EFFECTS OF A POTENT AND SPECIFIC P-GLYCOPROTEIN INHIBITOR ON THE BLOOD-BRAIN BARRIER DISTRIBUTION AND ANTINOCICEPTIVE EFFECT OF MORPHINE IN THE RAT

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ABSTRACT:

Previous data suggest that the analgesic effect of morphine may be modulated by P-glycoprotein (P-gp) inhibition. The effects of the P-gp inhibitor GF120918 on brain distribution and antinociceptive effects of morphine were examined in a rat cerebral microdialysis model. Pretreatment with GF120918 increased both the area under the concentration-time curve of unbound morphine in brain extracellular fluid (ECF) and morphine-associated antinociception. The area under the concentration-time curve ratio for unbound morphine in brain ECF versus unbound morphine in blood was significantly higher in GF120918-treated rats compared with control rats (1.21 ± 0.34 versus 0.47 ± 0.05, respectively; p < .05). Modulation of morphine brain-blood distribution was confirmed by quantitating brain tissue morphine in a separate group of rats; GF120918 increased the brain tissue:serum concentration ratio approximately 3-fold. The half-life of unbound morphine in brain ECF was approximately 3-fold longer in GF120918-treated rats compared with controls (p < .05). The fraction unbound of morphine in whole blood was not altered significantly in the presence of GF120918 (0.651 ± 0.039) as compared with controls (0.662 ± 0.035). Concentrations of unbound morphine-3-glucuronide in blood and brain ECF were increased in GF120918-treated rats versus controls. An integrated pharmacokinetic/pharmacodynamic model was developed to characterize the unbound blood and brain ECF morphine concentration profiles and concentration-effect relationships. The results of this study indicate that alteration of morphine antinociception by a potent P-gp inhibitor appears to be mediated at the level of the blood-brain barrier.

P-glycoprotein (P-gp),2 a product of the multidrug resistance (MDR) gene, is a transmembrane glycoprotein that is expressed in MDR tumor cells. This plasma membrane protein pump extrudes various chemotherapeutic agents from tumor cells and is one mechanism of MDR (Pastan and Gottesman, 1991). Expression of P-gp has been observed in specialized epithelial and endothelial cells with either secretory or excretory functions (Thiebaut et al., 1987; Cordon-Cardo et al., 1990; Thorgeirsson et al., 1991; Speeg et al., 1992a,b). P-gp located in brain capillary endothelial cell membranes has been thought to function as a component of the blood-brain barrier (BBB; Tsuji et al., 1992; Sakata et al., 1994; Samoto et al., 1994; Chikhale et al., 1995).

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2 Abbreviations used are: P-gp, P-glycoprotein; AUC, area under the concentration-time curve; AUE, area under the effect-time curve; BBB, blood-brain barrier; ECF, extracellular fluid; f u, fraction unbound; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; MRT, multidrug resistance/resistant; MRT, mean residence time; ; V ss, steady-state volume of distribution; CNS, central nervous system; %MPR, percentage of maximum possible response.

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P-gp has limited substrate specificity; a cationic nitrogen group is a characteristic common to many compounds transported by P-gp (Zamora et al., 1988). The physicochemical characteristics of morphine, as well as recent experimental evidence, are consistent with the hypothesis that morphine is a substrate for P-gp (Dahlstrom et al., 1986; Callaghan and Riordan, 1993; Schinkel et al., 1995; Zamora et al., 1988). Indeed, the relatively limited ability of morphine to penetrate the BBB (Oldendorf et al., 1972) is consistent with extrusion at the blood-brain interface, and recent data have demonstrated enhanced analgesia when morphine was coadministered with a P-gp inhibitor (Letrent et al., 1998). In addition, in vitro experiments indicated that morphine is transported by P-gp in brain capillary endothelium; the apparent BBB permeability of morphine was altered by P-gp inhibitors (Letrent et al., 1999). Inhibition of P-gp-mediated efflux from brain capillary endothelial cells may have significant implications for central nervous system (CNS) morphine disposition, thus P-gp inhibition may modulate the analgesic effect of morphine. Additional mechanisms by which P-gp inhibitors could alter the systemic disposition of morphine include inhibition of P-gp-mediated biliary excretion, renal excretion, and intestinal transport.

