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Prosocial effects of prolactin in male rats: Social recognition, social approach and social learning

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ABSTRACT

Prolactin (PRL) and oxytocin (OT) are pituitary hormones essential for lactation, but also promote sexual behavior. OT stimulates social behaviors, such as recognition, approach, and learning, but less is known about PRL in these behaviors. Since PRL and OT have complementary functions in reproduction, we hypothesized that PRL increases social recognition, approach, and learning. Male Long-Evans rats received ovine PRL (oPRL; 0.5, 2.0 or 5.0 mg/kg), the PRL antagonist bromocriptine (0.1, 3.0 or 5.0 mg/kg) or saline 20 mins before testing for recognition of familiar vs. unfamiliar stimulus males. Saline controls preferred the unfamiliar male ($p < 0.05$), while bromocriptine blocked this preference. oPRL did not increase preference. To measure social approach, we determined if PRL restores approach 2 h after defeat by an aggressive male. Defeated rats avoided the aggressive male. 2 mg/kg oPRL, before or after defeat, restored approach towards the aggressive male ($p < 0.05$). In non-defeated rats, oPRL or 3 mg/kg bromocriptine had no effect. To determine if PRL increases social learning, we tested social transmission of food preference. Rats choose between two unfamiliar flavors, one of which they have previously been exposed to through interaction with a demonstrator rat. Vehicle controls preferred chow with the demonstrated flavor over the novel flavor. oPRL-treated rats were similar. Bromocriptine-treated rats failed to show a preference. When tested one week later, only oPRL-treated rats preferred the demonstrated flavor. The results suggest that PRL is required for social recognition and learning, and that increasing PRL enhances social memory and approach, similar to OT.

1. Introduction

Prolactin (PRL) and oxytocin (OT) are pituitary hormones essential for lactation [reviewed in (Crowley, 2015)]. PRL promotes milk synthesis (Koprowski and Tucker, 1973), and its release from the anterior pituitary is regulated by tonic dopamine inhibition [reviewed in Ben-Jonathan and Hnasko, 2001]. OT is released from the posterior pituitary to control milk let-down (Nickerson et al., 1954). Although lactation is limited to females, PRL and OT also function as neuromodulators in both males and females. PRL and OT increase social behaviors related to reproduction, including maternal behavior (Bridges et al., 1990; Pedersen and Prange, 1979) and lordosis in female rats (Arletti and Bertolini, 1985; Drago and Lissandrello, 2000), penile erections in male rats (Argiolas et al., 1986; Drago and Lissandrello, 2000), and pair-bonding in monogamous tamarin monkeys (Snowdon et al., 2010; Snowdon and Ziegler, 2015). Furthermore, OT has also been shown to increase non-sexual social behavior in male rats, such as social recognition (Ferguson et al., 2001; Popik et al., 1992), social approach (Lukas et al., 2011), and social learning (Popik and Van Ree, 1993). Since PRL and OT have complementary peripheral and central functions in reproduction, it is reasonable to expect that they may have complementary functions in social behavior in both males and females.

Recent studies have shown that OT increases social behaviors in rodents and humans. OT restores social approach following a social defeat in male rats (Lukas et al., 2011), and increases social learning in rats during the social transmission of food preference task (STFP, Popik and Van Ree, 1993). Likewise, OT knockout mice have social memory impairments, which can be restored by OT infusion into the medial amygdala (Ferguson et al., 2001). In humans, intranasal OT increases social risk-taking, such as cooperation (Declerck et al., 2010), and trust (Kosfeld et al., 2005) regardless of deceit (Baumgartner et al., 2008). However, according to the social salience hypothesis of Shamay-Tsoory and Abu-Akel (2016), the effects of OT on social behavior depend on context, and can enhance negative responses towards out-group

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members. Moreover, OT is rewarding even in the absence of social stimuli as indicated by self-administration (Donhoffner et al., 2016) and conditioned place preference (CPP, Kent et al., 2013; Liberzon et al., 1997).

PRL is a pleiotropic hormone with over 300 functions [reviewed in Grattan, 2015], including both peripheral actions (e.g. immune function, salt and water balance) and central effects (e.g. food intake, neurogenesis). PRL release changes with stress (Euker et al., 1975), seasonality [reviewed in Curlewis, 1992] and circadian rhythms (Bertani et al., 2010). Moreover, PRL modulates reproduction even in species that do not lactate, such as fish [reviewed in Whittington and Wilson, 2013]. In birds, fish and mammals PRL promotes parental behavior, including olfactory recognition of offspring (Larsen and Grattan, 2010; Mak and Weiss, 2010) and paternal behavior [reviewed in Schradin and Anzenberger, 1999]. PRL also enhances opposite-sex odor preferences and attractiveness in meadow voles (Ferkin et al., 1997; Leonard and Ferkin, 1999).

We hypothesize that PRL and OT have similar central effects on non-sexual social behavior. If so, PRL should promote social recognition, social approach, social learning, and reward, similar to OT. The present study tested the effects of PRL on social behavior in male rats. While basal PRL levels in females are approximately double those in males (Kinsley et al., 1989), PRL has biological relevance in both males and females (Drago and Lissandrello, 2000). Furthermore, OT promotes social behavior in males. Rats were treated either with exogenous PRL to raise PRL levels in circulation, or with the dopamine D2 receptor agonist bromocriptine to block PRL release.

