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Review

Transport of drugs from the nasal cavity to the central nervous system

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1. Introduction

The blood–brain barrier that segregates the brain interstitial fluid from the circulating blood consists of two plasma membranes in series, the lumenal and the antilumenal membranes of the brain capillary epithelium, separated by about 0.3 mm of endothelial cytosol. The cells of the capillary endothelium are closely connected via intercellular connections; the tight junctions that act as zips closing the inter-endothelial pores that normally exist in endothelial membranes. This makes the blood–brain barrier resistant to the free diffusion of molecules across the membrane and prevents most molecules from reaching the central nervous system from the blood stream. Several different approaches have been attempted in order to circumvent the blood–brain barrier and to deliver drugs efficiently to the brain for therapeutic or diagnostic applications. Since lipid soluble drugs, with a molecular weight less than 600 Da, will readily diffuse through the blood–brain barrier a normal approach to increase the permeability of a drug is to create a more lipophilic molecule, often in the form of a prodrug that is converted to the parent drug once in the brain. Other approaches include the binding of drugs to carrier molecules such as transferrin or to a polycationic molecule such as cationised proteins that will bind preferentially to the negatively charged endothelial surface (Pardridge, 1991).

In the last decade, much interest has been given to the exploitation of the nasal route for delivery of drugs to the brain via a specific site, the olfactory region (Pardridge, 1991; Thorne et al., 1995). Recent times have also seen review articles dealing with this topic (Pardridge, 1991; Mathison et al., 1998). Indeed, it was realised early in the last century that the olfactory region of the nose can be a major site for entry of viruses into the brain. Hence, it has been shown by several groups that the nasal route can be involved in the contraction of the neurotropic poliomyelitis virus and that the virus reached both the olfactory lobe of the brain and the cerebrospinal fluid (CSF) (Landsteiner and Levaditi, 1910; Flexner, 1912; Fairbrother and Hurst, 1930; Faber and Gebhardt, 1933; Sabin and Olitsky, 1936, 1937a, 1938; Faber, 1938). In addition to poliomyelitis virus, Sabin and Olitsky (1937b) also showed that the vesicular stomatitis virus could enter the CNS via the nasal cavity. It was later demonstrated by Bodian and Howe (1940) that viruses can move from the nose to the brain via the olfactory neurons. This was confirmed in a study by Reiss et al. (1998) in mice who showed that vesicular stomatitis virus, applied to the nasal neuroepithelium, initially replicated in the olfactory receptor neurons and was transmitted along the olfactory nerve to the CNS within 12 h. The virus replicated invasively in the olfactory bulb and reached the olfactory ventricle by 4–5 days post infection and the hindbrain by day 8.

Studies in animals have reported that tracer materials, such as potassium ferrocyanide and iron ammonium citrate (Faber, 1937), albumin (Kristensson and Olsson, 1971), horseradish peroxidase (Stewart, 1985), the conjugate wheat germ agglutinin–horseradish peroxidase (Shipley, 1985; Baker and Spencer, 1986) and colloid gold (Gopinath et al., 1978), are also able to be transported across the olfactory epithelium into the CNS. A number of studies have also been published on the transport of heavy metals from the nasal mucosa into the CNS via olfactory pathways (Evans and Hastings, 1992; Tjalve et al., 1996; Gianutsos et al., 1997). Similarly, a large number of studies have been performed where low molecular weight drugs and peptides (such as estradiol, progesterone, cephalexin, dihydroergotamine and cocaine) have been shown to reach the CSF, the olfactory bulb and in some cases other parts of the brain after nasal administration (Anand Kumar et al., 1974, 1976, 1979, 1982; Hussain et al., 1981; Sakane et al., 1991a,b, 1994, 1995; Wang et al., 1998; Chou and Donovan, 1998a; Chow et al., 1999).

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Studies have also been directed to investigate the mechanism of transport of drugs across the olfactory epithelial membrane and the salient physicochemical characteristics of the drug affecting CNS uptake (Sakane et al., 1994, 1995; Chou and Donovan, 1998a). Most studies have been carried out in animal models, such as the rat and the monkey, and only few studies have been published where evidence of transport of drugs from nose to brain in humans has been given (Pietrowsky et al., 1996a,b; Kern et al., 1997, 1999; Okuyama, 1997; Derad et al., 1998).

The present review sets out to discuss the barriers involved in the passage of drugs from the nose to the brain and the likely transport pathways that drugs might take. Furthermore, studies in the literature describing the transport of drugs to the CNS in different animal models and man will be reviewed together with a discussion of the feasibility of deep brain penetration following nasal administration.

2. The human nose

The structure and the function of the human nose have been comprehensively described by various authors including Mygind (1978) and Hilger (1989). Therefore, only details that are relevant for the understanding of the morphological and physiological factors affecting the nasal absorption of drugs, and in particular the transport of drugs from the nasal cavity to the central nervous system, will be provided in this review.

2.1. The human nasal structure and function

The nasal cavity is subdivided along the centre into two halves by the nasal septum. The two cavities open to the facial side through the anterior nasal apertures and to the rhinopharynx via the posterior nasal apertures. The total surface area of the nasal cavity in man is about 150 cm^2 and the total volume about 15 ml. Each of the two nasal cavities can be subdivided into three regions; namely the nasal vestibule, the olfactory region and the respiratory region. The olfactory region in man covers an area of about 10 cm^2 and is positioned on the superior turbinate and opposite the septum. The respiratory region is dominated by the presence of the large inferior turbinate, the middle turbinate, which has similarities to a polyp and further back in the nose, the superior turbinate. Fig. 1 shows an outline of the human nasal cavity with a clear indication of the position of the olfactory region.

2.1.1. The respiratory region

In the respiratory region, which is considered the major

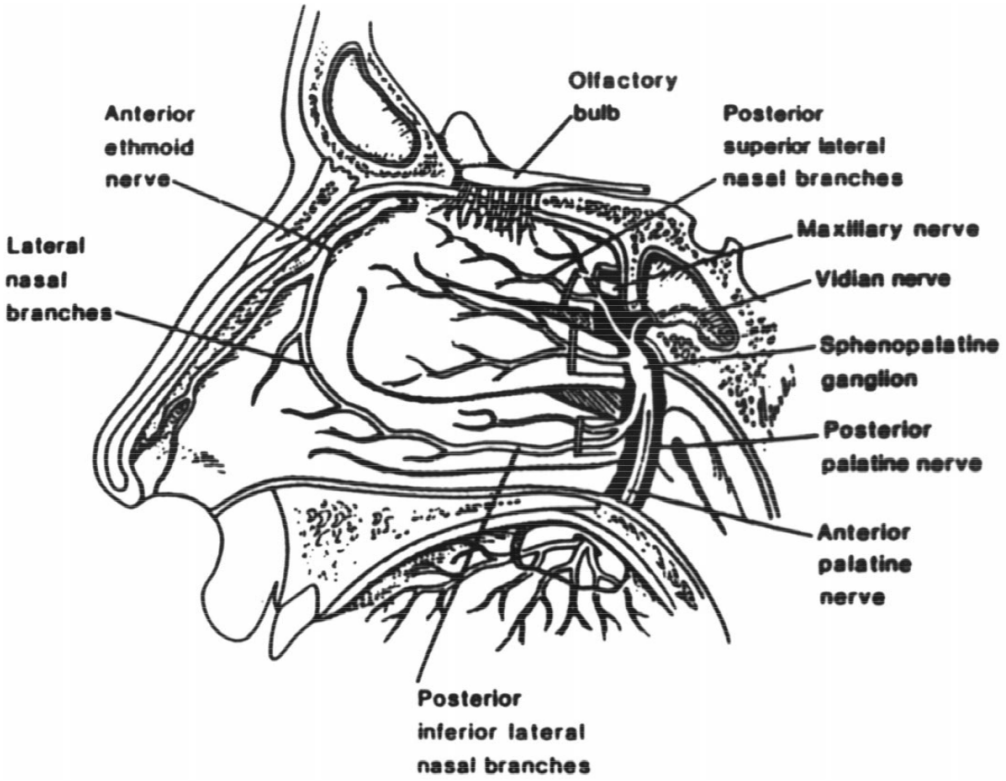

Fig. 1. Outline of human nasal cavity indicating the nasal turbinates, the olfactory region and the olfactory bulb. With permission from W.B. Saunders, Philadelphia, PA, USA.

site for drug absorption into the systemic circulation, the mucosa consists of an epithelium resting on a basement membrane and a lamina propria (Fig. 2). The anterior part of the respiratory region is covered with squamous epithelium, which changes to a transitional epithelium and converts in the posterior part of the cavity to a pseudostratified columnar epithelium. The pseudostratified epithelium, also named the respiratory epithelium, consists of four dominant cell types; ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells. Of these the basal cells are situated on the basal membrane and do not extend to the apical epithelial surface, as do the other three cell types. A total of 15–20% of the respiratory cells is covered by a layer of long cilia of size 2–4 μm. The cilia move in a coordinated way to propel mucus across the epithelial surface towards the pharynx. The respiratory cells are also covered by about 300 microvilli per cell. Microvilli increase the surface area of the cell considerably, which in turn promotes the transport of substances and water between the cells. Goblet cells are interspersed between the columnar cells and are the main entities responsible for the secretion of the mucus covering the epithelial cell layer. The mucus layer consists of a low viscosity sol layer that surrounds the cilia and a more viscous gel layer forming a layer on top of the sol layer and covering the tips of the cilia.

