Impact of P-glycoprotein at the blood-brain barrier on the uptake of heroin and its main metabolites: behavioral effects and consequences on the transcriptional responses and reinforcing properties

Maria Seleman, Hélène Chapy, Salvatore Cisternino, Cindie Courtin, Maria Smirnova, Joël Schlatter, Fouad Chiadmi, Jean-Michel Scherrmann, Florence Noble, Cynthia Marie-Claire

Psychopharmacology
DOI 10.1007/s00213-014-3490-9

ORIGINAL INVESTIGATION

Impact of P-glycoprotein at the blood-brain barrier on the uptake of heroin and its main metabolites: behavioral effects and consequences on the transcriptional responses and reinforcing properties

Marianne Seleman · Hélène Chapy · Salvatore Cisternino · Cindie Courtin · Maria Smirnova · Joël Schlatter · Fouad Chiadmi · Jean-Michel Scherrmann · Florence Noble · Cynthia Marie-Claire

Received: 6 September 2013 / Accepted: 5 February 2014

Abstract
Rationale Transport across the BBB is a determinant of the rate and extent of drug distribution in the brain. Heroin exerts its effects through its principal metabolites 6-monoacetylmorphine (6-MAM) and morphine. Morphine is a known substrate of P-glycoprotein (P-gp) at the blood-brain-barrier (BBB) however, little is known about the interaction of heroin and 6-MAM with P-gp.
Objective The objective of this paper is to study the role of the P-gp-mediated efflux at the BBB in the behavioral and molecular effects of heroin and morphine.
Methods The transport rates of heroin and its main metabolites, at the BBB, were measured in mice by in situ brain perfusion. We then examined the effect of inhibition of P-gp on the acute nociception, locomotor activity, and gene expression modulations induced by heroin and morphine. The effect of P-gp inhibition during the acquisition of morphine-induced place preference was also studied.
Results Inhibition of P-gp significantly increased the uptake of morphine but not that of heroin nor 6-MAM. Inhibition of P-gp significantly increased morphine-induced acute analgesia and locomotor activity but did not affect the behavioral effects of heroin; in addition, acute transcriptional responses to morphine were selectively modulated in the nucleus accumbens. Increasing morphine uptake by the brain significantly increased its reinforcing properties in the place preference paradigm.
Conclusions The present study demonstrated that acute inhibition of P-gp not only modulates morphine-induced behavioral effects but also its transcriptional effects and reinforcing properties. This suggests that, in the case of morphine, transport across the BBB is critical for the development of dependence.

Keywords Morphine · Heroin · P-glycoprotein · Gene expression · Nociception · Locomotor activity

Abbreviations

P-gp	P-glycoprotein
6-MAM	6-Monoacetyl-morphine
Nac	Nucleus accumbens
IEG	Immediate early genes

Introduction

Drug abusers commonly use several routes of administration to ingest heroin, including injection, snorting and smoking. Ratings of euphoria are greater when heroin is administered

M. Seleman · H. Chapy · S. Cisternino · C. Courtin · M. Smirnova · J.-M. Scherrmann · F. Noble (✉) · C. Marie-Claire
Laboratoire de Neuropsychopharmacologie des Addictions Centre National de la Recherche Scientifique Unité Mixte de Recherche 8206, 4 avenue de l'Observatoire, 75006 Paris, France
e-mail: florence.noble@univ-paris5.fr

M. Seleman · H. Chapy · S. Cisternino · C. Courtin · M. Smirnova · J.-M. Scherrmann · F. Noble · C. Marie-Claire
Institut National de la Santé et de la Recherche Médicale, U 705, Paris, France

M. Seleman · H. Chapy · S. Cisternino · C. Courtin · M. Smirnova · J.-M. Scherrmann · F. Noble · C. Marie-Claire
Sorbonne Paris Cité, Faculté de Pharmacie, Université Paris Descartes, 4 avenue de l'Observatoire, 75006 Paris, France

J. Schlatter · F. Chiadmi
Hôpital Jean Verdier—AP-HP—Laboratoire de Toxicologie, 93140Bondy, Paris, France

Published online: 05 April 2014

intravenously than when smoked (Abreu et al. 2001; Comer et al. 1999; Marsch et al. 2001), and the severity of dependence and comorbid symptoms are influenced by the route of administration used (Wang and Liu 2012). In addition, both the dose and route of administration of heroin greatly impact withdrawal severity (Smolka and Schmidt 1999). However, although the route of administration seems also to influence the rate of progression to heroin dependence, it has little influence on the long-term level of dependence (Barrio et al. 2001). These observations indicate the significance of the rate of delivery of the drug to the brain during the early stages of dependence.

Therefore, in addition to the route of administration, transport across the blood-brain-barrier (BBB) may be a determinant of the rate and extent of drug distribution in the brain and the development of addiction. The characteristics of the BBB are determined mainly by the functional properties of the brain endothelial cells which are linked together by tight junctions impeding the paracellular diffusion of solutes. The BBB expresses a wide range of transporters that selectively control the transcellular transport of solutes and xenobiotics between the blood and the brain parenchyma (Abbott et al. 2010). P-glycoprotein (P-gp; Abcb1) is an ATP-binding cassette (ABC) transporter involved in the permeability of the BBB to drugs: it limits the net transport of many xenobiotics/substrates into the brain parenchyma by active, unidirectional efflux into the blood (Scherrmann 2005). The ways that P-gp at the BBB both restrain the entry of drugs into the brain and increase their clearance from the brain have been illustrated by pharmacokinetic studies with various opioids (Tournier et al. 2011). The reference opiate, morphine, is a known substrate of P-gp at the BBB as illustrated in vivo using P-gp inhibitors, and mice lacking the *Mdr1a* gene coding for P-gp (Dagenais et al. 2001; Zong and Pollack 2000). However, little is currently known about the interaction of heroin and its active metabolite 6-monoacetyl-morphine (6-MAM) with ABC transporters.

The rewarding effects of heroin, like those of morphine, are mediated by the mu opioid receptor in the brain (Hand et al. 1989; Kitanaka et al. 1998). However, heroin has a higher pharmacological potency in vivo than morphine (Martin and Fraser 1961; Smith and Beecher 1962), and a much lower systemic dose of heroin than morphine is needed to induce a similar rewarding effect (Bardo et al. 1995). These differences have long been attributed to the greater lipophilic properties of heroin allowing this substance to pass through the BBB much faster than the less lipophilic morphine (Oldendorf et al. 1972). However, after systemic administration, heroin is quickly metabolized first into 6-MAM and subsequently into morphine. These active metabolites both reach the extracellular brain fluid (pharmacokinetic biophase) where they bind and activate extracellular plasma membrane mu opioid receptors (Selley et al. 2001; Watson et al. 1996). Therefore, it has been proposed that heroin acts as a prodrug and exerts its effects mainly through its principal metabolites 6-MAM and morphine (Andersen et al. 2009).

