Published in final edited form as:

Behav Brain Res. 2015 February 1; 278: 424–428. doi:10.1016/j.bbr.2014.10.023.

Peripherally administered oxytocin modulates latent inhibition in a manner consistent with antipsychotic drugs

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Abstract

Background—Peripherally administered oxytocin (OT) has produced antipsychotic drug (APD)-like effects in animal tests that are predictive of APD efficacy. However, these effects have mainly been demonstrated using animal models of schizophrenia-like deficits in prepulse inhibition (PPI) of the startle reflex. Another schizophrenia-relevant abnormality that is the basis of a predictive animal test for APD efficacy is deficient latent inhibition (LI). LI is the normal suppression of a classically conditioned response when the subject is pre-exposed to the conditioned stimulus (CS) before it is paired with the unconditioned stimulus (UCS). Conditioned taste aversion (CTA), the normal avoidance of ingesting a food or liquid by animals when its taste is associated with an aversive experience, was used to test whether OT facilitates LI consistent with APDs.

Methods—Brown Norway rats, known to naturally display attenuated LI, were aversively conditioned on two consecutive exposures to flavored drinking water (0.1% saccharin) by pairing it with malaise-inducing lithium chloride injections. Concurrent with conditioning, rats received subcutaneous OT (0.02, 0.1, 0.5 mg/kg) or saline. Some rats were pre-exposed to the flavored water prior to its aversive conditioning (pre-exposed) while others were not (non pre-exposed). Two days after aversive conditioning the amount of flavored water consumed during a 20-minute session was recorded.

Results—As expected, LI, defined as greater consumption by pre-exposed vs. non pre-exposed rats was only weakly exhibited in Brown Norway rats and OT enhanced LI by reducing CTA in pre-exposed rats in a dose-dependent manner, with the 0.02 mg/kg dose producing the strongest effect.

Conclusions—The facilitation of LI by OT is consistent with the effects produced by APDs and provides further support for the notion that OT has therapeutic potential for schizophrenia.

Keywords

oxytocin; latent inhibition; schizophrenia; antipsychotic drugs; animal models

INTRODUCTION

Oxytocin (OT) is a nonapeptide that modulates several neurotransmitter systems in the brain via receptors located in brain regions implicated in schizophrenia such as the nucleus accumbens and hippocampus [1]. Although it has been reported that 1 % or less of peripherally administered OT crosses the blood brain barrier [2], a recent study demonstrated that intraperitoneal and intranasal administration OT in rodents produces a rapid increase in brain OT levels [3]. This is consistent with findings from a large number of studies, which show that peripheral administration of OT produces various effects that are centrally mediated. For example, peripherally administered OT alters various aspects of social behavior and fear in rodents [4,5]. Similarly, our laboratory has demonstrated that subcutaneously (SC) administered OT enhances prepulse inhibition of the startle reflex (PPI), a centrally mediated index of sensorimotor gating an important feature of normal information processing [6,7], consistent with OT activity in the brain.

PPI is deficient in schizophrenia and certain other neuropsychiatric disorders [8]. Peripherally administered OT enhances PPI deficits induced by psychotomimetic drugs and PPI deficits naturally exhibited in the Brown Norway (BN) rat strain [6,7]. The facilitation of reduced PPI is consistent with the effects of antipsychotic drugs (APDs) and suggests that OT may have therapeutic potential for schizophrenia. Indeed, several recent preliminary studies in humans in which OT was administered intranasally support this notion that OT has anti-schizophrenia properties [9]. However, to date the question of whether OT has therapeutic potential for schizophrenia and related disorders remains open. Thus there is a need for further pre-clinical and clinical studies to address this question.

Like PPI, latent inhibition (LI) is a schizophrenia-relevant brain process that is sensitive to drugs with APD efficacy. LI is the normal suppression of a classically conditioned response if the subject is pre-exposed to the conditioned stimulus (CS) before it is paired with the unconditioned stimulus (UC) [10]. It is thought to reflect an adaptive allocation of attention away from irrelevant stimuli and there is evidence that LI regulation is abnormal in schizophrenia [11]. Specifically, LI has been found to be absent or reduced in schizophrenia patients who are early in the course of the disorder, whereas in patients with a long-standing schizophrenia LI seems to be intact [11,12] but see [13]. Weiner and Arad [14] have proposed two ways LI is likely perturbed in schizophrenia, a deficit in LI and excessive persistence of LI, each with a distinct underlying mechanism. This may account for the apparent transitory nature of LI deficits in schizophrenia as these deficits manifest early in the course of the disorder but are masked in later stages by the emergence of excessive persistence of LI.

LI deficits as often seen in the acute stages of schizophrenia can be modeled in animals by various manipulations. For example, administration of dopamine enhancing drugs such as amphetamine concomitant with conditioning disrupts LI. In contrast, the Brown Norway (BN) rat has been found to exhibit low LI [15], in addition to other schizophrenia relevant phenotypes such as low PPI [7,16].

