# Uptake of Materials from the Nasal Cavity into the Blood and Brain

## Are We Finally Beginning to Understand These Processes at the Molecular Level?

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**Substances that enter the nasal cavity can access the bloodstream or central nervous system by processes including receptor cell uptake, transneuronal transport, and paracellular transport. Until recently, the molecular mechanisms by which agents move from the nasal cavity have not been described. Although the full complement of transporter proteins found in the nasal cavity has certainly not yet been identified, several recent observations have advanced this field substantially. We summarize here a representative sample of transporter proteins found in olfactory mucosa and/or nasal respiratory mucosa and the substrates that they transport into the brain and/or bloodstream.**

*Key words:* **ZIP8; ZIP14; DMT1; transporters; paracellular transport; olfactory; nasal; solute carriers**

## Background

Hediger *et al.* have described transporters as "the gatekeepers for all cells and organelles, controlling uptake and efflux of crucial compounds, such as sugars, amino acids, nucleotides, inorganic ions and drugs."[1] Certain transport proteins can also regulate the movement of ions from the cytoplasm into intracellular compartments.[2] From the many diverse molecules that enter the brain and/or bloodstream after intranasal exposure,[3,4] it is clear that toxic agents, such as metals, should also be considered in terms of the possible transport proteins that can facilitate their uptake.

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## Metal Transporters

### Solute Carrier Gene Superfamily

The solute carrier (SLC) genes include those encoding passive transporters, ion-coupled transporters, and exchangers.[1] To date, the SLC gene superfamily has 46 known families, with 360 protein-encoding genes. Of these, five families have divalent cation transport function. The SLC11 family mediates proton-coupled cation influx; the SLC30 family is characterized as cation diffusion facilitator effluxors; the SLC31 family mediates $Cu^{2+}$ influx; and the 14 members of the SLC39 family are responsible for the influx of several divalent metal ions, including $Zn^{2+}$, $Fe^{2+}$, and $Mn^{2+}$. Finally, the SLC41 family mediates $Mg^{2+}$ influx.[1,2,5] We briefly discuss SLC gene products described in nasal epithelia.

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## Divalent Metal Transporter 1

Both the olfactory and the trigeminal nerves contribute to the uptake of metals, such as $Cd^{2+}$ and $Mn^{2+}$, into the central nervous system (CNS) from the nasal cavity.[3,4,6,7] To date, the best-characterized metal transporter in the nasal cavity is divalent metal transporter 1 (DMT1; encoded by *SLC11A2*), which transports $Fe^{2+}$, $Mn^{2+}$, and other transition metals.[7] DMT1 has been localized by immunohistochemistry to supporting and basal cells in the olfactory mucosa.[8] A comprehensive understanding of the role of DMT1 in $Mn^{2+}$ transport has been made possible by use of a DMT1 mutant rat model, the Belgrade rat, together with the fact that *DMT1* is induced in response to anemia. Using these model systems to manipulate DMT1 levels led to the observations that DMT1 plays an important role in uptake of $Mn^{2+}$ into both the brain and the bloodstream from the nasal cavity, but it is less important in the uptake of $Mn^{2+}$ into the brain from the bloodstream.[8]

