OT synthesizing neurons of the PVN (Sofroniew, 1983; Knobloch et al., 2012). Within the brain, OT acts as a neurotransmitter/neuromodulator and is known to play a role in numerous social functions of female rodents including maternal care (Bosch and Neumann, 2012), sexual receptivity (Bale et al., 2001), pair bonding (Lim and Young, 2006), as well as social recognition and social memory (Engelmann et al., 1998). Although sexual dimorphisms in the brain OT system exist (De Vries, 2004; Smeltzer et al., 2006; Carter, 2007; Dumais et al., 2013), the prosocial effects of OT are not limited to females and also occur in male rodents where brain OT is similarly important for the regulation of sexual behavior (Argiolas and Melis, 2004), social preference (Lukas et al., 2011), and social cognition (Popik and van Ree, 1991). Like rodents, OT has been shown to have a facilitatory influence on various aspects of human social behavior (Heinrichs et al., 2009; McCall and Singer, 2012).

In addition to its effects on sociability, OT is an important regulator of anxiety (Neumann and Landgraf, 2012). For example, OT knockouts present with an anxious phenotype indicating an involvement of endogenous OT (Mantella et al., 2003). Endogenous OT is also directly involved in anxiolysis during the postpartum period (Bosch and Neumann, 2012) as well as in males after mating (Waldherr and Neumann, 2007). Moreover, in rats and mice, OT administered peripherally or centrally attenuates anxiety (Uvnas-Moberg et al., 1994; McCarthy et al., 1996; Windle et al., 1997; Bale et al., 2001; Ring et al., 2006; Blume et al., 2008; Yoshida et al., 2009; Ayers et al., 2011; Mak et al., 2012). The anxiolytic effects of OT are also evident in humans where intranasal administration of OT has been shown to suppress anxiety responses in healthy and clinical populations (Heinrichs et al., 2003; Guastella et al., 2010; de Oliveira et al., 2012). In general, the ability of exogenous OT to reduce anxiety appears to occur regardless of sex (Neumann, 2008) although some sex-specific effects have been reported in rodents (Slattery and Neumann, 2010) and humans (Weisman et al., 2013).

The brain regions where OT acts to modulate anxiety remain to be fully elucidated. Previous work has implicated the PVN of males (Waldherr and Neumann, 2007; Blume et al., 2008) and amygdala of females (Bale et al., 2001; Neumann, 2002) as sites mediating the anxiolytic actions of OT. However, these areas are likely to be part of a widespread network that may also include the medial prefrontal cortex (mPFC). Lesion, inactivation, and molecular approaches have shown that the prelimbic (PL) subregion of the mPFC plays a role in regulating anxiety-like behavior as assessed in a variety of rodent behavioral paradigms including the elevated plus maze (EPM), open field (OF), and social interaction (SI) test (Maaswinkel et al., 1996; Gonzalez et al., 2000; Lacroix et al., 2000; Sullivan and Gratton, 2002; Shah and Treit, 2003; Shah et al., 2004; Resstel et al., 2008; Stack et al., 2010; Stern et al., 2010). The mPFC also contains OT-sensitive neurons (Ninan, 2011), abundantly expresses OT receptors (Insel and Shapiro, 1992; Gould and Zingg, 2003; Liu et al., 2005; Smeltzer et al., 2006), and may receive long range axonal projections from OT producing neurons in the hypothalamus (Sofroniew, 1983; Knobloch et al., 2012). Taken together, these findings suggest that the mPFC may be a target where OT acts to diminish anxiety. To investigate this possibility, OT was administered bilaterally into the PL region of the mPFC of female rats and its effects on anxiety-like behavior assessed. In addition, to determine if the effects of OT on anxiety-like behavior are sex dependent and to evaluate the specificity of OT, male and female anxiety-like behavior was tested following delivery of either OT or the closely related neuropeptide arginine vasopressin (AVP) into the PL mPFC. Finally, the importance of endogenous OT in the regulation of anxiety-like behavior was evaluated in male and female rats in which the OT receptor was blocked with an OT receptor antagonist (OTR-A).

2. Methods

2.1. Animals

Age-matched adult (9—12 weeks of age) virgin female (225—250 g) and male (300—350 g) Sprague-Dawley rats from Taconic (Germantown, NY) were used. Rats were housed individually in a temperature and humidity controlled room and maintained on a 12 h/12 h light/dark cycle (lights on at 06:00 h) with access to food and water ad libitum. All procedures were conducted in accordance with The Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and approved by The Ohio State University Institutional Animal Care and Use Committee.

Throughout the experiment, stages of estrous were monitored in all females through daily vaginal swabs. Samples of cells were obtained with a sterile cotton swab saturated in 0.9% saline and applied to a glass slide. After drying, slides were stained with 1% aqueous Toluidine Blue and cell types characterized under 10× magnification (Everett, 1989). Only those females that had normal 4—5 d estrous cycles were used.

2.2. Surgical procedures

After approximately 7 d of acclimation to the colony, rats were anesthetized with a 2—4% isoflurane gas/air mixture and aligned on a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Body temperature was maintained throughout the surgery with a warming pad. Bilateral cannula guides (pedestal mounted 22-gauge stainless steel tubes with 1.5 mm separation and cut 3.5 mm below the pedestal; Plastics One, Roanoke, VA) were secured in a stereotaxic holder and lowered into the prelimbic region (PL) of the mPFC (AP: +3.2 mm, ML: ±0.75 mm, DV: −3.2 mm) (Paxinos and Watson, 1998). The cannulae were secured by stainless steel screws and dental cement. A bilateral stainless steel obturator (0.35 mm diameter; Plastics One) extending