Enhanced Antinociception of the Model Opioid Peptide [D-Penicillamine^{2,5}] Enkephalin by P-Glycoprotein Modulation

Cuiping Chen[1] and Gary M. Pollack[1,2]

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Purpose. This study was conducted to examine the influence of P-glycoprotein (P-gp) modulation on the pharmacodynamics of the model opioid peptide DPDPE.

Methods. Mice (n = 5–7/group) were pretreated with a single oral dose of the P-gp inhibitor GF120918 (25 or 250 mg/kg) or vehicle. ^{3}H-DPDPE (10 mg/kg) or saline was administered 2.5 hr after pretreatment. Antinociception was determined, and blood and brain tissue were obtained, 10 min after DPDPE administration.

Results. A significant difference ($p < 0.001$) in DPDPE-associated antinociception was observed among mice pretreated with a 25- (83 ± 16% MPR) or 250- (95 ± 5% MPR) mg/kg dose of GF120918 in comparison to mice pretreated with vehicle (24 ± 14% MPR) or mice receiving GF120918 without DPDPE (12 ± 8% MPR). A significant difference ($p < 0.01$) in brain tissue DPDPE concentration also was observed among treatment groups [25 ± 6 ng/g (vehicle), 37 ± 11 ng/g (25 mg/kg GF120918), 70 ± 8 ng/g (250 mg/kg GF120918)]. In contrast, blood DPDPE concentrations were not statistically different between groups (678 ± 66, 677 ± 130, and 818 ± 236 ng/ml for vehicle, GF120918 [25 mg/kg], and GF120918 [250 mg/kg], respectively). A single 100-mg/kg i.p. dose of (+)verapamil increased the brain:blood DPDPE concentration ratio by ~70% relative to saline-treated control mice (0.139 ± 0.021 vs. 0.0814 ± 0.0130, $p < 0.01$), a change in partitioning similar to that observed with the low dose of GF120918. These data provide further support for a P-gp-based mechanism of brain:blood DPDPE distribution.

Conclusions. The present study demonstrates that GF120918 modulates blood-brain disposition and antinociception of DPDPE. Coadministration of a P-gp inhibitor with DPDPE may improve the pharmacologic activity of this opioid peptide.

KEY WORDS: [D-Penicillamine^{2,5}]enkephalin; P-glycoprotein; blood-brain barrier; antinociception; opioids.

INTRODUCTION

[D-penicillamine^{2,5}]enkephalin (DPDPE) is a δ-opioid receptor-selective pentapeptide with a conformationally restricted structure (1–3). DPDPE was developed as a potential analgesic in the hope that δ-receptor selectivity would minimize side effects (respiratory depression, constipation and nausea) associated with opioids. However, the sojourn of DPDPE in the systemic circulation was unexpectedly short for a metabolically stable peptide, and partitioning into brain tissue was low despite relatively favorable lipophilicity (3–8). Each of these factors served to decrease the magnitude and duration of antinociception produced by DPDPE.

Earlier experiments in this laboratory focused on systemic disposition, blood-brain translocation, and antinociception of DPDPE in rodents (6,7,9). In rats, DPDPE undergoes saturable hepatobiliary elimination, although the locus of the saturable process (uptake vs. excretion) is not clear (7). Experiments in CD-1 mice suggested that a saturable efflux system might be involved in the distribution of DPDPE between brain and blood (9). Taken together, these results suggested that transport system(s) in brain and bile canaliculi could be responsible for the short duration of action of DPDPE.

P-glycoprotein (P-gp), a member of the ATP binding cassette superfamily of transport proteins (10), is overexpressed in many tumors, resulting in marked reduction of intracellular concentrations of chemotherapeutic agents with a variety of chemical structures (11,12). P-gp also is present in several normal tissues, including brain microvessel endothelium and bile canaliculi (13). Although the physiologic functions of P-gp in normal tissues have not been well studied, and therefore still are not clear, extruding peptides from cells may be one function of P-gp (14,15).

