Anesthesiology
1996; 84:392–6

Palmitoyl Carnitine Increases the Transmeningeal Flux of Hydrophilic but Not Hydrophobic Compounds In Vitro

Christopher M. Bernards, M.D., Christian Kern, M.D.†*

Background: **Increasing drug penetration through the spinal meninges should improve epidural analgesia/anesthesia by increasing the rate and extent to which epidurally administered drugs redistribute to the spinal cord. Palmitoyl carnitine has been shown to improve drug penetration through the intestinal mucosa. The purpose of this study was to determine whether palmitoyl carnitine improves drug penetration through the spinal meninges.**

Methods: **The transmeningeal flux of morphine, mannitol, bupivacaine, and sufentanil was determined through the spinal meninges of *Macacca nemestrina* monkeys before and after addition of palmitoyl carnitine. Flux was determined using a previously established *in vitro* diffusion cell model.**

Results: **Palmitoyl carnitine significantly increased the transmeningeal flux of mannitol, morphine, and bupivacaine by 942 ± 102%, 401 ± 43%, and 12 ± 4%, respectively. However, palmitoyl carnitine had no significant effect on the transmeningeal flux of sufentanil. The penetration-enhancing effect of palmitoyl carnitine was shown to depend on the dose of palmitoyl carnitine added and on the octanol:buffer distribution coefficient of the study drugs but not on the concentration of the study drug.**

Conclusions: **The mechanism by which palmitoyl carnitine increases transmeningeal flux is unclear but may be a result of palmitoyl carnitine's ability to decrease packing order of lipid bilayers in the arachnoid mater cell membranes. Regardless of the mechanism, palmitoyl carnitine's ability to selectively increase the transmeningeal flux of hydrophilic compounds *in vitro* offers the possibility of improving the spinal bioavailability of this group of epidurally administered drugs *in vivo*. (Key words: Anesthetics, local: bupivacaine. Mannitol. Monkeys: *Macacca nemestrina*. Opioids: morphine; sufentanil. Sites of action: drug flux; spinal meninges.)**

* Associate Professor.

† Senior Research Fellow.

Received from the Department of Anesthesiology, University of Washington, Seattle, Washington. Submitted for publication July 5, 1995. Accepted for publication October 17, 1995. Supported by National Institute of Drug Abuse grant RO1DA-07313. Monkey tissues were obtained from the Regional Primate Research Center at the University of Washington, supported by National Institutes of Health grant RR00166.

Address reprint requests to Dr. Bernards: Department of Anesthesiology, Box 356540, University of Washington, Seattle, Washington 98195. Address electronic mail to: chrisb@u.washington.edu.

SPINALLY active drugs administered into the epidural space must cross the spinal meninges to reach their sites of action in the spinal cord. The available experimental evidence demonstrates that the arachnoid mater is the principal meningeal permeability barrier[1] and that diffusion is the principal mechanism by which drugs cross the meninges.[2–4] Thus, developing methods to improve drug diffusion through the arachnoid mater offers the possibility of improving epidural analgesia/anesthesia by increasing the rate and extent to which epidurally administered drugs redistribute to the spinal cord. We demonstrated previously that drug flux through the spinal meninges can be increased by coadministration of an appropriate adjuvant. In particular, hydroxypropyl-β-cyclodextrin was shown to significantly increase the transmeningeal flux of sufentanil.[5] However, cyclodextrin was ineffective at increasing the transmeningeal flux of more hydrophilic compounds.

Acylcarnitines are a ubiquitous group of amphipathic molecules that are used by mammalian cells to shuttle fatty acids across the inner mitochondrial membrane.[6,7] Palmitoyl carnitine has been shown to increase the absorption of drugs across the intestinal mucosa *in vitro*[8] and *in vivo*.[9,10] The purpose of this study was to determine if palmitoyl carnitine is similarly able to increase the diffusion of drugs through the spinal meninges.

To address this question, we used a previously described diffusion cell model[1,2,4,5] to quantitate the transmeningeal flux of morphine, sufentanil, mannitol, and bupivacaine across monkey spinal meninges before and after the addition of palmitoyl carnitine. Multiple does of palmitoyl carnitine and of the study drugs were investigated to determine if the effects were dose-related.

Materials and Methods

Studies were approved by the University of Washington Animal Care and Use Committee and guidelines of the American Association for Accreditation of Laboratory Animal Care were followed throughout.

