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Transport of hydroxyzine and triprolidine across bovine olfactory mucosa: Role of passive diffusion in the direct nose-to-brain uptake of small molecules

Karunya K. Kandimalla [1], Maureen D. Donovan *

College of Pharmacy, The University of Iowa, Iowa City, IA 52242, USA

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Abstract

Hydroxyzine and triprolidine have both been reported to reach the CNS following nasal administration. The objective of this study was to investigate their in vitro permeation across bovine olfactory mucosa in order to further characterize the biological and physicochemical parameters that influence direct nose-to-brain transport. In vitro experiments were conducted using Sweetana-Grass (Navicyte®) vertical diffusion cells to evaluate the effect of directionality, donor concentration and pH on the permeation of hydroxyzine and triprolidine across excised bovine olfactory mucosa. These studies demonstrated that the $J_{m\text{-}s}$ (mucosal–submucosal flux) and $J_{s\text{-}m}$ (submucosal–mucosal flux) of hydroxyzine and triprolidine across the olfactory mucosa were linearly dependent upon the donor concentration without any evidence of saturable transport. Hydroxyzine inhibited the efflux of P-gp substrates like etoposide and chlorpheniramine across the olfactory mucosa. Both hydroxyzine and triprolidine reduced the net flux ($J_{s\text{-}m} - J_{m\text{-}s}$) of etoposide with IC_{50} values of 39.2 and 130.6 μM, respectively. The lipophilicty of these compounds, coupled with their ability to inhibit P-gp, enable them to freely permeate across the olfactory mucosa. Despite the presence of a number of protective barriers such as efflux transporters and metabolizing enzymes in the olfactory system, lipophilic compounds such as hydroxyzine and triprolidine can access the CNS primarily by passive diffusion when administered via the nasal cavity.

Keywords: Olfactory mucosa; P-glycoprotein; Efflux; Membrane transport proteins; CNS; Diffusion; Nasal absorption; Etoposide; Hydroxyzine; Triprolidine

1. Introduction

Direct nose to brain delivery of therapeutic and diagnostic agents has gained considerable attention in recent years. Several tracer studies have indicated that the cranial subarachnoid space, lymphatics of the

* Corresponding author. Tel.: +1 319 335 9697; fax: +1 319 335 9349.
E-mail address: maureen-donovan@uiowa.edu (M.D. Donovan).
[1] Present address: College of Pharmacy and Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, USA.

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olfactory submucosa, and olfactory perineuronal space are connected by open pathways which allow for the direct nose to brain transport of solutes (Erlich et al., 1986; Kida et al., 1993) However, the mechanisms of drug transport through these pathways have not been clearly elucidated. There is clear evidence that some solutes gain access to the CNS following nasal administration, while other physicochemically similar solutes are restricted from entering. Chou and Donovan (1997) reported that both hydroxyzine and triprolidine were detected in the cerebrospinal fluid (CSF) after intra-arterial and nasal administration. The t_{max} for hydroxyzine in the CSF following nasal administration was very short (5 min) and the ratio of AUC values (nasal administration/intra-arterial administration) in the CSF was 4, suggesting that hydroxyzine had preferential distribution into the CSF following intranasal delivery. In comparison, the t_{max} of triprolidine in CSF following intranasal administration was 30 min, which coincided with the t_{max} following intra-arterial administration. The ratio of AUC values (nasal administration/intra-arterial administration) in the CSF for triprolidine was 0.56, indicating that triprolidine did not preferentially distribute into the CSF following nasal administration. Hydroxyzine and triprolidine are chemically similar to chlorpheniramine and chlorcyclizine (Table 1), compounds which have been reported to be restricted from entering the CSF following nasal administration by efflux transporters such as P-gp (Kandimalla and Donovan, 2005b). Cetirizine, an active metabolite of hydroxyzine, also has limited CNS distribution as demonstrated by its very low brain concentration following intravenous administration (Chen et al., 2003). While structurally similar (Table 1), cetirizine and hydroxyzine differ significantly in their lipophilicities; the log $D_{oct/pH\ 7.4}$ value for cetirizine is 1.09 while for hydroxyzine the value is 2.87 (Polli et al., 2003). Additionally, cetirizine has been shown to be a P-gp substrate, and the low brain distribution of cetirizine has been attributed to P-gp mediated efflux at the blood–brain barrier (Chen et al., 2003; Polli et al., 2003).

These examples demonstrate that both the physicochemical properties of the drug and other factors, including transporter affinity, play a significant role in determining the net distribution of a drug. This study focuses on identifying the key factors that are responsible for preferential nose-to-brain transport by studying the flux of hydroxyzine and triprolidine across excised olfactory mucosal tissues. The interaction between p-glycoprotein and these drug molecules was also investigated to determine whether active efflux of either compound was responsible for the CNS disposition patterns observed.

2. Experimental

2.1. Animal tissues

Bovine olfactory mucosa was obtained from the Roehrkasse Meat Co. (Williamsburg, IA), Ruzicka's Meat Processing (Solon, IA) or Bud's Custom Meats (Riverside, IA). Within 15 min after the animals were decapitated, longitudinal incisions along the lateral walls of the nasal cavity and a vertical incision along the ocular plane were made to expose the olfactory regions of the bovine nasal cavity. Olfactory turbinates located on the roof of the nasal cavity were removed (Popesko, 1984). The excised tissues were rinsed thoroughly with Krebs–Ringer buffer (KRB) and transported in fresh KRB maintained on ice. Studies were conducted within 4 h of the procurement time. The mucosal tissues were determined to be viable after the transport studies using either a Live-and-Dead cell assay (LIVE/DEAD® viability/cytotoxicity kit, Molecular Probes, Eugene, OR), Measuring the flux of a well-characterized paracellular marker (Lucifer yellow) across the mucosal tissue after the completion of transport experiments, or measuring the electrical resistance across the mucosal membrane to validate tissue integrity (EVOM, World Precision Instruments, Sarasota, FL).