P-gp may be involved in brain opioid egress. Mice lacking the *mdr1a* gene [*mdr1a(-/-)*] accumulated higher brain concentrations of loperamide, a μ-receptor agonist, than wild-type mice, suggesting that opioids may be substrates for P-gp (16). Chinese hamster ovary cells overexpressing P-gp also accumulated significantly less morphine, a μ-receptor agonist, than cells that did not overexpress P-gp (17). Three synthetic opioids (meperidine, pentazocine and methadone) increased accumulation of vinblastine, a P-gp substrate, in cells overexpressing P-gp (17). In previous studies in this laboratory, *mdr1a(-/-)* mice evidenced significant improvement in DPDPE-associated antinociception, and enhanced DPDPE accumulation in brain, compared with wild-type mice (18). In addition, pretreatment of rats with the P-gp inhibitor GF120918 increased morphine antinociception (19). The results of these studies suggest that P-gp may modulate the disposition and pharmacologic effects of opioids.

Recent efforts have been directed towards developing P-gp modulators for co-administration with chemotherapeutic agents to reverse multidrug resistance (20,21). Several agents known to be P-gp modulators have been used to reverse multidrug resistance (22). GF120918 (N-{4-[2-(1,2,3,4-tetrahydro-6,7-)dimethoxy-2-isoquinolinyl)-ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide), an acridone carboxamide derivative (23), has been shown to be one of the most potent of the second-generation P-gp modulators; it is active *in vitro* at concentrations of approximately 20 nM (24) compared with 10 μM for verapamil (25).

Since previous studies in this laboratory have suggested that DPDPE is a P-gp substrate, use of a combination of GF120918 and DPDPE would likely alter the disposition and/or pharmacologic effect of DPDPE. Accordingly, the present study was conducted to examine the impact of GF120918 on blood-brain disposition and antinociception of DPDPE.

[1] Division of Drug Delivery and Disposition, School of Pharmacy, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7360.

[2] To whom correspondence should be addressed. (e-mail: gary_pollack@unc.edu)

ABBREVIATIONS: DPDPE, [D-penicillamine^{2,5}]enkephalin; MPR, maximum possible response; P-gp, P-glycoprotein.

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METHODS

Materials

[Tyr^{2,6}-^{3}H]-DPDPE (1332 Gbq/mmol) was obtained from Dupont New England Nuclear (Boston, MA). DPDPE was provided by Chiron Mimotopes Peptide Systems (San Diego, CA) under the direction of NIDA. GF120918 was a gift of Glaxo Wellcome Inc. (Research Triangle Park, NC). All other reagents were of the highest grade available from commercial sources.

Animals and Assessment of Antinociception

Male FVB and *mdr1a(-/-)* mice (4–5 weeks old, Taconic, Germantown, NY) and CD-1 mice (25–30 g, Charles River, Inc., Raleigh, NC) were housed individually or 2–4 (CD-1 only) per wire-mesh cage. Mice had free access to food and water and were maintained on a 12-hr light/dark cycle for 1 week before the experiment. All procedures adhered to the "Principles of Laboratory Animal Care" (NIH publication #85-23), and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

The hotplate latency test (26) was used to determine antinociception. Latency was defined as the time between placement of the mouse on the hotplate (55°C; Columbus Instruments, Columbus, OH) and observation of hindpaw licking or jumping. Mice with a control latency ≤25 s were used. A maximum test latency of 60 s was used to avoid tissue damage. Antinociception (percent of maximum possible response; % MPR) was calculated as:

$$\%\text{MPR} = \frac{\text{test latency} - \text{control latency}}{60 - \text{control latency}} \times 100$$

Surgical Preparation

Mice were anesthetized with i.p. ketamine (85 mg/kg) and xylazine (0.3 mg/kg). A silicone rubber cannula (0.012 in id, 0.015 in od) was implanted (~1 cm) in the right jugular vein under a stereoscope, and was secured with surgical silk (5/0). The other end of the cannula was exteriorized at the back of the mouse and secured in a rubber patch stitched to the skin. Patency was maintained with heparinized saline (20 U/ml). Studies were conducted 24 hr after surgery.

Selection of Administration Routes of GF120918

Initial experiments were conducted in CD-1 mice to optimize the route of GF120918 administration, and to assess possible antinociceptive effects of GF120918 or vehicles. Mice were divided into two groups for each route to receive vehicle plus DPDPE or GF120918 plus DPDPE. In all cases, saline or ^{3}H-DPDPE was administered as an iv bolus (10 or 15 mg/kg; 4 μCi) after pretreatment with GF120918 or vehicle.

