The blood-brain barrier (BBB) is composed of several specialized elements which act together to regulate the internal milieu of the brain (Smith, 1989; Farrell and Risau, 1994; Davson and Segal, 1996) by controlling the exchange of compounds between two barrier structures—one located between the blood and brain extracellular fluid (ECF), termed the blood/brain barrier, and the second between the blood and cerebrospinal fluid (CSF), known as blood/CSF barrier. Diphenhydramine ([2-(diphenylmethoxy)-N,N-dimethylethylamine (DPHM)]) is a potent histamine H₁-receptor antagonist (Douglas, 1980) widely used for its antiallergic properties, as well as for its antiemetic, sedative, local anesthetic, and hypnotic effects (Runge et al., 1992; Ernst et al., 1993; Pontasch et al., 1993). Like other “first-generation” antihistamines, DPHM occupies central H₁-receptors to result in drowsiness, sedation, incoordination and with higher doses, convulsions, and death (Douglas, 1980; Nicholson, 1983; Koepf et al., 1987; Gengo et al., 1989). However, there are limited data on the CNS levels of the drug or on the mechanisms of transfer involved. Results from previous studies in rats, guinea pigs, and rabbits suggest that DPHM enters the brain tissue and CSF extremely rapidly to achieve CNS concentrations exceeding those in plasma (Glazko and Dill, 1949a,b; Takasato et al., 1984; Goldberg et al., 1987). Because only ~2.5% of DPHM (pKₐ ~9.0) is un-ionized at the physiological pH, the above results cannot be explained by the passive diffusion of this un-ionized form through the blood-brain and blood-CSF barriers. Moreover, there is evidence for saturable BBB transporter mechanisms for lipophilic, amine drugs (Partridge et al., 1973, 1984; Specter, 1988; Yamazaki et al., 1994a,b,c). This includes mephenyprazine, a histamine H₁-antagonist. The available data suggest that these compounds cross the blood-brain and blood-CSF barriers by both simple diffusion of the un-ionized lipid-soluble form and by carrier-mediated transport of the ionized form (Partridge et al., 1984; Goldberg et al., 1987; Yamazaki et al., 1994a). In addition, there is evidence that various substances can inhibit the actions of this transport process. In vivo (rat carotid injection technique), brain uptake of mephenyprazine is inhibited by DPHM (Yamazaki et al., 1994a). Further-
more, both in vivo and in vitro (bovine brain capillary endothelial cells) studies demonstrate that propanolol (PRN) inhibits mepramine uptake (Yamazaki et al., 1994; Yamazaki et al., 1994). Together, these data led us to hypothesize that PRN could inhibit brain (and perhaps CSF) DPHM uptake. The purpose of our studies then was to use in vivo microdialysis (MD) in chronically instrumented adult ewes to investigate the transport processes of DPHM across the adult ovine blood-brain-barrier using two different experiments. The first involved stepped infusions of DPHM at five different dosing rates to assess blood-brain CSF and blood-brain ECF drug concentration relationships in relation to variations in drug dose and hence plasma drug levels. The second involved coadministration of DPHM and PRN to examine whether PRN alters blood-brain CSF and blood-brain ECF DPHM relationships.

**Materials and Methods**

**Animals and Surgical Preparation.** A total of six nonpregnant Dorset Suffolk cross-bred ewes were used in these studies. All studies were approved by the University of British Columbia Animal Care Committee, and the procedures performed on sheep conformed to the guidelines of the Canadian Council on Animal Care. The sheep were between 2 to 4 years old with a body weight of 74.6 ± 22.0 kg (mean ± S.D.). Surgery was performed aseptically under isoflurane (1–2%) and nitrous oxide (60%) anesthesia (balance O2) after induction with i.v. sodium pentothal (1 g) and intubation of the ewe. Polyvinyl or silicone rubber catheters (Dow Corning Corp., Midland, MI) were implanted in both the carotid artery and jugular vein. In addition, flexible MD probes (CMA 20; CMA/Microdialysis, Solna, Sweden) were implanted in the lateral ventricle and ipsilateral parietal cortex for collection of CSF and ECF, respectively. The tubing of the MD probes was tunneled subcutaneously and exteriorized via a small incision on the back of the neck of the ewe. The antibiotics Triveitin (180 mg of trimethoprim, 900 mg of sulfoxadine; Schering Canada Inc., Pointe Claire, QC, Canada) and ampicillin (500 mg) were administered to the ewe on the day of the surgery and for 3 days postoperatively. After surgery, the animals were kept in holding pens with other sheep and were allowed free access to food and water. The ewes were allowed to recover for 3 days before experimentation.

