Nasal Administration of Compounds Active in the Central Nervous System

Exploring the Olfactory Pathway

BY

MARIA DAHLIN
The nasal administration of drugs offers advantages over administration by intravenous injection. Drugs can be rapidly absorbed through the nasal mucosa, resulting in a rapid onset of action, and also avoiding degradation in the gastrointestinal tract and first-pass metabolism in the liver. Targeting the brain via nasal administration offers potential for the development of new drugs. The olfactory receptor cells are in direct contact with both the environment and the central nervous system (CNS). The olfactory pathway thus circumvents the blood brain barrier (BBB) which prevents many systemically administered drugs from entering the brain.

The studies used compounds active in the CNS and the experiments were performed in rodents. The nasal bioavailability of (S)-UH-301, NXX-066 and [³H]-dopamine was investigated in a rat model; uptake into the cerebrospinal fluid (CSF) was compared after nasal and intravenous administration. The concentrations of (S)-UH-301 and NXX-066 in plasma and CSF were measured with high performance liquid chromatography. The possible transfer of dopamine and neurotensin along the olfactory pathway after nasal administration to mice was studied using brain tissue sampling and autoradiography. The radioactivity content in blood, CSF and dissected brain tissue samples after administration of [³H]-dopamine and [³H]-neurotensin was assessed using liquid scintillation, and thin layer chromatography (TLC) was used to investigate the metabolic fate of [³H]-dopamine.

The results of this thesis suggest that nasal administration of CNS-active compounds with low oral bioavailability is an interesting and workable alternative to intravenous injection. The small lipophilic compounds (S)-UH-301 and NXX-066 were rapidly and completely absorbed after nasal administration, although hard evidence of direct transfer from the nose remains elusive. Radioactivity measurements in the olfactory bulb following nasal administration of [³H]-dopamine and [³H]-neurotensin indicate that some transfer occurred. The TLC results showed the presence of unchanged dopamine in the olfactory bulb but it is less clear from initial results with neurotensin which radioactive products of this molecule reached the olfactory bulb, and further studies are required.

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PAPERS DISCUSSED
This thesis is based on the following Papers, which will be referred to by Roman numerals in the text.


III Levels of dopamine in blood and brain following nasal administration to rats. Dahlin, M., Jansson, B. and Björk, E., In manuscript.


V Exploring the olfactory pathway for nasal transfer of neurotensin to the brain in mice. Dahlin, M., Jansson, B., Bergman, U., Björk, E. and Brittebo, E., In manuscript.

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INTRODUCTION

The anatomy and physiology of the nasal passage indicate that nasal administration has potential practical advantages for the introduction of therapeutic drugs into the systemic circulation. Drugs can be rapidly absorbed through the highly vascular nasal mucosa, and they also avoid degradation in the gastrointestinal tract and first-pass metabolism in the liver. The concentration-time profiles achieved after nasal administration are often similar to those after intravenous administration, resulting in a rapid onset of pharmacological activity (Hussain, 1998). For example, Bjerre et al. (1996) showed that the sedative propiomazine, for which a rapid onset of action is desirable, is absorbed within 5 minutes after nasal administration to rats. Another attractive feature of nasal administration is the ease of patient administration compared to the more invasive alternatives.

Currently, several drugs for systemic administration have registered nasal dosage forms in Sweden. Desmopressin (a vasopressin analogue and potent antidiuretic), nafarelin (used as pre-treatment in in vitro fertilisation) and oxytocin (for secretion of milk in response to suckling during breast feeding or contraction of the uterine muscle to hasten childbirth) are all small polypeptides consisting of 9 amino acids that are available as a nasal dosage form. Nicotine is also available in a nasal dosage form for use in assisting smoking cessation.

In the last decade, there has been much interest in the nasal route for delivery of drugs to the brain via the olfactory region in order to circumvent the blood brain barrier (BBB). Targeting the brain via the nasal administration of drugs offers potential for drug development since the olfactory receptor cells are in direct contact with both the environment and the central nervous system (CNS). The absence of a strict nose-brain barrier could, then, allow air-borne substances, viruses, metals or drugs to be delivered directly into the CNS.

One of the first to demonstrate the presence of the olfactory pathway for non-microbial and non-viral agents was Faber, who placed Prussian blue dye in the nasal cavity of rabbits and observed the dye in the perineural space of the olfactory nerve and in the subarachnoid space of the brain as early as 1937. The nose-brain pathway, as a conduit for transmission of agents into the CNS, is an area of ongoing research; about 35 to 40 compounds have been reported to reach the CNS after nasal administration in experimental animals to date (Mathison et al., 1998). In recent studies, nerve growth factor (Frey II et al., 1997), local anaesthetics (Chou and Donovan, 1998a), inorganic mercury (Henriksson and Tjälve, 1998), taurine (Brittebo and Eriksson, 1995), dihydroergotamine (Wang et al., 1998), carboxylic acids (Eriksson et al., 1999) and 2’, 3’-didehydro-3’-deoxythymidine (Yajima et al., 1998) have been transported into the CNS via the nasal route.
ANATOMY AND PHYSIOLOGY OF THE NOSE

Anatomy and function

The nasal cavity is divided into two symmetrical halves by the nasal septum, a central partition of bone and cartilage; each side opens at the face via the nostrils and connects with the mouth at the nasopharynx (fig. 1). The nasal vestibule, the respiratory region and the olfactory region are the three main regions of the nasal cavity (Chien et al., 1989). The lateral walls of the nasal cavity include a folded structure which enlarges the surface area in the nose to about 150 cm² (Proctor, 1973). This folded structure includes three turbinates: the superior, the median and the inferior (fig. 1).

In the main nasal airway, the passages are narrow, normally only 1–3 mm wide (fig. 1), and this narrow structure enables the nose to carry out its main functions (Proctor, 1982). During inspiration, the air comes into close contact with the nasal mucosa and particles such as dust and bacteria are trapped in the mucus. Additionally, the inhaled air is warmed and moistened as it passes over the mucosa; this conditioning of the inhaled air is facilitated by the fluid secreted by the mucosa and the high blood supply in the nasal epithelium (Chien et al., 1989; Proctor, 1973). The submucosal zone of the nasal passage is extremely vascular and this network of veins drains blood from the nasal mucosa directly to the systemic circulation, thus avoiding first-pass metabolism (Mygind et al., 1982). Another, perhaps more familiar, major function of the nose is olfaction; the olfactory region is located on the roof of the nasal cavity.

The nasal cavity is covered with a mucous membrane which can be divided into non-olfactory and olfactory epithelium areas (Geurkink, 1983). The non-olfactory area includes the nasal vestibule, which is lined with skin-like cells, and the respiratory region, which has a typical airway epithelium.

Figure 1. Anatomy of the nose. To the left is the lateral wall of the nasal cavity with the olfactory region at the roof of the cavity, just below the cribriform plate of the ethmoid bone. To the right is a cross-section of the nose showing the narrow nasal airway passage and the folds of the turbinates.
The respiratory region

The nasal respiratory epithelium is generally described as a pseudo-stratified ciliated columnar epithelium. This region is considered to be the major site for drug absorption into the systemic circulation. The four main types of cells seen in the respiratory epithelium are ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells (fig. 2). Although rare, neurosecretory cells may also be seen but, like basal cells, these cells do not protrude into the airway lumen (Petruson et al., 1984).

![Diagram of respiratory epithelium](image)

**Figure 2.** The respiratory epithelium of the nasal cavity, showing the four main types of cells. Modified from Mathison et al. (1998).

The proportions of the different cell types vary in different regions of the nasal cavity. In the lower turbinate area, about 15-20% of the total number of cells are ciliated and 60-70% are non-ciliated epithelial cells. The numbers of ciliated cells increase towards the nasopharynx with a corresponding decrease in non-ciliated cells (Popp and Martin, 1984). The high number of non-ciliated cells indicates their importance for absorption across the nasal epithelium. Both columnar cell types have numerous (about 300–400 per cell) microvilli (Mygind, 1975). The large number of microvilli increases the surface area and this is one of the main reasons for the relatively high absorptive capacity of the nasal cavity. The role of the ciliated cells is to transport mucus towards the pharynx.

Basal cells, which vary greatly in both number and shape, never reach the airway lumen. These cells are poorly differentiated and act as stem cells to replace other epithelial cells (Jahnke, 1972). About 5-15% of the mucosal cells in the turbinates are goblet cells, which contain numerous secretory granules filled with mucin. In conjunction with the nasal glands, the goblet cells produce a secretion, which forms the mucus layer (Petruson et al., 1984).
The olfactory region

In humans, the olfactory region is located on the roof of the nasal cavities, just below the cribriform plate of the ethmoid bone, which separates the nasal cavities from the cranial cavity (fig 1). The olfactory tissue is often yellow in colour, in contrast to the surrounding pink tissue (Chien et al., 1989). Humans have relatively simple noses, since the primary function is breathing, while other mammals have more complex noses better adapted for the primary function of olfaction. In a morphometric analysis of rodent nasal cavities, Gross et al. (Gross et al., 1982) indicated that, in mice and rats, respectively, about 47% and 50% of the total nasal epithelium consists of olfactory epithelium. In humans, however, the neuroepithelium covers an area of 2-10 cm², i.e. around 3% (Morrison and Costanzo, 1990). These size differences in the olfactory area reflect the importance of the sense of smell for the different species. Many common animal models are classified as macrosmatic (i.e. the olfactory epithelium occupies a large area of the total nasal epithelium) while humans are classified as microsmatic (Reznik, 1990).

The human olfactory organ is similar in organisation and cell morphology to that of most vertebrate species (Morrison and Costanzo, 1992). The olfactory epithelium rests upon a thick connective tissue, lamina propria, which contains blood vessels, olfactory axon bundles and Bowman’s glands. Like the epithelium of the respiratory region, the olfactory epithelium comprises pseudo-stratified columnar cells of three principal types: olfactory receptor cells, supporting cells and basal cells (fig.4). The basal cells are flattened to an elongated ovoid shape and are located close to the epithelial side of the basal lamina. The olfactory neurons are interspersed between the supporting cells that form a distinct layer in the upper third of the olfactory epithelium (Urai and Maronpot, 1990).

