Evidence for intranasal oxytocin delivery to the brain: recent advances and future perspectives

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Abstract

The neuropeptide oxytocin plays an evolutionarily conserved role in mammalian social behavior. To experimentally manipulate central levels of oxytocin, animal studies have adopted direct intracerebroventricular (ICV) delivery to the brain, given that only small amounts of peripherally circulating oxytocin are thought to transfer from the periphery to the central compartment. Despite striking effects on social behavior in animals, ICV drug delivery is largely impractical for human therapies. Intranasal oxytocin delivery provides a non-invasive alternative to increase central oxytocin activity, and has shown promise as a treatment for psychiatric illnesses characterized by social dysfunction. Intranasal oxytocin delivery is purported to increase central oxytocin concentrations via channels surrounding trigeminal and olfactory nerve fibres, which may facilitate increased activity at central oxytocin receptors. This review outlines the evidence for intranasal oxytocin delivery increasing central concentrations or activity, identifies current knowledge gaps, and highlights future research opportunities. Recent efforts to enhance intranasal oxytocin delivery via improved intranasal delivery technology and dose-ranging studies are also discussed.

Introduction

Oxytocin is a neuropeptide known for its role in uterine contraction during labor and milk ejection during nursing. However, emerging work has revealed its role in centrally-mediated social behaviors. Since Kosfeld and colleagues [1] first reported that intranasal oxytocin increases trust in humans over a decade ago, there has been a surge of publications reporting its effects on human social cognition and behavior. For example, research indicates that intranasal oxytocin administration improves theory of mind, memory for social cues, and increases gaze to the eye region [for a review, see 2]. These findings, among others, have sparked interest in the use of intranasal oxytocin to treat psychiatric disorders characterized by social dysfunction, with several meta-analyses supporting small but statistically significant effects on certain facets of social behavior and cognition [3-6]. Oxytocin potentially offers a novel treatment that may improve health-related quality-of-life in several severe mental illnesses via improvements in social interaction and communication [7,8].

A central premise of oxytocin research is that intranasally administered oxytocin reaches the central nervous system and increases central concentrations of oxytocin allowing increased activity at central oxytocin receptors. This transport is purported to occur via ensheathed channels surrounding olfactory and trigeminal nerve fibers after nasal spray deposition onto the nasal epithelium. The purpose of this article is to outline the evidence for intranasal oxytocin delivery to the brain. It is hoped that by encouraging a better understanding of this evidence, researchers will recognize and pursue the potential advantages of intranasal transport to improve central delivery and ultimately improve treatment of brain diseases. We will also cover recent efforts to improve nose-to-brain delivery, and offer future perspectives for the field.

Direct oxytocin delivery to the brain modulates social behavior in rodents

Oxytocin's cognitive and behavioral effects have been largely attributed to central oxytocin receptor activity [9]. As peripherally administered oxytocin is thought to enter the central compartment across the blood-brain barrier (BBB) only in very small amounts [10], researchers have used direct oxytocin

delivery to the brain to study its biobehavioral effects in animals. There is a large body of animal research reporting that intracerebroventricular (ICV) oxytocin delivery, which would diffuse oxytocin throughout the brain [11], modulates social behavior. For instance, ICV administration of oxytocin facilitates partner preference in prairie voles [12], induces maternal behavior in virgin female rats [13], and facilitates social memory in rodents [14-16]. More recent work has administered oxytocin to specific brain regions in animals. For example, oxytocin injection into the medial amygdala of mice lacking the oxytocin gene rescues social amnesia [17]. Injection into the central amygdala in rats has also been reported to have anxiolytic [18] and anti-aggressive effects [19]. Conversely, oxytocin antagonists block social behavior [20,21], highlighting the critical role of the oxytocin system.

