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The distribution of local anesthetics into the CSF following intranasal administration

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Abstract

The distribution of therapeutic agents into the CNS following nasal administration has been found to be highly compound dependent (Kumar et al., 1979, Recent Advances in Reproduction and Regulation of Fertility, Elsevier, Amsterdam, pp. 49–56; Hussain et al., 1990, J. Pharm. Sci., 79, 771–772; Chou and Donovan, 1997, Biopharm. Drug Dispos., 18, 335–346). In order to gain additional insight into the chemical specificity of transport between the nasal cavity and the CNS, a series of local anesthetics with similar chemical structures were used as model compounds to investigate drug disposition following intranasal administration. The selected local anesthetics were administered to male, Sprague–Dawley rats either intranasally or intra-arterially. Drug concentrations were determined from CSF and plasma samples collected from the cisterna magna and femoral artery, respectively. The plasma levels achieved after intranasal administration were comparable to those measured after the intra-arterial administration of an equivalent dose for three of the four compounds studied. Procaine, the compound with the lowest distribution coefficient, showed much lower plasma concentrations following intranasal administration. The relative bioavailability of procaine obtained following nasal administration was approximately 43% compared to 100% for the other selected local anesthetics. The ratios of the AUC_{CSF} values obtained after nasal administration to those obtained after parenteral administration were found to be: tetracaine > bupivacaine > lidocaine > procaine. This rank order was well correlated to the distribution coefficients of the local anesthetics. Also of note, the AUC_{CSF}/AUC_{plasma} ratios of local anesthetics containing ester functionalities were much higher than for those containing amide linkages regardless of the route of administration. While the distribution of these compounds into the CSF followed the expected pattern based on a partitioning model, the time courses of drug concentrations within the CSF differed significantly depending both on the compound and the route of administration.

Keywords: CSF; Nasal administration; Local anesthetics; Blood–brain barrier; CNS disposition

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1. Introduction

The barrier for entry of certain substances into the central nervous system (CNS) following intranasal delivery appears to be less restrictive as compared with other routes of delivery. Various substances with diverse physicochemical properties and molecular weights, such as neuroanatomical markers (Shipley, 1985; Baker and Spencer, 1986), inorganic metal ions (Czerniawska, 1970; Gopinath et al., 1978; Hastings and James, 1991; Evans and Hastings, 1992), viruses (Eseri and Tomlinson, 1984; McLean et al., 1987), and some therapeutic agents (Kumar et al., 1974a,b, 1979, 1982; Sakane et al., 1991a,b; Frey et al., 1995; Gizurarson et al., 1996; Chou and Donovan, 1997) have been shown to be transported into the brain from the nasal cavity, apparently bypassing the blood brain barrier (BBB). The olfactory epithelium of the nasal membrane is considered to be the most likely portal for substances penetrating into the CNS from the nasal cavity. The dendrites of the olfactory neuroepithelial cells project through the nasal membrane where they directly contact the external environment. Substances may be taken up by the olfactory dendrites and transported along the olfactory nerve through the cribriform plate into the brain. For example, Hastings and James (1991) instilled cadmium, a heavy metal which is normally excluded from the CNS by the BBB, into one nostril of the rat and found that the cadmium levels were significantly elevated in the ipsilateral olfactory bulb but not in the contralateral olfactory bulb or forebrain area. These observations strongly support the theory of transport along the olfactory pathway.

It has also been demonstrated that the direct pathway from the nasal cavity into the CNS is quite selective. Kumar et al. (1979) reported that peak drug levels in the CSF after intranasal administration of progesterone were not only much higher than those obtained after intramuscular administration, the t_{max} in the CSF was also significantly shorter than that in the serum. In contrast, the t_{max} for norethisterone (NET) did not precede the serum t_{max} following nasal spray administration. The elimination of NET from the CSF was also significantly slower than that of progesterone. Hydroxyzine, a piperazine-derivative antihistamine, showed preferential absorption into the CSF after being instilled into the nasal cavity while chlorcyclizine, another member of the piperazine family, could only be detected in the plasma, not in the CSF (Chou and Donovan, 1997). The dissimilar distribution patterns between the structurally similar compounds indicate that there is significant selectivity based on chemical properties or structure for transport between the nasal cavity and the CSF.