These various observations raise the issue of the influence of the uptake of opioids across the BBB and as a consequence the selection of the most appropriate experimental model: morphine is used as a reference compound to study addiction in experimental animal models whereas drug abusers mostly use heroin. We report a study designed to investigate whether the different rates of penetration of morphine and heroin through the BBB to the brain affect their neuropharmacological effects. First, an in situ brain perfusion technique was used to assess the rates of transport of morphine, heroin, and 6-MAM and the effects of the inhibition of P-gp by PSC833. This analogue of cyclosporine A is a potent and selective P-gp inhibitor (Achira et al. 1999; Kusunoki et al. 1998; Watanabe et al. 1995). Then, the consequences of PSC833 pretreatment on the acute locomotor and nociceptive effects of morphine and heroin were studied. There is an extensive literature of genes regulated by acute morphine in mice striatum. However, it has been shown that some of them have distinct patterns of regulation after acute morphine or heroin (Piechota et al. 2010). Among these candidates, eight genes were chosen to study the effects of P-gp inhibition on gene expression regulation induced by the two drugs. Finally, we studied the influence of P-gp inhibition on the acquisition of morphine reinforcing properties in the place preference paradigm.

Materials and methods

Animals

Male Swiss mice (20–22 g at the beginning of the experiments) (Charles River, L'arbresle, France) were housed in groups of 12 in a room with a 12-h light/12-h dark cycle and a controlled-temperature environment (20–21 °C). Mice were allowed to get used to the housing facilities over a period of 1 week. Body weight was determined on the day of experimentation. Food and water were provided ad libitum. Animal experiments were carried out in accordance with the European Communities Council Directives of 24/11/1986 (86/609/EEC) with French law, and under the supervision of the faculty ethics committee. Standard principles of laboratory care were followed and every effort was made to minimize the number of animals used and their discomfort.

Drugs and pharmacological treatments

Morphine and heroin were purchased from Francopia (Paris, France). 6-MAM was purchased from Euromedex (Souffelweyersheim, France). PSC 833 (6-[(2*S*,4*R*,6*E*)-4-methyl-2-(methylamino)-3-oxo-6-octenoic acid]-7-L-valine-

cyclosporin A), also known as valspodar, was a generous gift from Novartis Pharma (Basel, Switzerland). Morphine and heroin were dissolved in saline solution (0.9 % NaCl) and were administered intravenously (i.v.). PSC 833 was dissolved in ethanol/PEG400/water (10/80/10 *v*/*v*) and injected intraperitoneally (i.p.) at a dose of 20 mg/kg, 30 min before the opiate. In all cases, the injection volume was 0.1 ml/10 g. For mass spectrometry, morphine-*d*3, 6-MAM-*d*3, and heroin-*d*9 freebase in methanol (100 μg/mL) were purchased from Cerilliant (LGC Standards, Molsheim, France). All other chemicals were of analytical grade.

In situ brain perfusion

The drug uptake at the BBB was measured in mice by in situ brain perfusion, a technique in which the blood is completely replaced by an artificial perfusion fluid for a short time, as previously described (Dagenais et al. 2000). This allows measurement of the intrinsic ability of a compound to cross the luminal BBB without the confounding influence of peripheral metabolism, systemic elimination and protein vascular binding. Mice were anesthetized with ketamine (140 mg/kg ip)+ xylazine (8 mg/kg, ip). Briefly, the right common carotid artery was exposed and the external carotid artery was ligated at the bifurcation of the common and internal carotid arteries. The right common carotid artery was catheterized and the catheter connected to a syringe containing the perfusion fluid fitted with an infusion pump. The thorax was opened, the heart was cut, and perfusion started immediately at a flow rate of 2.5 ml/min. The perfusion fluid consisted of bicarbonate-buffered physiological saline containing 128 mM NaCl, 24 mM $NaHCO_3$, 4.2 mM KCl, 2.4 mM NaH_2PO_4, 1.5 mM $CaCl_2$, 0.9 mM $MgCl_2$, and 9 mM D-glucose. The solution was gassed with 95 % O_2-5 % CO_2 for pH control (7.4) and warmed to 37 °C in a water bath. Heroin (30 μM), 6-MAM (30 μM), or morphine (30 μM) was added to the perfusion fluid with or without addition of PSC833 (5 μM) immediately before the start of brain perfusion experiments. Perfusion times were 30 s for heroin, and 60 s for 6-MAM and morphine; brain perfusion was terminated by decapitating the mouse after these times.

The brain was rapidly removed from the skull and the right hemisphere weighed and frozen in liquid nitrogen. Cold 0.1 N hydrochloric acid was added to the samples (2 mL/g of brain tissue), and the mixture homogenized by sonication; the resulting brain samples were stored at −80 °C until determination of morphine, 6-MAM and heroin concentrations by chromatography-mass spectrometry (GC-MS).

Sample preparation for gas-chromatography-mass spectrometry analysis

The frozen samples were thawed at room temperature for 15 min, centrifuged for 5 min at 10,000 rpm and 200-μL aliquots of the supernatant were transferred to a 5-mL polypropylene tube. Four milliliters of sodium acetate buffer (pH 5.0, 100 mM) and 0.1 mL of each internal standard stock solution (1 μg/mL) of morphine-*d*3, 6-MAM-*d*3, and heroin-*d*9 were added. A Gilson automated GX-271 Series GPC Clean-up System (Gilson, Villiers, France) was used for extractions. Samples were vortexed and transferred to the autosampler vial for extraction, and loaded onto a Phenomenex Strata X Drug B Clean Screen® solid-phase extraction column. The column was washed with water (2 mL), 0.1 N hydrochloric acid (2 mL) and acetonitrile (3 mL), and then dried under vacuum for 10 min. The analytes were collected in a 5-mL glass tube by elution with 3 mL of freshly prepared ethyl acetate, isopropanol, and 25 % ammonium hydroxide (70/20/10 *v*/*v*). The solvent was then evaporated under a gentle stream of nitrogen. The residue was derivatized by the addition of 20 μL BSTFA with 1 % TMCS and 20 μL of ethyl acetate; to ensure complete derivatization, the samples were incubated with this mixture at 65 °C for 20 min.

GC-MS quantification of morphine, heroin and 6MAM

The Thermo Focus DSQ II GC-MS system was used to assay opioids. The ions selected by SIM were *m*/*z* 429, 236, 414 for morphine and *m*/*z* 432, 239 for morphine-*d*3, *m*/*z* 399, 340, *m*/*z* 287 for 6-MAM and *m*/*z* 402, 343 for 6-MAM-*d*3, and *m*/*z* 369, 327 for heroin and *m*/*z* 378, 334 for heroin-*d*9. The limits of detection were 0.5 ng/mL and limits of quantification were 10 ng/mL. The system was equipped with an Uptibond® UB5 premium column (30 m×0.25 mm×0.25 μm). The samples were injected in the splitless mode. The flow rate of the carrier gas helium was 0.8 mL/min. The injector temperature was set at 250 °C. The oven temperature programs were set as follows: The initial temperature was 90 °C (held 0.5 min) and was increased at 30 °C/min to the first temperature hold at 220 °C (held for 3 min), and then increased at 15 °C/min to the final temperature hold at 290 °C (held for 15 min).

