ELSEVIER

International Journal of Pharmaceutics 195 (2000) 197–205

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Nasal absorption of (S)-UH-301 and its transport into the cerebrospinal fluid of rats

Maria Dahlin, Erik Björk *

Department of Pharmacy, Division of Pharmaceutics, Biomedical Centre, Uppsala University, Box 580, SE-751 23 Uppsala, Sweden

Received 4 May 1999; received in revised form 26 October 1999; accepted 3 November 1999

Abstract

Targeting the brain via nasal administration of drugs has been studied frequently over the last few years. In this study, the serotonin-1a receptor antagonist *(S)*-5-fluoro-8-hydroxy-2-(dipropyl-amino) tetralin ((S)-UH-301) hydrochloride was used as a model substance. The systemic absorption and transport of (S)-UH-301 into male Sprague–Dawley rat cerebrospinal fluid (CSF) were investigated after nasal and intravenous administration. Blood and CSF samples were obtained at regular time intervals from the arteria carotis and by cisternal puncture, respectively, after administration to both nostrils (total 12 μmol/kg) or into the vena jugularis (6 μmol/kg). The concentrations of (S)-UH-301 in plasma and CSF were measured by HPLC with electrochemical detection. The maximum plasma concentration of intranasal (S)-UH-301 occurred in about 7 min and the absolute bioavailability seemed to be complete ($F = 1.2 \pm 0.4$). Initially, no increased concentrations of (S)-UH-301 were seen in CSF after nasal compared to intravenous administration i.e. it appeared that no direct transport of (S)-UH-301 from the nasal cavity, along the olfactory neurons and into the CSF occurred. However, a prolonged duration of the concentration was seen after nasal administration of (S)-UH-301 and after about 20 min the CSF_{na}:CSF_{iv} concentration ratio (corrected for different dosage) exceeded 1.

Keywords: Nasal administration; 5-HT-1a receptor antagonist; Rat; Olfactory pathway; Cerebrospinal fluid (CSF); Brain targeting

1. Introduction

Nasal delivery of drugs has been studied extensively over the last 20 years. This administration route offers a variety of benefits for systemic drug delivery and is consequently an attractive non invasive alternative for drugs, normally administered by injection. Physiologically, the nose provides a permeable mucosa that is richly vascularised, thus facilitating rapid absorption of drugs and a rapid onset of action. The nasal environment also has a lower level of enzymatic activity, compared to the gastrointestinal tract, and first pass metabolism in the liver is avoided by administering drugs by this route. Nasal administration is also more convenient than the invasive routes. Thus, nasal administration is an attractive method of systemic delivery for many different drugs (Chien et al., 1989; Hussain, 1998).

* Corresponding author. Tel.: +46-18-4714388; fax: +46-18-4714223.

E-mail address: erik.bjork@galenik.uu.se (E. Björk)

0378-5173/00/$ - see front matter
PII: S0378-5173(99)00392-0

The blood brain barrier (BBB) restricts the transport of materials from the blood to the central nervous system (CNS), and the cerebrospinal fluid (CSF) which surrounds it, in order to maintain the environment required by the brain. However, Erlich et al. (1986) showed that there was no significant barrier between the nasal mucosa and the brain at the rabbit cribriform region, and that the CSF reached the submucosal region rapidly via open pathways in this model. There is also evidence indicating the existence of a direct pathway from the olfactory mucosa, along the olfactory neurons, into the brain. Several investigators have shown that metals [such as manganese, cadmium (Tjälve et al., 1996) and colloidal gold (Gopinath et al., 1978)], wheat germ agglutinin-horseradish peroxidase (Shipley, 1985; Thorne et al. 1995), progesterone (Kumar et al., 1974, 1982), zidovudine (Seki et al., 1994), taurine (Brittebo and Eriksson, 1995), cephalexin (Sakane et al., 1991), insulin (Sigurdsson et al., 1997), nerve growth factor (Frey et al., 1997), antihistamines (Chou and Donovan, 1997) and viruses (Perlman et al., 1990; Barnett and Perlman, 1993; Huneycutt et al., 1994)) can enter the brain by this route.