The epithelial cells are closely connected on the apical surface, surrounded by intercellular junctions whose specialised sites and structural components are commonly known as the junctional complex. Each complex is composed of three regions; the zonola occludens closest to the apical surface, further down the zonola adherens and last the macula adherens. The zonola occludens (ZO) forms a tight band around the upper part of the cell and is also known as the tight junction. The ZO contains the integral protein ZO-1 and controls the diffusion of ions and neutral molecules through the intercellular spaces. Tight junctions and selective paracellular permeability has recently been reviewed by Balda and Matter (1998).

It has been shown by electrophysiological analysis that the size of the largest molecule that can permeate the ZO varies between epithelial tissues in the body. Generally, the ZO limits the degree of permeability of molecules with a hydrodynamic radii larger than 3.6 Å. Tight junctions are impermeable to molecules with a radius larger than 15 Å (Madara and Dharmsathaphorn, 1985; Madara et al., 1986). It has also been shown that the extracellular environment has an effect on the permeability of the epithelial membrane in that the integrity of the tight junction is dependent on the concentration of extracellular calcium ions (Stevenson et al., 1988). Compounds, such as calcium chelators, are known to be able to open up tight junctions by chelating the calcium ions (Citi, 1992). However, it should be noted that this effect might be associated with changes in other junctional elements. Chelators have been shown to induce global changes in

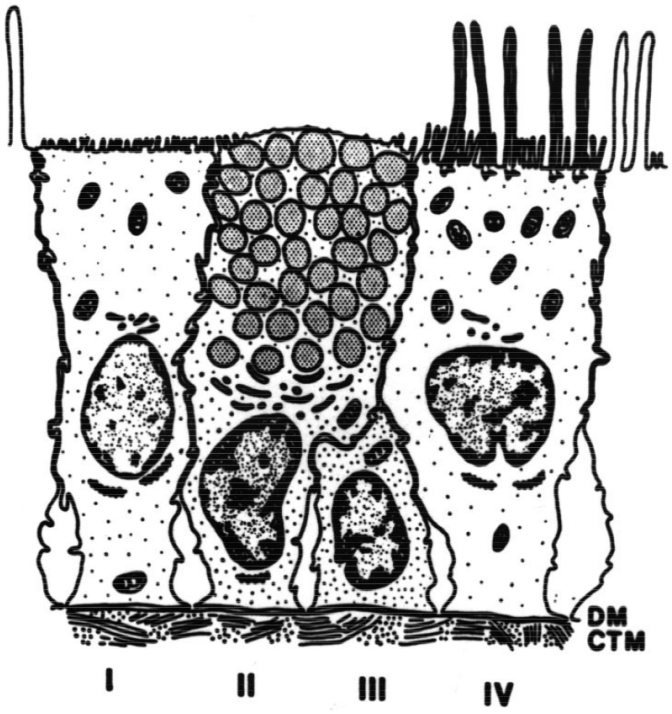

Fig. 2. Schematic illustration of the various cell types in the nasal respiratory epithelium. (I) Non-ciliated columnar epithelial cell with microvilli; (II) goblet cell with mucus granules and Golgi apparatus; (III) basal cell; (IV) ciliated columnar cell with mitochondria. DM, double membrane; CTM, connective tissue membrane. With permission from Blackwell Science Publishers, Oxford, UK.

cells, such as the disruption of adherent junctions, diminished cell adhesion and disruption of actin filaments (Citi, 1992).

The blood supply to the respiratory region of the nasal cavity comes from the external and the internal carotid arteries through a dense network of capillaries in the lamina propria. The blood from the main part of the nasal cavity is drained via the sphenopalatine foramen into the pterygoid plexus or via the superior ophthalmic vein, whereas blood from the anterior part of the nose is drained via the facial vein.

2.1.2. The olfactory region

The science of olfaction to include the olfactory region in vertebrates has been very adequately described in a recent publication by Serby and Chobor (1992). The olfactory epithelium is dedicated to the detection of smells and both the cellular composition and organisation of the epithelial layers maximise the accessibility of air to neuronal structures bearing odorant detectors. In man the epithelium is restricted to a small area in the roof of the nasal cavity of about 10 cm^2 as compared to an area of about 150 cm^2 in dogs, which reflects the major importance of the sense of smell to the dog. The olfactory region is situated between the nasal septum and the lateral wall of each of the two nasal cavities and just below the cribriform plate of the ethmoid bone separating the cranial cavity from the nasal cavity (Fig. 1). Since the olfactory mucosa is above the normal path of the airflow, odorants normally reach the sensitive receptors by diffusion. The process of sniffing enhances the diffusion process by drawing air currents upward within the nasal cavity so that a greater percentage of the molecules comes into contact with the receptor neurones. The olfactory epithelium is a modified form of respiratory epithelium in that it is a pseudo-stratified epithelium that consists of three cell types; the olfactory receptor cells, supporting epithelial (sustentacular) cells and basal cells. Beneath the basement membrane is the lamina propria, which contains blood vessels, olfactory axon bundles, trigeminal and autonomic nerve fibres and Bowman's glands, which secrete the mucus, that cover, the epithelial surface. Fig. 3 shows an outline of the olfactory epithelium indicating the various types of cells present and the connection of neurones to the olfactory bulb.

The olfactory receptor cells are bipolar neurones which are located in the middle stratum of the olfactory epithelium and interspersed among the sustentacular cells. 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 a dozen extremely long modified non-motile cilia. Chemical detectors, presumably receptor protein molecules, are located in the plasma membrane of these specialised cilia that float at the tissue/mucus/air interface (Krieger and Breer, 1999). At the basal aspect of the neuron cell body, each receptor gives

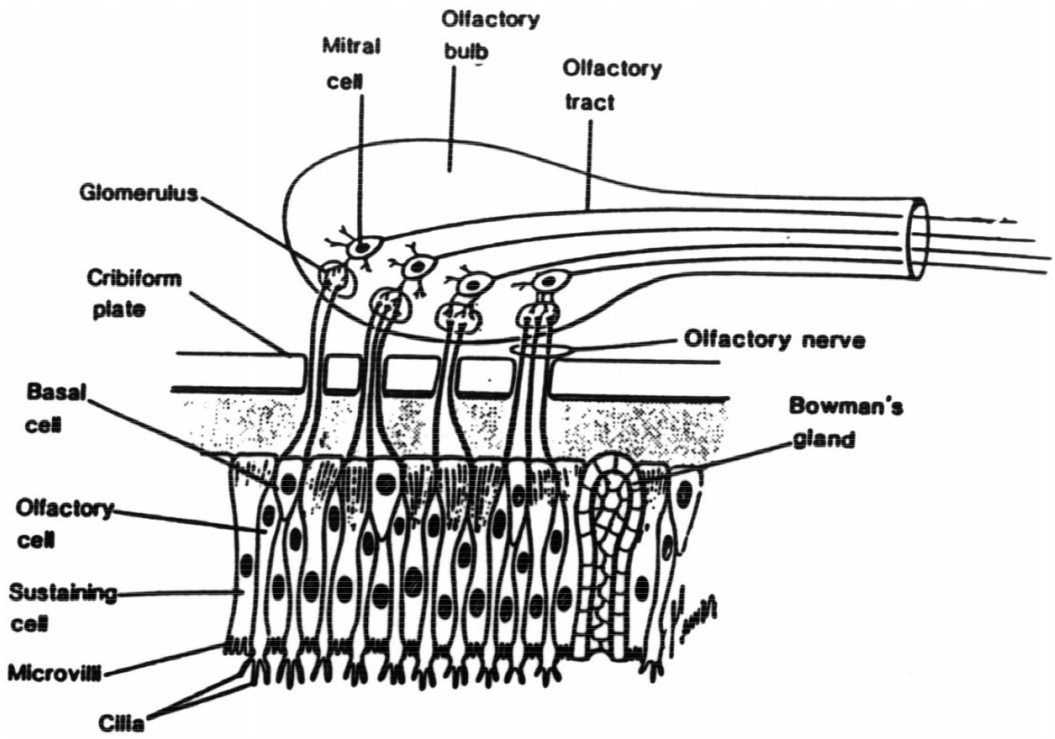

Fig. 3. Schematic illustration of the various cell types in the olfactory region in the vault of the human nose. With permission from W.B. Saunders, Philadelphia, PA, USA.

rise to a single fine non-myelinated 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 (Schwann cells) and cross into the cranial cavity through small holes in the cribriform plate of the ethmoid bone. The bundles of nerve fibres travel along the surface of the olfactory bulb, eventually synapsing with mitral cell dendrites and other neuronal targets in the glomeruli of the bulb (Fig. 3).

The sensory neurones, due to their anatomical organisation, are in direct contact with volatile odorants and also exposed to detrimental airborne substances, which include chemicals and viral and bacterial pathogens. This results in neuronal death being a feature of normal olfactory epithelium. In mice, the lifetime of olfactory receptor neurones has been estimated to be of the order of 30–90 days (Mackay-Sim and Kittel, 1991). The dying neurones are replaced by new sensory neurones. This neurogenesis is a specific feature of the olfactory epithelium and not found elsewhere in the adult nervous system.

The sustentacular cells are elongated columnar cells with their tapered bases resting on the basement membrane and many long microvilli extending from their luminal surface to form an entanglement with the cilia of the receptor cells. Most sustentacular cells have an unbranched stem but some have been shown to have finger-like processes that wrap themselves around the adjoining receptor cells (Moran et al., 1982). The function of the sustentacular cells is poorly understood but the cells most likely provide mechanical support for the receptor cells and have been suggested to be a source of xenobiotic metabolising enzymes (Harkema, 1991). The basal cells are small, conical cells similar in position to those in the respiratory epithelium. The cells are able to differentiate into neuronal receptor cells that replace the dying cells as described above.