Initial BBB transport kinetic parameters

Calculations were previously described (Cattelotte et al. 2008). Two variables describe the transport of a compound across the BBB: the apparent brain volume of distribution (V_{brain}, μL/g) and the initial brain transport parameter K_{in} (μL/s/g) also called brain clearance. The V_{brain} was calculated from the amount of drug in the right hemisphere corrected from the vascular space (V_v 14 μL/g):

$$V_{brain} = \left(X_{tot} - V_v.C_{perf}\right)/C_{perf}; K_{in} = V_{brain}/T,$$

where C_{perf} (nmol/μL) is the opioid concentration in the perfusion fluid, X_{tot} (nmol/g) the amount of opioid in the tissue sample (vascular+extravascular), and T the perfusion time

(second). Less than 10 % of heroin was transformed into 6-MAM in the brain tissue sample and no degradation was detected in the perfusion fluid after the 30 s of the brain perfusion. The total heroin concentration in the brain was then calculated from the sum of the molar concentrations of heroin and 6-MAM in the brain sample. Morphine was not detected in heroin or 6-MAM brain perfusion experiments. The coefficient of brain extraction (E, %) was calculated as the ratio of the K_{in} of the studied compound to the K_{in} of diazepam (42.3 μl/s/g), which can be considered to be a marker of vascular flow (Cattelotte et al. 2008).

Locomotor activity

The locomotor activity of mice was measured in an actimeter (Imetronic, Bordeaux, France) immediately after the drug injections. The Imetronic actimeter is composed of eight cages of transparent plastic of equal size (19×11×14 cm) under low illumination (<5 lx). One mouse was placed in each box and its movements were recorded. Displacements were measured by photocell beams projected across the long axis and above the floor. Horizontal locomotor activities are expressed in scores (mean±S.E.M), the score being the number of interruptions of the photocell beams recorded during the 180 min of the experiment. Locomotor activity was recorded for 180 min. The areas under the curve (AUCs) were used to determine the doses of morphine and heroin inducing equivalent locomotor activity in our conditions (data not shown): this led to doses of 10 mg/kg i.v for morphine and 5 mg/kg i.v for heroin being used.

Hot plate test

The test was based on that described by Eddy and Leimbach (1953). A glass cylinder (16 cm high, 16 cm diameter) was used to keep the mouse on the heated surface (52±0.5 °C) of the plate. The latency period until the mouse jumped was timed with a stop-watch (cut-off time=240 s). First, the lack of antinociception of PSC833 alone was verified (data not shown). Results are expressed as percentage of maximum possible effect (% MPE)±S.E.M. Analgesic dose 50 (AD_{50}) values, were calculated as doses that produced an analgesic effect equal to 50 % MPE (Tallarida and Murray 1986).

Conditioned Place Preference

The place preference apparatus consisted of two conditioning compartments separated by a neutral compartment. For this test, a dose of morphine (2 mg/kg) that does not produce conditioned place preference in our conditions was used (data not shown). The movement and location of animals were recorded by computerized monitoring software (Videotrack, Viewpoint, France). Briefly, the protocol consisted of three phases: (1) Preconditioning phase (1 day): Drug-naive animals were allowed free access to both compartments for 18 min, and the time spent in each compartment was recorded. (2) Conditioning phase: This phase lasted 3 days during which each conditioning chamber was closed. On the morning of days 2, 3 and 4, animals were pretreated with PSC833 or vehicle and then 30 min later with morphine and were placed individually in one of the conditioning environments for 5 min. In the afternoon of days 2, 3, and 4, animals were given saline in the opposite compartment. Control animals were given vehicle or PSC833 and then 30 min later saline twice a day and were subjected to an alternating sequence between the two compartments. (3) Postconditioning phase (1 day): This phase was carried out on day 5 exactly as the preconditioning phase. Results are expressed as scores (mean ±S.E.M.), calculated as the difference between postconditioning and preconditioning time spent in the drug-associated compartment.

Dissection, RNA extraction, and reverse transcription

A separate group of mice was used for transcriptional studies. Mice were killed by cervical dislocation 30 min after injection of the drugs. The brain was quickly removed, frozen in isopentane at −50 °C, and placed in an acrylic matrix (David Kopf Instruments, Phymep, France) allowing reproducible slicing of 1-mm-thick coronal Sections. A section of 2 mm was cut, corresponding approximately to bregma +0.26 mm to −0.46 mm. Dorsal striatum and nucleus accumbens were then dissected free-hand on ice from the slices, frozen in isopentane and stored at −80 °C until processing.

Total RNA was extracted from bilateral structures with an RNeasy® Qiagen mini kit plus (dorsal striatum) or micro kit plus (nucleus accumbens) according to the lipid tissue protocol of the manufacturer (Qiagen, Courtaboeuf, France). Total RNA was assayed with a NanoDrop® ND-1000 spectrophotometer. Reverse transcription of RNA (1 μg) was performed as previously described in a final volume of 20 μl (Belkai et al. 2009); the resulting cDNAs were stored at −20 °C.

Real-time quantitative PCR (Q-PCR)

The primer nucleotide sequences (Table 1) were chosen with the assistance of Oligo 7.55 software (MedProbe, Oslo, Norway). The genes analyzed in this study were selected on the basis of documented early modulation by acute morphine and/or heroin (Korostynski et al. 2006; Piechota et al. 2010). Gene expression was assessed by real-time quantitative RT-PCR. Amplification was detected by SYBR® Green fluorescence with a 7900HT sequence detection system (Applied Biosystems, France). Thermocycling was carried out in a final volume of 20 μl as previously described (Belkai et al. 2009). A calibration curve using cDNA from an untreated mouse

Table 1 Primer sequences used for SYBR Green-based real-time quantitative polymerase chain reaction

Gene	*Forward primer*	*Reverse primer*
Rplpo	GGGGGCCACCTGGAGAACAAC	CAGCAGCTGGCACCTTATTG
Fos	GGCAAAGTAGAGCAGCTATCTCCT	TCAGCTCCCTCCTCCGATTC
Egr1	GAGCCCGCACCCAACAGTG	TGGGGCTCAGGAAAAATGTCA
Arc	GCCGCCAAACCCAATGTGA	GTCGCCCGTGGGCACATAG
Dusp5	CGTCGCCTACAGACCAGCC	GTAGGCACTTCCAAGGTAGAGGA
Pdyn	GCAGGGGCAGGGAAGAGT	GATGGGCAGGGATCACAG
Penk	ACGCCCCGAGTGGTGGAT	GCCCCCGTATCTTTTCTC
Rnd3	GATCGGAGCAGCCACTTACATAG	GGTGGCGACGTGAAAAATGT
Plekhg4	TCTTCCTGCGTGAGCTAGAGG	AACGAGGTTTGTTCTTGCTGTAG

brain was used for quantification. The *Rplp0* transcript was assayed and results for the genes of interest in each sample were normalized to the *Rplp0* content. Results are thus expressed as the value for the transcript of the gene of interest/*Rplp0* transcript and normalized to the mean value for the saline control group.

Statistical analysis

Graphpad Prism 4.03 software (Graphpad Inc., CA, USA) was used for statistical analyses. Student's *t* test was used to identify PSC833 inhibition effects on the brain transport parameter (K_{in}). Locomotor activity, antinociception, conditioned place preference and real-time quantitative RT-PCR data were analyzed using one-way ANOVA between subjects, followed by Newman-Keuls tests for post hoc comparisons. In all cases, tests were two tailed and the level of significance was set at $p<0.05$.

