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Selective and non-selective OT receptor agonists induce different locomotor behaviors in male rats via central OT receptors and peripheral V1a receptors

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ABSTRACT

Oxytocin (OT) continues to inspire much research due to its diverse physiological effects. While the best-understood actions of OT are uterine contraction and milk ejection, OT is also implicated in maternal and bonding behaviors, and potentially in CNS disorders such as autism, schizophrenia, and pain. The dissection of the mechanism of action of OT is complicated by the fact that this peptide activates not only its cognate receptor but also vasopressin type 1a (V1a) receptors. In this study, we evaluated OT and a selective OT receptor (OTR) agonist, FE 204409, in an automated assay that measures rat locomotor activity. The results showed: 1) Subcutaneous (sc) administration of OT decreased locomotor behavior (distance traveled, stereotypy, and rearing). This effect was reversed by a V1a receptor (V1aR) antagonist ([Pmp1,Tyr(ME)2]AVP, sc), suggesting that OT acts through peripheral V1aR to inhibit locomotor activity. 2) A selective OTR agonist (FE 204409, sc) increased stereotypy. This effect was reversed by an OTR antagonist dosed icv, suggesting a central OTR site of action. Our findings identify distinct behavioral effects for OT and the selective agonist FE 204409, adding to the growing body of evidence that the V1aR mediates many effects attributed to OT and that peptides administered systemically at supra-physiological doses may activate receptors in the brain. Our studies further emphasize the importance of utilizing selective agonists and antagonists to assess therapeutic indications.

1. Introduction

For several decades the physiological and pharmacological effects of the neuropeptide oxytocin (OT) have been the focus of much research. Currently, OT is attracting interest for its therapeutic potential in pain (Eisenach et al., 2015; González-Hernández et al., 2017; Tzabazis et al., 2017), addictive disorders (Lee et al., 2016), autism (Young and Barrett, 2015), and schizophrenia (Rich and Caldwell, 2015; Shilling and Feifel, 2016). While the primary physiological functions of OT in the periphery are uterine contraction during labor and stimulation of milk ejection, this hormone has also been reported to have cardiovascular (Gutkowska et al., 2014), behavioral (Winslow and Insel, 2002; Neumann and Slattery, 2016), and sensorial (Eliava et al., 2016) effects.

Determining the mechanism of action of OT is complicated by the fact that this hormone activates not only the OT receptor (OTR) but also the vasopressin type 1a receptor (V1aR) due to the high level of sequence homology between OT and AVP (Barberis et al., 1998). By employing selective OTR and V1aR ligands, several studies have shown that effects induced by OT, and thought to be mediated by the OTR, are mediated by the V1aR (Anacker et al., 2016; Gupta et al., 2008; Kubo et al., 2017; Loichot et al., 2001; Qiu et al., 2014; Schorscher-Petcu et al., 2010; Song et al., 2014; Song and Albers, 2017). An example of such effect is the OT-induced analgesia and scratching in OTR knockout mice, which is absent in V1aR knockout mice (Schorscher-Petcu et al., 2010). Also, in a rodent model of infraorbital pain, the antinociception produced by OT dosed in the trigeminal ganglion was abolished by the V1aR antagonist SR49059 (Kubo et al., 2017). This same antagonist potently blocked OT- and AVP-induced contractions in erectile and ejaculatory tissues (Gupta et al., 2008). Moreover, social behaviors promoted by OT, such as flank marking in Syrian hamsters (*Mesocricetus auratus*) (Song et al., 2014) and decreased partner preference formation in meadow voles (*Microtus pennsylvanicus*) (Anacker et al., 2016), were also shown to be mediated by V1aR, rather than OTR. While these findings highlight the importance of using selective agonists for in vivo studies, much research continues using only the non-selective OT.

Another point of controversy in the literature is whether or not systemically-administered OT can penetrate the brain and, if not, whether centrally-mediated behaviors can be elicited indirectly by OT

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acting in the periphery. Multiple investigations have shown that OT does not readily cross the blood-brain barrier (Jones and Robinson, 1982; Kang and Park, 2000; Mens et al., 1983); yet, the hormone has been shown to effect centrally-mediated behaviors following peripheral administration. For instance, when given systemically at supra-physiological doses (0.1 to 3 mg/kg), OT decreases locomotor behavior in rats (Angioni et al., 2016; Hicks et al., 2012, 2016; Klenerova et al., 2009; Leong et al., 2016; Petersson et al., 1998; Uvnäs-Moberg et al., 1994). This effect has been attributed to OTR activation in the brain since OT administered centrally at much lower doses (2–200 ng/site) produces a similar decrease in locomotion (Uvnäs-Moberg et al., 1992; Angioni et al., 2016). While it may be possible that small amounts of OT enter the brain via the circumventricular organs (McEwen, 2004), which link the central nervous system with peripheral blood flow, peripheral OT may elicit central effects indirectly through its effect on autonomic function – increasing blood pressure and decreasing heart rate and body temperature (Agren et al., 1995; Hicks et al., 2014; Uvnäs-Moberg et al., 1994; Xu and Wiesenfeld-Hallin, 1994).

The goal of the current study was two-fold. First, to confirm previously reported locomotor effects for OT in male rats and determine which receptor type (OTR or V1aR) and their location (peripheral or central) mediate such effects. Second, to evaluate the effects of a selective OTR agonist (FE 204409) on locomotor activity in male rats, and determine the involvement of peripheral and central OTRs.

A portion of the results presented herein have been published in abstract/poster format.

