REVIEW

Intranasal Delivery to the Central Nervous System: Mechanisms and Experimental Considerations

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ABSTRACT: The blood–brain barrier (BBB) limits the distribution of systemically administered therapeutics to the central nervous system (CNS), posing a significant challenge to drug development efforts to treat neurological and psychiatric diseases and disorders. Intranasal delivery is a noninvasive and convenient method that rapidly targets therapeutics to the CNS, bypassing the BBB and minimizing systemic exposure. This review focuses on the current understanding of the mechanisms underlying intranasal delivery to the CNS, with a discussion of pathways from the nasal cavity to the CNS involving the olfactory and trigeminal nerves, the vasculature, the cerebrospinal fluid, and the lymphatic system. In addition to the properties of the therapeutic, deposition of the drug formulation within the nasal passages and composition of the formulation can influence the pathway a therapeutic follows into the CNS after intranasal administration. Experimental factors, such as head position, volume, and method of administration, and formulation parameters, such as pH, osmolarity, or inclusion of permeation enhancers or mucoadhesives, can influence formulation deposition within the nasal passages and pathways followed into the CNS. Significant research will be required to develop and improve current intranasal treatments and careful consideration should be given to the factors discussed in this review. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:1654–1673, 2010

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INTRODUCTION

Despite the immense network of the cerebral vasculature, systemic delivery of therapeutics to the central nervous system (CNS) is not effective for greater than 98% of small molecules and for nearly 100% of large molecules. The lack of effectiveness is due to the presence of the blood–brain barrier (BBB), which prevents most foreign substances, even many beneficial therapeutics, from entering the brain from the circulating blood. While certain small molecule, peptide, and protein therapeutics given systemically reach the brain parenchyma by crossing the BBB, generally high systemic doses are needed to achieve therapeutic levels, which can lead to adverse effects in the body. Therapeutics can be introduced directly into the CNS by intracerebroventricular or intraparenchymal injections; however, for multiple dosing
Intranasal delivery has come to the forefront as an alternative to invasive delivery methods to bypass the BBB and rapidly target therapeutics directly to the CNS utilizing pathways along olfactory and trigeminal nerves innervating the nasal passages.3–5

The primary goal of this review is to discuss the present understanding of the pathways and mechanisms underlying intranasal drug delivery to the CNS. With this background in mind, experimental considerations and formulation strategies for enhancing intranasal drug delivery and targeting to the CNS will be discussed. This review will also briefly highlight the diversity of therapeutic drugs that have been shown to be delivered to the CNS intranasally, the details of which have been recently published in several comprehensive reviews.3–5

The intranasal route of administration is not a novel approach for drug delivery to the systemic circulation. The novelty lies in using this non-invasive method to rapidly deliver drugs directly from the nasal mucosa to the brain and spinal cord with the aim of treating CNS disorders while minimizing systemic exposure. Early research demonstrated that tracers, such as wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), were transported within olfactory nerve axons to reach the olfactory bulbs in the CNS.10 These findings were subsequently confirmed in a quantitative study comparing intranasal and intravenous administration of WGA-HRP.11 Direct intranasal delivery of therapeutics to the brain was first proposed and patented in 1989 by William H. Frey II of the Alzheimer’s Research Center.12,13 Subsequently, numerous reports have shown that therapeutics given by the intranasal route are delivered to the CNS and have the potential to treat neurological diseases and disorders.3,5,6,14

Intranasal administration of insulin, which is currently under investigation for the treatment of Alzheimer’s disease, was initially developed as a noninvasive alternative to subcutaneous insulin injections used by diabetic patients. Insulin, like many therapeutic peptides and proteins, is not effective when given orally because of the rapid degradation that occurs in the gastrointestinal tract resulting in a poor pharmacokinetic profile. In order to enter the systemic circulation, intranasal formulations of insulin have required the use of enzyme inhibitors, mucoadhesives, and absorption enhancers to overcome barriers present in the nasal passages that limit systemic bioavailability. Nasal irritation from these additives, in addition to high and frequent dosing regimens, resulted in limited clinical success with intranasal insulin for diabetes management.15

Several decades after initial investigations of intranasal insulin, use of the intranasal method was proposed for direct delivery of insulin to the brain along olfactory pathways for the treatment of Alzheimer’s disease and other brain disorders.16 Using this method, researchers discovered profound improvements in memory and mood in normal individuals following intranasal administration of insulin17 and an insulin analog.18 Intranasal insulin did not alter blood insulin or glucose levels to cause these effects, consistent with observations noted in earlier investigations. Instead, the protein rapidly gains direct access to the CSF following intranasal administration19 and, similar to insulin-like growth factor-I (IGF-I), also likely gains direct access to the brain itself from the nasal mucosa.4 Intranasal insulin is now being considered as a treatment for Alzheimer’s disease, considered by some to involve “diabetes of the brain” or “type 3 diabetes,”20 and clinical investigations are underway in patients with the disease. Intranasal insulin dose-dependently improves memory after acute treatment,21,22 and improves attention, memory, and cognitive function after 21 days of intranasal treatment.23

In addition to insulin, other peptides and proteins administered by the intranasal route are proving to have beneficial effects in humans. For example, an eight amino acid peptide fragment of activity-dependent neuroprotective protein (ADNP) is in Phase II clinical trials for the treatment of mild cognitive impairment and schizophrenia and is also in development for treating Alzheimer’s disease.24 The weight regulatory peptide, melanocortin, reaches the CSF in humans within minutes of intranasal administration, without affecting blood concentrations,19 and decreases body weight in normal volunteers after chronic intranasal administration for 6 weeks.25 The peptide hormone, oxytocin, has been intranasally delivered to humans, resulting in significant changes in centrally mediated behaviors, such as increased trust.26,27
decreased fear and anxiety,\textsuperscript{25,29} and improved social behavior,\textsuperscript{30–32} and social memory.\textsuperscript{33}