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; these subtypes are widely distributed in the brain. The subtype 5-HT-1a is found in both pre and post synaptic areas. The greatest concentration of 5-HT-1a presynaptic receptors (autoreceptors), occurs in the raphe nucleus, while postsynaptic 5-HT-1a receptors are more concentrated in the hippocampus. 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 (low serotonin concentrations) and agony (high concentrations) (Barrett and Vanover, 1993; Baldwin and Rudge, 1995).

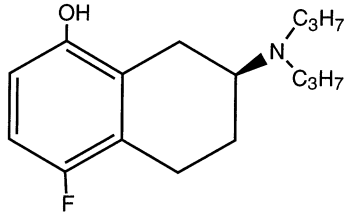

Fig. 1. Chemical structure of the 5-HT-1a receptor antagonist (S)-UH-301.

The hydrochloride salt of (S)-5-fluoro-8-hydroxy-2-(dipropylamino) tetralin ((S)-UH-301, Fig. 1), a 5-HT-1a receptor antagonist (Hillver et al., 1989), was used as a model substance in this study. The selective 5-HT-1a receptor agonist 8-hydroxy-2-(dipropylamino) tetralin hydrochloride (8-OH-DPAT) (Arvidsson et al., 1981) was used as an internal standard. (S)-UH-301 exhibits anticonvulsant, hypophagic and anxiolytic-like activities in rodents (Moreau et al., 1992) and also completely antagonises several of the effects of 5-HT induced by (R)-8-OH-DPAT in rats (Björk et al., 1991).

The two main objectives of this study were (1) to investigate the systemic absorption of the model substance (S)-UH-301 hydrochloride from the nasal cavity and; (2) to compare the uptake of (S)-UH-301 into the CSF after nasal and intravenous administration. It was assumed that if the CSF concentrations of (S)-UH-301 were higher after nasal administration than after intravenous administration, a direct pathway from the nasal olfactory area into the brain must exist for this molecule.

2. Materials and methods

2.1. Drugs and reagents

(S)-UH-301 hydrochloride and 8-OH-DPAT were donated by Astra Arcus AB, Sweden. Heparin (500 IU/ml) was acquired from Løwens, Denmark, and thiobutabarbital sodium (Inactin) was obtained from Byk Gulden, Germany. Solvents were of HPLC grade and all other chemicals were of analytical grade.

2.2. Characterisation of (S)-UH-301

Since no physicochemical data were available on (S)-UH-301, a few initial exploratory experiments were carried out. The water solubility of the hydrochloride salt of (S)-UH-301 was determined from a Beer's law plot constructed using (S)-UH-301 hydrochloride concentrations ranging from 2 to 250 μM. The UV absorbance of the solutions at these concentrations was measured with a single beam spectrophotometer (Hitachi U1100) at a wavelength of 205 nm. A saturated solution of (S)-UH-301 hydrochloride in distilled water was prepared and kept at room temperature while stirring for 12 h. After filtration, the absorbance of the diluted (1:1000) supernatant was measured. The solubility of (S)-UH-301 was determined by extrapolation, using Beer's law.

An automated titrator (Sirius PCA 101) was used to measure both log *P* (octanol/water) and p*K*a values for (S)-UH-301. 0.5 ml octanol and 19.5 ml water were used for, and 20 ml water was used for p*K*a. For each determination, three analyses were carried out.

2.3. Animals

The study was approved (C 84/94) by The Swedish National Board for Laboratory Animals (the local ethical committee in Uppsala). Male Sprague–Dawley rats (Møllegaard, Denmark), with a mean weight of 327 (range 256–412) g, were used in the experiments. The animals were housed at 22°C with a 12 h light/dark cycle and given a standard pellet diet (Lactamin R36) and tap water ad libitum.