For iv GF120918, mice (n = 3/group) received DPDPE (15 mg/kg) 15 min after administration of vehicle (1,2-propanediol/H_2O, 3:2 v/v) or GF120918 (5 mg/kg). Hotplate latency was determined immediately before administration of vehicle or GF120918 (control latency), and prior to (test latency for vehicle or GF120918) and at 10 min after (test latency for DPDPE) administration of DPDPE. Blood samples were obtained immediately after measurement of hotplate latency, and mice were sacrificed to obtain brain tissue. For ip GF120918, mice received DPDPE 2 hr after vehicle (corn oil, n = 3) or GF120918 (100 mg/kg, n = 5). Hotplate latency was determined, and samples were collected, at the intervals described above. For po GF120918, mice were pretreated with vehicle [Tween 80 (1%) and methylcellulose (0.5%), n = 4] or GF120918 (250 mg/kg, n = 6), one dose per day for 4 days (19). Hotplate latency was determined daily at 2.5 hr after each pretreatment of GF120918 or vehicle. Hotplate latency also was determined at timed intervals up to 9 hr to examine potential antinociceptive effects of GF120918 or vehicle at different times post-dose; this experiment was conducted only on day 1. Surgical preparation was performed on day 3; on day 4, mice received DPDPE 2.5 hr after the last dose of GF120918 or vehicle. Hotplate latency was determined at 5, 10, 30, and 40 min after administration of DPDPE, immediately followed by blood sampling and collection of brain tissue (≥2 samples per time point).

Blood-Brain Disposition and Antinociception of DPDPE in FVB Mice

Based on the preceding experiments (see Results), the oral route of GF120918 administration was selected. FVB mice, the wild-type controls for *mdr1a(-/-)* mice (18), were assigned to 1 of 4 groups: GF120918 (25 mg/kg) plus DPDPE (n = 6), GF120918 (250 mg/kg) plus DPDPE (n = 6), vehicle plus DPDPE (n = 7), or GF120918 (250 mg/kg) plus saline (n = 6). A single oral dose of GF120918 or vehicle was administered 2.5 hr before an i.v. bolus of ^{3}H-DPDPE (10 mg/kg; 4 μCi). Antinociception was assessed 10 min after administration of DPDPE, immediately followed by blood sampling and collection of brain tissue.

Influence of (+)verapamil on Brain:Blood Distribution of DPDPE

To determine whether the effects of GF120918 on DPDPE distribution between brain tissue and blood was due to P-gp inhibition *per se*, an additional experiment was performed with (+)verapamil as a P-gp inhibitor. [^{3}H]DPDPE (10 mg/kg, 1.5 μCi sc) was administered to FVB mice (26–37 g). At 30 min post-dose, mice (n = 7/group) received saline (0.2 ml) or (+)verapamil (100 mg/kg i.p.). A group (n = 6) of *mdr1a(-/-)* mice served as a positive control. Mice were sacrificed by decapitation at 120 min after the DPDPE dose; [^{3}H]DPDPE in brain tissue homogenate (saline, 1:2 w/v) and serum was determined by liquid scintillation spectrometry.

Sample Analysis

Samples were stored at −20°C pending analysis. Blood (10–50 μl) was mixed with 5 ml scintillation cocktail (Bio-Safe II, RPI, Mount Prospect, IL) before measurement of radioactivity by liquid scintillation spectrometry. Whole brain tissue was blotted dry and weighed. Two volumes of saline were added before homogenization with a blade homogenizer. Aliquots of homogenate (100 μl) were pretreated as described for blood samples. Previous experiments in this laboratory have shown that DPDPE is not metabolized, and that total radioactivity after administration of ^{3}H-DPDPE corresponds to intact parent peptide (7,9).

Statistical Analysis

All data are presented as mean ± SE. Analysis of variance (ANOVA) and Student's t-test, where appropriate, were used to determine the statistical significance of differences between experimental groups. The 0.05 level of probability was used as the criterion of significance.

