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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

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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

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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.