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Variable modulation of opioid brain uptake by P-glycoprotein in mice

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Abstract

The efflux transporter P-glycoprotein (P-gp) is an important component of the blood–brain barrier (BBB) that limits accumulation of many compounds in brain. Some opioids have been shown to interact with P-gp *in vitro* and *in vivo*. Genetic or chemical disruption of P-gp has been shown to enhance the antinociceptive and/or toxic effects of some opioids, although the extent of this phenomenon has yet to be understood. The purpose of this study was to assess quantitatively the influence of *mdr1a* P-gp on initial brain uptake of chemically diverse opioids in mice. The brain uptake of opioids selective for the mu (fentanyl, loperamide, meperidine, methadone, and morphine), delta (deltorphin II, DPDPE, naltrindole, SNC 121) and kappa (bremazocine and U-69593) receptor subtypes was determined in P-gp-competent (wild-type) and P-gp-deficient [*mdr1a*(−/−)] mice with an *in situ* brain perfusion model. BBB permeability of the opioids varied by several orders of magnitude in both mouse strains. The difference in brain uptake between P-gp-competent and P-gp-deficient mice ranged from no detectable effect (meperidine) to ≥8-fold increase in uptake (DPDPE, loperamide, and SNC 121). In addition, loperamide efflux at the BBB was inhibited by quinidine. These results demonstrate that P-gp modulation of opioid brain uptake varies substantially within this class of compounds, regardless of receptor subtype. P-gp-mediated efflux of opioids at the BBB may influence the onset, magnitude, and duration of analgesic response. The variable influence of P-gp on opioid brain distribution may be an important issue in the context of pharmacologic pain control and drug interactions.

Keywords: Analgesia; Blood–brain barrier; Efflux; Opioids; Permeability; P-glycoprotein

The ATP-dependent efflux transporter P-gp is expressed in a number of barrier tissues involved in the biodisposition of xenobiotics, and is encoded by the *mdr1a* and *mdr1b* genes in rodents and the *MDR1* gene in humans [1]. Transgenic mice lacking the *mdr1a* gene, which is the only isoform of P-gp expressed in intestinal epithelium and the blood–brain barrier (BBB), have provided insight into the physiologic role of P-gp [2]. P-gp now is recognized as an important component of the BBB that limits accumulation of many compounds in brain via active efflux across the luminal membrane of capillary endothelium [3]. P-gp also is expressed in the spinal cord [4] and choroid plexus [5], and although data are limited, P-gp may modulate substrate penetration at these sites [6,7]. P-gp has broad substrate specificity and interacts with a range of chemically diverse substrates, including chemotherapeutic agents, calcium channel blockers, antiarrythmics, immunosuppressants, and HIV protease inhibitors [8].

Callaghan and Riordan [9] demonstrated that some natural and synthetic opioids interact with P-gp in multidrug resistant cells *in vitro*. P-gp-mediated efflux of morphine at the BBB has been demonstrated with a variety of *in vitro* and *in vivo* approaches [10–15]. Loperamide elicits potent centrally mediated opiate-like effects and evidences increased brain accumulation in P-gp-deficient mice as compared to transport-competent animals [16]. Our group has demonstrated increased antinociceptive response and brain accumulation of the peptidic delta agonist DPDPE in P-gp-deficient mice and upon chemical disruption of P-gp with a potent inhibitor [17,18]. In a similar fashion, P-gp was shown to contribute to the peripheral selectivity of the kappa agonist asimadoline by limiting brain accumulation and sedation in mice [19]. Furthermore, antinociception mediated by morphine, methadone and fentanyl, but not meperidine and morphine-6-glucuronide, is increased in P-gp-deficient mice [20]. An efflux system that may be related to P-gp has been identified for fentanyl in an *in vitro*

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Abbreviations: BBB, blood–brain barrier; DPDPE, [D-Pen^{2,5}]-enkephalin; P-gp, P-glycoprotein; CNS, central nervous system.