RESULTS

Selection of Administration Route for GF120918

This experiment was conducted to select an administration route for GF120918, and to assess potential effects of GF120918 or vehicles on DPDPE antinociception. Mice pretreated with iv GF120918 (5 mg/kg) on average had slightly higher antinociception than mice receiving vehicle, although the difference was not statistically significant (94 ± 7% MPR vs. 75 ± 14% MPR, n = 3; $p > 0.2$). The vehicle used for iv administration of GF120918 [1,2-propanediol/H_2O (3:2, v/v)] had a sedative effect, and antinociception after the vehicle was significantly higher than after saline (75 ± 14% MPR vs. 5 ± 3% MPR, $p < 0.005$). GF120918 pretreatment tended to increase the blood concentration of DPDPE, although the difference was not statistically significant due to the number of animals studied ($p > 0.1$). Similarly, neither brain tissue concentrations nor the brain:blood concentration ratio differed statistically between groups (Table I).

Mice pretreated with a single ip dose of GF120918 (100 mg/kg) evidenced significantly higher antinociception compared to controls (91 ± 9% MPR vs. 10 ± 6% MPR, $p < 0.005$). Vehicle (corn oil) or GF120918 (100 mg/kg) alone did not produce significant antinociception (8 ± 6% MPR and 9 ± 4% MPR, respectively). Blood and brain DPDPE, as well as the brain:blood ratio (0.29 ± 0.01 vs. 0.18 ± 0.05; $p > 0.05$), were comparable between test and control groups (Table I).

Oral pretreatment with vehicle or GF120918 (250 mg/kg) did not produce antinociception through day 3, although a statistically significant effect was observed on day 4 (Fig. 1). Antinociception determined during the first dosing interval did not differ between vehicle and GF120918 (−10.0 ± 2.0% MPR vs. −3.4 ± 1.2% MPR). Mice pretreated with GF120918 for 4 days before iv DPDPE (2.5 hr after the last dose of GF120918) had an increased magnitude and longer duration of antinociception compared to controls. Antinociception dissipated within 30 min after DPDPE administration in control mice, but still

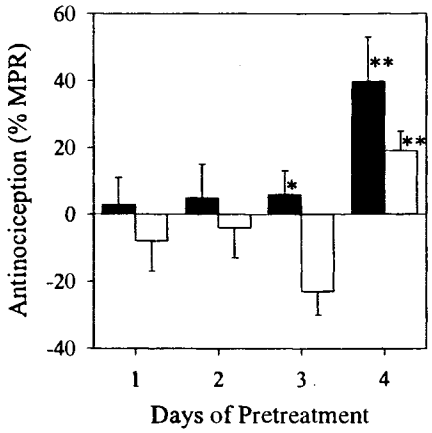

Fig. 1. Antinociceptive effect of vehicle (*open bars*, n = 4) and GF120918 (250 mg/kg/day; solid bars, n = 6) during daily p.o. administration in CD-1 mice. Data are expressed as mean ± SE. * $p < 0.05$ vs. corresponding control group; ** $p < 0.05$ vs day 1.

was significant (37 ± 15% MPR) in the test group (Fig. 2). Likewise, brain tissue DPDPE in mice receiving GF120918 were significantly higher than in mice receiving vehicle (144 ± 8 vs. 110 ± 10 ng/g; $p < 0.05$). However, no difference was observed in the blood concentration-time profile between the two treatment groups (Fig. 2).

Antinociception and Blood-Brain Disposition of DPDPE in FVB Mice

FVB mice had been used as controls in previous experiments (18) to assess the effect of lack of the *mdr1a* gene on the disposition and pharmacologic activity of DPDPE. In order to compare the relative impact of a P-gp modulator vs. *mdr1a* gene knockout on the pharmacokinetics and pharmacodynamics of DPDPE, FVB mice were employed in the present experiment in which a single oral dose of GF120918 (25 or 250 mg/kg) or vehicle was administered. Consistent with results from the preliminary experiments described in the preceding section, a significant difference ($p < 0.001$) in antinociception among treatment groups was observed (Table II). Antinociception was significantly higher ($p < 0.05$) in mice receiving either dose of GF120918 plus DPDPE than in mice receiving 250 mg/kg GF120918 alone or vehicle plus DPDPE. The small degree of antinociception produced by GF120918 alone did not differ