2.2. Chemicals and reagents

Chlorpheniramine maleate, diethyl amine, etoposide, heptanesulfonic acid, hydroxyzine·2HCl, triprolidine HCl and dimethyl sulfoxide (DMSO) were obtained from the Sigma Chemical Co. (St. Louis, MO). Krebs–Ringer buffer (KRB) salts, HPLC solvents and other reagents were obtained from Fisher Scientific (Chicago, IL). KRB was prepared by adding 0.5 mM $MgCl_2$, 4.56 mM KCl, 119 mM NaCl, 0.7 mM Na_2HPO_4, 1.3 mM NaH_2PO_4, 10 mM D-glucose, 2.5 mM $CaCl_2$ and 15 mM $NaHCO_3$ to deionized water.

Table 1
Structure and selected physicochemical properties of antihistamine compounds

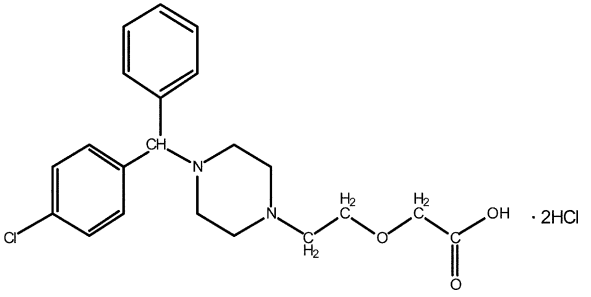
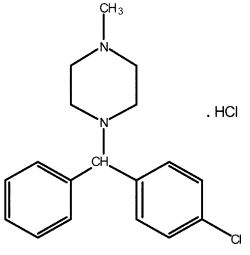
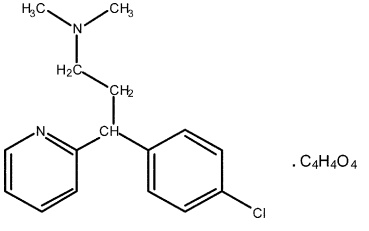
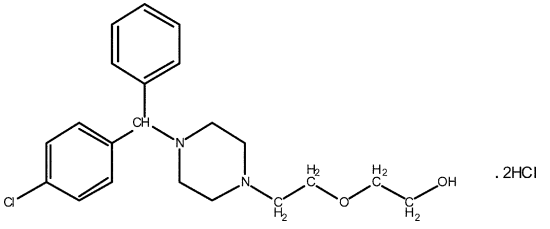
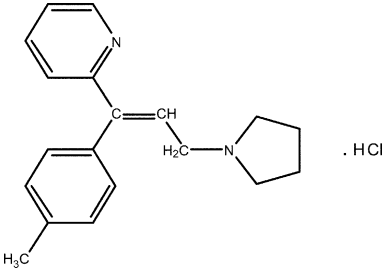
Compound	Chemical structure	Molecular weight	pK_{a1}; pK_{a2}; pK_{a3}	log *D*
Cetirizine·2HCl		314.9	2.2 (Newton et al., 1982); 2.9 (Newton et al., 1982); 8.0 (Newton et al., 1982)	1.09[a] (Polli et al., 2003)
Chlorcyclizine·HCl		337.3	2.1 (Newton et al., 1982); 7.6 (Newton et al., 1982)	2.91[b] (Chou and Donovan, 1997)
Chlorpheniramine maleate		390.9	4.0 (Higuchi and Kato, 1966); 7.2 (Perrin, 1965)	0.35[b] (Chou and Donovan, 1997)

Table 1 (*Continued*)

Compound	Chemical structure	Molecular weight	pK_{a1}; pK_{a2}; pK_{a3}	log *D*
Hydroxyzine·2HCl		447.8	1.9 (Newton et al., 1982); 7.4 (Newton et al., 1982)	2.37[b] (Chou and Donovan, 1997) 2.87[a] (Polli et al., 2003)
Triprolidine·HCl		314.9	3.6 (Benezra and Yang, 1979); 9.3 (Benezra and Yang, 1979)	1.98[b] (Chou and Donovan, 1997)

log *D* = log distribution coefficient.

[a] Distribution coefficient between octanol and water at pH 7.4.

[b] Distribution coefficient between chloroform and Sorensen's phosphate buffer (pH 6.8).

2.3. Solubility studies

Excess hydroxyzine·2HCl was added to a vial containing 2 ml of 0.2 M phosphate buffer. After the addition of the drug, the pH of the solutions was adjusted with 1 N HCl or 1 N NaOH to a pre-determined value ranging between 5.0 and 7.5. The suspensions were mixed continuously using a Labquake® shaker (Labindustries Inc., Berkeley, CA) for 48 h at room temperature. The contents were centrifuged at 3000 × *g* for about 10 min, and 1 ml of the supernatant was collected and filtered using Millex-HV (0.45 μm) filter units. The filtrate was appropriately diluted and analyzed by HPLC.

2.4. In vitro transport studies

The flux of various compounds across the bovine olfactory mucosa was determined using Sweetana-Grass (Navicyte®) (Harvard Apparatus, Holliston, MA) vertical diffusion cells. The mucosal tissue was mounted between the donor and the receiver compartments of the diffusion cells maintained at 37 °C by a recirculating water bath (Lauda RM6, Brinkmann Instruments Co., New York, NY). Prior to each experiment, the tissues were equilibrated for 30 min in either Krebs–Ringer bicarbonate buffer (KRB) or in an inhibitor solution (KRB + inhibitor). At the start of the experiment, KRB or the inhibitor solution was replaced with 1 ml each of donor (KRB + drug or KRB + drug + inhibitor) and fresh receiver solutions (KRB or KRB + inhibitor). Carbogen (95% O_2 + 5% CO_2) constantly bubbling (3–4 bubbles/s) through the donor and receiver solutions mixed and oxygenated the contents of the diffusion cells. Samples (200 μl) were removed from the receiver chamber at regular time intervals and analyzed for the quantity of permeant by HPLC. The volume removed was replaced with fresh, pre-warmed receiver solution.