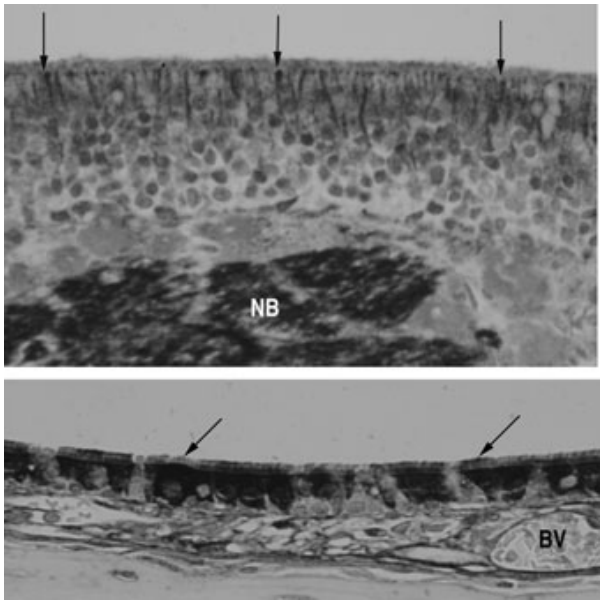


**Figure 1.** (**Top**) Photomicrograph of olfactory mucosa stained with anti-ZIP14 antibody, with the brownish–black reaction product representing sites of localization of ZIP14 in this tissue. Note staining in the subepithelial nerve bundles (NB) and in the apical dendrites of olfactory sensory neurons (arrows). Magnification, ×40. (**Bottom**) Photomicrograph of nasal respiratory epithelium, showing abundant ZIP14 immunoreactivity in the ciliated respiratory epithelial cells (slanted arrows). A subepithelial blood vessel (BV) is labeled at magnification ×40.

## ZIP8 and ZIP14

We have recently observed that the closely related proteins ZIP8 and ZIP14 (encoded by *SLC39A8* and *SLC39A14*, respectively) are expressed in both the nasal respiratory epithelium and the olfactory mucosa. Both ZIP8 and ZIP14 transport various divalent cations,[9–11] and ZIP8 has been characterized as a $Mn^{2+}$/bicarbonate symporter.[9] Both ZIP8 and ZIP14 are readily detectable in olfactory receptor neuron dendrites and nerve bundles of the olfactory mucosa, as well as in ciliated cells of the respiratory epithelium (Fig. 1). Thus, we propose that divalent metal ion uptake by ZIP8 and ZIP14 via the respiratory epithelium could contribute to metal deposition into the bloodstream, whereas uptake via olfactory receptor neurons is responsible for metal deposition in the brain. This proposal is supported by recent observations that transection of the olfactory nerve nearly abolishes $Mn^{2+}$ uptake from the nasal cavity and into the olfactory bulb.[7]

## ZnT1

The zinc transporter ZnT1 (encoded by *SLC30A1*) has been localized by immunohistochemistry to olfactory bulb glomeruli, including axons entering glomeruli from olfactory sensory neurons.[12] ZnT1 is proposed to reduce cellular toxicity due to high intracellular concentrations of $Zn^{2+}$ by mediating $Zn^{2+}$ efflux out of cells or into intracellular compartments.[2] This observation prompts the question of whether ZnT1 is responsible for $Zn^{2+}$ uptake into the brain or functions exclusively to regulate intracellular $Zn^{2+}$ concentrations.

## Other Transporters in the Olfactory Mucosa

### Organic Anion Transporters

*SLC22A20* encodes organic ion transporter 6 (OAT6), which has been localized to non-neuronal cells in the olfactory mucosa. OAT6 appears to transport small organic anions, such as estrone sulfate, that have previously been identified as odortype components in mouse urine.[13,14] *In vitro* studies demonstrate that accumulation of estrone sulfate mediated by mouse OAT6 was significantly stimulated by glutarate, indicating that OAT6 functions as an organic anion/dicarboxylate exchanger.[15] The role that OAT6 might play in olfaction is unclear; OAT6 might modulate the availability of odorant organic anions at the mucosal surface for presentation to olfactory neurons or facilitate delivery of odorants to a distal site of chemosensation.[13]

### Organic Cation Transporters

Dopamine is poorly bioavailable upon oral ingestion, but delivery to the CNS after intranasal administration is efficient. At least three dopamine transporters participate in this process: dopamine transporter (DAT; encoded by *SLC6A3*[16]) and organic cation transporter 1 (OCT1) and OCT2 (encoded by *SLC22A1* and *SLC22A2*, respectively). Use of high concentrations of inhibitors of these molecules still allows for some dopamine transport from the nasal cavity and into the brain, so additional dopamine transporters probably exist in nasal tissues.[17,18]

## Does All Transport from the Nasal Cavity to the Brain Require a Transporter?

The preceding discussion might suggest that one or more transporters are required for uptake of all molecules from the nasal cavity. However, highly lipophilic compounds, such as hydroxyzine and triprolidine, can access the CNS by diffusion across nasal epithelia, despite the complement of efflux transporters and metabolic enzymes in nasal epithelia.[19–21]