In animals, detailed pharmacokinetic and pharmacodynamic studies have shown that a broad spectrum of therapeutics not only reach specific areas of the brain, but also have effects on CNS-mediated behaviors within a short time frame, making the case for a rapid, extracellular pathway into the brain following intranasal administration. Small, lipophilic molecules, such as cocaine,\textsuperscript{34} morphine,\textsuperscript{35,36} raltitrexed,\textsuperscript{37} and testosterone,\textsuperscript{38,39} are able to reach the brain after intranasal administration in rodents. Intranasal studies with these drugs demonstrate that in addition to a portion of the drug being absorbed into the blood from the nasal mucosa, the drug gains access to the brain via direct pathways from the nasal cavity. Cocaine effects are observable within minutes of nasal administration, even before being detectable in the blood, indicating that an alternative pathway into the brain exists.\textsuperscript{34} Benzoylecgonine, the polar metabolite of cocaine, also reached the brain after intranasal administration via direct pathways, to a greater extent than cocaine.\textsuperscript{40} Intranasal administration of larger therapeutics, such as the protein hormone, leptin, results in direct delivery to the CNS\textsuperscript{41} with significant reductions in food intake in rats.\textsuperscript{42,43} Recently, intranasal leptin was shown to have anti-convulsant effects in rodent models of epilepsy.\textsuperscript{44,45} The largest therapeutic protein reported to be delivered to the brain after intranasal administration in animals is nerve growth factor (NGF, 27.5 kDa), which reached multiple brain regions in rats, with the greatest concentrations in the olfactory bulbs.\textsuperscript{46,47} Further, intranasal administration of NGF demonstrated neuroprotective effects in cerebral ischemic rats\textsuperscript{48} and reduced tau hyperphosphorylation and A\textsubscript{\textbeta} accumulation in mouse model of Alzheimer’s disease.\textsuperscript{49,50} Recently, it was shown that intranasal administration of an oligonucleotide inhibited brain tumor growth and increased survival in rats.\textsuperscript{51} Further, different sizes of plasmid DNA, ranging from 3.5 to 14.2 kb, were successfully delivered to the brain intact after intranasal administration in rats.\textsuperscript{52} A recent report demonstrated that mesenchymal stem cells and glioma cells were delivered to the brain within 1 h of intranasal administration to rodents, indicating that intranasal delivery may facilitate the use of stem cells for treating CNS disorders.\textsuperscript{53}

While there are numerous examples of the success and potential of intranasal delivery to rapidly target a great diversity of CNS therapeutics to the brain and spinal cord, direct transport following intranasal administration is not always evident. Researchers from Leiden University maintain that for several different therapeutics evaluated in their lab, including hydroxycovalam (vitamin B12), melatonin, and estradiol, no evidence has been found for direct transport into the CSF following intranasal compared to intravenous administration.\textsuperscript{54–56} Using microdialysis, other researchers have observed limited distribution of lidocaine,\textsuperscript{57} fluorescein labeled dextran,\textsuperscript{58} and stavudine\textsuperscript{59} following intranasal compared to intravenous administration. Interestingly, while van den Berg et al.\textsuperscript{55} concluded that intranasal estradiol held no advantage in drug targeting to the CSF over intravenous administration,\textsuperscript{56} other groups have shown that intranasal estradiol, as well as an estradiol prodrug, significantly target the brain relative to the intravenous route.\textsuperscript{60,61} Born et al.\textsuperscript{19} have shown that melatonin and vitamin B12 reach the CSF in humans within minutes of nasal administration without changing blood concentration. These contrasting conclusions for similar drugs may be due to differences in methodologies employed in studies and raise important issues relating to experimental and formulation factors that can significantly influence the outcome of studies. Understanding the pathways and mechanisms underlying intranasal delivery to the CNS is critical to advance the development of intranasal treatments for neurological diseases and disorders.

**PATHWAYS AND MECHANISMS**

While the exact mechanisms underlying intranasal drug delivery to the CNS are not entirely understood, an accumulating body of evidence demonstrates that pathways involving nerves connecting the nasal passages to the brain and spinal cord are important. In addition, pathways involving the vasculature, cerebrospinal fluid, and lymphatic system have been implicated in the transport of molecules from the nasal cavity to the CNS. It is likely that a combination of these pathways is responsible, although one pathway may predominate, depending on the properties of the therapeutic, the characteristics of the formulation, and the delivery device used.
Olfactory Nerve Pathways

Therapeutics can rapidly gain access to the CNS following intranasal administration along olfactory nerve pathways leading from the nasal cavity directly to the CNS. Olfactory nerve pathways are a major component of intranasal delivery, evidenced by the fact that fluorescent tracers are associated with olfactory nerves as they traverse the cribriform plate, drug concentrations in the olfactory bulbs are generally among the highest CNS concentrations observed, and a strong, positive correlation exists between concentrations in the olfactory epithelium and olfactory bulbs. Olfactory pathways arise in the upper portion of the nasal passages, in the olfactory region, where olfactory receptor neurons (ORNs) are interspersed among supporting cells (sustentacular cells), microvillar cells, and basal cells (Fig. 1, Box A). ORNs mediate the sense of smell by conveying sensory information from the peripheral environment to the CNS. Beneath the epithelium, the lamina propria contains mucus secreting Bowman’s glands, axons, blood vessels, lymphatic vessels, and connective tissue. The dendrites of ORNs extend into the mucous layer of the olfactory epithelium, while axons of these bipolar neurons extend centrally through the lamina propria and through perforations in the cribriform plate of the ethmoid bone, which separates the nasal and cranial cavities (Fig. 1, Box B). The axons of ORNs pass through the subarachnoid space containing CSF and terminate on mitral cells in the olfactory bulbs. In addition to ORNs, chemo sensory neurons located at the anterior tip of the nasal cavity in the Grueneberg ganglion lead into the olfactory bulbs.

