Impact of Nicotine Transport across the Blood–Brain Barrier: Carrier-Mediated Transport of Nicotine and Interaction with Central Nervous System Drugs

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Nicotine, an addictive substance, is absorbed from the lungs following inhalation of tobacco smoke, and distributed to various tissues such as liver, brain, and retina. Recent in vivo and in vitro studies suggest the involvement of a carrier-mediated transport process in nicotine transport in the lung, liver, and inner blood–retinal barrier. In addition, in vivo studies of influx and efflux transport of nicotine across the blood–brain barrier (BBB) revealed that blood-to-brain influx transport of nicotine is more dominant than brain-to-blood efflux transport of nicotine. Uptake studies in TR-BBB13 cells, which are an in vitro model cell line of the BBB, suggest the involvement of H+/organic cation antiporter, which is distinct from typical organic cation transporters, in nicotine transport at the BBB. Moreover, inhibition studies in TR-BBB13 cells showed that nicotine uptake was significantly reduced by central nervous system (CNS) drugs, such as antidepressants, anti-Alzheimer’s disease drugs, and anti-Parkinson’s disease drugs, suggesting that the nicotine transport system can recognize these molecules. The cumulative evidence would be helpful to improve our understanding of smoking-CNS drug interaction for providing appropriate medication.

Key words nicotine; tissue distribution; transporter; blood–brain barrier (BBB); central nervous system (CNS) drug

1. INTRODUCTION

Tobacco smoke contains a number of chemical compounds, and produces negative health effects. Tobacco kills more than 6 million people worldwide each year.1) The number of smokers is predicted to remain more than 1 billion in 2025,2) indicating the importance of efforts to achieve smoking cessation and understanding of smoking–drug interaction for appropriate drug medication.

Nicotine, an addictive substance in tobacco, induces its neural effects through the nicotinic acetylcholine receptor (nAChR). In the brain, nicotine induces release of neurotransmitters such as dopamine, and activates the rewarding system, which is one reason that people continue to smoke. It has been reported that nicotine addiction is vulnerable to changes in nicotine pharmacokinetics,3) suggesting that their understanding would provide important information for smoking cessation therapy.

In addition to its undesired effects including addiction, nicotine exhibits some beneficial effects such as neuroprotective and anti-nociceptive effects.4,5) It has been demonstrated that nicotine improves working memory and enhances cognitive performance.6,7) Moreover, a number of studies have suggested that nicotine exerts potential as a therapeutic agent for various diseases including depression, attention-deficit/hyperactivity disorder, schizophrenia, Tourette’s syndrome, Parkinson’s disease (PD), and Alzheimer’s disease (AD).8–11) However, application of nicotine as a therapeutic agent is limited because of the poor pharmacokinetic properties such as a short half-life in plasma, and adverse reactions such as cardiovascular and addictive effects.

Although much is known about nicotine’s pharmacokinetics, the transport mechanism of this substance across biological membranes is not fully understood. This review presents an overview of recent findings on transport mechanism of nicotine across the plasma membrane and focuses on interaction of central nervous system (CNS) drugs with nicotine at the blood–brain barrier (BBB), which predominantly regulates cerebral distribution of drugs from the circulating blood.

2. TRANSPORT MECHANISM OF NICOTINE IN TISSUES

During inhalation of tobacco smoke, nicotine rapidly enters the circulating blood via pulmonary alveoli, and is distributed to various tissues in the body. Nicotine is a weak base compound having pKₐ of 3.12 and 8.02,12) and mainly exists in positive-charged form at physiological pH. Ionized form of nicotine is not easy to transport by passive diffusion across the plasma membrane, which consists of a lipid bilayer. Nicotine is largely
Table 1. Kinetic Parameters of Nicotine Transport in Several Tissue Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol/(min·mg protein))</th>
<th>$V_{max}/K_m$ (µL/(min·mg protein))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat isolated hepatocytes</td>
<td>141</td>
<td>1.78</td>
<td>12.6</td>
</tr>
<tr>
<td>TR-iBRB2 cells</td>
<td>492</td>
<td>35.3</td>
<td>71.7</td>
</tr>
<tr>
<td>TR-BBB13 cells</td>
<td>92.4</td>
<td>16.7</td>
<td>181</td>
</tr>
<tr>
<td>A549 cells</td>
<td>50.4</td>
<td>4.11</td>
<td>81.5</td>
</tr>
</tbody>
</table>

