The Use of Microdialysis for the Study of Drug Kinetics: Central Nervous System Pharmacokinetics of Diphenhydramine in Fetal, Newborn, and Adult Sheep

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Received November 22, 2006; accepted May 3, 2007

ABSTRACT:

The central nervous system (CNS) pharmacokinetics of the H₁ receptor antagonist diphenhydramine (DPHM) were studied in 100- and 120-day-old fetuses, 10- and 30-day-old newborn lambs, and adult sheep using in vivo microdialysis. DPHM was administered i.v. at five infusion rates, with each step lasting 7 h. In all ages, cerebrospinal fluid (CSF) and extracellular fluid (ECF) concentrations were very similar to each other, which suggests that DPHM between these two compartments is transferred by passive diffusion. In addition, the brain-to-plasma concentration ratios were ≥3 in all age groups, suggesting the existence of a transport process for DPHM into the brain. Both brain and plasma DPHM concentrations increased in a linear fashion over the dose range studied. However, the ECF/unbound plasma and CSF/unbound plasma drug concentration ratios were significantly higher in the fetus and lambs (2–6) than in the adult (3). The factors f_{CSF} and f_{ECF}, the ratios of DPHM areas under the curves (AUCs) in CSF and ECF to the plasma DPHM AUC, respectively, decreased with age, indicating that DPHM is more efficiently removed from the brain with increasing age. The extent of plasma protein binding of the drug increased with age. This study provides evidence for a transporter-mediated mechanism for the influx of DPHM into the brain and also for an efflux transporter for the drug, whose activity increases with age. Moreover, the higher brain DPHM levels in the fetus and lamb compared with the adult may explain the greater CNS effects of the drug at these ages.

The perinatal period of development is a time of rapid physiological and anatomical changes that can profoundly affect drug disposition. Therefore, caution should be exercised for drug use during pre- and postnatal development due to a poor understanding of age-related changes in drug response and pharmacokinetics (Piper et al., 1987). Of all the issues related to xenobiotic use in the developing fetus and newborn, the effect of drugs on CNS development probably deserves the most attention because exposure to exogenous substances during this dynamic period of neurological growth can potentially cause deleterious effects on the developing brain (Rurak, 1992). Considering the fact that most drugs are lipophilic in nature, these compounds have the ability to cross biological membranes including the blood-brain barrier (BBB). Diphenhydramine, 2-(diphenylmethoxy)-N,N-dimethylethylamine (DPHM), is a potent histamine H₁ receptor antagonist (Douglas, 1980) widely used for its anti allergic properties, as well as for its antiemetic, sedative, local anesthetic, and hypnotic effects (Runge et al., 1992; Ernst 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; Gengo et al., 1989).

In pregnancy, DPHM is used for conditions such as nausea and vomiting, insomnia in the first trimester (Magee et al., 2002), a pregnancy-related urticaria in the third trimester, cough and colds, and allergy (Piper et al., 1987). These findings suggest that a significant number of human fetuses may be exposed to this drug at some time during their gestation. In addition, secretion into breast milk represents a potential route of neonatal exposure to DPHM (Schatz, 2002). The drug readily crosses the placenta and alters fetal behavioral state and may also do this in the newborn (Rurak et al., 1988). The DPHM-elicited behavioral effects occur at plasma concentrations lower than those required in adults (Rurak et al., 1988). Although the mechanisms underlying this phenomenon are unknown, it may involve a greater exposure of the fetal and neonatal brain to the drugs. Because of ethical and technical constraints, a study of this issue in...
humans is not possible. However, the fetal and newborn lamb are useful models for the human fetus and infant because of the similarities in the ontogenesis of both the BBB and behavioral functions in the two species (Mollgard and Saunders, 1986; Rurak, 1992). In addition, use of this species can overcome limitations in the available sampling volume of biological fluids associated with smaller animal models and thus allows for more detailed studies. Therefore, studies in sheep can provide useful information on drug exposure in the fetal, newborn, and adult brain. The purpose of this study is to assess blood-brain ECF and blood-CSF drug concentration relationships as a function of pre- and postnatal age and in relation to variations in drug dose and hence plasma drug levels. By applying in vivo microdialysis (MD) in chronically instrumented fetal and newborn lambs and adult ewes, we were able to collect serial samples from the lateral ventricle and cerebral cortex and therefore elucidate DPHM pharmacokinetics in the CNS from these animals at different ages.

