Morphine and Alfentanil Permeability Through the Spinal Dura, Arachnoid, and Pia Mater of Dogs and Monkeys

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Little information exists about which spinal meninx is the principal permeability barrier between the epidural space and the spinal cord or about what physicochemical properties of drug molecules govern their meningeal permeability. To better understand these aspects of epidural pharmacokinetics, the authors measured the permeability of morphine and alfentanil through the different components of the spinal meninges—dura mater, arachnoid mater, and pia mater—of dogs and monkeys in vitro. Live meningeal tissue from either species (dura mater alone, pia mater alone, or intact dura–arachnoid–pia) was placed between two fluid reservoirs of a temperature-controlled diffusion cell. The permeability of the tissues to each opioid was determined by placing the opioid in one of the reservoirs of the diffusion cell and measuring the rate at which the drug diffused through the tissue and appeared in the second reservoir. The arachnoid mater was found to be the major meningeal diffusion barrier between the epidural space and the spinal cord. Alfentanil was 3.7 times more permeable than morphine through all three meninges, suggesting that increased lipid solubility increases meningeal permeability. However, neither lipid solubility nor molecular weight adequately explained the difference in permeability between morphine and alfentanil. The authors conclude that this in vitro model has significant utility for studies aimed at predicting in vivo meningeal permeability and hence the potency and rapidity of action of any opioid administered by the epidural route. (Key words: Arachnoid mater, Dura mater, Meninges, Pia mater, Spinal cord: permeability. Opioids: alfentanil; morphine.)

Epidural administration of opioids for selective spinal analgesia requires that the drugs diffuse from the epidural space to their sites of action at opioid receptors of the spinal cord dorsal horn. En route from the epidural space, opioids must traverse all three spinal meninges—dura mater, arachnoid mater, and pia mater. The relative importance of these three quite different tissues to drug transfer is unknown, nor is it known what physicochemical properties govern meningeal permeability of drug molecules.

The transfer of opioids across the meninges is a critical step in producing selective opioid analgesia, and understanding the pharmacokinetics of that transfer is important to understanding the time course of epidural analgesia. However, although many studies have qualitatively demonstrated the passage of local anesthetic and opioid molecules across the meninges,1–3 quantitative studies of meningeal permeability have not been reported for any drug. This lack of quantitative information results in large part from the inherent complexities of in vivo epidural analgesia studies. To quantitate the meningeal permeability of any drug molecule, it is necessary to know the concentration gradient of drug across the meningeal tissue as well as the surface area of tissue available for diffusion. It is not possible to know either of these in vivo because of the unpredictable spread of opioid solutions in the epidural space, the uptake of drug into the epidural vasculature, and the uptake of drug into epidural fat.

Recognizing the limitations of in vivo studies, previous investigations have attempted to quantitate the diffusion of drug molecules across cadaveric human dura mater in vitro.4–6 However, these studies provided incomplete information for predicting the in vivo behavior of epidural drugs because they failed to address the contribution of the pia mater and arachnoid mater to the meningeal permeability barrier. This is an important omission because the meninges are not a single homogeneous membrane, but a complex set of three very different tissues. In addition, prior studies are flawed because the previously frozen cadaveric tissue used for these studies cannot be expected to behave like living tissue.

Therefore, we have developed an in vitro model of meningeal permeability that uses live meningeal tissue from dogs and monkeys. We have used this model to determine the permeability of morphine and alfentanil across all three lumbar meningeal tissues and have identified which meningeal tissue is the principal barrier to diffusion of these opioids from the epidural space to the spinal cord.

Materials and Methods

Studies were approved by our animal care committee, and guidelines of the American Association for Laboratory Animal Care were followed throughout.

Tissue Sources

Monkey (Macaque nemestrina) tissue was obtained from animals scheduled to be killed as part of the tissue distri-
bution program of the University of Washington Regional Primate Research Center. The animals (eight female, four male) weighed 2–7 kg. All meningeal specimens were removed from animals anesthetized with thiopental/ketamine and without muscle relaxants.

Dog tissue was obtained from animals used in acute experiments by other investigators. None of these acute experiments involved the spinal cord or meninges or the use of opioids. Tissue was removed from these animals anesthetized with halothane (1–2%)/N₂O (66%). The dogs (four male, two female) weighed 18–22 kg.

