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The Effects of Oxytocin and its Analog, Carbetocin, on Genetic Deficits in Sensorimotor Gating

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Abstract

Converging evidence from preclinical and clinical studies suggest that oxytocin has therapeutic potential for schizophrenia and other neuropsychiatric disorders. Prepulse inhibition of the startle reflex (PPI) is a measure of sensorimotor gating, an important brain function involved in filtering environmental information. We previously demonstrated that systemically administered oxytocin reversed psychostimulant-induced PPI deficits in rats suggesting that oxytocin can produce antipsychotic-like central effects. That finding was supported by a recent trial in humans, which found that intranasal oxytocin reduced symptoms of schizophrenia. The goal of this study was to extend this line of investigation by testing the effects of oxytocin, and a structural analog of oxytocin, carbetocin, on nonpharmacological deficits in PPI. In experiment 1, Brown Norway (BN) rats, a rat strain that has naturally low PPI, were given either saline or one of three doses of oxytocin (0.04 - 1.0 mg/kg, sc). In experiment 2, BN rats were given either saline, one of three doses of carbetocin (0.04 - 1.0 mg/kg) or oxytocin (1 mg/kg). PPI and acoustic startle response (ASR) of rats were tested. Oxytocin significantly increased PPI ($P < 0.01$) and decreased ASR levels ($P < 0.01$) in BN rats in a dose-dependent fashion. In contrast, carbetocin had no effect on PPI levels or ASR. The facilitation of BN PPI by oxytocin is similar to what we have previously observed with clozapine and thus further supports oxytocin having antipsychotic properties. In contrast to oxytocin, our data do not support the use of carbetocin as an antipsychotic drug.

Keywords

Schizophrenia; antipsychotics; prepulse inhibition; Brown-Norway; animal models

Introduction

Oxytocin is a neurohypophyseal peptide with widespread central and peripheral effects (Macdonald and Macdonald, 2010). Studies in animals and humans have demonstrated that it plays a powerful role in regulating social cognition and affiliation (Harony and Wagner,

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2010; Insel, 2010; Sala et al., 2011). Converging evidence suggests that oxytocin may play a role in a number of neuropsychiatric disorders and that drugs targeting the central oxytocin system may have therapeutic benefit for these brain disorders.

Schizophrenia is one such disorder. Patients diagnosed with schizophrenia may have irregularities in oxytocin function, which may serve to explain the affective and social disturbances reported in this severe psychiatric disorder (Beckmann et al., 1985; Goldman et al., 2008; Linkowski et al., 1984). Recently, high oxytocin serum levels have been associated with less severe positive symptoms of psychosis in female patients with schizophrenia and with increased prosocial behaviors in male and female patients with this disorder (Rubin et al., 2010).

Prepulse inhibition (PPI) of the startle reflex is a measure of sensorimotor gating, a neural process critical for intact cognitive functioning. PPI is deficient in a number of neuropsychiatric disorders associated with cognitive dysfunction including schizophrenia (Geyer et al., 2001; Swerdlow et al., 1994) and autism (Perry et al., 2007) and similar disruption of PPI can be induced in animals by psychotomimetic drugs such as amphetamine and PCP. Antipsychotic drugs tend to reverse these drug-induced deficits. Therefore, PPI has become rigorously studied and used as the basis of animal models with predictive validity for drugs with therapeutic potential in schizophrenia (Braff, 2010; Geyer et al., 2001). Further supporting the endogenous antipsychotic-like activity of oxytocin, Caldwell et al. (Caldwell et al., 2009) showed that mice lacking oxytocin receptors are more susceptible than normal mice to PCP-induced disruption of PPI.

In a proof-of-concept test of whether exogenous oxytocin may have therapeutic potential in schizophrenia and whether it can ameliorate sensorimotor gating deficits, we found that, similar to the well-known effects of atypical antipsychotics, systemic oxytocin reversed PPI deficits induced by amphetamine and the PCP analog dizocilpine (Feifel and Reza, 1999). Lee and colleagues (Lee et al., 2005) reported that PCP-induced social interaction deficits are also reversed by oxytocin. Encouraged by these findings, we recently conducted the first controlled, proof-of-concept study of oxytocin in patients with chronic schizophrenia. In a double-blinded, placebo-controlled study, we found that oxytocin given intranasally to schizophrenia subjects who were symptomatic, despite being on stable antipsychotic regimens, significantly improved positive and negative symptoms characteristic of the disorder (Feifel et al., 2010).

The demonstration of the therapeutic efficacy of oxytocin in humans with schizophrenia now provides an opportunity for backwards validation of the predictive validity of other preclinical animals for schizophrenia treatments. Brown Norway (BN) rats have reduced PPI under certain parametric conditions and it has been suggested that these animals represent an appropriate model of schizophrenia, since they exhibit behavioral characteristics frequently reported in schizophrenia patients (Palmer et al., 2000; Swerdlow et al., 2008).