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model of the BBB [21]. Also, the Met-enkephalin analog metkephamid is effluxed by a verapamil-sensitive mechanism suggestive of P-gp in Caco-2 cell monolayers [22]. In addition, transport studies suggest that methadone is a P-gp substrate in the rat gut [23]. Recently, our group reported the interaction of nonpeptidic delta agonists with P-gp at the murine BBB [26].

Despite an increasing number of reports indicating that opioids interact with P-gp, the evidence often has been indirect (i.e. inhibition of binding or transport of another substrate, use of nonspecific inhibitors, antinociceptive response without concurrent measurement of brain accumulation). Furthermore, although *in vitro* studies have suggested the involvement of P-gp in the disposition of various opioids [24], unequivocal demonstration of opioid transport by P-gp at the blood–brain interface *in vivo* remains scarce. Moreover, the use of different model systems and experimental conditions makes it difficult to assess quantitatively the interaction of opioids with P-gp on a comparative basis. As centrally mediated opioid antinociception involves spinal and supraspinal mechanisms in CNS sanctuaries protected by P-gp [25], we hypothesized that P-gp modulates access of different opioids to relevant receptors to variable extents. In the present study, we utilized a recently developed *in situ* brain perfusion model that exploits the availability of transgenic and mutant *mdr1a*(−/−) mice lacking BBB P-gp [26] to assess the influence of P-gp on initial brain uptake of opioids selective for mu, delta and kappa receptor subtypes in mice.

1. Materials and methods

1.1. Materials

Probe substrates were obtained from the following sources: [^{3}H]-deltorphin II (49.5 Ci/mmol), [^{3}H]-DPDPE (45 Ci/mmol), and [^{14}C]-inulin (2.21 mCi/g) (NEN Life Science Products); [^{3}H]-meperidine (3 Ci/mmol) (Moravek Biochemicals); [^{3}H]-morphine (80 Ci/mmol) and [^{3}H]-naltrindole (60 Ci/mmol) (American Radiolabeled Chemicals); [^{3}H]-SNC 121 (53 Ci/mmol) (Tocris Cookson); [^{3}H]-U-69593 (65 Ci/mmol) (Amersham Pharmacia Biotech); (±)-bremazocine, loperamide, and *R*-(−)-methadone (Research Biochemicals Inc.); fentanyl, (±)-verapamil, (±)-methoxyverapamil, and quinidine (Sigma-Aldrich). All other chemicals and reagents were of analytical grade quality, and were obtained from commercial sources.

1.2. Animals

Adult CF-1 mice (*mdr1a*(+/+) and *mdr1a* (−/−), 30–40 g, 6–8 weeks of age) were obtained from Charles River Laboratories and maintained in a breeding colony in the School of Pharmacy, The University of North Carolina at Chapel Hill. With the exception of SNC 121, for which only female mice were used, all brain perfusion experiments were performed with males; gender has little or no effect on the brain uptake of selected compounds [27]. Animals were housed in a temperature- and humidity-controlled room with a 12 hr/12 hr light/dark cycle and given food and water *ad libitum*. The experimental protocol was approved by the institutional animal care and use committees of The University of North Carolina at Chapel Hill and AstraZeneca R&D Montréal; procedures were conducted according to the ''Guide for the Care and Use of Laboratory Animals'' (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC, USA, 1996).