2.5. Direction and concentration dependence of transport

To determine if the transport of hydroxyzine and triprolidine across the olfactory mucosa was carrier mediated, their fluxes were measured in both the mucosal–submucosal and submucosal–mucosal directions over concentration ranges of 0.27–17.8 mM for hydroxyzine and 0.2–25.4 mM for triprolidine. Mucosal–submucosal flux ($J_{m\text{-}s}$) was determined by mounting the bovine olfactory mucosa so that the mucosal surface of the tissue faced the donor phase, while in the experiments determining submucosal–mucosal flux ($J_{s\text{-}m}$), the submucosal surface faced the donor phase. At low donor concentrations [0.25–2.5 mM], the pH of the donor and receiver solutions was maintained at 7.0 ± 0.1 when studying hydroxyzine. However, due to lower solubility of hydroxyzine at higher pH values, the pH of the donor solutions was reduced to 6.3 ± 0.2 for higher donor concentrations [2.5–18 mM]. The pH of triprolidine donor and receiver solutions was maintained at 6.5 ± 0.2, a value comparable to that used for hydroxyzine.

2.6. Inhibition of transport studies

The activities of hydroxyzine and triprolidine as P-gp inhibitors were investigated using the P-gp substrates etoposide (212 μM) (Makhey et al., 1998) and chlorpheniramine (320 μM) (Kandimalla and Donovan, 2005b). In the case of etoposide, the donor and receiver solutions also contained 0.1% DMSO to increase solubility. This concentration of DMSO has been shown to have minimal effect on tissue permeability (Yang et al., 2000). Net etoposide transport ($J_{s\text{-}m} - J_{m\text{-}s}$) across the olfactory mucosa was measured in the presence of various concentrations of hydroxyzine and triprolidine ranging from 0.01 to 1000 μM. The pH of the donor solutions was maintained at 6.3 ± 0.2 when hydroxyzine was included and 6.5 ± 0.2 when triprolidine was present.

2.7. Effect of donor pH on transport

The effect of donor pH on the permeability of hydroxyzine and triprolidine across the bovine olfactory mucosa was determined in the m-s direction. The pH of the donor and receiver solutions was adjusted to a desired value, ranging between 5.0 and 7.5, using 1 N HCl or 1 N NaOH. The concentrations of hydroxyzine and triprolidine in the donor solutions were 2.2 and 3.2 mM, respectively. At the end of each experiment, the pH of the donor and the receiver compartments was measured and found to be within ±0.2 units of the initial value.

The pH of the donor and receiver solutions is customarily adjusted to a value which simulates physiologic conditions. However, Neuhoff et al. (2003) demonstrated that a pH gradient between the donor (~6.5) and receiver (~7.4) chambers may result in higher drug efflux ratios ($J_{s\text{-}m}/J_{m\text{-}s}$), which can falsely indicate active apical efflux. To avoid this potential artifact, the donor and receiver chambers were maintained at the same pH for all non-pH-gradient transport studies. However, experiments were conducted to study the effect of a pH gradient on the $J_{m\text{-}s}$ of hydroxyzine and triprolidine across the olfactory mucosa; the pH of the donor solutions was maintained at 6.2 ± 0.2 for hydroxyzine and 6.0 ± 0.2 for triprolidine, while the pH of the receiver solutions was maintained at 7.3 ± 0.1.

2.8. HPLC analysis

Chlorpheniramine, etoposide, hydroxyzine and triprolidine analyses were carried out on a HPLC system consisting of a Hitachi L-6200 Intelligent Pump and L-4000 UV detector (Hitachi, San Jose, CA), Shimadzu CR-501 Chromatopac integrator (Shimadzu, Columbia, MD) and a Waters 712 WISP auto-sampler (Waters Corp., Milford, MA). Chlorpheniramine separation was performed with a Luna C8 column (5 μm, 250 mm × 4.60 mm, Phenomenex, Torrance, CA) at 254 nm using a mobile phase composed of 45% methanol and 55% NaH_2PO_4 (0.05 M). The mobile phase pH was adjusted to 3 with H_3PO_4. Etoposide was detected at 230 nm using a Luna C8 column (5 μm, 250 mm × 4.60 mm, Phenomenex, Torrance, CA) with a mobile phase of 40% acetonitrile, 1% glacial acetic acid and 59% water. Hydroxyzine was measured using a Luna Phenyl-Hexyl column (5 μm, 250 mm × 4.60 mm, Phenomenex, Torrance, CA) with a mobile phase of 75% acetonitrile and 25% water containing 9 mM decanesulfonic acid as an ion-pairing agent (Chou and Donovan, 1997). The UV wavelength was set at 230 nm. The triprolidine assay was performed with a Luna C18 column (5 μm, 250 mm × 4.60 mm, Phenomenex, Torrance, CA) using a mobile phase of 65% methanol in water containing 0.2% diethyl amine (Chou and Donovan, 1997). The UV detector was set at a wavelength of 235 nm. The flow rate in each case was 1 ml/min.