Table 2. Inhibitory Effect of Several Compounds on $[^3H]$Nicotine Uptake

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Percentage of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TR-BBB13 cells</td>
</tr>
<tr>
<td>Control</td>
<td>100±4</td>
</tr>
<tr>
<td>Nicotine</td>
<td>13.5±0.8**</td>
</tr>
<tr>
<td>Verapamil</td>
<td>3.58±0.58**</td>
</tr>
<tr>
<td>Amantadine</td>
<td>3.91±0.33**</td>
</tr>
<tr>
<td>Quinidine</td>
<td>4.03±0.71**</td>
</tr>
<tr>
<td>Propranolol</td>
<td>4.11±0.49**</td>
</tr>
<tr>
<td>Pyrillamine</td>
<td>4.21±0.34**</td>
</tr>
<tr>
<td>Clonidine</td>
<td>5.58±0.18**</td>
</tr>
<tr>
<td>Desipramine</td>
<td>N.D.</td>
</tr>
<tr>
<td>Timolol</td>
<td>N.D.</td>
</tr>
<tr>
<td>Thiamine</td>
<td>N.D.</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>N.D.</td>
</tr>
<tr>
<td>Proscoterol</td>
<td>N.D.</td>
</tr>
<tr>
<td>Serotonin</td>
<td>N.D.</td>
</tr>
<tr>
<td>TEA</td>
<td>101±14</td>
</tr>
<tr>
<td>Choline</td>
<td>103±4</td>
</tr>
<tr>
<td>MPP+</td>
<td>107±10</td>
</tr>
<tr>
<td>t-Carnitine</td>
<td>137±13</td>
</tr>
<tr>
<td>PAH</td>
<td>136±14</td>
</tr>
</tbody>
</table>

[1H]Nicotine uptake was performed at 37°C in the presence or absence (control) of inhibitors (1 ms). *p<0.05, **p<0.01, significantly different from control. TEA, tetramethylammonium; MPP+, 1-methyl-4-phenylpyridinium; PAH, p-aminobenzoic acid., N.D.; not determined. This table was prepared by reference to previous reports.26–28,35]

accumulated in several tissues such as lung, liver, and kidney rather than circulating blood,15) suggesting the involvement of the binding of nicotine to tissue proteins and/or active transport system(s) in tissue distribution of nicotine.

Nicotine has been reported as a substrate and/or inhibitor of organic cation transporters such as organic cation transporter family (OCTT1-3/SCL22A1-3), organic cation/carnitine transporter family (OCTN1-2/SCL22A4-5), multidrug and toxin extrusion protein 1 (MATE1/SLC47A1), and the plasma membrane monoamine transporter (PMAT/SCL29A4).16–23 Spector and Goldberg22) suggested the existence of an active transport system of nicotine in isolated rabbit choroid plexus. In addition, it has been reported that a carrier-mediated transport process contributes to nicotine uptake into pig kidney epithelial cell line (LLC-PK1), human choriocarcinoma cell line (JAR), and human intestinal epithelial cell line (Caco-2).23–25 Fukada et al.15) have also demonstrated that nicotine is greatly accumulated into rat kidney via the specific transport system. Recent studies have clarified that nicotine is transported via carrier-mediated transport processes in human alveolar epithelial cell line (A549) and isolated rat hepatocytes, and immortalized rat retinal capillary endothelial cell line (TR-iBRB2), which are in vitro models of the lung, liver, and inner blood–retinal barrier, respectively.26–28 The kinetic parameters of nicotine transport in these cells are displayed in Table 1. The $K_m$ values are similar among these cells (Table 1), and nicotine transport was inhibited by hydrophobic cationic drugs such as pyrilamine and verapamil, whereas typical substrates of the above organic cation transporters such as tetraethylammonium (TEA), 1-methyl-4-phenylpyridinium (MPP+), and t-carnitine had little effect on nicotine transport26–28 (Table 2). These findings suggest that a novel organic cation transporter, which has not yet been identified, may play a significant role in nicotine transport in the lung, liver, and inner blood–retinal barrier (Fig. 1). More recently, Takano et al.29,30) reported that a carrier-mediated transport system, which is sensitive to hydrophobic cationic compounds, may be involved in nicotine uptake by rat primary cultured alveolar epithelial cells and epithelial cancer cell line such as NCI-H441 and MCF-7. These lines of evidence clearly suggest the importance of specific transport system(s) on nicotine tissue disposition.