Figure 3. The olfactory epithelium of the nasal cavity showing the three principal cell types. Modified from Mathison et al. (1998).
The olfactory receptor cells are specialised for the detection of odorants. It is estimated that there are 10 to 20 millions of these cells in humans (Geurink, 1983). The olfactory neurons are bipolar, with dendrites projecting into the airway lumen. Near the epithelial surface, the dendrites terminate in ciliated olfactory knobs of various shapes, which usually extend above the epithelial surface. The number of cilia vary, but there are about 10–25 extending from each knob (Morrison and Costanzo, 1992). The olfactory neurons form a layer of approximately five to six cells thick and are distributed between supporting cells. The nuclei are prominent throughout the middle third of the epithelium. The basal end of each sensory cell tapers to a slender axon that passes through the basal lamina into the lamina propria. Here they group into small bundles to form glomeruli, the fila olfactoria, which pass through the cribriform plate of the ethmoid bone into the olfactory bulb where they synapse with second order neurons (Uraihi and Maronpot, 1990). The axons of the olfactory neurons do not make synaptic connections until they reach the olfactory bulb.

The olfactory organ is unique in the CNS in that it is the only part that is in direct contact with the environment. The neurons are exposed to volatile odorants, but also to detrimental airborne substances, including chemicals and viral and bacterial pathogens. As a consequence, neuronal death is a normal feature of the olfactory epithelium. However, the olfactory epithelium also has an ability to regenerate damaged or lost neurons. The life span of an olfactory receptor is approximately one month. It is likely that apoptosis (regulated cell death) is important in maintaining a balance between cell proliferation and death, although it has been shown that cells live considerably longer if they are not exposed to pollutants (Jones and Rog, 1998).

**ABSORPTION ACROSS THE NASAL EPITHELIUM**

The pathways for absorption across the nasal respiratory epithelium are no different from those across other epithelia in the body. The four main absorption routes are transcellular and paracellular passive absorption, carrier-mediated transport and absorption through transcytosis. Transcellular passive diffusion is the main mode of absorption for most drugs but, for large or ionised molecules, the paracellular route can provide an opportunity for absorption (Kimura et al., 1991).

**Barriers to drug absorption**

The nasal membrane is the first line of defence against inhaled microorganisms, allergens and irritating substances from the environment. There are various barriers in the nasal membrane, for protection from these unwanted substances, that must be overcome by drugs before they can be absorbed into the systemic circulation. The nasal membrane is a physical barrier and the mucociliary clearance is a temporal barrier to drug absorption across the nasal epithelium. Mucus traps the particles of dust, bacteria and drug substances and is transported towards the nasopharynx at a speed of 5–8 mm/min, where it is swallowed. It takes about 15-25 minutes to clear the nose from particles (Lioté et al., 1989).

The role of the enzymatic barrier is to protect the lower respiratory airways from toxic agents; the nasal mucosa contains many enzymes, for example cytochrome P-450-
dependent monooxygenase (Hadley and Dahl, 1982; Brittebo, 1982) carboxyl esterase (Bogdanffy et al., 1987) and amino peptidase (Stratford and Lee, 1986). Although nasal delivery avoids hepatic first-pass metabolism, the nasal mucosa provides a pseudo-first-pass effect. However, because of the lower activity per mg respiratory mucosa compared to the gastrointestinal tract and the liver (Longo et al., 1988; Stratford and Lee, 1986) and the higher drug to enzyme ratio, it is easier to overcome the degradation problem when using the nasal route.

Factors affecting nasal drug absorption

The extent of absorption of a drug from the nasal cavity depends partly on the size of the drug molecules, a factor that is most important for hydrophilic compounds. An almost linear relationship between the molecular weight and the bioavailability of water soluble drugs (190–70 000 Da) and dextran of different weights (1260–45 500 Da) has been shown (Fisher et al., 1987; Fisher et al., 1992). McMartin et al. (1987) linked the extent of absorption of compounds with their molecular weight. The nasal route appears to be suitable for the efficient rapid delivery of molecules of molecular weight <1000. This means that the bioavailability of larger polypeptides like insulin will be too low when they are administered nasally. However, formulation additives (absorption enhancers) may increase the bioavailability of these compounds, and several research groups are now employed in the search for suitable enhancer systems for larger molecules. The main problem is to achieve high absorption enhancement without causing irreversible damage to the nasal cavity, such as affecting the cell membrane or altering the defence mechanisms in the nose.

Lipophilic drugs like propranolol (Hussain et al., 1980) and nicotine (Jung et al., 2000) are well absorbed from the nasal cavity, providing plasma concentration-time profiles similar to those obtained after intravenous administration. A linear relationship between the rate constant of absorption and the log P (octanol/water) has been demonstrated earlier with progesterone (Corbo et al., 1989a) in rabbits.

The pKₐ of a substance and the pH in the surrounding area are the two factors that decide the ratio of dissociated to undissociated molecules of a drug. Several studies have shown that the amount of absorbed drug is increased with an increasing fraction of undissociated molecules (Hirai et al., 1981; Hussain et al., 1985).

THE OLFACTORY PATHWAY

The central nervous system

The BBB represents a very complex endothelial interface, which separates the blood compartment from the extracellular fluid compartment of the brain parenchyma. The BBB consists of a monolayer of polarised endothelial cells connected by complex tight junctions, which act as zips closing the inter-endothelial pores that normally exist in endothelial membranes.

The BBB is the primary obstacle to the delivery of drugs to the brain. The lipid solubility, molecular mass and charge of the drug molecules will affect the extent to
which they are absorbed from the blood into the CNS. Small lipid-soluble drug molecules (molecular weight<700 Da), such as anaesthetics and steroid hormones, diffuse readily through the BBB. The higher the lipid solubility of a drug, the greater will be its ability to penetrate or diffuse across the BBB (Oldendorf, 1974). However, the brain needs substances such as glucose and lactate (which are water-soluble nutrients) and peptides like insulin and transferrin. These substances are transported over the BBB with special carrier-mediated transport systems (Pardridge, 1993).

The ventricular and subarachnoid spaces of the brain are filled with cerebrospinal fluid (CSF). The CSF is not a filtrate of plasma but rather a secretory fluid produced mainly by the choroid plexus. Each choroid plexus comprises a secretory epithelium that is perfused by blood at a local high perfusion rate. The ependyma is the lining membrane of the choroid plexus and the lateral ventricles; this membrane consists of cubic cells joined in close apposition by apical junctional complexes, thus forming a barrier to the CSF. However, the blood-CSF barrier is not as formidable as the BBB, since many compounds that are restricted by the BBB can fairly easily pass the cellular ependymal layer (Pardridge, 1993).

Transport of agents from the brain to the nose

Early investigations indicated that there is a direct connection between the subarachnoid space of the CNS and the nasal mucosa. According to Faber (1937), Schwalbe was the first to demonstrate (in 1869) that dyes injected into the subarachnoid space are transported to the nasal mucosa and then further to the lymph nodes. After this observation, other investigators have shown, with different types of tracers, that injection into the CSF leads to drainage into the nasal mucosa (Arnold et al., 1973; Casley-Smith et al., 1976; Yoffey, 1958). Erlich et al. (1986) showed that no significant barrier to CSF drainage is present in the rabbit cribriform region and that CSF reaches the submucosal region in the nose rapidly via open pathways. These results were confirmed in studies with Indian ink in a rat model (Kida et al., 1993) and in humans post mortem (Löwhagen et al., 1994). More recently, retrograde transport of nerve growth factor (NGF) from the olfactory bulb to the olfactory epithelium in mice was proven after injection of $[^{125}I]$-NGF into the bulb (Miwa et al., 1998). $[^{125}I]$-NGF was found in the olfactory epithelium 18 hours after administration and it was suggested that bulbar NGF might act as a neurotrophic factor in olfactory epithelial cells.

Transport of agents from the nose to the brain

The olfactory epithelium may serve as a portal of entry for endogenous compounds, as well as viruses and foreign chemicals into the brain. It has long been recognised that the olfactory region in the nose is a potentially important site for entry of viruses and bacteria into the brain. In 1937, Rake used distribution tests and direct microscopical examination to show that the bacteria pneumococci and S. enteritidis entered the CNS via the olfactory mucosa and the perineural space after nasal instillation to mice. Different strains of mouse hepatitis virus (Barnett and Perlman, 1993; Perlman et al., 1990) have also been reported to travel along the olfactory neurons into the CNS; unilateral surgical ablation of this pathway prevented spread of the virus via the olfactory tract on the side of the lesion (Perlman et al., 1990).
**Transport mechanisms along the olfactory pathway**

The olfactory pathways have been reviewed by several authors (Illum, 2000; Jackson et al., 1979; Mathison et al., 1998). Mathison et al. broadly classified the pathways into two possible routes from the olfactory mucosa in the nasal cavity into the CNS along the olfactory neurons: the olfactory nerve pathway (axonal transport) and the olfactory epithelial pathway.

Agents that are able to enter the olfactory receptor cells, by endocytic or pinocytotic mechanisms, could utilise the olfactory nerve pathway and thus be transported by intracellular axonal transport to the olfactory bulb (Illum, 2000). Mouse hepatitis (Barnett and Perlman, 1993) and vesicular stomatitis viruses (Huneycutt et al., 1994) and agglutinin-conjugated horseradish peroxidase (Thorne et al., 1995) have been shown to enter the brain by axonal transport.

Axonal transport of endogenous substances, in either the anterograde or retrograde direction, is a well-known phenomenon. Anterograde transport may be either fast (20–400 mm/day) or slow (0.1–4 mm/day), depending on the substance that is being transported (Vallee and Bloom, 1991). Further, the transport rate also varies in different animal models. Retrograde transport, which can involve pinocytotic vesicles, lysosomal organelles and mitochondria, occurs at a rate similar to that of the fast anterograde transport.

Transport of gold particles along the olfactory nerve pathway is slow in both monkeys (de Lorenzo, 1970) and rabbits (Czerniawska, 1970). Czerniawska showed that the radioactive isotope $^{198}$Au penetrates the CSF directly from the nasal olfactory region; radioactive activity was highest in CSF taken from the cribriform plate and the base of the olfactory bulb. De Lorenzo used electron microscopy to estimate that the particles moved at a rate of 2.5 mm/hour in the olfactory nerve.

Airborne neurotoxic metals like cadmium, nickel, mercury and manganese have been shown to enter the CNS via the olfactory epithelium in the nose. The levels of cadmium were 40 times higher in the bulb ipsilateral to the exposed side than in the contralateral bulb after nasal instillation of $^{109}$Cd to rats (Evans and Hastings, 1992). When cadmium was administered intratracheally or intraperitoneally, only low levels of $^{109}$Cd were found in the olfactory bulbs. One research group has reported that nickel, manganese (Tjälve et al., 1996) and mercury (Henriksson and Tjälve, 1998) are transported along the olfactory pathway. The mechanism of transport for these metals was not elucidated, but it was mentioned that the metals may have adhered to some endogenous neuronal constituents undergoing axonal transport within the olfactory nerves (Henriksson, 1999).