EXPERIMENTAL SET-UP

Tissue Preparation

Spinal cords of 12 *M. nemestrina* monkeys (six for which alfentanil was used and six for which morphine was used) and six mongrel dogs (morphine was used in all) were removed en block and an incision was made simultaneously through all three meningeal layers (dura, arachnoid, and pia) along the ventral surface of the spinal cord. Together, the dura, arachnoid, and pia mater were carefully reflected from the spinal cord, with preservation of their normal anatomic relationships. The meningeal tissue used for these experiments came from the L1–2 or L2–3 cord segments and did not include nerve roots or portions of dural cuff.

All three meninges, in their normal anatomic relationships, were placed between two halves of a temperature-controlled diffusion cell with a 0.785 cm² connecting port (fig. 1). Ten milliliters bicarbonate buffered mock cerebrospinal fluid (CSF) (pH = 7.38–7.42; 295 mOsm) was placed in fluid reservoirs on either side of the meningeal tissue. Each fluid reservoir was vigorously stirred by a magnetic stirrer to minimize unstirred layer effects. Oxygen (95%) and carbon dioxide (5%) were bubbled through each fluid reservoir to maintain normal pH and to oxygenate meningeal cells. Mock CSF contained 72 mg/dl glucose to meet energy requirements of the meningeal cells.

Flux Measurements

After allowing 20 min for the chambers to equilibrate to 37° C, we added the study drug to fluid reservoir 1 on the dura mater side of the diffusion cell (morphine sulfate = 0.1 mg base; alfentanil HCl = 0.05 mg base). Thereafter, at 5-min intervals for 45 min, 100-μl samples were removed simultaneously from reservoir 1 and reservoir 2 (on the pia mater side of the diffusion cell) and frozen for later measurement of drug concentrations.

After measurement of morphine flux (but not alfentanil) through all three meninges (dura–arachnoid–pia mater), both diffusion cell reservoirs were thoroughly rinsed five times with 15-ml volumes of mock CSF to remove any remaining morphine. We then repeated the morphine experiment, as described above, using the same tissue sample to verify that the results were reproducible and to demonstrate that the tissue was stable in vitro. The repeat experiment started approximately 1.5 h after the beginning of the first experiment.

After duplicate measurement of morphine flux and single measurement of alfentanil flux through all three meninges, we opened the diffusion cell and separated the pia–arachnoid mater from the dura mater. The cell was then reassembled with only the dura mater in place. The reservoirs were rinsed five times with 15-ml volumes of mock CSF, and the experiment was repeated as described above to measure morphine and alfentanil flux through the dura mater alone.

After the measurement of morphine (but not alfentanil) flux through the dura mater alone, the cell was again rinsed thoroughly and the measurement of morphine flux through the dura mater alone was repeated. As before, this was done to verify reproducibility and determine that the tissue did not deteriorate over time.

CALCULATION OF PERMEABILITY

Flux (Q) was determined from drug concentration data by plotting the amount of drug in reservoir 2 at each time point. The slope of the line relating concentration versus time data was determined with the use of least-squares linear regression and is equal to the test drug’s flux through the meninges. Permeability was then determined by application of the flux equation:

$$Q_t = P \cdot [C1 + C2] \cdot A$$
where:
\[ Qt = \text{flux at any given time} \]
\[ P = \text{permeability} \]
\[ (C1 - C2) = \text{concentration gradient across the meninges at any time } t \ (\mu g/ml) \]
\[ A = \text{cross-sectional area of meninges available for diffusion, i.e., area of port connecting two halves of diffusion} \]

Solving for permeability:
\[ P = \frac{Q}{(C1 - C2)_t} \cdot A \]

All experiments were conducted over a time period during which the concentration gradient of the drug under study decreased by less than 3% from its value at time zero. This condition assures that flux is predominantly unidirectional and that the concentration gradient across the tissues is nearly constant throughout the experiment. As a result, the concentration gradient across the tissues can at all times be closely approximated by the initial concentration gradient at time zero (i.e., \( C1 - C2 \approx C1 \)).

Therefore,
\[ P = \frac{Q}{C1} \cdot A \]

The permeability of dura--arachnoid--pia mater and of dura mater alone to each drug was then estimated from the following: 1) the experimentally determined flux; 2) the measured initial drug concentration in reservoir 1; and 3) the measured port area connecting the two reservoirs.