2.4. Animal experiments

The experiments were performed as reported by Björk and Edman (1988) where care is taken to maintain the normal functions of the cavity by minimising disturbance of the mucosa via mechanical manipulation. The rats were anaesthetised with an intraperitoneal injection of thiobutabarbital sodium (150 mg/kg). During the experiment, the rat body temperature was maintained at 37°C with a heating pad. The trachea and arteria carotis were cannulated with polyethylene tubes, PE 200 and PE 50, respectively, and the experiments were started 30 min after surgery. (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 administered solution was 5.9. The intravenous injection (6 μmol/kg) was given as a bolus dose through a catheter (PE 50) into the external vena jugularis. Half the nasal dose (total dose 12 μmol/kg) was administered as 25–30 μl through each nostril, using polyethylene tubes (PE 90) attached to a micropipette.

Blood samples of 200 μl were withdrawn from the arteria carotis prior to administration and 3, 7, 10, 15, 20, 30, 60 and 120 min after, from seven rats who had received intravenous and five who had received intranasal (S)-UH-301 hydrochloride. The plasma was separated after the addition of one drop of heparin (24 gauge needle) by centrifugation at 7000 rpm for 10 min After the experiments, the animals were killed with an overdose of pentobarbital (100 mg/ml).

The CSF samples were taken by cisternal puncture (Waynforth and Flecknell, 1980; Persson et al., 1989). Briefly, the rats were placed in a specially made stand, which fixed the head at a specific forward angle. After exposing the atlanto–occipital membrane, 100–150 ml of the CSF was withdrawn through the membrane by gentle suction through a 30 gauge needle, attached to a disposable syringe connected to polyethylene tubing (PE 50). The CSF samples were withdrawn before and 3, 5, 10, 15, 30 and 60 min after, administration of (S)-UH-301 hydrochloride. Collection of CSF was terminated, as soon as blood appeared and the blood-tainted portion of CSF was prevented from reaching the collecting tube. If blood appeared immediately during sampling, the result was excluded. Blood samples were withdrawn from the arteria carotis 2 min after each CSF sampling, for correlation with the absorption data. Measurements were made on three rats at each time point. After the experiments, the animals were killed with an overdose of pentobarbital.

All samples, both plasma and CSF, were stored at −80°C until analysis.

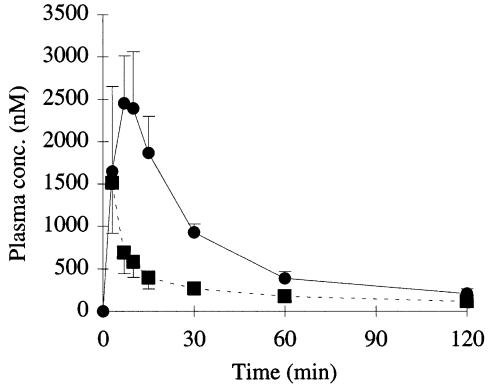

Fig. 2. Concentration–time profiles of (S)-UH 301 in plasma after (■) intravenous (6 μmol/kg) ($n = 7$) or (●) nasal (12 μmol/kg) ($n = 5$) administration of the hydrochloride salt to rats. The data are expressed as mean ± S.D. The initial intravenous concentrations were obtained by extrapolation.

2.5. Analysis

In order to extract (S)-UH-301 and 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 min in a rotating mixer and 8 min 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, $\mu = 0.1$) and 100 μl was injected into the HPLC system.

Standard and control samples for the CSF analysis were made from artificial CSF (Elliot's B solution) according to Martindale (30th edition). 50 μl of the untreated CSF samples were injected into the HPLC system.

An ODS C-18 analytical column (100 × 4.6 mm, 3 μm, YMC Europe GmBH) and precolumn (15 × 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% acetonitril 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). The peak heights were used for quantification of (S)-UH-301 in both plasma and CSF. The ratio (S)-UH 301/8-OH-DPAT was used for calculation of the concentration of (S)-UH-301 in the plasma samples.