1.3. In situ brain perfusion

The details of the *in situ* mouse brain perfusion have been described elsewhere [26]. Briefly, mice were anesthetized with intraperitoneal ketamine/xylazine (140/8 mg/kg). The right hemisphere was perfused through the right common and internal carotid arteries following ligation of the external carotid artery. The cardiac ventricles were severed immediately before brain perfusion (Krebs-bicarbonate buffer gassed to pH 7.4 with 95% O_2 and 5% CO_2 at 37°) for 20–180 s (2.5 mL/min) via a syringe pump. Radiolabeled substrates (≤0.3 μCi/mL) were added to the perfusate in order to obtain obtain ≥1000 dpm per tissue sample, with [^{14}C]-inulin being used as a vascular space marker. Aqueous solutions of unlabeled (±)-bremazocine, fentanyl and *R*-(−)-methadone, and a solution of loperamide in dimethylsulfoxide (DMSO), were diluted 100-fold (200-fold for loperamide) in perfusion buffer to concentrations of 1–2 μM. Unlabeled verapamil was added to the perfusate at 0.5 μM as an internal standard for the influence of P-gp [26]. Chemical inhibition of P-gp in the BBB was investigated with loperamide (2 μM) using quinidine as the inhibitor (co-perfusion at 4, 20, and 100 μM). The perfusions were terminated by decapitation. Multiple time point experiments (20, 40, and 60 s or 20, 60, and 100 s, depending on the substrate) were performed for meperidine, U-69593, naltrindole and SNC 121. Single time point experiments were performed for methadone and fentanyl (60 s), (±)-bremazocine and loperamide (100 s), morphine (120 s), and DPDPE and deltorphin II (180 s). The single time point was chosen for these compounds in order to improve accuracy and maximize brain exposure while maintaining linear conditions. The brain was removed from the skull and dissected on ice to isolate the right hemisphere. Samples for HPLC–MS analysis were frozen in liquid nitrogen and maintained at −80° until analysis.

1.4. Analysis of radioactivity

Samples containing radiolabeled substrates were collected and weighed in tared 8-mL glass scintillation vials.

Perfusate (~100 mg) was obtained from the tip of the catheter by activation of the pump. The right hemisphere (~150 mg) was digested with 0.7 mL Solvable (Packard) at 50°. Samples were mixed with 5 mL Ultima Gold (Packard). Total ^{14}C and ^{3}H were determined simulataneously in a Packard 1600TR liquid scintillation analyzer.

1.5. HPLC–MS analysis

Samples containing (±)-bremazocine, fentanyl, loperamide, and *R*-(−)-methadone were analyzed by HPLC–MS on an 1100 Series Liquid Chromatograph/Mass Selective Detector (LC/MSD; Agilent Technologies) with an atmospheric pressure ionization-electrospray (API-ES) chamber. Mass detection of (±)-bremazocine (*m/z* 316.3), fentanyl (*m/z* 337.2), loperamide (*m/z* 477.2), *R*-(−)-methadone (*m/z* 310.2), verapamil (*m/z* 455.4), and the analytical internal standard methoxyverapamil (*m/z* 485.5) was performed in positive single ion monitoring mode.

Perfusate samples (0.5–1.0 mL) were acidified with glacial acetic acid (1% v/v) and vortex-mixed (45 s). An aliquot (100 μL) was added to an equal volume of internal standard (500 nM in 1% acetic acid) and diluted 5-fold with 10–40% acetonitrile in 0.04% formic acid. Brain tissue was weighed in a tared 5-mL polypropylene tube and homogenized with 400 μL internal standard solution (100 nM in 1% acetic acid), followed by ultrasonic cell disruption (10–12 s). Proteins were precipitated with 1.0 mL ice-cold acidified acetonitrile (0.1% v/v acetic acid). The suspension was mixed by vortex (45 s), and a 400-μL aliquot was centrifuged (1600 × *g*, 5 min). The supernatant (300 μL) was transferred to a clean polypropylene tube and evaporated under a stream of nitrogen. The sample was reconstituted in 100 μL 10–40% acetonitrile in 0.04% formic acid.

Aliquots (30 μL) were injected on a 75 mm × 4.6 mm Luna column (3 μm C-18; Phenomenex) at 40°. The mobile phase (1 mL/min) consisted of a mixture of 0.04% formic acid (solvent A) and acetonitrile (solvent B) as follows: 100% solvent A (0–2 min), linear gradient 0–75% solvent B (2–10 min), 75% solvent B (10–12 min), and equilibration (4 min) with 100% A prior to the next injection. Calibration curves were established using brain hemispheres perfused for 120 s with drug-free buffer and spiked with known amounts of analytes (~10–1000 pmol).