Table I. Effect of Intravenous (5 mg/kg) or Intraperitoneal (100 mg/kg) GF120918 on Disposition and Antinociception of DPDPE (15 mg/kg)[a]

	Intravenous administration		Intraperitoneal administration	
	Vehicle (n = 3)	GF120918 (n = 3)	Vehicle (n = 3)	GF120918 (n = 5)
Blood DPDPE (ng/ml)	1300 ± 120	3210 ± 990	1680 ± 500	1210 ± 80
Brain DPDPE (ng/g)	49 ± 11	45 ± 12	30 ± 10	35 ± 20
Brain:blood ratio	0.038 ± 0.009	0.014 ± 0.006	0.018 ± 0.005	0.029 ± 0.010
Antinociception (%MPR)	75 ± 14	94 ± 7	10 ± 6	91 ± 9[b]

[a] Data are presented as mean ± SE.
[b] Significantly different from corresponding control group ($p < 0.001$).

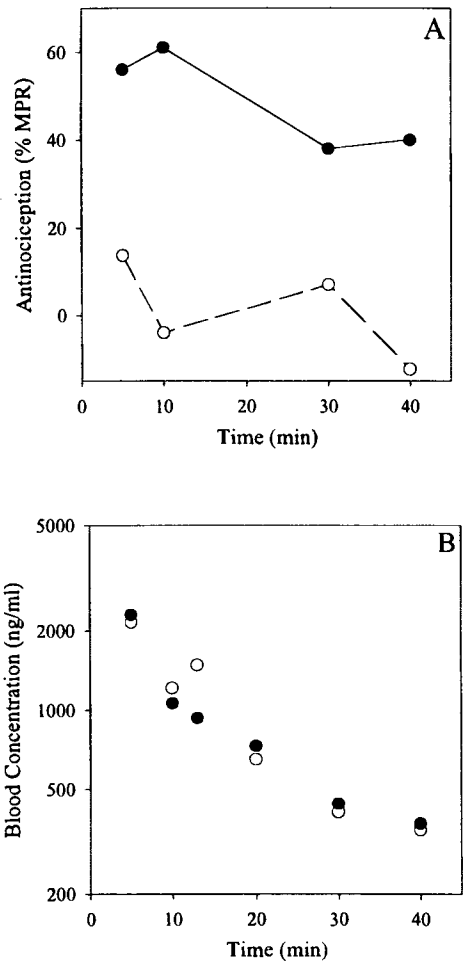

Fig. 2. Antinociceptive effect vs. time profile (A) and blood concentration-time profile (B) of DPDPE in CD-1 mice after pretreatment with multiple oral doses of vehicle (○) or 250-mg/kg GF120918 (●). Data are presented as average of 2 or 3 samples per time point.

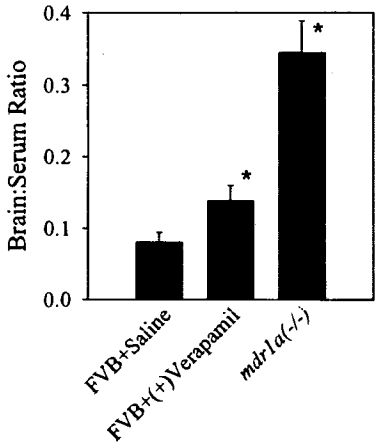

Fig. 3. Effect of (+)verapamil or genetic manipulation of P-gp activity on brain to serum partitioning of DPDPE. Bars indicate mean ± SE. * $p < 0.05$ vs saline group.

statistically from that produced by saline (12 ± 8% MPR vs. 5 ± 3% MPR, $p > 0.05$). A difference in brain tissue concentrations of DPDPE was observed among treatment groups; no statistical difference was observed in blood concentrations or the brain tissue to blood concentration ratio among groups (Table II). However, when GF120918-treated mice (regardless of dose) were compared to controls, the difference in brain:blood ratio (0.119 ± 0.028 vs. 0.0395 ± 0.0106 respectively) was statistically significant ($p < 0.05$).

Influence of (+)verapamil on Brain:Blood Distribution of DPDPE

The DPDPE brain:serum concentration ratio in (+)verapamil-treated FVB mice is compared to that in saline-treated FVB mice and *mdr1a(-/-)* mice in Fig. 3. (+)Verapamil increased the brain:serum ratio of [^{3}H]DPDPE by ~70% relative to saline-treated control mice (0.139 ± 0.021 vs. 0.0814 ± 0.013, $p < 0.01$). The DPDPE brain:serum ratio was ~4-fold higher in *mdr1a(-/-)* mice (0.345 ± 0.044) compared to saline-treated FVB mice ($p < 0.0001$), consistent with previous studies in the gene-deficient mice (18).

DISCUSSION

Previous experiments have shown that brain concentrations of DPDPE and DPDPE-associated antinociception were higher in *mdr1a(-/-)* mice than genetically competent mice, suggesting that P-gp modulates DPDPE translocation between blood and brain and influences DPDPE pharmacodynamics (18). The present study was undertaken to examine the effect of chemical modulation of P-gp on blood-brain disposition and antinociception of DPDPE.

Table II. Comparison of Disposition and Antinociception of DPDPE Between FVB Mice Pretreated with GF120918 (p.o.) and *mdr1a(−/−)* Mice[a]

	Vehicle (n = 7)	GF120918 25 mg/kg (n = 6)	GF120918 250 mg/kg (n = 6)	FVB[b] (n = 5)	*mdr1a(−/−)*[b] (n = 5)
Blood DPDPE (ng/ml)	678 ± 65	566 ± 51	703 ± 152	670 ± 140	773 ± 142
Brain DPDPE (ng/g)	24.6 ± 5.1	37.0 ± 6.0	70.0 ± 7.8[c]	30 ± 12	108 ± 32[c]
Brain:blood ratio (%)	3.95 ± 1.06	8.00 ± 2.99	12.0 ± 3.4	4.48 ± 1.01	14.0 ± 2.4[c]
Antinociception (%MPR)	24.1 ± 13.4	83.3 ± 16.7[c]	95.0 ± 5.0[c]	23.2 ± 8.8	100 ± 0[c]

[a] Data are presented as mean ± SE (n = 3–6).
[b] Data are from Chen and Pollack (18).
[c] Significantly different compared with corresponding control ($p < 0.05$).

Preliminary experiments were conducted to select an appropriate administration route for GF120918. Previous studies indicated that a 5-mg/kg iv dose of GF120918 could restore sensitivity of MDR P388/Dox tumors to doxorubicin (24). A 5-mg/kg i.v. dose of GF120918 in the present study increased DPDPE antinociception (Table I). However, mice receiving the vehicle (1,2-propanediol) prior to DPDPE also evidenced higher antinociception compared to mice receiving DPDPE alone (75 ± 14 vs. 16 ± 9% MPR, $p < 0.05$). Therefore, use of the iv formulation was abandoned in favor of other routes of administration.

Mice pretreated with a 100-mg/kg ip dose of GF120918 in corn oil exhibited significantly increased antinociception compared to mice receiving vehicle prior to DPDPE (91 ± 9 vs. 10 ± 6% MPR, $p < 0.001$). Corn oil alone did not produce antinociception, suggesting that GF120918 could modulate the pharmacologic effect of DPDPE (Table I). Improvement in DPDPE-associated antinociception by GF120918 was not due to alterations in systemic disposition or blood-brain translocation; blood or brain DPDPE in the test group did not differ significantly from controls (1680 ± 500 vs. 1210 ± 80 ng/ml for blood; 30 ± 10 vs. 35 ± 20 ng/g for brain). While ip GF120918 was effective in altering DPDPE pharmacodynamics, absorption of GF120918 from the peritoneal cavity appeared to be incomplete; particles of GF120918 were observed in the peritoneal cavity after the animals were sacrificed. Thus, further experimentation was directed towards po administration of GF120918 to provide more consistent systemic delivery.

A single dose of the vehicle used for oral administration of GF120918 did not exert an antinociceptive effect (Fig. 1). In addition, GF120918 did not alter hotplate latency up to 9 hr after a 250-mg/kg dose; antinociception associated with vehicle and GF120918 was not statistically different (−10.0 ± 0.8 vs. −3.4 ± 0.5% MPR). A 2.5-hr interval was used between pretreatment and drug testing to allow relatively complete absorption and distribution of GF120918 (K.R. Brouwer, personal communication) prior to DPDPE administration. A separate preliminary experiment showed that mice receiving multiple po doses of GF120918 prior to DPDPE had antinociceptive effects associated with DPDPE comparable to those in mice receiving pretreatment with a single po dose of GF120918 (95 ± 5 vs. 91 ± 9% MPR, $n = 3$). Therefore, a single oral dose of GF120918 was selected.