2.6. Calculation

In order to calculate area under the plasma concentration–time curve (AUC) from 0 to 120 min, the initial intravenous concentrations were obtained by extrapolating the data from the first two sampling points. The individual AUC values for (S)-UH-301 were calculated according to the trapezoidal rule. The nasal bioavailability (F) was calculated according to the following equation:

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

where $\mathrm{AUC_{na}}$ and $\mathrm{AUC_{iv}}$ denote the means of individual AUC values from the nasal and intravenous groups, respectively. Results are expressed as mean ± S.D.

The statistics were calculated according to Student's t-test.

3. Results

The solubility of the hydrochloride salt of (S)-UH-301 (MW 301.8 g/mol) in water was 77 g/ml. The two pKa values obtained from the automated titrator were 9.4 and 11.0. (S)-UH-301 is a small molecule (MW 265.4 g/mol) (Fig. 1) which appears to be relatively lipophilic ($\log P$ 4.03 ± 0.02).

The absorption of (S)-UH-301 from the nasal cavity into the systemic circulation was rapid and complete. The maximum concentration was achieved after about 7 min (Fig. 2) and the extent of nasal bioavailability appeared to be 100%. The mean AUC values after intravenous and nasal administration were 36 100 ± 12 200 and 87 400 ± 11 200 nM/min, respectively, and consequently $F = 1.2 \pm 0.4$.

The concentration–time profiles in CSF (Fig. 3) showed no increased concentration of (S)-UH-301 after nasal, compared to intravenous administration. However, a prolonged duration of the concentration was seen after nasal administration. This was illustrated in Fig. 4 by plotting the CSF_{na}:CSF_{iv} concentration ratios versus time. The ratios were corrected for the different dosage. After about 20 min, the ratio exceeded 1, 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 min and this level was sustained throughout the experiments.

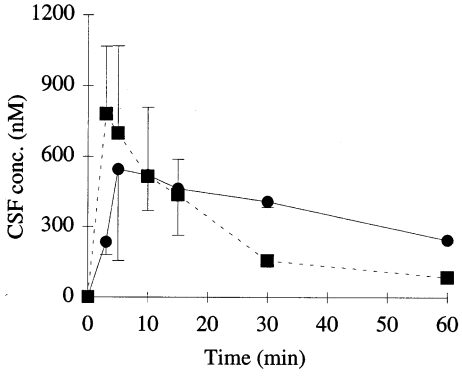

Fig. 3. Concentration–time profiles of (S)-UH-301 in CSF after (■) intravenous (6 μmol/kg) or (●) nasal (12 μmol/kg) administration of the hydrochloride salt to rats ($n = 3$ for all time points except for the intravenous route at 30 min and the nasal route at 60 min, where $n = 2$). The data are expressed as mean ± S.D.

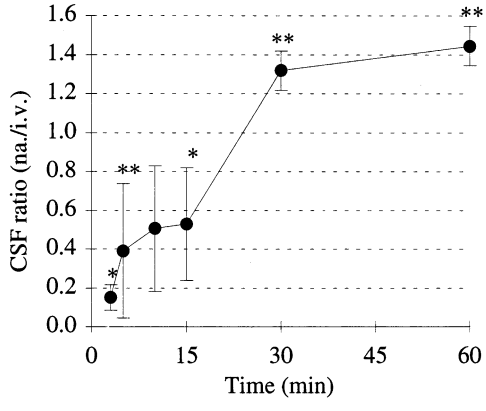

Fig. 4. The concentration ratios (nasal/intravenous) in CSF after nasal (12 μmol/kg) and intravenous (6 μmol/kg) administration of (S)-UH-301 hydrochloride to rats. The ratios are corrected for differences in dosages and expressed ± S.D. Any significant difference from unity was calculated according to Student's t-test: $*P < 0.05$; $**P < 0.01$.