In the olfactory epithelial pathway, the substance must first cross the olfactory epithelium. The general transport mechanisms across the olfactory epithelium are similar to those across other types of epithelium, as described above. The substance could be absorbed by passive diffusion through the supporting cells or Bowman’s glands or it could be transported by a paracellular route through the tight junctions between the supporting. After entering the lamina propria, adjacent to the olfactory...
neurons, the substance could then enter the perineural space and reach the CNS (fig. 4) (Mathison et al., 1998).

**Figure 4.** A schematic figure showing the anatomical connection between the olfactory mucosa in the nose and the CSF in the subarachnoid space outside the olfactory bulb. Modified from Mathison et al. (1998).

This extracellular pathway relies on the anatomical connection between the nasal submucosa and the subarachnoid space. The perineural space around the olfactory neurons is an extension of the subarachnoid space and the fluid in the perineural space is in direct contact with the CSF. Transport of substances into the CNS via the epithelial pathway could thus be more rapid than that via axonal transport. It is likely that smaller compounds that appear rapidly in the CSF after nasal administration have been transported through this pathway. However, Frey II et al. (1997) showed that NGF, with a molecular weight of 37 kD, was transported into the CNS within 20 minutes of nasal administration in a rat model. The rapid appearance of $[^{125}\text{I}]$-NGF in the olfactory bulb indicated that transport was more likely to have taken place through the intercellular clefts and extracellular transport to the CSF and brain, rather than via axonal transport along the olfactory neurons.

**Factors affecting transport along the olfactory pathway**

The molecular weight of a substance is, as mentioned above, one deciding factor in whether or not it will be transported along the olfactory pathway, as with absorption across other epithelia in the body. In studies in rats, Sakane and co-workers have demonstrated a linear relationship between the transport of compounds from the nose
into the CSF and their molecular weight (Sakane et al., 1995), degree of dissociation (Sakane et al., 1994) and lipophilicity (Sakane et al., 1991a).

In these studies, direct uptake into the CSF of various molecular weights of dextrans labelled with fluorescein isothiocyanate after nasal administration was dependent on molecular weight. Dextrans with molecular weights ≤20 kDa were directly transported to the CSF, while those weighing 40 kDa were not found in the CSF.

Nasal administration of sulphasomidine in perfusions of varying pH resulted in more extensive transport of undissociated drug molecules into the CSF (Sakane et al., 1994). The ratio of the drug concentration in the CSF to that in the nasal perfusion fluid was dependent on the un-ionised fraction of the drug, i.e. drug transport from the nasal cavity into the CSF conforms to the pH partition theory.

For drugs with comparatively low lipophilicity, transport into the CSF is dependent on the partition coefficient. In one study, the concentration of various sulphonamides in the CSF increased linearly with the partition coefficient (between isoamyl alcohol and the phosphate buffer, pH 7.4) (Sakane et al., 1991a). Similar results were shown by (Chou and Donovan, 1998a) who studied the distribution of local anaesthetics with similar chemical structures in rats. The rank order of these local anaesthetics, according to the ratios of the area under the concentration-time curve (AUC) values in the CSF for the two administration routes (nasal/parenteral), correlated well with their ranking by distribution coefficient.

**Drug transport along the olfactory pathway in animal models**

The nose-brain pathway, as a conduit for transmission of agents into the CNS, is an area of ongoing research. Table 1 lists drugs and drug-related compounds that are reported to reach the CNS after nasal administration in different species.

Wang et al. (1998) studied the brain uptake of tritium-labelled dihydroergotamine ([3H]-DHE) after nasal and intravenous administration in rats. Dihydroergotamine is used for the treatment of migraine headache and, because of low oral bioavailability, it is usually administered intravenously or intramuscularly. In the same study, [14C]-inulin was used as a non-BBB-permeable marker. Both [3H]-DHE and [14C]-inulin were transported directly into the brain. [3H]-DHE penetrated the BBB, but the level of radioactivity in the olfactory bulb was significantly (approximately four times) higher 30 minutes after nasal administration than it was after intravenous administration.

In one of the first studies by Sakane et al. (1991b), the authors compared the uptake into the CSF after intranasal, intraduodenal and intravenous administration of the water-soluble antibiotic cephalexin in a rat model. The plasma concentrations were similar after 15 and 30 minutes for the three routes but the levels of the drug in the CSF were significantly higher at both times after nasal administration. Because of the higher concentration in CSF after 15 minutes, Sakane et al. postulated that cephalexin was transported from the nasal cavity to the CSF by passive diffusion, i.e. via the olfactory epithelium pathway.
Chou and Donovan (1998b) studied the disposition of lidocaine within the CNS of the rat after nasal and intraarterial administration. Since the systemic bioavailability of lidocaine is 100% after nasal administration (Chou and Donovan, 1998a), the CSF/plasma concentration ratios for the two administration routes (nasal and intraarterial) should be equal. However, the ratio for nasally administered lidocaine was 1.54 when measured using the direct CSF sampling technique and 1.07 when using a microdialysis probe in the cisterna magna. The changes in disposition pattern between the two administration routes indicated that other factors or pathways in addition to the systemic circulation may play a role in the transport of lidocaine into the brain following nasal administration.

The use of other administration routes has been examined in order to improve the compliance and therapeutic efficacy of zidovudine (AZT) over that seen with oral administration in patients with AIDS and neuropathies. Seki et al. (1994) examined nasal absorption of AZT and its subsequent transport to the CSF in rats. Both rapid absorption and high CSF concentrations were observed after nasal application; the nasal bioavailability was 60% compared to intravenous administration. Though not fully proven for this drug, the high CSF/plasma concentration ratio 15 minutes after nasal administration could reflect a direct pathway into the CSF. The levels of zidovudine in both plasma and CSF were increased when the drug was co-administered with probenecid.

Although other low molecular weight compounds like progesterone (Anand Kumar et al., 1974a) and various benzodiazepines (Gizurarson et al., 1996; Henry et al., 1998) are able to penetrate the BBB, nasal administration has shown that they can also enter the CNS by this route. An investigation with midazolam demonstrated that delivery via nasal spray resulted in peak plasma concentrations approximating only 7% of the intravenous route (Henry et al., 1998). However, peak CSF concentrations following nasal spray yielded CSF concentrations nearing 30% of that obtained with intravenous administration. At each sampling point, higher CSF concentrations were found after intravenous administration but the amount of midazolam in the CSF increased over time after nasal route while the level decreased after intravenous administration. The authors thought that these results add to the theory that midazolam do not need to enter the systemic circulation in order to enter the CNS after nasal administration. Gizurarson et al. (1996) reported (unpublished results) that the highest concentration of diazepam was found just behind the olfactory bulb 10 minutes after nasal administration. The results indicated that diazepam had been further transported through the olfactory tract to the thalamus or the limbic system in the brain.

Experiments with the nasal delivery of the protein dimer NGF to rats (Chen et al., 1998; Frey II et al., 1997) have shown that the nose could be a potential administration route for this larger drug in patients with Alzheimer’s disease (AD). The BBB normally prevents NGF from entering the brain but within 20 minutes of nasal administration of \[^{125}\text{I}]\text{-NGF}\) to rats, the drug appeared in the olfactory bulb, with accumulation increasing linearly with the dose. The study indicated that \[^{125}\text{I}]\text{-NGF}\) was transported directly into the brain along the olfactory pathway; its rapid appearance in the olfactory bulb indicated that it was probably transported to the CSF through the intercellular...
clefts and by extracellular transport rather than by axonal transport along the olfactory neurons.

Another drug, which has potential in the treatment of AD is the endogenous monosialoganglioside, GM1 (Kumbale et al., 1999). Choline acetyltransferase (ChAT) activity decreases in patients with AD, and GM1 has been shown to enhance ChAT activity in vitro and in vivo. Since GM1 was detected in the CSF immediately after nasal administration, it was suggested that the olfactory epithelium pathway was the most likely route for the direct transport of GM1 into the CSF of rats.

**Table 1. Drugs and drug-related compounds reported to reach the CNS after nasal administration in different animal models.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Sample</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviral lacZ vector</td>
<td>Mouse</td>
<td>–</td>
<td>Histochemical</td>
<td>(Draghia et al., 1995)</td>
</tr>
<tr>
<td>β-Alanine (as carnosine)</td>
<td>Hamster</td>
<td>Mouse Brain tissue</td>
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<td>Species</td>
<td>Sample</td>
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\[D4T = 2', 3'-didehydro-3'-deoxythymidine, MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropropyridine, WGA-HRP = wheat germ agglutinin-horseradish peroxidase, FITC = fluorescein isothiocyanate, ELISA = enzyme-linked immunosorbent assay, HPLC = high performance liquid chromatography, ECF = extracellular fluid\]

The delivery of other proteins to the brain has been demonstrated using protein tracers such as albumin labelled with Evans blue, (Kristensson and Olsson, 1971), wheat germ agglutinin-horseradish peroxidase (WGA-HRP) (Shipley, 1985; Thorne et al., 1995), horseradish peroxidase (HRP) (Bal in et al., 1986; Kristensson and Olsson, 1971) and, more recently, radioactively labelled insulin (Gizurarson et al., 1996). The distribution of \([^{125}I]\)-insulin between blood and brain was investigated in mice after intraolfactory instillation and subcutaneous administration. The amount of radioactivity in the brain, measured with a gamma-counter, were significantly higher following intraolfactory than subcutaneous administration. In 1995, Thorne et al. designed a study to quantitatively assess the anterograde transport of WGA-HRP and HRP to the brain via olfactory neurons and to evaluate the capacity of the potential drug delivery method to achieve biologically significant protein concentrations in the brain. According to the authors, the results indicated that the transport of protein along the olfactory pathway into the brain
after nasal administration was sufficiently high to attain therapeutic levels (Thorne et al., 1995).

