

LABORATORY REPORT

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Barrier Properties of the Spinal Meninges Are Markedly Decreased by Freezing Meningeal Tissues

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THE increased interest in epidural anesthesia and analgesia and the diverse nature of the drug classes now administered into the epidural space (e.g., α -agonists, local anesthetics, opioids, steroids, conopeptides, and others) has renewed interest in understanding the processes governing drug movement between the epidural space and the spinal cord. One common approach to studying drug transfer across the spinal meninges has been the use of a classical diffusion cell model, in which a section of the meninges is mounted *in vitro* into a diffusion cell apparatus. The rate at which a study drug diffuses through the meninges from a donor reservoir to the recipient reservoir is quantified to the permeability of the coefficient of the drug. Some of these studies have used fresh spinal meninges obtained from living animals,¹⁻⁴ whereas others have relied on cadaveric human tissue previously frozen for variable periods of time before study.⁵⁻⁸

It has never been shown that the barrier properties of frozen meningeal tissue are the same as those of fresh tissue; still, diffusion cell studies using fresh and frozen meningeal tissues continue to be submitted to anesthesia journals for publication (personal observation, C.M.B.). To address this concern, we directly compared the permeability of morphine through fresh and dry-frozen spinal meninges. The permeability of morphine across frozen cadaver meninges has been reported previously^{5,6}; morphine shows low permeability, presumably caused

by its low lipid solubility. We measured the permeability of morphine through fresh spinal meninges obtained from anesthetized monkeys and then repeated this measurement through the same tissues after they were dry-frozen at -20°C .

Methods

The methods for obtaining fresh meningeal tissue and for determining permeability coefficients have been described in detail previously.¹⁻⁴ Briefly, thoracic spinal cords of seven *Macaca nemestrina* monkeys were carefully removed *en bloc* and placed in PBS within 10-30 min of the animal's death by barbiturate overdose. After ventral incision through all three meningeal layers, the dura, arachnoid, and pia mater were reflected from the spinal cord, preserving their normal anatomic relation. Specimens of approximately 2 cm^2 were placed carefully between two halves of a temperature-controlled diffusion cell. The specimens were oriented so that the dura mater side was exposed to the donor reservoir and the pia mater side was exposed to the recipient reservoir.

Fluid reservoirs on either side of the tissue were filled with bicarbonate buffered mock cerebrospinal fluid; oxygen (95%) and carbon dioxide (5%) were bubbled vigorously through the mock cerebrospinal fluid to maintain normal pH level and to provide mixing. After allowing 30 min for equilibration to 37°C , the study drug was added to the epidural side of the meninges. For this experiment, unlabelled morphine sulfate (0.1-mg base) and $x\text{ uCi }^{14}\text{C}$ -labeled morphine sulfate (specific activity = $82 \times 10^3\text{ uCi/mg}$; New England Nuclear, Boston, MA) were added to the donor reservoir at $t = 0$. Thereafter at 5-min intervals, 100- μl samples were removed from donor and recipient reservoirs and placed in liquid scintillation vials for subsequent determination of morphine concentration. Decays/min were counted in a Packard TriCarb 2000 liquid scintillation counter (Packard Instruments, Downers Grove, IL; uncertainty of measurement 4% for 2,500 total counts). At the end of the

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experiment, the fluid reservoirs were emptied and the cell chambers were rinsed once gently with fresh mock cerebrospinal fluid. The diffusion cells were wrapped in plastic with the meningeal tissue position undisturbed and were dry-frozen at -20°C for 2–15 days. The cells were then brought out to equilibrate at room temperature for 1 h, refilled with mock cerebrospinal fluid at room temperature, reequilibrated to 37°C , and studied again, as described before.

The permeability coefficient of morphine was determined as previously described in detail.² Briefly, morphine flux was determined as the slope of a plot of morphine concentration (C) versus time (from $t = 30$ to $t = 90$ min), and the permeability coefficient was calculated from the equation $P = Q/AC$, where P = permeability coefficient, Q = flux and A = meningeal surface area. Least-squares linear regression was used to determine the slope of the plot described before.

Statistical Analysis

Differences in the permeability coefficient of morphine between fresh and frozen tissue was evaluated by Fisher's paired Student's t test. Differences in the permeability coefficient of morphine among tissues frozen for different periods of time were assessed by analysis of variance. Differences were considered significant at a $P \leq 0.05$.

Results

An example of morphine flux curves for one tissue sample before and after dry-freezing are shown in figure 1. The r value for linear regression lines used to determine morphine flux averaged 0.97 ± 0.02 for the fresh meningeal tissue and 0.96 ± 0.06 after the tissue was frozen, indicating excellent fit to a linear model in both sets of experiments.