When administered concurrent with the pairing of the CS and UC in animal models of deficient LI (drug-induced or natural deficits), first and second generation APDs enhance LI [14]. This APD-induced facilitation of LI is thought to be mediated by the regulation of brain dopamine [17]. Psychotropic drugs that lack APD activity in humans generally do not facilitate LI in animal models [18] suggesting that LI deficit animal models have predictive validity for the therapeutic effects of APDs.

In order to further investigate the therapeutic potential of OT for schizophrenia, we tested its effects on LI in BN rats.

EXPERIMENTAL METHODS

Animals

Thirty-seven BN rats (170 – 300 grams at testing) (Harlan, San Diego, CA) were housed individually 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 studies described in this publication were carried out in accordance with The Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Procedures

An overview of the LI procedure is shown in Table 1. Throughout this experiment, all rats were water deprived except for 20 minutes a day during which they were allowed to drink ad-libitum from bottles filled with 100 ml of water. At the end of the 20 minutes, water bottles were removed and the amount of water consumed measured. Animals were allowed unrestricted access to food at all times in their home cages. Rats were acclimated to this drinking schedule for 2 consecutive days and then were split into nine groups, matched based upon their average water intake. Four of the groups chosen at random were designated to be pre-exposed (PE) to the conditioned stimuli (flavored water) and the remaining five groups were not pre-exposed (NPE).

On the third day (D3), rats assigned to the PE groups received water flavored with 0.1 % saccharin for their 20-minute drinking session, while the NPE rats continued to receive unflavored water. The fourth day (D4) was the first conditioning day and on that day all rats were given-flavored water for 20 minutes. Thirty minutes before being presented with the flavored water the four PE groups and four of the NPE groups were randomly assigned to receive either subcutaneous (SC) saline or one of three doses of SC OT (0.02, 0.1, 0.5 mg/kg). The doses of OT were based on earlier studies that our lab conducted examining the effects of various doses of SC administered OT on motor activity and drinking under fluid deprived conditions. Doses above 0.5 mg/kg reduced drinking (Unpublished data). Thus, we avoided testing doses higher than 0.5 mg/kg. Twenty minutes later, immediately after the drinking session, each rat in these groups was administered one of the four oxytocin doses, then a SC injection of lithium chloride (0.15 M, 1.33% body weight), a dose that produces a transient malaise [19]. The final NPE group received two injections of SC saline after the drinking session and served as a non-conditioned control group.

The next day (D5) all rats were allowed to drink regular water during their 20-minute drinking session. Day 6 (D6) served as both a LI test day and a second conditioning day. On that day all rats were again given flavored water for their 20-minute drinking session. Immediately subsequent to the drinking session, all rats received an SC injection of saline or OT followed immediately by SC lithium chloride or saline injections, consistent with the treatment they received after flavored water drinking on D4. This was done to maintain aversive conditioning to the flavored water. The next day (D7) rats were again allowed to drink regular water during their 20-minute drinking session and the following day (D8) served as a second LI test day when all rats received flavored water.

Statistical analysis

To confirm successful conditioned taste aversion, a Student's t-test was used to compare flavored drinking consumption on each test day by the saline-treated NPE groups that received lithium chloride on conditioning day(s) to saline-treated NPE rats that did not receive lithium chloride.

To analyze LI across the drug groups, the flavored water drinking data from PE and NPE rats was subjected to a 3-way ANOVA with OT treatment and Pre-exposure status as between subject factors and Test day as a within subject factor. Data of any factor that did not significantly interact with our factor of interest, OT treatment, was averaged across its levels and eliminated as a factor in subsequent post-hoc analyses, which included one-way ANOVAs and group-wise comparisons using Bonferonni corrected t-tests or Dunnett's post hoc tests, as appropriate. LI was defined as significantly greater flavored water drinking (i.e., weaker conditioned taste aversion) in NPE compared to PE rats in the same drug group [19].

To further assess LI, the LI index was calculated for each PE rat. We employed a method similar to [15], i.e., the amount of flavored water consumed by each PE rat on test days minus the mean amount consumed by the corresponding NPE group. ANOVA was used to analyze LI index across the four drug groups. Significant results were followed by Dunnett's post hoc tests.

RESULTS

Table 2 illustrates drinking by all experimental groups on each of the two test days (D6 and D8). Consistent with successful conditioned taste aversion, the saline treated NPE group that received LiCl on conditioning days drank significantly less during both drinking test sessions than the NPE group that did not receive LiCl ($P < 0.01$).

