Neuropharmacology 105 (2016) 61–71

Contents lists available at ScienceDirect

Neuropharmacology

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

Reversal of social deficits by subchronic oxytocin in two autism mouse models

Brian L. Teng [a, b, 1], Viktoriya D. Nikolova [a, c], Natallia V. Riddick [a, c], Kara L. Agster [a, c, 2], James J. Crowley [d], Lorinda K. Baker [a, c], Beverly H. Koller [d], Cort A. Pedersen [a, c], Michael B. Jarstfer [a, b], Sheryl S. Moy [a, c, *]

[a] Carolina Institute for Developmental Disabilities, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA
[b] Eshelman School of Pharmacy, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA
[c] Department of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA
[d] Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 23 August 2015
Received in revised form
28 November 2015
Accepted 29 December 2015
Available online 31 December 2015

Keywords:
Autism spectrum disorders
Clozapine
Oxytocin
Risperidone
Schizophrenia
Sociability

ABSTRACT

Social deficits are a hallmark feature of autism spectrum disorder (ASD) and related developmental syndromes. Although there is no standard treatment for social dysfunction, clinical studies have identified oxytocin as a potential therapeutic with prosocial efficacy. We have previously reported that peripheral oxytocin treatment can increase sociability and ameliorate repetitive stereotypy in adolescent mice from the C58/J model of ASD-like behavior. In the present study, we determined that prosocial oxytocin effects were not limited to the adolescent period, since C58/J mice, tested in adulthood, demonstrated significant social preference up to 2 weeks following subchronic oxytocin treatment. Oxytocin was also evaluated in adult mice with underexpression of the *N*-methyl-D-aspartate receptor NR1 subunit (encoded by *Grin1*), a genetic model of autism- and schizophrenia-like behavior. Subchronic oxytocin had striking prosocial efficacy in male *Grin1* knockdown mice; in contrast, chronic regimens with clozapine (66 mg/kg/day) or risperidone (2 mg/kg/day) failed to reverse deficits in sociability. Neither the subchronic oxytocin regimen, nor chronic treatment with clozapine or risperidone, reversed impaired prepulse inhibition in the *Grin1* knockdown mice. Overall, these studies demonstrate oxytocin can enhance sociability in mouse models with divergent genotypes and behavioral profiles, adding to the evidence that this neurohormone could have therapeutic prosocial efficacy across a spectrum of developmental disorders.

1. Introduction

Oxytocin is a neuropeptide hormone with a long-recognized role in maternal responses and mother-infant bonding. Clinical studies in subjects with autism spectrum disorder (ASD) have found that acute oxytocin can improve social function and decrease motor stereotypy and other forms of repetitive behavior (Andari et al., 2010; Guastella et al., 2010; Hollander et al., 2003, 2007). Further, Hall et al. (2012) observed that acute oxytocin could ameliorate indicators of social anxiety in male adolescents and adults with fragile X syndrome. One recent study using a 5-week regimen with intranasal oxytocin in young children (3–8 years in age) with ASD found improved social responsivity, although no concomitant reduction in abnormal repetitive behavior (Yatawara et al., 2015). These initial reports also suggest that oxytocin might not have the same potential for adverse events as found with more powerful psychoactive agents, such as risperidone or fluoxetine, used to treat co-morbid symptoms in ASD (Mahajan et al., 2012; West et al., 2009; Yatawara et al., 2015). However, not all clinical trials using intranasal application of oxytocin to ameliorate social deficits or other symptoms have proven successful (Anagnostou et al., 2012; Cacciotti-Saija et al., 2015; Dadds et al., 2014),

* Corresponding author. Carolina Institute for Developmental Disabilities, CB#7146, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.
E-mail address: ssmoy@med.unc.edu (S.S. Moy).
[1] Present address: Brian L. Teng, Ph.D., Biogen Idec, Corporate Strategy Division, Cambridge, MA 02142, USA.
[2] Present address: Kara Agster Saddoris, Ph.D., Department of Psychology and Neuroscience, University of Colorado, Boulder, CO 80309-0345, USA.

http://dx.doi.org/10.1016/j.neuropharm.2015.12.025

indicating the need for further investigation of oxytocin as a therapeutic agent.

Our research group has reported that peripheral administration of oxytocin can alleviate sociability deficits in two mouse models of autism-like behavior, the BALB/cByJ and C58/J inbred strains (Teng et al., 2013). Previous work has shown that BALB/cByJ and the related substrain, BALB/cJ, are characterized by a lack of social preference in a three-chambered choice task and by anxiety-like behavior in an elevated plus maze (Brodkin et al., 2004; Moy et al., 2007; Sankoorikal et al., 2006). Our previous study showed that, while acute oxytocin treatment did not reverse social deficits, a subchronic regimen of four injections, given across 8–9 days, led to significant sociability in adolescent BALB/cByJ mice, tested 24 h following the final dose (Teng et al., 2013).

Our group has also investigated oxytocin effects in the C58/J inbred strain, which has low sociability in a three-chambered task, deficits in social transmission of food preference, and overt repetitive behavior (Moy et al., 2008b, 2014; Muehlmann et al., 2012; Ryan et al., 2010; Silverman et al., 2012). We found that a subchronic oxytocin regimen had prosocial effects in adolescent male and female C58/J mice, with increases in social preference emerging one or two weeks following treatment (Teng et al., 2013). Acute, but not subchronic, administration of oxytocin led to significant decreases in abnormal repetitive behavior.