**Experimental Protocols.** This was a single-site, nonrandomized, open-label, two-period, single sequence study with the stepped infusion experiment followed by the DPHM-PRN coadministration study. There was a washout period of at least 2 days between the study sessions. The details of the two study periods are described below.

**DPHM step-infusions.** The protocol involved bolus i.v. loading doses of DPHM (to hasten the achievement of steady state), followed by i.v. infusion of the drug using an infusion pump (model 600-000; Harvard Apparatus Inc., Dover, MA) at five different rates, with each infusion rate lasting 7 h. The DPHM loading dose was 0.15 mg/kg, and the infusion rates were 1.5, 5.5, 9.5, 13.5, and 17.5 μg/kg/min. During the infusions, arterial blood samples (3 ml) were collected hourly. MD sampling began at the onset of the infusion. The microdialysis pump (model PHD2000; Harvard Apparatus Inc., Holliston, MA) infusion rate was 2 μl/min, and 60-min cumulative samples of CSF and ECF were collected throughout the duration of the experiment. Both blood and MD samples were collected up to 18 h after the end of the last infusion step.

**Coadministration of DPHM and PRN.** DPHM was infused at rate 4 (i.e., 13.5 μg/kg/min) as described in DPHM step-infusions above for 8 h. After 4 h, PRN was confusomed (1.5 mg/kg loading dose, 20.0 μg/kg/min) (Jones and Ritchie, 1978; Mihaly et al., 1982; Czuba et al., 1988) for the remaining 4 h of the DPHM infusion. Blood and MD samples were collected as described above during the infusion and for up to 12 h after infusion.

**Retrodialysis.** MD probe recovery was determined using the retrodialysis technique (De Lange et al., 1998). The MD dialysate (degassed, sterile lactated Ringer solution) contained a calibrator ([3H]-DPHM) at a concentration of 400.0 ng/ml. The probe recovery rate was determined by comparing the input and output concentrations of the calibrator as follows:

\[
\text{Recovery} = \frac{[\text{Calibrator}_{\text{input}}] - [\text{Calibrator}_{\text{output}}]}{[\text{Calibrator}_{\text{input}}]} \tag{1}
\]

Free-fraction drug concentration (C\text{CSF} or C\text{ECF}) at the MD sampling site was equal to the diphenhydramine concentration in the output dialysate (DPHM\text{(dialysate)})/recovery rate.

**Physiological Recording.** Arterial pressure was measured using strain-gauge manometers (Ohmeda Inc., Madison, WI) and heart rates from a cardiotochometer (Astro-Med, West Warwick, RI). All variables were recorded on a Grass K2G polygraph (Astro-Med) and on a computerized data acquisition system (chart v4.2; ADInstruments, Grand Junction, CO).

**Plasma Protein Binding of DPHM.** Determination of plasma protein binding/unbound fraction (C\text{pu}) of DPHM was achieved using the equilibrium dialysis procedure described by Yoo et al. (1990) in the 7-h steady-state plasma sample from each infusion step of the five-step infusion studies. In the case of the DPHM-PRN coadministration study, a sample collected at 8 h of the infusion was used for measurement.

**Drug Analysis.** The concentrations of DPHM (C\text{p}, C\text{CSF}, C\text{ECF}, and C\text{CSF}) in all samples were measured using a gas chromatograph/mass spectrometric assay capable of simultaneously measuring DPHM and [3H]-DPHM with a limit of quantitation of 2.0 ng/ml (Tonn et al., 1993).

**Statistical and Data Analysis.** All pharmacokinetic modeling was performed using WinNonlin, version 1.1 (Scientific Consulting Inc., Apex, NC).