Figure 1 illustrates the mean drinking values from the two test sessions. There was a significant effect of Test Day [$F(1,25) = 5.647$, $P < 0.05$] as drinking of flavored water on the second test day was generally lower than the first test as expected due to the strengthened CTA produced by the second conditioning session. There were no significant interactions (2- or 3-way) between Test day and OT treatment or Pre-exposure status. There was a significant effect of OT treatment [$F(3,32) = 7.604$, $P < 0.01$], Pre-exposure [$F(1,32)$

= 29.322, $P < 0.001$], and a significant OT x Pre-exposure interaction [$F(3,32) = 6.520$, $P < 0.01$] suggesting that OT had a differential effect on PE versus NPE rats.

This differential effect was confirmed by one-way ANOVAs conducted separately for the averaged drinking over both test days in PE and NPE rats, which revealed a significant effect of OT on the PE groups [$F(3,16) = 12.216$, $P < 0.001$] but not the NPE groups [$F(3, 15) = 0.341$, $P = 0.796$]. There was an overall increase in flavored water consumption (less CTA) in OT-treated PE rats but not in NPE rats. Dunnett's post hoc tests revealed that for the PE groups, the rats that received the low dose of OT exhibited significantly greater drinking compared to the saline-treated rats ($P < 0.01$).

Bonferroni corrected t-tests of NPE and PE groups for each OT dose revealed that BN rats treated with saline did not display significant LI (PE drinking not significantly different from NPE) consistent with previous reports that BN rats have deficient LI [15]. In contrast, rats that received the low and mid OT doses drank significantly more ($P < 0.01$ and $P < 0.05$, respectively) flavored water compared to the NPE group.

Figure 2 displays the LI index across the four drug groups. A one-way ANOVA comparing the LI index across the four OT doses was statistically significant [$F(3, 15) = 11.706$, $P < 0.01$], reflecting a LI index that was significantly greater at the low PD dose ($P < 0.05$) compared to saline-treated rats

DISCUSSION

Our main finding that OT significantly increased LI in a dose-dependent manner suggests that OT regulates the central processes involved in LI. OT significantly increased flavored water drinking in rats that had been aversively conditioned to flavored water (CTA) after being exposed to it in a neutral context (pre-exposed group), whereas it had no significant effect on flavored water drinking in rats that had been aversively conditioned to flavored water during their first two exposures to it (non pre-exposed group). In other words, OT enhanced the pre-exposure induced attenuation of CTA, without altering CTA itself. This indicates that it had a selective effect on the LI process and not the underlying associative learning process. This LI enhancing effect was most distinctly observed with the lowest dose of OT tested in this experiment.

Given that facilitation of LI is considered a predictive test of anti-schizophrenia drug efficacy, the current results are consistent with other preclinical tests suggesting OT exhibits APD-like therapeutic efficacy [6,7,20]. For example, OT also exhibits APD-like effects in animal models of PPI deficits. In addition to reversing the PPI disrupting effects of amphetamine and the NMDA antagonist MK801 [6], peripheral OT has been shown to reverse natural PPI deficits in BN rats [7]. Caldwell et al., [20] reported that OT knockout mice were more vulnerable to the PPI disrupting effects of the NMDA antagonist PCP, suggesting that OT acts as an endogenous APD. To date, the only preclinical study that has reported OT's APD potential that did not rely on PPI based predictive animal models was performed by Lee et al. [21] who found that peripheral administration of OT attenuated the social withdrawal deficits produced by chronic PCP in rats, a phenomenon proposed to

model the negative symptoms of schizophrenia. Given the strong interest in OT as a putative novel treatment for schizophrenia and other neuropsychiatric disorders, it is a weakness of the current literature that there have only been four published studies, which have investigated this potential therapeutic role of OT in established animal models and all but one of them have been based on studying OT effects on PPI deficits.

The current study is an important addition to the current body of published literature and provides additional preclinical evidence that OT or OT analogs may have therapeutic benefits for schizophrenia and other disorders associated with dysregulation of information gating. The APD-like preclinical effects of OT seen in this study and the others already discussed are consistent with several recent small clinical studies showing that intranasal OT significantly improves positive and negative symptoms, as well as certain cognitive functions in schizophrenia patients [9].

The overwhelming majority of studies to date that have reported APD facilitation of deficient LI in animals have used psychotomimetic drugs, namely amphetamine, to induce LI deficits [18,22]. In contrast, the current study took advantage of a known natural LI deficit in BN rats. These results indicate the BN rat, which has several natural schizophrenia-like phenotypes including low PPI [7], dysregulated LI [15], and working memory deficits [23] is a useful tool to study the APD-like potential of novel putative treatments for schizophrenia.