The permeability coefficient for morphine through fresh tissue was $0.64 \pm 0.14 \text{ cm/min} \times 10^{-3}$ and for frozen tissue was $3.2 \pm 1.8 \text{ cm/min} \times 10^{-3}$ ($P = 0.001$ for the difference of the mean) This represents a permeability increase of $500 \pm 235\%$ for the tissue after freezing. No significant difference in permeability coefficients was found among tissues frozen for 2, 6, 7, or 15 days.

Discussion

The data clearly indicate that freezing the spinal meninges markedly increases the *in vitro* permeability of

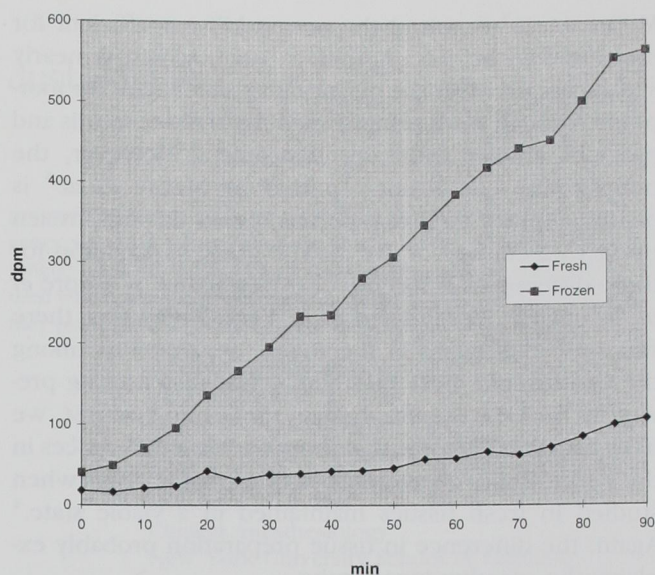


Fig. 1. Permeability of morphine sulfate through fresh and frozen-thawed spinal meninges in one tissue sample. Radiolabeled morphine sulfate (0.1-mg base in $100 \mu\text{l}$; SA 82×10^3 uCi/mg) added to the reservoir at $t = 0$. Samples from reservoir B, $100 \mu\text{l}$, taken every 5 min, and depositions/min counted by liquid scintillitography.

morphine when compared to fresh tissue. Of note, the value reported for morphine permeability through fresh tissue in these experiments is identical to that reported in earlier studies from this laboratory ($0.64 \pm 0.17 \text{ cm/min} \times 10^{-3}$), indicating that the model is inherently reproducible.

The principal barrier to diffusion in the meninges has been shown histologically^{9,10} and functionally² to be within the arachnoid layer. The arachnoid contains a barrier cell layer with frequent tight junctions that effectively seal plasma membranes of adjoining cells.^{9,10} Stripping of the arachnoid layer in *M nemestrina* increases the meningeal permeability of opiates nearly 10-fold.² In cadaveric frozen tissue, cell death and/or tissue disruption, caused by ice crystal formation might certainly alter the microanatomy of the arachnoid barrier cell layer and cause a substantial increase in the apparent permeability of epidural drugs.

The results of this study may also explain some seeming inconsistencies in the literature regarding the barrier properties of the spinal meninges. For example, Moore *et al.*,⁵ using previously frozen cadaveric human tissues, concluded that molecular weight was the principle determinant of meningeal permeability. In contrast, using the model described in this study, previous reports from this laboratory showed that molecular weight plays no role in determining meningeal permeability.⁴ Also,

Moore *et al.*⁵ reported the permeability coefficient for morphine to be $3.6 \text{ cm/min} \times 10^{-3}$, which is nearly sixfold greater than the permeability coefficient for morphine through fresh primate meningeal tissue in this and previous studies from our laboratory.² However, the permeability coefficient reported by Moore *et al.*⁵ is nearly identical to that reported herein through frozen tissue ($3.2 \text{ cm/min} \times 10^{-3}$). The likely explanation for these differences is the use of frozen tissue by Moore *et al.*⁵ Similarly, McEllistrem *et al.*⁶ concluded that there was little difference in meningeal permeability among the opioids and local anesthetics they tested using previously frozen cadaveric human tissues. In contrast, we have previously shown that there are clear differences in the permeability characteristics of the same drugs when studied in fresh tissues maintained in a viable state.⁴ Again, the difference in tissue preparation probably explains the seeming incongruity.

In summary, studies of meningeal permeability and the factors governing it might best be performed *in vitro*, where uncontrollable variables such as vascular uptake, drug absorption into epidural fat, and others are not a factor. However, *in vitro* studies are only useful to the extent that the tissue being investigated behaves as it does *in vivo*. The results of this study suggest that frozen meningeal tissues do not behave as they would *in vivo* and are inappropriate for studies of drug permeability.

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