**Five-step infusion study.** Volume of distribution (V\text{d}) and total body clearance (CL\text{t}) were calculated using the following respective equations (Gibaldi and Perrier, 1982):

\[
\text{Vd} = \text{Rate of DPHM infusion}/[\text{C}_{\text{pr}} \cdot \beta] \tag{2}
\]

\[
\text{CLt} = \text{Rate of DPHM infusion}/[\text{C}_{\text{pr}}] \tag{3}
\]

where C\text{pr} is the plasma total steady-state DPHM concentration, and β is the terminal elimination constant.

**Results**

**Free-fraction drug concentration (C\text{CSF} or C\text{ECF}) at the MD sampling site was equal to the diphenhydramine concentration in the output dialysate (DPHM\text{dialysate})/recovery rate.**
CCSF/CPu and CECF/CPu ratios ranged from 2 to 3. There was no significant difference between the steady-state CSF and ECF concentrations across the five steps. This similarity in the two CNS compartments is especially evident upon examination of Fig. 1. Due to the high level of plasma protein binding (86.1 ± 2.3%), the plasma-free DPHM concentrations were lower than that in the CSF and ECF. This relationship is demonstrated clearly in Table 3. The steadystate concentrations were lower than that in the CSF and ECF. This was because of failure of ECF probes in three of the animals. Otherwise, n = 6.

### FIG. 1
Plasma, CSF, and ECF DPHM concentrations achieved with the five-step infusion study. All three concentrations increased in a linear manner corresponding to the increases in infusion rates. DPHM was present in the CSF and ECF within 15 min after starting the infusion, reaching 80 to 90% of the step 1 steady-state concentration.

### TABLE 1
Summary of microdialysis probe recovery rate in the five-step infusion and DPHM-PRN coadministration experiments

<table>
<thead>
<tr>
<th>Infusion Step</th>
<th>CCSF/CPu (%)</th>
<th>CECF/CPu (%)</th>
<th>CCSF/CPt (%)</th>
<th>CECF/CPt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46.3 ± 4.0</td>
<td>47.9 ± 4.0</td>
<td>46.1 ± 3.8</td>
<td>45.8 ± 2.7</td>
</tr>
<tr>
<td>2</td>
<td>8.3 ± 4.2</td>
<td>11.2 ± 3.6</td>
<td>40.3 ± 3.6</td>
<td>44.8 ± 3.6</td>
</tr>
<tr>
<td>3</td>
<td>3.4 ± 2.1</td>
<td>2.6 ± 1.6</td>
<td>4.2 ± 2.0</td>
<td>3.4 ± 2.3</td>
</tr>
<tr>
<td>4</td>
<td>3.8 ± 2.6</td>
<td>3.5 ± 2.3</td>
<td>3.9 ± 3.5</td>
<td>44.5 ± 4.0</td>
</tr>
</tbody>
</table>

* a Probe recovery rates were determined using eq. 1.

### TABLE 2
Steady-state DPHM total plasma (CPt), unbound plasma (CPu), CSF, and ECF concentrations for the five infusion steps

<table>
<thead>
<tr>
<th>Infusion Step</th>
<th>CPt (µg/ml)</th>
<th>CPu (µg/ml)</th>
<th>CCSF</th>
<th>CECF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.4</td>
<td>20.0</td>
<td>20.2</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>169.3</td>
<td>18.9</td>
<td>54.4</td>
<td>15.9</td>
</tr>
<tr>
<td>3</td>
<td>306.6</td>
<td>112.8</td>
<td>119.6</td>
<td>66.8</td>
</tr>
<tr>
<td>4</td>
<td>453.8</td>
<td>158.0</td>
<td>165.6</td>
<td>108.7</td>
</tr>
<tr>
<td>5</td>
<td>599.3</td>
<td>221.9</td>
<td>214.0</td>
<td>109.0</td>
</tr>
</tbody>
</table>

** Average concentration for each step was derived from the last sample of each infusion step.
** Free plasma concentrations were determined by equilibrium dialysis of the last sample of each infusion step.
** Significantly different from their corresponding CPu values (one-way ANOVA).