2.9. Data analysis

The flux of various compounds across the olfactory mucosa in either the mucosal–submucosal ($J_{m\text{-}s}$) or submucosal–mucosal ($J_{s\text{-}m}$) direction was calculated using the following equation:

$$J_{m\text{-}s \text{ or } s\text{-}m} = \frac{dQ/dt}{A} \quad (1)$$

where dQ/dt is the slope of the steady state portion of the cumulative amount of drug transported versus time plot and A is the diffusional cross-sectional area of the mucosal membrane (0.64 cm^2).

The apparent permeabilities of the compounds in the mucosal–submucosal ($P_{m\text{-}s}$) and submucosal–mucosal ($P_{s\text{-}m}$) directions were calculated using the following equation:

$$P_{m\text{-}s \text{ or } s\text{-}m} = \frac{J_{m\text{-}s} \text{ or } J_{s\text{-}m}}{C_0} \quad (2)$$

where C_0 is the initial donor concentration.

One-way ANOVA followed by Tukey's multiple comparison testing was performed to compare the means. A value of $p < 0.05$ was considered statistically significant.

To determine the inhibitory effect of hydroxyzine and triprolidine on P-gp mediated efflux, the net flux ($NF = J_{s\text{-}m} - J_{m\text{-}s}$) of etoposide (212 μM) was plotted versus the log donor concentration ($\log C$) of hydroxyzine or triprolidine. Inhibitory parameters (IC_{50} and K_i) were calculated by fitting the following equations to the data using GraphPad Prism version 3.03 (GraphPad Software, San Diego, CA):

$$NF = NF_{min} + \frac{NF_{max} - NF_{min}}{1 + 10^{\log C - \log IC_{50}}} \quad (3)$$

where NF is the net flux of etopside across bovine olfactory mucosa (Motulsky and Christopoulos, 2003). NF_{max} is the maximum net flux of etoposide in the absence of the inhibitor. NF_{min} is the minimum net flux of etoposide at the highest inhibitor concentration.

K_i, which is a measure of the affinity of the inhibitor for the transporter, was calculated by:

$$K_i = \frac{IC_{50}}{(K_m + [\text{etoposide}])/K_m} \quad (4)$$

where K_m is the affinity constant of etoposide (Motulsky and Christopoulos, 2003). The K_m of etopo-

side efflux across the bovine olfactory mucosa has been previously calculated to be 260.5 μM (Kandimalla and Donovan, 2005a).

3. Results

3.1. Solubility

Hydroxyzine exhibited pH dependent solubility, most likely due to the limited solubility of its free base form (Table 2). The solubility of hydroxyzine at pH 6.1 was found to be 26.5 ± 1.7 mM, whereas the solubility decreased precipitously to 1.6 ± 0.2 mM at pH 7.3. In contrast, the aqueous solubility of triprolidine was found to be greater than 50 mM at all pH values between 5.8 and 7.5 (Table 2).

3.2. Effect of donor pH on transport

Since the pH of the nasal mucosa and CSF are constantly buffered to pH values of 6.0 ± 0.5 (Washington et al., 2000) and 7.3 (Milhorat et al., 2004), respectively, it is likely that drugs will be exposed to a range of pH conditions following intranasal administration. In order to study the potential impact of the pH-dependent solubility on transport, the effect of donor pH on the m–s permeabilities of hydroxyzine and triprolidine across the olfactory mucosa was determined. The donor concentrations used for hydroxyzine and triprolidine were 2.2 and 3.2 mM, respectively. The permeability of hydroxyzine increased with increasing donor and receiver phase pH between 5.3 and 6.8, but decreased at pH ∼ 7.3 (Table 2). Changes in the donor pH over a similar range did not result in any statistically significant differences in the permeability of triprolidine (Table 2).

3.3. Effect of pH gradient on transport

The effect of a pH gradient between the donor and receiver chambers on the mucosal–submucosal permeability of hydroxyzine and triprolidine was determined. Triprolidine permeability was reduced by ∼66%, whereas no significant effect on the mucosal–submucosal permeability of hydroxyzine under the pH gradient conditions was observed (Table 3).

3.4. Concentration dependence and polarization of transport

The $J_{m\text{-}s}$ and $J_{s\text{-}m}$ of hydroxyzine across the olfactory mucosa at pH 7.0 ± 0.1, where the pH-dependent fluxes were near maximal, were linearly dependent upon the donor concentration (Fig. 1a). For high donor concentrations >2.5 mM, the pH was reduced to 6.3 ± 0.2 to enhance hydroxyzine solubility; a linear profile was observed for concentrations up to 18 mM (Fig. 1b). Moreover, the $J_{m\text{-}s}$ of hydroxyzine was not significantly different from the $J_{s\text{-}m}$ at any donor concentration tested (Fig. 1a and b). Triprolidine demonstrated transport behavior similar to hydroxyzine. The $J_{m\text{-}s}$ and $J_{s\text{-}m}$ of triprolidine were both linearly dependent on donor concentration, and the $J_{m\text{-}s}$ was not