A correlation between the extent of transport into the CSF from the nasal membrane and the compound's partition coefficient has been found for some sulfonamides (Sakane et al., 1991b). These results suggest that the lipophilicity of drug molecules may play an important role for some compounds in determining distribution patterns from the nasal cavity.

The hydrochloride salts of four local anesthetics: procaine, tetracaine, bupivacaine, and lidocaine were studied in an attempt to further characterize the transport of drug compounds from the nasal cavity to the CSF. The local anesthetics utilized in these studies are structurally similar, but their physicochemical properties change dramatically with increasing length of the alkyl side chains or with substitution by various functionalities. The distribution coefficient values for local anesthetics are approximately two orders of magnitude greater than those for the sulfonamides and one order of magnitude greater than those for the antihistamines studied previously. Therefore, studying the distribution of local anesthetics into the CSF following nasal administration may lend some insight as to whether the correlation of the distribution into the CSF from the nasal cavity with lipophilicity holds over a wide range, or is useful only for relatively hydrophilic or hydrophobic compounds. In addition, intranasal lidocaine has been shown to be useful for migraine treatment (Maizels et al., 1996). Characterization of the distribution of this compound, and the other local anesthetics, may provide supportive evidence for the mechanism of action of this therapy or may assist in the identification of other useful anti-migraine compounds for clinical investigation.

2. Materials and methods

2.1. Chemicals

Procaine hydrochloride, tetracaine hydrochloride, bupivacaine hydrochloride and prilocaine hydrochloride were purchased from Sigma (St. Louis, MO). Lidocaine hydrochloride was purchased from Pfaltz and Bauer (Stamford, CT). HPLC grade acetonitrile was obtained from EM Science (Gibbstown, NJ). Sodium hydroxide and 1-decanesulfonic acid were also purchased from Sigma. Potassium phosphate (monobasic) was obtained from Fischer (Fairlawn, NJ). Phosphoric acid, sulfuric acid and anhydrous ethyl ether were obtained from Mallinckrodt (Paris, KY). All chemicals and solvents were used without further purification.

2.2. Animal preparation

The animal experiments adhered to the 'Principles of Laboratory Animal Care' (NIH publication # 85-23, revised 1985) and were approved by the University of Iowa Committee on the Use and Care of Animals. Male Sprague–Dawley rats (350–400 g) were anesthetized with an intramuscular dose of 50% (w/v) urethane (1.5 g/kg). Urethane has shown no inhibitory effect on either retrograde and anterograde axoplasmic transport, thus it appears superior to other anesthetics for use in these studies (Rogers et al., 1980). Full surgical anesthesia was obtained by a supplementary dose of a mixture of ketamine (47 mg/kg) and xylazine (7 mg/kg). The femoral arteries of the anesthetized animals were cannulated with heparinized PE-50 tubing to allow for drug administration and blood collection. To insure that all drug absorption took place via the nasal mucosa, the nasal cavity was isolated from the respiratory and gastrointestinal tracts using a modification of the method of Hussain et al. (1980). The animal was then moved to a stereotaxic frame. The cisterna magna was cannulated to collect CSF samples. Due to the extremely low total volume of CSF in the rat, the volume of CSF withdrawn with each sample was replaced by an infusion of artificial CSF into the lateral ventricle (Chou and Donovan, 1997). A 500 μl volume of blood and a 50 μl volume of CSF were obtained at each collection interval. The infusion rate of artificial CSF into the lateral ventricle was varied according to the sample collection frequency so that CSF samples could be obtained over the entire experimental period. Blood volumes were replaced with 500 μl of heparinized (10 U/ml) Ringer's solution.