Translating animal research to human trials

As oxytocin is an evolutionarily conserved system, researchers have investigated if these social effects found in animals also extend to humans. Some clinical trials have demonstrated effects on social cognitive outcomes. In a crossover study of children with autism spectrum disorders (ASD), six weeks of intranasal oxytocin treatment was found to improve parent-reported social behavior [22]. A recent four-week crossover trial also suggests that intranasal oxytocin improves social functioning in children with ASD, with causality further supported by the observation that the effect was particularly pronounced in children with lower pre-treatment peripheral oxytocin concentrations [23]. However, clinical trials in patients suffering from ASD or schizophrenia, both diseases characterized by deficits in social functioning, have produced inconsistent results [24,25]. Intranasal oxytocin therapy has also been suggested as a treatment in Prader-Willi syndrome (PWS), a complex neurodevelopmental genetic disorder characterized by a deficit in oxytocin-producing neurons in the hypothalamus, but the results so far in clinical trials with traditional spray delivery are conflicting. Broadly speaking, these translational difficulties might be attributable to either experimental design or mechanistic issues. Regarding experimental design, the specific choice of outcome measure may contribute to disparate results, as oxytocin may only have an effect on higher order social cognition [6]. Oxytocin studies have

also been statistically underpowered, in general, to detect replicable effects [26] or statistical equivalence [27] with the instruments utilized.

On a mechanistic level, translational issues have been attributed by some authors to species differences in central oxytocin receptor distribution [28]. However, recent work suggests that humans and rodents have similar receptor distribution across the brain [29]. If central networks are similar, then translational failures may reflect difficulties in transporting oxytocin to the brain. Given the difficulties in transporting oxytocin across the BBB due to its molecular weight, most animal studies typically administer oxytocin via ICV injection in lieu of intravenous or oral administration. As ICV delivery is impractical for human trials, researchers have turned to intranasal nasal delivery as an apparently straightforward and inexpensive means to attempt to potentially circumvent the BBB.

Using the nose to deliver therapeutics to the brain

The central premises behind intranasal delivery rest on careful study of nasal cavity physiology (Fig. 1) and a body of animal research, which has examined the transport of other peptide molecules. Almost every human oxytocin administration study of behavior or cognition has used the intranasal route for delivery to the brain. The nasal cavity provides unique access to the brain as it is the only accessible surface of the body with direct innervation to the central compartment. There are two distinct barriers preventing drugs to enter brain tissues; the BBB, incorporating capillary endothelium with high resistance tight junctions between the cells, and the blood-cerebrospinal fluid barrier, which limits brain tissue access of drugs in the CSF [30]. Thus, using CSF concentrations to index central drug transport may be an unreliable method [30,31], as intranasally administered drugs might reach brain tissues by bypassing the CSF altogether [32]. The upper part of the nose is innervated with olfactory nerve endings extending through the mucosa (cranial nerve I, originating in the cerebrum), whereas the upper and posterior regions of the nasal cavity are richly innervated by trigeminal (cranial nerve V, originating in the brainstem) and olfactory nerve fibers (Fig. 1). Intranasally delivered molecules deposited in this upper posterior region are then purported to travel to the CSF

Figure 1. Key anatomical structures in the nasal cavity relevant for oxytocin transport to the brain. Reproduced with permission from [32].

or directly to the central brain compartment along ensheathed channels surrounding the olfactory and trigeminal nerve fiber pathways. Intranasally administered peptides are therefore in principle not only transported to the CSF, but also to the amygdala and hippocampus [33], which are rich in oxytocin receptors [29].