### TABLE 3
Steady-state CCSF/CPt and CECF/CPt ratios for each infusion step

<table>
<thead>
<tr>
<th>Infusion Step</th>
<th>CCSF/CPt</th>
<th>CECF/CPt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>0.4 ± 0.3</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>0.5 ± 0.3</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.3</td>
</tr>
</tbody>
</table>

* a The CCSF/CPt and CECF/CPt ratios were calculated by dividing the concentrations of the last sample of CSF with the last sample of plasma in each step. All model fitting was carried out using a weighting factor of 1/predicted y² because it provides more accurate estimates at lower DPHM concentrations. The drug was extensively distributed in the animals, as shown by the high Vd value (27.9 ± 17.4 l/kg) (Table 6). Two-compartment pharmacokinetics were observed in the DPHM elimination profiles of all three fluids, with the CNS compartments...
In contrast, CECF and CCSF concentrations increased during PRN for step 4 and 110 the end of the infusion. During steps 4 and 5, which involved the symptoms of agitation including restlessness, tremor, excessive bleating. The elimination half-life in plasma with PRN coadministration, whereas Vd was lower, but these changes were not statistically significant. The elimination half-life in plasma after PRN coinfusion (101.9 mm Hg for step 5) was not significantly different from baseline. No significant changes from baseline were observed during all five infusion steps.

In the DPHM-PRN study, no significant difference was observed in mean arterial pressure after PRN coinfusion (101.9 mm Hg) compared with DPHM administration alone (102.4 mm Hg). However, a significant drop in mean heart rate was observed with PRN coadministration (77.7 ± 3.47 beats per minute) compared with DPHM alone (95.5 ± 2.37 beats per minute), and this fall in heart rate persisted for the full 20-h duration of the experiment (Fig. 3).

**Discussion**

For both the blood-brain and blood-CSF barriers, the most important element is the tight junctions in the brain capillary endothelial cells and in the epithelial cells of the choroid plexus, respectively (Saunders et al., 1999). The tight junctions primarily restrict the entry of proteins and other large hydrophilic molecules into the CNS (Davson and Segal, 1996; Habgood et al., 2000). For lipophilic compounds not significantly bound to plasma proteins, there is a good correlation between the BBB permeability coefficient and the octanol/water partition coefficient, provided the molecular mass is <400 Da (Levin, 1980). However, numerous transporters are present in brain endothelial cells, which transfer substances into the CNS at rates higher than could occur via simple diffusion. There is also evidence for saturable transporter mechanisms in the BBB for a number of lipophilic amine drugs, including PRN and the histamine H1-antagonist mepyramine (Pardridge et al., 1984; Yamazaki et al., 1994b). There are two possible pathways for transport across the BBB: via brain or choroidal blood. The second route involves efflux via bulk flow of CSF draining into either the lymphatic system or venous blood. The latter phenomenon is termed the sink effect, whereby the brain concentrations of different compounds under steady-state conditions are different from each other and lower than the unbound concentration in blood (Davson and Segal, 1996), as a consequence of the continuous removal of the substances via the CSF.

In this study, we applied the microdialysis technique to investigate the blood-brain CSF and blood-brain ECF DPHM relationships in two different experiments. In the first experiment, the results from the five-step infusion showed that brain concentrations increased correspondingly to increases in dose, suggesting that the transfer of DPHM...
into the CNS was a concentration-dependent process. However, considering the high degree of plasma protein binding (86.1 ± 2.3%), the CNS DPHM concentrations were actually higher than the free DPHM concentration in plasma (Tables 2–5). This suggested that the entry of DPHM into the CNS was most likely due to an active transport process, because if passive diffusion was the only driving force, then free plasma DPHM concentrations should be comparable with the CNS levels. In fact, our findings indicated that DPHM concentrations in plasma were higher than the free DPHM concentration by 0.2, respectively), which were not significantly different from each other. Two-compartment pharmacokinetics were observed in the DPHM elimination profiles of all three fluids in the five-step infusion studies, with the CNS compartments declining at the highest rates (Table 6). This was reflected in the half-life $t_{1/2,CSF,ECF}$ of 10.8 ± 5.4 versus $t_{1/2,ECF}$ of 5.3 ± 4.2 h values. The more rapid elimination of DPHM from the brain compared to plasma is associated with higher concentrations of the drug in the CNS compared with the unbound plasma concentration (Table 2). This is probably due to active transport of the ionized DPHM into the brain, as discussed previously, so that more of the total circulating concentration of the drug is available to the brain compared with other organs and tissues.