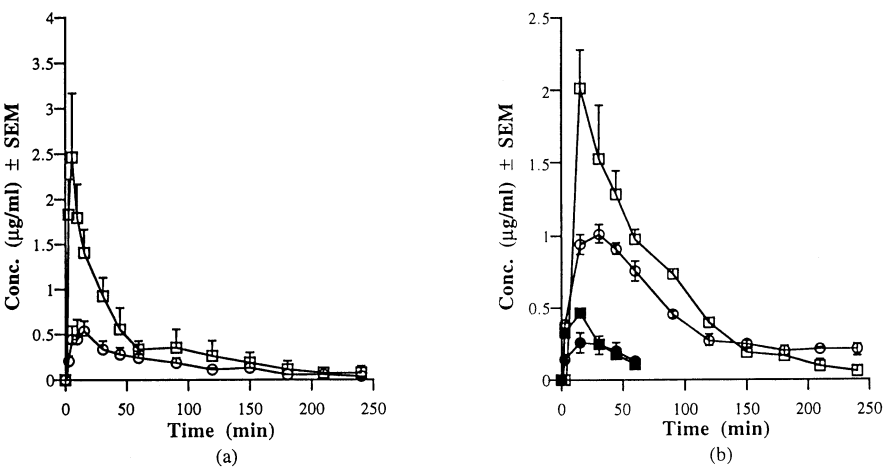

Fig. 1. Procaine concentrations in (a) plasma and (b) CSF following intra-arterial (□, ■) and nasal (○, ●) administration. (open symbol: 17.14 mg/kg, closed symbol: 2.86 mg/kg). Data represent the mean ± SEM ($n = 3, 4$).

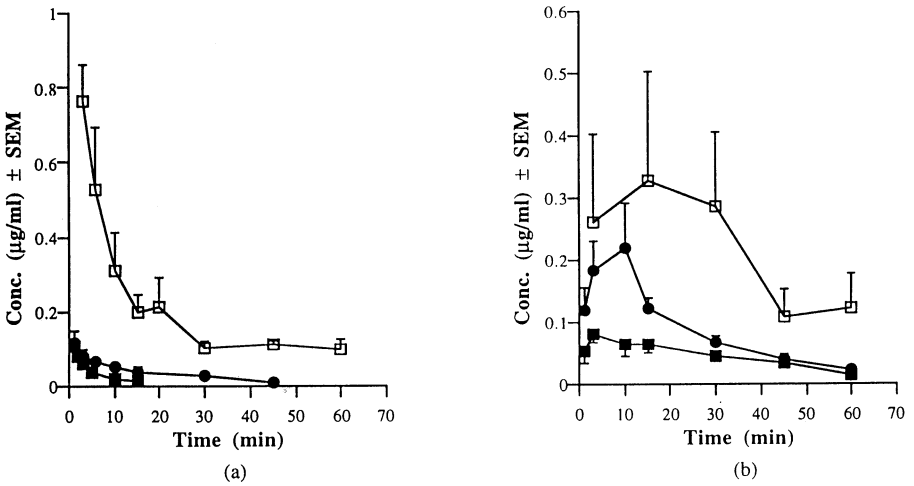

Fig. 2. Tetracaine concentrations in (a) plasma and (b) CSF following intra-arterial (□, ■) and nasal (○, ●) administration. (open symbol: 17.14 mg/kg, closed symbol: 2.86 mg/kg. Data represent the mean ± SEM ($n = 3–5$).

2.2.1. Nasal absorption studies

All drug solutions were prepared in pH 6.8, 0.1 M Sørensen's phosphate buffer. A volume (143 μl/kg) of drug solution was placed into each nostril by carefully inserting a length of PE-10 tubing attached to a volumetric syringe. The doses for each of the local anesthetics were: procaine hydrochloride, 2.9 or 17.1 mg/kg; tetracaine hydrochloride, 2.9 or 17.1 mg/kg; bupivacaine hydrochloride, 2.3 mg/kg; and lidocaine hydrochloride, 2.9 or 17.1 mg/kg. These doses were selected based on the LD_{50} of each compound and the analytical sensitivities of the HPLC assays used to measure drug in the biological samples.

2.2.2. Intra-arterial administration

For intra-arterial administration, doses equivalent to those administered intranasally were administered via the femoral artery.