1.6. Calculation of apparent brain distributional volume and initial uptake clearance

In experiments involving radioactivity, [^{14}C]-inulin was co-perfused with the tritium-labeled opioids to estimate vascular volume (V_{vasc}, mL·100 g^{-1}) as:

$$V_{vasc} = \frac{X^*}{C^*} \quad (1)$$

where, X^* and C^* represent [^{14}C]-inulin in the brain (dpm 100 g^{-1}) and perfusate (dpm mL^{-1}), respectively. In the (±)-bremazocine, fentanyl, loperamide, and *R*-(−)-methadone experiments, a vascular volume of 1 mL·100 g^{-1} was assumed [26].

Apparent brain distributional volumes for each opioid (V_{brain}, mL·100 g^{-1}) were calculated as:

$$V_{brain} = \frac{X_{brain}}{C_{perf}} \quad (2)$$

where, X_{brain} is opioid in brain (dpm·100 g^{-1} or nmol·100 g^{-1}) corrected for vascular contamination ($X_{total} - V_{vasc}C_{perf}$) and C_{perf} is opioid in perfusate (dpm·mL^{-1} or nmol·mL^{-1}).

In multiple time point experiments, initial brain uptake clearances (Cl_{up}, mL·100 g^{-1}·min^{-1}) were determined by nonlinear least-squares regression (WinNonlin Professional 3.1, Pharsight) with the following differential equation:

$$\frac{dV_{brain}}{dt} = Cl_{up} - Cl_{eg}K_p \quad (3)$$

where, Cl_{eg} is the egress component (i.e. from brain tissue to perfusate) of intercompartmental clearance between blood and brain, and K_p represents the brain-to-perfusate partition coefficient.

In single time point experiments, Cl_{up} was calculated from

$$Cl_{up} = \frac{X_{brain}/T}{C_{perf}} \quad (4)$$

where, T is the perfusion time (min).

1.7. Data analysis

Data are presented as mean ± SD for four animals, unless specified otherwise. Two-tailed Student's *t*-tests were used to determine the statistical significance of differences between experimental groups. In the case of unequal variance between experimental groups (as determined with the Brown–Forsythe test), *t*-tests were adjusted with Satterwaite's correction. In multiple time point uptake experiments, the statistical significance of differences in V_{brain} was determined at each time point using Bonferroni *t*-tests with a correction for three comparisons. The P-gp effect on brain uptake was defined as the ratio of average Cl_{up} in P-gp-deficient mice to that of P-gp-competent mice. The SD of the P-gp effect was derived from the individual SDs of the numerator (x) and denominator (y): [28]

$$SD_{x/y} = \frac{x}{y}\sqrt{\left(\frac{SD_x}{x}\right)^2 + \left(\frac{SD_y}{y}\right)^2} \quad (5)$$

2. Results

2.1. Time dependence of brain uptake

Brain uptake of meperidine, U-69593, naltrindole, and SNC 121 at multiple time points in P-gp-competent and

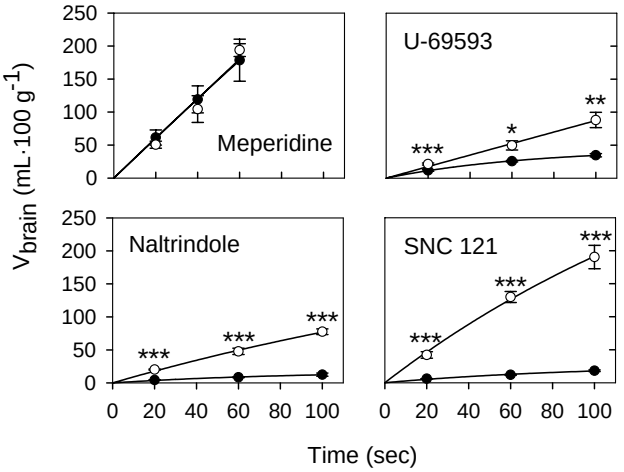

Fig. 1. Time-dependent uptake of meperidine, U-69593, naltrindole, and SNC 121 in the right hemisphere (expressed as apparent brain distributional volume, V_{brain}) of wild-type (solid) and *mdr1a*(−/−) P-gp deficient (open) mice. Data are presented as mean ± SD (N = 4 per point). Solid lines represent the best fit of the data by nonlinear least-squares regression with Eq. (3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for *mdr1a*(−/−) P-gp deficient vs. wild-type mice at individual time points using Bonferroni *t*-tests.

