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Role of dopamine transporter (DAT) in dopamine transport across the nasal mucosa

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Abstract

Dopamine is a catecholamine neurotransmitter necessary for motor functions. Its deficiency has been observed in several neurological disorders, but replacement of endogenous dopamine via oral or parenteral delivery is limited by poor absorption, rapid metabolism and the inability of dopamine to cross the blood-brain barrier. The intranasal administration of dopamine, however, has resulted in improved central nervous system (CNS) bioavailability compared to that obtained following intravenous delivery. Portions of the nasal mucosa are innervated by olfactory neurons expressing dopamine transporter (DAT) which is responsible for the uptake of dopamine within the central nervous system. The objective of these studies was to study the role of DAT in dopamine transport across the bovine olfactory and nasal respiratory mucosa. Western blotting studies demonstrated the expression of DAT and immunohistochemistry revealed its epithelial and submucosal localization within the nasal mucosa. Bidirectional transport studies over a 0.1–1 mM dopamine concentration range were carried out in the mucosal–submucosal and submucosal–mucosal directions to quantify DAT activity, and additional transport studies investigating the ability of GBR 12909, a DAT inhibitor, to decrease dopamine transport were conducted. Dopamine transport in the mucosal–submucosal direction was saturable and was decreased in the presence of GBR 12909. These studies demonstrate the activity of DAT in the nasal mucosa and provide evidence that DAT-mediated dopamine uptake plays a role in the absorption and distribution of dopamine following intranasal administration.

Keywords: Dopamine transporter; Nasal absorption; Olfactory transport

Introduction

Dopamine is a catecholamine neurotransmitter associated with the coordination of motor functions and is important in the brain reward systems. Studies have shown that dopamine is transported centrally by a protein carrier called the dopamine transporter (DAT) (Chen et al., 2000; Chen and Reith, 2000) while it is transported by the organic cation transporter family (OCTs) in the periphery (Breidert et al., 1998).

DAT is a twelve transmembrane domain protein carrier responsible for transport of dopamine into neurons. It is responsible for the termination of dopamine activity by both the pre-synaptic and post-synaptic uptake of dopamine. DAT is a facilitative transporter, and the conditions controlling the direction of transport are still under active investigation (Chen et al., 2000, 2002; Chen and Reith, 2000; Appell et al., 2004). For each substrate molecule transported, DAT also co-transports two Na^+ and one Cl^- ions in the same direction as the substrate molecule (Surratt et al., 1994; Syringas et al., 2001).

Considerable efforts have been focused on determining the role of dopamine and DAT in Parkinson's disease. There is a degradation of dopaminergic neurons in Parkinson's disease resulting in a decrease in dopamine storage efficiency due to the decreased DAT population on the degraded dopaminergic neurons (Watanabe et al., 2005). Exogenously administered dopamine could produce temporary relief of symptoms, and while dopamine itself would be a useful drug to treat Parkinson's symptoms, several factors preclude its use. First, dopamine is subject to extensive hepatic metabolism when

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administered orally. Also, dopamine is almost completely ionized at physiological pH resulting in poor blood-brain barrier penetration.

Dopamine has shown enhanced uptake into the CNS following nasal administration (Anand Kumar et al., 1974; Dahlin et al., 2000, 2001). Olfactory neurons, which are known to express DAT (Revay et al., 1996), innervate the olfactory mucosa, and cerbrospinal fluid (CSF) from the brain circulates through the regions surrounding these neurons. Both the neurons and the CSF provide potential entryways into the brain from the nasal cavity while the ciliated epithelium forms a barrier limiting access to these pathways. It was the objective of these studies to investigate the presence of DAT in the nasal mucosa and determine its role in dopamine transport across the olfactory and nasal respiratory tissues. Bovine tissues were selected for investigation since the olfactory and nasal respiratory tissues are easily distinguishable, and numerous investigations of olfactory neuronal structure and function have been conducted using these tissues (Menco, 1980; Carr et al., 1998). Since the nasal respiratory mucosa does not contain olfactory neurons, the presence of DAT in this tissue was also investigated. DAT expression has been reported in other non-neuronal tissues including regions of the gastrointestinal system and the kidneys (Sharif et al., 1989; Surratt et al., 1994; Mezey et al., 1996, 1999), and the comparison of the olfactory and respiratory mucosa with respect to the role of DAT expression, location and role in dopamine transport was an important aim of these studies.

Materials and methods

Materials needed to prepare Krebs Ringers buffer (KRB), loading buffer, and transfer buffer along with Lucifer Yellow were obtained from Sigma–Aldrich Chemical Co (St. Louis, MO). KRB contained 0.49 mM $MgCl_2$, 4.56 mM KCl, 119.78 mM NaCl, 0.70 mM Na_2HPO_4, 1.5 mM NaH_2PO_4. H_2O, 9.99 mM dextrose, 2.52 mM $CaCl_2$. $2H_2O$ and 15.00 mM $NaHCO_3$. KRB-sodium metabisulfite solution contained KRB with 0.11 mM $Na_2S_2O_5$ to control the oxidation of dopamine. GBR 12909 (as the hydrochloride salt) was synthesized as described previously (Ironside et al., 2002).

Rabbit anti-rat DAT antibody (Alpha Diagnostics International Inc., San Antonio, TX) was used as a primary antibody for Western blotting and immunohistochemistry. The secondary antibody, horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody, was donated by Dr. Craig Morita, Department of Immunology, The University of Iowa.

Western blotting

Tissues were homogenized with KRB containing protease inhibitors (Protease Inhibitors Cocktail, #P8340, Sigma Diagnostics Inc, St. Louis, MO) and centrifuged at 200 ×*g* for 15 min (Sorvall, GMI Inc., Ramsey, MN). This and all subsequent centrifugation steps were conducted at 4 °C. The supernatant was spun at 600 ×*g* for 30 min and the resulting supernatant was centrifuged at 10,000 ×*g* for 45 min. The final supernatant was centrifuged at 100,000 ×*g* for 1 h. The pellet was solubilized by heating with loading buffer (4.5 g sodium dodecyl sulfate, 1.69 g Tris base, 15 g sucrose, 0.46 g dithiothreitol, 6 mg bromophenol blue and distilled water to 50 mL) at 100 °C for 10 min. This mixture was centrifuged for 10 min at 13,000 ×*g* and the supernatant was subject to 10% SDS-polyacrylamide gel electrophoresis (Biorad, Hercules, CA) followed by transfer to nitrocellulose membranes (Biorad, Hercules, CA). The blots were blocked with blocking buffer (5% w/v skimmed milk powder, 0.15% v/v Tween-20, 10 mM Tris base and 100 mM NaCl) and treated with the primary antibody against DAT (1:500) for 1 h. The blots were then washed with a washing buffer (0.15% v/v Tween-20, 10 mM Tris base and 100 mM NaCl) for 15 min and treated with the secondary antibody (1:20,000). The presence of antigen–antibody complex was detected using an enhanced chemiluminescence (ECL) kit from Pierce Biotechnology (Rockford, IL). Rat kidney extract (Santa Cruz Biotechnology, Santa Cruz, CA) was used as the positive control.

Microscopy

Bovine nasal tissues were cryo-frozen and 10 μm thick sections were cut and dried overnight at 4 °C. The sections were brought to room temperature and hydrated with phosphate buffered saline (PBS) for 2 min. The sections were washed with distilled water for 2 min followed by incubation with the primary antibody (1:2000) overnight. The sections were rinsed with PBS twice (5 min each) and then exposed to the secondary antibody (1:10,000) for 30 min followed by two 5 min rinses with PBS. The sections were treated with a diaminobenzidine and hydrogen peroxide mixture (Diaminobenzidine Kit, Vector Laboratories, Burlingame, CA) for ~5 min. The sections were rinsed with distilled water and treated with methyl green for 2 min. The counterstain was washed away using a 100% ethanol rinse for 1 min followed by two butanol rinses, each for 1 min. This was followed by two rinses with xylene (2 min each). The final sections were coverslipped and examined using an Olympus BX 50 microscope (Olympus Microscopes and Imaging Systems Inc., Melville, NY). Control sections were treated similarly using an irrelevant rabbit IgG to rule out any non-specific binding of the primary antibody.

Transport studies

Bovine tissues

The use of bovine olfactory and respiratory explants for drug transport and metabolism studies has previously been validated, and the presence and activity of transporters (e.g. *P*-glycoprotein) in these tissues has also recently been demonstrated. (Schmidt et al., 2000; Kandimalla and Donovan, 2005a,b,c) Bovine olfactory and respiratory explants were harvested from the nasal cavities of freshly sacrificed cows from local abattoirs (Roehrkassee Meat Lockers, Williamsburg, IA and Bud's Custom Meats Co, Riverside, IA) and transported to the lab on ice. Transport studies were carried out within 4 h of harvest. Mucosal explants of 2 cm^2 were cut and placed over the