2.3. Analytical procedures

CSF samples were stored at −20°C immediately after collection without further treatment. Blood samples were centrifuged at 7000 rpm, and the plasma (100 μl) was decanted and stored at −20°C. CSF and plasma samples were thawed prior to HPLC analysis. CSF samples were analyzed directly without further treatment. Each plasma sample was extracted using a modification of the method of Klein et al. (1994). A 100 μl volume of plasma along with 10 μl of internal standard were placed into a 5 ml conical centrifuge tube. 100 ml of 2 M NaOH were added to the tube, vortexed briefly, and then 5 ml of anhydrous ethyl ether were added. The mixture was vortex-mixed for 1 min and centrifuged at 1000 × *g* for 5 min. The ether phase was transferred to a clean 5 ml conical centrifuge tube followed by the addition of 250 μl of 0.0125 M H_2SO_4. The sample was shaken for 1 min and once again centrifuged at 1000 × *g* for 5 min. An aliquot of the aqueous layer was injected onto an HPLC system consisting of a Spectra Physics SP8700 ternary solvent delivery system, an SPD-6A UV spectrophotometric detector, and a CR-4A Chromatopac integrator (Shimadzu, Kyoto, Japan). A WISP 710B autosampler (Waters Chromatography, Milford, MA) was also used with this HPLC system.

Procaine was measured using an Intersil ODS-2 column (MetaChem, Torrance, CA), with a mobile phase of 5% acetonitrile in acidified water (pH 3.0) containing 0.03 M KH_2PO_4 (linear response range: 0.1–2.0 μg/ml) (Nakazono et al., 1991). For lidocaine, a μBondapak® C18 column (Waters Chromatography, Milford, MA) was

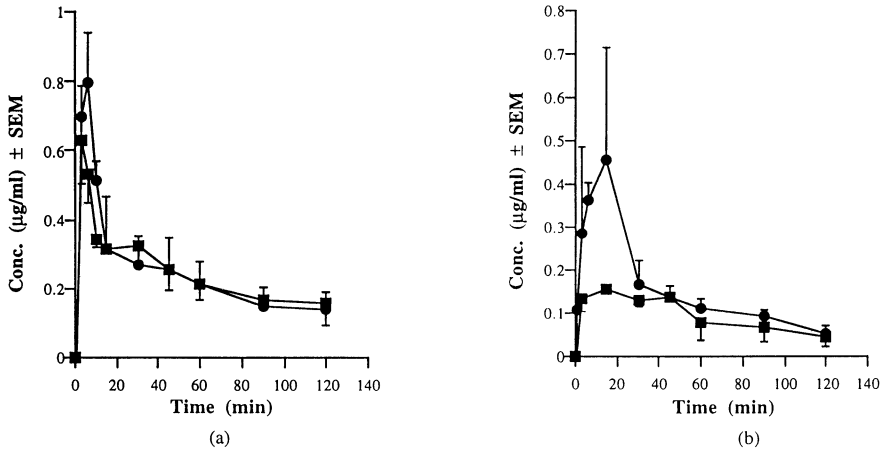

Fig. 3. Bupivacaine concentrations in (a) plasma and (b) CSF following intra-arterial (■) and nasal (●) administration (dose: 2.29 mg/kg). Data represent the mean ± SEM ($n = 3$).

used, with a mobile phase of 25% acetonitrile in acidified water (pH 3.0) containing 3 mM decanesulfonic acid as an ion-pairing agent (linear response range: 0.05–3.0 μg/ml). The separations for tetracaine and bupivacaine were performed with a Protein and Peptide C18 column (The Separation Group, Hesperia, CA) using a mobile phase of 18% acetonitrile in acidified water (pH 3.0) containing 0.03 M KH_2PO_4 (tetracaine linear response range: 0.05–1.2 μg/ml, bupivacaine linear response range: 0.2–1.6 μg/ml). The UV wavelength for detection was 210 nm for local anesthetics with amide functionalities (bupivacaine and lidocaine) and 310 nm for those containing ester linkages (procaine and tetracaine). Lidocaine was used as the internal standard for procaine while prilocaine was used for lidocaine. Tetracaine and bupivacaine were used as the internal standards for each other, respectively.

2.4. Data analysis

Results from the HPLC analyses were plotted as drug concentration in the CSF or plasma versus time. The AUC values for each curve were calculated from time zero to the last data point using the linear trapezoidal rule. Statistical differences between nasal and parenteral treatment were determined using the Student's t-test with $p = 0.05$.