2. Materials and methods

2.1. Receptor pharmacology assays

The agonist and antagonist activity of compounds at rat receptors were evaluated using cell-based receptor reporter gene assays. Assays utilized CHO-K1 cells transiently expressing the rat OT receptor (rOTR) and HEK-293 cells transiently expressing rat V1a receptors (rV1aR). A luciferase reporter gene under the control of transcriptional regulatory elements responsive to receptor activation was also introduced to the cells (previously described in Boss et al., 1996). CHO-K1 and HEK-293 cells were maintained in standard growth media (DMEM-F12 containing 5% (v/v) heat-inactivated fetal bovine serum (FBS-HI) and 2 mM L-glutamine or DMEM containing 10% (v/v) FBS-HI and 4 mM L-glutamine, respectively). One day following transfection, rOTR and rV1aR expressing cells were plated into 96 well plates (4–5 × 10^4 cells/well) and incubated overnight (37 °C, 5% CO_2).

2.2. Luciferase reporter gene assay for EC50 determination

Cells were incubated with logarithmically-spaced concentrations (1 pM −10 μM) of OT or FE 204409 for 5 h at 37 °C under 5% CO_2 in phenol-red free growth medium. Compound stock solutions were initially prepared in 100% dimethylsulfoxide (DMSO) and then serially diluted to 10× final concentrations in phenol-red free Dulbecco's Modification of Eagle's Medium (DMEM) containing 10% heat-inactivated fetal bovine serum. The final concentration of DMSO in the assay was < 0.1% (v/v). Each compound was assayed in duplicate and a vehicle control and reference agonist were included in each experiment. Expression of the luciferase reporter gene was quantified using a luminescent substrate (Luclite Reporter Gene Assay System, PerkinElmer).

2.3. Luciferase reporter gene assay for IC50 determination

Cells were incubated with logarithmically-spaced concentrations (0.1 nM −10 μM) of antagonist for 10 min at 37 °C under 5% CO_2 in phenol-red free growth medium. Following pre-treatment, 0.3 nM OT (for rOTR) or 1 nM AVP (for rV1aR) agonist was added to the cells and the plates incubated for an additional 5 h at 37 °C under 5% CO_2. Compound stock solutions were initially prepared in 100% dimethylsulfoxide (DMSO) and then serially diluted to 10× final concentrations in phenol-red free Dulbecco's Modification of Eagle's Medium (DMEM) containing 10% heat-inactivated fetal bovine serum. The final concentration of DMSO in the assay was < 0.1% (v/v). Each compound was assayed in duplicate and a vehicle control and reference antagonist were included in each experiment. Expression of the luciferase reporter gene was quantified using a luminescent substrate (Luclite Reporter Gene Assay System, PerkinElmer).

2.4. Data analysis

Mean luminescent counts per second (LCPS) were calculated from replicate values and a single-binding site, four parameter concentration response model from XIfit (IDBS) was used to perform non-linear regression analysis. Agonist potency was expressed as EC50 (the concentration that produces a half-maximal response) in nM. For multiple independent experiments, data are presented as the geometric mean values with 95% confidence intervals (CI). Antagonist potency was expressed as Ki (inhibition constant, nM), which was calculated from IC50 values using the Cheng-Prusoff equation:

$$Ki = IC50/(1 + [Agonist]/Agonist\ EC50)$$

where [Agonist] was the concentration of the agonist used in each antagonist reporter gene assay and Agonist EC50 was the concentration of the agonist that produced 50% of the maximal possible effect. For multiple independent experiments, the Ki value was reported as the geometric mean with 95% CI.

Efficacy (Emax (%)) of agonist compounds is expressed relative to the response of a reference full-agonist run within the same experiment (carbetocin for the rOTR and AVP for the rV1aR).

Antagonist efficacy (Efficacy Antag (%)), maximal inhibition of the agonist response, is expressed relative to the response of a reference antagonist run within the same experiment (barusiban for the rOTR and HOPhAc-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-NH_2 for the rV1aR).

2.4.1. Animals

Adult male Sprague Dawley rats weighing 250–300 g on arrival were housed in ventilated chambers in a climate-controlled room under a 12-h light/dark schedule with access to food and water ad libitum. Animals fitted with intracerebroventricular (icv) cannulas (Charles River, San Diego, CA) were singly housed to prevent disruption of the surgical site, and appropriate housing enrichment was supplied; non-instrumented animals (Envigo, Livermore, CA) were housed in groups of two. All animals were allowed to acclimate to the animal facility for 7 days before behavioral testing was initiated. Adult female Sprague Dawley rats (Envigo, Livermore, CA) weighing 150–250 g were used for uterine contraction studies. All experimental procedures were conducted in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the Ferring Research Institute IACUC committee.

2.4.2. Drugs

The following drugs were used: Oxytocin (OT); FE 204409 (a novel and selective OTR agonist discovered by Ferring Research Institute); [Pmp1,Tyr(Me)2]AVP (a mixed OTR/V1aR antagonist, known as “Manning Compound”) (Kruszynski et al., 1980); and barusiban (carba-6-[D-Trp2,aIle4,MeOrn-ol7]dOT-(1-7), a selective OTR antagonist) (Wisniewski et al., 1999). All compounds were synthesized at Ferring Research Institute by solid phase peptide synthesis, purified by reverse phase HPLC, and formulated to achieve desired concentrations taking into consideration free base peptide content as well as purity.

2.4.2.1. Dosing. OT was administered subcutaneously (sc) at doses of 0.2 and 1 mg/kg. FE 204409 was administered sc at doses of 0.03, 0.3,