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Review Article

Is nose-to-brain transport of drugs in man a reality?

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Abstract

The blood–brain barrier that segregates the brain interstitial fluid from the circulating blood provides an efficient barrier for the diffusion of most, especially polar, drugs from the blood to receptors in the central nervous system (CNS). Hence limitations are evident in the treatment of CNS diseases, such as Parkinson's and Alzheimer's diseases, especially exploiting neuropeptides and similar polar and large molecular weight drugs. In recent years interest has been expressed in the use of the nasal route for delivery of drugs to the brain, exploiting the olfactory pathway. A wealth of studies has reported proof of nose-to-brain delivery of a range of different drugs in animal models, such as the rat. Studies in man have mostly compared the pharmacological effects (e.g. brain functions) of nasally applied drugs with parenterally applied drugs and have shown a distinct indication of direct nose-to-brain transport. Recent studies in volunteers involving cerebrospinal fluid sampling, blood sampling and pharmacokinetic analysis after nasal, and in some instances parenteral administration of different drugs, have in my opinion confirmed the likely existence of a direct pathway from nose to brain.

Introduction

In the last decade increasing interest has been expressed in the possibility of circumventing the blood–brain barrier for the delivery of drugs to the central nervous system by exploiting the potential direct transport pathway from nose to brain via the olfactory region. Such a pathway has been proven to exist in animal models, but it is still debatable whether a similar transport takes place in man. Hence, it is still debatable whether such delivery of drugs to the brain could be exploited therapeutically for diseases of the central nervous system (Mathison et al 1998; Illum 2000; Pardridge 2001; Thorne & Frey 2001; Minn et al 2002). This would be especially beneficial for drugs that do not cross the blood–brain barrier easily due to their physicochemical characteristics.

The vasculature of the central nervous system (CNS) is characterized by the existence of the blood–brain barrier that separates the brain interstitial fluid from the circulating blood. Apart from protecting the brain from agents in the blood that could impair neurological functions, the blood–brain barrier controls influx and efflux of substances to provide the brain with necessary nutrients and maintain proper homeostasis. The cells of the capillary epithelium in the brain are closely connected by complex tight junctions. These tight junctions completely encircle each endothelial cell like a belt and join both adjacent cells and contiguous borders of the same cell. In addition, each brain capillary is composed of two lipid membranes separated by 300 nm of endothelial cytosol, the luminal membrane facing the blood and the antiluminal membrane, facing the brain (Pardridge 1991).

Lipid soluble molecules are absorbed rapidly and efficiently across the nasal membrane into the systemic blood stream via the transcellular pathway with a plasma profile resembling that of an intravenous injection and with a bioavailability of up to 100%. Due to this rapid absorption such molecules do not normally show direct nose-to-brain transport, although this might be dependent on the site of deposition in the nasal cavity (Illum 2003). Once such lipophilic molecules reach the blood stream they can diffuse freely through the blood–brain barrier and reach the CNS. This diffusion is

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qualified by the degree of lipid solubility and molecular size, with smaller molecules passing through the membrane more easily than larger ones (Temsamani 2002).

Less lipophilic or polar molecules are not as readily absorbed across the nasal membrane into the systemic circulation, with bioavailabilities being in the order of 10% or less for low molecular weight and less than 1% for large molecular weight polar molecules such as peptide drugs (Illum 2000). Such molecules normally pass the nasal membrane via the paracellular pathway, through the tight junctions. This pathway is less efficient than the transcellular pathway and very dependent on the molecular weight of the molecule. Once in the systemic circulation, the hydrophilic molecules do not pass the blood–brain barrier easily unless aided by some form of receptor or carrier mediated transport mechanism (Schwartz et al 1990), whether naturally occurring (as is the case for insulin) or by a specific drug delivery approach (Pardridge 2001). Polar molecules do not rapidly diffuse across the nasal membrane into the systemic circulation and so they have a better chance of reaching the olfactory mucosa and from there being transported across into the CNS. This has been demonstrated in many animal studies (Illum 2000).