**Calculation of Pia--Arachnoid Permeability**

Because resistance through each tissue is the inverse of permeability and resistances in series are additive, we calculated the apparent permeability of each drug through the pia--arachnoid tissue as the difference between experimentally determined permeability through dura alone and through intact dura--arachnoid--pia. That is,
\[ R_{total} = \ast R_{dap} = \ast R_d + R_{ap} \]

Therefore:
\[ R_{ap} = \ast R_{dap} - \ast R_d \]

and
\[ P_{ap} = \frac{1}{R_{ap}} \]

where
\[ R = \text{resistance} = \frac{1}{\text{permeability}} \]
\[ \text{dap} = \text{dura--arachnoid--pia} \]
\[ a = \text{arachnoid} \]
\[ d = \text{dura} \]
\[ * \text{indicates measured quantity} \]

**Measurement of Pia Mater Permeability and Calculation of Arachnoid Permeability**

In six additional monkeys (three male, three female; weight, 2.2–7.0 kg), we measured the permeability of morphine through pia mater alone to determine whether the pia mater or the arachnoid mater accounted for most of the resistance to diffusion through the combined pia--arachnoid meninx.

The pia mater was carefully separated from the dura and arachnoid. The pia was then placed in the diffusion cell, and morphine permeability was determined as described above except that radiolabeled morphine (2.5 \( \mu \)Ci \(^3\)H-morphine and 0.1 mg morphine base) was used to determine morphine concentration in each sample.

The arachnoid mater permeability was calculated as follows:
\[ R_{total} = \ast R_{dap} = \ast R_d + \ast R_p + R_a \]

Therefore:
\[ R_a = \ast R_{dap} - (\ast R_d + \ast R_p) \]

For these calculations, the total resistance (i.e., dura--arachnoid--pia resistance) and resistance through the dura mater were measured in the same meningeal specimen from each of six monkeys. The value for pial resistance was calculated from the average pial permeability from six different monkey specimens.

**Drug Analysis**

Morphine was analyzed by a modification of the method of Todd et al. Briefly, this method involves addition of an internal standard, alkalization of the sample, and extraction into chloroform–isopropanol (95:5). The chloroform–isopropanol is evaporated and the residue redissolved in methanol–acetonitrile–KH2PO4 buffer. The analysis is performed with the use of a Varian (Walnut Creek, CA) 5060® HPLC with the Bioanalytical Systems (West Lafayette, IN) LC48® electrochemical detector. Extraction recoveries for morphine average 90.3%, the detection limit is 0.5 ng/ml, and the coefficient of variation is 4.3%.

Alfentanil was analyzed by a modification of the technique of Woestenborghs et al. Briefly, this involves addition of an internal standard, alkalization of the sample, and extraction into heptane–isooamy alcohol (98.5:1.5). Alfentanil is then extracted from the heptane–isooamy alcohol mixture by addition of sulfuric acid. The sample is then alkalized with ammonium hydroxide, extracted back into heptane–isooamy alcohol, and the organic phase is evaporated to dryness. The residue is redissolved in methanol and analyzed on a Hewlett-Packard (Palo Alto, CA) gas chromatograph mass spectrometer (model 59970B). Extraction recoveries for alfentanil average 94%, the detection limit is 0.1 ng/ml, and the coefficient of variation is 3.8%.

Samples containing \(^3\)H-morphine were placed in borosilicate glass vials containing 5–10 ml Hydrofluor® (National Diagnostics, Manville, NJ) scintillation fluid. Samples were counted in a Hewlett-Packard liquid scintillation
counter (Tri-Carb 2000®, Packard Instrument, Downers Grove, IL) for sufficient time that the standard deviation of depositions per minute (DPM) was 2% or less. Background counts from blank mock CSF were subtracted from total DPM to produce corrected DPM.

**Statistical Analyses**

The differences in mean values of meningeal permeability between drugs in the same tissue and across different tissues for the same drug were assessed for statistical significance by pooled t test. Differences were considered significant at the $P \leq 0.05$ level. All experimental results are reported as mean ± standard deviation.

**Results**

The correlation coefficients (r) for regression lines used to determine the flux of each opioid through the tissue under study were in each case greater than 0.95.

Table 1 lists the results of morphine permeability experiments performed in duplicate. There was no significant difference between the first and second permeability measurement in any tissue of either species. For this reason, the results reported below for morphine permeability in the dog and monkey represent the mean of duplicate measurements in each of six monkey and six dog tissue samples.