The unique characteristics of the ORNs contribute to a dynamic cellular environment critical for intranasal delivery to the CNS. Due to the direct contact with toxins in the external environment, ORNs regenerate every 3–4 weeks from basal cells residing in the olfactory epithelium. As a result, proteins characteristic of the BBB (i.e., proteolytic enzymes, tight junction proteins, efflux transporters), which are present in the nasal passages, may not be fully functional during the maturation of ORNs. The nasal barrier to the CNS could be considered “leaky” from the constant turnover of the ORNs. Special Schwann cell-like cells called olfactory ensheathing cells (OECs) envelope the axons of ORNs and have an important role in axonal regeneration, regrowth, and remyelination. The OECs create continuous, fluid-filled perineurial channels that, interestingly, remain open, despite the degeneration and regeneration of ORNs.

Given the unique environment of the olfactory epithelium, it is possible for intranasally administered therapeutics to reach the CNS via extracellular or intracellular mechanisms of transport along olfactory nerves. Extracellular transport mechanisms involve the rapid movement of molecules between cells in the nasal epithelium, requiring only several minutes to 30 min for a drug to reach the olfactory bulbs and other areas of the CNS after intranasal administration. Transport likely involves bulk flow mechanisms within the channels created by the OECs (Fig. 1, Box B). Drugs may also be propelled within these channels by the structural changes that occur during depolarization and axonal propagation of the action potential in adjacent axons. Intracellular transport mechanisms involve the uptake of molecules into ORNs by passive diffusion, receptor-mediated endocytosis or adsorptive endocytosis, followed by slower axonal transport, taking several hours to days for a drug to appear in the olfactory bulbs and other brain areas. Intracellular transport in ORNs has been demonstrated for small, lipophilic molecules such as gold particles, aluminum salts, and for substances with receptors on ORNs such as WGA-HRP.

Intracellular mechanisms, while important for certain therapeutics, are not likely to be the predominant mode of transport into the CNS. The vast majority of published intranasal studies demonstrate rapid delivery, with high CNS concentrations and effects observed almost immediately after or within an hour of intranasal administration, consistent with rapid extracellular mechanisms of transport. Further, receptor-mediated transport mechanisms involve specific interactions and cannot account for the broad spectrum of therapeutics shown to be delivered to the CNS following intranasal administration. While some large molecules, such as galanin-like peptide (GALP), exhibit saturable transport pathways into the CNS, for other large molecules such as NGF and insulin-like growth factor-I (IGF-I), intranasal delivery into the brain is nonsaturable and not receptor mediated.
Figure 1. Pathways of drug distribution in the nasal cavity and central nervous system. Following intranasal administration, drugs (blue circles) come into contact with the nasal mucosa, which is innervated by olfactory and trigeminal nerves. The nasal mucosa is comprised of the nasal epithelium, which contains various cell types, and the underlying lamina propria, which contains blood vessels, axons, glands, and connective tissue. (A) In the respiratory region, ciliated epithelial cells and mucous secreting goblet cells in the epithelium form the basis of mucociliary clearance mechanisms that remove foreign substances from the mucous layer towards the nasopharynx for elimination. Trigeminal nerve endings residing in the respiratory and olfactory epithelium convey chemosensory information to the CNS. In the olfactory region, olfactory receptor neurons are interspersed among supporting cells and basal cells to form the olfactory epithelium. Drugs can be transported through the nasal mucosa to the CNS by entering perivascular channels (dashed lines surrounding blood vessels) in the lamina propria or via extracellular or intracellular mechanisms involving olfactory and trigeminal nerves (dashed arrows). The blood supply to the respiratory epithelium is relatively greater compared to the olfactory epithelium, making it an ideal site for systemic absorption of nasally applied drugs. (B) After reaching the lamina propria, drugs can enter channels created by olfactory ensheathing cells surrounding the olfactory nerves, where they can access the cerebrospinal fluid (CSF) and olfactory bulbs (dashed arrows). (C) From the CSF, drugs can be distributed via bulk flow mechanisms and mix with brain interstitial fluid throughout the brain (dashed arrows). Drugs can also enter perivascular spaces after reaching the brain to be rapidly distributed throughout the CNS. Drugs that entered perivascular spaces from the nasal mucosa can also exit these spaces in the brain. These same pathways in the reverse direction are involved in the clearance of solutes from the CNS to the periphery.
**Trigeminal Nerve Pathways**

An often overlooked but important pathway connecting the nasal passages to the CNS involves the trigeminal nerve, which innervates the respiratory and olfactory epithelium of the nasal passages and enters the CNS in the pons.\(^{70,95}\) Interestingly, a small portion of the trigeminal nerve also terminates in the olfactory bulbs.\(^{96}\) The cellular composition of the respiratory region of the nasal passages is different from that of the olfactory region, with ciliated epithelial cells distributed among mucus secreting goblet cells (Fig. 1, Box A). These cells contribute to mucociliary clearance mechanisms that remove mucus along with foreign substances from the nasal cavity to the nasopharynx. The trigeminal nerve conveys sensory information from the nasal cavity, the oral cavity, the eyelids, and the cornea, to the CNS via the ophthalmic division (V1), the maxillary division (V2), or the mandibular division (V3) of the trigeminal nerve.\(^{70,95}\) Branches from the ophthalmic division of the trigeminal nerve provide innervation to the dorsal nasal mucosa and the anterior portion of the nose, while branches of the maxillary division provide innervation to the lateral walls of the nasal mucosa. The mandibular division of the trigeminal nerve extends to the lower jaw and teeth, with no direct neural inputs to the nasal cavity. The three branches of the trigeminal nerve come together at the trigeminal ganglion and extend centrally to enter the brain at the level of the pons, terminating in the spinal trigeminal nuclei in the brainstem. A unique feature of the trigeminal nerve is that it enters the brain from the respiratory epithelium of the nasal passages at two sites: (1) through the anterior lacerated foramen near the pons and (2) through the cribriform plate near the olfactory bulbs, creating entry points into both caudal and rostral brain areas following intranasal administration. While there are no published reports of ensheathing cells and channels associated with the trigeminal nerve comparable to those observed with the olfactory nerves, these anatomical features may be present along the trigeminal nerve. It is also likely that other nerves that innervate the face and head, such as the facial nerve, or other sensory structures in the nasal cavity, such as the Grueneberg ganglion, may provide entry points for intranasally applied therapeutics into the CNS.