At the luminal surface, the plasma membranes of adjoining receptor cells and sustentacular cells are connected by typical junctional complexes in line with those described for the respiratory epithelium (Engstrom et al., 1989). The olfactory region is supplied with blood from principally the anterior and posterior ethmoidal branches of the ophthalmic artery supply. Venous drainage is via the same principal veins as for the rest of the nasal cavity.

3. The central nervous system

The central nervous system (CNS) is protected from trauma by the skull and vertebra. The brain is surrounded by the subarachnoid space in which runs the cerebrospinal fluid (CSF). This space is again surrounded by the meninges which consists of three membranes; the dura mater, which lies directly beneath the skull, the pia mater that lies directly over the brain, and in between the arachnoid. Between the pia mater and the arachnoid is the subarachnoid space (Fig. 4).

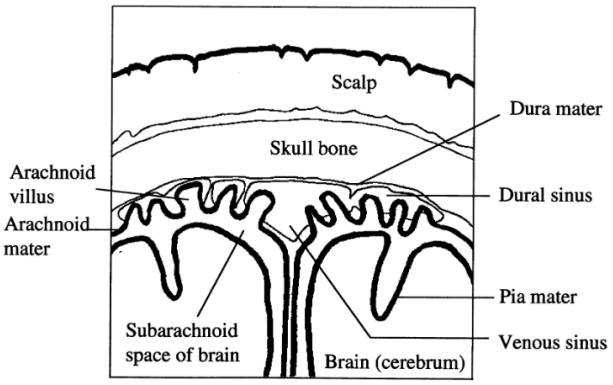

Fig. 4. Relationship of meninges and cerebrospinal fluid to brain and spinal cord. Frontal section in the region between the two cerebral hemispheres of the brain, depicting the meninges in greater detail.

The CSF is produced almost entirely by secretion at the four choroid plexi, especially at the fourth and lateral ventricles. CSF is not a plasma filtrate but a secretory fluid produced by the choroid plexi. Each choroid plexus comprises a secretory epithelium that is perfused by blood at a local high perfusion rate. The epithelium is polarised, with the apical and basolateral membranes facing the ventricular and vascular surfaces, respectively. The CSF is secreted by the epithelium across the apical membrane. The cells have tight junctions, which constitute the blood–CSF barrier. The CSF flows from the choroid plexi and circulates over the surfaces and convexities of the brain in a rostral to caudal direction and leaves the subarachnoid space at the arachnoid villi to be absorbed into the blood. The volume of CSF present is dependent on age and varies from 40 ml in infants up to 160 ml in adults (Allison and Stach, 1978). In comparison, the mouse brain and the rat brain contains only 35 μl and 150 μl of CSF fluid, respectively (Rieselbach et al., 1962; Davson et al., 1987). The rate of CSF fluid production, which equals the rate of CSF absorption into the peripheral bloodstream at the arachnoid villi, varies from 21 ml/h in humans to 0.18 ml/h in rats and 0.018 ml/h in mice (Table 1). It can hence be calculated that for a rat the entire CSF volume would be totally replaced every hour (i.e. 24 times a day)

Table 1
Production rates and volumes of CSF in different species[a]

Species	Production rate (ml/h)	Volume in brain (ml)
Mouse	0.018	0.035
Rat	0.18	0.15
Rabbit	0.6	2.3
Monkey	2.5	–
Sheep	7.1	14.2
Man	21.0	100.0

[a] From Davson et al. (1987).

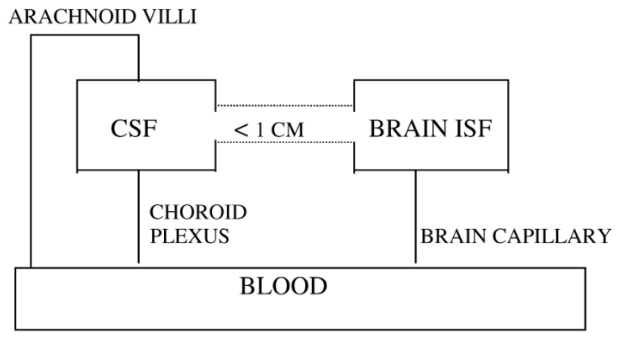

Fig. 5. The relationship between the cerebrospinal fluid and the interstitial fluid/brain tissue and their functional interaction with the bloodstream. Adopted from Pardridge (1991).

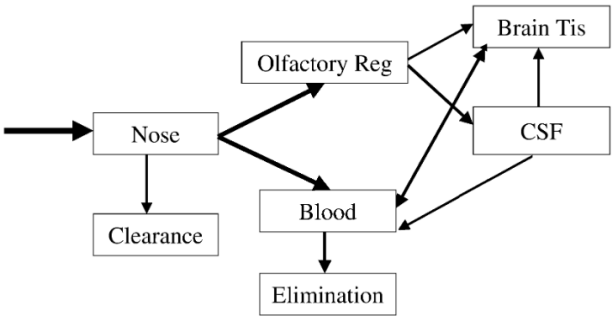

Fig. 6. The nose to brain transport routes.

whereas in humans the CSF is turned over every 5 h, 4–5 times a day. Thus, the CSF fluid is constantly formed at the choroid plexi and subsequently drained into the peripheral bloodstream at the arachnoid villi. These differences may have significant impact on nose to brain delivery studies, especially in terms of the possibility of the diffusion of drugs from the CSF deeper into the brain tissue, and should be considered when choosing an appropriate animal model.

The diffusion of drugs from the surface of the brain into the brain tissue is slow with the rate of diffusion, expressed as diffusion coefficient (D), inversely related to the molecular weight of the drug. The time it takes for a molecule to diffuse a given distance is related to the square of the distance (Pardridge, 1991). The time (for even a small molecule such as glucose (M_w 180 Da, 3.5 Å size)) to diffuse 5 mm is about 11.7 h with a D of 6×10^{-6} cm^2/s. For a protein such as albumin (M_w 68 kDa, 50 Å size) the time is 4.2 days with a D of 0.7×10^{-6} cm^2/s.

According to Pardridge (1991), the distinct difference in CSF bulk flow properties and the diffusional flow rates of drugs in brain tissue (and ISF) creates a functional barrier between the CSF and the cells of the brain tissue to include the ISF. This prevents complete equilibration between the two fluid compartments and a significant drug concentration difference exists between CSF and brain ISF. A cartoon of these two central extracellular compartments of the brain and their functional interaction with the bloodstream is given in Fig. 5. Hence, although no anatomical barrier exists between the CSF and the brain it can be concluded that a drug administered nasally which successfully reaches the CSF (and available drug receptors at this site) cannot automatically be considered to distribute further into the brain parenchyma.

4. Transport pathways from nose to brain

The different routes by which a drug delivered nasally can reach the CSF and the brain are shown schematically in Fig. 6, where the thickness of the arrows indicates the likelihood of drugs exploiting the route in question. When drugs are administered nasally the drug will normally be rapidly cleared by the mucociliary clearance system (Illum et al., 1994). Some of the drug (for lipophilic drugs up to 100% but normally much less) will be absorbed into the bloodstream from where it reaches the systemic circulation directly and subsequently is eliminated from the bloodstream via normal clearance mechanisms (Hussain et al., 1980). The drug can reach the brain from the blood by crossing the blood–brain barrier (the so-called systemic pathway to the brain) but can also be eliminated from the CSF into the blood. Of particular interest to this review is the fact that the drug can also be absorbed from the nose via the olfactory region into the CSF and possibly further into the brain. The amount of drug absorbed or lost via the different pathways has been shown to be highly dependent upon the characteristics of the drug, especially lipophilicity and molecular weight, but also the drug formulation (Sakane et al., 1991b, 1995).

In order for a drug to travel from the olfactory region in the nasal cavity to the CSF or the brain parenchyma, it has to transverse the nasal olfactory epithelium and, depending on the pathway followed, also the arachnoid membrane surrounding the subarachnoid space. In principle, one can envisage three different pathways across the olfactory epithelium; (i) transcellularly especially across the sustentacular cells, most likely by receptor mediated endocytosis, fluid phase endocytosis or by passive diffusion, the latter pathway most likely for more lipophilic drugs, (ii) paracellularly through tight junctions between sustentacular cells or the so-called clefts between sustentacular cells and olfactory neurones, (iii) by the olfactory nerve pathway where the drug is taken up into the neuron cell by endocytosic or pinocytotic mechanisms and transported by intracellular axonal transport to the olfactory bulb.

One of the first indications of the existence of the olfactory nerve pathway into the nasal cavity for non-microbial or non-viral agents administered was published by Faber (1937) who administered various materials such as potassium ferrocyanide and iron ammonium citrate nasally to rabbits. However, the mechanism of transport

was not fully elucidated. De Lorenzo (1970) placed ^{198}Au-labelled gold particles intranasally in squirrel monkeys. The particles were traced with electron microscopy and after 15 min were found to have travelled to the tips of the olfactory receptors. They reached the olfactory bulb in 30–60 min. The rate of progression in the olfactory neuron was estimated as 2.5 mm/h. This was confirmed by Czerniawska (1970) who injected rabbits with colloidal ^{198}Au under the mucosa of the olfactory region. The gold was found to reach the CSF within 1 to 2 h. Gopinath et al. (1978) later found in rhesus monkeys, that similar gold particles not only entered the olfactory rods but also the sustentacular cells. In the sustentacular cells the particles were found either as discrete particles or aggregated into electron-dense masses in the mitochondria. The gold particles were also found in the endothelial cells of the blood vessels in the lamina propria. Such particles were thought to originate from the base of the sustentacular cells. Gold particles were not seen in the intercellular spaces (tight junctions or clefts) of the olfactory mucosa. *The Lancet* reported an experiment performed by Perl and Good (1987) where rabbits were exposed nasally to aluminium lactate in solution. These researchers found that the aluminium lactate travelled to the olfactory bulb and the cerebral cortex where it was found inside granulomas of macrophages, lymphocytes and occasional plasma cells. A possible association between the occurrence of Alzheimer's disease and the inhalation of aluminosilicates present in polluted air was suggested.