P-gp-deficient mice, expressed as apparent distributional space (V_{brain}), is shown in Fig. 1. For these compounds, nonlinear least-squares regression of the data with Eq. (3) indicated that the egress component (Cl_{eg}) was negligible even in P-gp-competent mice (<10% relative to Cl_{up}). This indicated that brain uptake of these opioids was mostly unidirectional over 60–100 s of brain perfusion. In preliminary experiments, BBB permeability of deltorphin II, DPDPE and morphine was relatively low. Consequently, perfusion time was increased to 120 or 180 s to improve accuracy, and a single time point determination was performed. Due to the labor-intensive nature of HPLC–MS analysis, a single time point determination was performed for (±)-bremazocine, fentanyl, loperamide and *R*-(−)-methadone. Since relatively good brain uptake had been reported for methadone in rats [29], and given the high lipophilicity of fentanyl [30], a perfusion time of 60 s was selected; a 100-s time point was selected for (±)-bremazocine and loperamide to maximize brain exposure while maintaining unidirectional uptake conditions.

Table 2
P-gp effect for verapamil (0.5 μM) in the presence and absence of unlabeled opioids in the perfusate

Verapamil brain perfusions	P-gp effect for verapamil
60 s saline control	5.24 ± 0.81
+ Fentanyl (∼1 μM)	5.26 ± 0.63
+ *R*-(−)-Methadone (∼2 μM)	4.94 ± 0.86
100 s saline control	6.71 ± 1.26
+ (±)-Bremazocine (∼1 μM)	5.29 ± 0.78
100 s DMSO (0.5%) control	4.45 ± 0.51
+ Loperamide (∼1 μM)	4.65 ± 0.64

Data are presented as mean ± SD of the ratio of four individual experiments at a single time point in *mdr1a*(−/−) P-gp deficient and wild-type mice.

2.2. Brain uptake clearances in P-gp-competent (wild-type) and P-gp deficient [mdr1a(−/−)] mice

Opioid initial brain uptake clearances in the presence and absence of *mdr1a* P-gp are reported in Table 1. Of the 11 opioids examined, 10 evidenced varying degrees of P-gp effect, ranging from a 24% (fentanyl, morphine) to more than 8-fold (SNC 121, loperamide, DPDPE) increase in brain uptake in P-gp-deficient mice. No relationship was apparent between P-gp effect and either the receptor subtype or the rate of brain uptake in the absence of P-gp.

2.3. Brain uptake of verapamil in the presence and absence of (±)-bremazocine, fentanyl, loperamide, meperidine, or R-(−)-methadone in the perfusate

The P-gp effect observed for verapamil in the presence and absence of unlabeled opioids is presented in Table 2.

Table 1
Initial brain uptake clearances (Cl_{up}, mL·100 g^{-1}·min^{-1}) of opioids during *in situ* perfusion in mice