This review sets out to discuss recent relevant studies concerning the potential of drugs applied to the nasal cavity being at least partially transported via the olfactory pathway to the CNS. These studies have been published in the literature or have been provided as information at scientific meetings and largely concern investigations in man. Support in the understanding of the subject will be provided in the form of a brief overview of nasal morphology and physiological function.

The human nose

To comprehend fully the intricacies of nasal drug delivery and to evaluate whether nose-to-brain transport of drugs is a reality, it is important to have an understanding of the relevant morphological structures and physiological factors affecting these functions. Comprehensive reviews dealing with the morphology and physiology of the nose, to include the olfactory mucosa, have been published (Mygind 1978; Moran et al 1982; Hilger 1989) and hence only limited necessary details will be given here.

Structure and function of the human nose

An outline of the human nose is shown in Figure 1. The total surface area of the nasal cavity is approximately 150 cm^2 in a man and normally less in a woman. The cavity is divided longitudinally into two non-connected parts by the nasal septum. The two cavities open anteriorly to the facial site through the narrow (0.3 cm^2 in diameter) nasal apertures or "the nasal valve" at the top of the nostril and posteriorly to the rhinopharynx via the posterior nasal apertures. Each of the two nasal cavities are largely subdivided into three regions i.e. the nasal

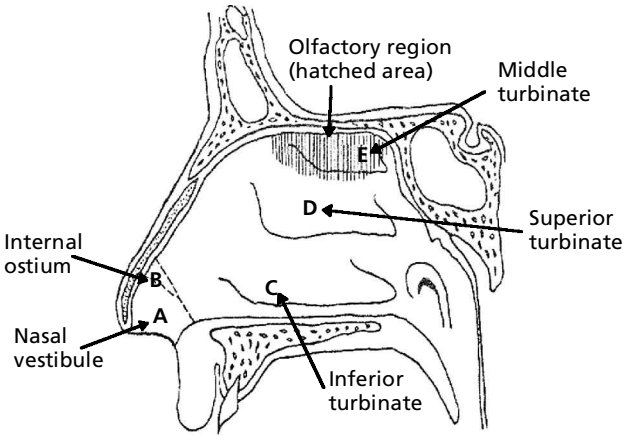

Figure 1 Schematic representation of the lateral wall of the human nasal cavity.

vestibule, the respiratory region and the olfactory region. The nasal vestibule (0.6 cm^2) is covered with stratified squamous epithelium (very similar to skin) and is the part of the nose one can reach with an index finger. The olfactory region in man is situated in the roof of the nasal cavity lying partly on the nasal septum and partly on the superior and middle turbinates. The olfactory mucosa covers a relatively small area of approximately 4 cm^2 or 3–5% of the area of the total nasal cavity (Morrison & Constanzo 1992). However, it has been suggested that the tips of the olfactory sensory neurons can stretch further into the nasal cavity and hence be accessible over a larger area (personal communication, N. Jones). As a comparison, in the dog the olfactory mucosa constitutes 77% and in the rat 50% of the total nasal area (Illum 1996).

The respiratory epithelium

The anterior part of the nasal cavity is covered with squamous epithelium that gradually changes posteriorly into the respiratory epithelium comprising a pseudostratified columnar epithelium. The cells of the respiratory epithelium are covered with microvilli. These provide this part of the nasal cavity with a relatively high absorptive capacity, due to an increase in the surface area, and make this the major site for systemic drug absorption. The respiratory epithelium consists of four major cell types, namely the ciliated (approximately 15–20% of the respiratory cells) and the non-ciliated columnar cells, the goblet cells and the basal cells. The cilia project 2–4 μm from the surface of the cells, are mobile and through a co-ordinated movement (synchronized beating, 1000 strokes min^{-1}) are able to propel the mucous layer, covering the respiratory epithelium, anteriorly towards the nasopharynx. Mucus is mainly derived from the goblet cells, interspersed between the columnar cells and is the major component of the mucous layer. The mucous layer consists of a low viscosity sol layer that surrounds the cilia and a more viscous gel

layer on top of the cilia. Hence, materials deposited on the mucous layer will gradually be cleared from the nasal cavity by this mucociliary clearance mechanism. For non-mucoadhesive materials this will generally result in a half-time of clearance of approximately 15–20 min (Illum 2000).