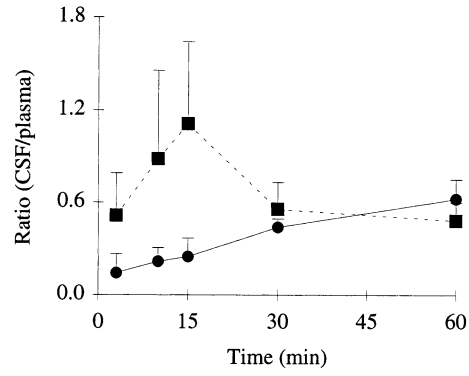

Fig. 5. Ratios between concentrations of (S)-UH-301 in CSF and plasma after (■) intravenous and (●) nasal administration of the hydrochloride salt to rats over time. Ratios are expressed ± S.D.

The ratios between the concentrations of (S)-UH-301 in the CSF and in plasma over time were plotted in Fig. 5. Because the blood samples were taken 2 min after cisternal puncture for these animals, plasma concentrations for the same time points as the CSF values were extrapolated from the systemic absorption graph (Fig. 2). The plasma samples obtained after the CSF samples, correlated well with the absorption data (data not shown).

The AUC values of (S)-UH-301 in the CSF and plasma, and the ratios (C/P) between these values are listed in Table 1. The ratios of AUC_{CSF} to AUC_{plasma} for the time periods between 0 and 30 and 30 and 60 min were 0.26 and 0.50 for the nasal data and 0.60 and 0.53 for the intravenous data. The changes over time are 92 and −13%, respectively, for the two time periods, i.e. the amount of (S)-UH-301 in the CSF increased, relative to that in the plasma with time after nasal administration and decreased after intravenous administration.

4. Discussion

Several investigators have shown that different drugs can be rapidly absorbed to achieve high concentrations in plasma after nasal administration (Hussain et al., 1980; Yajima et al., 1996). This is also the case with (S)-UH-301. The physicochemical characteristics, such as molecular size and partition coefficient, allow (S)-UH-301 to be transported rapidly across most membranes. A linear relationship between the rate constant of absorption and the log *P* (octanol/water) has been demonstrated earlier with progesterone (Corbo et al., 1989). The peak plasma concentration of (S)-UH-301 after nasal administration appeared at 7 min. McMartin et al. (1987) linked fast absorption of compounds with their molecular weight. The nasal route is suitable for efficient rapid delivery of many molecules of molecular weight < 1000.

The large S.D. in the calculation of the absolute bioavailability of (S)-UH-301 ($F = 1.2 \pm 0.4$) is mainly due to deviations in the intravenous AUC values, because of the rapid decline in plasma concentrations. Small differences in the time between administration and sampling, during the initial phase can cause large differences in plasma concentrations.

The nose–brain pathway, as a conduit for transmission of agents into the CNS, is an area of ongoing research. A number of substances, about 35–40, including viruses, metals, dyes and drugs, have been reported to gain direct access to the CSF and/or brain after nasal administration (Mathison et al., 1998). However, this does not appear to be the case for (S)-UH-301, since no increased uptake into the CSF was seen after nasal administration, compared with intravenous administration, i.e. there was no evidence that (S)-UH-301 was transported from the nasal cavity, along the olfactory neurons into the brain. These results are similar to results from two other studies: one with nasally administered progesterone (Kumar et al., 1982) and another with a cognition enhancer (Hussain et al., 1990). Hussain et al. suggest that the nose–brain pathway may only be significant for poorly absorbed substances. For substances that are rapidly and completely absorbed into the systemic circulation after nasal administration, any transport along the olfactory neurons into the CNS is slow and insignificant.

In order to decide if a substance has been transported into the CNS, it is important to determine the concentration at several time points. Although the peak concentration of (S)-UH-301 in CSF appeared after only 3–5 minutes, the concentrations were followed for a total of 60 min. In a study with zidovudine (Seki et al., 1994), the CSF concentrations in rats were measured once, 15 min after administration. The concentrations of zidovudine in the CSF were not significantly higher following nasal administration than after intravenous infusion, and no direct nose–brain pathway was proven. However, if several CSF samples had been taken at different time points and AUC values had been calculated, the conclusions may have been different. Sakane et al. (1991) demonstrated significantly higher concentrations of cephalexin in rat CSF 15 and 30 min