Compound	Opioid receptor subtype	Wild-type mice	P-gp deficient mice	P-gp effect
Meperidine	μ	185 ± 38	180 ± 33	0.98 ± 0.27
Fentanyl	μ	184 ± 24	228 ± 9*	1.24 ± 0.17
Morphine	μ	1.04 ± 0.03	1.29 ± 0.08**	1.24 ± 0.08
U-69593	κ	39.2 ± 3.0	52.6 ± 8.8[a]	1.34 ± 0.25
Bremazocine	κ	44.1 ± 5.5	66.3 ± 3.8***	1.50 ± 0.21
Deltorphin II	δ	0.166 ± 0.037	0.263 ± 0.010**	1.58 ± 0.36
Methadone	μ	41.7 ± 5.8	109 ± 17***	2.61 ± 0.55
Naltrindole	δ	12.5 ± 2.4	55.4 ± 5.1[a]	4.44 ± 0.93
SNC 121	δ	17.0 ± 1.8	147 ± 15[a]	8.60 ± 1.26
Loperamide	μ	9.86 ± 1.73	103 ± 6***	10.4 ± 1.9
DPDPE	δ	0.0547 ± 0.0293	0.636 ± 0.067***	11.6 ± 6.4

P-gp effect is defined as the ratio between Cl_{up} in *mdr1a*(−/−) P-gp deficient and wild-type mice. Data are presented as mean ± SD of four individual experiments at a single time point or from multiple time point experiments (N = 4 per point at three time points) $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

[a]Statistical significance of differences in V_{brain} between *mdr1a*(−/−) P-gp deficient and wild-type mice at individual time points is reported in Fig. 1.

The concentration of verapamil in the perfusate (0.5 μM) is one order of magnitude below the apparent self-inhibition constant for P-gp-mediated efflux in the BBB [27]. At a concentration of 1–2 μM, none of the opioids tested resulted in a statistically significant modulation of the P-gp effect for verapamil (i.e. Cl_{up} in P-gp-competent and P-gp-deficient mice was unchanged; data not shown). However, 50 μM methadone, but not meperidine, increased the brain uptake of verapamil 2-fold in P-gp-competent mice at 60 s (84.0 ± 4.1 vs. 41.8 ± 1.3 mL $100\ g^{-1}\ min^{-1}$, $P < 0.001$). Other opioids were not evaluated for their ability to inhibit verapamil efflux at a higher concentration.

2.4. Chemical inhibition of loperamide and verapamil efflux at the BBB by quinidine

The brain uptake of loperamide (2 μM) and verapamil (0.5 μM) in the absence and presence of quinidine in the perfusate during *in situ* perfusion in wild-type mice is shown in Fig. 2. A significant increase in brain uptake was observed for both compounds at a quinidine concentration of 20 μM or more. At 100 μM quinidine, the brain uptake of loperamide and verapamil was increased by factors of 9.0 ± 2.5 and 5.1 ± 2.9, respectively, suggesting full inhibition of P-gp (Tables 1 and 2). Preliminary experiments utilizing P-gp-deficient mice indicated that neither verapamil nor quinidine administration affected the BBB integrity (data not shown); thus the increase in Cl_{up} caused by quinidine administration appears to be primarily a P-gp-mediated event.

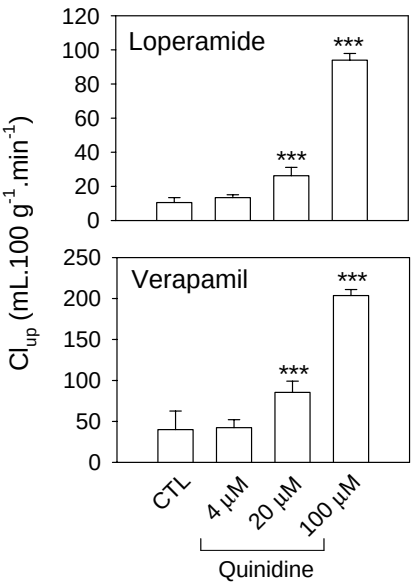

Fig. 2. Initial brain uptake clearances (Cl_{up}, $mL \cdot 100\ g^{-1} \cdot min^{-1}$) of loperamide (2 μM) and verapamil (0.5 μM) in the absence (control) and presence of increasing concentrations of quinidine during *in situ* perfusion (100 s) in wild-type mice. $^{***}P < 0.001$ vs. control using Bonferroni *t*-tests.