Shipley (1985) confirmed the existence of a neuronal pathway from nose to brain by administering gelfoam implants soaked in wheat germ agglutinin–horseradish peroxidase (WGA–HRP) solution to the nose of rats. The WGA–HRP was found to have travelled a substantial distance within the brain, with labelled neurons being visualised by light microscopy in the raphe nuclei in the midbrain and pons and throughout the entire expanse of the olfactory cortex to the caudal pole of the cerebral hemisphere. Broadwell and Balin (1985) performed similar experiments in mice and squirrel monkeys with HRP and WGA–HRP and observed the HRP reaction product in the superficial fiber layer of the main olfactory bulb bilaterally within 45 to 60 min. The coloration product was evident throughout the intercellular clefts in the olfactory epithelium. At a much later time the olfactory fiber and glomerular layers of the olfactory bulbs were also labelled densely with the reaction product. However, this was attributed to the slower anterograde axoplasmic transport in the olfactory sensory neurones. An identical axonal transport was seen for the WGA–HRP. Similar results were shown by Itaya (1987) who observed secondary order neuron labelling of the accessory olfactory bulb and by Balin et al. (1986).

Apart from a study by Hastings and Evans (1991), who evaluated the transport of cadmium from the nasal cavity to the brain by the olfactory neurones, Thorne et al. (1995) were the first to determine quantitatively the intraneural transport of HRP and WGA–HRP after nasal administration. These authors measured the quantity of protein accumulated in the olfactory bulb 48 h after nasal or intravenous administration in the rat model. They found that WGA–HRP given nasally as a 1.0% solution resulted in a concentration of 140 nM in the olfactory bulb tissue whereas HRP given in the same concentration by the same route and WGA–HRP given in the same concentration by an intravenous injection only resulted in background levels in the olfactory bulb. This showed that WGA–HRP was not able to penetrate significantly the blood–brain barrier to enter the olfactory bulb parenchyma. It also indicated that the transport pathway of HRP may be different to that of WGA–HRP. The authors suggested that the WGA–HRP was taken up into the neuron by adsorptive or receptor-mediated endocytosis and transferred in organelles associated with the Golgi system within the cell to the axon terminal for release at the first order synapse in the glomeruli of the olfactory bulb. It was also suggested that HRP could have exploited two potential pathways due to the lack of binding sites on the plasmalemma; namely fluid phase endocytosis into the neuron followed by degradation in lysosomes (a pathway that was also suggested by Broadwell and Balin (1985)), or transport through the open intercellular clefts. However, the authors considered the availability of clefts large enough to let through HRP, to be limited in number.

It has been shown that the transneuronal pathway is very slow and that agents reach the CNS as late as 24 h after administration in the nasal cavity (Kristensson and Olsson, 1971). Hence, the transneuronal pathway cannot explain the rapid appearance of drug in the CSF and the brain seen for a range of small molecular weight drugs such as dihydroergotamine (Wang et al., 1998), cocaine (Chow et al., 1999), lignocaine (Chou and Donovan, 1998b), antihistamines (Chou and Donovan, 1997) and cephalexin (Sakane et al., 1991a).

Faber (1938) very early observed that if Prussian blue was placed in the nasal cavity of animals it could later be observed in the perineural spaces of the olfactory nerve and in the subarachnoid space (in the CSF) of the brain, apart from also appearing in the nasal lymph vessels and the cervical lymph nodes. This extracellular transport of agents would involve transport via patent intercellular tight junctions or clefts in the epithelium into the CSF and would rely on a direct anatomic connection between the submucosa and the subarachnoid extensions, the perineural space, surrounding the olfactory nerves as they penetrate the cribriform plate. The agent is thought to enter the perineural space, either due to the perineural epithelium surrounding the olfactory axon being loosely adherent ('open-cuff' model), or to enter through the epithelium cell junction if the perineural epithelium is closely adhered to the axon ('closed-cuff' model) (Jackson et al., 1979). The extracellular transport pathway is considered to be very

fast and possibly accounts for most of the rapid transport of small molecular weight drugs from the nasal cavity into the CSF (Frey et al., 1997).

Frey et al. (1997) and Chen et al. (1998) also showed that larger molecular weight drugs, such as the protein nerve growth factor (M_w 37 kDa), could be transported rapidly to the CNS in a rat model. Hence, after intranasal administration of ^{125}I labelled nerve growth factor (^{125}I-NGF), the radiolabeled drug (less than 20% free label) appeared within 20 min in the olfactory bulb and to a lesser extent in other parts of the brain. The concentration of radiolabel in the blood was similar for both intranasal and intravenous administration studies but the radiolabel in the CNS, after intravenous administration, was only 0.001 nM as compared to up to 2.0 nM after nasal administration. The authors suggested that the rapid appearance of the drug in the olfactory bulb, the cerebrum and the brain stem was consistent with the drug being transported via intercellular clefts in the olfactory epithelium or extracellularly along the neurones to reach the CSF and the brain. The fact that no receptors for NGF are found on primary olfactory neurons supports the suggestion that the transport is not intracellular via the axonal pathway. The efficiency of the NGF delivery to the brain was found to be much less than that found by Sakane et al. (1991a) for the low molecular weight drug, cephalexin (M_w 401 Da) of 13 μM in the CSF. This could most likely be due to limitations in the diameter of the open clefts or tight junctions.

It can be concluded that the pathway employed for delivery of a particular drug from the nose to the brain is highly dependent on various factors such as the existence of specific receptors on the olfactory neurons, the lipophilicity and the molecular weight of the drug. Generally, the rapid appearance of a drug in the CSF, and parts of the brain, indicates a primary use of routes other than the olfactory nerve pathway. A slow transport of drug into the CNS indicates that the main pathway taken is via the olfactory nerve. It is likely that drugs may use more than one pathway in the transport from nose to CNS.

5. Transport of drugs from nose to brain

A range of publications has emerged in which the transport of various drugs (mostly low molecular conventional drugs) from the nasal cavity to the CSF or the brain has been investigated. Most studies have been performed in the rat model but studies in mice, rabbits and monkeys have also been reported. It is important to consider, when interpreting results obtained in animals, that the olfactory region, of, for example, the rat and other commonly used animal models, is covering a large part of the nasal mucosa, whereas in humans the olfactory epithelium covers only a small area in the roof of the nasal cavity. Hence, it is most likely that the olfactory transport of drugs will be much more pronounced in rats than for the same compounds in humans. Also, the commonly used technique of placing the rat in an anaesthetised state on its back during application of the test formulation will most likely contribute to the efficient bathing of the olfactory region and thereby to an enhanced transport. As far as known, no studies have investigated the comparative transport of drugs from nose to brain in different animal models. However, for the absorption of drugs across the respiratory epithelium to the systemic circulation, it has been widely recognised that the animal model used is of considerable significance (Illum, 1996).

5.1. Experimental methods used in nose to brain transport studies

In the most basic studies, for example performed in mice and rats, the animals are dosed with the drug nasally and parenterally, plasma samples are taken for a dedicated time period and the animals then killed at certain time points. The nasal dosing of these animals is normally performed in anaesthetised subjects placed on their backs. The dose is given as nose drops in volumes as high as 100 μl, given over extended periods of time. Such a high dosing volume and the position of the animal during dosing can promote the drug formulation reaching and covering the olfactory region. It should be noted, however, that volumes of 50 μl and larger will fill the nasal cavity of rats and most likely the surplus will disappear into the back of the throat. It has further been reported that some anaesthetics can have an inhibitory effect on the retrograde and anterograde axoplasmic transport. Urethane was shown to have no such effect on this transport (Chou and Donovan, 1998b). In some studies, such as the ones by Gizurarson et al. (1996) and Sigurdsson et al. (1997), invasive techniques were used for the application of the dose to the olfactory region e.g. by sub-olfactory epithelial injection through the palate. As clearly stated in the discussion of these papers, this will influence the characteristics of the transport of the drug.

In most studies the brain is removed and then either counted as a whole tissue for radioactivity or measured for drug content (Hussain et al., 1990; Gizurarson et al., 1996), or sliced into vertical slices and counted for radioactivity (Javaid and Davis, 1993) or separated into the various brain sections and counted individually (Gozes et al., 1996; Wang et al., 1998). In some cases the brain has been perfused-fixed before its dissection into different parts and radioactivity counted (Frey et al., 1995; Chen et al., 1998).