The permeability of morphine was greater through the dura mater alone than through the pia–arachnoid mater in both the dog (dura: $7.62 \cdot 10^{-5} \pm 2.2 \cdot 10^{-5}$ cm/min; pia–arachnoid: $1.7 \cdot 10^{-5} \pm 7.5 \cdot 10^{-6}$ cm/min; $P < 0.05$) and the monkey (dura: $9.4 \cdot 10^{-5} \pm 2.9 \cdot 10^{-5}$ cm/min; arachnoid–pia: $6.6 \cdot 10^{-4} \pm 2.3 \cdot 10^{-4}$, $P < 0.05$). The permeability of morphine through all three meninges (dura–arachnoid–pia) did not differ from the permeability through the pia–arachnoid mater in either the dog (dura–arachnoid–pia: $1.8 \cdot 10^{-3} \pm 5.3 \cdot 10^{-4}$ cm/min; arachnoid–pia: $1.7 \cdot 10^{-2} \pm 7.5 \cdot 10^{-4}$, $P > 0.05$) or the monkey (dura–arachnoid–pia: $6.2 \cdot 10^{-4} \pm 2.1 \cdot 10^{-4}$; arachnoid–pia: $6.6 \cdot 10^{-4} \pm 2.3 \cdot 10^{-4}$; $P > 0.05$). The permeability of morphine through monkey pia mater was $6.9 \cdot 10^{-5} \pm 1.8 \cdot 10^{-5}$ (fig. 2).

**Table 1. Morphine Permeability Measurements Performed in Duplicate**

<table>
<thead>
<tr>
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<th>Permeability (cm/min $\times 10^{-5}$)</th>
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<tbody>
<tr>
<td></td>
<td>1st Measurement</td>
</tr>
<tr>
<td>Monkey</td>
<td></td>
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<tr>
<td>Dura–arachnoid–pia</td>
<td>0.63 ± 0.3</td>
</tr>
<tr>
<td>Dura</td>
<td>9.35 ± 2.86</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
</tr>
<tr>
<td>Dura–arachnoid–pia</td>
<td>1.05 ± 0.44</td>
</tr>
<tr>
<td>Dura</td>
<td>6.85 ± 1.87</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

NS = not significant.

Morphine was less permeable through all three meninges of the monkey (dura–arachnoid–pia) than through all three meninges of the dog ($P < 0.05$). As well, morphine was less permeable through the pia–arachnoid of the monkey than through the pia–arachnoid of the dog ($P < 0.05$). There was no difference in morphine permeability through the dura mater alone of either species ($P > 0.05$) (fig. 2).

The permeability of alfentanil was greater than the permeability of morphine through all three meninges (alfentanil: $2.3 \cdot 10^{-3} \pm 5.8 \cdot 10^{-4}$ cm/min; morphine: $6.2 \cdot 10^{-4} \pm 2.1 \cdot 10^{-4}$, $P < 0.05$) and through the pia–arachnoid mater (alfentanil $3.0 \cdot 10^{-3} \pm 9.4 \cdot 10^{-4}$ cm/min; morphine: $6.6 \cdot 10^{-4} \pm 2.3 \cdot 10^{-4}$, $P < 0.05$) of the monkey. There was no difference between morphine and alfentanil in their permeability through monkey dura mater (alfentanil: $1.0 \cdot 10^{-2} \pm 2.2 \cdot 10^{-3}$ cm/min; morphine: $9.4 \cdot 10^{-3} \pm 2.9 \cdot 10^{-3}$, $P > 0.05$) (fig. 3).

**Fig. 2.** Permeability of morphine through the meninges of the dog and monkey. Arachnoid mater and pia–arachnoid mater values are calculated from the measured permeability through dura–arachnoid–pia mater, dura mater, and pia mater. *$P < 0.05$ compared to permeability through monkey pia mater and dura mater. **$P < 0.05$ compared to dog dura mater.

**Fig. 3.** Permeability of morphine and alfentanil through the meninges of the monkey. Pia–arachnoid values are calculated from the measured permeability through dura–arachnoid–pia mater, and dura mater. *$P < 0.05$ compared to morphine permeability through the same monkey meninges.
Discussion

Our data indicate that the principal diffusion barrier for morphine and alfentanil lies in the pia–arachnoid mater, and not the dura mater, of both the dog and the monkey. The dura mater is very permeable to both drugs and is thus relatively unimportant as a determinant of overall meningeal permeability. That this is true in such dissimilar species suggests that it is likely to be true in humans as well. Because morphine and alfentanil have very different physicochemical properties (see below), we hypothesize that the pia–arachnoid is the principal diffusion barrier for all drugs placed in the epidural space.