Further assessment of the effect of GF120918 on the disposition and action of DPDPE was conducted with FVB mice in order to compare the impact of chemical inhibition of P-gp with previous experiments in *mdr1a* gene-deficient mice. Consistent with results from *mdr1a(-/-)* mice (18), significantly higher antinociception and brain concentrations of DPDPE were observed in mice pretreated with GF120918. The impact of pretreatment with a 250-mg/kg oral dose of GF120918 on accumulation of DPDPE in brain was comparable to the lack of the *mdr1a* gene; an ~3-fold increase in brain tissue DPDPE concentration was observed in *mdr1a(-/-)* mice compared to FVB mice, consistent with the increase in mice pretreated with GF120918 (70 ± 28 vs. 25 ± 6 ng/g in control mice). Similarly, the brain tissue to blood DPDPE concentration ratio in *mdr1a(-/-)* mice was ~3-fold higher than in wild-type controls, and po administration of GF120918 produced an ~3-fold increase in brain tissue partitioning of DPDPE compared to vehicle-treated controls (0.119 ± 0.028 vs. 0.0315 ± 0.0106, respectively). In contrast to GF120918, (+)verapamil produced a modest, although statistically significant, increase in the brain:blood ratio of DPDPE (Fig. 3), providing further evidence that DPDPE is a substrate for P-gp at the blood-brain interface.

Comparison of the effects of GF120918 and *mdr1a* gene deficiency on modulation of DPDPE antinociception is not clear-cut. A 10-mg/kg i.v. dose of DPDPE in *mdr1a(-/-)* mice elicited maximal response, suggesting that the actual effect might have been above the cut-off value. In contrast, antinociception after pretreatment with GF120918 was below the cut-off value. It is possible that this difference is a function of available P-gp, such that larger doses of GF120918 might result in mice becoming phenotypically indistinguishable from *mdr1a(-/-)* animals. Further work is required to elucidate the relationship between modulation of DPDPE antinociception and the dose of GF120918 administered as a pretreatment.

The impact of P-gp expression on the pharmacodynamics of DPDPE does not appear to be limited to brain:blood partitioning. Based on whole organ DPDPE concentrations, the EC_{50} for DPDPE-associated antinociception was 12.3 ± 0.2 ng/g in *mdr1a(-/-)* mice vs. 160 ± 14 ng/g in wild-type controls (18). Since no evidence exists that the δ-opioid system differs between these two mouse strains, this observation suggests that, in the presence of functional P-gp, whole organ concentrations of DPDPE are not representative of concentrations at the receptor biophase. The results of the present study are consistent with this hypothesis. Based upon the response vs. brain tissue DPDPE concentration relationship established in FVB mice (18), a brain tissue concentration of 25 ng/g should produce minimal antinociception. In the present study, the observed antinociception in control mice (24.1% MPR) did not differ statistically from baseline ($p > 0.2$). In contrast, brain tissue concentrations observed in mice pretreated with GF120918 (37 and 70 ng/g for the 25- and 250-mg/kg dose of inhibitor, respectively) should have produced <10 %MPR (18); the degree of pharmacologic activity observed in these animals (83.3 ± 16.7 and 95.0 ± 5.0% MPR, respectively) was substantially higher, and similar to the activity predicted (>80% MPR) based on the response vs. brain tissue concentration relationship in *mdr1a(-/-)* mice (18). Since GF120918 alone did not produce measurable antinociception, and since mice pretreated with GF120918 exhibited brain tissue DPDPE and antinociception similar to *mdr1a(-/-)* mice, the two studies taken together indicate that changes in P-gp function have a pharmacokinetic, rather than a pharmacodynamic, influence on DPDPE antinociception. The mechanism by which P-gp exerts this influence is unknown. Experiments are underway to elucidate the interactions between P-gp and opiate systems in the mammalian CNS.

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