Materials and Methods

Animals and Surgical Preparation. All studies were approved by the University of British Columbia Animal Care Committee, and the procedures performed on the sheep conformed to the guidelines of the Canadian Council on Animal Care.

Fetuses. Time-dated pregnant Dorset Suffolk crossbred ewes (term, ~145 days) were operated on between 95 and 105 days for the 100-day-old group (n = 3) and between 115 and 125 days of gestation for the 120-day-old group (n = 7). The mean maternal weight was 81.5 ± 9.3 kg. Food was withheld for ~18 h before surgery, but the animals were allowed free access to water. Approximately 30 min before surgery, a 6-mg i.v. dose of atropine (Abbott Laboratories, Montreal, QC, Canada) was administered via the jugular vein to control salivation. Surgery was performed aseptically under isoflurane (1 to 2%) and nitrous oxide (60%) anesthesia (balance O2) after induction with i.v. sodium pentothal (1 g) and intubation of the ewe. Silicone rubber catheters (Dow Corning Corp., Midland, MI) were implanted in a fetal femoral artery and vein, fetal trachea, amniotic cavity, and a maternal femoral artery and vein. In addition, through 1.5-mm holes drilled through the skull, flexible MD probes (CMA/20; CMA, Stockholm, Sweden) were implanted in the lateral ventricle and ipsilateral parietal cortex for collection of CSF and ECF, respectively. The MD probe input and output catheters were extended with FEP catheters (CMA) and tunneled s.c. and exteriorized via a small incision on the back of the neck for access. Postsurgical treatments of the ewes were the same as described above for fetuses and lambs. After surgery, 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 at least 3 days before experimentation.

Experimental Protocols. The protocol involved a bolus i.v. loading dose of the drug (to hasten the achievement of steady state), followed by i.v. infusion of the drug at five different rates, with each infusion rate lasting 7 h. All DPHM (diphenhydramine hydrochloride; Sigma Chemical Co., St. Louis, MO) doses were prepared in 0.9% sodium chloride solution and were sterilized by filtering through a 0.22-μm nylon syringe filter (MSI, Westboro, MA) into a capped empty sterile injection vial. For the 100-day-old fetus, the DPHM loading dose was 0.5 mg/kg and the infusion rates were 17, 76.5, 136, 195.5, and 255 μg/kg/min. For the 120-day-old fetus, the DPHM loading dose was 0.5 mg/kg and the infusion rates were 13.6, 61.2, 108.8, 156.4, and 204 μg/kg/min. For the 10-day lamb, the DPHM loading dose was 0.7 mg/kg and the infusion rates were 5.25, 19.25, 33.25, 47.25, and 61.25 μg/kg/min. For the 30-day lamb, the DPHM loading dose was 0.7 mg/kg and the infusion rates were 6, 22, 38, 54, and 70 μg/kg/min. In adult ewes, 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. The various dosages were determined based on two sources: 1) results obtained from previous pharmacokinetic studies performed in newborn lambs and pregnant and adult sheep (Yoo et al., 1993; Kumar et al., 2000; Wong et al., 2000), which indicated that the systemic clearance of DPHM was high in the fetus and newborn and decreased in adult sheep, and 2) results obtained from preliminary studies. The doses were adjusted so that they would target a plasma concentration range of ~35 to 450 ng/ml. This particular plasma concentration range was used because it was associated with behavioral changes in fetal lambs in a study performed previously (Rurak et al., 1988).

During the infusions, arterial blood samples (3 ml adult; 0.5 ml fetus and lamb) were collected at ~5, 5, 15, and 30 min and at 1, 2, 3, 4, 5, 6, 7, 7.083, 7.25, 7.5, 8, 9, 10, 11, 12, 13, 14, 14.083, 14.25, 14.5, 15, 16, 17, 18, 19, 20, 21, 21.083, 21.25, 21.5, 22, 23, 24, 25, 26, 27, 28, 28.083, 28.25, 28.5, 29, 30, 31, 32, 33, 34, 35, 35.083, 35.25, 35.5, 36, 36.5, 38, 40, 43, 46, 49, and 53 h. Samples (0.5 ml) were also collected from the fetus at intervals for assessment of blood gas and metabolic status. Because of the relatively small total blood volume in the fetus (~125–300 ml, Kwan et al., 1995), maternal blood (8 ml) was infused into the fetus every 7 h for replacement. Microdialysis sampling began at the onset of the infusion.