Intranasal drug delivery along trigeminal pathways was first clearly demonstrated for \(^{125}\)I-IGF-I, where high levels of radioactivity were observed in the trigeminal nerve branches, trigeminal ganglion, pons, and olfactory bulbs, consistent with delivery along both trigeminal and olfactory nerves.\(^{4}\) Because one portion of the trigeminal neural pathway enters the brain through the cribriform plate alongside the olfactory pathway, it is difficult to distinguish whether intranasally administered drugs reach the olfactory bulb and other rostral brain areas via the olfactory or trigeminal pathways or if both are involved. Intranasal studies with other proteins and peptides, including interferon-\(\beta\) (IFN-\(\beta\)), hypoxi-tin-1,\(^{66,68}\) hypocretin-1,\(^{69,94}\) and peptoids,\(^{67}\) found similar results of high levels of radioactivity in the trigeminal nerve. It is important to note that these results came from one lab and that drug concentrations in the trigeminal nerve are not commonly measured in intranasal delivery studies. Other researchers have found significant drug distribution to caudal brain areas such as the brainstem and cerebellum after intranasal delivery, suggesting the involvement of the trigeminal nerves, though researchers were likely unaware of this pathway.\(^{63,93,97}\)

**Vascular Pathways**

Traditionally, the intranasal route of administration has been utilized to deliver drugs to the systemic circulation via absorption into the capillary blood vessels underlying the nasal mucosa. The nasal mucosa is highly vascular, receiving its blood supply from branches of the maxillary, ophthalmic and facial arteries, which arise from the carotid artery.\(^{70,98}\) The olfactory mucosa receives blood from small branches of the ophthalmic artery, whereas the respiratory mucosa receives blood from a large caliber arterial branch of the maxillary artery.\(^{99}\) The relative density of blood vessels is greater in the respiratory mucosa compared to the olfactory mucosa (Fig. 1, Box A), making the former region an ideal site for absorption into the blood.\(^{99}\) The vasculature in the respiratory region contains a mix of continuous and fenestrated endothelia,\(^{100,101}\) allowing both small and large molecules to enter the systemic circulation following nasal administration.

Delivery to the CNS following absorption into the systemic circulation and subsequent transport across the BBB is possible, especially for small, lipophilic drugs, which more easily enter the blood.
stream and cross the BBB compared to large, hydrophilic therapeutics such as peptides and proteins. It is also possible that rather than being distributed throughout the systemic circulation, drugs can enter the venous blood supply in the nasal passages where they are rapidly transferred to the carotid arterial blood supply feeding the brain and spinal cord, a process known as counter-current transfer. However, delivery through the systemic circulation results in problems related to drug elimination via hepatic and renal mechanisms, and is limited by other factors including: the BBB, drug binding to plasma proteins, degradation by plasma proteases, and potential peripheral side effects.

Increasing evidence is emerging suggesting that mechanisms involving channels associated with blood vessels, or perivascular channels, are involved in intranasal drug delivery to the CNS (Fig. 1, Box A and Box C). Perivascular spaces are bound by the outermost layer of blood vessels and the basement membrane of the surrounding tissue. These perivascular spaces act as a lymphatic system for the brain, where neuron-derived substances are cleared from brain interstitial fluid by entering perivascular channels associated with cerebral blood vessels (Fig. 1, Box C). For example, radiolabeled tracers, India ink, and amyloid beta, have been shown to be cleared from the brain via perivascular spaces. Perivascular transport is due to bulk flow mechanisms, as opposed to diffusion alone, and arterial pulsations are also a driving force for perivascular transport. The resulting “perivascular pump” can account for the rapid distribution of therapeutics throughout the brain. Intranasally applied drugs can move into perivascular spaces in the nasal passages or after reaching the brain and the widespread distribution observed within the CNS could be due to perivascular transport mechanisms.

Several intranasal studies show high levels of drug present in the walls of cerebral blood vessels and carotid arteries, even after removal of blood by saline perfusion, suggesting that intranasally administered drugs can gain access to perivascular spaces.

Pathways Involving the Cerebrospinal Fluid and Lymphatics

Pathways connecting the subarachnoid space containing CSF, perineurial spaces encompassing olfactory nerves, and the nasal lymphatics are important for CSF drainage and these same pathways provide access for intranasally applied therapeutics to the CSF and other areas of the CNS. Several studies document that tracers injected into the CSF in the cerebral ventricles or subarachnoid space drain to the underside of the olfactory bulbs into channels associated with olfactory nerves traversing the cribriform plate and reach the nasal lymphatic system and cervical lymph nodes. Drugs can access the CNS via these same pathways after intranasal administration, moving from the nasal passages to the CSF to the brain interstitial spaces and perivascular spaces for distribution throughout the brain. These drainage pathways are significant in a number of animal species (sheep, rabbits, and rats) accounting for approximately 50% of CSF clearance. However, in humans, solutes are primarily cleared into the blood due to pressure differences at arachnoid granulations present on blood vessels in the subarachnoid space. Pathways between the nasal passages and the CSF are still important and functional in humans, evidenced by the fact that therapeutics are directly delivered to the CSF following intranasal delivery, without entering the blood to an appreciable extent.