3. Results

Drug concentration profiles in the plasma and CSF following intra-arterial or intranasal administration are shown in Figs. 1–4. The data represent the average ± SEM of the concentration measured. The disposition profiles for local anesthetics containing ester functionalities differ from those containing amide linkages, regardless of the route of administration. The disappearance of ester-linked local anesthetics from blood was much faster than that of the amide-linked local anesthetics.

Of the compounds studied, procaine showed the slowest absorption into the systemic circulation and the CSF following intranasal administration. The relative bioavailability of procaine obtained following nasal administration was approximately 43% while bioavailabilities of nearly 100% were measured for the other three compounds. The t_{max} values for procaine in the plasma and CSF were 15 and 15–30 min after nasal administration, respectively (Fig. 1). Procaine concentrations in the CSF were found to be much higher than those measured in plasma following nasal administration, although the peak level achieved in the CSF did not precede that achieved in plasma. Measurable concentrations of procaine in plasma could not be detected in the animals that received the 2.86 mg/kg dose via

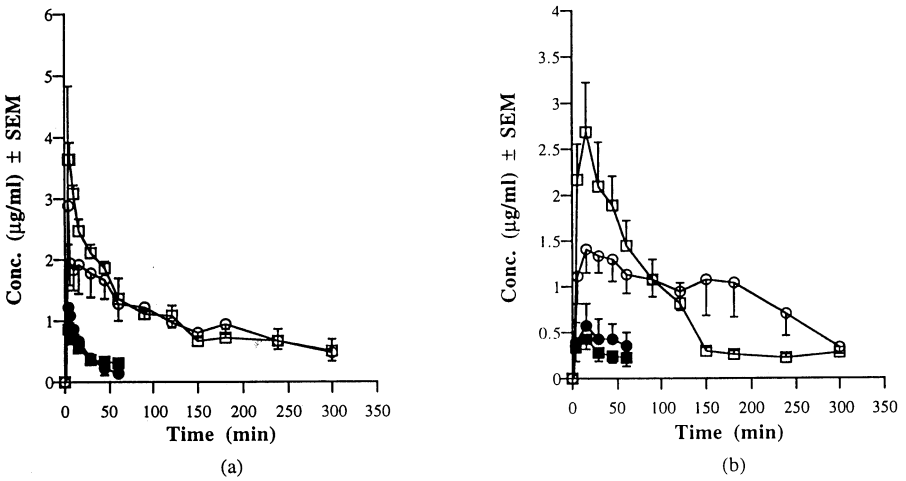

Fig. 4. Lidocaine concentrations in (a) plasma and (b) CSF following intra-artial (□, ■) and nasal (○, ●) administration. (open symbol: 17.14 mg/kg, closed symbol: 2.86 mg/kg). Data represent the mean ± SEM ($n = 3, 4$).

either route. In the CSF, concentrations found after nasal administration did not surpass those measured following intra-arterial administration.

In comparison to the extended t_{max} of procaine, the peak concentrations of tetracaine and bupivacaine in plasma were achieved within 3 min following nasal administration. Tetracaine and bupivacaine levels in the plasma, after intranasal administration, were comparable to those obtained after intra-arterial administration, while the CSF levels after nasal administration were significantly higher than those obtained after intra-arterial administration (Figs. 2 and 3). The plasma levels obtained in the rats receiving the 2.86 mg/kg dose of tetracaine decreased far more rapidly than those obtained in the rats receiving a higher dose of drug. In addition, animals receiving the high dose of tetracaine via the nasal cavity survived for no longer than 2 min following administration. Tetracaine concentrations in the CSF obtained at 1 min following nasal administration in these animals were found to be significantly higher than those achieved after intra-arterial administration.

The C_{max} of lidocaine in the CSF following intranasal administration did not exceed that achieved after intra-arterial administration. The $AUC_{CSF,\,IN}/AUC_{CSF,\,IA}$ ratio for lidocaine was found to be greater than one (Table 1), however; likely due to the slower elimination rate of lidocaine from the CSF following nasal delivery. It can be seen that the distribution of lidocaine into the CSF and its elimination from the CSF after nasal administration were slower than after parenteral administration (Fig. 4).