The rapid efflux of DPHM from CNS could be due to the bulk flow of CSF (sink effect) and also involvement of a transporter-mediated efflux mechanism for the drug. The validity of the above assumption can be assessed by examining the results from the DPHM-PRN experiment. The CSF DPHM concentration increased significantly during PRN coadministration and for both CSF and ECF the percentage increase in drug levels was statistically significant (Table 4). In addition, both the $f_{CSF}$ and $f_{ECF}$ increased after the coadministration of propranolol (from 0.40 ± 0.20 to 0.69 ± 0.03 and 0.95 ± 0.05, respectively), with $f_{ECF}$ being significantly increased. The trend for an increase in the CSF and ECF DPHM elimination half-life after PRN is also consistent with reduced clearance of the drug from the brain. However, these changes were not statistically significant. Although the PRN infusion was not continued into the elimination phase, the persistence of the PRN-elicted bradycardia for the entire duration of the experiment (Fig. 3) indicates that there was sufficient PRN still present during the elimination period for this action and thus perhaps also for an effect on DPHM transfer across the BBB. Overall, the findings suggest lower DPHM clearances from the CNS after PRN coadministration.

As mentioned earlier, besides the bulk flow of CSF, efflux of substances (transporter-mediated or not) can occur via brain or choroidal blood back to the systemic circulation. To date, there is no information showing that PRN lowers CSF formation or its secretion rates and that it has any interference with the passive diffusion process of substances back to the cerebral circulation. In addition, besides a slight decrease in heart rate, PRN causes no systemic or cerebral physiologic changes in sheep (O’Brien et al., 1999). In another study, PRN infusion did not significantly change choroid plexus blood flow in sheep (Townsend et al., 1984). Results from these studies are consistent with our current findings in that only heart rate, but not arterial pressure, was affected by PRN. Therefore, the lowered brain clearances are probably due to lowered rates of efflux of the drug.
Propranolol can be involved in this process in one or both of the following manners—by directly inhibiting the efflux mechanism and/or by competing with DPHM for the efflux process. Both of these actions could be responsible for the observed lowered rates of CNS clearances; however, the exact mechanism cannot be elucidated by our current experiments.

In contrast to the situation with DPHM concentrations in CSF and ECF, the plasma total DPHM level fell by 12.8% during PRN coadministration. This may have been due to an increase in systemic clearance of the drug, given the trend for an increase in this variable during PRN and for a decrease in t1/2 (Table 6). DPHM has a high hepatic extraction in sheep, and thus its hepatic clearance is largely dependent on hepatic blood flow (Kumar et al., 1999). However, PRN has been reported to decrease hepatic blood flow in humans (Zoller et al., 1993; Orszulak-Michalak, 1995); thus, the mechanisms involved in the decreased plasma DPHM concentration are unclear.

In summary, using in vivo microdialysis in chronically instrumented adult ewes, we have demonstrated that DPHM enters the brain rapidly after administration by passive diffusion and an active transfer process. Drug concentrations are markedly higher in the brain relative to unbound plasma levels and this may, in part, explain the significant CNS effects of the drug. DPHM clearances from brain ECF and CSF were similar and faster than plasma clearance, as indicated by the relatively short half-lives in the brain. The rapid efflux of DPHM from the CNS could be due to the bulk flow of CSF (sink effect) and also a transport-mediated efflux mechanism for the drug. The DPHM-PRN coadministration study suggests that PRN inhibits an efflux rather than influx mechanism. This latter finding was rather unexpected and warrants further investigation.

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Bouw MR, Xie R, Tunblad K, and Hammarlund-Udenaes M (2001) Blood-brain barrier transport of the drug, given the trend for an increase in this variable during PRN and for a decrease in t1/2 (Table 6). DPHM has a high hepatic extraction in sheep, and thus its hepatic clearance is largely dependent on hepatic blood flow (Kumar et al., 1999). However, PRN has been reported to decrease hepatic blood flow in humans (Zoller et al., 1993; Orszulak-Michalak, 1995); thus, the mechanisms involved in the decreased plasma DPHM concentration are unclear.

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