In certain studies the CSF is collected, either as a one off sample at the end of the experiment (Anand Kumar et al., 1982; Chen et al., 1998) or as several samples during the period of the study (Chou and Donovan, 1998a). The collection of CSF is most often performed by cisternal puncture with a fine needle connected to tubing, where an incision is made in the skin over the occipital bone. Collection is terminated when blood appears (Anand

Kumar et al., 1982; Chen et al., 1998). Volumes of 150 μl or larger can be collected in this way (Seki et al., 1994). Some papers report technical difficulties in obtaining consistent volumes of CSF using the cisternal cannulation method, due to slow flow rate of the CSF and blood appearing in the sample (Kumbale et al., 1999). It has also been reported by Sakane et al. (1991a,b) that due to the location of the CSF on the surface of the brain the initial CSF fraction obtained often has a lower drug concentration than in the later fractions collected. This problem was overcome by some researchers by sampling an early and a late fraction of the CSF (Seki et al., 1994). A volume larger than 70 μl was recommended in order to have representative samples. In experiments where CSF is collected throughout the entire study period, the CSF has been replaced by infusion of artificial CSF into the lateral ventricle (Chou and Donovan, 1997).

CSF drug concentration has also been measured by a novel microdialysis technique, where the dialysis probe is inserted into the cisterna magna (Chou and Donovan, 1998b). In this technique the anaesthetised rat was secured on a stereotaxic frame and the allanto-occipital membrane exposed by making a blunt dissection through the allanto-occipital muscle. A hole was made in the membrane using a 21G needle to access the cisterna magna. The microdialysis probe was then placed in the hole reaching 3.5 mm below the membrane so that the entire surface area was bathed in the CSF. The implanted probe was perfused with artificial CSF at a flow rate of 3 μl/min for 1 h prior to drug administration and continuously during the whole experimental period. This microdialysis method permits continuous monitoring of the drug concentration in the CSF without removing any body fluids. It can also be used for monitoring drug concentration in, for example, the olfactory bulb and other regions of the brain of the animal. In the experiment reported by Chou and Donovan (1998b) the results obtained for CSF concentration of lignocaine were similar, whether measured by microdialysis or by direct sampling of CSF. However, the disappearance of the lignocaine after 120 min following nasal administration was faster when measured by microdialysis. This was thought to be due to regional differences in drug concentrations.

Some experiments on nose to brain transport have been reported in monkeys (Anand Kumar et al., 1974; Gopinath et al., 1978). These animals have been dosed in the conscious state and after the end of the experiment the animals have been killed and the brain and other parts examined. In other experiments the monkeys were anaesthetised and sampling of CSF performed throughout the experiment (David et al., 1981; Anand Kumar et al., 1982). In this way it was possible to perform cross-over studies in a group of animals. Clearly, the monkey model can be considered to be more realistic for providing information on the human transport process than the rodent models described above.

5.2. Transport of low molecular weight drugs from nose to CNS in animal models

It has long been known that cocaine is absorbed rapidly from the nasal mucosa. Moreover, the euphoria derived from the sniffing of cocaine in conscious subjects has been reported to occur rapidly (within 3 to 5 min) (Bromley and Hayward, 1988). It was speculated that the reason for such a rapid effect of cocaine on the CNS was the presence of a direct pathway for cocaine from the nasal cavity to the brain and the capacity for the drug to concentrate selectively in specific regions of the brain. In order to evaluate this theory Javaid and Davis (1993) examined the cocaine concentration in serum and discrete brain regions after intraperitoneal administration in rats. They found that the absorption pattern was similar to that seen in humans after nasal administration with a peak cocaine level in the brain obtained after 10 min. There was no indication of cocaine disposition in selective regions of the brain. Chow et al. (1999) have subsequently compared the uptake of cocaine in different brain regions after nasal and intravenous administration in rats. These authors confirmed the similar distribution of the cocaine in the various regions of the brain after intravenous administration found by Javaid and Davis (1993). However, after nasal administration, the cocaine content in samples collected within 60 min after administration showed the highest concentration in the olfactory bulb, followed by the olfactory tract and then the remaining parts of the brain. For direct comparison between brain uptake, following the two routes of administration, the concentrations were normalised in relation to their respective plasma cocaine concentrations. It could be seen that for the early time points (0–1 min) there was a significantly higher ratio between 'AUC olfactory bulb/AUC plasma' after nasal administration as compared to intravenous injection of cocaine. However, due to the rapid and extensive systemic absorption of the drug after nasal administration, most of the brain deposition of the drug resulted from access across the blood–brain barrier. This could be seen in the very similar ratios obtained after nasal and intravenous injection after 1 min.

A similar result was found for the nasal administration of a cognition enhancer in rats by Hussain et al. (1990) who showed that the ratio of brain to plasma concentrations of the drug were similar for administration via the nasal route and by intravenous injection. The drug was rapidly absorbed into the systemic circulation with a bioavailability of 50%. The first samples were taken 2 min after administration and consequently the authors would have missed the elevated levels seen in the brain for cocaine as opposed to plasma up to 1 min after dosing.

Progesterone administered nasally provides 100% bioavailability with a plasma profile very similar to an intravenous injection. The CSF:plasma ratio for this drug was found to be higher for nasal than for intravenous injection but lower than for intravenous infusion (Anand

Kumar et al., 1982). These results could, however, be influenced by the time taken for the infusion to be given. The results are in line with results obtained by the same authors in previous studies, where oestradiol and progesterone were administered nasally and by intravenous injection to rhesus monkeys (Anand Kumar et al., 1974; David et al., 1981). Here the authors concluded that the amount of labelled drug that reached the CSF was higher for nasally administered drugs than for drugs given by injection. This was especially pronounced for progesterone. The experiments were only performed in a small number of monkeys for each leg and the studies were only in one case carried out as cross-over experiments. Hence, different levels of endogenous hormone could have influenced the results.

Gizurarson et al. (1996) reported that the highest concentration of diazepam that occurred 10 min after nasal administration was not in the olfactory bulb as was expected, but just behind the olfactory bulb. This suggested that the drug, in this short time interval, had travelled through the olfactory bulb continuing through the olfactory tract to the thalamus or to the limbic system. A preferential brain distribution of diazepam was also claimed in rats by Einer-Jensen (1998) who sampled the blood from the carotid artery after nasal and intravenous administration. However, the rationale for the suggested mechanism was related to the possible existence of a direct transfer of drug between the nasal veins and the brain arteries.

Char et al. (1992) compared the plasma and brain levels after nasal and intravenous administration of dextromethorphan hydrochloride (DH) in rats. This drug has good blood–brain permeability. The results showed that the plasma and brain concentrations of DH after nasal administration were 78.8% and 65.7%, respectively, as compared to an intravenous injection. These results were based on AUCs obtained from 2 to 120 min after administration. They are in line with the results obtained for the drugs, such as cocaine, mentioned above. In these experiments the CSF was not sampled and the region of the brain investigated was only the anterior part of the cerebrum. No information was given about the levels of drug in the olfactory bulb. Also in this work, the first sample point was 2 min after administration.

Zidovudine is a drug used for treatment of AIDS patients with CNS dysfunctions. Its nasal bioavailability has been estimated to about 60%. A study by Seki et al. (1994) in rats compared the CSF and plasma levels of the drug after nasal and intravenous infusion. It was found that at 15 min after administration of the drug, the ratio of CSF to plasma was higher after nasal than after intravenous infusion. A direct transport from the nasal cavity to the CSF was suggested.

Cephalexin, a drug with a low permeability to the blood–brain barrier, was shown by Sakane et al. (1991a) to preferentially enter the CSF after nasal administration as compared to intravenous and intraduodenal administration in the rat model. After nasal administration the levels in the CSF were hundred folds higher than for the other routes of administration. The doses were chosen to ensure similar plasma levels for all routes of administration. The concentration of drug in the CSF was higher at 15 min than at 30 min after nasal administration, whereas there was no difference between the two time points after intravenous or intraduodenal application. The levels of drug in the brain parenchyma or the olfactory lobe were not measured.

The same authors investigated the effect of drug lipophilicity on nasal transport to the CSF (Sakane et al., 1991b). In this experiment the nasal cavity of rats was perfused (single pass infusion, 10 mM drug in phosphate buffer pH 7.4, 1 ml/min flow rate) with the drug solution in question. The drugs studied were sulphanilic acid (SA), sulphamethizole (SMZ), sulphisoxazole (SIX) and sulphisomidine (SID) with the respective partition coefficients, between isoamyl alcohol and phosphate buffer, of 0.012, 0.250, 0.261 and 0.892. The corresponding octanol/water partition coefficients are not readily available except for SMZ, which was measured to be 0.540. Hence, the range of lipophilicities was in the lower end of the scale for the chosen drugs. The CSF uptake after intravenous administration was studied as a comparison. Although the plasma levels of the drugs were much lower after nasal administration, as compared to intravenous administration, the CSF levels were significantly higher, with the uptake being directly related to the lipophilicity of the drugs (Fig. 7). The SA showed the lowest and the SID the highest level of uptake in the CSF. It was also pointed out by the authors that the concentration of drug in the CSF was only

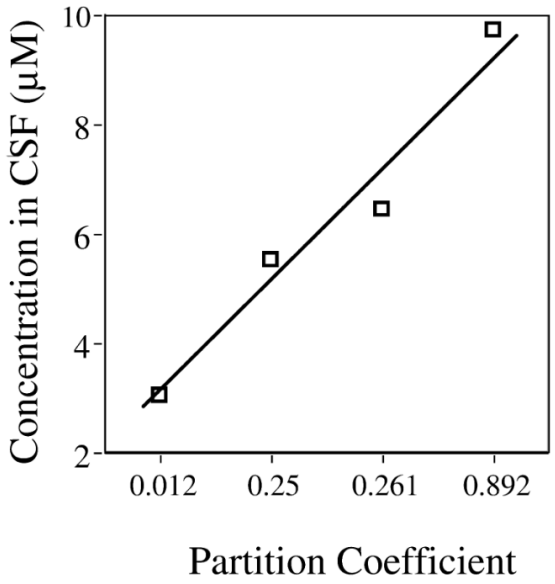

Fig. 7. The effect of compound partition coefficient on transport from nose to CSF. Adopted from Sakane et al. (1991b).

a thousandth of that in the nasal perfusion medium. This was suggested to be due to the rapid absorption of the drug from the nasal cavity to the plasma. However, the rapid transfer of drug from the CSF to the plasma (as discussed above) could also have an effect on the levels measured after 60 min.