Tissue Preparation

Monkey (*Macacca nemestrina*) tissues were obtained from animals scheduled to be killed as part of the tissue distribution program of the University of Washington Regional Primate Research Center. All animals were anesthetized with thiopental and ketamine before removal of the meningeal specimens.

The spinal cords were exposed from T5 to L5 by laminectomy. The spinal cord was removed *en bloc* and all three meningeal layers were carefully reflected from the spinal cord, preserving their normal anatomic relationships. From this sheet of intact meningeal tissues, specimens measuring approximately 4 cm^2 were cut for mounting in the diffusion cell.

Flux Measurements

The intact spinal meninges were placed between two halves of a temperature-controlled (37°C) diffusion cell. Ten milliliters of mock cerebrospinal fluid (NaCl 140 mEq, $NaHCO_3$ 25 mEq, KCl 2.9 mEq, $MgCl_2$ 0.4 mEq, urea 3.5 mEq, glucose 4.0 mEq, $CaCl_2$ 2.0 mEq; pH = 7.38–7.42; 292–298 mOsm) were placed in the fluid reservoirs on either side of the meningeal tissue. Air and carbon dioxide (5%) were bubbled through each fluid reservoir to maintain normal pH and to provide oxygen to the meningeal cells.

After allowing at least 20 min for the chambers to equilibrate to 37°C, one or two study drugs and the corresponding ^{3}H-and/or ^{14}C-labeled radiotracer were added to the donor reservoir on the dura mater side of the diffusion cell. In most experiments, the flux of two different drugs were measured simultaneously. The drugs studied were morphine (2.6 μM), sufentanil (2.6 μM), mannitol (0.26, 2.6, and 26 μM) and bupivacaine (150 μM) The radiotracers used were ^{3}H-morphine (specific activity 62 μCi/mmol; radiochemical purity 98.7%, New England Nuclear), ^{3}H-sufentanil (specific activity 9 μCi/mmol; radiochemical purity 99%, Janssen Pharmaceutica, Belgium), ^{14}C-mannitol (specific activity 56.7 μCi/mmol; radiochemical purity 98%, New England Nuclear), and ^{14}C-bupivacaine (specific activity 3.57 μCi/mmol; radiochemical purity 98.4%, New England Nuclear). After addition of the study drugs and radiotracers, 200-μl samples were removed from the donor and recipient reservoirs at 10-min intervals for 100 min. The samples were placed in borosilicate scintillation vials for later scintillation counting to determine drug concentration.

At t = 100 min, 0.05, 0.5, 2.5, or 5 mM palmitoyl carnitine was added to the donor reservoir and 200-μl samples were collected from both reservoirs for an additional 100 min. These samples were also placed in borosilicate scintillation vials for later scintillation counting to determine drug concentration. The palmitoyl carnitine concentrations studied were 0.05, 0.5, 2.5, and 5 mM for mannitol and 5 mM only for morphine, sufentanil, and bupivacaine.

Drug flux was determined from drug concentration data by plotting the amount of drug in the recipient reservoir at each time point. The slope of the line relating concentration *versus* time data was determined by least-squares linear regression and is equal to the test drug's flux through the meninges. Because of the unavoidable delay in reaching the new flux rate after addition of palmitoyl carnitine (fig. 1), flux was determined from the samples collected between 30 and 100 min (before adding palmitoyl carnitine) and between 130 and 200 min (after adding palmitoyl carnitine). Thus, the reported flux values represent steady-state conditions and not initial flux conditions.

Drug Analysis

Hydrofluor scintillation fluid (5–10 ml; National Diagnostics, Manville, NJ) was added to each sample and the samples were counted in a Packard liquid scintillation counter (Tricarb 2000, Packard Instruments, Downers Grove, IL) for 10 min or until the standard deviation of depurations per minute was ≤2%. Background radioactivity was determined by counting mock cerebrospinal fluid without added radiotracer and was subtracted from the depurations per minute of each sample.

Statistical Analysis

Differences in drug flux before and after addition of palmitoyl carnitine were assessed by Student's paired *t* test. The effect of palmitoyl carnitine dose and mannitol dose on drug flux was assessed by analysis of variance. Differences were considered statistically significant at the $P \leq 0.05$ level. All results are reported as mean ± standard error.