Table 1
Mean area under curve (AUC) values from concentration–time profiles (nM/min) in plasma and CSF, after intravenous (6 μmol/kg) and nasal (12 μmol/kg) administration of (S)-UH-301 hydrochloride to rats

	Nasal				Intravenous			
	AUC		Ratio		AUC		Ratio	
Time (min)	Plasma	CSF	C/P[a]	Change (%)	Plasma	CSF	C/P[a]	Change (%)
0–30	49 600	12 800	0.26		20 700	12 500	0.60	
30–60	19 800	9800	0.50	92	6700	3500	0.52	−13

[a] $C/P = AUC_{CSF}/AUC_{plasma}$.

after nasal administration than seen after intravenous and intraduodenal administrations. However, the concentration of cephalexin in plasma increased rapidly after nasal administration and so, if CSF samples been taken earlier than 15 min after administration, even higher CSF concentrations may have been reached.

The prolonged duration of (S)-UH-301 in plasma after nasal administration in this study may have been the result of delayed absorption of (S)-UH-301 across the nasal membrane into the systemic circulation. Perhaps, because of its high lipophilicity ($\log P \sim 4$) (S)-UH-301 was accumulated in the mucosa and then slowly delivered into the blood. Slow drug transport directly from the nasal cavity into the CNS, could also be the reason for the increasing amounts of (S)-UH-301 in the CSF after about 20 min (Fig. 4). The increasing C/P ratio shown in Table 1 also demonstrates the increasing amounts of (S)-UH-301 in the CSF over time. However, the C/P ratios after nasal administration were not higher than the same values after intravenous administration. Although (S)-UH-301 was totally absorbed into the systemic circulation after nasal administration, the uptake into the CSF was higher after intravenous administration. The reason for this is not known. The high initial plasma concentration after intravenous administration may have caused high, rapid transport of (S)-UH-301 through the BBB by passive diffusion.

Rats have been widely used for nasal drug delivery studies. The rat model used in these experiments is a modification of the in vivo model described by Gizurarson (1990). Instead of sealing the nasopalatine tract with adhesive glue, the normal functions of the cavity were maintained to the fullest possible extent (Björk and Edman, 1988).

Many common animal models, including rats, are classified as macrosmatic, i.e. the olfactory epithelium occupies a large area (50% for the rat) (Gross et al., 1982) of the total nasal epithelium. Man, however, is classified as microsmatic (Reznik, 1990), with a total olfactory area of around 3% (Morrison and Costanzo, 1990). Further, in humans, the olfactory region is located in the roof of the cavity, while the olfactory area in rats is spread throughout the whole cavity. It is important to take these anatomical differences between species under consideration when results are interpreted and compared.

After nasal administration of (S)-UH-301, only $\approx 0.002\%$ of the administered dose was detected in the CSF. Many CNS-active substances are potent drugs but unfortunately these substances are often associated with unwanted side effects. If it was possible to administer drugs nasally and utilise the nose–brain pathway, doses and side-effects could be reduced. This pathway could prove to be a useful route for CNS-active substances, which do not normally pass the BBB in sufficient amounts.

The conclusions from this study with the 5-HT-1a receptor antagonist (S)-UH-301 are, that while this molecule is well absorbed after nasal administration, no enhanced uptake into the CSF was detected compared to intravenous bolus injection.

Acknowledgements

AstraZeneca R&D Södertälje, Sweden, supported this work. The authors would like to thank Dr Conny Bogentoft and Prof. Peter Edman for valuable scientific discussions.

References

Arvidsson, L.E., Hacksell, U., Nilsson, J.L G., et al., 1981. 8-hydroxy-2-(di-*n*-propylamino) tetralin a new centrally acting 5-hydroxytryptamine agonist. J. Med. Chem. 24, 921–923.

Arvidsson, L.E., Hacsell, U., Glennon, R.A., 1986. Recent advances in central 5-hydroxytryptamine agonists and antagonists. Prog. Drug Res. 30, 365–471.

Baldwin, D., Rudge, S., 1995. The role of serotonin in depression and anxiety. Int. Clin. Psychopharm. 9 (4), 41–45.