All blood samples collected were placed into EDTA-containing Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) and centrifuged at 2000g for 10 min. The plasma supernatant layer was removed and placed into clean borosilicate test tubes with polytetrafluoroethylene-lined caps. MD dialysate samples were collected directly into clean borosilicate test tubes. Plasma and MD samples were stored frozen at ~20°C until the time of analysis.

Retrodialysis. Microdialysis sampling began at the onset of the infusion. The microdialysis pump (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. 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 ([H3]DPHM) at a concentration of 400 ng/ml. The probe recovery rate can be determined by comparing the input and output concentrations of the calibrator as follows:

\[
\text{Recovery} = \frac{[\text{Calibrator}_{\text{input}}] - [\text{Calibrator}_{\text{output}}]}{[\text{Calibrator}_{\text{input}}]} \quad (1)
\]

Free-fraction drug concentration (C_{CSF} or C_{ECF}) at the MD sampling site = \([\text{DPHM}]_{\text{dialysate}}/\text{Recovery Rate}\).
Plasma Protein Binding of DPHM. Determination of plasma protein binding/unbound fraction ($C_{p0}$) of DPHM was achieved using the equilibrium dialysis procedure described by Kumar et al. (2000) in steady-state plasma samples from each infusion step of the five-step infusion studies.

Drug Analysis. The concentrations of DPHM ($C_{pl}$, total plasma DPHM concentration), $C_{CSF}$ (CSF DPHM concentration), and $C_{ECF}$ (ECF DPHM concentration) in all samples were measured using a gas chromatographic-mass spectrometric assay capable of simultaneously measuring DPHM and $[^{3}H]_{DPH}$ph with a limit of quantitation of 2.0 ng/ml (Tonn et al., 1993).

Data Analysis. All pharmacokinetics modeling was performed using WinNonlin, version 1.1 (Scientific Consulting Inc., Apex, NC). Volume of distribution ($V_d$), and total body clearance ($CL_T$) were calculated using the following respective equations (Gibaldi and Perrier, 1982):

$$VD_T = \text{Rate of DPHM infusion}/C_{pl}\cdot \beta$$

(2)

$$CL_T = \text{Rate of DPHM infusion}/V_d$$

(3)

where $V_{d,T}$ is the plasma total steady-state DPHM concentration, and $\beta$ is the terminal elimination constant.

The extent of DPHM transfer into the brain in this study was calculated by relating the CSF and ECF total area under the plasma concentration versus time curve ($AUC_{CSF}$) values to the plasma $AUC_{pl}$ value to yield the $f_{CSF}$ and $f_{ECF}$ ratios. Specifically, using $f_{CSF}$ as an example, the CSF $AUC_{CSF}$ was divided by the plasma $AUC_{pl}$ as follows:

$$f_{CSF} = AUC_{CSF}/AUC_{pl}$$

(4)

The $f_{ECF}$ value was calculated in the same manner using $AUC_{ECF}$ (total area under the ECF concentration versus time curve). This method of characterizing drug transfer across the BBB has been used in numerous other MD studies for many different drugs (Wang et al., 1993; Wong et al., 1993; de Lange et al., 1994; Potsehka et al., 2002).

Statistical Analysis. Data were plotted using SigmaPlot 9.0 (SPSS Inc., Chicago, IL). All data are reported as mean ± S.E.M. Statistical analyses were performed using NCSS 2000 (NCSS, Kaysville, UT). Pharmacokinetic parameters and concentrations were compared using one-way ANOVA followed by a Duncan’s multiple comparison test. For statistical analysis of the difference in ECF and CSF DPHM concentrations, the paired $t$ test was used. In occasions where normal distribution of data was not observed, a Kruskal-Wallis test was used instead of ANOVA. The significance level was $p < 0.05$ in all cases.