A similar study was performed later by Chou and Donovan (1998a) who studied the distribution of local anaesthetics, procaine hydrochloride, tetracaine hydrochloride, bupivacaine hydrochloride and lignocaine hydrochloride, into the CSF after nasal administration and confirmed the dependence of CSF uptake on the partition coefficient of the drug. The log DCs (octanol/water) were −0.092 for procaine HCl, 1.55 for lignocaine HCl, 2.18 for tetracaine HCl and 2.22 for bupivacaine HCl. The pK_as for the compounds were of the order of 8 to 9. Intra-arterial administration of the drugs was used as a control. The sampling of the CSF was done by cannulation of the cisterna magna. For most of the drugs, except procaine HCl, the plasma levels obtained after nasal administration were comparable to those obtained after arterial injection. The relative bioavailability for procaine HCl was 43% compared to 100% for the others. The rank order of the ratios of AUCcsf/AUCplasma for the compounds were tetracaine HCl>bupivacaine HCl>lignocaine HCl> procaine HCl. The order was well correlated with the log DC of the compounds, however, the time courses of the drug concentrations within the CSF differed significantly depending both on the compound and the route of administration.

Sakane later investigated the relationship between the degree of dissociation of a drug and the uptake into the CSF after nasal perfusion administration in the rat model (Sakane et al., 1994). These authors used the drug sulphisomidine (PC 0.892, isoamyl alcohol/phosphate buffer) which was shown previously to have a relatively high uptake into the CSF. The drug was perfused in solutions of pH values of 5.5, 6.5, 7.4, 8.7 and 9.4. The drug has a pK_a of 7.5, which therefore provides a range of conditions from fully undissociated drug to fully dissociated drug. An intravenous control was also applied in this study. It was found that nasal absorption increased with the degree of undissociated. This was also the case for CSF uptake. Hence, this study shows that the transport of a drug into the CSF obeys the well-known pH-partition theory and that the transport is most likely governed by passive diffusion across the olfactory epithelium.

In the latest study by the same authors the relationship between molecular weight and drug transport to the CSF after nasal perfusion and intravenous injection was investigated in the rat model (Sakane et al., 1995). The authors used fluorescein isothiocyanate-labelled dextran as a model drug. The molecular weights were in the range 4400 (FD4), 9400 (FD10), 18 900 (FD20) and 40 500 (FD40) Da. None of the dextrans were detected in the CSF after intravenous administration. All the various dextrans of differing molecular weights, except FD40, were detected in the CSF after nasal administration and the concentration decreased with increase in molecular weight. The study showed that molecules as large as 20 000 Da could be transported from the nasal cavity to the CSF. However, for these relatively large model drugs the level of CSF uptake was of the order of three times lower than that seen for the low molecular weight drugs (0.01–0.1%). The brain parenchyma uptake was not investigated.

Kao (1995) studied the nasal transport of various esters, such as butyl and methyl, of the carboxyl group of L-dopa in the rat model. It was found that the nasal administration of the ester prodrugs produced significantly higher levels of L-dopa in the CSF and the olfactory bulb than did equimolar doses of the prodrugs given intravenously (Fig. 8). That L-dopa can access the brain after nasal administration had previously been suggested by De Souza Silva et al. (1997) and Silva et al. (1997), who detected increased extracellular levels of dopamine in the neostriatum in rats by means of a microdialysis technique.

Chou and Donovan (1997) investigated the distribution of a range of low molecular weight antihistamines (chlorcyclizine hydrochloride, chlorpheniramine maleate, hydroxyzine dihydrochloride, triprolidine hydrochloride) into the CSF following nasal delivery to the rat. The log distribution coefficient (log DC) between buffer and chloroform for the drugs ranged from 0.35 for chlorpheniramine maleate to 2.91 for chlorcyclizine hydrochloride. The drugs had similar pK_as and were administered in similar concentrations to the nasal cavity and all the drugs were ionised at the pH of the administration buffer. The authors showed no distinct correlation between log DC for the various drugs and their brain uptake. Moreover, for two of the drugs, the ones with the highest and lowest log DC

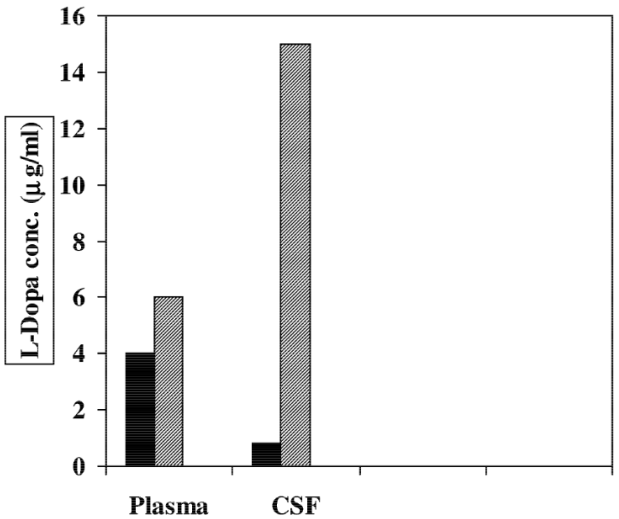

Fig. 8. Plasma and CSF levels in rats at 30 min following nasal and intravenous administrations of L-dopa butylester at 20 mg/kg L-dopa equivalent dose. ■, i.v. administration; □, i.n. administration. Adopted from Kao (1995).

values, no CSF transport was seen although for the highest log DC a low plasma concentration was detectable. For the two drugs with intermediate log DCs the highest CSF uptake was seen together with highest plasma concentrations. The authors concluded that, for this range of molecules, the transport to the CSF seemed to be controlled by a combination of molecular and biological properties; none of which singularly determined the tissue distribution.

The disposition of lignocaine within the CNS of the rat, after nasal and intra-arterial administration, was characterised by Chou and Donovan (1998a,b) using both conventional methods and a novel in vivo microdialysis sampling technique. The authors found that the ratio of AUCn/AUCa were higher than unity for only the cisterna magna sample and lower than unity for the olfactory bulb and the cerebellum. The CSF concentration, after nasal administration and conventional sampling, was found to be considerably higher than after arterial administration, with the ratio of 1.54 between the AUCs. The systemic bioavailability of lignocaine after nasal administration was 100% and it would be expected that all ratios would be equal to unity. Hence, this study also provides confirmation of the existence of a direct pathway from nose to brain.

It was recently shown by Wang et al. (1998) that dihydroergotamine (DHE), which is used for treatment of migraine headaches, is transported preferentially to the olfactory bulb. The study was carried out in the rat model. The rats were given similar doses of [^{3}H]DHE and [^{14}C]inulin nasally and intravenously. The inulin is known not to cross the blood–brain barrier and was used as an internal standard. The concentrations of drug in various brain sections were measured. CSF was not collected. The DHE only showed limited transport from the nose to the plasma (bioavailability 14%). For most brain tissues the concentration of DHE after nasal administration was similar or lower than after intravenous administration. However, in the olfactory bulb the DHE concentration was markedly higher (about fourfold) for the nasally administered DHE. Even higher concentrations were found in the various brain tissues for inulin when administered nasally. Here again, these results show clearly the existence of a direct pathway for these drugs to the CNS from the nasal cavity.

This review of the literature concerning the transport of small molecular drugs from the nasal cavity to the CNS in animal models has shown that uptake will indeed take place preferentially but also that the extent of uptake is highly dependent on the physico-chemical properties of the drug in question. Hence, it can be predicted that, at least in animal models, a drug with a moderate lipophilicity, i.e. one that is not so lipophilic so as to give a rapid and total transport into the systemic circulation, will show a higher CSF and olfactory bulb concentration after nasal administration than after parenteral administration. In some cases the drug can also be seen to move further into the brain parenchyma. The degree of uptake is dependent on the molecular weight, the degree of lipophilicity and the degree of dissociation. Thus the most likely pathway followed by such drugs will be passive diffusion across the epithelium (the more lipophilic drugs) or through the tight junction of the cells (the more hydrophilic drugs). Since the passage of these drugs into the CSF and olfactory bulb is seen to occur so rapidly, it is unlikely that the neuronal pathway is of significance.

Therefore, the above examples suggest that the direct pathway from nose to brain may only be significant for compounds which are poorly absorbed from the nasal cavity to the systemic circulation or have low blood–brain barrier transport properties and that the drug that finds its way from the nasal cavity (if any) can be found in the highest concentrations in the CSF and to a lesser degree in the olfactory bulb, as compared to the rest of the brain.

5.3. Transport of large molecular weight drugs from nose to CNS in animal models

The intravenous injection of human β-endorphin has been shown to result in the release of prolactin and growth hormone in rat and man. However, in order to achieve a pharmacological effect high doses have to be given via this route. Rao et al. (1986) administered β-endorphin to adult bonnet monkeys at a dose of 20 μg/kg body weight by nasal spray and compared the resultant prolactin release with that obtained after an intravenous injection of the same dose. After nasal administration a significant increase in prolactin levels was seen within 30 min (Table 2). This level was maintained for 60 min and at 120 min had declined to control levels. After intravenous injection the prolactin levels did not increase until 60 min after administration and then only to lower levels than that seen after nasal administration. It was suggested by the authors that β-endorphin achieved a direct access to the hypothalamic circulation from the nasal cavity. No suggestions were made for the chosen pathway.