A number of intranasal studies demonstrate that drugs gain direct access to the CSF from the nasal cavity, followed by subsequent distribution to the brain and spinal cord. Many intranasally applied molecules rapidly enter the CSF, and this transport is dependent on the lipophilicity, molecular weight, and degree of ionization of the molecules. Assessing distribution into the CSF can provide information on the mechanism of intranasal delivery. For example, observing a decreasing concentration gradient from the CSF to brain tissues or observing drug distribution to brain areas distant from the olfactory bulbs are consistent with distribution via the CSF. However, trigeminal-mediated transport also plays a role in distribution of intranasally administered drugs to brain areas distant from the olfactory bulbs. It is difficult to experimentally separate contributions of different pathways into the CNS after intranasal administration.

**EXPERIMENTAL CONSIDERATIONS**

It is important to consider the different methodologies used in intranasal studies, since factors...
such as head position, method of delivery, including surgical interventions (for animal studies), and delivery volume, can all influence drug deposition in the nasal cavity and the pathway a drug follows to the CNS after intranasal administration.

**Head Position**

The majority of preclinical work has been carried out in anesthetized mice and rats, with animals positioned in the supine position. In experiments evaluating dye deposition in the nasal passages, Thorne et al.\(^ {11} \) found that nose drops administered to animals lying on their backs resulted in consistent deposition in the olfactory epithelium. Optimal delivery to the CNS along neural pathways required targeting of the drug to the upper-third of the nasal cavity.\(^ {13} \) van den Berg et al.\(^ {131} \) found that different head positions can alter absorption into the blood and CSF following nasal administration to rats when a tube inserted into the nostrils was used to deliver the drug solution. A supine position with the head angle at 70° or 90° was found to be most suitable for efficient delivery to the CSF using this method of intranasal administration. This head position would also likely favor drainage into the esophagus and trachea, which is why most researchers position animals with the head at 0° (horizontal). For chronic dosing regimens, Hanson et al.\(^ {94} \) developed an intranasal method for delivery in unanesthetized mice. The efficiency of delivery to brain tissues is approximately fivefold less with awake intranasal administration because of the reduced time that mice are held in position on their backs.\(^ {94} \) Rats generally do not tolerate intranasal delivery in the unanesthetized state; however there are some reports of effective, minimal stress, intranasal delivery techniques in freely moving rats.\(^ {132} \) Repeated use of isoflurane or ketamine anesthesia can be effective for chronic dosing in rats.

In nonhuman primates and clinical studies, different head positions can also influence the deposition of nasal drops in the nasal cavity.\(^ {133,134} \) When the head is tilted back, a liquid latex dye was shown to deposit primarily on the floor of the nasal cavity.\(^ {133} \) When the head is extended off the side of a bed to tilt the head back further (Mygind’s position) or when the head is positioned on the side and down (Ragan position), the dye reached the respiratory region of nasal cavity.\(^ {133} \) The most promising position for targeting the olfactory region is with the “praying to Mecca” position, with the head-down-and-forward, however this position can be uncomfortable for patients, which could result in compliance issues.

**Administration Technique**

Differences in administration techniques employed by researchers can affect deposition within the nasal epithelium and delivery along pathways to the CNS. For intranasal drug administration in anesthetized mice and rats, several researchers administer nose drops over a period of 10–20 min using a pipettor for delivering drops to alternating nostrils every 1–2 min to allow the solution to be absorbed into the nasal epithelium.\(^ {4,50,66,67,69,135–137} \) This noninvasive method is preferred as it does not involve inserting the pipette tip into the nostril. Instead, drops are placed at the opening of the nostril, allowing the animal to sniff the drop into the nasal cavity. For rats, which are obligatory nose breathers, the opposite nostril is occluded while introducing the nose drop to allow the drop to be “sniffed” forcefully to deliver the formulation to the respiratory and olfactory epithelia.\(^ {11} \) Other administration methods in anesthetized rats involve sealing the esophagus and inserting a breathing tube into the trachea to prevent the nasal formulation from being swallowed and to eliminate issues related to respiratory distress.\(^ {34,46,41,138} \) Flexible tubing can be inserted into the nostrils for localized delivery of a small volume of the drug solution to the respiratory or olfactory epithelia, depending on the length of the tubing.\(^ {34,54,55,63,131,139–141} \) When using tubing for intranasal administration, care must be taken to avoid damaging the nasal mucosa and to avoid delivery through the nasopharynx into the mouth and throat. It is also important to note that the length of the tubing can affect deposition in the respiratory and olfactory epithelia and delivery to the CNS and blood.\(^ {140} \)

In clinical studies, nasal delivery devices, such as sprays, nose droppers or needle-less syringes, can target the drug to different regions of the nasal cavity. OptiMist\(^ {\text{TM}} \) is a breath actuated device that targets liquid or powder nasal formulations to the nasal cavity, including the olfactory region, without deposition in the lungs or esophagus.\(^ {142} \) This device is promising for targeting therapeutics to the olfactory epithelium for direct transport into the CNS along olfactory nerves; however studies so far have only evalu-
ated deposition patterns and clearance rates in the nasal cavity. The ViaNase™ device can also be used to target a nasal spray to the olfactory and respiratory epithelia of the nasal cavity. Nasal drops tend to deposit on the nasal floor and are subjected to rapid mucociliary clearance, while nasal sprays are distributed to the middle meatus of the nasal mucosa. For intranasal insulin, use of a needle-less syringe or the ViaNase™ electronic atomizer have been shown to be effective to improve memory in patients with Alzheimer’s disease. Efficient delivery to the CNS can be achieved in humans by selecting the right combination of head position, formulation and delivery device to target the therapeutics to specific regions of the nasal cavity.