Results

The average age of the fetuses on the day of their experiments was 103.5 ± 1.2 days for the 100-day-old fetus group and 124.1 ± 0.5 days for the 120-day-old fetus group. For the lambs, the average age on the day of their experiments was 11.5 ± 0.7 and 33.8 ± 0.5 days for the 10-day- and 30-day-old lamb groups, respectively. For the adults, the average age on the day of their experiments was 5.2 ± 1.1 years old. Estimated mean fetal body weight for the 100-day-old fetal group was 1.1 ± 0.1 kg, and for the 120-day-old fetal group was 2.4 ± 0.1 kg. Fetal weights in utero were estimated from the weight at birth and the time between the experiment and the birth as described by Gresham et al. (1972). Mean lamb body weight for the 10-day-old lamb group was 7.1 ± 0.7 kg and for the 30-day-old lamb group was 12.2 ± 0.5 kg, and mean adult ewe body weight was 74.6 ± 8.3 kg. Fetal blood gas and pH values during the experiment were within the normal range and did not change significantly (data not shown).

MD probe recovery rates ranged between 39.2 ± 4.2 to 48.0 ± 1.1% across the different age groups (Table 1). However, the failure rate was high (~1/3 of the probes failed to work shortly after surgeries), and only animals that had functional probes are listed in Table 1. Most animals had one CSF and one ECF probe; however, three of the 30-day-old lambs had both probes implanted in the brain tissue (i.e., ECF) because of difficulties in locating the lateral ventricles for CSF probe insertion. Figure 1 presents semilogarithmic plots of mean DPHM CSF, ECF, and plasma concentrations versus time for all age groups, whereas the mean concentrations for each infusion step are given in Table 2. From the profiles, it can be seen that CSF, ECF, and plasma concentrations increased in proportion to increases in infusion rate. The mean difference ($±$S.E.M.) in DPHM concentration between CSF and ECF was 38.2 ± 14.2 ng/ml in all five infusion steps across all age groups, and this difference was not significantly different from zero; thus, the concentrations in the two fluid compartments were identical. Steady-state concentrations were reached at the 4th h of each infusion step, since no significant differences were observed among the concentrations in all three fluids beyond this point (ANOVA, $p > 0.05$).

Upon examination of the overall $C_{CSF}/C_{pl}$ and $C_{ECF}/C_{pl}$ ratios (Tables 2 and 3), a trend existed where the brain concentrations started higher than plasma concentrations in the fetal groups, then became roughly equal to each other in the newborn lamb groups, and eventually dropped to levels lower than the plasma concentrations in the adult group. The ratios for total drug started at approximately 2 to 3 in the fetal groups dropped to between 1 and 2 in the postnatal lambs and were below 1 in the adult group. Equilibrium dialysis was performed on the steady-state plasma samples to determine the extent of plasma protein binding (Table 4) and therefore yield the plasma concentrations of unbound DPHM. Because plasma protein binding increased with advancing age, and it is the free drug that crosses the BBB, the steady-state CSF ($C_{CSF}$) and ECF ($C_{ECF}$) concentrations were compared with the unbound plasma levels ($C_{pl}$) to further examine the brain/blood concentration relationships at each infusion step (Table 3). When the ratios were calculated using the unbound plasma DPHM concentrations, the values at all ages were significantly higher than plasma concentrations in the fetal groups, then became roughly equal to each other in the newborn lamb groups, and eventually dropped to levels lower than the plasma concentrations in the adult group. The ratios for total drug started at approximately 2 to 3 in the fetal groups dropped to between 1 and 2 in the postnatal lambs and were below 1 in the adult group. Equilibrium dialysis was performed on the steady-state plasma samples to determine the extent of plasma protein binding (Table 4) and therefore yield the plasma concentrations of unbound DPHM. Because plasma protein binding increased with advancing age, and it is the free drug that crosses the BBB, the steady-state CSF ($C_{CSF}$) and ECF ($C_{ECF}$) concentrations were compared with the unbound plasma levels ($C_{pl}$) to further examine the brain/blood concentration relationships at each infusion step (Table 3). When the ratios were calculated using the unbound plasma DPHM concentrations, the values at all ages were significantly
greater than 1 (Table 3). There was a significant increase in the extent of protein binding between the 120-day-old fetus and 10-day-old lamb groups (Table 4). After birth, a significant increase in the level of plasma protein binding also occurred from 30-day-old lambs to adults. In contrast to the decreasing trend in the overall $C_{\text{CSF}}/C_{\text{Pn}}$ and $C_{\text{ECF}}/C_{\text{Pn}}$ ratios, the overall $C_{\text{CSF}}/C_{\text{Pn}}$ and $C_{\text{ECF}}/C_{\text{Pn}}$ ratios increased after birth [i.e., relative to the 120-day fetus values before dropping to adult values (Table 3)].
higher than 1, suggesting that DPHM is transported into the CNS in sheep against a concentration gradient. Many transporters are present at the BBB to deliver a range of substances into the CNS (Oldendorf, 1973), including sodium-independent organic cation transporters (Koepsell, 1998). A diverse group of organic cations, including endogenous bioactive amines (i.e., acetylcholine, choline, dopamine, epinephrine, norepinephrine, guanidine, and thiamine) and therapeutic drugs (i.e., cimetidine, amiloride, morphine, quinidine, and verapamil) are actively transported by the organic cation transporter system (Koepsell, 1998; Wu et al., 1998; Zhang et al., 1998; Lee et al., 2001).

There is also evidence for saturable transporter mechanisms in the BBB for a number of lipophilic amine drugs including propranolol, lidocaine, amphetamine, rimantadine, amantadine, pentazocine, and the histamine H1 antagonist mepyramine (Pardridge and Connor, 1973; Pardridge et al., 1984; Spector, 1988; Yamazaki et al., 1994; Suzuki et al., 2002a,b). Moreover, in rat studies involving the carotid injection technique (Suzuki et al., 2002a) and the in situ brain perfusion method (Suzuki et al., 2002b), the transport of pentazocine into the brain was inhibited by several compounds, including DPHM, mepyramine, and propranolol. This led the authors to suggest that these compounds (including DPHM) use a common cationic carrier-mediated influx system; however, its precise identity is not yet known.

Both CNS and plasma DPHM concentrations showed corresponding increases with dose. Furthermore, a significant trend existed where CNS concentrations started higher than plasma concentrations in the fetal groups, approximated each other in the lamb groups, and finally became lower than plasma concentrations in the adult group (Fig. 1 and Table 2). Examination of $C_{CSF}/C_{P}$ and $C_{ECF}/C_{P}$ ratios reveals a similar trend (Tables 2 and 3). Because only the unbound drug in plasma can cross the BBB, the relationships between brain and free plasma concentrations were also examined. Although the $C_{CSF}/C_{P}$ and $C_{ECF}/C_{P}$ ratios increased in the 10- and 30-day-old lambs compared with the fetal values (Table 3), this was primarily caused by the increase in protein binding that occurred after birth (Table 4). This resulted in a decrease in free DPHM concentration. Therefore, the increase seen in [Brain]/[Free Plasma] ratios for the postnatal lambs was due to division by smaller values (i.e., lower free DPHM concentrations), rather than an increase in transfer of DPHM into the CNS. A significant increase in protein binding was also observed in the adults. However, the [Brain]/[Free Plasma] ratios in

**Discussion**

**CSF, ECF, and Plasma Relationships in Relation to Age.** CSF and ECF concentrations were not significantly different from each other in all age groups throughout the dose ranges studied (Fig. 1 and Table 2). This rapid equilibrium in concentrations suggests that transfer of DPHM between the two compartments involves a passive diffusion process. In fact, no transporters have been identified to date on the neuroepithelium, which is the cell layer separating the ECF and CSF compartments (Davson and Segal, 1996). In addition, this homogeneous distribution of the drug over the brain suggests lack of local metabolism (Kerr et al., 1984). As shown in Table 3, the $C_{CSF}/C_{P}$ and $C_{ECF}/C_{P}$ ratios from the different age groups were
the adult are lower than that in the 120-day fetal group because of much lower brain concentrations of DPHM in the adult compared with the fetus. For example, step-5 CSF and ECF in the adult was 29.9 and 29.4%, respectively, of the corresponding 120-day fetal values. Overall, trends from these ratios indicate that brain clearance of DPHM increased with age, which will be discussed further later with comparison of pharmacokinetic parameters.

Goldberg et al. (1987) have examined transport of DPHM in the CNS using a brain perfusion technique in rats. The concentration of DPHM was rapidly eliminated from these compartments. Goldberg et al. (1987) also observed rapid rate of efflux of DPHM from rabbit choroid plexus, determined using a brain homogenate method. Because DPHM is, to a great extent, ionized at physiologic pH, simple diffusion of the drug back to cerebral circulation alone does not seem adequate to explain this observation. Besides diffusion, substances can leave the CNS via two other mechanisms. One comprises efflux (transporter-mediated or not) via brain or choroidal blood. The second route involves efflux via bulk flow of CSF draining into either the lymphatic system or venous blood through the arachnoid villi in the superior sagittal sinus (Saunders and Dzialogiewska, 1998). The latter phenomenon is responsible for what is termed the sink effect. Whereas CSF flow is a normal physiological process that happens regardless of the substance, transporter-mediated efflux is a substrate specific process. As noted previously, there is evidence for a cationic carrier-mediated influx system for pentazocine, DPHM, and other cationic substances (Suzuki et al., 2002a,b). Moriki et al. (2005) have provided evidence that P-glycoprotein acts as BBB efflux transporter for propranolol, which inhibits P-glycoprotein (Bachmakov et al., 2006), significantly increases the brain/plasma ratios of DPHM in adult sheep (Au-Yeung et al., 2006). The identity of CNS efflux transporter for DPHM in the adult, which is what we observed (Table 5), is not involved in DPHM transport into the CNS using a brain perfusion technique in rats. The concentration of DPHM in the CSF at steady state was approximately twice the unbound plasma concentration, which is similar to our results from the previous study (Kumar et al., 2000). On the other hand, Vdss of DPHM increased with age, which will be discussed further later with comparison of pharmacokinetic parameters.

### TABLE 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>Parameter</th>
<th>Age Group</th>
<th>120-day Fetus</th>
<th>10-day Lamb</th>
<th>30-day Lamb</th>
<th>Adult</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100-day Fetus</td>
<td>120-day Fetus</td>
<td>10-day Lamb</td>
<td>30-day Lamb</td>
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<tr>
<td>Plasma</td>
<td>CLT (ml/min/kg)</td>
<td>106.5 ± 19.6$^{1}$</td>
<td>110.0 ± 4.6$^{1}$</td>
<td>126.4 ± 3.6$^{2}$</td>
<td>145.4 ± 5.9$^{3}$</td>
<td>31.5 ± 5.5$^{4}$</td>
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<td>Vdss (l/kg)$^*$</td>
<td>14.8 ± 1.7$^{1}$</td>
<td>13.9 ± 0.8$^{1}$</td>
<td>9.7 ± 0.6$^{2}$</td>
<td>9.1 ± 0.2$^{3}$</td>
<td>26.8 ± 7.0$^{3}$</td>
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<td>$t_{1/2}$ (h)$^*$</td>
<td>4.6 ± 1.1$^{1}$</td>
<td>3.6 ± 0.4$^{1}$</td>
<td>4.9 ± 0.9$^{2}$</td>
<td>4.5 ± 1.3$^{3}$</td>
<td>10.8 ± 2.0$^{3}$</td>
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<td></td>
<td>$f_{CSF}$</td>
<td>0.3 ± 0.1$^{1}$</td>
<td>0.4 ± 0.04$^{1}$</td>
<td>1.2 ± 0.4$^{2}$</td>
<td>0.7 ± 0.2$^{3}$</td>
<td>0.5 ± 0.1$^{4}$</td>
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<tr>
<td></td>
<td>$f_{ECF}$</td>
<td>1.9$^{b}$</td>
<td>2.5 ± 0.2$^{a}$</td>
<td>1.1 ± 0.3$^{b}$</td>
<td>0.6$^{c}$</td>
<td>0.4 ± 0.1$^{f}$</td>
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<td></td>
<td>$f_{ECF}$</td>
<td>1.2</td>
<td>1.6 ± 0.2$^{a}$</td>
<td>2.2 ± 0.8$^{b}$</td>
<td>2.2</td>
<td>3.6 ± 0.4$^{d}$</td>
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<td>$f_{ECF}$</td>
<td>0.1</td>
<td>0.9 ± 0.1</td>
<td>1.8 ± 0.3$^{c}$</td>
<td>1.7</td>
<td>0.6 ± 0.1$^{d}$</td>
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<td></td>
<td>$f_{ECF}$</td>
<td>1.0</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 0.8$^{g}$</td>
<td>2.4 ± 1.3$^{h}$</td>
<td>5.3 ± 4.2$^{i}$</td>
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<td></td>
<td>$f_{ECF}$</td>
<td>0.9</td>
<td>0.8 ± 0.2</td>
<td>1.5 ± 0.6$^{i}$</td>
<td>1.6 ± 0.6$^{j}$</td>
<td>0.5 ± 0.3$^{j}$</td>
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</table>