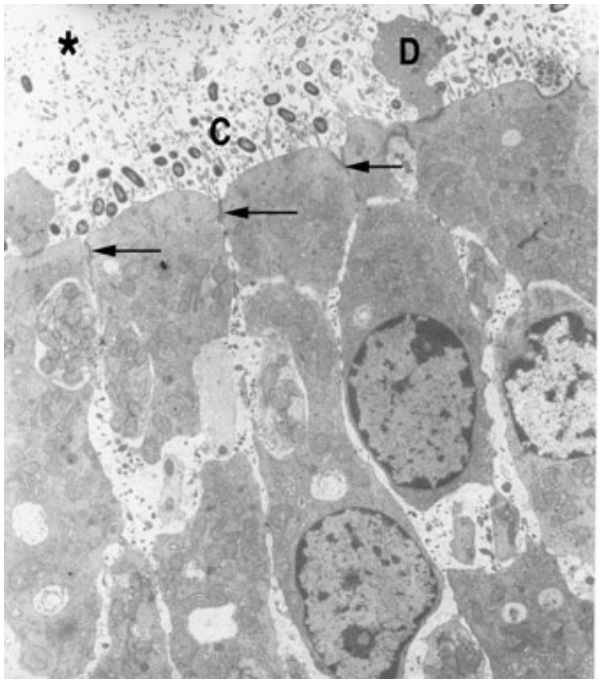


**Figure 2.** Transmission electron micrograph showing tight junctions (arrows) at the apical surfaces of olfactory epithelial cells. The nasal airway (*), an olfactory dendrite (D), and obliquely sectioned olfactory cilia (C) are also shown. Magnification, ×20,000.

## Paracellular Transport: The Next Frontier?

Tight junctions are critical barrier features in tissues throughout the body. In the olfactory epithelium, tight junctions are found at the apical surface of cells, adjacent to the nasal airways (Fig. 2). Disruption of tight junctions can allow entry of pathogenic bacteria and viruses into various tissues[22,23] by a process called paracellular transport. Studies with fluorescein isothiocyanate–labeled dextran beads suggest that the size cutoff for paracellular transport from the nasal cavity and into cerebrospinal fluid is approximately 20 kD.[24] Various strategies, including use of detergents or polycations to disrupt cell–cell connections, can enhance absorption of materials from the nasal cavity.

Detergents and related chemicals damage and irritate nasal epithelia, but polycations are safe for nasal epithelia.[25]

Olfactory epithelial tight junctions consist of multiple proteins, including zona occludens 1 (ZO-1), occludin, and claudin-5 (CLDN5).[26,27] Claudins are an important constituent of tight junctions, and expression of the approximately 20 different claudin proteins varies among body tissues. CLDN5 is also an important component of the blood–brain barrier, and *Cldn5*-deficient mice have abnormally permeable blood–brain barriers.[28] Several compounds can dysregulate CLDN5, including α-carrageenan,[29] cAMP,[30] and bradykinin.[31]

Another claudin protein that has been studied extensively is CLDN16 (also known as paracellin 1 [PCLN1]). PCLN1 contributes to divalent ion paracellular transport, specifically $Mg^{2+}$ resorption in the kidney.[32] Mutations in human *PCLN1* are associated with hypomagnesemia, hypercalciuria, and nephrocalcinosis,[33] and CLDN16 is downregulated by the immunosuppressive drug ciclosporin, causing $Mg^{2+}$ wasting in patients treated with this drug.[34] Because of our interest in divalent metal transport into the brain from the nasal cavity, we asked whether CLDN16 was present in olfactory mucosal tight junctions. Reverse transcription of mouse olfactory mucosal RNA, followed by polymerase chain reaction with mouse *Cldn16*-specific primers, did not detect *Cldn16* in olfactory mucosa, whereas a robust response was observed in the kidney (data not shown). Thus, as other investigators have observed, claudins are expressed in highly tissue-specific distributions, and CLDN5 therefore may eventually prove to be a highly relevant molecule for manipulation of olfactory mucosal paracellular transport.

## Summary

Our knowledge of transporters and barrier molecules in the olfactory mucosa has advanced greatly in recent years. These observations should make formulation of more drugs for intranasal delivery possible, either by taking advantage of newly identified transporters or by pharmacological manipulation of olfactory mucosal tight junctions to enhance drug delivery.[35] The latter could be important in improving the treatment of Parkinson's disease; certain intranasally administered compounds readily access deeper brain regions, such as the striatum,[36] a brain region profoundly affected in Parkinson's disease.

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### Conflicts of Interest

The authors declare no conflicts of interest.

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