Volume

While differences in delivery volumes can affect the deposition within the nasal cavity and distribution to the CNS, no systematic studies have been published that evaluate the effect of solution volume on the efficiency of intranasal delivery to the CNS. Delivery volume is important in terms of covering the surface area of the nasal passages in order for the drug to reach the respiratory and olfactory epithelia for transport to the CNS along trigeminal and olfactory neural pathways. The olfactory system in rats is far more extensive as compared to humans, with the olfactory region in rats occupying approximately 50% of the surface area of the nasal cavity and with a nasal cavity volume of 0.26 cm³. In the majority of intranasal studies, rats receive a total volume of 40–100 μL given as 6–10 μL nose drops using a pipettor or given all at once using flexible tubing. The volume of the nose drop can also affect deposition in the nasal passages, where a small volume drop (i.e., 2 μL) will likely result in deposition primarily in the respiratory epithelium and a large volume drop (i.e., 20 μL) will result in deposition in the nasopharynx and could lead to respiratory distress. If tubing is used for drug administration, lower volumes of 20–40 μL are used for intranasal delivery because there is less surface area to cover. In mice, a total volume of 24 μL is administered in 3–4 μL nose drops. This total volume is less than the volume of the nasal cavity in mice (0.032 cm³). In humans, the nasal cavity has a volume of 25 cm³ and the olfactory region occupies 8% of the nasal cavity surface area. Intranasal drug delivery volumes of 0.4 mL administered in 100 μL aliquots clearly result in CNS effects in humans and 200 μL administered in 100 μL aliquots may also be sufficient.

Despite anatomical differences between rodents and humans, similar pathways are involved in intranasal delivery to the CNS, at least for IFNβ-1b in rats and primates and for melatonin in rats and humans. Translation into the clinic is currently underway, with clinical trials of intranasal treatments for Alzheimer’s disease demonstrating success. This is a testament to the fact that the same direct pathways into the CNS utilized in animals are also important and functional in humans.

Assessment of CNS Distribution

Assessing concentrations and distribution to different brain areas provides insight into pathways followed to the CNS after intranasal administration. For example, greater distribution in the olfactory bulbs and frontal cortex compared to the cerebellum and brainstem would be consistent with pathways involving the olfactory nerves following intranasal administration. Whole brain measurements of drug concentration generally underestimate the extent of distribution because of dilution effects and do not provide any information about pathways and mechanisms underlying delivery to the CNS after nasal administration. In many studies, drug concentrations in the CSF act as a surrogate for brain exposure, particularly in studies conducted in humans, even though concentrations in brain and CSF compartments are not necessarily the same.

Perhaps of greater importance is the evaluation of drug targeting, which evaluates the relative distribution of the drug to therapeutic target sites (i.e., brain or specific brain area) compared to exposure to nontarget sites (i.e., blood, spleen or other peripheral tissues). Intravenous delivery is used as a control for evaluating blood-mediated delivery to the CNS. Since concentrations observed in the CNS after intranasal administration could be due to absorption into the nasal vasculature followed by distribution from the systemic circulation, intravenous delivery controls for distribution into the CNS from the blood. Comparing ratios of brain concentrations to blood concentrations after intranasal and intravenous administration provides an assessment of direct transport to the brain. Brain-to-blood ratios that are greater with intranasal compared to intravenous administration indicate that direct
pathways other than the vasculature are important for transport from the nasal cavity to the CNS. An alternative to determining brain-to-blood concentration ratios includes designing experiments such that blood exposure after intranasal and intravenous administration are similar (i.e., similar AUC), which allows for direct comparisons of concentrations between different routes of administration. Drug targeting efficiency (DTE, determines the fraction of the brain AUC observed after intranasal administration involving pathways other than the vasculature. Intranasal compared to intravenous administration generally results in greater brain-to-blood ratios and drug targeting efficiency. These measures are helpful in comparing findings across different studies conducted in different labs and are useful for assessing the effects of formulations on enhancing intranasal delivery to the CNS.

FORMULATION CONSIDERATIONS

Protective barriers in the nasal mucosa contribute to the low efficiency of delivery observed following intranasal administration, with typically less than 1% of the administered dose reaching the brain. Research efforts have focused on the development of formulation strategies to overcome the barriers present in the nasal mucosa to improve intranasal delivery efficiency and targeting to the CNS. Nasal mucociliary clearance mechanisms are in place to remove foreign substances towards the nasopharynx, which is accomplished by dissolution of substances in the mucus layer and transport by ciliated cells in the nasal epithelium. Efflux transport proteins, such as p-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP1), are expressed in the nasal mucosa and can significantly limit the uptake of substrates into the brain. In addition, there is evidence of drug metabolizing enzymes and tight junction proteins in the nasal epithelium, which can limit the efficiency of intranasal delivery to the CNS. The nasal vasculature can also be a limiting factor as it clears inhaled toxins and intranasally applied therapeutics into the systemic circulation for detoxification and elimination. Common themes in formulation approaches to overcome these barriers involve improving drug solubility, increasing permeability across the nasal epithelium, reducing clearance from the nasal passages, or a combination approach. While recently published reviews discuss formulation considerations for intranasal delivery, here we focus on how changes in formulation parameters can affect CNS distribution and drug targeting after intranasal administration.

Formulation Strategies to Improve Drug Solubility

In order for a therapeutic to have adequate absorption and bioavailability in the CNS after intranasal administration, it should have sufficient solubility at the site of delivery in the nasal epithelium. Drugs can be encapsulated in carriers, such as cyclodextrins, microemulsions, and nanoparticles, to overcome these issues for intranasal delivery to the CNS. Cyclodextrin inclusion complexes containing a hydrophobic cavity and a hydrophilic shell improve the solubility of poorly water-soluble drugs, enhancing brain uptake after intranasal administration. Galanin-like peptide (GALP) mixed with alpha-cyclodextrin resulted in enhanced delivery to all brain regions by two- to threefold, with the greatest uptake in the olfactory bulbs and hypothalamus, while GALP mixed with betacyclodextrin resulted in enhanced uptake of GALP specifically to the olfactory bulbs compared to a simple intranasal solution. It may be possible that alpha-cyclodextrin modulates the transport of GALP in perivascular spaces, which could explain the increased concentrations observed throughout the brain. Beta-cyclodextrin appears to specifically enhance intranasal delivery to the CNS along olfactory pathways. These results indicate that in addition to improving drug solubility, cyclodextrins added to intranasal formulations can allow for targeting to specific brain regions.