Olfactory receptor neurons extend to the mucous layer in the olfactory epithelium [34]. Primarily, these neurons detect odorants via odorant receptors, which initiate the olfactory signalling cascade [35]. Olfactory nerve fibres traverse through small openings in the cribriform plate, pass through the subarachnoid space, and terminate in the olfactory bulbs (Fig 1). Perineural channels of protective olfactory ensheathing cells, which surround bundles of approximately 1000 olfactory nerve fibres each, may offer a transport route for intranasally administered molecules as they provide an uninterrupted route from the nasal mucosa to the olfactory bulbs [36]. Research in rodents and monkeys demonstrates that molecules transported using this extracellular route take 45-90 minutes to

appear in the olfactory bulb [37,38], and possibly much faster [39,40]. Intranasally delivered molecules may also travel to the brain via intracellular mechanisms, but this is unlikely as intracellular transport would take hours, and elevated CSF levels have been reported to occur 40-75 minutes after molecule administration [41,42]. Intranasal administration of wheat germ agglutinin-horseradish peroxidase (WGA-HRP), which at 40,000 daltons has considerably more mass than oxytocin (1007 daltons), is transported to the olfactory bulb in rats after intranasal administration [43]. Labelled molecules were also found in the olfactory epithelium and olfactory nerve layers, suggestive of transport via olfactory nerve fibres. As WGA-HRP was not meaningfully detected after intravenous administration, this supports direct nose-to-brain travel via olfactory nerve fibres.

The nasociliary branch of the ophthalmic division of the trigeminal nerve, which originates at primary trigeminal sensory nuclei via the superior orbital fissure, innervates the nasal cavity [44] (Fig. 1). Some trigeminal ganglion cells with endings in the nasal epithelium also reach into the olfactory bulb via the cribriform plate [45]. After intranasal administration of labelled Insulin-like growth factor-I in rats, traces of the label are found in both the olfactory bulbs and the brainstem [46]. Intranasal administration of hypocretin-1 is also detected in the trigeminal nerve [47].

Evidence for intranasal administration for central oxytocin delivery in humans

Despite controversies regarding nose-to-brain transportation of oxytocin [e.g., 48] there is evidence for the nose-to-brain transport in animal studies of molecules with similar properties [32,49]. The investigation of the transportation of oxytocin molecules from the nose to the central nervous system has been hampered by the lack of an oxytocin radiotracer. However, other less direct methods provide support for nose-to-brain delivery. While brain imaging studies [50-52] can be offered as evidence for nose-to-brain oxytocin effects, such studies only indicate that intranasal oxytocin can modulate neural activity, not that this change in activity occurred via transport of the molecule from the nose to the brain. Therefore, other approaches are needed. There are two plausible alternatives for direct nose-to-brain delivery. First, intranasal oxytocin, which raises peripherally circulating levels of oxytocin

[42,53-55], may cross the BBB in small amounts [10], but these quantities may still be biologically relevant [56]. If this is the case, then intravenous administration should also demonstrate effects. Indeed, early evidence that has yet to be replicated suggests that intravenous oxytocin reduces repetitive behaviors [57] and improves memory for social stimuli in ASD [58]. The second alternative to nose-to-brain delivery that would explain central activity is that intranasal oxytocin delivered to peripheral circulation leads to afferent feedback to the brain from peripheral organs rich in oxytocin receptors [59,60]. For instance, oxytocin receptors in the gastrointestinal tract [61,62] or the cardiovascular system [63] may provide feedback to the brain.

Research has shown that oxytocin concentrations in CSF increase after intranasal administration in rodents [64], macaques [41,65,66], and humans [42]. Increases in CSF oxytocin concentrations after intranasal oxytocin appear to be accompanied by concomitant increases in peripheral levels [67]. A study examining the dose-dependent effects of intranasal and intravenous oxytocin administration on CSF oxytocin levels in macaques found only the highest intranasal dose (5IU/kg) increased CSF oxytocin levels, despite no increase in plasma oxytocin levels [68].