In a study in rhesus monkeys, (Anand Kumar et al., 1974a) showed that tritium-labelled oestradiol and progesterone are able to enter the CSF by a direct route after nasal administration. The CSF/plasma radioactivity ratios showed that the concentrations of hormones in the CSF in monkeys receiving the nasal spray were much higher than in those receiving injections. In contrast, Öhman et al. (1980) demonstrated an initial increase in 17ß-oestradiol concentrations in the CSF after nasal administration, but the hormone levels reached were about the same as after an intravenous injection. The small initial increase may have been due to the transport of free hormone from plasma to CSF. The CSF profiles in another study in rhesus monkeys, using the sex hormone ethinyloestradiol (Madrid and Langer, 1991), showed a sustained presence of the drug after nasal administration compared to intravenous injection, while the blood levels were similar after both administration routes.

**Drug transport along the olfactory pathway in humans**

CSF drainage via the nasal route in man post mortem was demonstrated by Löwhagen et al. (1994) and a few studies showing access to the human brain after nasal administration of drugs have been published. For example, a research group provided functional evidence of the facilitated access of arginine-vasopressin (Pietrowsky et al., 1996a) and cholecystokinin-8 (Pietrowsky et al., 1996b) into the brain by this route. The substances were administered nasally or intravenously to healthy subjects and the event-related potentials (ERP) were recorded during the subject’s performance on an oddball task. The P3 component of the ERP increased after nasal but not after intravenous administration and it was suggested that the substances were delivered to the brain by a direct pathway from the nose.

Intranasal administration of angiotensin II to healthy volunteers showed that the drug directly influences the CNS regulation of blood pressure (Derad et al., 1998). It was shown that the blood pressure profiles differed with the route (intravenous or intranasal) of administration of angiotensin II, and that the plasma concentrations of vasopressin were increased after intranasal but not after intravenous angiotensin II administration. The same research group also showed that nasal administration of insulin (Kern et al., 1999), an active fragment of adrenocorticotrophin (Smolnik et al., 1999), and a corticotrophin-releasing hormone (Kern et al., 1996) resulted in effects not seen after intravenous administration assuming a direct deliver into the CNS of the compounds.

Although highly interesting, effect studies give no clear-cut evidence regarding the role of transfer of peptides to the CNS. In another human study (Okuyama, 1997), cerebral radioactivity increased significantly after spraying a radioactive mixture of 99mTc-diethylene-triaminopenta-acetic acid and hyaluronidase into the noses of anosmic patients.
COMPOUNDS USED IN THE THESIS

The hydrochloride salt of (S)-5-fluoro-8-hydroxy-2-(dipropylamino) tetralin, (S)-UH-301 (fig. 5), a serotonin-1a receptor antagonist (Hillver et al., 1989), was used as a model substance in Paper I. Serotonin (5-hydroxytryptamine, 5-HT) is a transmitter substance in the CNS, but only about 1% of the total amount of serotonin in the body is located in the brain, despite the important role it plays in the regulation of, for example, sleep and mood (Arvidsson et al., 1986). Several subtypes of 5-HT receptors have been identified and the subtype 5-HT-1a is found in both pre- and postsynaptic areas. Activation of postsynaptic receptors increases the incidence of serotonergic signals while activation of the presynaptic 5-HT-1a autoreceptors on the cell body decreases the synthesis of serotonin. The dynamic balance of serotonin in the CNS appears to affect the development of both depression and agony (Baldwin and Rudge, 1995; Barrett and Vanover, 1993).

Figure 5. Chemical structures of the CNS-active substances discussed in this thesis. (S)-UH-301 (Paper I), NXX-066 (Paper II), dopamine (Papers III and IV) and neurotensin (Paper V).

The substance used in Paper II, (3aS)-cis-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethylpyrrolo (2, 3b) indol-5-yl 3, 4 dihydro-2-isoquinolinicarboxylate (NXX-066, fig.5), acts as a potent inhibitor of acetylcholinesterase (AChE). Alzheimer’s disease, the most common cause of dementia, affects millions of people over the age of 65 in the western world, and an increase in the occurrence of AD is expected in the future as the proportion of older people in the population grows. Despite the apparent progress in research, successful treatment of AD remains elusive. The goal of caring for patients with AD is thus to enhance function, maintain quality of life and preserve autonomy (Daly, 1999). Inhibition of (AChE), however, is a promising approach, and the most common method under investigation for the treatment of AD (Giacobini, 1993). Physostigmine appears to improve memory function in patients with AD, and nasal administration of this cognition enhancer to rats was shown to be a feasible alternative to parenteral administration (Hussain and Mollica, 1991).
Dopamine (fig. 5) is currently used to treat acute cardiovascular diseases. Because of high first-pass metabolism after oral administration, the drug is usually only given by intravenous infusion. Dopamine is also an important part of the treatment of Parkinson’s disease. Since dopamine does not cross the BBB in appreciable amounts, the immediate precursor, levodopa, is used to target the brain. Levodopa passes the BBB easily and is decarboxylated rapidly to dopamine within the brain. About 95% of the drug is converted to dopamine by dopa decarboxylase in the peripheral tissues and only a small proportion enters the brain. Because of this, levodopa is nearly always combined with a peripheral dopa decarboxylase inhibitor (Rang et al., 1999). Administration of large doses of levodopa can cause adverse effects in patients with Parkinson’s disease. Physiological variables such as gastric emptying and protein-rich meals may markedly affect the amount of orally administered levodopa entering the brain and the speed with which it enters (Koller and Ruedea, 1998).

Neurotensin (fig. 5), a 13-amino acid peptide originally isolated from bovine hypothalami, has a wide spectrum of pharmacological effects (Carraway and Leeman, 1973). The peptide is present throughout the animal kingdom, suggesting its participation in important processes basic to animal life (Vincent, 1995). Central and peripheral injections of neurotensin produce completely different pharmacological effects, indicating that it does not normally cross the BBB. Examples of biological activities of neurotensin reported in vivo after peripheral injection are vasodilation (rat, dog) or hypertension (guinea pig), inhibition of gastric secretion, and increased secretion of pituitary hormones. Central injection of neurotensin resulted in hypothermia, an analgesic effect, enhancement of sedative activity and increased dopamine release (Leeman and Carraway, 1982; Vincent, 1995). Like many other neuropeptides, neurotensin is a messenger of intracellular communication which works as a neurotransmitter or neuromodulator in the brain (Vincent, 1995).

THE AIMS OF THE THESIS

The main objectives of this thesis were to study the nasal absorption of CNS-active compounds and explore the olfactory pathway as a possible conduit for these compounds from the nose into the brain.

The specific aims were to study the following aspects of nasal administration:

- To investigate the nasal bioavailability of (S)-UH-301, NXX-066 and dopamine in a rat model and compare their uptake into the CSF after nasal or intravenous administration.
- To study the uptake of dopamine into the CNS following nasal administration to rats and mice.
- To study the possible transfer of dopamine and neurotensin along the olfactory pathway after nasal administration to mice using brain tissue sampling and autoradiography.
EXPERIMENTAL

Materials

Chemicals
The substances in Papers I and II, (S)-5-fluoro-8-hydroxy-2-(dipropylamino)tetralin hydrochloride ((S)-UH-301), 8-hydroxy-2-(dipropylamino)tetralin hydrochloride (8-OH-DPAT), (3aS)-cis-1, 2, 3, 3a, 8, 8a-hexahyro-1, 3a, 8-trimethyl-pyrrolo-[2, 3b]-indol-5-yl 3, 4 dihydra-2-isoquinolincarboxylate (NXX-066) and (3aS)-cis-1, 2, 3, 3a, 8, 8a-hexahydro-1, 3a, 8-trimethyl-pyrrolo [2, 3b] indol-5-ol, (1-methyl-1, 2,3,4-tetrahydroisoquinolinyl) carbamate (NXX-453), were donated by AstraZeneca R&D Södertälje (Sweden). The radioactively labelled [2, 5, 6 3H]-dopamine (Papers III and IV) was obtained from Amersham Pharmacia Biotech (Sweden) and [3,11-tyrosyl-3,5-3H(N)]-neurotensin (Paper V) was obtained from Du Medical Scandinavia AB, Sollentuna (Sweden).

Heparin (500 IU/ml) was acquired from Løwens, Denmark, and thiobutabarbitral sodium (Inactin) was obtained from Byk Gulden (Germany) and Research Biochemical International (USA). Ketamine (Ketalar® 50 mg/ml) and xylazine (Rompun® vet. 20 mg/ml) were purchased from Apoteket AB (Sweden). Hionic-Fluor™ and Soluene®-350 were purchased from the Packard Instrument Company (USA) and Solvable™ and Ultima Gold™ were obtained from Chemical Instruments AB, Sweden. Ultrapure deionised water (Milli Q UF Plus, Millipore, France) was used for preparation of the solutions. Solvents were of HPLC grade, and all other chemicals were of analytical and commercially available grade.

Animals
Male Sprague Dawley rats from Møllegaard, Denmark and B&K Universal, Sweden were used in Paper I and Papers II and III, respectively. The female NMRI mice used in Papers IV and V were obtained from B&K Universal, Sweden. The animals were housed in the animal house at the Biomedical centre in Uppsala, at 22°C with a 12-hour light/dark cycle and were fed with a standard pellet diet with free access to tap water. The experiments were performed in specially designed rooms in the animal house.

The studies in this thesis were carried out in compliance with approval numbers C 84/94 (I, II), C 211/99 (III), C 94/97 (IV) and C153/98 (V), issued by the animal research ethics committee in Uppsala.

Methods

Drug solutions
(S)-UH-301 hydrochloride was dissolved in physiological saline solution; 5 mg/ml for intravenous use and 25 mg/ml for nasal use. The pH of the solution was 5.9 and the doses were 6 µmol/kg and 12 µmol/kg for intravenous and nasal administration, respectively.

NXX-066, which has a solubility in water of 0.094 mg/ml, was dissolved in an acetate buffer (pH 5), in which the solubility was 6.5 mg/ml. The concentrations of the
solutions used for nasal and intravenous administration were 6.4 mg/ml and 3.0 mg/ml, respectively.

The radioactively labelled dopamine was dissolved in 0.02 M acetic acid:ethanol (1:1) and neurotensin was dissolved in ethanol. The solutions were evaporated to dryness under a gentle stream of nitrogen at room temperature before use. In Paper III, \[^3H\]-dopamine was redissolved in PBS (pH 7.4) and 50 µCi was administered both nasally and intravenously. In Papers IV and V, \[^3H\]-dopamine and \[^3H\]-neurotensin were redissolved in 0.1 M phosphate buffer (pH 7.4) and 5 µCi was administered. The intravenous solutions of \[^3H\]-dopamine were prepared in physiological saline and 5 µCi was injected.