3. Discussion

The results of the present study demonstrate variable P-gp modulation of opioid brain uptake by *in situ* brain perfusion in P-gp-competent (wild-type) and P-gp-deficient [*mdr1a*(−/−)] mice. Over short perfusion times, this approach estimates unidirectional influx across the intact BBB. It is important to note that it may be possible to underestimate the brain uptake if the data are outside of the linear range. However, under these experimental conditions, unidirectional uptake conditions were maintained; therefore, single time point determination is appropriate. In addition, the brain perfusion technique eliminates the confounding factors of systemic disposition such as plasma protein binding, the formation of metabolites and variable pharmacokinetic behaviors. Hence, a direct comparison of BBB transport parameters can be made between compounds. Under the experimental conditions used in this study, the maximal possible value for initial brain uptake clearance (Cl_{up}) is perfusate flow, which has been estimated to $255\ mL \cdot 100\ g^{-1} \cdot min^{-1}$ [26]. Apparent brain distributional volumes (V_{brain}) describe the extent of brain distribution. For comparison, the brain water space is approximately $70\ mL \cdot 100\ g^{-1}$.

For this study, opioids were selected on the basis of receptor subtype specificity and differing physicochemical properties. Because only a limited number of opioids have been reported to interact with P-gp, the selection was arbitrary to some extent, recognizing that lack of transport by P-gp for a given opioid could be relevant information. The *R*-(−) enantiomer of methadone was selected because it is primarily responsible for the analgesic effect at mu opioid receptors [31]. Brain uptake varied by several orders of magnitude within this compound set (Table 1). For the first time, it was demonstrated that bremazocine, deltorphin II, U-69593, naltrindole, and SNC 121 are substrates of *mdr1a* P-gp, with the nonpeptidic delta agonist SNC 121 having a relatively high P-gp effect (8.60 ± 1.26). For fentanyl, loperamide, methadone, morphine, and DPDPE, previous investigations provide behavioral correlates to the present observations [12,13,15–18,20]. For instance, Thompson *et al.* [20] reported increased morphine, fentanyl, and methadone analgesia in P-gp-deficient mice. However, increased brain accumulation was demonstrated only for morphine. Because pharmacologic dose–response or concentration–response relationships are not linear, the influence of genetic P-gp disruption on behavioral responses cannot be viewed as a quantitative measure of alterations in BBB transport. The present investigation provides a uniform and quantitative assessment of the influence of P-gp on the brain uptake of several opioids. In conjunction with behavioral studies, this information may be useful to predict the magnitude of drug interactions resulting from inhibition of P-gp in the BBB.

The current dataset was too small to establish statistically meaningful correlations between physicochemical

descriptors and brain uptake. However, regardless of the P-gp status, there were general trends such as increasing brain uptake with log D 7.4, and inverse relationship with hydrogen bonding potential and solvation free energy of neutral species (data not shown). While such an investigation was beyond the scope of this study, it is reasonable to assume that classic physicochemical predictors of passive BBB transport may not apply in the face of P-gp-mediated efflux. The possibility that the rate of diffusion across the BBB, together with affinity and capacity of *mdr1a* P-gp, determines the P-gp effect for a given substrate is currently under investigation. The structural requirements for efficient P-gp transport are poorly defined [32], making potential interactions with P-gp difficult to predict and emphasizing the need for model systems in which such interactions can be assessed.

In experiments with unlabeled opioids, the prototypical P-gp substrate verapamil was included in the perfusate as a positive control for P-gp function to serve as an internal standard [26]. At relatively low concentrations (1–2 μM), these opioids did not decrease the P-gp effect for verapamil (Table 2), which would have indicated P-gp inhibition (i.e. increased verapamil Cl_{up} in the presence of P-gp, but no change in its absence). Because high methadone concentrations (50 μM) increased verapamil Cl_{up} in P-gp-competent mice, relatively poor affinity for P-gp may explain the lack of inhibition at lower opioid concentrations. Thus, it appears unlikely that these compounds would inhibit P-gp at analgesic concentrations. However, it may be relevant to determine if oral administration of opioids can inhibit intestinal P-gp (Cf, high local concentration). It should be noted that lack of change in verapamil Cl_{up} may have resulted from the interaction of opioids and verapamil at different P-gp binding sites. Despite the ability of meperidine to inhibit the interaction of vinblastine with P-gp *in vitro* (75 μM) [9], it did not influence the brain uptake of verapamil in this study (50 μM), and transport of meperidine was not limited by P-gp (Fig. 1). The latter observation is in agreement with a recent report that the analgesic effect of meperidine was unaltered in P-gp-deficient mice [20].