Results

The P-gp inhibitor PSC833 modulates the uptake of morphine but not 6-MAM or heroin at the BBB

We used in situ brain perfusion rather than conventional systemic pharmacokinetics to study transport across the BBB and to assess the effects of P-gp inhibition on uptake of morphine, 6-MAM and heroin by the mouse brain. This method allows measurement of the initial kinetics in trans-influx zero conditions prior to the establishment of any equilibrium, and in similar kinetic conditions, for the diverse drugs tested. The short perfusion time with the artificial perfusion fluid avoids significant drug degradation and allows exposure of the BBB to the compounds tested to be controlled qualitatively and quantitatively. Following in situ brain perfusion, brain tissue samples were assayed for morphine, 6-MAM, heroin, and the fluid flow marker diazepam; the concentrations of morphine, 6-MAM and heroin were 0.47 %, 16.3 % and 29.3 %, respectively, of that of diazepam. The measured K_{in} for heroin was thus 1.8-fold that of 6-MAM and 62-fold that of morphine (Fig. 1). In co-perfusion experiments with the P-gp inhibitor PSC833, the morphine transport rate across the BBB was increased by 1.5-fold ($p<0.01$) (Fig. 1a); P-gp inhibition by PSC833 had no significant effect on the transport of 6-MAM and heroin (Fig. 1b).

Inhibition of P-gp induces a left shift of the dose-effect curve of morphine but not that of heroin in the hot plate test

We then investigated the effect of PSC833 on the morphine- and heroin-induced antinociceptive responses in the hot plate test, a classical paradigm used to assess effects of opiates on acute pain. The dose-response curves were established from experiments performed 5 min after i.v. drug administration.

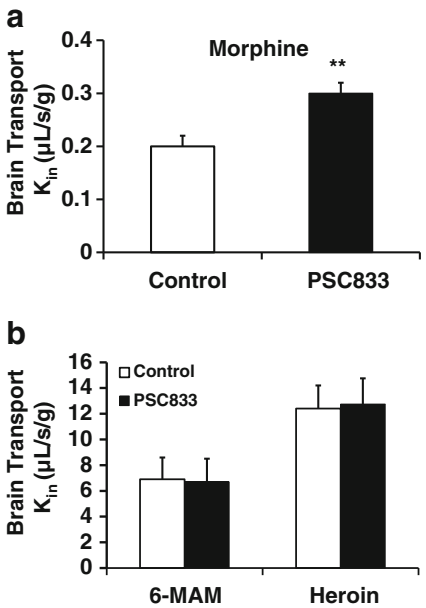

Fig. 1 Brain transport (K_{in}; µL/s per gram) of morphine (panel **a**), 6-MAM and heroin (panel **b**) with and without co-perfusion of the P-gp inhibitor PSC833 (5 µM) measured by in situ brain perfusion in mice. Results are expressed as mean values±s.d. (n=4–6 mice per group). **$p<0.01$ as compared with the appropriate control group

One-way ANOVA showed a significant effect of each morphine ($p<0.0001$) and heroin ($p<0.0001$) treatment. Post hoc Newman-Keuls tests indicated that pretreatment with PSC833 significantly increased the AD_{50} of morphine (1.9 times $p<0.05$) from 1.5 to 0.8 mg/kg (Fig. 2) but that PSC833 had no effect on the AD_{50} of heroin.

Pretreatment with PSC833 lengthens the duration of the morphine-induced but not heroin-induced antinociceptive response

To determine whether pretreatment with PSC833 alters the duration of morphine or heroin antinociceptive responses, time course studies were undertaken. Mice were pretreated with PSC833 or vehicle as a control, and then injected with morphine and heroin at the AD_{50} determined in the previous experiments (1.5 and 0.05 mg/kg i.v, respectively). One-way ANOVA confirmed that at these doses, morphine ($p<0.0001$) and heroin ($p<0.0001$) treatment had significant antinociceptive effects. Newman-Keuls post hoc tests revealed that the antinociceptive effect of morphine declined more slowly in PSC833-pretreated mice than in vehicle-pretreated mice (Fig. 3): % MPE at 15 and 25 min were significantly higher in PSC833 pretreated animals than vehicle-pretreated animals (1.63-fold, $p<0.01$ and 1.67-fold, $p<0.01$). Twenty-five min after i.v. administration of the drug, vehicle-morphine treated mice showed no antinociceptive effects whereas PSC833-morphine mice still exhibited 60 % antinociception relative to controls. In the case of heroin,

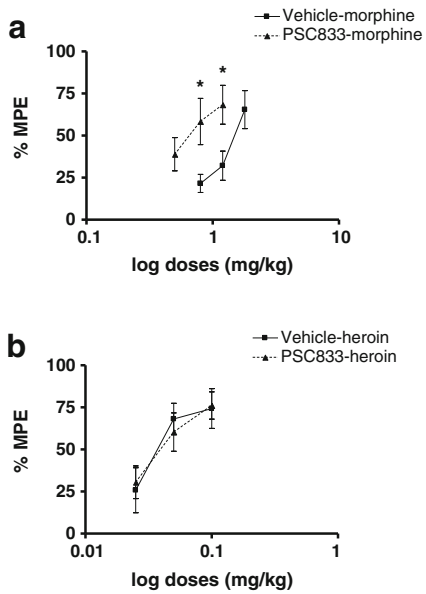

Fig. 2 Effect of PSC833 pretreatment on morphine and heroin antinociceptive log dose-response curves in the hot plate test. Data are plotted as percent of maximum possible effect (%MPE) versus log dose in milligrams per kilogram. Each point represents the mean±S.E.M. (n=9–11 per group). *$p<0.05$, with the vehicle-morphine treated group used for reference

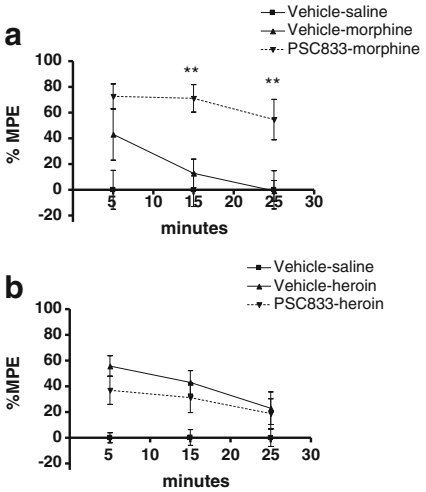

Fig. 3 Inhibition of P-gp modulates the time course of the effects of morphine (1.2 mg/kg) but not heroin (0.05 mg/kg) on acute pain in the hot plate test. **$p<0.01$ as compared to the vehicle-morphine treated group. Results are expressed as percent of maximum possible effect (MPE)± S.E.M. (n=9–11 per group)

PSC833 pretreatment had no significant effects on the duration of analgesic activity of the drug (Fig. 3).

Pretreatment with the P-gp inhibitor PSC833 modulates morphine-induced but not heroin-induced locomotor activity

To investigate the role of P-gp on another opioid-induced acute behavior in mice, we assessed the effects of PSC833 on each morphine- and heroin-induced locomotor activity. One-way ANOVA showed a significant treatment effect ($p<0.0001$). Post hoc analyses revealed that PSC833 alone had no effect on locomotor activity but administration of morphine- and heroin-induced hyperlocomotion (2.6- and 2.8-fold, respectively) as compared to control mice (Fig. 4). Morphine-induced hyperlocomotion was greater in PSC833 than in vehicle-pretreated animals (2.65-fold) but PSC833 pretreatment had no effect on heroin-induced hyperlocomotion.