The ratios of AUC_{CSF} obtained after nasal administration of the lower dose of each of the local anesthetics to those obtained after parenteral administration were found to be: tetracaine > bupivacaine > lidocaine > procaine. Procaine was the only compound whose ratio was less than one. Another ratio, AUC_{CSF}/AUC_{plasma}, indicated that the local anesthetics containing ester linkages were present in the CSF for much longer times than in plasma. Procaine and tetracaine are subject to pseudocholinesterase activity present in the plasma. While the plasma levels observed in the rats receiving the lower dose of procaine dropped rapidly, those receiving the higher doses dropped gradually, likely due to saturation of the enzymes at the higher dose. As a result, procaine and tetracaine concentrations in the CSF were not directly proportional to systemic drug concentrations, regardless of route of administration. It is likely that lower esterase concentrations exist in the CSF as compared to the blood, thus resulting in a slower elimination of susceptible compounds from this compartment. Nakazono et al. (1991)

Table 1
AUC values for local anesthetics following nasal (IN) and intra-arterial (IA) administration

Compound	Dose (mg/kg)	AUC[a] (μg min/ml) (plasma)		AUC (μg min/ml) (CSF)		Ratio of AUC (CSF/plasma)		Ratio of AUC (IN/IA)	
		IA	IN	IA	IN	IA	IN	Plasma	CSF
Procaine	17.1	96.0 (33.7)	40.8 (9.2)	127.0 (13.7)	96.5 (5.1)	1.3	2.4	0.43	0.76
Procaine	2.9	ND	ND	15.6 (0.3)	12.1 (2.9)	—	—	—	0.78
Tetracaine	17.1	10.9 (1.9)	—	13.2 (5.6)	—	—	—	—	—
Tetracaine	2.9	0.9[b] (0.1)	0.9[b] (0.1)	2.7[c] (0.4)	5.3[c] (0.9)	—	—	1.00	1.96
Bupivacaine	2.3	29.2 (2.6)	28.9 (7.4)	11.5 (2.3)	19.6 (4.3)	0.39	0.68	0.99	1.69
Lidocaine	17.1	311 (25.7)	317 (32.5)	202 (17.4)	312 (93.0)	0.65	0.98	1.02	1.54
Lidocaine	2.9	28.4 (2.6)	29.7 (9.2)	17.6 (2.4)	25.8 (5.9)	0.62	0.87	1.05	1.47

[a] AUC was calculated from 0 to 3–5 h for higher dose and 0–1 h for lower dose. Values in parentheses represent ± SEM, ND: not detectable.
[b] 0–15 min.
[c] 0–60 min.

also demonstrated that the ester-type local anesthetics distributed into the brain following bolus intravenous administration irrespective of their short half-lives in plasma. They found that the ratios of brain concentrations of ethyl *p*-aminobenzoate (benzocaine) and *n*-butyl *p*-aminobenzoate to the plasma concentrations achieved after IV bolus ranged from 1 to 3.5.

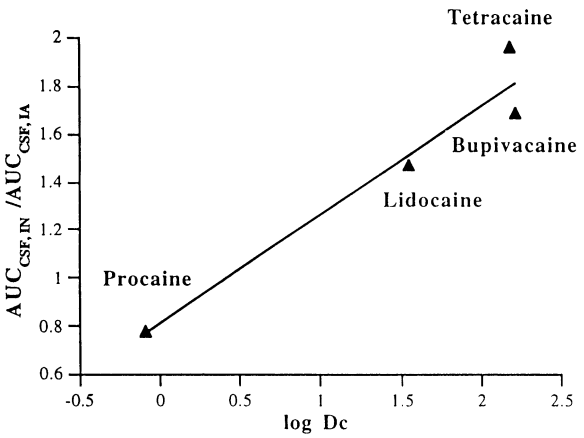

Fig. 5. Relationship between $AUC_{IN/IA}$ ratio and distribution coefficient of local anesthetics.