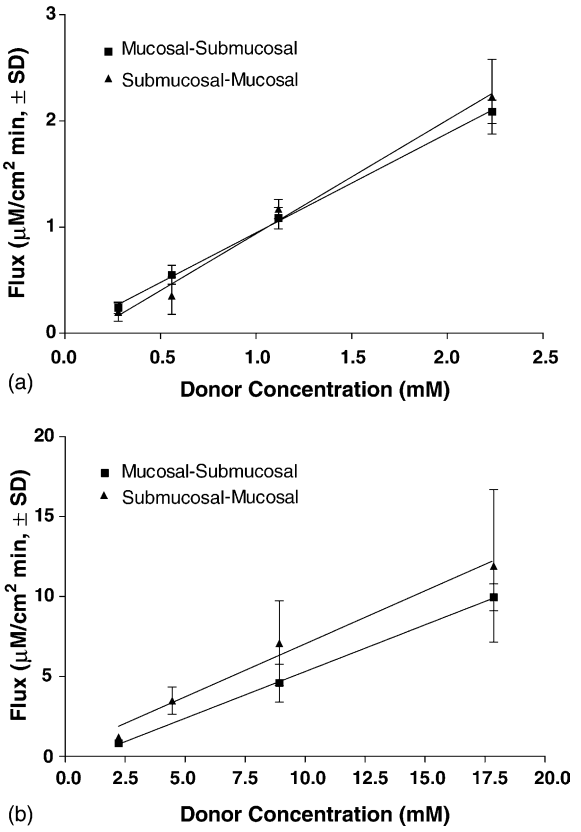


Fig. 1. Effect of donor concentration on the flux of hydroxyzine across bovine olfactory mucosa in either mucosal–submucosal or submucosal–mucosal directions at (a) pH 7.0 ± 0.1; $n = 3$; (b) pH 6.3 ± 0.2; $n = 3$–4.

Table 2
Effect of donor pH on the solubility and mucosal–submucosal flux of hydroxyzine and triprolidine

Compound	pH (±0.2)	Solubility (mM) $n = 3$	Permeability ($\times 10^6$ cm/s) $n = 3$–5[a]
Hydroxyzine·2HCl	5.3	–	9.0 (1.1)
	5.7	–	13.9 (4.4)
	6.1	26.5 (1.7)	13.4 (3.8)
	6.5	11.6 (0.3)[†]	–
	6.8	–	15.7 (2.3)[‡]
	7.1		15.6 (1.4)[‡]
	7.3	1.6 (0.2)[†,**]	12.4 (0.9)
Triprolidine·HCl	5.8	>50.0	11.3 (5.5)
	6.3	>50.0	12.2 (4.8)
	6.5	–	16.8 (5.0)
	7.5	>50.0	17.3 (3.4)

–, Values were not determined. Values in parentheses are S.D.
[a] Hydroxyzine and triprolidine flux values were determined at donor concentrations of 2.2 mM and 3.2 mM, respectively.
[**] Significantly lower than the solubility of hydroxyzine at pH 6.5 (ANOVA, $p < 0.001$).
[†] Significantly lower than the solubility of hydroxyzine at pH 6.1 (ANOVA, $p < 0.001$).
[‡] Significantly higher than the permeability of hydroxyzine at pH 5.3 (ANOVA, $p < 0.05$).

Table 3
Effect of pH gradient between mucosal and submucosal surfaces on the mucosal–submucosal transport of hydroxyzine and triprolidine

Compound	Donor pH (±0.2)	Receiver pH (±0.2)	Permeability ($\times 10^6$, cm/s)
Hydroxyzine·2HCl (2.2 mM)	6.1	6.1	13.4 (3.7[a])
	6.2	7.3	9.8 (6.0[a])
Triprolidine·HCl (3.2 mM)	6.5	6.5	16.8 (5.0[c])
	6.0	7.3	7.8 (1.5[b,@])

Values in parentheses are S.D.
[a] $n = 3$.
[b] $n = 6$.
[c] $n = 9$.
[@] Significantly different from the control (Student's t-test, $p < 0.05$).

statistically different from $J_{s\text{-}m}$ over the concentration range studied (Fig. 2).

3.5. Inhibition of efflux transport

In the presence of hydroxyzine, the $J_{m\text{-}s}$ of etoposide and chlorpheniramine across the olfactory mucosa increased and the $J_{s\text{-}m}$ decreased (Fig. 3). Without the inhibitor present, net flux ($J_{s\text{-}m} - J_{m\text{-}s}$), which is a measure of carrier mediated efflux, was found to be 0.032 ± 0.002 μM/(cm^2 min) for etoposide (212 mM) (Kandimalla and Donovan, 2005a) and 0.20 ± 0.04 μM/(cm^2 min) for chlorpheniramine (320 μM) (Kandimalla and Donovan, 2005b). The net flux of both compounds diminished significantly in the presence of 1 mM hydroxyzine. The inhibitory effects of various concentrations of hydroxyzine and triproli-

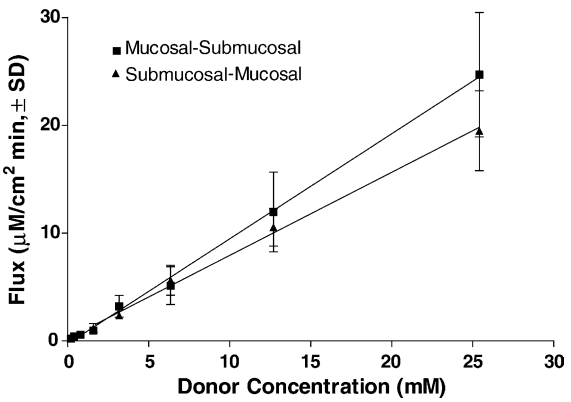


Fig. 2. Effect of donor concentration on the flux of triprolidine across bovine olfactory mucosa in either mucosal–submucosal or submucosal–mucosal directions at pH 6.5 ± 0.2; $n = 3$–6.