Epithelial cell barrier – tight junctions

The epithelial cells on the apical surface of the membrane are closely connected by intercellular junctions. The structural components and specialized sites of these junctions are generally known as the junctional complex. They are composed of three regions and are, in successive order from the apical surface towards the basal surface, the zona occludens (ZO) also known as the tight junction, the zonola adherens and the macula adherens (Madara 2000). These complexes create a regulatable semipermeable diffusion barrier between cells. It is clear that the tight junction is a dynamic structure that is selectively permeable to certain hydrophilic molecules (ions, nutrients and drugs). The permeability of the tight junction varies between the epithelial tissues in the body but is generally limited for molecules with a hydrodynamic radius larger than 3.6 Å and negligible to molecules with a radius larger than 15 Å (Stevenson et al 1988). It is difficult to relate these sizes to exact molecular weights since the size of a molecule, and especially peptides and proteins, will be determined by the physicochemical environment, and possible secondary and tertiary structures of the molecules. However, it has been shown in the literature that for molecules of a molecular weight of approximately 1000 Da and larger, the transport through tight junctions is normally very restricted (McMartin et al 1987).

The tight junction comprises a series of transmembrane and cytosolic proteins that interact not only with each other but also with the membrane and the cytoskeleton e.g. occludins, claudins and junctional adhesion molecule (Anderson & Van Itallie 1995; Denker & Nigram 1998) (Figure 2). The topology of occludin suggests that the amino and the carboxyl termini of this protein are situated in the cytoplasm of the cell with two extracellular loops projecting into the paracellular space between adjacent cells. The loops of the extracellular occludin from two neighbouring cells may interact in the extracellular space to promote sealing of the paracellular space. The cytoplasmic occludin interacts with tight junction-associated proteins present in the cytoplasm (ZO-1, ZO-2 and ZO-3) (Ward et al 2000). For example, the N-terminal of the ZO-1 interacts with the C-terminal tail of occludin and its C-terminal interacts with F-actin of the cytoskeleton and thereby couples the tight junction to the scaffold of the cytoskeleton. The ZO-2 interacts with the C-terminal of the occludin and the N-terminal of ZO-1. The claudins have been suggested to be major structural components of tight junction strands in line with occludins. The third transmembrane protein junctional adhesion molecule is different structurally to the occludins and claudins, and is immunoglobulin-like in form.

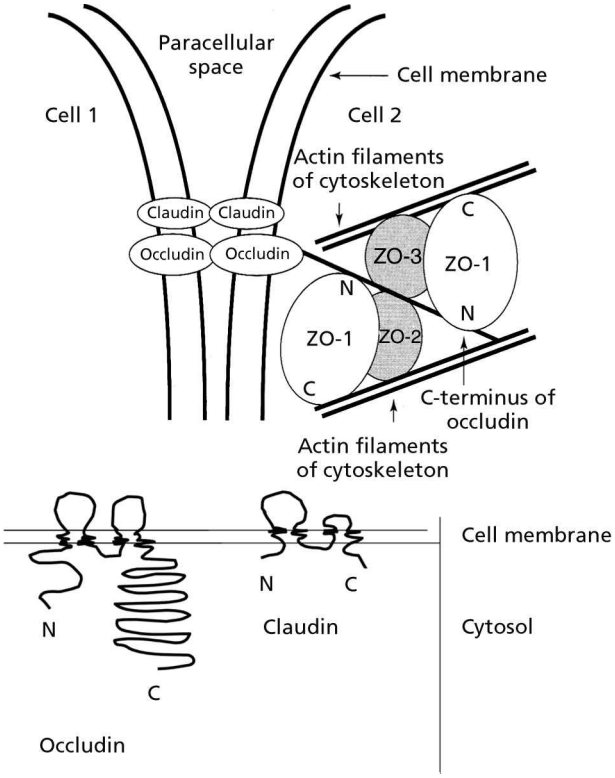

Figure 2 Schematic representation of the tight junction and the interaction of the transmembrane and cytosolic proteins (adapted from Ward et al (2000)).