To directly examine whether intranasally administered oxytocin crosses the BBB from the peripheral circulation, researchers can compare behavioral responses between centrally targeted administration and peripheral administration. For instance, Williams and colleagues [12] examined partner preference in prairie voles after administering oxytocin both centrally and peripherally, reporting that only central administration facilitated partner preference. Regarding humans, we recently compared the social cognitive and neural effects of single-dose intranasal administration (8IU and 24IU doses) of intravenous oxytocin (1IU) and placebo using a double-dummy crossover design. That is, participants were administered each treatment during 4 separate visits and at each visit, participants were administered an intranasal and intravenous solution, with solution contents determined by randomization. Despite both intravenous and intranasal oxytocin eliciting comparable peripheral concentrations after administration, only the 8IU intranasal dose modulated social cognition [55] and dampened right amygdala response to angry faces [50] (Fig. 2) compared to placebo

Figure 2. Dampened amygdala response to emotional stimuli after intranasal oxytocin. Right amygdala activity (z-scores) during the presentation of angry faces (A), happy faces (B), ambiguous faces (C), and shapes (D). Right amygdala activity was reduced after the administration of 8 IU oxytocin in comparison with placebo during the presentation of angry faces (A). Violin plots illustrate the distribution of the z-score normalized data by showing the probability density of the data at different values (plot tails trimmed to the range of data) with means and standard errors. * $p = 0.002$ ($q = .05$, revised FDR threshold value of $p = .008$). Reproduced with permission from [50].

administration. While there were no direct measures of central oxytocin concentration, these results suggest that intranasal administration appears to elicit disproportionate behavioral and neural effects when compared with intravenous administration. If the primary activity of oxytocin were a consequence of crossing the BBB from peripheral circulation, then similar behavioral and neural effects should have been seen after IV oxytocin administration. The comparable peripheral oxytocin concentrations following IN and IV administration helped largely rule out the notion that the central effects were mediated via effects on peripheral organs which elicited afferent feedback. However, future work should also block potential peripheral actions of oxytocin with antagonists [48].

Dosage

Most human oxytocin studies use a 24IU dose despite very few dose ranging studies to determine the optimal dose. The use of this dose is not surprising — if prior studies found an effect using this dose why use something different? There is evidence for a modest, yet significant effect on aspects of social

cognition with 24IU using a traditional nasal spray pump for drug delivery [3]. However, lower doses may offer a stronger and more consistent response [14,15,69,70]. Our own dose ranging study comparing two doses (8IU and 24IU) found the lower dose was more efficacious in eliciting neural [50] and behavioral [55] responses, which may be explained by vasopressin cross-reactivity [56]. We observed similar outcomes in a population of adults with ASD, where a lower 8IU dose (but not a 24IU dose) increased emotion salience compared to placebo [71]. It is not clear why there was no response with 24IU in this study; however, the use of a novel Exhalation Delivery System (EDS) for administration of oxytocin (more details below), an approach designed to improve nasal deposition in the target regions essential for nose-to-brain transport, may have contributed to this. Further research is needed to determine the most efficacious dose in humans, and to which extent the delivery system influences dose vs. response.

Delivery device and deposition

Site of deposition in the nose may significantly influence the efficiency of nose-to-brain delivery and the proportions of the drug transported to the brain versus absorbed systemically. For instance, when an angiotensin antagonist drug was delivered by pipette to the olfactory region of rats (15mm into the nasal cavity), twice the amount of drug was detected in the brain as compared to when the drug was deposited in the anterior respiratory region, which is only 7mm into the nasal cavity [72]. The olfactory region of humans compared to most other mammals is substantially smaller in relation to body weight [73] and more difficult to reach for reasons including the nasal valve barrier [32]. The prime function of the nose is to filter, humidify, and warm inspired air before inhalation to the delicate lining of the lungs [74]. The anterior narrow triangular shaped valve and the complex slender nasal passages of the nasal labyrinth provide the aerodynamics features required to fulfil these important physiological functions [75]. Traditional devices used to deliver drugs intranasally, such as mechanical spray pumps and propellant driven metered-dose inhalers, deliver the majority of the drug anterior to the nasal valve [76] (Fig 3.). The fraction that passes the valve mainly travels along the

A.