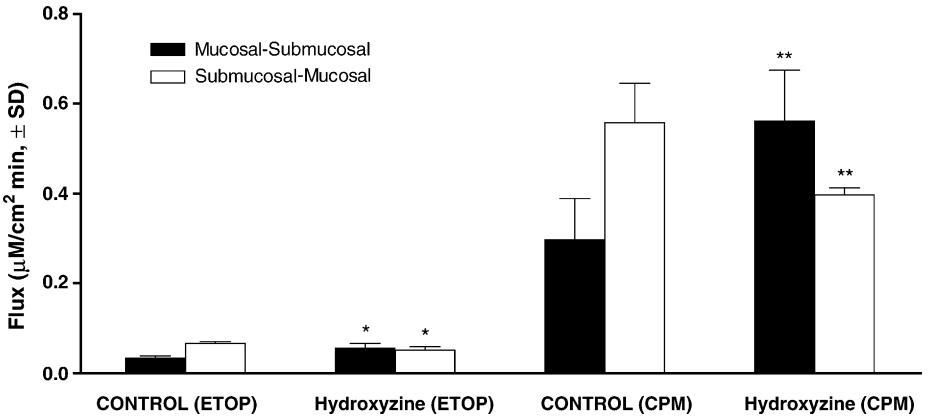


Fig. 3. Effect of hydroxyzine on the flux of etoposide and chlorpheniramine across bovine olfactory mucosa at pH 6.5 ± 0.2 (etoposide) and 7.1 ± 0.1 (chlorpheniramine) Donor phase contents: control (ETOP) = 212 μM etoposide; hydroxyzine (ETOP) = 1 mM hydroxyzine + 212 μM etoposide; control (CPM) = 300 μM chlorpheniramine, hydroxyzine (CPM) = 1 mM hydroxyzine + 300 μM chlorpheniramine. $^{*}J_{m\text{-}s}$ and $J_{s\text{-}m}$ are significantly different from $J_{m\text{-}s\ (control,\ ETOP)}$ and $J_{s\text{-}m\ (control,\ ETOP)}$, respectively. $^{**}J_{m\text{-}s}$ and $J_{s\text{-}m}$ are significantly different from $J_{m\text{-}s\ (control,\ CPM)}$ and $J_{s\text{-}m\ (control,\ CPM)}$, respectively; $n = 3$–7.

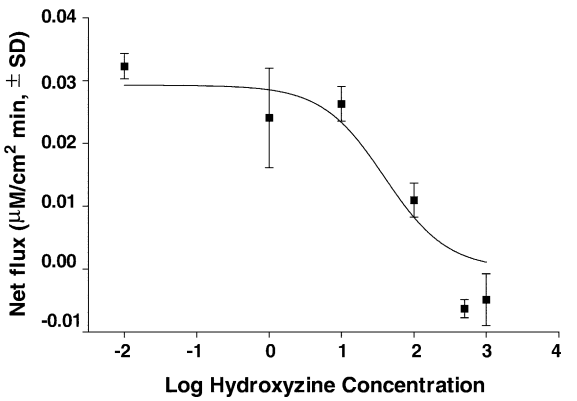


Fig. 4. Inhibitory effect of hydroxyzine (0.01–1000 μM) on the net flux of etoposide (212 μM) at pH 6.5 ± 0.2; $n = 3$–5.

dine on the net flux of etoposide across the olfactory mucosa were also determined (Figs. 4 and 5), and the IC_{50} values were estimated to be 109.3 μM for hydroxyzine and 145.2 μM for triprolidine. The K_i values for hydroxyzine and triprolidine were calculated to be 60.2 and 79.97 μM, respectively.

4. Discussion

The saturability of solute transport and the polarization of transport across a mucosal membrane have been employed as evidence for the existence of carrier-mediated solute transport. In vitro transport studies conducted with hydroxyzine and triprolidine did not demonstrate polarized flux or saturable submucosal–mucosal transport across the bovine olfactory mucosa, suggesting that the flux of these two compounds is predominantly driven by passive diffusion. These in vitro transport behaviors suggest that the previously reported nose-to-brain transport of hydroxyzine and triprolidine was likely the result of passive diffusion (Chou and Donovan, 1997). Similar passive transport of compounds from nose-to-brain has been

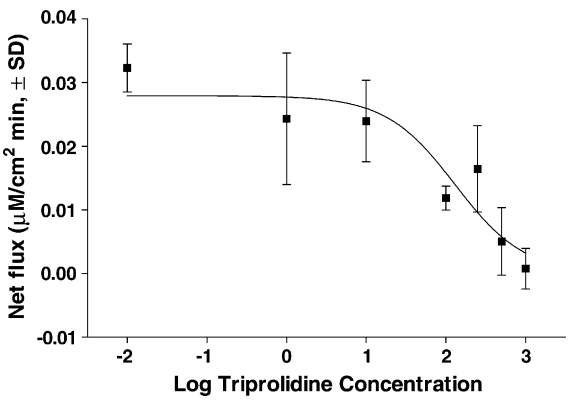


Fig. 5. Inhibitory effect of triprolidine (0.01–1000 μM) on the net flux of etoposide (212 μM) at pH 6.5 ± 0.2; $n = 3$–5.

previously described for lipophilic compounds such as progesterone and diazepam (Illum, 2000) and several sulfonamides (Sakane et al., 1991, 1994).

Hydroxyzine ($pK_{a2} = 7.4$) exhibits pH dependent changes in solubility in the pH ranges present within the nasal cavity, the blood and the CSF. The free base form of hydroxyzine is sparingly soluble in water, but the ionized form has reasonable solubility in lower pH environments. The mucosal to submucosal permeability increased with an increasing fraction of hydroxyzine free base in solution (pH 5.3–6.8) and decreased at pH 7.3, which correlates to the drastic decrease in the solubility of hydroxyzine between pH values 6.8 and 7.2. Interestingly, significant mucosal–submucosal permeability of hydroxyzine across the bovine olfactory mucosa was observed at pH 5.3 where 99% of the molecules exist in the ionized form. Studies conducted by Avdeef et al. (1998) have demonstrated that the ionized form of some drug molecules can partition across phosphotidylcholine bilayers, although to a lesser extent than the unionized forms. Additional evidence for the lipophilicity of cationic hydroxyzine was reported by Van Balen et al. (2001) who showed that the partition coefficient of cationic hydroxyzine ($\log PC = 2.8$) in a liposome/water system is almost as high as the partition coefficient of its neutral form ($\log PC = 3.4$).