More recently, Frey et al. (1995) have investigated the nasal absorption of nerve growth factor into the brain via the olfactory bulb. The drug was labelled with ^{125}I and administered nasally and intravenously to rats. The doses given were chosen to provide similar AUCs for the time

Table 2
Effect of intranasal and intravenous administration of β-endorphin on serum prolactin levels in female bonnet monkeys[a]

	Prolactin ng/ml serum Minutes after administration			
	0	30	60	120
Vehicle control i.n.	47.2	51.2	57.2	45.0
β-endorphin i.n	41.2	80.8	77.6	35.4
β-endorphin i.v.	35.1	36.2	69.5	34.7

[a] From Rao et al. (1986).

versus plasma concentrations of the drug given by the two routes and were 7.4 nmol nasally and 21 pmol parenterally. The olfactory epithelium, olfactory bulb, cerebellum, brain stem, and four approximately equal coronal brain sections and the blood were analysed for drug content. After nasal, but not intravenous, administration the radiolabel appeared rapidly (<20 min) in the olfactory bulb and to a lesser extent in the other parts of the brain. The accumulation of the radiolabel in the olfactory bulb was a linear function of the nasal dose administered. The authors suggested that the rapid appearance of the drug in the various brain sections was more consistent with entry of the label through clefts in the olfactory epithelium than by transport via the axonal pathway; which would be a much slower process. This theory was later supported by further work of the same authors as described above (Frey et al., 1997).

The nasal delivery of insulin and the potential for transport to the brain was investigated by Gizurarson et al. (1996) in a mouse model. A [^{125}I]insulin solution was administered to mice by (I) injection through the palate into the olfactory region, (II) administration on the nostrils with subsequent inhalation, (III) instillation by tube into the olfactory region and (IV) as III but using a shorter tube. A subcutaneous injection was used as a control. The brain was removed after sacrifice of the animal 10 min after nasal administration and the activity associated with the brain tissue counted. For all nasal administration methods the brain/blood ratio was higher than for the subcutaneous injection. The concentration in the brain tissue was highest for the direct injection into the olfactory region (method I) but also significantly higher for the subcutaneous injection for administration method IV. There was no distinction made between uptake into the various brain regions to include olfactory lobe and CSF. Hence, it is difficult to judge how far into the parenchymal tissue the insulin had travelled. It is likely that most of the activity was in the olfactory lobe and the CSF. The same authors later investigated factors influencing the insulin transport from nose to brain, again the mouse model was used (Sigurdsson et al., 1997). They found that the nose to brain transport of insulin was not influenced by a pre-injection of insulin by the subcutaneous route, which was not surprising in view of the low transport to the brain from this route of delivery seen in their first study. It was also found that the accumulation of insulin in the brain was linearly related to the dose administered nasally. The authors suggested that insulin absorption was highest near the isoelectric point (pH 5.3–5.4) although only at a pH value of 6.5 on the pH-absorption profile was the absorption significantly higher than that at higher and lower pH values.

Neurodegenerative diseases, where the neuronal cells disintegrate, result in deterioration in cognitive functions as seen in Alzheimer patients. Vasoactive intestinal peptide (VIP) has been shown to protect neurones and play an important part in learning and memory. A synthetic lipophilic analogue (St-Nle^{17})VIP has been found to exhibit similar protective characteristics in that it can completely prevent cell death caused by the administration of β-amyloid peptide, which normally cause 70% loss in the number of neurones in the rat cerebral cortical cultures treated with this peptide. In a rat model, subjected to treatment with a cholinergic blocker, (St-Nle^{17})VIP was administered nasally and intra-cerebroventricularly (Gozes et al., 1996). Concentrations of the drug were found mainly in the brain cortex and in the brain stem after nasal administration. It was also shown that the drug given via both routes prevented impairments in spatial learning and memory associated with cholinergic blockade. These results suggest that the (St-Nle^{17})VIP entered the brain intact after nasal administration. (St-Nle^{17})VIP does not easily cross the blood–brain barrier.

Chen et al. (1998) investigated the potential of delivering nerve growth factor (NGF) to the brain along the olfactory neural pathway for the treatment of Alzheimer's disease. The NGF was administered as nose drops to anaesthetised rats and the concentration of the drug in the brain was determined using an ELISA assay. It was found that the NGF reached the brain within an hour achieving the highest concentration in the olfactory bulb and less (although still significant) in other brain regions. After intravenous administration little or no drug was found in any regions of the brain due to its inability to pass the blood–brain barrier. As suggested above, the likely pathway taken from the olfactory region to the brain was via an extraneuronal route.

The same group of researchers recently published studies in rats which evaluated the delivery of monosialoganglioside (GM1) to the brain from the olfactory region (Kumbale et al., 1999). The total nasal dose (100 μl) was administered in small volumes over a period of 30 min. As controls and as comparative formulations the GM1 was also administered by intravenous injection, either as a simple solution or in lipid nanospheres or as a positively charged lipid complex. The latter two formulations were also administered by the nasal route. Apart from blood samples, CSF was collected by cisternal puncture. The effect of sampling time and volume of sampling of CSF was investigated. A volume of 70 μl collected at 30 min was selected as giving the highest CSF concentration. All three nasal formulations were shown to be transported to the CSF. Low levels of GM1 were found in the CSF after intravenous administration of a simple solution of the drug, indicating that little or no transport took place across the blood–brain barrier. None of the three nasal formulations differed significantly in terms of quantities transported within the time scales of the experiment, although the trend showed increased transport for the positive GM1 complex formulation (Fig. 9). The highest brain concentrations were detected after intravenous injection of GM1 in the lipid nanospheres. The lipid nanosphere formulation and the positively charged complex formula-

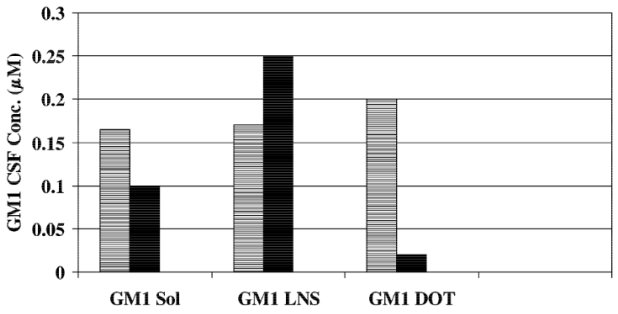

Fig. 9. CSF levels of GM1 after nasal □ and i.v. administration ■ of 3.25 μmol GM1. Sol, solution; LNS, lipid nanospheres; DOT, lipid complex. Adopted from Kumbale et al. (1999).

tion were included in the study in order to investigate whether a mucoadhesive formulation that would bind to the olfactory mucosa could enhance the transport to the brain. Unfortunately, the paper lacks information concerning the release of the GM1 from these formulations and information concerning their ability (as large particles) to cross the membrane by transcytosis. The authors indicated that the most likely transport mechanism for all GM1 formulations was free GM1 moving via the intercellular clefts in the membrane.

Parkinsonian syndrome is characterised by progressive degeneration of the substantia nigra dopaminergic neurons and severe depletion of dopamine level in the stratum and can be induced in animal models by injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. It has been shown that the animal can be protected against lesions induced by this neurotoxin by acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) present in the dopaminergic neurons (Kucheryanu et al., 1999). Kucheryanu et al. (1999) found that mice with an induced Parkinsonian syndrome given nasal applications of aFGF and bFGF, either 30 min before the start of the induction of the neurotoxin or 3 or 5 days after the beginning of the induction, showed a significantly increased motor activity and other forms of locomotor activity and suppressed rigidity on days 5 and 10 after the beginning of the neurotoxin administration as compared to a saline control. Also tremors were significantly reduced in the animals. It was suggested that the growth factors were delivered directly to the brain from the nasal cavity. Unfortunately, the study lacked an intravenous control, hence it cannot totally be ruled out that the drug reached the brain via the bloodstream.

The various studies and results discussed above indicate clearly that large molecular weight drugs such as peptides and proteins, can also be transported from the olfactory region in the nasal cavity to the CSF, the olfactory bulb and in some cases further into the brain parenchyma. What is noteworthy is the speed with which the drug appears in the CSF and other brain regions; often within 10 min after nasal administration. This rapid transport indicates that the pathway taken, at least in the initial stages of transport, is by extracellular routes such as the clefts or tight junctions between the epithelial cells.

5.4. Evidence for nose to brain transport of drugs in humans

The studies described above show conclusively that small molecular weight drugs of suitable lipophilicity and larger molecules are transported from the olfactory region in the nasal cavity to the CSF, to the olfactory bulb and in some cases further into the brain tissue in animal models. Most studies have been performed in the rat model that most likely over estimates the degree of transport for the reasons discussed above. Other studies have been performed in monkey models which should give better indication of the degree and rate of transport of such drugs to the regions of the brain in man.

Only few studies provide evidence for the transport of drugs from nose to CNS in man. Naturally enough, most studies that have been published do not measure directly the rate and degree of transport of the drug but rather indirect measures in the form of pharmacodynamic effects on the CNS. The studies discussed below were mainly conducted by the group of Fehm and Born in the Department of Internal Medicine, University of Lubeck, Germany.