**Anaesthesia and administration of the drugs**

Prior to the operations and administration of drugs the rats were anaesthetised with an intraperitoneal injection of thiobutabarbital sodium (150 mg/kg). In order to facilitate breathing, the trachea was cannulated with polyethylene tubes (PE-200). Thirty minutes after the operation, the experiments were initiated by administration of the drug solution. In Papers I and II, the nasal administrations were given by volume according to the rat body weight, using PE-90 tubes attached to a micropipette. A volume of 25-30 µl was given to each nostril. In Paper III, a fixed volume of 20 µl radioactive \[^3H\]-dopamine solution was applied to the right nostril. During the experiments, the rat body temperature was maintained at (37°C) with a heating pad.

Mice were anaesthetised with an intraperitoneal injection of a combination of ketamine and xylazine (100 mg and 10 mg/kg body weight, respectively). The nasal doses were given unilaterally to the right nostril (5 µl) using polyethylene tubes (PE-10) attached to a micropipette. The mice were then placed on their right sides on the heating pad (38°C) until they woke up. The intravenous doses were injected into the tail vein.

**Absorption studies**

Absorption studies were performed in Papers I–III; in addition to the operation above, the carotid artery was cannulated with a PE-50 tube in order to obtain blood. The intravenous bolus doses were injected through a catheter (PE 50) into the external jugular vein (Papers I–II) or injected into a tail vein (Paper III). The blood samples (about 200 µl) were withdrawn from the catheter, prior to drug administration and at scheduled time points.

In Papers I and II, after the addition of one drop of heparin (24-gauge needle), the plasma was separated by centrifugation at 7000 rpm for 10 minutes. After the experiments, the animals were killed with an overdose of pentobarbital (100 mg/ml) and all samples, both plasma and CSF, were stored at -80°C until analysis.

In Paper III, aliquots of 100 µl blood were transferred to glass scintillation vials and 1 ml of tissue solubiliser (Solvable™) was added. After incubation (55°C, 1 hour), 0.1 ml EDTA (0.1M) and 0.4 ml hydrogen peroxide (30%) were added to remove the colour from the samples. After another incubation followed by cooling, 10 ml of liquid scintillation cocktail (Ultima Gold™) was added and the radioactivity was measured.
over 10 minutes/sample in a liquid scintillator analyser (Tri-Carb 1900CA, Packard Instruments Company, U.S.A.).

CSF sampling

CSF sampling was used in Papers I–III. Using a stand to fix the animal’s head, the CSF samples were taken by cisternal puncture (Persson et al., 1989; Waynforth and Flecknell, 1980). About 100–150 µl CSF was withdrawn through the puncture site at the cisterna magna, before and at each scheduled time after administration of the drug solutions, by gentle suction through a 30-gauge needle attached to a syringe connected to polyethylene tubing (PE 50). Collection of CSF was terminated as soon as blood appeared and the blood-tainted portion of CSF was prevented from reaching the collecting tube.

In Papers I and II, blood samples were withdrawn from the arteria carotis about 2 minutes after each CSF sampling, for correlation with the absorption data. In Paper III, these blood samples were obtained before the brain tissue sampling as described below.

HPLC analysis of plasma and CSF samples

In Papers I and II, high performance liquid chromatography (HPLC) was used to measure the drug content in the plasma and CSF samples. In order to extract (S)-UH-301 and the internal standard 8-OH-DPAT to an organic phase, 100 µl of the plasma sample, 400 µl 8-OH-DPAT (400 nM), 500 µl TRIS buffer pH 8.5 (0.2 M) and 4 ml organic solvent mixture (hexane-diethylether-butanol, 70-20-5 v/v) were added to test tubes. After 8 minutes in a rotating mixer and 8 minutes of centrifugation at 1500 rpm, 3 ml of the organic phase was transferred to new test tubes. The organic phase was evaporated to dryness, under a gentle stream of nitrogen at 40°C. The residue was redissolved in 250 µl sodium phosphate buffer pH 2.0 (0.1 M, µ=0.1) and 100 µl was injected into the HPLC system. An ODS C-18 analytical column (100 x 4.6 mm, 3 µm, YMC Europe GmBH) and precolumn (15x 2.3 mm, 7 µm, Brownlee CN) were used to separate the components of the solution. Electrochemical detection of (S)-UH-301 and 8-OH-DPAT was accomplished using an amperometric controller (BAS LC-4B, LC-17 flowcell) at a potential of 1.2 V. The mobile phase consisted of 28% acetonitrile and 0.75 mM sodium octylsulphate in sodium phosphate buffer pH 2.0. The flow rate was 0.5 ml/min (LKB 2150 HPLC pump).

Extraction of NXX-066 and the internal standard NXX-453 into an organic phase was begun with 100 µl of the plasma sample, 100 µl NXX-453 (313.3 nM), 200 µl sodium hydrogen carbonate solution (0.5 M) and 2 ml organic solvent mixture (diethylether/n-heptane 30/70 v/v %) which were added to test tubes. After extraction for 2 minutes in a tumble mixer, freezing in an ethanol-dry ice bath separated the phases. The supernatant was transferred to a new tube and evaporated to dryness under a gentle stream of nitrogen at 40°C. The extraction was repeated and the residue was redissolved in 200 µl phosphate buffer (0.01 M; pH 2). The plasma samples (170 µl) were injected (Kontron 460, Kontron, Switzerland) into the chromatographic system and separation was achieved using a reversed phase column (Zorbax Sb-CN, 75 x 4.5, 3.5 µm) and a precolumn (CN 12.5 x 4, 5 µm) at 35°C (Jones 7950, Jones Chromatography, U.K.).
The mobile phase consisted of 23 v/v % acetonitrile and 0.5 mM dimethyloctylamine in ammonium formate buffer pH 4.4 and was delivered at a flow rate of 1.3 ml/min (Shimadzu LC 9A, Shimadzu, Japan). Detection of NXX-066 and NXX-453 was accomplished using a fluorescence detector (Waters 470, Waters Division of Millipore, U.S.A.) at wavelengths of 245 for excitation and 346 nm for emission. The CSF samples were assayed directly without any pretreatment. Before injection (80 µl) into the chromatography system, 50 µl NXX-453 (313.3 nM) was added to 50 µl CSF.

In Papers I and II, plasma from untreated rats and artificial CSF (Elliot’s B solution, according to Martindale, 30th edition) were used for standard and control samples. These samples were treated in the same way as the drug samples. The peak heights were used for quantification and the drug/internal standard ratio was used for calculation of the concentration of (S)-UH-301 or NXX-066 in plasma and CSF samples. In Paper I, no internal standard was added to the CSF samples.

**Brain tissue sampling**

In the dopamine study in rats (Paper III), the animals were killed at a scheduled time by exposure to gaseous CO₂, approximately 2 minutes after CSF sampling, and were then decapitated. Blood was collected from the trunk. The skull was cut open and the olfactory bulbs, lateral olfactory tract, cerebrum and cerebellum were carefully excised. The oesophagus and the olfactory mucosa from the septum of both cavities were also excised. All samples were weighed and dissolved in tissue solubiliser (Solvable™) and the tissue samples were incubated (55°C) for 3 hours. The blood samples were treated as described above. Scintillation cocktail (Ultima-Gold™) was added to each sample prior to assessment of the radioactivity in the liquid scintillator analyser for 10 minutes.

In the mice studies (Papers IV and V), dopamine or neurtensin were administered nasally to mice, which were then killed at a scheduled time post-dose, as described above. After decapitation, the skull was cut open and the olfactory bulbs, lateral olfactory tract, anterior and posterior portions of cortex and the remaining brain were carefully excised. The oesophagus and trachea were also dissected. All samples were weighed and dissolved in tissue solubiliser (Soluene®-350) and scintillation cocktail (Hionic-Fluor™) was added to each sample. The radioactivity was measured for 10 min/sample in the liquid scintillator analyser.

**Tape section autoradiography**

Tape section autoradiography was used in Papers IV and V and the experiments were performed as originally described by Ullberg (1977). The heads or whole bodies were embedded in aqueous carboxymethyl cellulose and frozen in a cyclohexane/dry-ice bath. Series of transversal (head only) or sagittal (whole body) sections (20 µm; Jung Cryomacrocot, Leica) were collected on tape at various levels and processed for autoradiography using Hyperfilm-[³H] (CEA for Amersham, Sweden). In Paper V, some of the freeze-dried tissue sections were stepwise extracted with 5% trichloroacetic acid, 50% ethanol, absolute ethanol, heptane (twice), absolute ethanol and 50% ethanol for 1 minute each and then rinsed in running water for 5 minutes before they were apposed to the film. The remaining radioactivity then represents radioactivity bound to the tissue. The exposure of the film was performed at -20°C.
Thin layer chromatography

In order to investigate the fate of $[^3]$H-dopamine in the samples, thin layer chromatography (TLC) was used in Papers III and IV. Two hours after nasal administration of dopamine, the animals were killed (n=2) as described above. The olfactory bulb and olfactory mucosa were dissected and immediately homogenised in ice-cold tubes. The homogenisation medium consisted of dopamine hydrochloride (0.5 mg/ml), 3, 4-dihydroxyphenylacetic acid (DOPAC) (0.5 mg/ml) and sodium metabisulfite (10 mg/ml) in 0.1 M perchloric acid. The homogenates were centrifuged (10 000 g, 4°C) for 10 minutes and 20 µl of each supernatant was applied in a 2 cm line on the TLC plate (Silica gel 60 F$_{254}$ on plastic sheet, Merck). The plate was air dried 90 minutes before development in n-butanol: acetic acid: water (4:1:1) for 3 hours (Chobotská et al., 1998). The plates were cut into strips corresponding to the various bands and placed in scintillation vials with 1 ml sodium metabisulfite (10 mg/ml) in perchloric acid (0.1 M) overnight. A liquid scintillation cocktail was added and the tubes were placed in the dark at room temperature overnight before counting in a liquid scintillator analyser (Tri-Carb 1900CA, Packard Instruments Company, U.S.A.).