As for triprolidine ($pK_{a2} = 9.3$), nearly all of the molecules are in the ionized form at physiological pH (pH 7.4). Hence, the solubility of the unionized species does not significantly affect the flux at pH values between 5.8 and 7.4. This was confirmed by the observation that triprolidine permeability across the bovine olfactory mucosa did not change significantly with changes in the donor pH.

Although both hydroxyzine and triprolidine appear to permeate across the olfactory mucosa by passive diffusion, they possess obvious differences in physicochemical properties (pK_a and solubility) and pharmacokinetic disposition patterns following intranasal delivery. These differences are also compounded by the presence of a pH gradient between the olfactory mucosa and CSF, to which the compounds respond differently. According to Neuhoff et al. (2003), the permeability ($P_{a\text{-}b}$) of weak bases, such as atenolol and metoprolol, is generally decreased in the presence of a pH gradient between the apical (lower pH) and basolateral (higher pH) surfaces, whereas the permeability of a neutral molecule like digoxin is unaffected. Similarly, the olfactory mucosal permeability of triprolidine ($pK_{a2} = 9.3$), which has a pK_a close to that of atenolol ($pK_a = 9.6$) (Neuhoff et al., 2003) and metoprolol ($pK_a = 9.7$) (Neuhoff et al., 2003) also decreased in the presence of a pH gradient between the olfactory mucosal and submucosal surfaces. However, the pH gradient had no significant effect on the permeability of hydroxyzine whose pK_{a2} (7.4) is close to the pH of the receiver solution (7.3 ± 0.1). At this pH, approximately 50% of the hydroxyzine molecules in solution remain unionized and in the donor compartment (pH 6.2 ± 0.2) <10% of the molecules are unionized. The higher permeability of the unionized species across the olfactory mucosa may have compensated for the decrease in the $P_{m\text{-}s}$ expected for hydroxyzine in the presence of a pH gradient. Assuming that these in vitro observations are predictive of in vivo disposition, the lower permeability of triprolidine in the presence of a pH gradient may account for the minimal CSF distribution and the longer t_{max} resulting from triprolidine entering the brain from the vascular compartment rather than via the olfactory pathway.

Carrier mediated efflux of hydroxyzine and triprolidine cannot be completely ruled out based on these transport studies, however. Lentz et al. (2000) demonstrated that the evaluation of P-gp-mediated efflux based on directional permeabilities is effective only when the compounds exhibit moderate transcellular permeability (e.g. cimetidine; $P_{a\text{-}b} = 1.71 \times 10^{-6}$ cm/s and $P_{b\text{-}a} = 3.54 \times 10^{-6}$ cm/s in Caco-2 cells). On the other hand, efflux transport was not distinguishable in the case of nicardipine (Herzog et al., 1993), a lipophilic compound with high trancellular permeability ($P_{a\text{-}b} = 19.8 \times 10^{-6}$ cm/s and $P_{b\text{-}a} = 24.1 \times 10^{-6}$ cm/s in Caco-2 cells). Both hydroxyzine and triprolidine are highly lipophilic with permeabilities similar to nicardipine ($P_{m\text{-}s} = 10\text{–}17 \times 10^{-6}$ cm/s) and significantly greater amounts of the drugs may permeate transcellularly compared to the amount effluxed by a carrier like P-gp. Hence, it is not surprising that polarized transport was not evident from the in vitro studies conducted with the olfactory mucosa.

Other investigators have also reported that hydroxyzine is an inhibitor for P-gp. Kan et al. (2001) demonstrated that etoposide efflux in the rat intestine decreased significantly in the presence of hydroxyzine (1 mM) and quinidine (1 mM) resulting in a

two- and four-fold increase in etoposide transport from the luminal side to the serosal side, respectively. The same concentration of hydroxyzine was also shown to be effective in diminishing the net flux of chlorpheniramine and etoposide across the bovine olfactory mucosa (Fig. 3). Both hydroxyzine and triprolidine were shown to inhibit etoposide efflux (Figs. 4 and 5) and, based on the IC_{50} and K_i-values determined from these studies, it can be concluded that hydroxyzine is a more potent inhibitor of the efflux transporter than triprolidine.

Etoposide is considered as a substrate of efflux transporters such as P-glycoprotein, multi-drug resistance-related protein (MRP1) and breast cancer-related protein (BCRP) (Hamilton et al., 2001; Allen et al., 2003). Kandimalla (2003) demonstrated that the efflux of etoposide was inhibited by quinidine, a mixed inhibitor of P-gp and MRP1 (Hamilton et al., 2001). Studies have indicated that the efflux of etoposide is mediated predominantly by P-gp rather than MRP1 (Hamilton et al., 2001) or BCRP (Allen et al., 2003). From these observations it can be inferred that the efflux of etoposide across bovine olfactory mucosa, which is effectively inhibited by hydroxyzine and triprolidine is most likely mediated by P-gp.

In conclusion, these transport studies demonstrate that lipophilic compounds like hydroxyzine and triprolidine can freely permeate across the olfactory mucosa. Despite the presence of a number of protective barriers such as efflux transporters and metabolizing enzymes in the olfactory system, these studies demonstrate the vulnerability of olfactory mucosa to xenobiotics. Compounds with appropriate physicochemical properties for good passive permeability can easily traverse the olfactory mucosa, access the CSF and potentially produce significant CNS effects.

References

Allen, J.D., Van Dort, S.C., Buitelaar, M., van Tellingen, O., Schinkel, A.H., 2003. Mouse breast cancer resistance protein (Bcrp1/Abcg2) mediates etoposide resistance and transport, but etoposide oral availability is limited primarily by P-glycoprotein. Cancer Res. 63, 1339–1344.