Pietrowsky et al. (1996a) conducted a double blind, crossover study in 15 healthy male volunteers who received, on three different occasions, either 20 IU of arginine-vasopressin (AVP) intranasally or 1.5 IU AVP by intravenous injection or a saline solution intranasally. The event-related potentials (ERP) were recorded during the subject's performance of an auditory attention task (oddball task). The recording of the ERP started about 45 min after the drug was administered and lasted for 45 min. Intranasal administration of AVP substantially increased the P3 component of the ERP, whereas this effect was not seen after intravenous administration of the drug, nor after the placebo nasal treatment (Fig. 10). The authors concluded that the results provided functional evidence for a facilitated access of the AVP to the CNS after nasal administration as compared to intravenous injection in man. It was suggested that the pathway taken by the AVP was extracellular, due to the lack of receptors for vasopressin on the olfactory bulb and to the fast onset of the effect of the drug.

The same authors provided similar evidence of nose to brain transport of the peptide, cholecystokinin-8 (CCK) in a placebo controlled, double blind, cross-over study in 20 healthy volunteers (men and women) (Pietrowsky et al., 1996b). CCK was administered intranasally (10 μg) and intravenously (0.25 and 2.5 μg). The placebo control was saline given nasally or by intravenous injection. The auditory event-related potentials (AERP) were recorded during a subject's performance of an auditory attention

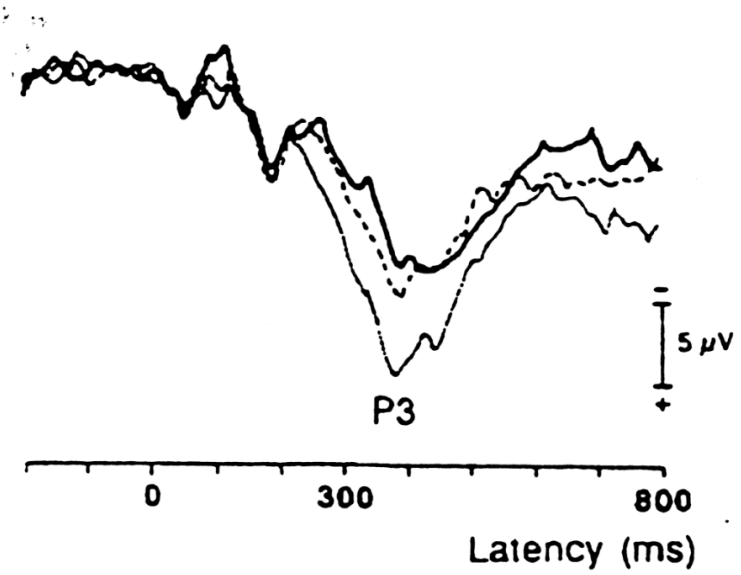

Fig. 10. Averaged auditory evoked potentials to target stimuli of an attended oddball task from a single subject. Thick solid line, placebo; thin solid line, intranasal administration of arginine-vasopressin 20 IU; dashed line, intravenous administration of arginine-vasopressin 1.5 IU. Adopted from Pietrowsky et al. (1996a).

task (oddball task). The recording of the ERP started about 30 min after the drug was administered and lasted for 15 min. Plasma CCK concentrations after nasal administration of CCK were comparable to those after intravenous administration of 0.25 μg CCK, but substantially lower than those after intravenous administration of 2.5 μg CCK. Intranasal administration of CCK markedly increased the P3 complex of the AERP, whereas this effect was not seen after intravenous administration of 0.25 μg of CCK, nor after the placebo treatments. This effect was more pronounced in women than in men. As with the study on vasopressin, the authors concluded that the results pointed to a direct pathway from the nasal olfactory region to the brain, most likely an extracellular pathway, although it could not be excluded that some effect could be due to transport of the drug across the blood–brain barrier.

Derad et al. (1998) investigated whether nasally administered angiotensin II (ANG II) affected central nervous functions of cardiovascular control in a different way to that from intravenously administered ANG II. In a balanced cross-over design, 12 healthy men were treated with 2.5 μg ANG II intravenously and 400 μg ANG II intranasally and a placebo. The plasma levels of ANG II, vasopressin, norepinephrine and epinephrine were assessed every 10 min and the blood pressure, heart rate and systemic vascular resistance measured by a Dinamap and by continuous non-invasive body plethysmography. For intravenous and nasal administration of ANG II similar plasma levels of ANG II were seen. Also, both methods of administration resulted in comparable acute rises in blood pressure. However, subsequent blood pressure profiles were different for nasal and intravenous administration. After intravenous ANG II administration the blood pressure remained enhanced at an intermediate level, whereas after nasal administration it returned to normal or even below normal levels. Intranasal ANG II also counteracted the decrease in norepinephrine levels observed after intravenous administration of ANG II and enhanced plasma concentrations of vasopressin. The authors concluded that this diverging pattern of effects bore similarities to the effects after an intracerebroventricular administration of ANG II in animals, suggesting that nasal administration resulted in a direct central nervous action of ANG II.

The effects of insulin administered nasally to 18 volunteers (double blind within subject crossover design) on the auditory evoked potentials (AEP) indexing cortical sensory processing were recorded while the subjects performed a vigilance task (oddball paradigm) during the baseline phase and after 60 min of nasal treatment with insulin or placebo (Kern et al., 1999). Blood glucose and serum insulin levels were not affected by the nasal insulin. However, compared to placebo the nasal insulin reduced the amplitudes of the N1 and P3 components of the AEP and increased P3 latency. The reduction in P3 amplitude was most pronounced over the frontal recording site. The results suggested that nasally administered insulin directly entered the brain from the nasal cavity and exerted distinct influences on the central nervous functions in humans.

Brain potentials and attention after acute and subchronic nasal administration of adrenocorticotropin 4-10 (ACTH 4-10) and desacetyl-α-MSH in humans were studied by Smolnik et al. (1999). A double-blind placebo controlled experiment was performed in 54 healthy young subjects. Event-related brain potentials (ERP) were recorded while the subjects performed an auditory selective attention task. Moreover, a modified Stroop interference test including

motivational (food, sex) and non-motivational words was performed. The acute nasal administration of the ACTH 4-10 (1 mg) reduced the processing negativity of the ERP over the frontal and central cortical areas indicating diminished focusing of attention, whereas subchronic (1 mg/day over 6 weeks) did not affect the processing or the Stroop performance. The results confirmed an acute decrease in focusing of attention after ACTH 4-10 nasal administration. These effects were suggested by the authors to reflect a direct action of the peptide on the respective brain functions.

Recently, the first attempt at radioisotopic assessment of the integrity of the nose–brain barrier was published. In a study by Okuyama (1997) a mixture of ^{99m}Tc–DTPA and hyaluronidase was sprayed onto the olfactory mucosa of anosmic patients and the cerebral radioactivity measured. A significant rise in cerebral radioactivity was observed 5 min after application of the radioisotope.

The above studies, albeit mostly from one research group, have given clear evidence that even large molecules, such as the peptide insulin, can be transported rapidly from the nasal cavity to the CNS in man where they can exert specific effects on various brain functions. Such effects are not seen after parenteral administration or placebo treatment. Due to the rapid onset of action it is most likely that the molecules are transported by an extracellular transport pathway.

6. Conclusion

It is evident from the results described and discussed above that when drugs are administered to the nasal cavity of rodent and primate animal models a proportion is transported through the olfactory epithelial region to the CSF, the olfactory lobes of the brain or, in some cases, further into the parenchyma of the brain. It has been shown that the rate and degree of transport and deposition are highly dependent on the physico-chemical properties of the drugs, especially the molecular weight and the lipophilicity. It is also evident that the choice of pathway is drug dependent and can be related to the existence (or not) of receptors on the olfactory neurones. This was seen in particular for HRP and WGA–HRP, which exploit different pathways through the epithelium (WGA–HRP has binding sites on the plasmalemma).

The results from studies performed in animal models have been confirmed to some extent in human studies. Evidence has been provided that suggests that even large molecules, such as the peptide insulin, can be transported rapidly from the nasal cavity to the CNS and by so doing can exert specific effects on various brain functions. For most drugs studied the rapid appearance of the drug in the CNS or the rapid onset of effect (often within 10 min) indicates that the initial pathway used is by an extracellular route. However, since most of the published experiments last for but a few hours only, it cannot be ruled out that intracellular pathways can also take effect at later times.

A question can be raised as to the importance of the 'nose to brain' transport mechanism. Is it a route of transport to the brain that could be exploited for drugs that have poor blood–brain barrier permeability or should it be considered an undesirable consequence of nasal delivery which should be avoided?

It is evident that for drugs, where the target receptors are situated in the CNS and the therapeutic effect is related to brain functions, such as in Parkinson's disease, treatment of Alzheimer's disease or the treatment of pain, an increased transport of drug to the brain could be considered beneficial. However, for other situations, where nasal delivery is used only for its convenience and a means of obtaining high bioavailability, the direct delivery of drugs to the CNS could possibly result in side effects. However, it should be remembered, that the quantities of drugs reported to access the brain are very low indeed, with concentrations in the CSF and olfactory lobes quoted as nM or from 0.01 to 0.1% bioavailability. In many cases, for the effective exploitation of the nose to CNS delivery route for drugs it will be necessary to find ways of increasing the quantity of drug delivered. Apart from the study published by Kumbale et al. (1999), who compared the brain delivery of lipid nanospheres and positively charged complex formulations of GM1, little effort seems to have been directed to the possibility of enhancing the transport process by formulating the drugs with an appropriate delivery system. This is an area that warrants further investigation in the future.

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