Results

Figure 1 shows raw data from representative experiments. After converting depurations per minute to mM,

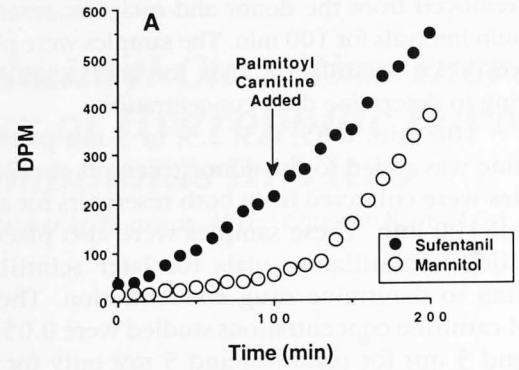

Fig. 1. Raw depurations per minute (DPM)/sample data from two representative experiments. (*A*) Simultaneous measurements of sufentanil and mannitol flux through the same piece of tissue before and after addition of palmitoyl carnitine. (*B*) Simultaneous measurements of bupivacaine and morphine flux through a second piece of tissue before and after addition of palmitoyl carnitine.

linear regression was used to determine drug flux (mM/min) from these plots before and after addition of palmitoyl carnitine. The correlation coefficient (r) for the regression lines averaged 0.98 ± 0.0012 (range 0.91–1.0) indicating excellent fit of the data to a linear model.

Table 1 shows the effect of 5-mM palmitoyl carnitine on drug flux through the spinal meninges. The flux of mannitol, morphine, and bupivacaine increased significantly after addition of palmitoyl carnitine. In contrast, the flux of the more hydrophobic compound, sufentanil, was not significantly altered by addition of palmitoyl carnitine. Figure 2 more clearly shows the relationship between the hydrophilic character of the study drugs and palmitoyl carnitine's effect on drug flux. The ability of palmitoyl carnitine to increase drug flux decreased linearly ($P = 0.0001$) as the log octanol:buffer distribution coefficient of the study drugs increased (correlation coefficient = 0.989).

The effect of palmitoyl carnitine concentration on drug flux was determined by measuring mannitol flux before and after addition of 0.05 mM (n = 7), 0.5 mM (n = 6), 2.5 mM (n = 4), or 5 mM (n = 8) palmitoyl carnitine. Figure 3 demonstrates that the magnitude of the change in mannitol flux after addition of palmitoyl carnitine increased significantly ($P = 0.0001$) as the palmitoyl carnitine dose increased (correlation coefficient = 0.999).

The effect of study drug concentration on the palmitoyl carnitine-mediated increase in drug flux was determined by measuring mannitol flux at three different mannitol concentrations (0.26 mM, n = 7; 2.6 mM, n = 8; and 26 mM, n = 8). Figure 4 demonstrates that mannitol concentration had no significant effect ($P = 0.7150$) on the percentage increase in mannitol flux after addition of palmitoyl carnitine (5 mM).

Discussion

The results indicate that palmitoyl carnitine significantly increases the *in vitro* transmeningeal flux of a variety of drugs in a dose dependent manner. This finding is consistent with the work of Sutton *et al.*, who demonstrated that 5 mM palmitoyl carnitine increases cefoxitin permeability through the intestinal mucosa by 20-fold *in vitro*.[8] *In vivo* studies have similarly demonstrated that palmitoyl carnitine induces a dose-dependent increase in the intestinal permeability of a variety of compounds.[9,10] In contrast to the current investigation, those studies of drug penetration through the intestinal mucosa did not demonstrate a relationship between hydrophobicity of the study drugs and the effect of palmitoyl carnitine. Whether this difference reflects tissue differences, species differences, or differences in the chosen study drugs is unclear.

Because the arachnoid mater accounts for nearly 90% of the resistance to drug diffusion through the spinal meninges,[1] palmitoyl carnitine must exert its penetration-enhancing effects on this tissue. However, the exact mechanism by which palmitoyl carnitine increases transmeningeal flux is not clear. LeCluyse *et al.* used brush border membranes labeled with 1,6-diphenyl-1,3,5-hexatriene to demonstrate that palmitoyl carnitine partitions into membranes and thereby decreases the degree of structural order in the hydrophobic core of the lipid bilayer.[11] This observation is consistent with