Further, our studies of morphine’s permeability through monkey pia mater indicate that the arachnoid mater, not the pia mater, is the principal diffusion barrier of the spinal meninges. In fact, the arachnoid mater accounts for 84% of the overall resistance of the meninges to morphine penetration. This finding is consistent with the histologic characteristics of the meninges. The pia and dura mater consist largely of collagen and elastic fibers, whereas the arachnoid mater is formed by overlapping tiers of flattened cells. The cells of the arachnoid mater are connected by frequent zonule occludentes, zonule adherents, and maculae adherentes; these cellular junctions likely account for the greater physiologic barrier presented by the arachnoid mater compared with the pia or dura mater.

Moore et al. reported the permeability coefficient for morphine through previously frozen cadaveric human dura mater to be $3.6 \times 10^{-3}$ cm/min. This is less than the permeability reported here for either the monkey ($9.4 \times 10^{-3} \pm 2.9 \times 10^{-3}$ cm/min) or the dog ($7.62 \pm 2.2 \times 10^{-3}$ cm/min). The variation in dural permeability between this study and the study by Moore et al. may result from species differences or may reflect the fact that this study used live tissue, whereas the study by Moore et al. did not. The fact that morphine was less permeable through intact monkey meninges than through intact dog meninges is likely the result of species differences. There are no detailed morphologic studies comparing dog and monkey meninges to allow speculation as to the anatomic reasons for the observed difference in permeability.

Because we have quantified the meningeal permeability of only two drugs, we are unable to draw firm conclusions about which physicochemical properties of drug molecules govern their meningeal permeability. However, some general inferences are possible. The molecular weight of alfentanil (416) is 40% greater than the molecular weight of morphine (286), yet alfentanil is 3.7 times more permeable through monkey meninges than is morphine. This would suggest that molecular weight is not the primary determinant of a molecule’s meningeal permeability. The octanol–water partition coefficient of alfentanil is 130 times greater than that of morphine (130 vs. 1). This suggests that greater lipid solubility may enhance meningeal permeability, but certainly there is not a linear relationship between the octanol–water partition coefficient and meningeal permeability. Additional physicochemical factors such as molecular volume or molecular radius may be important in determining the meningeal permeability of a drug molecule.

We have developed our in vitro model of meningeal permeability to use as a tool for studying the pharmacokinetics of opioid transfer from the epidural space, through the meninges, and into the spinal cord. A criticism of our model is that it assumes that, in vivo, drugs move from the epidural space to the spinal cord solely by passive diffusion through the meninges. However, some authors have speculated that there also exists a vascular route by which drugs traverse the meninges. They hypothesize that drugs diffuse through the wall of segmental arteries within the epidural space and are then distributed directly to the spinal cord through the anterior and posterior radicular arteries. This is an attractive hypothesis, but it is not supported by the anatomy or histologic characteristics of spinal cord blood supply. First, arterial walls are highly specialized structures that function to separate their intravascular contents from the extravascular space. It seems highly unlikely that large, charged molecules like morphine and fentanyl (two of the most commonly used epidural opioids) would be capable of diffusing through the wall of a segmental artery to gain access to the arterial blood supply of the spinal cord. Second, there are no segmental arteries in the lumbar epidural space (where the bulk of epidural opioids are most often placed) that contribute to spinal cord blood flow. Finally, in a study by Bromage et al. in which $^{14}$C-lidocaine was placed in the epidural space of dogs, autoradiographs of the animals’ spinal cords located the drug circumferentially in the periphery of the spinal cord. However, most spinal cord blood flow is to the gray matter in the center of the cord, thus one would have expected to find the lidocaine located preferentially in this area if it were distributed by a vascular route.

An additional criticism of our study is that the process of separating one meninx from another may disrupt tissue integrity, resulting in inaccurate measurements of permeability. However, the dura mater is held to the pia–arachnoid by occasional denteate ligaments that were easily severed without damage to either the dura or pia–arachnoid. The arachnoid is attached more diffusely to the pia by cellular extensions of the arachnoid. The arachnoid was removed by gently peeling it off the pia mater, and inspection of the pia mater under a dissecting microscope showed no evidence of damage. In addition, the fact that the variability in measured pia mater permeability is no greater than the variability of the dura or dura–arach-
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References