Inhibition of P-gp at the BBB modulates morphine-induced but not heroin-induced transcriptional effects in the nucleus accumbens and dorsal striatum

Real-time PCR was used to determine whether the P-gp inhibition by PSC833 30 min before the acute injection of morphine (10 mg/kg i.v.) and heroin (5 mg/kg i.v.) affected the expression of eight early-responding genes in the nucleus accumbens and the dorsal striatum (*Fos*, *Egr1*, *Arc*, *Dusp5*, *Pdyn*, *Penk*, *Rnd3*, and *Plekhg4*). Only genes that showed modulations are presented here (Fig. 5). The *Dusp5* mRNA level was significantly lower (0.67-fold) in the nucleus accumbens following heroin treatment than following saline treatment, and this difference was not altered by pretreatment

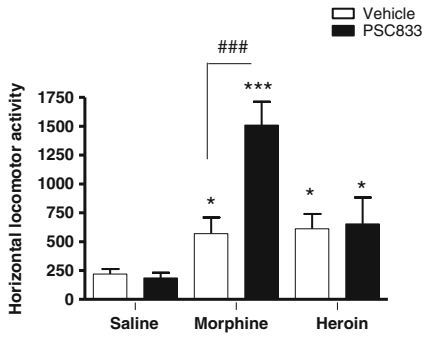

Fig. 4 Effect of P-gp inhibition on morphine (10 mg/kg) and heroin (5 mg/kg)-induced horizontal locomotor activity over 180 min. Results are expressed as means (± S.E.M.) of locomotor activity in activity counts. *$p<0.05$; ***$p<0.001$ as compared to the vehicle-saline treated group; $^{###}p<0.001$ as compared to the vehicle-morphine treated group ($n=10–13$ per group)

with PSC833 (Fig. 5a). However, in the case of morphine, a significant decrease in the level of *Dusp5* mRNA was observed only after pretreatment with PSC833 (0.68-fold). Similarly, the *Rnd3* mRNA level in this structure was lower following heroin treatment (0.8-fold) than in saline controls; this effect was not modified by PSC833, whereas the inhibitor significantly altered the effect of morphine on *Rnd3* mRNA (0.78-fold; $p<0.05$). In our conditions, the six other genes tested were not significantly regulated in the nucleus accumbens after acute injection of either morphine or heroin, although for the *Arc* and *Penk* genes, there was a tendency to decreased and increased expression, respectively (Fig. 5a).

The amount of *Penk* mRNA in the dorsal striatum was significantly higher in heroin-treated animals, with or without PSC833 pretreatment (1.51- and 1.52-fold, respectively), than in saline-treated controls (Fig. 5b). There was a slight but not significant decrease of *Arc* mRNA level in this structure following heroin or morphine treatment. Neither morphine nor heroin treatments had any significant effect on the expression in this structure of the other genes tested, in our conditions.

Modulation of morphine-induced place preference by PSC833 pretreatment

As P-gp inhibition appeared to affect only morphine, but not heroin, transport across the BBB, we used the P-gp inhibitor PSC833 to determine whether the inhibition of P-gp modifies the reinforcing properties of morphine in the Conditioned Place Preference (CPP) paradigm. PSC833 alone had no effect and a non-effective dose of morphine (2 mg/kg i.v.) was used. One-way ANOVA revealed a significant treatment effect ($p=0.0021$). Post hoc comparisons showed a significant difference between the PSC833/morphine group and controls (vehicle/saline vs PSC833/morphine: $p<0.01$) and a significant effect of PSC833 pretreatment on the acquisition of morphine-induced conditioned place preference (vehicle/morphine vs PSC833/morphine: $p<0.05$) (Fig. 6).

Discussion

We report for the first time pharmacokinetic and pharmacodynamic evidence for P-gp inhibition at the BBB having substantial consequences not only on the acute behavioral effects of morphine but also on its molecular response and reinforcing properties. Our work demonstrates that P-gp inhibition: (i) increases the rate of morphine uptake by the brain; (ii) modifies the acute analgesic and locomotor effects of morphine; (iii) alters acute transcriptional responses selectively in the nucleus accumbens; and (iv) increases the time spent in the drug-associated compartment in the conditioned place preference test. We also show that inhibition of P-gp did not affect the transport across the BBB of heroin or 6-MAM, and that inhibiting P-gp had no effect on either the acute behavioral or transcriptional responses to heroin. These differences between the opioid compounds tested provide new information about the role of the BBB in controlling the rate of drug delivery to the brain, and the different neuropharmacological effects of these drugs.

It is generally believed that morphine is the active agent responsible for the behavioral effects of heroin. Consequently, morphine has been widely used as the reference opiate to study the effects and mechanisms of action of heroin. However, 6-MAM is the first metabolite to appear in vivo and its role has been underestimated: 6-MAM displays higher efficacy than morphine for mu opioid receptor-mediated G-protein activation (Oldendorf et al. 1972; Selley et al. 2001). Following subcutaneous injection of heroin into mice, 6-MAM concentrations in the brain are higher than those of heroin and correlate better with acute heroin-induced locomotor activity, suggesting that 6-MAM is the metabolite responsible for major acute effects of heroin (Oldendorf 1992). We found that the uptake rate of heroin into the brain is faster than that of morphine. In view of the results reported by Andersen et al. (2009) it was of particular importance to also assess the rate of entry of 6-MAM into the brain; we found that 6-MAM enters the brain 35 times faster than morphine and 1.8 times slower than heroin. This is consistent with the recent pharmacokinetic model for heroin suggesting that the high levels of 6-MAM in the brain result from this compound crossing the BBB from the blood to the brain (Boix et al. 2013). The blood concentration of 6-MAM are high after subcutaneous (Andersen et al. 2009) or intravenous (data not shown) injection of heroin into mice. The fast BBB uptake of 6-MAM suggests that these high blood levels originate from the difference between the high deacetylation rate of heroin to produce 6-MAM in the blood and the lower rates of elimination and metabolization of 6-MAM into morphine. We also show here