The zona occludens is closely associated with the zonola adherens complex. The zonola adherens complex holds cells close together but does not form a tight barrier. The zonola adherens is made up of transmembrane proteins known as cadherins. Both zona occludens and zonola adherens structures act to anchor cytoskeleton components.

Many classical second messengers and protein kinases of signalling pathways such as tyrosine kinases, Ca^{2+} and protein kinase C (PKC) influence both the barrier properties and assembly of the tight junction. Hence increases in intracellular calcium can affect phosphorylation of myosin regulatory light chain contraction of perijunctional actin and cause increased paracellular permeability (Ward et al 2000). PKC plays a dual role in that it initiates tight junction synthesis under conditions that preclude tight junction synthesis (e.g. incubation in low calcium medium) and also appear to be involved in tight junction disruption in conditions that encourage tight junction formation (e.g. incubation in normal calcium medium). Hence PKC is strongly involved in the highly complex signal transduction process that regulates the tight junction. The phosphorylation of the tight junction proteins or the displacement (i.e. contraction or relaxation) of the perijunctional actin-myosin ring is generally the final effect of modulation of many of these signalling pathways. This has been shown by the fact that a disruption of the tight junction integrity by ATP depletion

induces a decrease in phosphorylation of the tight junction regulatory proteins. During ATP repletion the phosphorylation is increased again (Tsukamoto & Nigam 1999). Furthermore, the same signalling pathway that induces phosphorylation of the tight junction proteins may also modulate the actin cytoskeleton, which again has been shown to increase the transmembrane flux of sodium and mannitol. Recently, it has been shown that cationic polymer absorption enhancers, such as poly-L-arginine and chitosan, which predominantly work by transiently opening epithelial tight junctions, initiate this mechanism by activating the PKC signalling pathway (Natsume et al 2003).

The olfactory mucosa

The olfactory organ is unique in the CNS, since it is the only part in direct contact with the environment and hence exposed to volatile odorants and airborne (toxic) substances. The olfactory mucosa is located within the recesses of the skull, just under the cribriform plate of the ethmoid bone, approximately 7 cm from the nostril, at the top of the nasal cavity, lying partly on the nasal septum and partly on the superior turbinate (Figure 1). The olfactory region is not easily accessible anatomically in living human beings since to reach this area (for example in biopsy) an instrument must pass through a 1.5-mm crevasse between closely apposed nasal structures (turbinates and septum). The olfactory mucosa is above the normal airflow path, and hence odorants normally reach the sentive receptors on the neurons by diffusion. The size of the olfactory region in man has been quoted as 3.7 cm^2 (Jones 2001), 10 cm^2 (Proctor 1977) and as 2–10 cm^2 (Morrison & Constanzo 1990). The region is much smaller than, for example, that found in dogs (150 cm^2), indicating the importance of olfaction in the daily functions of dogs but not of man.

The olfactory epithelium is a modified (pseudostratified) respiratory epithelium. It comprises olfactory sensory neurons, sustentacular cells (also called supporting cells) that ensheath the receptor neurons providing mechanical support and maintain the normal extracellular potassium levels needed for neuronal activity, and basal cells, which are able to differentiate into neuronal receptor cells and replace these every 40 days (Figure 3). The underlying lamina propria contains olfactory nerve fascicles and the mucus secreting tubuloalveolar Bowman's glands. The olfactory receptor cells are bipolar neurons with a round cell body. A single dendritic process extends from the cell body to the free apical surface where it terminates as a small knob-like swelling from which extends numerous (10–23) long and non-motile cilia. The olfactory sensory neurons taper into an unmyelinated axon which penetrates the basal membrane to join other axons and form large bundles in the lamina propria. The unbranched axons are ensheathed by glial cells, also called Schwann cells, and cross into the cranical cavity through small holes in the cribriform plate and synapse in the olfactory bulb. Approximately 1500 olfactory receptor cells on the bipolar sensory neurons converge on one mitral cell or tufted cell in the olfactory bulb (12.2 mm, range 6–16 mm, long). The mitral and the tufted cells