Microemulsion and nanoemulsion formulations can improve drug solubility and opportunities for direct transport into the CNS. These oil-in-water dispersions demonstrate increased brain uptake for small molecule therapeutics such as clonazepam, sumatriptan, risperidone, zolmitriptan, and nimodipine. However, for clonazepam, sumatriptan succinate, and risperidone, the increased brain uptake was accompanied by increased uptake into the blood,
resulting in drug targeting efficiencies that were comparable to simple intranasal solutions. Increased systemic exposure can lead to adverse side effects, which could be problematic for certain therapeutics. Studies with nimodipine showed the greatest increase in targeting in the olfactory bulbs (4.5-fold); suggesting that delivery along olfactory pathways was enhanced with this microemulsion formulation approach. An emulsion-like formulation was recently patented for use with water-insoluble peptides and proteins, and preliminary data presented at the 2007 Society for Neuroscience meeting demonstrate that a lipid emulsion of growth differentiation factor 5 (GDF5) increased delivery to all regions of the CNS and to the trigeminal nerve, compared to an intranasal formulation of GDF5 in acidic buffer.

Polymeric nanoparticles, comprised of a hydrophobic core of polyactic acid (PLA) and a hydrophilic shell of methoxy-poly(ethylene glycol) (MPEG), have been evaluated for improving solubility and intranasal drug targeting to the CNS. Unlike the microemulsion formulation of nimodipine, nimodipine loaded into MPEG-PLA nanoparticles resulted in the greatest targeting increase to the CSF (14-fold) compared to a simple nimodipine solution, indicating that pathways involving the CSF were affected with this nanoparticle formulation. The regional differences in targeting between the microemulsion and nanoparticle nimodipine formulations could be due to differences in particle size. Dramatic increases in CSF targeting using nanoparticles are not always observed. For example, chitosan nanoparticles loaded with estradiol modestly improved targeting to the CSF by 1.3-fold compared to an intranasal solution. Taken together, these formulation approaches to improve solubility show promise for enhancing intranasal delivery efficiency to the CNS.

Formulations Affecting Membrane Permeability

In addition to solubility, efficient delivery to the CNS following intranasal administration is dependent on membrane permeability. For peptides and proteins or for hydrophilic compounds, where paracellular transport is hindered due to size and polarity, improving membrane permeability could enhance extracellular mechanisms of transport to the CNS along olfactory and trigeminal nerves. One approach to modifying membrane permeability within the nasal epithelium is by using permeation enhancers, such as surfactants, bile salts, lipids, cyclodextrins, polymers, and tight junction modifiers. These compounds are often accompanied by nasal toxicity and increased permeation into the nasal vasculature, which could be problematic for therapeutics with systemic side effects. While there has been considerable research studying the effect of permeation enhancers on systemic absorption after intranasal delivery, there have been few reports evaluating effects on CNS distribution. However, in situ nasal perfusion studies evaluating brain uptake of VIP showed that the permeation enhancer, lauroylcarnitine (LC), improved brain uptake compared to a formulation without the permeation enhancer. Effects of LC on VIP blood absorption were not reported in this study, so it is possible that the increased delivery to the brain could have been due to increased delivery to the blood.

Effects of changes in formulation parameters, such as osmolarity, on brain uptake of intranasal vasoactive intestinal peptide (VIP) were also evaluated. Changes in osmolarity of a formulation can cause cells to expand or shrink, enhancing intracellular or extracellular transport mechanisms along olfactory and trigeminal nerves to the CNS. A hypertonic nasal solution was found to reduce VIP brain uptake after intranasal administration compared to an isotonic solution. In addition to cell shrinking, it is possible that the hypertonic solution caused epithelial changes, such as increased mucus secretion, that hindered transport into the brain. No other studies have reported effects of osmolarity on CNS distribution of intranasally applied therapeutics.

The pH of the nasal formulation and ionization state of the drug can affect the efficiency of intranasal delivery to the CNS. Sakane et al. showed that delivery of sulphisomidine to the CSF following intranasal administration increased as the fraction of unionized drug increased. Similarly, brain uptake of VIP was greater when the peptide was in the unionized form at pH 9 compared to the positively charged peptide at pH 4. Green fluorescent protein conjugated to a cationization agent had limited uptake into the brain following intranasal administration, however when the pH was lowered to reduce the ionic interaction with the nasal epithelial cells, greater brain penetration was observed. Positively charged drugs may form electrostatic interactions with the negatively charged nasal
epithelial cells, effectively hindering transport beyond the nasal mucosa and into the brain. These findings may be drug-dependent since in a different study, negatively charged drugs were shown to have greater CNS bioavailability after intranasal administration compared to a neutral drug of similar size and lipophilicity. There have not been many systematic studies that evaluate the effect of osmolarity and pH of nasal formulations on extracellular or intracellular mechanisms of delivery to the CNS.