Table 1. Drug Flux before and after Addition of 5 mM Palmitoyl Carnitine

Drug	n	Flux before PC ($pM \cdot min^{-1} \cdot cm^{-2}$)	Flux after PC ($pM \cdot min^{-1} \cdot cm^{-2}$)	% Change in Flux after PC	Significance
Bupivacaine	13	343 ± 35	338 ± 31	12 ± 4	0.0304
Mannitol	8	1.07 ± 0.4	8.96 ± 2.1	942 ± 102	0.0025
Morphine	7	1.71 ± 0.2	8.12 ± 0.5	401 ± 43	0.0001
Sufentanil	8	4.84 ± 0.7	4.43 ± 0.06	−0.25 ± 14	NS

PC = palmitoyl carnitine; NS = not significant.

palmitoyl carnitine's known function to translocate fatty acids through the inner mitochondrial membrane. However, it is not clear exactly how disruption of the ordering of lipid bilayers results in greater drug permeability.

One possibility suggested by the data from this study is that the palmitoyl carnitine-induced decrease in membrane packing order increases the ease with which hydrophilic molecules partition into the lipid bilayer of the arachnoid mater cells. By lowering the activation energy required for hydrophilic molecules to traverse the lipid bilayer, transcellular flux of hydrophilic molecules would be expected to increase significantly. In contrast, this hypothesis would predict that palmitoyl carnitine would not significantly affect the flux of hydrophobic drugs because the ability of these drugs to cross the arachnoidal membranes should not be limited by their ability to partition into the hydrophobic environment of the lipid bilayer. Thus, that bupivacaine flux was minimally increased by palmitoyl carnitine and sufentanil flux was not significantly changed is consistent with this hypothesis.

An alternative explanation is that palmitoyl carnitine is toxic at the supraphysiologic concentrations employed and that drug flux increases because the arachnoid cells were damaged and the arachnoid mater became "leaky." However, because the fluxes of a hydrophobic drug (sufentanil or bupivacaine) and a hydrophilic drug (mannitol or morphine) were almost always measured simultaneously through the same piece of tissue, one would expect that damaged cells and leaking membranes would result in similar increases in the flux of both drugs. However, as demonstrated in figure 1, this was not the case. Also, that mannitol flux increased linearly as palmitoyl carnitine concentration increased indicates that it is the presence of palmitoyl carnitine that is responsible for the increase in mannitol flux, not the passage of time. In addition, we have demonstrated previously that the permeability coefficient for morphine in this prepara-

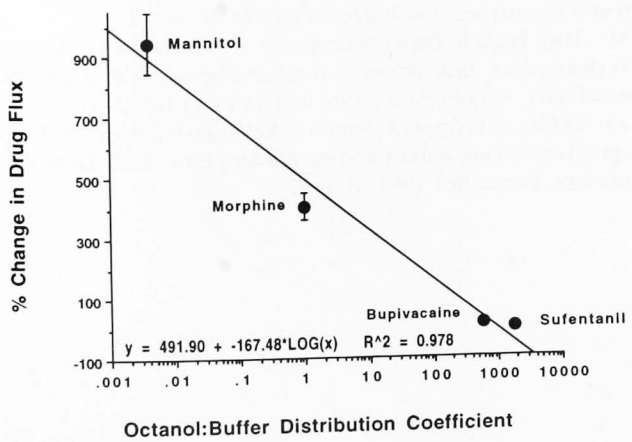

Fig. 2. The relationship between the octanol:buffer distribution coefficient of the study drugs and the percentage change in drug flux after addition of palmitoyl carnitine. The equation of the regression line through the data points and the determination coefficient (r^2) also is presented.

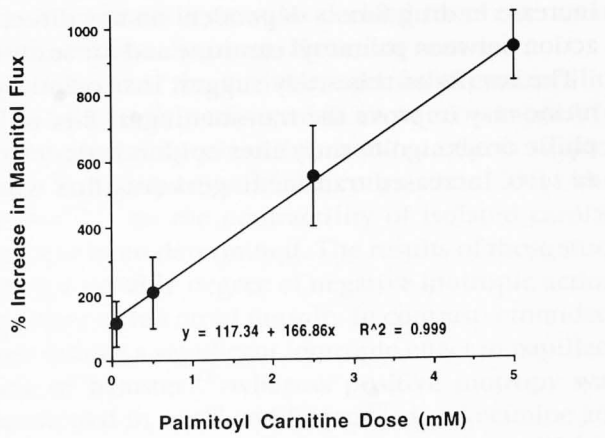

Fig. 3. The relationship between the dose of palmitoyl carnitine and the percentage change in drug flux after addition of palmitoyl carnitine. The equation of the regression line through the data points and the determination coefficient (r^2) also is presented.