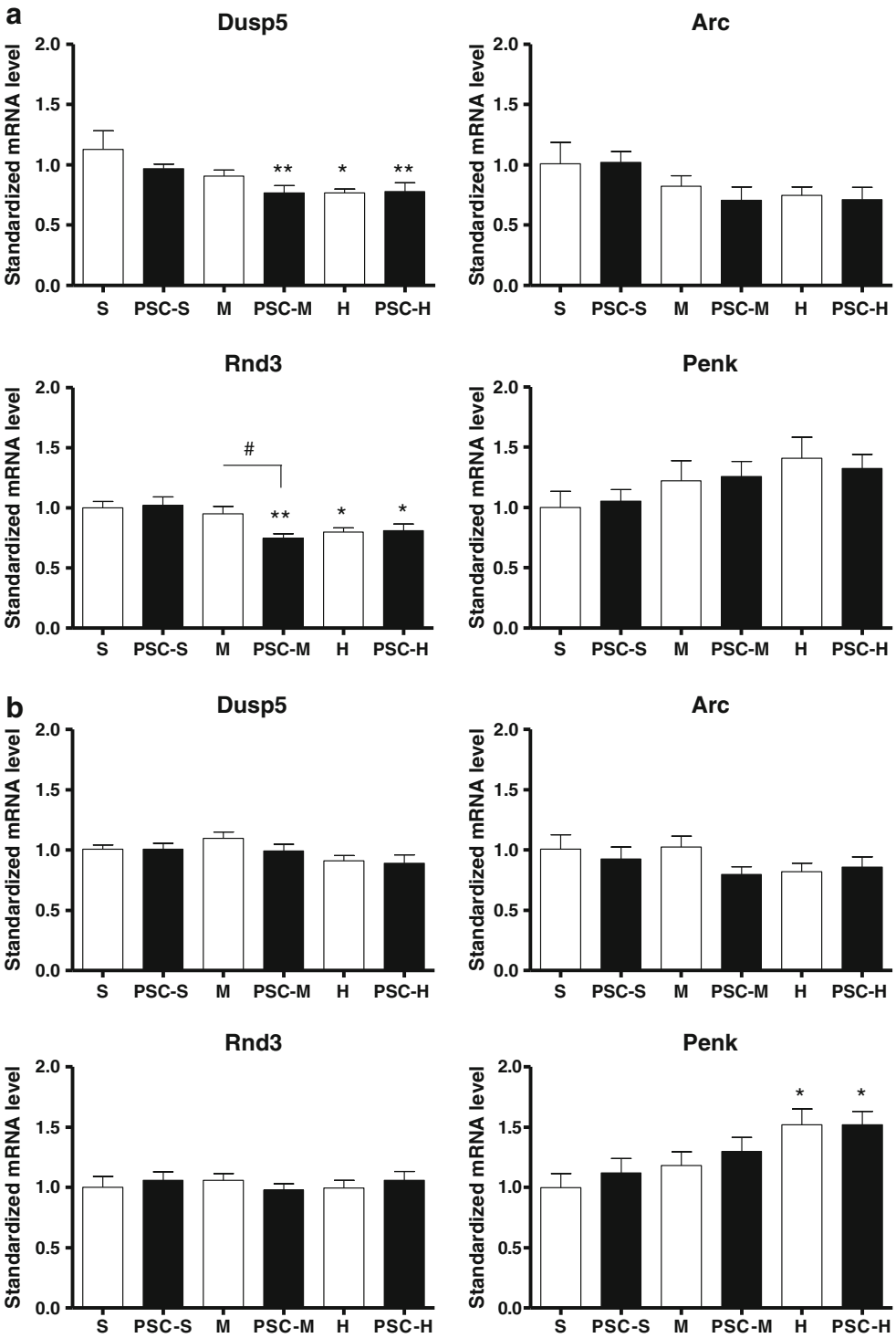

Fig. 5 Effect of PSC833 pretreatment on morphine (10 mg/kg)- and heroin (5 mg/kg)-induced mRNA regulation in the nucleus accumbens (**a**) and the striatum (**b**). Treatment groups are as follows: *S* vehicle-saline, *PSC-S* PSC833-saline, *M* vehicle-morphine, *PSC-M* PSC833-morphine, *H* vehicle-heroin, and *PSC-H* PSC833-heroin. Mice were sacrificed 30 min after i.v. injection of the drugs. Data are expressed as mean±S.E.M. of the ratio of the abundance of the transcript of the gene of interest to that of Rplp0 (n=10–12 per group). For each gene, mRNA levels in the samples were normalized such that the value in the S group was 1. *p<0.05; **p<0.01 as compared to the vehicle-saline treated group; $^{\#}p$<0.05 as compared to the vehicle-morphine treated group

that P-gp slows the transport of morphine, but not heroin or 6-MAM, through the BBB. This is consistent with previous results showing a capacity-unlimited transport of morphine by P-gp at the BBB (Cisternino et al. 2004; Dagenais et al. 2004) and greater morphine transport in P-gp-deficient than wild-type mice (Cisternino et al. 2001; Schinkel et al. 1995; Xie et al. 1999).

To better understand the behavioral consequences of the involvement of P-gp in regulating morphine transport across the BBB, we assessed morphine- and heroin-induced acute antinociceptive and locomotor effects. We observed no effect of P-gp inhibition on heroin-induced behaviors. Previous studies have shown that 6-MAM is the main compound responsible for the behavioral effects of heroin (Andersen

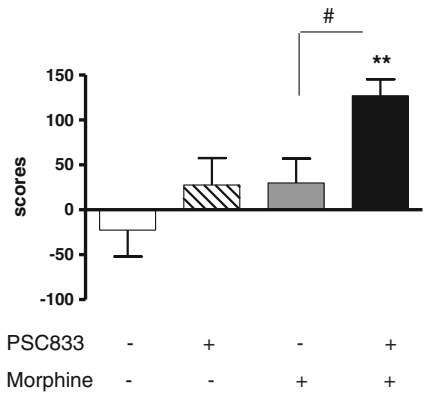

Fig. 6 Effect of PSC833 on the acquisition of morphine-induced CPP. Mice were given PSC833 (20 mg/kg) or vehicle 30 min before morphine (2 mg/kg) or saline during the conditioning phase. Data are expressed as mean±S.E.M, and score values are calculated as the difference between postconditioning and preconditioning time spent in the compartment associated with the drug (n=10–12 per group). $^{**}p<0.01$ as compared to the vehicle-saline treated group; $^{\#}p<0.05$ as compared to the vehicle-morphine treated group

et al. 2009; Boix et al. 2013). Our results are in agreement with this since we also observed no effect of PSC833 on the brain uptake of heroin and 6-MAM. Using the hot plate test, we observed, in the case of morphine, that PSC833 pretreatment significantly shift the dose-response curve to the left. These results are consistent with published findings for the effects of inhibitors of the efflux pumps at the BBB on the response to morphine: in rats, the antinociceptive effect of morphine is increased by GF120918, an inhibitor of both P-gp and Bcrp (Letrent et al. 1999). The antinociceptive efficacy of morphine is also higher in mice lacking P-gp than in controls (King et al. 2001; Thompson et al. 2000; Zong and Pollack 2000).

The rate of cocaine and nicotine delivery to the brain can modulate the regulation of immediate early genes (IEG), including *Fos* and *Arc* which can serve as markers of neural activity (Samaha et al. 2004, 2005). We tested whether exposure to morphine and heroin had different effects on gene expression. We assayed the mRNAs of eight genes in two cerebral structures central to the development of dependence: the dorsal striatum and nucleus accumbens (Di Ciano et al. 2008; Galtress and Kirkpatrick 2010). We found that inhibiting P-gp influenced the transcriptional response to morphine. The effects were gene-specific and differed between the two structures analyzed. The effects are more pronounced in the nucleus accumbens where, for two of the tested genes, the modulations of expression induced by morphine after pretreatment with PSC833 were similar to those induced by heroin. Nucleus accumbens is mostly involved in Pavlovian learning and motivation while dorsal striatum appears to be involved in goal-oriented conditioning and motor behaviors. These functional differences are underpinned by the variation in the density of mu opioid receptors and the divergent glutamatergic and dopaminergic afferents (Wickens et al. 2007) in these two cerebral structures. The differences in the gene expression profiles observed here result from the activation of selective pathways in the nucleus accumbens and suggest that uptake rate might have an effect mainly on the motivational and learning component of dependence.