Conti et al. (Conti et al., 2005) reported that these deficits were not ameliorated by haloperidol or clozapine but we found that both clozapine and the putative antipsychotic, PD149163, a brain-penetrating neurotensin-1 agonist significantly increased BN PPI. The

purpose of this study was to determine whether systemically administered oxytocin could significantly increase PPI in BN rats. In addition, we tested the effects of the oxytocin analog Butyryl-Tyr(Me)-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH₂ (Carbetocin, CBT) designed to resist enzymatic cleavage and thus improve the stability of oxytocin in the peripheral circulation. Carbetocin, which is approved for use in many countries outside the United States for the control of postpartum intrauterine hemorrhage (Page, 2010) has been shown to have a longer plasma half-life than oxytocin and longer duration of uterine myometrium contractions (Engstrom et al., 1998). In addition, carbetocin has been shown to modulate certain behaviors when administered peripherally in rats (Chaviaras et al., 2010; Klenerova et al., 2009a, b).

Experimental Procedures

Seventy male Brown Norway (BN) rats (170 – 300 grams at testing; Harlan Laboratories, San Diego) were housed in groups of two or three in clear plastic chambers in a climate-controlled room under a 12h light/dark schedule, with food and water provided ad libitum. Behavioral testing was begun 7 days after the animals arrival, and was conducted during the light phase of their light/dark schedule. All experimental procedures were conducted in accordance with the University of California, San Diego guidelines for animal care and experimentation.

Sensory gating behavior was obtained by individually testing animals in a startle chamber (San Diego Instruments, San Diego, CA) which consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a 12.5 × 25.5 cm Plexiglas frame within a ventilated enclosure. The apparatus was housed in a sound-attenuated room with 65 dB background noise, and acoustic stimuli were presented via a speaker mounted 24 cm above the animal. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion within the cylinder.

Experiment 1

On day 1, baseline PPI and startle response were assessed following a 5-minute acclimation period in the apparatus. Three days following initial testing, the animals were assigned to one of four drug-dosing groups, based on their baseline PPI data (n=8-10 per group). Animals were given subcutaneous (SC) injections of 0 (saline), 0.04, 0.2, or 1 mg/kg of oxytocin (VWR International, Bachem Americas, Inc.) dissolved in saline (1 ml/kg). Doses of oxytocin were selected based on their demonstrated ability to reverse PPI deficits in a previous report (Feifel and Reza, 1999). Behavioral testing was conducted 30 minutes after drug administration. Following a 5-min acclimation, rats were given a 15 minute PPI test session, and were presented with one of five possible acoustic stimuli conditions: (A) a 40 msec 120 dB startle pulse with no prepulse (pulse-only condition); pulses preceded 100 msec by a prepulse of (B) 4 dB, (C) 8 dB or (D) 12 dB above background (E) a neutral (no sound) stimuli condition. Stimuli were presented in pseudorandom order with an average 15-second inter-trial interval.

In Experiment 2, a separate set of rats were tested as described above except that they were assigned to receive one of five SC treatments, 0 (vehicle), 0.04, 0.2 or 1 mg/kg carbetocin

(VWR International, Bachem Americas, Inc.) or 1 mg/kg of oxytocin (VWR International, Bachem Americas, Inc.) dissolved in saline (1 ml/kg).

Data Analysis

Startle amplitude was defined as the degree of motion detected by the accelerometer, and startle responses and PPI were recorded for all stimuli presentations. Acoustic startle response (ASR) data were analyzed by one-way ANOVA. Pearson's analysis was used to investigate correlations between variables. P values less than 0.05 were considered to indicate statistical significance. Data are presented as (Mean $\pm$ SEM) and were analyzed with SPSS 13.0.

PPI for each animal was calculated as a percentage of the pulse-only startle magnitude using the following formula: $[1 - (\text{startle magnitude after prepulse-pulse pair} / \text{startle magnitude after pulse only})] \times 100$. PPI data were analyzed by 2-way ANOVAs in which prepulse intensity (4, 8 or 12 dB) was a within-subject factor, and Drug (saline and three oxytocin doses) was a between-subject factor. In Experiment 1, ANOVAs were also employed to address potential drug effects at each of the three prepulse intensities, with Drug as a between factor. Dunnett's post-hoc tests were used to compare each of the three oxytocin-treated groups to saline-treated animals in Experiment 1 and each of the four carbetocin groups to saline in Experiment 2. T-test with Bonferroni corrections for multiple comparisons ($\alpha = 0.017$) was used to compare oxytocin- to saline-treated groups in Experiment 2 (PPI).

In Experiment 2, Carbetocin was analyzed by 2-way ANOVA (Drug X Prepulse Intensity). Oxytocin was analyzed by t-test (vs. saline at three prepulse intensities) with α set to 0.017.