In the present studies, we investigated whether oxytocin would exert prosocial effects in adult C58/J mice, similar to our findings in adolescents. Motivation for social affiliation and regulation of social interactions can differ between adolescents and adults (Spear, 2011; see also Ernst et al., 2006). For example, Morales and Spear (2014) reported that, in a two-chamber test box, adolescent rats had higher levels of social interaction and a higher frequency of crossovers toward an unfamiliar social partner than adult rats. These data support a greater sensitivity to the rewarding aspects of novel social stimuli during adolescence, and raise the possibility that oxytocin might be most effective at this stage of development, while long-term social deficits in adults could be more recalcitrant to reversal.

The present studies also extended the evaluation of oxytocin to a third model of ASD-like behavior and synaptopathology, the *Grin1* knockdown mouse. Although the mechanistic basis for ASD is not known, genetic analyses in human populations have implicated several genes important for synaptic function, including *GRIN1*, which encodes the obligatory NMDAR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor (Abrahams and Geschwind, 2008; Voineagu et al., 2011; Zeidán-Chuliá et al., 2014); however, not all studies have found an positive association between ASD and *GRIN1* (e.g. Sanders et al., 2015; Tarabeux et al., 2011). There is growing evidence that alterations in NMDA receptor signaling play a role in ASD and other neurodevelopmental disorders (for recent reviews, see Burnashev and Szepetowski, 2015; Lee et al., 2015), including reports that autism candidate genes, such as *NEUROLIGIN-1* and *SHANK3*, serve as regulators of NMDA receptor function (Budreck et al., 2013; Duffney et al., 2013). Mice with reduced *Grin1* expression recapitulate many ASD features, including overt social deficits, inappropriate social interaction, abnormal repetitive behavior, self-injurious responses, and impaired sensorimotor gating (Billingslea et al., 2014; Duncan et al., 2004; Finlay et al., 2015; Gandal et al., 2012; Milenkovic et al., 2014; Mohn et al., 1999; Moy et al., 2008a, 2012, 2014; Saunders et al., 2013). We determined the effects of oxytocin on social deficits, reduced prepulse inhibition, and hyperactivity in *Grin1* knockdown mice. We also examined whether chronic regimens with atypical antipsychotics, initiated in early adolescence or young adulthood, have prosocial efficacy in the *Grin1* knockdown model.

2. Methods and materials

2.1. Animals

C58/J mice were offspring of breeding pairs obtained from Jackson Laboratories (Bar Harbor, ME). *Grin1*$^{neo/neo}$ mice engineered with a neomycin resistance gene (neo) in intron 20 of the *Grin1* locus and *Grin1*$^{+/+}$ littermate controls were generated from heterozygous breeder pairs, as previously described (Mohn et al., 1999; Moy et al., 2012). Experimenters conducting the behavioral tests were blind to genotype.

Mice were maintained in groups of 2–4 animals per polycarbonate mouse cage, in a room under a 12-h light/dark cycle (lights off at 7pm). ProLab RMH 3000 chow and water were provided ad libitum. All animal procedures were conducted in strict compliance with the animal welfare policies set by the National Institutes of Health and the University of North Carolina (UNC), and were approved by the UNC Institutional Animal Care and Use Committee.

2.2. Drug treatment regimens

2.2.1. Oxytocin

Oxytocin (Bachem, Torrance, CA) was dissolved in saline containing 0.002% glacial acetic acid. All injections were administered IP (intraperitoneal) in a volume of 10.0 ml/kg. For the subchronic regimen, mice were given four injections of vehicle or oxytocin (1.0 or 2.0 mg/kg) across 8–9 days, with at least 48 h between each injection (i.e. mice were injected on sequential weekdays WFMW or WFTTh). Experimenters conducting the behavioral tests were blind to drug treatments.

2.2.2. Chronic clozapine and risperidone regimens in Grin1 mice

Chronic regimens with clozapine (30 days; 66 mg/kg/day) or risperidone (21 days; 2.0 mg/kg/day) were initiated with a preliminary ramping up of drug dose to minimize sedative or other side effects at the beginning of treatment. Doses were selected to reflect therapeutic dosage in humans, determined by clinical levels of dopamine D_2 receptor occupancy (Kapur et al., 2003; Wadenberg et al., 2001).

Slow-release pellets were utilized for chronic clozapine administration because of difficulties in higher-dose drug solubility for osmotic minipumps (Kapur et al., 2003) and issues with variable plasma levels during administration in drinking water (Perez-Costas et al., 2008). At 11–14 weeks of age, mice were briefly anesthetized by isoflurane and implanted, using a trocar injector, with subcutaneous 30-day slow-release clozapine or sham tablets (Innovative Research of America, Sarasota, FL). The target dosage of 66 mg/kg/day was reached by incremental stages across 8 days, with 2–3 total pellet implants per subject. Following each implant, the trocar injection site was sealed using Tissuemend (Jeffers Inc., Dothan, AL).

For the initial acclimation to risperidone (Sigma–Aldrich, St. Louis, MO), adolescent mice (starting at age 33–38 days) received 3 IP injections of either saline vehicle containing 1% glacial acetic acid (adjusted to pH 5.5) or risperidone (0.3 mg/kg), with 2–3 days between each injection. One day following the third injection, mice were briefly anesthetized by isoflurane and implanted with a subcutaneous osmotic minipump (Model 1002; Alzet; Braintree Sci. Inc., Braintree, MA) containing either risperidone (2.0 mg/kg/day) or vehicle, for a 14-day delivery. At the end of the 14-day period, mice were again anesthetized, and the depleted 14-day pump was replaced by a new 7-day pump (Model 1007D) for the final phase of the 21-day regimen. This pump replacement allowed dosage to be adjusted for increased body weight during the chronic risperidone