3. INFLUX AND EFFLUX TRANSPORT OF NICOTINE ACROSS THE BBB

It is well documented that nicotine is rapidly transported from blood to brain in animals because it is used as a cerebral blood flow marker.31–33 In humans, it has been reported that tobacco smoking causes a rapid raise of nicotine concentration in the brain, where the concentration reaches more than
50% of its maximum level within 15 s after a single puff from cigarettes. To enter the brain, it is considered that nicotine rapidly crosses the BBB, which is formed by tight-junction of brain capillary endothelial cells. We have performed kinetics studies of nicotine transport across the BBB. Brain-to-blood efflux clearance of nicotine in rats was calculated to be $137 \mu\text{L/(min·g brain)}$ by combined intracerebral microinjection technique and brain slice uptake.

Fig. 1. Schematic Diagram of Carrier-Mediated Transport of Nicotine in the Lung, Liver, Brain, and Retina

Nicotine is absorbed from alveolar epithelium of the lung, and distributes to the liver, brain, and retina. Recent studies suggest that carrier-mediated transport processes play important roles in nicotine transport in alveolar epithelial cells, brain capillary endothelial cells, and retinal capillary endothelial cells. CNS, central nervous system; BBB, blood–brain barrier; BRB, blood–retinal barrier. (Color figure can be accessed in the online version.)

Fig. 2. Carrier-Mediated Transport of Nicotine at the BBB

A, Comparison of the brain uptake index (BUI) relation in transporters and the lipophilicity trend line. The lipophilicity trend line indicates the correlation between the BUI and the logarithm of distribution coefficient between n-octanol and pH 7.4 buffer (log DC) of the compounds transported by passive diffusion. Open circles and triangles are substrates for SLC transporters and P-glycoprotein, respectively. B, $[^3\text{H}]$Nicotine uptake by TR-BBB13 cells showing concentration dependence ($K_m=92.4 \mu\text{M}$), suggesting the involvement of carrier-mediated transport in nicotine transport across the BBB. Figure 2A was prepared by reference to Hosoya K, Yamamoto A, Akanuma S, Tachikawa M. Lipophilicity and transporter influence on blood–retinal barrier permeability: a comparison with blood–brain barrier permeability. *Pharm. Res.*, **27**, 2715–2724 (2010). with permission from Springer, and Fig. 2B was adapted from Tega Y, Akanuma S, Kubo Y, Terasaki T, Hosoya K. Blood-to-brain influx transport of nicotine at the rat blood–brain barrier: Involvement of a pyrilamine-sensitive organic cation transport process. *Neurochem. Int.*, **62**, 173–181 (2013) with permission from Elsevier.
method.\textsuperscript{35} On the other hand, integration plot analysis after intravenous injection of \(^{[3}H\)nicotine showed that blood-to-brain influx clearance of nicotine in rats was 272 \(\mu\)L/(min·g brain), which is 2-fold greater than brain-to-blood efflux clearance.\textsuperscript{35} These results suggest the dominant influx transport of nicotine across the BBB, and the expression at the BBB of specific transport system(s) which involve asymmetrical transport of nicotine.