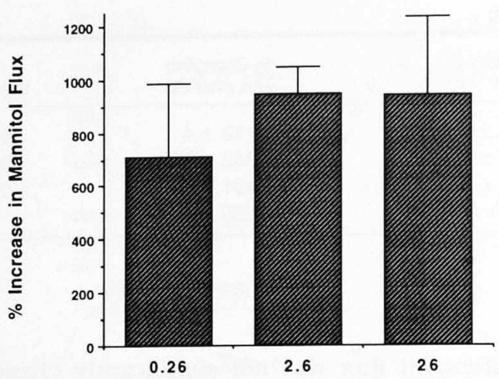

Fig. 4. The relationship between mannitol dose and the percentage increase in mannitol flux after addition of 5 mM palmitoyl carnitine.

tion is stable for at least 3 h.[1] Finally, studies investigating the effect of palmitoyl carnitine on drug flux through intestinal tissue found that the penetration-enhancing effects of palmitoyl carnitine were reversible[8,10] and that there was no electron micrographic evidence of cell damage at the same palmitoyl carnitine concentrations used in the current study.[10] Thus, tissue damage would not seem to be a plausible explanation for the observed increase in drug flux.

That mannitol dose had no effect on the magnitude of the flux increase after addition of palmitoyl carnitine indicates that the flux increase was not dependent on a particular stoichiometric relationship between palmitoyl carnitine and mannitol. This observation would seem to rule out the possibility that palmitoyl carnitine functions as a "carrier" for the study drugs or that the increase in drug flux is dependent on any direct interaction between palmitoyl carnitine and the study drugs.

The results of this study suggest that palmitoyl carnitine may improve the transmeningeal flux of hydrophilic drugs significantly after epidural administration *in vivo*. Increased transmeningeal drug flux would be expected to result in greater drug delivery to the spinal cord and reduced drug redistribution from the epidural space to the systemic circulation. As a result, the therapeutic index for spinally active drugs would be expected to be increased. However, before use of palmitoyl carnitine can be advocated, the results of this study must first be replicated in an *in vivo* animal model and formal toxicity studies must be conducted.

References

1. Bernards C, Hill H: Morphine and alfentanil permeability through the spinal dura, arachnoid and pia mater of dogs and monkeys. ANESTHESIOLOGY 1990; 73:1214–9

2. Bernards C, Hill H: Physical and chemical properties of drug molecules governing their diffusion through the spinal meninges. ANESTHESIOLOGY 1992; 77:750–6

3. Bernards C, Sorkin L: Radicular artery blood flow does not redistribute fentanyl from the epidural space to the spinal cord. ANESTHESIOLOGY 1994; 80:872–8

4. Bernards C, Hill H: The spinal nerve root sleeve is not a preferred route for redistribution of drugs from the epidural space to the spinal cord. ANESTHESIOLOGY 1991; 75:827–32

5. Bernards C: Effect of (hydroxypropyl)-b-cyclodextrin on the flux of morphine, fentanyl, sufentanil, and alfentanil through the spinal meninges of the monkey. J Pharm Sci 1994; 83:620–2

6. Bremer J: Carnitine in intermediary metabolism. The metabolism of fatty acid esters of carnitine by mitochondria. J Biol Chem 1962; 237:3628–32

7. Bremer J: Carnitine—metabolism and functions. Physiol Rev 1983; 63:1420–80

8. Sutton S, Forbes A, Cargill R, Hochman J, LeCluyse E: Simultaneous in vitro measurement of intestinal tissue permeability and transepithelial electrical resistance (TEER) using sweetana-grass diffusion cells. Pharm Res 1992; 9:316–9

9. Sutton S, LeCluyse E, Cammack L, Fix J: Enhanced bioavailability of cefoxitin using palmitoyl l-carnitine. I. Enhancer activity in different intestinal regions. Pharm Res 1992; 9:191–4

10. Fix J, Engle K, Porter P, Leppert P, Selk S, Gardner C, Alexander J: Acylcarnitines: drug absorption-enhancing agents in the gastrointestinal tract. Am J Physiol 1986; 251 (3 pt 1):G332–40

11. LeCluyse E, Appel L, Sutton S: Relationship between drug absorption enhancing activity and membrane perturbing effects of acylcarnitines. Pharm Res 1991; 8:84–7