An interesting feature of the results of this study is that the OT dose that most effectively restored LI is lower (0.02 mg/kg) than the dose we had previously found to facilitate PPI (1.0 mg/kg). However, there are other examples in the literature that demonstrate greater dose sensitivity for APD-induced LI facilitation than PPI facilitation. For example, the lowest dose of haloperidol reported to facilitate baseline PPI is 0.1 mg/kg [8], whereas doses of haloperidol as low as 0.03 mg/kg facilitate LI [17]. Haloperidol also facilitated LI at higher doses, whereas OT did not.

OT's affinity for vasopressin receptors may be a contributing factor to the dose-response relationship we found. Exogenous OT has been reported to produce some of its *in vivo* effects via vasopressin-1A receptor activation [24,25]. For example, Song et al. recently reported that OT regulated social communication in Syrian hamsters by activation of vasopressin-1A receptors [25]. While vasopressin's regulation of LI has not been reported, it may exert a disrupting effect on it. If this were the case, increasing activation of vasopressin receptors, with the higher doses of OT tested in this study, may have acted to counteract the LI enhancing effects of OT on the OT receptor. Future studies should investigate the effect of vasopressin on LI.

Dopamine transmission plays a critical role during the conditioning process in the manifestation of LI and is thought to produce the facilitation of deficient LI by first and second generation APDs administered during the conditioning process [17]. Therefore, OT's APD-like effects on LI seen in this study are likely also due to inhibition of dopamine transmission. In this regard, OT has been shown in several previous studies to inhibit the effects of pro-dopamine drugs. For example, OT has been reported to attenuate

amphetamine-induced PPI disruption [6], mesolimbic dopamine release, and hyperactivity and stereotypy induced by cocaine [1].

In summary, this study provides evidence that peripherally administered OT regulates LI in a way that is consistent with established APDs and adds to the body of evidence suggesting that OT has therapeutic efficacy for schizophrenia.

Acknowledgments

DF and PDS are supported in part by NIMH R01MH080910.

D. Feifel: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DF is a named inventor on an oxytocin method of use patent application. J. Hillman: None. M. Maisel: None. J. Winfield: None. G. Melendez: None. P. D. Shilling: None

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Figure 1.

The effects of oxytocin on latent inhibition. Values represent Mean $\pm$ SEM volume (ml) of flavored water consumed during the two 20-minute test sessions. NPE = non-pre-exposed, PE= pre-exposed. ***Represents a significant difference from the non-conditioned saline group ($p < 0.001$). ## Represents a significant difference from the corresponding NPE drug group ($p < 0.01$), # ($p < 0.05$). \$ Represents a significant difference from the saline PE group ($P < 0.05$).

Figure 2.

The effects of OT on LI Index. LI index for each drug treatment group was calculated as the average amount of flavored water consumed on the two test days by each PE rat minus the mean amount consumed by the corresponding NPE group. Values = Mean ± SEM. ** Represents a significant difference from saline, $P < 0.01$.

Table 1

Overview of the experimental design. D3, D5, D7: on these days all rats received regular water during their 20-minute daily drinking session.

DAY	D1 & D2	D3	D4	D6	D8
Procedure	Water Restriction Training All rats have 20 min. access to water.	Pre-exposure PE rats have 20 min. access to flavored water. NPE and NC rats have 20 min access to regular water.	Conditioning & Drug Treatment #1 All rats have 20 min. access to flavored water. All rats then given IP injections of LiCl except NC rats (saline injections). All rats then given SC injection of a dose of OT or saline.	LI Testing #1 All rats receive flavored water for 20 minutes. ----- Conditioning & Drug Treatment #2 Rats given same IP and SC injections as on D4.	LI Testing #2 All rats receive flavored water for 20 min.

Abbreviations: NC = non conditioned group, NPE = non-pre-exposed group, PE = pre-exposed group, SAC = saccharine, LiCl = lithium chloride

Table 2

Flavored water consumption during 20-minute drinking session on the two test days.

OT Dose (mg/kg)	n	Exposure Status	Test Day 1 – D6 (ml)	Test Day 2 – D8 (ml)
Sal (NC)	4	NPE	13.75 (1.70)	15.50 (2.52)
Sal	5	NPE	4.20 (1.24)**	3.60 (0.81)***
Sal	5	PE	6.80 (1.28)	5.60 (2.40)
0.02	4	NPE	5.50 (1.50)	3.75 (0.85)
0.02	4	PE	12.25 (0.25)	11.00 (2.08)
0.10	4	NPE	6.00 (0.91)	2.25 (0.63)
0.10	4	PE	8.00 (1.08)	5.50 (0.65)
0.50	3	NPE	5.67 (2.03)	4.00 (0.58)
0.50	4	PE	5.50 (0.96)	4.50 (0.29)

Values represent Mean $\pm$ SEM (ml). Doses are in mg/kg;

NPE=non-pre-exposed; PE= pre-exposed. Significantly different from NC saline group.

P < 0.001,

**
P < 0.01.

Abbreviations: OT = oxytocin, NC = non-conditioned control, Sal = saline