Calculations

In Papers I-III, the plasma area under curve (AUC) was calculated by the standard trapezoidal method without extrapolation to infinity. The initial intravenous concentrations were obtained by extrapolating, using the two first sampling points. The absolute nasal bioavailability (F) was calculated by dividing the average of the individual AUC values from the nasal group by the average from the intravenous group according to the following equation:

$$F = \frac{AUC_{na} \times Dose_{iv}}{AUC_{iv} \times Dose_{na}}$$

where AUC$_{na}$ and AUC$_{iv}$ denote the means of individual AUC values from the nasal and intravenous groups, respectively. Noncompartmental analysis was used (Paper II) for calculating the individual terminal half-life and distribution volume for NXX-066 in plasma.

Statistics

Results are presented as mean values ± standard deviations (S.D.). The Student’s t-test was used to test the significance between two means. A value of p<0.05 was considered statistically significant. In Papers IV and V, when comparison involved more than two mean values, a one-way analysis of variance (ANOVA) was used, followed by the Fisher PLSD tests for comparison between individual means.
RESULTS

Nasal absorption

In Papers I–III, the nasal bioavailability of the three compounds, (S)-UH-301, NXX-066 and dopamine, was determined in rats. The absorption of (S)-UH-301 (Paper I) from the nasal cavity into the systemic circulation was rapid and complete. The maximum concentration was achieved after about 7 minutes (fig. 6 a) and the extent of nasal bioavailability appeared to be 100%. The mean AUC values after intravenous and nasal administration were 36100±12200 and 87400±11200 nM•min, respectively, and consequently F=1.2±0.4.

Nasal administration of NXX-066 (Paper II) resulted in extremely rapid and complete absorption into the systemic circulation followed by a rapid decline in the plasma concentrations (fig. 6 b). The intravenous and nasal concentration-time profiles of NXX-066 were similar, coinciding after about 15 minutes, and the elimination could be described biexponentially. The mean AUC values after intravenous and nasal administration were 18800±5500 and 19000±2000 nM•min, respectively, and the absolute nasal bioavailability of NXX-066 was consequently calculated as 100±30%.

![Figure 6. Concentration-time profiles of (S)-UH-301 (Paper I) and NXX-066 (Paper II) in plasma after (○) intravenous or (●) nasal administration to rats. The initial intravenous concentrations were obtained by extrapolation. All data are expressed as means ± S.D. (a) (S)-UH-301; nasal (n=7) 12 µmol/kg or intravenous (n=5) 6 µmol/kg administration of the hydrochloride salt. (b) NXX-066; intravenous (n=6) or nasal (n=7) administration of 3 µmol/kg.](image)

The pharmacokinetic parameters for NXX-066 in plasma did not differ significantly between the two administration routes (p<0.05). The average half-life of elimination from plasma for the intravenous and nasal routes was 70±14 and 75±22 minutes, respectively. The volume of distribution was comparatively large: 13.2±4.1 and 13.0±2.7 l/kg for the intravenous and nasal routes, respectively.

The blood radioactivity-time profiles of [3H]-dopamine (Paper III) after intravenous and nasal administration are shown in figure 7. The maximum concentration was measured at the first sampling point after 15 minutes and the nasal bioavailability of [3H]-dopamine was 68±30%.
Uptake into the CSF
The concentration-time profiles of (S)-UH-301 in CSF (fig. 8 a) showed no increased concentration of the drug after nasal compared to intravenous administration. However, this concentration was retained for longer after nasal administration. This was illustrated (fig. 8 b) by plotting the nasal/intravenous CSF concentration ratios versus time. The ratios were corrected for the different dosages. After about 20 minutes, the ratio exceeded one, i.e. the concentrations of nasally administered (S)-UH-301 in the CSF were higher than those after intravenous administration. A plateau was reached after 30 minutes and this level was sustained throughout the experiments.

Figure 7. The mean radioactivity (dpm) of blood samples obtained after (■) intravenous (n=4) or (●) nasal (n=6) administration of [3H]-dopamine (50 µCi) to rats. All data are expressed as means ± S.D. (Paper III)

Figure 8. (a) Concentration-time profiles of (S)-UH 301 in CSF after (□) intravenous (6 µmol/kg) or (●) nasal (12 µmol/kg) administration of the hydrochloride salt to rats (n=3 for all time points except for the intravenous route at 30 min and the nasal route at 60 min where n=2). (b) Nasal/intravenous concentration ratios in CSF. The ratios are corrected for differences in dosages. Any significant difference from unity was calculated according to Student’s t-test: * P<0.05, ** P<0.01. All data are expressed as means ± S.D. (Paper I).
Only low concentrations of NXX-066 were detected in the CSF, and the drug was not detected at all in the last two samples obtained 30 and 60 minutes after both intravenous and nasal administrations (fig. 9). The concentration of NXX-066 in the CSF was significantly higher (p<0.05) 3 minutes after intravenous administration than after nasal administration.

The radioactivity profile in CSF over time after nasal administration of [³H]-dopamine is illustrated in figure 10 a; the highest amount of radioactivity was recorded 60 minutes after administration. However, since there was no difference between the samples after 30 minutes, it seems that a plateau is reached. Between 60 and 240 minutes there was a significant decrease in radioactivity in the CSF (p<0.01). The CSF/blood radioactivity ratio (fig.10 b) increased over time up to 120 minutes, at which point a plateau was reached (there was no significant difference between the last two points).

The plasma concentration-time profile obtained from blood samples taken about 2 minutes after the collection of CSF in Papers I–II and from the trunk after decapitation in Paper V correlated well with the data from systemic delivery of the drugs. This
indicated that turning the animal before CSF sampling did not influence absorption through the nasal membrane.

**Brain tissue sampling**

The uptake of [³H]-dopamine by the selected brain tissue samples 30 minutes after nasal administration to rats (Paper III) was significantly higher (p>0.01) than after intravenous administration (fig. 11 a). The radioactivity levels after nasal administration were about 2.3 times higher than after intravenous administration in the CSF and about 6.8 times higher in the right olfactory bulb. Similarly, the radioactivity in the mucosa from the right nasal cavity (administration side) in the rats receiving nasal [³H]-dopamine was significantly higher than in rats receiving the tail vein injection. Conversely, there were no differences in radioactivity in oesophagus, left mucosa and blood samples from rats receiving the drug by either route (fig. 11 b).

![Figure 11. The amount of radioactivity in samples 30 minutes after unilateral nasal (right side) or intravenous administration of [³H]-dopamine (50 µCi) to rats (n=3–4). (a) Mean radioactivity in CSF, lateral olfactory tract (LOT), left and right olfactory bulbs, cerebellum and encephalon. (b) Mean radioactivity in oesophagus, and left and right mucosa. Note that different scales are used on the y axes. Data are expressed as means ± S.D. ** p < 0.01 by Student’s unpaired t-test; iv = intravenous; na = nasal (Paper III).](image)

The radioactivity of the tissue samples after nasal administration of [³H]-dopamine to mice is illustrated in figure 12. The radioactive content of the right olfactory bulbs was significantly higher than that of the left bulbs up to 4 hours after administration (fig 12 a). However, at the last sampling point (8 hours), the radioactivity concentration in the right bulbs had decreased to almost the same level as in the left bulbs, i.e. no statistically significant difference between the bulbs was detected. The right/left ratio of the radioactivity content of the olfactory bulbs reached a value of 6 as early as 30 minutes after administration. The ratio had increased to 27 after 4 hours; i.e. the amount of radioactivity in the right bulb (the side of administration) was 27 times higher than in the left bulb.
The amount of radioactivity in selected brain tissue samples after unilateral nasal administration (right side) of [3H]-dopamine (5 µCi) to mice (n=5–7). (a) Mean radioactivity in the right and left olfactory bulbs after nasal administration (n =5–7; * p<0.05, ** p<0.01 by Student’s t-test for right vs left olfactory bulbs) and in whole brain tissue samples (including bulbs) 30 minutes after intravenous administration (n=3) of [3H]-dopamine (5 µCi). (b) Mean radioactivity in the lateral olfactory tract (LOT), posterior and anterior portions of cortex and the remaining parts of the brain after nasal administration (** p < 0.01 by the Fisher PLSD for the olfactory tract at 4 hours vs other time points and other brain tissue samples). Data are expressed as means ± S.D. Note that different scales are used on the y-axes (Paper IV).

The highest level of radioactivity (967 ± 672 dpm/mg) in the right bulbs appeared 4 hours after the unilateral nasal administration of [3H]-dopamine. Low levels of radioactivity were detected in the other regions of the brain (fig. 12 b). The radioactivity in the lateral olfactory tract 4 hours after administration was significantly higher than at other time points and than in the other brain tissue samples within the same group. In three mice which received intravenous [3H]-dopamine, the mean radioactivity in the brain tissue samples (including the olfactory bulbs) at 30 minutes post-dose was 22±5 dpm /mg.

Radioactivity content (dpm/mg) in the left and right olfactory bulbs over time after nasal administration of [3H]-neurotensin (5 µCi) to the right nostril in mice (n=3). Data are expressed as means ± S.D. * denotes significantly higher amounts of radioactivity (P<0.05) in the right olfactory bulb compared to the left bulb (Paper V).

In Paper V, there was a significantly higher level of radioactivity in the right olfactory bulb compared with the left bulb at 1, 4 and 24 hours after nasal administration of [3H]-neurotensin (fig. 13). The activity in the bulb was approximately the same at the two
first sampling times, but increased significantly between two and four, and between 4 and 24 hours. The ratio between the right and left bulb was approximately 2 after 1 hour and increased to 6 after 24 hours. The radioactivity in the left olfactory bulb was unchanged over time. All other tissues showed activities in the same order of magnitude as the left olfactory bulb.

**Tape section autoradiography**

In paper IV, autoradiograms of mice killed 1, 2 and 4 hours after nasal administration of \(^{3}H\)-dopamine (fig. 15; 2 hours) showed that there was a high level of radioactivity in the olfactory mucosa of the right nasal cavity (site of administration). Low or negligible levels of radioactivity occurred in the mucosa of the left nasal cavity.

One to 4 hours after administration of dopamine, radioactivity was mainly located in the outer layer of the right olfactory bulb. Selective uptake of radioactivity was seen in the right axonal nerve layer and the glomerular layer of the right olfactory bulb. There was a decreasing level of radioactivity towards the centre of the olfactory bulb. Twenty-four hours after administration, no radioactivity was observed in the right olfactory bulb. High levels of radioactivity were observed in the mucosa of the nasopharynx and oesophagus 1 to 4 hours after the nasal administration of \(^{3}H\)-dopamine.