B.

Figure 3. (A) Spray deposition in the same participant. Initial gamma deposition (0–2 min after delivery) in the same healthy individual following self-administration with a traditional spray and three Exhalation Delivery System device variants, the standard and the nose to brain variants of the multidose liquid device and the powder device. (B) Spray deposition after Exhalation Delivery System delivery. Mean initial nasal gamma deposition in seven healthy participants 2 min after self-administration with the Exhalation Delivery System device variant. Approximately 30% and 80% of the delivered dose was deposited in the upper third and upper two-thirds, respectively. Approximately 15% was found in the lower third and a small fraction (~5%) was deposited or had been cleared to the nasopharynx 2 min after delivery. This figure displays the power device variant. Reproduced with permission from [32].

floor of the nose (Fig. 1), leaving the olfactory region and large parts of the respiratory mucosa untouched [76]. Indeed, Guastella and colleagues concluded that the current spray pumps were poorly suited to provide the delivery efficiency required for efficient and reliable nose-to-brain delivery [77]. In contrast, EDS devices significantly enhance delivery beyond the nasal valve to the upper posterior nasal segments innervated by the olfactory nerve and broadly to the respiratory mucosa with sensory innervation from the trigeminal nerve [78] (Fig. 3). The EDS device consists of a mouthpiece and a

sealing nosepiece that stents opens the valve region. When the user exhales into the mouthpiece, the soft palate elevates to seal off the nasal cavity from the oral cavity. The administered drug is released concurrently with the positive air pressure expanding the valve region, and the airflow helps carry drug deeply and broadly into target regions beyond the nasal valve. There is evidence to suggest that this approach to drug delivery may facilitate nose-to-brain drug activity. For example, a product using an EDS to deliver sumatriptan, and shown to have different pharmacokinetics than the same drug delivered via traditional spray pump, appears to produce clinical effects that are disproportionate to expectations based on only the blood pharmacokinetic profile, suggesting that part of the clinical response in the treatment of migraine is related to direct neurochemical effects [79]. In another study, this time with the easily absorbed sedative midazolam, EDS delivery of the molecule was associated with faster and more extensive sedation, similar to IV delivery that had much higher serum levels [80]. A unique nose-piece that may further optimize drug deposition after EDS delivery in the upper segments innervated by the olfactory nerve was used in the oxytocin studies described above [50,55,71].

Future perspectives

Successful radiolabeling of oxytocin or the development of specific ligands binding to oxytocin receptors approved for human use would be important steps to facilitate progress in the study of nose-to-brain drug transport. The inclusion of IV comparators, when the study design allows, will expand our knowledge of oxytocin delivery to the brain, or at least direct activity not attributable to serum levels. Research suggests an important role of the method of drug delivery on the effect produced and on dose and, possibly, dosing frequency. If specific brain regions are the target for the nasally delivered oxytocin, and if off-target peripheral activity may produce antagonistic effects, it may be crucial to optimize direct nose-to-brain transport and limit systemic absorption. Reduced systemic absorption is also important to reduce the risk of short-term adverse events and long-term side effects that can limit the usefulness of the treatment. Dose-ranging and dose-response studies to determine the optimal dose

for the various potential clinical indications, carefully considering the delivery performance and efficiency of the device, are also required. Oxytocin has shown promise for the treatment of ASD [23], schizophrenia [81], post-traumatic stress disorder [82], alcohol dependence [83], and social anxiety [69]. Properly designed, long-term, larger, and well-controlled intervention trials are required to verify the therapeutic potential and role of intranasal oxytocin as a treatment modality in psychiatric and neurodevelopmental disorders.

Acknowledgements

The authors would like to thank Ramy Mahmoud for helpful comments on an earlier version of this manuscript. DSQ is supported by an Excellence Grant from the Novo Nordisk Foundation (NNF16OC0019856).

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