Avdeef, A., Box, K.J., Comer, J.E., Hibbert, C., Tam, K.Y., 1998. pH-metric logP10 determination of liposomal membrane-water partition coefficients of ionizable drugs. Pharm. Res. 15, 209–215.

Benezra, S.A., Yang, C.-H., 1979. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances. Academic, New York, pp. 509–528.

Chen, C., Hanson, E., Watson, J.W., Lee, J.S., 2003. P-Glycoprotein limits the brain penetration of nonsedating but not sedating H1-antagonists. Drug Metab. Dispos. 31, 312–318.

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

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.

Hamilton, K.O., Topp, E., Makagiansar, I., Siahaan, T., Yazdanian, M., Audus, K.L., 2001. Multidrug resistance-associated protein-1 functional activity in Calu-3 cells. J. Pharmacol. Exp. Ther. 298, 1199–1205.

Herzog, C.E., Tsokos, M., Bates, S.E., Fojo, A.T., 1993. Increased Mdr-1/P-glycoprotein expression after treatment of human colon-carcinoma cells with P-glycoprotein antagonists. J. Biol. Chem. 268, 2946–2952.

Higuchi, T., Kato, K., 1966. Ion-pair extraction of pharmaceutical amines II: extraction profile of chlorpheniramine. J. Pharm. Sci. 55, 1080–1084.

Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1–18.

Kan, W.M., Liu, Y.T., Hsiao, C.L., Shieh, C.Y., Kuo, J.H., Huang, J.D., Su, S.F., 2001. Effect of hydroxyzine on the transport of etoposide in rat small intestine. Anticancer Drugs 12, 267–273.

Kandimalla, K.K., Donovan, M.D., 2005a. Localization and differential activity of P-glycoprotein in the bovine olfactory and respiratory mucosae. Pharm. Res. 22, 1121–1128.

Kandimalla, K.K., Donovan, M.D., 2005b. Carrier mediated transport of chlorpheniramine and chlorcyclizine across bovine olfactory mucosa: implications on nose-to-brain transport. J. Pharm. Sci. 94, 613–624.

Kida, S., Pantazis, A., Weller, R.O., 1993. CSF drains directly from the subarachnoid space into nasal lymphatics in the rat. Anatomy, histology and immunological significance. Neuropathol. Appl. Neurobiol. 19, 480–488.

Lentz, K.A., Polli, J.W., Wring, S.A., Humphreys, J.E., Polli, J.E., 2000. Influence of passive permeability on apparent P-glycoprotein kinetics. Pharm. Res. 17, 1456–1460.

Makhey, V.D., Guo, A., Norris, D.A., Hu, P., Yan, J., Sinko, P.J., 1998. Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells. Pharm. Res. 15, 1160–1167.

Milhorat, T.H., Uchida, K., Yamada, M., Hayashi, T., Mine, Y., Kawase, T., 2004. Possible harmful effects on central nervous system cells in the use of physiological saline as an irrigant during neurosurgical procedures. Surg. Neurol. 62, 96–105.

Motulsky, H.J., Christopoulos, A., 2003. Fitting Models to Biological Data Using Linear and Nonlinear Regression: A Practical Guide to Curve Fitting. GraphPad Software Inc., San Diego.

Neuhoff, S., Ungell, A.-L., Zamora, I., Artursson, P., 2003. pH-dependent bidirectional transport of weakly basic drugs across Caco-2 monolayers: implications for drug–drug interactions. Pharm. Res. 20, 1141–1148.

Newton, D.W., Murray, W.J., Lovell, M.W., 1982. pK_a determination of benzhydrylpiperazine antihistamines in aqueous and aqueous methanol solutions. J. Pharm. Sci. 71, 1363–1366.

Perrin, D.D., 1965. Dissociation Constants of Organic Bases in Aqueous Solution. Butterworths, London.

Polli, J.W., Baughman, T.M., Humphreys, J.E., Jordan, K.H., Mote, A.L., Salisbury, J.A., Tippin, T.K., Serabjit-Singh, C.J., 2003. P-glycoprotein influences the brain concentrations of cetirizine (ZyrtecR), a second-generation non-sedating antihistamine. J. Pharm. Sci. 92, 2082–2089.

Popesko, P., 1984. Atlas of Topographical Anatomy of the Domestic Animals. W.B. Saunders, Philadelphia, p. 31.

Sakane, T., Akizuki, M., Yamashita, S., Nadai, T., Hashida, M., Sezaki, H., 1991. The transport of a drug to the cerebrospinal fluid directly from the nasal cavity: the relation to the lipophilicity of the drug. Chem. Pharm. Bull. 39, 2456–2458.

Sakane, T., Akizuki, M., Yamashita, S., Sezaki, H., Nadai, T., 1994. Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the dissociation of the drug. J. Pharm. Pharmacol. 46, 378–379.

Van Balen, G.P., Caron, G., Ermondi, G., Pagliara, A., Grandi, T., Bouchard, G., Fruttero, R., Carrupt, P.-A., Testa, B., 2001. Lipophilicity behavior of the zwitterionic antihistamine cetirizine in phosphatidylcholine liposomes/water systems. Pharm. Res. 18, 694–701.

Washington, N., Steele, R.J., Jackson, S.J., Bush, D., Mason, J., Gill, D.A., Pitt, K., Rawlins, D.A., 2000. Determination of baseline human nasal pH and the effect of intranasally administered buffers. Int. J. Pharm. 198, 139–146.

Yang, J.J., Kim, K.J., Lee, V.H., 2000. Role of P-glycoprotein in restricting propranolol transport in cultured rabbit conjunctival epithelial cell layers. Pharm. Res. 17, 533–538.