**Figure 14.** (A) Autoradiogram of a mouse skull (horizontal section) 2 hours after unilateral nasal administration (right side) of \(^{3}H\)-dopamine (5 µCi). (B) Corresponding hematoxylin-eosin stained tissue section at the same order of magnification. There is a high level of radioactivity in the right olfactory mucosa, the right axonal nerve layer and the glomerular layer of the olfactory bulb. Radioactivity is present only at low levels in the other brain regions. The arrowheads in both figures point to the border between the olfactory mucosa in the nasal cavity and the olfactory bulb. The different regions in part B of the figure are nasal cavity (n), olfactory bulbs (o), brain (b) and eye (e) (Paper IV).

The autoradiograms in Paper V, after nasal administration of \(^{3}H\)-neurotensin (not shown) appear very much like the one for dopamine. The level of radioactivity was highest in the right side of the nasal cavity (site of administration) with little or no labelling of the left side. A high level of radioactivity was also localised to the
peripheral layers of the ipsilateral olfactory bulb. There was no obvious decrease in radioactivity in the ipsilateral olfactory bulb with time, while the radioactivity in the right nasal cavity decreased over time. The level of labelling in the solvent-extracted tissue sections was less intense, but the distribution of radioactivity was similar to the non-extracted tissue sections.

**Thin layer chromatography**

TLC was used to study the metabolic fate of $[^3]$H-dopamine in the various samples in Papers III and IV. Dopamine and DOPAC, one of the major metabolites of dopamine, was added to the homogenates. In Paper III, the radioactivity in each segment of the TLC plate after application of the extract from the homogenates of the blood, CSF, olfactory bulbs and right olfactory mucosa indicated that metabolism of dopamine had taken place in all samples. Nevertheless, in most samples the sum of the dopamine and DOPAC spots accounted for nearly 100% of the total radioactivity in the tissue samples 30 minutes after nasal administration (the values are listed in table 2).

The metabolism of dopamine was most pronounced in the CSF samples; in these samples, 14% of the radioactivity was located in the dopamine spot. However, 83% of the radioactivity coeluted with DOPAC and hence a total of 97% of the radioactivity was located in the CSF samples. Metabolism of $[^3]$H-dopamine in the olfactory mucosa was not that extensive, and therefore the metabolism to DOPAC must have occurred during movement to or in the CSF.

**Table 2.** The mean radioactivity ($n=2$) in segments of the TLC plate after application of the blood, CSF, olfactory bulb and right mucosa homogenate extracts from rats 30 minutes after nasal administration of $[^3]$H-dopamine (50 µCi). The amounts of radioactivity in the dopamine and DOPAC spots are presented as the percentage of the total radioactivity on the TLC plate (Paper III).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Percentage of radioactivity</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dopamine spot</td>
<td>DOPAC spot</td>
</tr>
<tr>
<td>Blood</td>
<td>32</td>
<td>58</td>
</tr>
<tr>
<td>CSF</td>
<td>14</td>
<td>83</td>
</tr>
<tr>
<td>Left olfactory bulb</td>
<td>55</td>
<td>41</td>
</tr>
<tr>
<td>Right olfactory bulb</td>
<td>59</td>
<td>31</td>
</tr>
<tr>
<td>Right mucosa</td>
<td>68</td>
<td>6</td>
</tr>
</tbody>
</table>

Most of the radioactivity transported into the olfactory bulbs was from unchanged $[^3]$H-dopamine (55% and 59% for left and right, respectively) and it appears that dopamine was transferred into the bulbs first, before entering the CSF. About 59% and 31% of the radioactivity in the right olfactory bulb coeluted with dopamine and DOPAC, respectively, and it was only in the olfactory bulbs that the activity of dopamine was higher than that of DOPAC. The radioactivity levels in the right olfactory mucosa (administration side) were 68% and 6% for dopamine and DOPAC, respectively.
In Paper IV, the profiles of the radioactivity in each segment of the TLC plate after application of the extract from the homogenates of the right olfactory bulb or mucosa are shown in figure 15. About 75% of the radioactivity in the olfactory bulb coeluted with dopamine. In the olfactory mucosa, conversely, only about 10% coeluted with dopamine.

![Figure 15](image-url) 

**Figure 15.** The profile of radioactivity (dpm/cm) in different regions of the TLC plates 2 hours after unilateral nasal (right side) administration of [3H]-dopamine (5 µCi) to a mouse. The concentration of radioactivity in the extracts of homogenate from (a) the right olfactory bulb and (b) the olfactory mucosa from the right nasal cavity are demonstrated (Paper IV).

**DISCUSSION**

The rat is widely used in animal experiments and is established as an excellent animal model to study nasal drug absorption of drugs. For most non-peptide drugs, results obtained in the rat can accurately predict the absorption profiles of the drugs in humans (Hussain, 1998). Three main models, some with minor modifications, have been reported in the literature (Gizurarson, 1990). The model used in Papers I-III is a modification of the in vivo surgical method (Hirai et al., 1981). In this method, first reported by (Björk and Edman, 1988), drainage from the nasal cavity is not prevented with an adhesive glue. Instead, care is taken to maintain the normal functions of the cavity by minimising disturbance of the mucosa via mechanical manipulation.

The absorption data in this thesis showed that the nasal route has potential for the administration of, in this case, CNS-active compounds with low oral bioavailability. Rapid absorption into the systemic circulation after nasal administration has been demonstrated with several drugs that are active in the CNS: physostigmine and arecoline (Hussain and Mollica, 1991), propiomazine (Bjerre et al., 1996), dextromethorphan (Char et al., 1992) and cocaine (Chow et al., 1999). Both (S)-UH-301 and NXX-066 were totally absorbed across the nasal mucosa, which is consistent with other studies using small lipophilic compounds (Corbo et al., 1989a; Fisher et al., 1992). The molecular weights of (S)-UH-301 and NXX-066 are 265.4 g/mol and 377.5 g/mol, and the log P values are 4.03 (Paper I) and 4.35 (Paper II), respectively. A linear relationship between the rate constant of absorption and the log P (octanol/water) has been
demonstrated earlier with progesterone (Corbo et al., 1989b). McMartin et al. (1987) linked the extent of absorption of compounds with their molecular weights, and the nasal route appears suitable for the efficient rapid delivery of many molecules of molecular weight<1000.

No direct evidence for transfer along the olfactory pathway was shown with (S)-UH-301 (Paper I). However, a prolonged duration of the concentration in the CFS was seen after nasal administration and, after about 20 minutes, the nasal/intravenous CSF concentration ratio exceeded one i.e. the concentrations of nasally administered (S)-UH-301 in the CSF were higher than those after intravenous administration. These results are similar to those found by Chou and Donovan (1998a) in a rat model of the uptake of lidocaine. The maximum concentration of lidocaine in the CSF following nasal administration did not exceed that achieved after intraarterial administration but the nasal/intraarterial AUC ratios in the CSF were found to be greater than one. According to the authors, this may have been a consequence of the dynamic equilibrium between biological compartments or the existence of a slow continuing absorption process from the nasal mucosa. Again, these observations may suggest the existence of other pathways between the nasal cavity and the CNS in addition to passive diffusion and partitioning from the blood (Chou and Donovan, 1998a).

Since the uptake of NXX-066 into the CSF was not higher after nasal administration than after intravenous administration (Paper II), there was no evidence that NXX-066 was transported from the nasal cavity along the olfactory neurons into the brain. Similar results, with no enhanced brain uptake after nasal administration, were shown with \([^{14}C]\)-dextromethorphan, which is a compound that penetrates the BBB to a large extent (Char et al., 1992). The nasal route was suggested to be a viable alternative to the parenteral route for administration of this drug. After nasal administration, the AUC in CSF was 65.9% of that after intravenous administration, whereas the plasma bioavailability after nasal administration was 78.8%. In this study, no CSF samples were taken and only the anterior part of the cerebrum was examined for radioactivity 2 minutes after administration. CSF sampling and/or measurements of the olfactory bulbs could perhaps have demonstrated a higher radioactivity content after nasal administration of \([^{14}C]\)-dextromethorphan. Despite the rapid systemic absorption of the drug, an unexplained initial absorption phase into the brain occurred after nasal administration, and the total brain uptake was lower than seen with intravenous administration (Char et al., 1992). A similar initial absorption phase into the CSF occurred with NXX-066 in this study. Although NXX-066 was totally absorbed into the systemic circulation after nasal administration, uptake into the CSF was significantly higher after intravenous administration at the first sampling time 3 minutes after administration (fig. 9). The reason for this is not known, but it is possible that the high initial plasma concentration of NXX-066 after intravenous administration may have caused rapid transport of the drug through the BBB by passive diffusion.

The absolute bioavailability of a cognition enhancing drug was increased from less than 10% after oral to more than 50% after nasal administration. The amount of the \([^{14}C]\)labelled drug was examined in the whole brain 2 minutes after administration and the brain/plasma AUC ratios were the same after intravenous and nasal administration. The
brain uptake was independent of the route of administration. The authors postulated that
the nose-brain pathway only enhances brain uptake for poorly absorbed substances like
proteins, peptides and metals. Substances which are well absorbed over the nasal
epithelium are cleared rapidly into the systemic circulation and transport from the nose
into the brain is relatively slow and insignificant (Hussain et al., 1990).

Because of high first pass metabolism after oral administration, dopamine is usually
given by intravenous infusion to treat acute cardiovascular disease. In beagle dogs the
nasal bioavailability of dopamine is 11.7% (Ikeda et al., 1992), increasing to 20 to
100% after addition of absorption enhancers. The nasal route has been suggested as a
potential route of administration for this drug. Species differences in absorption studies
of substances are common and the nasal bioavailability of dopamine in the rat (68±30%;
Paper III) seems to be more consistent with that of [³H]-dopamine in monkeys (Anand
Kumar et al., 1974b). Although the bioavailability was not reported in this paper, [³H]-
dopamine appears from the figures to have been almost totally absorbed into the
systemic circulation.

In paper III and IV, the results following intravenous administration of [³H]-dopamine
in rats and mice (fig. 11 and 12 a) support previous findings that dopamine has limited
ability to penetrate the BBB. Bearing this in mind, it is postulated that the increased
CSF/blood concentration ratio after nasal administration of [³H]-dopamine in rats
(Paper III) could be explained by transport of the drug through the olfactory pathway.
The CSF/blood concentration ratio (fig. 10 b) of [¹H]-dopamine increased over time up
to 120 minutes, at which point a plateau was reached. These results are in agreement
with a study by Anand Kumar et al. (1974b) on the nasal administration of [³H]-
dopamine in monkeys where radioactivity was detected in the CSF 15 min after nasal
but not after intravenous administration of [³H]-dopamine. The level of radioactivity in
the CSF reached a plateau 15 minutes after nasal administration of [³H]-dopamine and
the plateau was maintained throughout the experiment for 60 minutes.