A recent study comparing, in parallel, the transcriptional responses to morphine and heroin in the mouse striatum has shown that intraperitoneal injection of morphine and heroin modulate the same genes; however, the time course of heroin-induced modulations differs from that of morphine and the largest difference was at the first time point analyzed (1 h) (Piechota et al. 2010). Here, we used the intravenous route of administration and analyzed gene expression regulation 30 min after the injection of the drugs. This could explain the absence of modulation observed for some of the analyzed genes. However, we found that heroin significantly modulates the expression of more genes than morphine in our conditions. This is the first study to show that increasing morphine distribution into the brain increases the range of morphine-induced significant gene expression modulations to a level similar to that of heroin. These results demonstrate that acute transcriptional responses to morphine, in the nucleus accumbens, depend on its rate of uptake into the brain. This selective effect is particularly interesting in view of the central role of this structure in addiction. It has been shown that modulating the transcriptional response induced by morphine in the nucleus accumbens could modulate the behavior of animals. Thus, injections of IEGs antisense oligonucleotide into this structure modulate the acquisition, expression or reinstatement of morphine-induced CPP (Lv et al. 2011; Tolliver et al. 2000). The genes whose expression levels are significantly modulated by morphine in the nucleus accumbens after inhibition of P-gp are *Rnd3* and *Dusp5*. Rnd3 regulates actin cytoskeleton organization, cell migration and has been proposed to promote neurite and/or dendrite outgrowth (Chardin 2006). We have previously shown that it is an early common effector of several drugs of abuse in the striatum in mice (Marie-Claire et al. 2007). The observed morphine-induced downregulation of this mRNA in the nucleus accumbens suggests a more pronounced effect after pretreatment with PSC833 and is consistent with the reduction of dendritic spine described in the nucleus accumbens after morphine treatment (Robinson and Kolb 1999). Dual specificity phosphatases (Dusp) are involved in the inactivation of Mitogen-activated protein kinases such as extracellular signal-regulated kinase (ERK) (Owens and Keyse 2007). The observed significant morphine-induced downregulation of Dusp5 in the nucleus accumbens after inhibition of P-gp is coherent with the increase in phosphorylated ERK observed after morphine treatment in this structure (Valjent et al. 2004).

Drugs of abuse induce the expression of a complex pattern of IEG. This is thought to be an initial step in their ability to alter synaptic organization and produce persistent forms of

neurobehavioral plasticity that contribute to addiction (Hyman and Malenka 2001; Nestler 2001). We show here that inhibition of P-gp during the acquisition of morphine-induced place preference significantly enhances the rewarding properties of a sub-effective dose of the drug in this experimental paradigm. Therefore, our results demonstrate that the efflux of morphine at the BBB by P-gp not only influences of the molecular mechanisms underlying the acute effects of morphine in the striatum but also its positive reinforcing properties. This is consistent with the view that the ability of drugs to produce adaptations in the mesocorticolimbic system contributes to addiction (Robinson and Berridge 2000).

In conclusion, our study confirms the importance of P-gp to the rate of transport of morphine into the brain and consequently to its behavioral effects. The presence of the P-gp inhibitor PSC833 modulates morphine-induced transcriptional effect especially in the nucleus accumbens. In addition, we show that inhibiting the P-gp-dependent efflux of morphine increases the reinforcing properties of the drug. Rapid delivery into the brain might influence the rate of progression to dependence by facilitating the action of the drug to induce, in the striatum, forms of neurobiological plasticity that contribute to its rewarding properties. Interestingly, P-gp expression and activity may be affected in several clinical situations, including inflammation, infection or chronic exposure to drugs (Decleves et al. 2011; Roberts and Goralski 2008) and this could have an impact on the vulnerability of patients and/or the variability of the response to opioids.

Acknowledgments The authors thank Didier Fauconnier for technical assistance and the animal facility of the *Institut Medicament-Toxicologie-Chimie-Environnement* for providing resources. This work was supported by Centre National de la Recherche Scientifique (CNRS) UMR8206 and Institut National de la Santé et de la Recherche Médicale (INSERM) U705 and Université Paris Descartes. The authors have no financial interest to disclose.

References

Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ (2010) Structure and function of the blood-brain barrier. Neurobiol Dis 37: 13–25

Abreu ME, Bigelow GE, Fleisher L, Walsh SL (2001) Effect of intravenous injection speed on responses to cocaine and hydromorphone in humans. Psychopharmacology (Berlin) 154:76–84

Achira M, Suzuki H, Ito K, Sugiyama Y (1999) Comparative studies to determine the selective inhibitors for P-glycoprotein and cytochrome P4503A4. AAPS Pharm Sci 1:E18

Andersen JM, Ripel A, Boix F, Normann PT, Morland J (2009) Increased locomotor activity induced by heroin in mice: pharmacokinetic demonstration of heroin acting as a prodrug for the mediator 6-monoacetylmorphine in vivo. J Pharmacol Exp Ther 331:153–161

Bardo MT, Rowlett JK, Harris MJ (1995) Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 19:39–51

Barrio G, De La Fuente L, Lew C, Royuela L, Bravo MJ, Torrens M (2001) Differences in severity of heroin dependence by route of administration: the importance of length of heroin use. Drug Alcohol Depend 63:169–177

Belkai E, Scherrmann JM, Noble F, Marie-Claire C (2009) Modulation of MDMA-induced behavioral and transcriptional effects by the delta opioid antagonist naltrindole in mice. Addict Biol 14:245–252

Boix F, Andersen JM, Morland J (2013) Pharmacokinetic modeling of subcutaneous heroin and its metabolites in blood and brain of mice. Addict Biol 18:1–7

Cattelotte J, Andre P, Ouellet M, Bourasset F, Scherrmann JM, Cisternino S (2008) In situ mouse carotid perfusion model: glucose and cholesterol transport in the eye and brain. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab 28:1449–1459

Chardin P (2006) Function and regulation of Rnd proteins. Nat Rev Mol Cell Biol 7:54–62

Cisternino S, Rousselle C, Dagenais C, Scherrmann JM (2001) Screening of multidrug-resistance sensitive drugs by in situ brain perfusion in P-glycoprotein-deficient mice. Pharm Res 18:183–190

Cisternino S, Rousselle C, Debray M, Scherrmann JM (2004) In situ transport of vinblastine and selected P-glycoprotein substrates: implications for drug-drug interactions at the mouse blood-brain barrier. Pharm Res 21:1382–1389

Comer SD, Collins ED, MacArthur RB, Fischman MW (1999) Comparison of intravenous and intranasal heroin self-administration by morphine-maintained humans. Psychopharmacology (Berlin) 143: 327–338

Dagenais C, Rousselle C, Pollack GM, Scherrmann JM (2000) Development of an in situ mouse brain perfusion model and its application to mdr1a P-glycoprotein-deficient mice. J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab 20:381–386

Dagenais C, Zong J, Ducharme J, Pollack GM (2001) Effect of mdr1a P-glycoprotein gene disruption, gender, and substrate concentration on brain uptake of selected compounds. Pharm Res 18:957–963

Dagenais C, Graff CL, Pollack GM (2004) Variable modulation of opioid brain uptake by P-glycoprotein in mice. Biochem Pharmacol 67: 269–276

Decleves X, Jacob A, Yousif S, Shawahna R, Potin S, Scherrmann JM (2011) Interplay of drug metabolizing CYP450 enzymes and ABC transporters in the blood-brain barrier. Curr Drug Metab 12:732–741