Given its excretory function in the gut, liver, and kidney, P-gp can be the locus of drug interactions [8,33]. P-gp-mediated excretion is not likely to play a major role in the elimination of most opioids, which is largely dependent on hepatic metabolism [34]. However, inhibition of P-gp could increase systemic exposure to active or toxic metabolites. For instance, it has been suggested that morphine-6-glucuronide may be a substrate of P-gp *in vitro* [35], although recent studies in transgenic mice do not support such an interaction *in vivo* [20,36]. In addition, modulation of P-gp function in the gut may influence the oral absorption of opioids; rifampin induction of intestinal P-gp appears to decrease the bioavailability of oral morphine [37]. However, low oral bioavailability of opioids secondary to P-gp-mediated efflux should not be taken for granted. For example, methadone appears to be a substrate of P-gp in the rat gut [23], but the oral bioavailability in humans is ∼92% [38].

With the exception of deliberate attempts to overcome P-gp with chemical modulators [12,13,15,18], few clinically relevant P-gp-mediated interactions in the BBB have been reported. The sedative and analgesic effects of fentanyl were increased in mice by the P-gp modulators cyclosporine and PSC833 [39,40]. Recently, Sadeque *et al*. [41] reported respiratory depression after oral co-adminstration of loperamide and quinidine in humans. Lack of significant change in loperamide plasma concentrations suggested that this was due to enhanced CNS exposure following inhibition of P-gp in the BBB by quinidine. The results of the present study (Fig. 2) support this hypothesis. Nevertheless, it should be recognized that many clinically used drugs with the ability to modulate P-gp may not inhibit P-gp significantly at therapeutic concentrations.

It may be argued that the P-gp effect on initial brain uptake is not representative of steady-state situations *in vivo*, in which other factors (plasma protein binding, affinity for brain tissue, egress components) influence net brain accumulation. Distributional equilibrium of a solute into brain is a function of uptake and egress processes in direct and inverse proportion, respectively; a net increase in initial uptake results in a proportional increase at steady state. Conceptually, a P-gp-mediated decrease in opioid brain uptake implies decreased magnitude and duration of centrally mediated analgesia or side effects, consistent with recent experimental evidence [12,13,15–20]. It appears that the P-gp effect observed on brain uptake *in situ* does not fully account for the effect observed some time after intravenous administration [31], suggesting that an additional efflux component comes from brain-to-blood egress under the latter condition. Plasma protein binding may also increase the influence of P-gp on brain uptake for substrates with high intrinsic BBB permeability (e.g. verapamil [27]). Although the influence of P-gp on the brain uptake of some opioids may appear low in the current study, it can be expected that the effect under steady-state conditions would be more significant, as exemplified for morphine [31].

Whether the results of this study in mice are relevant to opioid brain uptake in humans cannot be established without demonstration that the compounds interact selectively with *MDR1* P-gp *in vitro*, for which there is some evidence [27], and that this interaction decreases transport across the intact human BBB. In terms of substrate specificity, qualitative similarities between murine *mdr1a* and human *MDR1* have been reported, but species differences may exist [16,42]. In conclusion, the present results provide direct evidence for variable modulation of ligand access to brain opioid receptors by P-gp in mice. This may prove to be a potentially important issue in the context of pharmacologic pain control and drug interactions, given that

interindividual variability in P-gp function (either genetic or through drug interactions) could potentially alter the degree of opioid-induced analgesia attained in humans.

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