Barnett, E.M., Perlman, S., 1993. The olfactory nerve and not the trigeminal nerve is the major site of CNS entry for mouse hepatitis virus, strain. JHM Virol. 194, 185–191.

Barrett, J.E., Vanover, K.E., 1993. 5-HT receptors as targets for the development of navel anxiolytic drugs: models, mechanisms and future directions. Psychopharmacology 112, 1–12.

Björk, E., Edman, P., 1988. Degradable starch microspheres as a nasal delivery system for insulin. Int. J. Pharm. 47, 233–238.

Björk, L., Cornfield, L.J., Nelson, L., et al., 1991. Pharmacology of the novel 5-hydroxytryptamine1a-receptor antagonist *(S)*-5-fluoro-8-hydroxy-2-(dipropylamino) tetralin: inhibition of (R)-8-hydroxy-2-(dipropylamino) tetralin-induced effects. J. Pharmacol. Exp. Ther. 258, 58–65.

Brittebo, E.B., Eriksson, C., 1995. Taurine in the olfactory system: effects of the olfactory toxicant dichlobenil. Neurotoxicology 16, 271–280.

Chien, Y.W., Su, K.S.E., Chang, S.-F., 1989. Nasal systemic drug delivery. In: Drugs and the Pharmaceutical Sciences. Marcel Dekker, New York.

Chou, K.J., Donovan, M.D., 1997. Distribution of antihistamines into the CSF following intranasal delivery. Biopharm. Drug. Dispos. 18, 335–346.

Corbo, D.C., Liu, J.-C., Chien, Y.W., 1989. Drug absorption through mucosal membranes: effect of mucosal route and penetrant hydrophilicityx. Pharm. Res. 6, 848–852.

Erlich, S.S., McComb, J.G., Hyman, S., Weiss, M.H., 1986. Ultrastructural morphology of the olfactory pathway for cerebrospinal fluid drainage in the rabbit. J. Neurosurg. 64, 466–473.

Frey, W.H., Liu, J., Chen, X., et al., 1997. Delivery of ^{125}I-NGF to the brain via the olfactory route. Drug Deliv. 4, 87–92.

Gizurarson, S., 1990. Animal models for intranasal drug delivery studies. Acta Pharm. Nord. 2, 105–122.

Gopinath, P.G., Gopinath, G., Kumar, T.C.A., 1978. Target site of intranasally sprayed substances and their transport across the nasal mucosa: a new insight into the intranasal route of drug delivery. Curr. Ther. Res. 23, 596–607.

Gross, E.A., Swenberg, J.A., Fields, S., Popp, J.A., 1982. Comparative morphometry of the nasal cavity in rats and mice. J. Anat. 135, 83–88.

Hillver, S.-E., Björk, L., Li, Y.-L., et al., 1989. (S)-5-fluoro-8-hydroxy-2-(dipropylamino) tetralin: a putative 5-HT1A-receptor antagonist. J. Med. Chem. 33, 1541–1544.

Huneycutt, B.S., Plakhov, I.V., Shusterman, Z., et al., 1994. Distribution of vesicular stomatitis virus proteins in the brains of BALB/c mice following intranasal inoculation: an immunohistochemical analysis. Brain. Res. 635, 81–95.

Hussain, A., Hirai, S., Bawarshi, R., 1980. Nasal absorption of propanolol from different dosage forms by rats and dogs. J. Pharm. Sci. 69, 1411–1413.

Hussain, A.A., 1998. Intranasal drug delivery. Adv. Drug. Del. Rev. 29, 39–49.

Hussain, M.A., Rakestraw, D., Rowe, S., Aungst, B.J., 1990. Nasal administration of a cognition enhancer provides improved bioavailability but not enhanced brain delivery. J. Pharm. Sci. 79, 771–772.

Kumar, T.C., David, G.F., Umberkoman, B., Saini, K.D., 1974. Uptake of radioactivity by body fluids and tissues in rhesus monkeys after intravenous injection or intranasal spray of tritium-labelled oestradiol and progesterone. Curr. Sci. 43, 435–439.