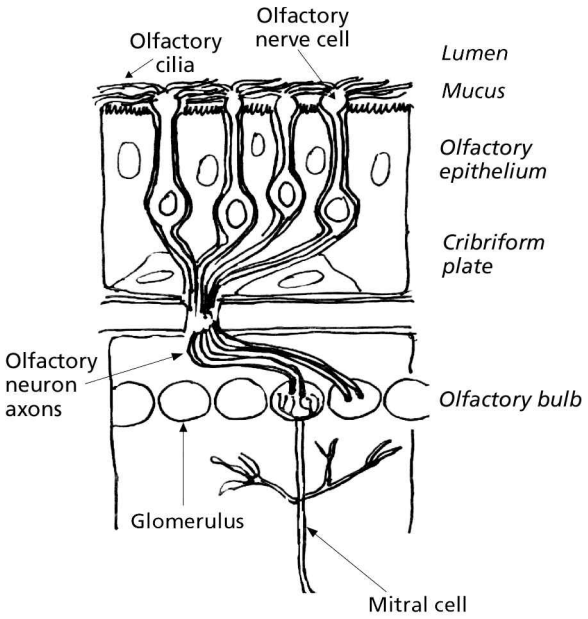

Figure 3 The structure of the olfactory epithelium (adapted from Firestein (2001)).

project a single primary dendrite to a single glomerolus and emit several dendrites within the external plexiform layer. From the olfactory bulb tract the main axons originate in the mitral or tufted cells and give off striae, which pass to the olfactory tubercle. The projections then go to the amygdala, the prepyriform cortex, the anterior olfactory nucleus and the entorhinal cortex as well as the hippocampus, hypothalamus and thalamus.

The olfactory epithelium is covered by a dense and viscous layer of mucus, which is secreted from the Bowman's glands and the supporting cells. Due to the non-motile cilia the mucus layer in the olfactory region is not cleared by a mucociliary clearance mechanism as in the respiratory epithelium. Over-production of mucus results in the mucus layer slowly moving into the respiratory region from where it is cleared by the normal mechanism of mucociliary clearance.

At the luminal surface in the olfactory epithelium the membranes of the adjoining receptor cells and supporting cells are connected by typical junctional complexes similar to those described for the respiratory epithelium (Engstrom et al 1989). The olfactory region is supplied with blood from the anterior and posterior ethmoidal branches of the ophthalmic artery supply and venous drainage is as for the respiratory system via the sphenopalatine foramen into the pterygoid plexus or via the superior ophthalmic vein.

Transport of drugs from nose to brain

The CNS

The CNS is protected against trauma by the cranium (skull) that encases the brain and the vertebral column that sur-

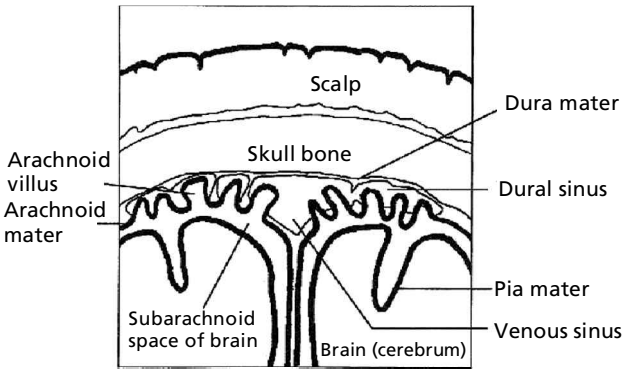

Figure 4 Relationship of meninges and cerebrospinal fluid to brain and spinal cord. Frontal section in the region between the two cerebral hemispheres of the brain, depicting the meninges in greater detail. (Adapted from Illum (2000).)

rounds the spinal cord. Three protective membranes, called the meninges, lie between the skull and the brain tissue (Pardridge 1991; Thorne & Frey 2001). Moving in the direction from the skull to the brain, these are the dura mater, the arachnoid mater and the pia mater. The dura mater consists of two layers, which are normally closely adherent. However, in some regions they are separated by blood-filled cavities, the dural sinuses or venous sinuses (Figure 4). Venous blood from the brain empties into these sinuses to be returned to the heart. The space between the arachnoid and pia mater, the subarachnoid space, is filled with cerebrospinal fluid (CSF) in which the brain is essentially suspended. Protrusions of arachnoid tissue, the arachnoid villi, penetrate through gaps in the overlying dura and project into the dural sinuses. It is across the surfaces of these villi that the CSF is reabsorbed into the blood circulating within the sinuses. The CSF is produced primarily by the four choroid plexi found in particular regions of the ventricle cavities of the brain. Once formed it flows through the four interconnected ventricles within the interior of the brain and through the spinal cord's narrow central canal, which is continuous with the last ventricle, and escapes from this fourth ventricle at the base of the brain to enter the subarachnoid space. When the CSF reaches the upper regions of the brain, it is re-absorbed into the venous blood through the arachnoid villi. It is known also that the CSF can drain from the subarachnoid space through the perivascular space surrounding the nerve bundles in the cribriform plate, and enters the olfactory submucosa where it drains into the nasal lymphatics (Pardridge 1991). This drainage constitutes less than 5% of the CSF.

Through the ongoing procedure of formation, circulation and re-absorption of the CSF, the entire volume of approximately 125–150 mL (in adults) is replaced more than three times a day (Sherwood 1989). In comparison the rat brain contains only 150 μL CSF and is replaced approximately 24 times a day. These differences in CSF renewal between rat and man could have a significant impact on interpretation of nose-to-brain drug delivery studies and together with the other anatomical differences depicted in Table 1 should always be carefully considered.

Knowledge of the manner in which drugs diffuse from the CSF into the brain parenchyma and the probability of this is important for the understanding of the significance of uptake of drug into the CSF after nasal application for treatment of CNS diseases. Unless receptors for the drug are present on the surface of the brain the drug will by necessity have to penetrate into the brain tissue. The rate of diffusion of drugs in the extracellular space of the brain can be expressed as $D^* = D/\lambda^2$, where D is the diffusion coefficient of the molecule in water and λ is tortuosity. Tortuosity is a dimensionless parameter reflecting the restrictions placed on the diffusion of the molecule by cellular elements and the connectivity of the extracellular spaces into which the molecule has access (Nicholson & Sykova 1998). Values of λ vary from 1.4 for small molecules to 2.5 for large molecules such as albumin. D is inversely related to the molecular size of the drug. It can be calculated that the time it takes for a small molecule such as glucose to diffuse 5 mm in the brain is approximately 11.7 h and for a molecule such as albumin 4.2 days!

The fact that there is a distinct difference between the bulk flow properties of the CSF and diffusional flow rates in the brain tissue creates a functional barrier between the CSF and the brain tissue (Pardridge 1991). This prevents complete equilibration between the two fluid compartments and consequently a significantly different drug concentration will normally exist between these two compartments.

Transport pathways

It is suggested in the literature that a drug administered nasally is able to reach the CNS (i.e. CSF and brain tissue)

Table 1 The characteristics of the rat animal model vs man in relation to nose-to-brain delivery of drugs.

The nasal cavity is approximately 180 cm^2 in man and approximately 10 cm^2 in rats.
The olfactory area constitutes approximately 3% of the nasal cavity in man, but 50% in rat.
The CSF volume is 160 mL in adult humans and 150 μL in rats.
The CSF volume is replaced every 5 h in man and every 1 h in rats.
The placement of the rat on its back in most experiments with easy access to the olfactory area influences CSF uptake.

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