Results

Experiment 1: Oxytocin

Two-way ANOVA revealed significant main effects of Drug [$F(3,28) = 8.336$, $P < 0.01$] as oxytocin dose-dependently restored PPI in BN rats (Figure 1). There was also a main effect of Prepulse Intensity [$F(1,56) = 55.533$, $P < 0.01$] as % PPI increased with increasing prepulse intensities. In addition, ANOVAs were employed to address potential drug effects at each of the three prepulse intensities, with Drug as a between factor. All three ANOVAs were significant (4 dB = [$F(3,31) = 3.377$, $P < 0.05$]; 8 dB = [$F(3,31) = 5.141$, $P < 0.01$], and 16 dB = [$F(3,31) = 8.318$, $P < 0.001$]) Dunnett's post-hoc tests revealed that treatment with 1 mg/kg oxytocin significantly elevated PPI compared to saline-treated rats at 8 dB ($P < 0.05$) and 12 dB ($P < 0.001$).

Oxytocin significantly decreased acoustic startle response as evidenced by a significant effect of Drug [$F(3,31) = 5.67$, $P < 0.01$]; Fig 1 (Inset), with the highest dose of oxytocin significantly reducing startle response compared to saline treatment ($P < 0.01$).

Experiment 2: Carbetocin/Oxytocin

Carbetocin PPI—There was a main effect of Prepulse Intensity [$F(2,68) = 59.442$, $P < 0.001$] as % PPI increased with increasing prepulses intensities (Figure 2). There were no other significant effects.

Oxytocin PPI—There was a main effect of Drug [$F(1,16) = 6.620$, $P < 0.05$] and Prepulse Intensity [$F(2,32) = 80.745$, $P < 0.001$] as % PPI increased with increasing prepulse intensities (Figure 2). In addition, there was a significant Drug X Prepulse interaction [$F(2,32) = 8.189$, $P < 0.01$] as oxytocin-treated rats exhibited significantly higher PPI compared to saline at 8 dB [$t(16) = -2.734$, $P < 0.017$] and 12 dB [$t(16) = -4.084$, $P < 0.003$].

Startle Magnitude: Carbetocin did not produce any significant effects on startle magnitude. In contrast, oxytocin significantly decreased startle magnitude compared to saline treatment [$t(16) = 2.759$, $P < 0.05$] (Figure 2 Inset).

Discussion

Our finding that systemically administered oxytocin increased the naturally low PPI exhibited by the BN rat is consistent with our previous report that oxytocin reversed PPI deficits induced either by the NMDA receptor antagonist dizocilpine (MK801) or by the indirect dopamine agonist amphetamine (Feifel and Reza, 1999). In this respect, oxytocin has similar effects as the atypical antipsychotics clozapine and quetiapine (Swerdlow et al., 2008) as well as the putative antipsychotic PD149163, which have also been shown to increase PPI in BN rats (Feifel et al., 2011). These data further add to the preclinical evidence supporting the notion that oxytocin has facilitatory effects on sensorimotor gating circuits in the brain and that it also has therapeutic potential in schizophrenia and perhaps other disorders. Since oxytocin has been shown to improve symptoms in patients with schizophrenia (Feifel et al., 2010) these findings strengthen the predictive validity of the BN rat as a model for antipsychotic efficacy.

Interestingly, we found that carbetocin did not have any significant effects on PPI in BN rats. In some respects, this is surprising since carbetocin has a nonapeptide structure that is very similar to oxytocin, and both compounds have similar affinity for the oxytocin receptor as do its two main metabolites carbetocin I and carbetocin II (Engstrom et al., 1998). On the other hand, studies on myometrial oxytocin receptors indicate that carbetocin may act as a partial oxytocin receptor agonist and that the two metabolites may act as antagonists (Engstrom et al., 1998). This distinct pharmacological property may underlie the differences between carbetocin and oxytocin, we detected in this study.

Although neither oxytocin nor carbetocin readily cross the blood brain barrier ($< 1.5\%$) (Ermisch et al., 1985; Dvorska et al., 1992), these two compounds produce somewhat distinct effects on behavior when administered systemically and this may be the result of small amounts of the total administered dose entering the brain. For example, Klenerova et al. (Klenerova et al., 2009a, b) found that 1 mg/kg of oxytocin but not carbetocin increased grooming in rats, whereas the same 1 mg/kg dose of oxytocin and carbetocin reduced and increased locomotor behavior, respectively. Chaviaras (Chaviaras et al., 2010) tested the

effect of carbetocin in the forced swim test and found that it had anti-depressant like effects on immobility. Our findings do not support carbetocin as a putative antipsychotic but further studies using different animals should be conducted to corroborate this.

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Figure 1. Prepulse inhibition (main) and acoustic startle response (inset) in Brown Norway rats treated with oxytocin. Significantly different than saline-treated rats represented by ** $P < 0.01$, * $P < 0.05$. Data represented as the mean $\pm$ SEM.

Figure 2. Prepulse inhibition (main) and acoustic startle response (inset) Brown Norway treated with carbetocin or oxytocin. Significantly different than vehicle-treated rats represented by ** $P < 0.01$, * $P < 0.05$. Data represented as the mean $\pm$ SEM.