**Strategies to Reduce Clearance and Increase Residence Time**

Mucociliary clearance mechanisms rapidly remove drugs from the delivery site, reducing contact with the nasal epithelium and delivery into the CNS after intranasal administration. Several approaches, including use of mucoadhesive agents, surface-engineered nanoparticles, efflux transporter inhibitors, and vasoconstrictors, have been utilized to reduce clearance, to prolong the residence time of the formulation at the delivery site, and to increase transport along direct pathways to the CNS. Increasing the residence time at the delivery site potentially enhances delivery into the CNS along olfactory and trigeminal nerves, the vasculature, or CSF and lymphatic channels. When mucoadhesives, which adhere to the mucous membranes lining the nasal mucosa, were added to microemulsion formulations discussed in the previous section, drug targeting to the CNS was significantly increased. Addition of a mucoadhesive (sodium hyaluronate) and an emulsifying agent (castor oil, Cremophor RH40) to a nasal formulation of fluorescein isothiocyanate increased uptake into different brain areas without affecting plasma levels. Certain mucoadhesives, such as acrylic acid derivatives, lectin, and low methylated pectin, form a viscous gel upon contact with the nasal epithelium, resulting in reduced clearance from the administration site. Chitosan, a cationic mucoadhesive, forms electrostatic interactions with the negatively charged surface of epithelial cells to reduce clearance from the nasal epithelium. Chitosan has the additional effect of reversibly opening tight junctions, with potential to increase extracellular transport along olfactory and trigeminal nerve pathways into the CNS. However, in vivo studies showed that compared to a simple intranasal solution, nasal formulations of a zwitterionic drug containing low methylated pectins or chitosan reduced uptake into the olfactory bulbs, while increasing uptake into the plasma, effectively reducing targeting to the olfactory bulbs. These additives affect delivery into the blood rather than increasing transport into the brain via direct pathways. Mucoadhesives used in combination with microemulsion formulations show the greatest potential in terms of enhancing brain uptake and drug targeting to the CNS.

Surface engineering of nanoparticles with ligands that bind to specific cell surfaces is a promising approach to reduce clearance and enhance targeted delivery to the CNS. For example, the lectin, ulex europeus agglutinin I (UEA I), binds to receptors located predominantly in the olfactory epithelium, while WGA recognizes sugar molecules and binds to receptors expressed throughout the olfactory and respiratory epithelia. UEA I nanoparticles could enhance delivery to the CNS along olfactory pathways, whereas WGA nanoparticles could enhance delivery to the CNS along multiple pathways, including neural and vascular pathways. Intranasal studies using UEA I or WGA conjugated PEG-PLA nanoparticles loaded with a fluorescent marker resulted in increased delivery to different brain areas, including the olfactory bulbs, olfactory tract, cerebrum, and cerebellum, compared to unmodified nanoparticles, without resulting in nasal ciliotoxicity. WGA nanoparticles, but not UEA I nanoparticles, also increased delivery into the blood. This finding is likely due to the nonspecific binding of WGA throughout the nasal epithelium compared to UEA I nanoparticles, which bypass the highly vascular respiratory epithelium. As a result, drug targeting to the CNS was greatest for the UEA I conjugated nanoparticle formulation. However, no regional differences in CNS distribution were observed with these formulation approaches. WGA conjugated nanoparticles carrying the therapeutic peptide, VIP, were shown to enhance brain uptake, with the greatest exposure observed in the cerebellum, without dramatically increasing blood absorption. This formulation also improved spatial memory in an Alzheimer’s mouse model compared to unmodified particles, indicating that surface engineered nanoparticles have therapeutic potential following intranasal administration.

Reducing clearance from the nasal cavity due to efflux from transport proteins or due to absorption into the nasal vasculature are additional strategies that have been explored to increase the residence time at the delivery site and to enhance
the efficiency of intranasal delivery to the CNS. Intranasal pretreatment with an inhibitor (rifampin) of the P-gp efflux transport protein prior to intranasal administration of a P-gp substrate (verapamil) resulted in significantly greater brain uptake as a result of reduced clearance from P-gp-mediated efflux. Reducing clearance into the blood from the site of delivery by using a vasoconstrictor could allow more of the drug to be available for direct transport into the CNS. Intranasal administration of hypocretin-1 with the vasoconstrictor, phenylephrine, resulted in reduced absorption of hypocretin-1 into the blood. The reduced clearance from the nasal epithelium into the blood led to increased deposition in the olfactory epithelium and increased delivery along olfactory nerve pathways to the olfactory bulbs. However, concentrations in the trigeminal nerve and in remaining brain areas were reduced with the vasoconstrictor nasal formulation. These findings are in contrast to a study evaluating a different vasoconstrictor (ephedrine), where drug concentrations in the blood and brain were increased, suggesting the need for additional studies to understand the effect of vasoconstrictors on mechanisms underlying intranasal delivery to the CNS.

THE FUTURE OF INTRANASAL DELIVERY TO THE CNS

This review has discussed the pathways and mechanisms involved in intranasal delivery to the CNS. In addition to olfactory pathways and vascular pathways into the CNS following intranasal administration, there is clear evidence that pathways involving trigeminal nerves, perivascular channels, the CSF, and lymphatic channels are also significant for transport from the nasal mucosa to the CNS. Drug transport within or along these pathways is governed by diffusion, bulk flow, perivascular pumping, and other mechanisms. This review has also highlighted how experimental factors including head position, delivery techniques, and volume can affect the deposition of the drug formulation within the nasal passages and the pathway a drug follows into the CNS following intranasal administration. Moreover, the characteristics of the drug formulation, such as the osmolarity, pH, or addition of enhancers, can influence deposition in the nasal cavity and transport pathways to the CNS. Emulsion-like formulations used in combination with mucoadhesive agents demonstrate great potential for enhancing targeted delivery to the CNS following intranasal administration.

Despite enormous progress that has been made over the last several decades since the introduction of the intranasal method to directly deliver therapeutics to the brain, considerable research remains in the area of intranasal delivery. Since neurological disease does not generally affect the brain in a global manner, additional formulation strategies will be required to improve the delivery efficiency and to target therapeutics to specific brain areas requiring treatment. For example, development of formulations that specifically target the trigeminal nerve could be used to specifically deliver therapeutics to the brainstem and cerebellum for treating Parkinson’s disease. Similarly, formulations designed to target the olfactory nerves could be used to deliver therapeutics to the olfactory bulbs and frontal cortex for treating Alzheimer’s disease, dementia, and personality disorders. The future of this field lies in designing studies to elucidate the underlying mechanisms of intranasal drug delivery to the CNS and using this knowledge to develop formulation strategies and delivery devices to improve the treatment of neurological and psychiatric diseases.

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REFERENCES