Figure 2A shows the relation between \textit{in vivo} brain permeability (brain uptake index; BUI) and lipophilicity (logarithm of distribution coefficient between \(n\)-octanol and pH 7.4 buffer: log DC) of compounds showing carrier-mediated transport. The lipophilicity trend line was obtained from the permeability of compounds undergoing passive diffusion. The nutrients and amino acid mimetic drugs such as D-glucose and l-3,4-dihydroxyphenylalanine (l-Dopa), which are substrates for glucose transporter type 1 (GLUT1/SLC2A1) and L-type amino acid transporter 1 (LAT1/SLC7A5), respectively, exhibited higher brain permeability than that predicted from the lipophilicity trend line\textsuperscript{36} (Fig. 2A). On the other hand, drugs including vincristine, verapamil, and digoxin, which are substrates for P-glycoprotein (P-gp/ABCB1), exhibited lower brain permeability than that predicted from the lipophilicity trend line\textsuperscript{36} (Fig. 2A). Since the log DC of nicotine has been reported 0.41,\textsuperscript{37} the BUI value of nicotine was calculated 34%. Carotid artery injection technique revealed that BUI value for typical P-gp substrates.\textsuperscript{36,40} In addition, it has been reported that nicotine transport was not changed between LLC-PK1 cells and P-gp stably expressed LLC-PK1 cells (LLC-GA5-COL150 cells).\textsuperscript{23} Furthermore, brain permeability of nicotine across the BBB was not different between wild-type and P-gp knock-out mice.\textsuperscript{41} These lines of evidence indicate that P-gp is not involved in nicotine transport at the BBB.

\[^{[3}H\)Nicotine uptake by TR-BBB13 cells, an \textit{in vitro} model cell line of the BBB,\textsuperscript{42} was time, temperature, and concentration dependent with a \(K_{\text{m}}\) of 92.4 \(\mu\)M\textsuperscript{35} (Fig. 2B), and the uptake clearance for the carrier-mediated transport process \(V_{\text{max}}/K_{\text{m}}\) in TR-BBB13 cells was greater than that of other cells (Table 1). The uptake was promoted by outward \(H^+\) gradient.\textsuperscript{39} In addition, hydrophobic cationic drugs, such as verapamil, pyrilamine, clonidine, propranolol, and quinidine, showed potent inhibitory effects whereas hydrophilic cationic compounds, which are substrates of organic cation transporters including OCTs, OCTNs, PMAT, and MATEs, such as TEA, MPP\(^+\), L-carnitine, and choline, had no inhibitory effects (Table 2). Pyrimethamine is known as a specific inhibitor of MATEs,\textsuperscript{43} which is an organic cation transporter driven by opposite \(H^+\) gradient.\textsuperscript{39} The IC\(_{50}\) value of pyrimethamine for human and mouse MATEs-mediated transports was reported 46–145 nM.\textsuperscript{49} In contrast, the IC\(_{50}\) value of pyrimethamine for nicotine transport in TR-BBB13 cells was 14.7 \(\mu\)M (Fig. 3A), which is more than 100 times greater than the reported value of human and mouse MATEs-mediated transport. These results suggest that nicotine transport system(s), which is sensitive to hydrophobic cationic drugs and distinct from typical organic cation transporters (OCTs, OCTNs, PMAT, and MATEs), plays an important role in blood-to-brain transport of nicotine across the BBB. Yamazaki \textit{et al.}\textsuperscript{44} reported that pyrilamine is transported to the brain across the BBB via carrier-mediated transport system. In addition, Okura \textit{et al.}\textsuperscript{45} revealed the involvement of novel \(H^+/\text{organic cation antipporter
in pyrilamine uptake by TR-BBB13 cells. Our study showed that pyrilamine exhibited competitive inhibition of [3H]-nicotine uptake with a $K_i$ of 15 $\mu$M (Fig. 3B), whose value was similar to the $K_m$ value in pyrilamine uptake (28 $\mu$M), suggesting that nicotine and pyrilamine share the same transport system, which is known as the H$^+$/organic cation antiporter.

4. INTERACTION OF CNS DRUGS ON NICOTINE TRANSPORT ACROSS THE BBB

It is known that smoking affects the pharmacokinetics of several CNS drugs. Since the BBB regulates the transport of compounds and drugs between the circulating blood and brain, it is conceivable that nicotine, which is uptaken by smoking, interacts with the transport system(s) at the BBB, and affects the distribution of the CNS drugs to the brain. Therefore we investigated the interaction of CNS drugs with nicotine transport system(s) in TR-BBB13 cells.