4. Discussion

Two transport pathways via the nasal olfactory epithelium into the CSF have been proposed by previous investigators (Gopinath et al., 1978; Jackson et al., 1979). Substances can be transported either through the extension of subarachnoid space surrounding the olfactory bundle or by axoplasmic flow in the olfactory nerve. These transport processes can be characterized by three distinct mechanisms: passive diffusion, carrier mediated transport, or bulk flow. Of these, carrier mediated transport shows the greatest dependency on the specific characteristics of the compounds while passive diffusion is most directly dependent upon general physicochemical parameters such as molecular weight, chemical structure, surface charge, and lipophilicity. Sakane et al. (1991b) demonstrated that the transport of sulfonamides, relatively hydrophilic compounds, from the nasal cavity into the CSF was dependent on their lipophilicity; the extent of their absorption into the CSF could be predicted by the pH-partition theory (Sakane et al., 1994). These observations suggest that the nasal cavity to CSF transport pathways for relatively small, hydrophilic compounds are dominated by passive diffusion. Anatomical connections also exist between the nasal cavity, subarachnoid spaces, and lymphatic systems (Bradbury and Westrop, 1983). Since

Table 2
Physicochemical parameters of selected local anesthetics

Compound	M_w	Solubility (mg/ml)	Log DC[a]	pK_a
Procaine HCl	272.8	1000	−0.092	8.89
Tetracaine HCl	300.8	133	2.18	8.38
Bupivacaine HCl	324.9	40	2.22	8.10
Lidocaine HCl	270.5	680	1.55	7.77

[a] Octanol/standard aqueous medium (150 mM NaCl, 5 mM 2-(*N*-morpholino) ethanesulfonic acid, 5 mM morpholinopropane sulfonic acid, and 5 mM (3-cyclohexylamino)-propane sulfonic acid in water), pH = 6.8 (Strichartz et al., 1990).

larger molecules and more lipophilic compounds tend to show preferential absorption into the lymph, distribution patterns of hydrophilic compounds following nasal administration may differ from those of lipophilic compounds. Upon investigation, however, the rank order of AUC_{CSF} ratios obtained after nasal administration to those obtained after parenteral administration were also found to be well correlated to the distribution coefficients (log DC) of the local anesthetics (Fig. 5, Table 2), compounds significantly more lipophilic than the sulfonamides.

The correlation between the AUC_{CSF} ratios and the distribution coefficients observed in these and previous studies provide supportive evidence that the transport pathway of certain drugs through the nasal membrane into the CSF differs from the transport pathway into the CSF following parenteral administration. For example, when a high dose of tetracaine was administered via the nasal cavity, the previously anesthetized animals did not survive as compared to those who received an equivalent dose of drug via the femoral artery which did. The CSF levels of tetracaine achieved after nasal administration were remarkably higher than either the plasma or the CSF levels achieved after intra-arterial administration. These findings provide additional evidence for the existence of transport pathways from nasal cavity to the CSF, this time for moderately lipophilic compounds, which bypass the vascular compartment and thus are capable of circumventing the BBB.

More detailed analysis of the kinetics of the local anesthetics in the plasma and CSF showed that the elimination rate of lidocaine from the CSF after nasal delivery was slower than after parenteral administration. This may be the consequence of a dynamic equilibrium between several biological compartments or the existence of a slow continuing absorption process from the nasal cavity. Again, these observations may also suggest that other pathways between the nasal cavity and the CNS exist in addition to passive diffusion and partitioning from the blood. The results of these studies are consistent with the proposals of Sakane et al. (1991b, 1994) and Frey et al. (1995) that the observed preferential absorption of some compounds from the nasal cavity into the CSF is through a non-axonal pathway with passive diffusion the most likely transport mechanism. This provides additional supportive evidence that the anatomical connections which exist between the nasal cavity and the CSF, and ultimately the CNS, afford some compounds the ability to access the CNS without the need to be transported from the systemic circulation across the BBB.

Rats were used as an animal model to evaluate the feasibility of using the nasal route for CNS targeting due to their previous use in nasal absorption studies and the ease with which the necessary surgical procedures could be accomplished. However, there are anatomical differences between the rat and human that may need to be taken into consideration when interpreting the results. The olfactory and respiratory epithelia of the rat, and many other common animal models, are interspersed throughout the entire nasal mucosa. In humans, in contrast, the olfactory epithelium is present only at the roof of the nasal cavity. Therefore, preferential CSF absorption following nasal delivery may be more pronounced in rats. To achieve comparable effects in humans, the use of delivery devices that can target drugs to the olfactory region may be necessary.

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