The brain tissue sampling in both rats (Paper III) and mice (Paper IV) and the
autoradiographic experiments (Paper IV) indicate that dopamine is transported along the
olfactory pathway into the olfactory bulb following nasal administration.
Dopamine did not seem to be transferred further into the brain, since selective uptake of
radioactivity was not seen in other regions of the brain (Paper IV). However, the liquid
scintillation data showed that the olfactory tract had a significantly higher radioactivity
content 4 hours after nasal administration than the other selected brain tissue samples
(not olfactory bulbs) (fig. 12 b). In Paper IV a trace dose of [³H]-dopamine was
administered nasally and totally 0.12% was detected in the brain after 4 hours. It may be
that higher doses of dopamine would be required in order for the drug to be transferred
further into the brain.

The results in the dopamine study in mice (Paper IV) showed significantly higher
amounts of radioactivity in the right than in the left olfactory bulb after unilateral (right
side) administration of [³H]-dopamine while no significant difference was shown
between the right and left olfactory bulbs in the rat model (Paper III). However,
significantly higher amounts of radioactivity were found in the right nasal mucosa than
in the left nasal mucosa at the first three sampling points, which indicates that the \([^3\text{H}]\)-dopamine in the left olfactory bulb could not exclusively be explained by transport from the left nasal cavity (Paper III). After nasal instillation of cadmium and manganese, drainage occurred from the cavity receiving the metal to the untreated side (Tjälve et al., 1996). There was a correlation between the levels of the metals present in the olfactory epithelium on both sides of the nasal septum and the levels of the metals taken up in the corresponding olfactory bulbs. A so-called septal window (Keleman and Sargent, 1946) between the two cavities could perhaps explain the drainage in the rat model. Further, in order to aid breathing during the experiments, the trachea were cannulated and the rats lay on their backs, while the mice were placed on their right sides after administration.

Several different experimental methods have been used to study the uptake of substances into the CSF and/or brain after nasal administration. As seen in table 1, the most common methods are collection of CSF (Chou and Donovan, 1998a; Yajima et al., 1998; Papers I, II and III) or brain tissue sampling (Dluzen and Kefalas, 1996; Frey II et al., 1997; Papers III, IV and V). Autoradiography (Brittebo and Eriksson, 1995; Henriksson and Tjälve, 1998; Papers IV and V) is a useful technique for exploring the olfactory pathways into the brain but has rarely been used in nasal drug delivery experiments. The large distribution volume of NXX-066 appears to be consistent with the lipophilic character of the molecule, and this suggests that it undergoes significant tissue binding, which could explain the low concentrations of NXX-066 in the CSF. Since there are no apparent barriers to the penetration of the BBB by NXX-066, the drug presumably diffuses into the more lipophilic environments of the brain tissue rather than remaining in the CSF. Thus, it could be useful to include a complementary method of sampling highly lipophilic compounds such as NXX-066 along with CSF sampling.

It is important to take samples or measurements at several time points when investigating whether a substance has been transferred into the CNS after nasal administration. In Paper I, the peak concentration of (S)-UH-301 in the CSF appeared after only 3-5 minutes, but the concentrations were followed for a total of 60 minutes and were still high about 20 minutes after nasal administration. In the \([^3\text{H}]\)-dopamine study in mice (Paper IV), 5 brain tissue samples were obtained over the 8 hours following administration. If samples had been collected for shorter period of time, e.g. only at 30 minutes or 1 hour after administration, the peak at 4 hours would have been overlooked.

In the \([^3\text{H}]\)-dopamine study in monkeys (Anand Kumar et al., 1974b), the last CSF sample was collected 1 hour after administration and the maximum level of radioactivity in the CSF may not have been noticed. In another study in which zidovudine was administered to rats (Seki et al., 1994), the concentration of drug in the CSF after nasal administration was no higher after 15 minutes than after intravenous administration, i.e. direct transport appeared not to have taken place. Samples of CSF taken at several different time points might have led to another conclusion. As seen with cocaine (Chow et al., 1999), it is also important to take the first sample soon after nasal administration when investigating the olfactory pathway for small and lipophilic compounds.
In Paper IV, the peak concentration of dopamine in the right olfactory bulb appeared 4 hours post-dose. In Paper III, the highest level of radioactivity in the olfactory bulbs was obtained 30 minutes after administration of [3H]-dopamine and in the CSF, levels increased to 60 minutes post-dose and then decreased. The difference in time to reach the peak levels in these two studies of nasally administered [3H]-dopamine may be explainable by the presence of two pathways for the transfer of substances from the olfactory mucosa into the CNS. The dopamine may have first reached the olfactory bulb by traversing the perineural space that is continuous with the subarachnoid space in the olfactory epithelial pathway. The appearance of the peak after 4 hours may have been the result of additional slower transport of dopamine within the axons.

The level of radioactivity in the ipsilateral olfactory bulb increased after unilateral nasal administration of [3H]-labelled neurotensin in mice (Paper V). The radioactivity in the ipsilateral olfactory bulb markedly exceeded that in the contralateral bulb during the study period (24 hours). Autoradiography of mice given a unilateral instillation of [3H]-neurotensin showed that radioactivity was preferentially located in the peripheral layers of the ipsilateral olfactory bulb up to 48 hours after administration. This prolonged time profile differs from the timing of olfactory transport of [3H]-dopamine, for which maximum radioactivity was measured in the right olfactory bulb at 4 hours (Paper IV).

These observations, together with the fact that most of the radioactivity in the ipsilateral olfactory bulb was not extractable with organic solvents, suggest that little of the transferred radioactivity was associated with unchanged neurotensin. The degradation of intravenously administered neurotensin is rapid. The plasma half-life in rats has been reported to be as short as 0.55 minutes (Aronin et al., 1982). Since peptidases are known to be present in the olfactory mucosa, rapid degradation of neurotensin after nasal administration cannot be excluded. Several reports regarding the olfactory axonal transport of amino acids, e.g. taurine (Brittebo and Eriksson, 1995) and β-alanine (Burd et al., 1982), have been published. Taurine is thought to be transported as an individual amino acid, and β-alanine is thought to be transported as the dipeptide carnosine.

The use of labelled compounds for initial studies of absorption and distribution are common but additional studies are usually needed. TLC was used in Papers III and IV to study the metabolic fate of [3H]-dopamine and the results indicated that unchanged [3H]-dopamine was transported into the olfactory bulb. The proportion of radioactive dopamine in the right olfactory mucosa (administration side) was 68% in the rat model (Paper III) and about 75% in the mouse model. Subsequently, unchanged dopamine was transferred into the olfactory bulb of rats and mice after nasal administration of [3H]-dopamine.

Peptides and proteins, which are metabolically unstable and are more difficult to analyse, complicate the experiments further. The use of radioactively labelled compounds has, however, been applied previously to peptides, e.g. in the study of the olfactory absorption of [125I]-insulin (Sigurdsson et al., 1997). Although ELISA has also been used for the detection of nerve growth factor (NGF) in brain tissue after nasal
administration (Chen et al., 1998), this method may detect more than just the intact and pharmacologically active peptides.

In the papers discussed, only small amounts of the compounds were delivered into the CNS after nasal administration, however, many CNS-active substances are biologically active in low concentrations. Encouraging findings of both Thorne et al. (1995) and Chen et al. (1998) studying WGA-HRP and recombinant human NGF, respectively, showed that it is possible to achieve therapeutically active concentrations of the proteins in the brain via the olfactory pathway after nasal administration.

So far, not many articles have been published regarding the influence of the formulation for the uptake of compounds into the CNS after nasal administration. Henry et al. (1998) compared the uptake of midazolam into the CSF after nasal administration with two devices: nasal atomiser and nasal drops. Higher bioavailability of midazolam in the CSF was obtained with the nasal atomiser. Kumbale et al. (1999) studied the brain delivery of GM1 after nasal administration using GM1-lipid nanospheres and GM1-DOTAP (a positively charged lipid) complex. Enhancing the delivery of drugs into the CNS along the olfactory pathway is an interesting area of research in the future.

Most studies investigating the pathway from the nose to the brain have been performed in rodents, but studies in monkeys have also been reported. As mentioned in the introduction many common animal models, including those involving rodents, are classified as macrosomatic while humans are understood to be microsmatic. Further, in humans, the olfactory region is located in the roof of the cavity while the olfactory area in rats is spread throughout the posterior part of the cavity. It is important to take these anatomical differences between species under consideration when results are interpreted and compared.

**CONCLUSIONS**

Taken together, the results of this thesis and earlier observations from other research groups suggest that nasal administration of CNS-active compounds with low oral bioavailability is an interesting and workable alternative to administration by intravenous injection. The small lipophilic compounds (S)-UH-301 and NXX-066 were rapidly and completely absorbed into the systemic circulation after nasal administration. These molecules appeared rapidly in the CSF, although hard evidence of direct transfer from the nose remains elusive.

Importantly, the results also indicate that it may be viable to administer CNS-active drugs, which would normally not reach the brain because of the BBB, via the olfactory pathway. Radioactivity measurements in the olfactory bulb following nasal administration of \[^{3}H\]-dopamine and \[^{3}H\]-neurotensin indicate that transfer occurred. The TLC results showed the presence of unchanged dopamine in the olfactory bulb but it is less clear from initial results with neurotensin, which radioactive products reached the olfactory bulb, and further studies are required.
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REFERENCES
Barnett, E.M. and Perlman, S., 1993. The olfactory nerve and not the trigeminal nerve is the major site of CNS entry for mouse hepatitis virus, strain JHM. Virology 194, 185-191.
Chou, K.-J. and Donovan, M.D., 1998a. The distribution of local anaesthetics into the CSF following intranasal administration. Int. J. Pharm. 168, 137-145.


Shipley, M.T., 1985. Transport of molecules from nose to brain: transneuronal anterograde and retrograde labeling in the rat olfactory systemby wheat germ


Smolnik, R., Mölle, M., Fehm, H.L. and Born, J., 1999. Brain potentials and attention after acute and subchronic intranasal administration of ACTH4-10 and desacetyl-α-MSH in humans. Neuroendocrinol. 70, 63-72.