GF120918 is a potent and specific inhibitor of P-gp in rats and humans (Hyafil et al., 1993; Witherspoon et al., 1996) under development by Glaxo Wellcome, Inc. Unlike several of the first generation P-gp modulators (e.g., verapamil and cyclosporin A), GF120918 inhibits P-gp in vivo without significant toxicities or side effects (Hyafil et al., 1993). GF120918 is consistently active in vitro
models of P-gp inhibition at concentrations of ~20 nM. The lack of sensitization to drugs that are not P-gp substrates, as well as the absence of effect on MDR-negative cell lines, such as wild-type MCF7 and lymphocytic cell lines, is evidence for the specificity of GF120918 (Hyafil et al., 1993). Previous experiments have demonstrated that GF120918 enhances antinociception associated with i.v. morphine in vivo; the alteration in pharmacologic response could not be attributed to changes in systemic concentrations of morphine (Letrent et al., 1998). In addition, GF120918 and other P-gp inhibitors altered morphine transport across brain capillary endothelial cells in vitro (Letrent et al., 1999). Taken together, these observations suggest that GF120918 increases morphine-associated antinociception by decreasing morphine efflux at the blood-brain interface. In vitro model systems are important for the study of cellular, biochemical, and molecular features of BBB transport in an isolated and controlled fashion. However, only an in vivo study can reproduce fully the complexity of morphine metabolism, BBB disposition, and pharmacologic effect.

The present study was designed to evaluate the effect of P-gp inhibition on blood-brain distribution and antinociceptive pharmacodynamics of morphine. A rat in vivo microdialysis model in conjunction with the radiant heat tail-flick assay was selected for use in these experiments to determine simultaneously brain extracellular fluid (ECF) and blood concentrations of morphine as well as morphine-associated pharmacologic activity.

**Experimental Procedures**

**Materials.** Morphine sulfate was purchased from Sigma Chemical Company (St. Louis, MO). [3H]-Morphine was purchased from New England Nuclear Life Sciences Products (Boston, MA) (>98.5% pure as determined by HPLC) and was used without further purification. N-[4-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]phenyl-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) was donated by Glaxo Wellcome, Inc. (Research Triangle Park, NC). Acetonitrile, trifluoroacetic acid, and ammonium sulfate were of analytical grade; hydroxypropylmethylcellulose and Tween 80 were of pharmaceutical grade. GF120918 suspension (300 mg base/ml) was stored in a tightly sealed glass container and protected from light. Morphine sulfate was dissolved in 0.9% sterile saline for injection to achieve a final morphine base concentration of 1 mg/ml.

**Microdialysis Probes and Perfusate.** CNS microdialysis probes and guide cannulae (CMA/12) and soft tissue microdialysis probes and introducers (CMA/20) were purchased from CMA/Microdialysis (Acton, MA). Both probe types had a 4-mm polycarbonate membrane with a 20-kDa molecular mass (CMA/20) were purchased from CMA/Microdialysis (Acton, MA). Both probe types had a 4-mm polycarbonate membrane with a 20-kDa molecular mass (CMA/20) were purchased from CMA/Microdialysis (Acton, MA). Both probe types had a 4-mm polycarbonate membrane with a 20-kDa molecular mass (CMA/20) were purchased from CMA/Microdialysis (Acton, MA).

Body temperature was maintained at 37°C with a heating pad and rectal temperature probe. A silicone rubber cannula filled with heparinized saline (20 U/ml) was placed into the left femoral vein and exteriorized at the back of the animal. The soft tissue microdialysis probe (CMA/20) was inserted into the right jugular vein with the inlet and outlet tubing exteriorized at the back of the animal. The rat was immobilized in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), the skull was surgically exposed, and a hole was trephined into the skull based on stereotaxic coordinates (Paxinos and Watson, 1986). The probe guide (CMA/12) was placed into the left frontal cortex (1.0 mm lateral to midline and 3.0 mm anterior to bregma). An additional hole was trephined to allow insertion of an anchoring screw. The probe guide was secured to the skull and anchoring screw with acrylic dental cement. A dummy cannula was used to plug the guide. Animals were allowed to recover for 4 days before experiments. Cannulae were flushed daily with heparinized saline to maintain patency. On the day of the experiment, the dummy cannula in the cortical probe guide was replaced with a microdialysis probe (CMA/12). The jugular and cortical probes were perfused with artificial cerebrospinal fluid at a rate of 1 µl/min throughout the experiment. During the study, each rat was housed in a bowl-shaped cage and tethered to a swivel to prevent tangling of the infusion tubing (CMA/120 System for Freely Moving Animals, CMA/Microdialysis, Acton, MA).

**Influence of GF120918 on Morphine Disposition and Antinociception.** A two-way parallel design was used to compare the disposition and action of morphine, as assessed by cerebral microdialysis and the radiant heat tail-flick assay, after an i.v. bolus dose of morphine in the presence or absence of the P-gp inhibitor GF120918. Rats in the GF120918 treatment group received 500 mg/kg GF120918 via oral gavage daily for 4 days before morphine administration. In a previous study, this dosing regimen altered the pharmacodynamics of morphine in rats (Letrent et al., 1998). On the day of the study the dummy cannula was removed, the cortical microdialysis probe was inserted slowly, the baseline tail-flick response was determined, and a predose microdialysate sample was obtained. Microdialysis probes were perfused (1 µl/min) for 2 h before morphine administration.

After equilibration of the microdialysis probes, morphine (1 mg/kg containing 100 µCi [3H]morphine) was administered as an i.v. bolus. Serial tail-flick determinations and microdialysate samples were obtained at 15, 45, 75, 105, and 135 min after the morphine dose. Tail-flick latency was assessed in duplicate utilizing a radiant heat tail-flick analgesia meter (model 0570–001L, Columbus Instruments International, Columbus, OH). The instrument was operated in the auto-detect mode with both optical sensors active; lamp intensity was set at 10, which produced a baseline tail-flick response in < 3 s. A point 5 cm from the distal end of the tail was exposed to the lamp via a shutter mechanism and the time that elapsed between the shutter opening and tail-flick was recorded. A maximum response time of 10 s was set to minimize damage to the tail during multiple evaluations.

After the last microdialysate samples were collected, in vivo probe recovery of morphine and morphine-3-glucuronide (M3G) was estimated by retrodialysis of [3H]morphine and unlabeled morphine (100 nM). For accurate determination of recovery, the concentration of [3H]morphine in the probe perfusate was at least 100-fold higher than the concentration of [3H]morphine and [3H]M3G determined in the last microdialysate collection. After determination of probe recovery, a venous blood sample was collected at 180 min and the animal was euthanized via decapitation to harvest the brain for analysis. The blood samples were allowed to clot in polypropylene microcentrifuge tubes and serum was harvested after centrifugation (2000g for 10 min). The serum, microdialysate, and brain tissue samples were stored in polypropylene microcentrifuge tubes at −20°C until chromatographic analysis.

**Influence of GF120918 on Brain Tissue Morphine.** To evaluate potential changes in BBB integrity secondary to placement of the microdialysis probe, the influence of GF120918 on blood-brain distribution of morphine was evaluated in a separate group of rats in the absence of cortical microdialysis probes. Twelve rats were randomized to receive either GF120918 (500 mg/kg) by gavage daily for 4 days or no treatment (control). A jugular vein cannula was placed the day before study (i.e., after 3 days of pretreatment) under ketamine/xylazine anesthesia. On the day of study a 1 mg/kg i.v. bolus dose of morphine (containing 10 µCi [3H]morphine) was administered. Blood samples were collected at either 30 or 60 min and the rats were decapitated to harvest...
obtained at the midpoint of each perfusion. After 90 min, GF120918 was added to the microdialysis probes. Recovery was estimated by retrodialysis of \([3 \text{ H}]\)morphine and unlabeled microdialysate and blood samples was continued for an additional 90 min. Probe recovery was estimated by retrodialysis of \([3 \text{ H}]\)morphine and unlabeled morphine \((1 \mu \text{M})\) in a separate aliquot of heparinized blood that did not contain morphine. Plasma was harvested after centrifugation (2000g for 10 min). The plasma and microdialysate samples were stored in polypropylene microcentrifuge tubes at \(-20^\circ\text{C}\) until chromatographic analysis.

**Morphine and M3G Analyses.** The concentrations of morphine and M3G in serum or microdialysate samples were determined by a modification of the HPLC method of Ouleyt and Pollack (1995). Solid-phase extraction of alkalized serum samples was performed with C8 Bond Elut columns (Varian, Harbor City, CA). Analytes were eluted with methanol, evaporated to dryness, and reconstituted in mobile phase (10% acetonitrile in 0.1% trifluoroacetic acid) and injected onto the HPLC system. Whole brain tissue was blotted dry, weighed, homogenized with 0.5 M ammonium sulfate buffer \((\text{pH}\ 9.3)\), and mixed with acetonitrile \((1:1, \text{v/v)}\). After centrifugation \((2000\text{g for 10 min)}\), the supernatant was removed, evaporated to dryness, reconstituted with mobile phase, and injected onto the HPLC system. Microdialysis samples required no sample preparation and were injected directly onto the HPLC system.

Chromatographic separation was achieved with a Spherisorb C6, 5 \(\mu\text{m}\) \((250 \times 4.6 \text{ mm})\) column (Phase Separations, Inc., Norwalk, CT) and constant flow gradient elution using a Shimadzu HPLC system (Columbia, MD). Fluorescence (FP-920 Fluorescence Detector, Jasco Corp., Tokyo, Japan) of the column eluent was monitored at an excitation wavelength of 220 nm and an emission cutoff of 350 nm; total effluent was collected in 2-mI fractions. The fractions associated with the M3G and morphine peaks (retention times of 6 and 10 min, respectively) were mixed with 15 ml of scintillation cocktail (Ultima Gold, Beckman Instrument Company, Meriden, CT) and analyzed by liquid scintillation spectrometry. Concentrations of morphine and M3G were determined by the amount of radioactivity associated with the morphine and M3G peaks given the specific activity of \([3 \text{ H}]\)morphine in the administered dose. Quality control samples were evaluated every seventh sample and differed from the expected result by \(<18\%\).

Unbound morphine and M3G concentrations in brain ECF and blood were calculated from microdialysate concentrations corrected for the probe recovery. Recovery-corrected morphine and M3G concentrations were obtained by dividing the morphine and M3G concentrations from the microdialysate samples by the fractional loss of morphine as determined by in vivo retrodialysis. A previous investigation has demonstrated similar recoveries of morphine and M3G (Barjavel et al., 1995).

**Data Analysis.** Antinociception was expressed as the percentage of maximum possible response \((\%\text{MPR})\) according to the following equation:

\[
\text{\%MPR} = \frac{\text{Test Latency} - \text{Baseline Latency}}{\text{Cutoff Time} - \text{Baseline Latency}} \times 100
\]

The midpoint of the 30-min collection periods was used as the sample time for brain ECF and blood microdialysate concentration-time profiles. Area under the concentration-time curve \((\text{AUC})\) and area under the effect-time curve \((\text{AUE})\) were calculated by the linear trapezoidal method. Extrapolation to infinite time according to the terminal rate constant was utilized for AUC estimation. Noncompartmental pharmacokinetic analysis was performed to determine clearance \((\text{CL})\), mean residence time \((\text{MRT})\), half-life \((T_{1/2})\), and steady-state volume of distribution \((V_{ss})\). The unbound fraction \((f_u)\) of morphine was calculated as the ratio of the microdialysate concentration to the corresponding serum concentration.

Integrated pharmacokinetic/pharmacodynamic models were developed to fit simultaneously unbound morphine concentrations in blood and brain ECF and antinociceptive activity \((\%\text{MPR})\) by nonlinear least-squares regression (WinNonlin version 1.1, PharSight Corp., Mountain View, CA) to estimate the pharmacokinetic and pharmacodynamic parameters for each rat. The relative performance of different models was assessed by residual analysis, Akaive’s Information Criterion (AIC) and graphical analysis.

Descriptive statistics for the pharmacokinetic and pharmacodynamic parameters were calculated. Two-tailed unpaired Student’s \(t\) tests were utilized to identify significant differences in parameters between treatment groups. Half-life parameters were log-normalized before statistical testing. Two-tailed two-way ANOVA and post hoc unpaired \(t\) tests were utilized to identify significant differences in the brain-to-serum concentration ratios between treatment groups and over time. One-tailed tests were utilized to evaluate differences in the parameter estimates from the pharmacokinetic/pharmacodynamic model. The criterion for statistical significance was \(p < .05\).

**Results.**

**Microdialysis.** The average recoveries of the jugular and cerebral cortex microdialysis probes were 31.8 \pm 4.3\% and 30.3 \pm 3.5\%, respectively. Mean unbound morphine and M3G blood concentration versus time profiles in GF120918-treated and control rats are presented in Fig. 1. A monoexponential or biexponential decline was observed for morphine and M3G concentrations in all rats. Mean unbound concentrations of morphine and M3G in brain ECF are presented in Fig. 2. Morphine and M3G could be detected in both blood and brain ECF microdialysate for up to 150 min postdose. The results of the noncompartmental pharmacokinetic analyses for unbound concentrations of morphine and M3G in blood are summarized in Table 1. The AUC<sub>0→t</sub>, CL<sub>renal</sub>, V<sub>ss</sub>, T<sub>1/2</sub>, and MRT of unbound morphine in blood were not significantly different between treatment groups. In contrast, the AUC<sub>u</sub> and apparent T<sub>1/2</sub> of unbound M3G in blood in GF120918-treated rats were more than 2-fold higher compared with control rats \((p < .05)\).

Noncompartmental pharmacokinetic parameters for unbound brain ECF morphine and M3G are summarized in Table 2. The T<sub>1/2</sub> of unbound morphine in brain ECF was 2- to 3-fold longer in GF120918-treated rats compared with control rats \((p < .05)\) and 3-fold longer than the systemic T<sub>1/2</sub> of unbound morphine in GF120918-treated rats \((p < .05)\). The ratios of the concentration of unbound morphine in brain ECF to the concentration of unbound morphine in blood over time in both control and GF120918-treated rats are shown in Fig. 3A. The AUC ratio for unbound morphine in brain to unbound morphine in blood was significantly higher in GF120918-treated rats as compared with control rats (Table 2). The concentration ratios for unbound M3G in brain ECF versus blood were approximately 0.48 and did not vary statistically with time (Fig. 3B). The AUC of unbound M3G in brain ECF was approximately 2-fold higher in GF120918-treated rats compared with controls; however, no significant differences in the apparent brain T<sub>1/2</sub> or brain ECF to blood ratios were observed for M3G.

**Antinociception.** Mean effect versus time profiles for GF120918-treated and control rats are presented in Fig. 4. Maximal antinociceptive response was observed at 15 min \((i.e., the first time point examined)\) for all animals. Antinociception declined more slowly in GF120918-treated rats than in control rats. Response at 45, 75, and 105 min was significantly higher in the GF120918-treated compared with control rats \((p < .05)\). Mean AUE (Table 3) also was increased approximately 2-fold by GF120918 pretreatment \((p < .05)\).

**Morphine Concentrations in Brain Cortex Homogenate.** The ratios of morphine concentrations in brain cortex homogenate to those in serum in a group of rats without microdialysis probes were deter-
mined at 30 and 60 min and compared with data obtained from rats at
the end of the preceding microdialysis experiment (180 min) (Table
4). The ratios increased over time in both control and GF120918-
treated rats; at 60 and 180 min, ratios in GF120918-treated animals
were more than 2-fold higher than those in control rats (ANOVA post
hoc unpaired t tests, p < .05). At the end of the microdialysis
experiment, concentration ratios based on unbound morphine were
similar to those based on morphine concentrations in tissue homog-
enate and serum.

Mean effect versus unbound morphine concentration in blood and
brain ECF for the different treatment groups is presented in Fig. 5.
The effect versus unbound blood morphine concentration relation-
ship was shifted to the left in GF120918-treated rats compared with con-
trols. In contrast, no difference in the effect versus brain ECF con-
centration relationship was observed between GF120918-treated rats
and controls. No obvious relationship could be discerned between
M3G concentrations in blood or brain and antinociception.

An integrated pharmacokinetic/pharmacodynamic model (Fig. 6)
was developed to characterize the unbound blood and brain ECF
morphine concentration profiles and concentration-effect relation-
ships (Fig. 7). All parameters were similar between treatment groups
except \( k_{21} \), the rate constant representing net egress of morphine from
brain ECF to blood. The rate constant \( k_{21} \) was approximately 2-fold
lower in the GF120918-treated compared with control rats \( p < .05 \).

**In Vitro Binding in Blood.** GF120918 did not significantly alter
the \( f_u \) of morphine in whole blood in vitro (\( f_u = 0.662 \pm 0.035 \) in
control versus \( 0.651 \pm 0.039 \) in the presence of GF120918).

**Discussion**

Morphine is the most potent natural opiate and is the most common
narcotic analgesic used today for the treatment of pain associated with
cancer. Morphine analgesia is produced by activation of opioid re-
ceptors within the CNS at both the spinal and supraspinal levels (Jaffe
and Martin, 1990). The concentration of morphine at any point in time
at active sites in the CNS will depend on the systemic disposition and
the CNS distribution of morphine. Changes in morphine disposition at
either of these locations may influence morphine CNS concentrations
and consequently the degree of antinociception.

In vitro studies in P-gp overexpressing Chinese hamster ovary cell
lines and MDR1-transfected cells, as well as in vivo studies in mice
genetically deficient in \( mdr1a \), have demonstrated that morphine may
be a substrate for P-gp (Callaghan and Riordan, 1993; Schinkel et al.,

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>GF120918</th>
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<tbody>
<tr>
<td>AUC ( \mu M \cdot \text{min} )</td>
<td>22.7 ± 4.8</td>
<td>32.3 ± 4.2</td>
</tr>
<tr>
<td>CL ( \text{mL/min/kg} )</td>
<td>160 ± 36</td>
<td>110 ± 15</td>
</tr>
<tr>
<td>( V_{ss} ) ( \text{L/kg} )</td>
<td>10.8 ± 2.6</td>
<td>11.4 ± 3.4</td>
</tr>
<tr>
<td>( T_{1/2} ) ( \text{min} ) (^a)</td>
<td>42.2 ± 0.8</td>
<td>61.1 ± 13.0</td>
</tr>
<tr>
<td>( MRT ) ( \text{min} ) (^b)</td>
<td>67.6 ± 2.2</td>
<td>104 ± 31.7</td>
</tr>
<tr>
<td>M3G AUC ( \mu M \cdot \text{min} )</td>
<td>14.7 ± 5.7</td>
<td>37.1 ± 5.6*</td>
</tr>
<tr>
<td>M3G ( T_{1/2} ) ( \text{min} ) (^b)</td>
<td>42.1 ± 4.4</td>
<td>89.6 ± 24.6*</td>
</tr>
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</table>

\(^a\) Mean ± S.D.; \( n = 3 \) per treatment.
\(^b\) Harmonic mean ± jackknife S.D.
* \( p < .05 \) (unpaired t test).
in the presence and absence of GF120918. Consistent with our previous report (Letrent et al., 1998), no significant differences in the systemic disposition (AUC$_{ss}$, CL$_{ss}$, $V_{ss}$, $T_{1/2}$, and MRT) of unbound morphine in blood were apparent between treatment groups. A trend toward reduced systemic clearance of morphine in the presence of GF120918 was observed. Unbound M3G in blood was elevated more than 2-fold in GF120918-treated rats. M3G may be a substrate for P-gp at the canalicular membrane and/or the renal tubule, primary sites of elimination of this conjugate. Recent evidence has demonstrated that morphine-6-glucuronide (M6G) uptake in brain capillary endothelial cells was increased approximately 2-fold in the presence of verapamil, a P-gp inhibitor (Huwyler et al., 1996). The uptake of M3G has not been evaluated, but M3G and M6G are physicochemically similar (Carrupt et al., 1991), suggesting that M3G also may be a substrate for P-gp.

Relative to the dose administered, a small amount of morphine enters the brain, and the concentration in the CNS is a fraction of that observed in other organs (Way and Adler, 1961). The results from the present microdialysis experiment indicate that the brain-to-blood ratio of unbound morphine increased from approximately 0.2 to 0.6 over 2 h after a single i.v. bolus dose in control rats. Determination of morphine in whole tissue revealed that the morphine brain-to-blood ratio is 0.2 at 30 min to ~0.4 at 180 min. Disposition of morphine in the rat brain has been examined previously (Dahlgren and Paalzow, 1978; Plomp et al., 1981; Bolander et al., 1983). Peak brain concentrations of morphine have been observed within the first 5 to 15 min after i.v. administration providing a brain:plasma concentration ratio of approximately 0.2; brain concentrations decrease rapidly thereafter (Dahlgren and Paalzow, 1975; 1978; Plomp et al., 1981; Bolander et al., 1983). The morphine brain-to-plasma ratio ranges from 0.6 to 1.0 at time points after 15 min

### TABLE 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>GF120918</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-\infty}$ (µM · min)</td>
<td>10.8 ± 3.3</td>
<td>39.7 ± 14.3*</td>
</tr>
<tr>
<td>$T_{1/2}$ (min)</td>
<td>69.6 ± 4.1</td>
<td>194.6 ± 64.2*</td>
</tr>
<tr>
<td>Brain blood AUC ratio</td>
<td>0.47 ± 0.05</td>
<td>1.21 ± 0.34*</td>
</tr>
<tr>
<td>M3G AUC$_{0-\infty}$ (µM · min)</td>
<td>9.7 ± 1.7</td>
<td>16.8 ± 4.8*</td>
</tr>
<tr>
<td>M3G $T_{1/2}$ (min)</td>
<td>118 ± 40</td>
<td>173 ± 39</td>
</tr>
<tr>
<td>M3G brain blood AUC ratio</td>
<td>0.68 ± 0.47</td>
<td>0.45 ± 0.06</td>
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*Mean ± S.D.; n = 3 per treatment.

### TABLE 3

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>GF120918</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{10}$ (min$^{-1}$)</td>
<td>0.0184 ± 0.0034</td>
<td>0.0185 ± 0.0072</td>
</tr>
<tr>
<td>$V$ (l/kg)</td>
<td>5.95 ± 1.72</td>
<td>5.79 ± 1.75</td>
</tr>
<tr>
<td>$k_{12}$ (min$^{-1}$)</td>
<td>0.164 ± 0.142</td>
<td>0.117 ± 0.105</td>
</tr>
<tr>
<td>$k_{21}$ (min$^{-1}$)</td>
<td>0.590 ± 0.160</td>
<td>0.263 ± 0.198*</td>
</tr>
<tr>
<td>$EC_{50}$ (µM)</td>
<td>0.0751 ± 0.0375</td>
<td>0.0538 ± 0.0387</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>3.55 ± 0.23</td>
<td>2.75 ± 1.42</td>
</tr>
<tr>
<td>AUE (%MPR · h)</td>
<td>70.5 ± 16.9</td>
<td>153 ± 38.2*</td>
</tr>
</tbody>
</table>

*Mean ± S.D.; n = 3 per treatment.

*p < .05 (unpaired t test).
In addition, regional differences in rat brain morphine concentrations have been reported; the highest concentrations of morphine have been observed in subcortical areas, the cerebellum and spinal medulla. The ability of morphine to penetrate the BBB is well recognized, but compared with many drugs the penetration is rather limited. Morphine uptake into the brain appears to be via passive diffusion. P-gp present in brain capillary endothelial cells functions to limit cellular exposure to substrates via an active efflux mechanism.

Previous studies in bovine brain capillary endothelial cell cultures, an in vitro model of the BBB, have demonstrated that the P-gp inhibitors GF120918, verapamil, and cyclosporin A enhanced accumulation of morphine and decreased morphine efflux in endothelial cells; the transendothelial cell permeability of morphine was consistent with net efflux. The data presented support the hypothesis that P-gp modifies morphine BBB permeability. The presence of the potent P-gp inhibitor, GF120918, significantly increased the morphine brain-to-blood ratio and more than doubled the AUC of unbound morphine in brain ECF relative to the AUC of unbound morphine in blood compared with control rats.

The relationship between antinociception and morphine concentrations in blood and brain tissue also were consistent with the alteration of BBB distribution of morphine by GF120918. The effect versus unbound blood morphine concentration relationship was shifted to the left in GF120918-treated rats compared with controls, consistent with our previous report that GF120918 pretreatment enhanced morphine antinociception independent of significant alterations in systemic disposition. In this previous study, a pharmacokinetic/pharmacodynamic model with a peripheral effect compartment was required to fit the blood concentration-effect profiles; GF120918-treated rats had significantly lower $k_{e0}$ values versus control rats, consistent with the hypothesis that inhibition of P-gp reduced the efflux of morphine from brain and thus decreased the rate of offset of morphine activity. In contrast, the relationship between effect and brain morphine microdialysate concentration in control and GF120918-treated rats in the present study were super-

### Table 4

<table>
<thead>
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<th>Time</th>
<th>Control</th>
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<tr>
<td></td>
<td>Brain to Serum Ratio</td>
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<td></td>
<td>Brain</td>
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<tr>
<td>30 min</td>
<td>20.4 ± 4.7</td>
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</tr>
<tr>
<td>180 min</td>
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<td>19.0 ± 2.5</td>
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*Mean ± S.D.; n = 3 per treatment.
Animals without microdialysis probes.
Symbols and bars represent mean ± S.D.

(Plomp et al., 1981; Bolander et al., 1983; Bhargava et al., 1992; Xie and Hammarlund-Udenaes, 1998). In addition, regional differences in rat brain morphine concentrations have been reported; the highest concentrations of morphine have been observed in subcortical areas, the cerebellum and spinal medulla (Bolander et al., 1983). The ability of morphine to penetrate the BBB is well recognized, but compared with many drugs the penetration is rather limited. Morphine uptake into the brain appears to be via passive diffusion. P-gp present in brain capillary endothelial cells functions to limit cellular exposure to substrates via an active efflux mechanism (Pastan and Gottesman, 1991). Brain capillary endothelial cells are thought to be the primary physical barrier between brain ECF and blood (Tsuji et al., 1992). Previous studies in bovine brain capillary endothelial cell cultures, an in vitro model of the BBB, have demonstrated that the P-gp inhibitors GF120918, verapamil, and cyclosporin A enhanced accumulation of morphine and decreased morphine efflux in endothelial cells; the transendothelial cell permeability of morphine was consistent with net efflux. The data presented support the hypothesis that P-gp modifies morphine BBB permeability. The presence of the potent P-gp inhibitor, GF120918, significantly increased the morphine brain-to-blood ratio and more than doubled the AUC of unbound morphine in brain ECF relative to the AUC of unbound morphine in blood compared with control rats.

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![Figure 5](image1.png)

**Figure 5.** Antinociceptive effect versus unbound morphine blood concentration profiles (A) and antinociceptive effect versus unbound morphine brain ECF concentration profiles (B) in control (▲) and GF120918-treated (●) rats (n = 3 per treatment).

![Figure 6](image2.png)

**Figure 6.** Representation of the pharmacokinetic/pharmacodynamic model used to fit morphine concentration and effect data.

![Table 4](image3.png)

**Table 4** Effect of GF120918 on brain tissue-to-serum morphine concentration ratios

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*Mean ± S.D.; n = 3 per treatment.
†Animals without microdialysis probes.
‡Animals with microdialysis probes.
* $p < .05$ (post hoc unpaired $t$ test).
approximately 2-fold higher in GF120918-treated rats; however, the ratio of M3G. The AUC of unbound M3G in brain ECF was approximately a simple sigmoid $E_{\text{max}}$ model was capable of describing these data, indicating that brain ECF is more representative of the biophase than systemic morphine concentrations consistent with previous investigations (Bhargava et al., 1993).

Penetration of morphine glucuronides into the brain is assumed to be low because the physicochemical properties of these conjugates are incompatible with transport through the BBB. Although morphine glucuronides are more hydrophilic than morphine (Murphy and Olsen, 1994), M3G and M6G may be “molecular chameleons,” folding the glucuronic acid group to present a relatively hydrophobic face. The rat does not form M6G (Coughtrie et al., 1989) and thus, reduction of systemic M3G clearance and not enhanced BBB permeability. The rat does not form M6G (Coughtrie et al., 1989) and thus, reduction of systemic M3G clearance and not enhanced BBB permeability. Reduced P-gp in GF120918-treated rats was due to decreased egress of morphine across the BBB. The potential role of P-gp in modulating opioid pharmacodynamics, particularly in the presence of altered P-gp activity or expression secondary to concomitant drugs or disease, warrants further investigation.

Acknowledgment. We thank Dr. P.L. Golden at the Medical University of South Carolina for her guidance with the brain microdialysis technique.

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**References**