Nicotine uptake by TR-BBB13 cells was strongly inhibited in the presence of tricyclic and tetracyclic antidepressants such as desipramine, amitriptyline, imipramine, maprotiline, mianserin, and amoxapine by more than 90% (Fig. 4). In addition, selective serotonin reuptake inhibitors (SSRI), such as fluvoxamine and paroxetine, serotonin and noradrenaline reuptake inhibitors (SNRI) such as duloxetine, and noradrenergic and specific serotonergic antidepressant such as mirtazapine showed potent inhibitory effect whereas milnacipran, an SNRI, had little effect (Fig. 4A). Therapeutic drugs for AD and PD, such as donepezil, memantine, pramipexole, trihexyphenidyl, and amantadine, significantly inhibited nicotine uptake by 77–85% (Fig. 4B). Concentration-dependent inhibitory effects of drugs on [3H]nicotine uptake by TR-BBB13 cells are summarized in Table 3. The $IC_{50}$ values of tricyclic antidepressants, such as amitriptyline and imipramine, and anti-AD drugs, such as donepezil and memantine, were less than 10 $\mu$M. Other antidepressants, such as paroxetine, maprotiline, fluvoxamine, and desipramine, also exhibited concentration-dependent inhibitory effects on [3H]nicotine uptake with $IC_{50}$ values 13–33 $\mu$M. These results suggest that CNS drugs such as antidepressants, anti-AD drugs, and anti-PD drugs are recognized by the nicotine transport system. On the other hand, drugs for migraine headache, which have indole structure, such as sumatriptan, naratriptan, rizatriptan, and zolmitriptan, showed weak to moderate inhibitory effects (Fig. 4B), suggesting that the indole structure does not have high affinity for the nicotine transport system.

The therapeutic concentration of the above CNS drugs is almost under 1 $\mu$M, while these $IC_{50}$ values were over 5 $\mu$M. Considering this point, the interaction of these CNS drugs with nicotine transport system seems weak in clinical use. Other CNS drugs with high affinity to nicotine transport system could interact with nicotine transport at the BBB. Nicotine concentration in circulating blood is reported to increase up to about 300 nM during inhalation of tobacco smoke. Although the inhibitory effect of nicotine on CNS drugs transport at the BBB has not been assessed, it is possible that nicotine in the circulating blood, uptaken by smoking, affects the distribution...
of antidepressants and anti-AD/PD drugs. Kurosawa et al.\(^{51}\) reported that varenicline, which is a selective partial agonist of \(\alpha_4\beta_2\) nicotinic acetylcholine receptor and used for smoking cessation, is transported from the circulating blood to the brain via a carrier-mediated transport process at the BBB, and varenicline and nicotine share the same transport system at the BBB since varenicline transport is strongly inhibited by nicotine. These results suggest that varenicline has the potential to inhibit nicotine transport at the BBB as well as cerebral nicotinic acetylcholine receptor, and it is possibly related to the therapeutic effect of varenicline for nicotine addiction if varenicline inhibits nicotine transport at the BBB. In addition, it is possible that nicotine affects varenicline distribution to the brain via a carrier-mediated transport process at the BBB. Further investigation is necessary to reveal whether nicotine inhibits these CNS drugs transport at the BBB for understanding of smoking–drug interaction.

5. CONCLUSION

After nicotine was first isolated from the tobacco plant in the 19th century, a large number of pharmacological and pharmacokinetic studies of this stimulant have been performed. Recently, evidence has emerged to suggest the significant contribution of a carrier-mediated transport process in tissue distribution of nicotine. The present review suggests that the \(H^+/\)organic cation antipporter contributes to blood-to-brain transport of nicotine across the BBB, which interacts with several CNS drugs such as antidepressants and anti-AD/PD drugs. Detailed study of nicotine transport is expected to improve our understanding of smoking–drug interaction, and may lead to providing safe and effective medication for smokers.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES