Psychoneuroendocrinology 66 (2016) 185–194

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Plasma and CSF oxytocin levels after intranasal and intravenous oxytocin in awake macaques

Sara M. Freeman[a], Sridhar Samineni[b], Philip C. Allen[b], Diane Stockinger[b], Karen L. Bales[a,c], Granger G.C. Hwa[b,*], Jeffrey A. Roberts[b]

[a] California National Primate Research Center, University of California-Davis, Davis, CA 95616, USA
[b] Valley Biosystems, Inc., West Sacramento, CA 95605, USA
[c] Department of Psychology, University of California-Davis, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 8 October 2015
Received in revised form
21 December 2015
Accepted 14 January 2016

Keywords:
Neuropeptide
Rhesus macaque
Cerebrospinal fluid
Nasal spray
ELISA

ABSTRACT

Oxytocin (OT) is a neuropeptide that mediates a variety of complex social behaviors in animals and humans. Intranasal OT has been used as an experimental therapeutic for human conditions characterized by deficits in social functioning, especially autism spectrum disorder and schizophrenia. However, it is currently under intense debate whether intranasal delivery of OT reaches the central nervous system. In this study, four female rhesus macaques were implanted with chronic intrathecal catheters and used to investigate the pharmacokinetic profile of OT in the central nervous system and the peripheral vasculature following intravenous (IV) and intranasal (IN) administration of OT. In a randomized, crossover design, OT was given to four awake monkeys at three different doses based on body weight (0.1 IU/kg; 1 IU/kg; 5 IU/kg). A time course of concurrent cerebrospinal fluid (CSF) and plasma samples were taken following administration. We found a dose-dependent effect of IV OT treatment on plasma OT levels, which peaked at 5 min post-dose and gradually returned to baseline by 120 min. In contrast, a change in CSF OT was only observed at the highest IV dose (5 IU/kg) at 15 min post-dose and gradually returned to baseline by 120 min. After IN administration, there was no significant change in plasma OT at any of the three doses. However, at the highest dose level, we found a significant increase in CSF OT at 15–30 min post- dose. The results of this study in light of recent, similar publications highlight the importance of methodological consistency across studies. This study also establishes a non-human primate model that can provide a stable platform for carrying out serial sampling from the central nervous system and peripheral vasculature concurrently.

1. Introduction

Oxytocin (OT) is a nine amino acid neuropeptide that has been shown to modulate social behavior in both animals and humans. Due to the ability of this peptide to promote prosocial behaviors in animals, such as pair-bonding and affiliation, there has been an increasing number of studies investigating the effects of OT on human behavior (Young and Flanagan-Cato, 2012). However, OT has been shown to be too large to effectively cross the blood–brain barrier (BBB) and access neural structures when delivered peripherally, such as after intravenous (IV) administration (Ermisch et al., 1985). Thus, the overwhelming majority of OT studies in humans have employed an intranasal (IN) dosing paradigm in order to (hypothetically) bypass the BBB by delivering OT directly to the olfactory and respiratory epithelia in the nasal cavity, where OT is thought to enter the brain via the olfactory neurons or trigeminal nerve endings (Quintana et al., 2014).

Administering intranasal OT (IN-OT) to healthy humans has been shown to affect a suite of social behaviors, such as trust (Kosfeld et al., 2005), eye contact (Guastella et al., 2008), emotion recognition (Domes et al., 2007), socially-reinforced learning (Hurlemann et al., 2010), and pair-bonding-related behaviors (Scheele et al., 2012, 2013). More recent efforts have expanded these findings by administering IN-OT to clinical populations with deficits in social function, such as individuals with autism spectrum disorder (ASD). Indeed, there are now several studies showing that IN-OT can ameliorate several aspects of social function in adults and children with ASD, including enhancing the saliency of human faces (Domes et al., 2013), improving emotion recognition (Guastella et al., 2010), and increasing feelings of social reciprocity and increasing gaze to the eyes (Andari et al., 2010).

* Corresponding author at: 1265 Triangle Court, West Sacramento, CA 95605, USA.
E-mail address: ghwa@valleybiosystems.com (G.G.C. Hwa).

http://dx.doi.org/10.1016/j.psyneuen.2016.01.014
0306-4530/

Despite the progress that has been made in the clinical application of OT, the pharmacokinetics of IN-OT within the central nervous system (CNS) remain unclear. Several studies have begun to characterize the plasma response to IN-OT in humans (Burri et al., 2008; Gossen et al., 2012; Kirkpatrick et al., 2014; Striepens et al., 2013), and one study has reported changes in CSF OT levels in humans after IN-OT treatment (Striepens et al., 2013). These studies have all reported significant increases in plasma OT after treatment with IN-OT at doses between 24–40 IU, with peaks around 15–30 min post-administration and a return to baseline levels by 75–90 min. One recent study delivered IN-OT with a novel, breath-powered device that closes the soft palate during administration and found significant increases in plasma OT levels at a much lower dose of 8 IU (Quintana et al., 2015). In the only study that has assessed the effect of IN-OT on CSF OT levels in humans, there was only a significant increase at 75 min post-administration (Striepens et al., 2013).

In order to extend the results from the human literature, there are three studies that have sought to characterize the effects of IN-OT treatment on cerebrospinal fluid (CSF) levels of OT in the non-human primate (NHP) (Chang et al., 2012; Dal Monte et al., 2014; Modi et al., 2014). However, there were limitations in their respective study designs as well as variation in methodologies across studies, which may underlie the differing outcomes reported by these studies. First, these studies were all based on acute cisterna or lumbar puncture to collect CSF samples. The method of acute dural puncture poses an inherent limitation on sampling frequency, because it can cause post-dural puncture headache, CSF leak, intracranial hypotension (Burns and Scheinfeld, 2013; Gaiser, 2006) or even venous sinus thrombosis (Wilder-Smith et al., 1997). Second, the samples were taken at only one or two time points post-administration. Furthermore, the way in which OT was delivered to the nasal passage differed among these previous studies, with some using a nasal spray (IN-OT), which is consistent with the method used in human studies, and others using aerosolized OT that is inhaled via a nebulizer and nose cone. Finally, two of the studies used monkeys that were anesthetized during dosing, and one used awake monkeys.

In the current study, we established an awake NHP model that allows for frequent and concurrent sampling of CSF and whole blood to measure the OT levels within the CNS and the peripheral vasculature following IN or IV administration of OT. The experiments were carried out in rhesus macaques that were chronically implanted with an intrathecal catheter so that the animals could be sampled repeatedly in the awake state over an extended period of time. We included IV dosing (IV-OT) because it is the principal route of OT administration for labor induction and postpartum hemorrhage (Prata et al., 2013; Stubbs, 2000) and the treatment of other conditions in humans (Hollander et al., 2003). Further, it has been reported that IV-OT can penetrate into the CNS in rodents (Mens et al., 1983).

2. Materials and methods

2.1. Animals

Four adult female rhesus monkeys *(Macaca mulatta)* between the ages of 6 and 8 years (mean body weight, 5.8–7.3 kg) were used for this study (Valley Biosystems, CA). The animals were fed a standard laboratory primate chow (TekLad, Madison, WI) twice daily and housed in accordance with Guide for the Care and Use of Laboratory Animals. All animal studies were performed as approved by the Valley Biosystems Institutional Animal Care and Use Committee. Animals were pair housed during the day except during day of dosing and sample collection, when they were housed in squeeze cages in 12:12 h light dark cycle room. Animals were monitored at all times for distress or other signs of adverse reactions. In a randomized, crossover study design, animals received OT via IV or IN routes with minimum of a one-week washout period between each administration. The same four animals were used for all IV and IN dosing procedures. Animals were dosed awake, and blood and CSF samples were drawn at predetermined time points (0 min [predose], 5, 15, 30, 60, and 120 min). All monkeys were implanted with chronic CSF catheters as described below. Because there is evidence that plasma OT changes during the course of the menstrual cycle in macaques (Falconer et al., 1980), this entire study took place during the nonbreeding season (March–September), when the macaque menstrual cycle is weak, irregular, and least likely to impact OT levels (Du et al., 2010). Based on the timing of the study and the randomized, crossover design, the effects of menstrual cycle on OT levels were not assessed.

2.2. Behavioral habituation and training for awake dosing

Animals were habituated to chair restraint using positive reinforcement for a minimum of 2 weeks prior to study. The animals did not show any obvious signs of stress during the dosing procedures.

2.3. Implantation of chronic CSF catheter

The animals were habituated and chair-trained for a minimum of two weeks prior to implantation. They were fasted overnight before surgery. On the day of surgery, the animals were immobilized with ketamine (10 mg/kg; Phoenix), dexmedetomidine (15 μg/kg; Pfizer) and Buprenorphine (0.03 mg/kg; Reckilt Benckider Pharmaceutics) intramuscularly. Animals were intubated and maintained on isoflurane (1–1.5%) anesthesia with monitoring of blood pressure, heart rate, respiration, and SPO_2. The skin was surgically prepped from mid cranium to the lumbar spine. An incision was made of the occipital ridge of the skull to approximately C4. Blunt dissection was used to expose atlantooccipital membrane at the level of cisterna magna. The atlantooccipital membrane was punctured with an 18 g needle and a custom-made polyurethane catheter was inserted. A second incision was made lateral to the lumbar spine, and the catheter was fed underneath the skin to this incision. The catheter was then connected to an access port. Once CSF flow was established, the catheter was secured to the surrounding muscle using 2–0 silk (Ethicon). Both skin incisions were closed with 3–0 Vicryl (Ethicon) in a subcuticular pattern. The animals were reversed with Antiseden (Pfizer) and allowed to recover and returned to their home cages. Post-operative analgesia included Buprenorphine (0.03 mg/kg), and either Rimadyl (2.2 mg/kg; Pfizer Animal Health) or ketofen (2 mg/kg; Fort Dodge). Cefazolin (25 mg/kg; Walgreens Critical Care) was given as a perioperative antibiotic. Animals were given a minimum of two weeks to recover from surgery prior to initiation of study. All CSF was collected from the port sterilely.

2.4. Intravenous OT administration

Animals were chair-restrained and given an intravenous bolus of OT at either 0.1, 1, or 5 IU/kg body weight using right cephalic vein. Based on average body weights over the course of the study, these doses are the equivalent of 0.58–0.73 IU, 5.5–7.3 IU, or 29–36.5 IU, depending on the individual animal's body weight. There was a minimum of a one-week washout period between each dose administration. Following IV-OT administration, timed venous blood samples were collected in EDTA tubes from the left arm cephalic vein. Concurrent blood (1.0–1.5 mL) and CSF (300 μL) samples were collected pre-dose (0 min) and at 5, 15, 30, 60, and 120 min post OT administration. Blood was kept on ice until cen-