Kumar, T.C.A., David, G.F.X., Sankaranarayanan, A., Puri, V., Sundram, K.R., 1982. Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion or intranasal spraying. Proc.Nat. Acad. Sci. 79, 4185–4189.

Mathison, S., Nagilla, R., Kompella, U.B., 1998. Nasal route for direct delivery of solutes to the central nervous system: fact or fiction? J. Drug Target 5, 415–441.

McMartin, C., Hurchinson, L.E.F., Hyde, R., Peters, G.E., 1987. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. J. Pharm. Sci. 76, 535–540.

Moreau, J.-L., Griebel, G., Jenck, F., et al., 1992. Behavioral profile of the 5-HT-1a receptor antagonist (S)-UH-301 in rodents and monkeys. Brain Res. Bull. 29, 901–904.

Morrison, E.E., Costanzo, R.M., 1990. Morphology of the human olfactory epithelium. J. Comp. Neurol. 297, 1–13.

Perlman, S., Evans, G., Afifi, A., 1990. Effect of olfactory bulb ablation on spread of a neurotropic coronavirus into the mouse brain. J. Exp. Med. 172, 1127–1132.

Persson, S., Post, C., Alari, L., Nyberg, F., Terenius, L., 1989. Increased neuropeptide-converting enzyme activities in cerebrospinal fluid of opiate-tolerant rats. Neurosci. Lett. 107, 318–322.

Reznik, G.K., 1990. Comparative anatomy, physiology, and function of the upper respiratory tract. Env. Health Perspect 85, 171–176.

Sakane, T., Akizuki, M., Yoshida, M., et al., 1991. Transport of cephalexin to the cerebrospinal fluid directly from the nasal cavity. J. Pharm. Pharmacol. 43, 449–451.

Seki, T., Sato, N., Hasegawa, T., Kawaguchi, T., Juni, K., 1994. Nasal absorption of zidovudine and its transport to cerebrospinal fluid in rats. Biol. Pharm. Bull. 17, 1135–1137.

Shipley, M.T., 1985. Transport of molecules from nose to brain: transneuronal anterograde and retrograde labeling in the rat olfactory systemby wheat germ agglutinin-horseradish peroxidase aaplied to the nasal epithelium. Brain Res. Bull. 15, 129–142.

Sigurdsson, P., Thorvaldsson, T., Gizuararson, S., 1997. Olfactory absorption of insulin to the brain. Drug Deliv. 4, 195–200.

Thorne, R.G., Emory, C.R., Ala, T.A., Frey, W.I., 1995. Quantitative analysis of the olfactory pathway for drug delivery to the brain. Brain Res. 692, 278–282.

Tjälve, H., Henriksson, J., Tallkvist, J., Larsson, B.S., Lindquist, N.G., 1996. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. Pharmacol. Toxicol. 79, 347–356.

Waynforth, H.B., Flecknell, P.A., 1980. Cisternal puncture (and intracisternal injection). In: Experimental and Surgical Techniques in the Rat. Academic Press, London, pp. 59–61.

Yajima, T., Hasegawa, T., Juni, K., Saneyoshi, M., Kawaguchi, T., 1996. Nasal absorption of 2′,3′-didehydro-3'-deoxythymidine (D4T) and its esters in rats. Biol. Pharm. Bull. 19, 1234–1237.

Lindquist, N.G., 1996. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. Pharmacol. Toxicol. 79, 347–356.

Waynforth, H.B., Flecknell, P.A., 1980. Cisternal puncture (and intracisternal injection). In: Experimental and Surgical Techniques in the Rat. Academic Press, London, pp. 59–61.

Yajima, T., Hasegawa, T., Juni, K., Saneyoshi, M., Kawaguchi, T., 1996. Nasal absorption of 2′,3′-didehydro-3'-deoxythymidine (D4T) and its esters in rats. Biol. Pharm. Bull. 19, 1234–1237.