Di Ciano P, Robbins TW, Everitt BJ (2008) Differential effects of nucleus accumbens core, shell, or dorsal striatal inactivations on the persistence, reacquisition, or reinstatement of responding for a drug-paired conditioned reinforcer. Neuropsychopharmacology 33:1413–1425

Eddy NB, Leimbach D (1953) Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. J Pharmacol Exp Ther 107:385–393

Galtress T, Kirkpatrick K (2010) The role of the nucleus accumbens core in impulsive choice, timing, and reward processing. Behav Neurosci 124:26–43

Hand TH, Stinus L, Le Moal M (1989) Differential mechanisms in the acquisition and expression of heroin-induced place preference. Psychopharmacology (Berlin) 98:61–67

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci 2:695–703

King M, Su W, Chang A, Zuckerman A, Pasternak GW (2001) Transport of opioids from the brain to the periphery by P-glycoprotein: peripheral actions of central drugs. Nat Neurosci 4:268–274

Kitanaka N, Sora I, Kinsey S, Zeng Z, Uhl GR (1998) No heroin or morphine 6beta-glucuronide analgesia in mu-opioid receptor knockout mice. Eur J Pharmacol 355:R1–R3

Korostynski M, Kaminska-Chowaniec D, Piechota M, Przewlocki R (2006) Gene expression profiling in the striatum of inbred mouse strains with distinct opioid-related phenotypes. BMC Genomics 7: 146

Kusunoki N, Takara K, Tanigawara Y, Yamauchi A, Ueda K, Komada F, Ku Y, Kuroda Y, Saitoh Y, Okumura K (1998) Inhibitory effects of a

cyclosporin derivative, SDZ PSC 833, on transport of doxorubicin and vinblastine via human P-glycoprotein. Jpn J Cancer Res: Gann 89:1220–1228

Letrent SP, Pollack GM, Brouwer KR, Brouwer KL (1999) Effects of a potent and specific P-glycoprotein inhibitor on the blood-brain barrier distribution and antinociceptive effect of morphine in the rat. Drug Metab Dispos Biol Fate Chem 27:827–834

Lv XF, Xu Y, Han JS, Cui CL (2011) Expression of activity-regulated cytoskeleton-associated protein (Arc/Arg3.1) in the nucleus accumbens is critical for the acquisition, expression and reinstatement of morphine-induced conditioned place preference. Behav Brain Res 223:182–191

Marie-Claire C, Salzmann J, David A, Courtin C, Canestrelli C, Noble F (2007) Rnd family genes are differentially regulated by 3,4-methylenedioxymethamphetamine and cocaine acute treatment in mice brain. Brain Res 1134:12–17

Marsch LA, Bickel WK, Badger GJ, Rathmell JP, Swedberg MD, Jonzon B, Norsten-Hoog C (2001) Effects of infusion rate of intravenously administered morphine on physiological, psychomotor, and self-reported measures in humans. J Pharmacol Exp Ther 299:1056–65

Martin WR, Fraser HF (1961) A comparative study of physiological and subjective effects of heroin and morphine administered intravenously in postaddicts. J Pharmacol Exp Ther 133:388–399

Nestler EJ (2001) Molecular neurobiology of addiction. Am J Addict 10: 201–217

Oldendorf WH (1992) Some relationships between addiction and drug delivery to the brain. NIDA Res Monogr 120:13–25

Oldendorf WH, Hyman S, Braun L, Oldendorf SZ (1972) blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. Science 178:984–986

Owens DM, Keyse SM (2007) Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. Oncogene 26: 3203–3213

Piechota M, Korostynski M, Solecki W, Gieryk A, Slezak M, Bilecki W, Ziolkowska B, Kostrzewa E, Cymerman I, Swiech L, Jaworski J, Przewlocki R (2010) The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. Genome Biol 11:R48

Roberts DJ, Goralski KB (2008) A critical overview of the influence of inflammation and infection on P-glycoprotein expression and activity in the brain. Exp Opin Drug Metab Toxicol 4:1245–1264

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95(Suppl 2): S91–S117

Robinson TE, Kolb B (1999) Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. Synapse 33:160–162

Samaha A-N, Mallet N, Ferguson SM, Gonon F, Robinson TE (2004) The rate of cocaine administration alters gene regulation and behavioral plasticity: implications for addiction. J Neurosci 24:6362–6370

Samaha AN, Yau WY, Yang P, Robinson TE (2005) Rapid delivery of nicotine promotes behavioral sensitization and alters its neurobiological impact. Biol Psychiatry 57:351–360

Scherrmann JM (2005) Expression and function of multidrug resistance transporters at the blood-brain barriers. Exp Opin Drug Metab Toxicol 1:233–246

Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P (1995) Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Investig 96:1698–1705

Selley DE, Cao CC, Sexton T, Schwegel JA, Martin TJ, Childers SR (2001) mu Opioid receptor-mediated G-protein activation by heroin metabolites: evidence for greater efficacy of 6-monoacetylmorphine compared with morphine. Biochem Pharmacol 62:447–455

Smith GM, Beecher HK (1962) Subjective effects of heroin and morphine in normal subjects. J Pharmacol Exp Ther 136:47–52

Smolka M, Schmidt LG (1999) The influence of heroin dose and route of administration on the severity of the opiate withdrawal syndrome. Addiction 94:1191–1198

Tallarida RJ, Murray RB (1986) Manual of pharmacological calculation, 2nd edn. Springer, New York

Thompson SJ, Koszdin K, Bernards CM (2000) Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. Anesthesiology 92:1392–1399

Tolliver BK, Sganga MW, Sharp FR (2000) Suppression of c-fos induction in the nucleus accumbens prevents acquisition but not expression of morphine-conditioned place preference. Eur J Neurosci 12:3399–3406

Tournier N, Decleves X, Saubamea B, Scherrmann JM, Cisternino S (2011) Opioid transport by ATP-binding cassette transporters at the blood-brain barrier: implications for neuropsychopharmacology. Curr Pharm Des 17:2829–2842

Valjent E, Pages C, Herve D, Girault J-A, Caboche J (2004) Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. Eur J Neurosci 19:1826–1836

Wang QL, Liu ZM (2012) Characteristics of psychopathology and the relationship between routes of drug administration and psychiatric symptoms in heroin addicts. Subst Abus Off Publ Assoc Med Educ Res Subst Abus 33:130–137

Watanabe T, Tsuge H, Oh-Hara T, Naito M, Tsuruo T (1995) Comparative study on reversal efficacy of SDZ PSC 833, cyclosporin A and verapamil on multidrug resistance in vitro and in vivo. Acta Oncol 34:235–241

Watson B, Meng F, Akil H (1996) A chimeric analysis of the opioid receptor domains critical for the binding selectivity of mu opioid ligands. Neurobiol Dis 3:87–96

Wickens JR, Budd CS, Hyland BI, Arbuthnott GW (2007) Striatal contributions to reward and decision making: making sense of regional variations in a reiterated processing matrix. Ann N Y Acad Sci 1104:192–212

Xie R, Hammarlund-Udenaes M, de Boer AG, de Lange EC (1999) The role of P-glycoprotein in blood-brain barrier transport of morphine: transcortical microdialysis studies in mdr1a (−/−) and mdr1a (+/+) mice. Br J Pharmacol 128:563–568

Zong J, Pollack GM (2000) Morphine antinociception is enhanced in mdr1a gene-deficient mice. Pharm Res 17:749–753