2. Materials and methods

2.1. Animals and housing

Male Long-Evans rats (ca. 225 g at arrival; Charles River Laboratories, Wilmington, MA) were used to test social recognition ($n = 24$), social approach ($n = 40$), social learning ($n = 24$) and CPP ($n = 24$) to measure reward. They were pair-housed, and maintained in a temperature- and humidity-controlled room on a reversed 14:10 light/dark cycle (lights off at 9 a.m.). Food and water were available ad libitum, except during behavioral testing. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (Council, 2011) and with the approval of the University of Southern California Institutional Animal Care and Use Committee (IACUC).

2.2. Experimental design

To determine if PRL promotes social behavior, rats received ovine PRL (oPRL; Sigma-Aldrich, St. Louis, MO), the PRL antagonist bromocriptine (Sigma-Aldrich) or saline vehicle 20 min before behavioral testing. Separate groups of rats were tested for social recognition, social approach, social learning, and CPP to measure reward. Initially, to establish behaviorally-effective doses of oPRL and bromocriptine, rats were tested for social recognition in response to oPRL at 0.5, 2.0, and 5.0 mg/kg, or to bromocriptine at 0.1, 3.0, and 5.0 mg/kg. Subsequently, to determine whether PRL promotes social approach, social learning, and CPP, rats received oPRL at 2.0 mg/kg or bromocriptine at 3.0 mg/kg, based on behaviorally-effective doses from the social recognition test. While we cannot rule out the possibility that different social behaviors respond to different doses of oPRL or bromocriptine, the remaining behavioral assays in this study are not amenable to repeated testing at different drug doses. All behavioral tests were videotaped and scored by an observer blind to the treatment groups.

oPRL was delivered by ip injection. While PRL synthesis can occur centrally in the hypothalamus, cerebral cortex and hippocampus [reviewed in Freeman et al., 2000], the majority of PRL in the rat brain originates from anterior pituitary release (Ben-Jonathan et al., 2008). PRL crosses into the cerebral spinal fluid via the choroid plexus [reviewed in Freeman et al., 2000]. Doses of oPRL were based on previous rodent studies. At 2.0 mg/kg, oPRL inhibits gastric emptying and increases plasma cholecystokinin, whereas a 0.5 mg/kg dose has no effect (Chang et al., 2012). At 5.0 mg/kg, oPRL decreases anxiety in female rats on the elevated plus maze (Torner et al., 2001).

Bromocriptine is a dopamine D2 receptor agonist with low blood brain barrier permeability (Markey et al., 1979), which has been used extensively as a PRL antagonist. Following iv injection, bromocriptine concentrations are 10-fold higher in the pituitary than in the striatum (Granveau-Renouf et al., 2000). Bromocriptine binds to D2 receptors on lactotrophs to inhibit PRL synthesis and release from the anterior pituitary (Israel et al., 1985). At 0.1 mg/kg, bromocriptine reduces serum PRL selectively in older female rats, but is ineffective in younger females (Cocchi et al., 1984). At 3.0 mg/kg, bromocriptine decreases serum PRL in male and female rats within 30 min of injection (Atterwill et al., 1989). At 5.0 mg/kg, bromocriptine reduces PRL and corticosterone in male rats (Kan et al., 2003).

2.3. PRL effects on social behaviors

2.3.1. Social recognition

To determine if PRL promotes social memory, we tested rats for social recognition as adapted from Engelmann et al. (2011). Rats prefer a novel stimulus rat over a familiar one, and this preference lasts for approximately 45 min (Noack et al., 2010). After 120 min, test rats fail to show a preference for the novel stimulus rat. In the present study, each test rat was isolated in a clean cage for 2 h. Twenty minutes before the first encounter with an unfamiliar stimulus rat (ovariectomized female), test rats received an injection of vehicle, oPRL, or bromocriptine ($n = 8$/group). Time spent investigating the stimulus rat was recorded, where investigation was defined when the test rat had its nose within 1 cm of the stimulus rat. After 4 min, the stimulus rat was removed. After 45 min (short-term) or 120 min (long-term), the test rat was exposed to the same stimulus rat and a novel stimulus rat for 4 min, and investigation of each stimulus rat was recorded. Each test rat was evaluated for short-term and long-term social recognition on 3 occasions with the same treatment (vehicle, oPRL or bromocriptine) at increasing doses of oPRL or bromocriptine. Data from individual vehicle-treated rats were averaged over the 3 tests. At least 2 days elapsed between successive tests, and stimulus rats were rotated to avoid familiarity.

2.3.2. Social approach

To determine if PRL promotes social approach, social approach following social defeat was evaluated according to Lukas et al. (2011). Initially, the test rat was placed in the home cage (43 × 24.5 × 20 cm) of a larger, aggressive male rat (defeater) for 30 min. Defeater rats were prescreened for aggressive behavior towards an intruder. Test rats showed freezing behavior during the first 10 min with the defeater for an average of 188.4 ± 32.0 s. Physical interaction with the defeater rat was terminated after 10 min by introduction of a wire-mesh screen to bisect the cage. After 20 min, test rats were removed to a clean cage. 90 min later, test rats were tested for social approach in a novel arena (81 × 81 × 46 cm). Initially, rats had 4 min with an empty enclosure (17 × 17 × 17 cm; object approach), followed by 4 min with an identical enclosure containing the defeater rat (social approach). Investigation was defined as the test rat being within 4 cm of the enclosure. The chamber was cleaned between trials. All test rats received 2 injections: 1 injection 20 min before defeat, and a second injection 20 min before exposure to the novel arena. Vehicle-treated rats ($n = 8$/group) received 2 injections of saline. Separate groups of rats ($n = 8$ each) received an injection of 2.0 mg/kg oPRL, either before defeat or before the novel arena, and a second injection of saline.

To determine if PRL promotes social approach in the absence of