Data are shown as mean ± S.E.M. $f_{CSF}$, the ratio of AUC_{CSF}/AUC_{net}; $f_{ECF}$, the ratio of AUC_{ECF}/AUC_{net}; $t_{1/2}$, elimination half-life; $t_{1/2b}$, distribution half-life. Values with different number superscripts (1–4) in each row are statistically different as determined by the Duncan’s multiple comparison test ($p < 0.05$); comparisons were made by ANOVA.

* Comparisons were made by Kruskal-Wallis test followed by Duncan’s multiple comparison test.

### TABLE 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein binding in steady-state plasma samples for each infusion step</th>
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<tbody>
<tr>
<td>Age Group</td>
<td>Step 1</td>
</tr>
<tr>
<td>100-day fetus (n = 3)</td>
<td>0.60 ± 0.09</td>
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<tr>
<td>120-day fetus (n = 7)</td>
<td>0.62 ± 0.08</td>
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<tr>
<td>10-day lamb (n = 5)</td>
<td>0.76 ± 0.04</td>
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<tr>
<td>30-day lamb (n = 4)</td>
<td>0.82 ± 0.05</td>
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<tr>
<td>Adult (n = 8)</td>
<td>0.86 ± 0.02</td>
</tr>
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</table>

Data are shown as mean ± S.E.M. Values with different numbers (1–3) are statistically different as determined by the Duncan’s multiple comparison test ($p < 0.05$).
fetal low-voltage electrocorticographic pattern, rapid eye movements, and fetal breathing (Rurak et al., 1988). These sedative-like effects occurred at fetal plasma drug concentrations (~36 ng/ml) lower than those that result in discernible CNS effects in adults (~50 ng/ml) (Carruthers et al., 1978). The fetal plasma concentration of 36 ng/ml is slightly higher than the concentration of 26.9 ng/ml achieved during the step-1 infusion in the 120-day-old fetal group in the current study (Table 2). The brain ECF concentration at step 1 was 51 ng/ml, and in adult ewes, this ECF concentration was not reached until infusion step 2, when the plasma DPHM level was 169.3 ng/ml. This is considerably higher than the plasma concentration of the drug associated with CNS effects in adult humans. Thus, greater exposure of the fetal brain to DPHM seems to explain its greater CNS effects.

In terms of the relevance of these observations to human, fetal exposure to DPHM after maternal bolus administration is extensive (i.e., AUC fetal/AUC maternal = 0.85) (Yoo et al., 1986). In humans, peak plasma concentrations following a 50-mg oral dose are between 40 to 80 ng/ml (Carruthers et al., 1978). Assuming that placental transfer of DPHM in human is similar to sheep, a 50-mg oral dose to a pregnant woman would result in peak plasma concentrations of 34 to 68 ng/ml in fetal plasma. This would result in fetal brain concentrations of ~70 to 140 ng/ml. Thus, alterations in the behavior of human fetuses are likely to occur with maternal administration of normal oral doses of the drug.

In summary, use of in vivo microdialysis probes has allowed us to study the disposition of DPHM in the CNS of sheep as a function of postconceptual age. Brain ECF and CSF concentrations of the drug were higher in the fetal and postnatal lambs than in the adult, and this may be due to immaturity of a CNS efflux transporter for the drug. Furthermore, greater exposure of the fetal brain to DPHM may be due to immaturity of a CNS efflux transporter for the drug.

References


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