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Current Neuropharmacology, 2017, *15*, 1156-1173

REVIEW ARTICLE

Opioids and the Blood-Brain Barrier: A Dynamic Interaction with Consequences on Drug Disposition in Brain

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Abstract: ***Background*:** Opioids are widely used in pain management, acting *via* opioid receptors and/or Toll-like receptors (TLR) present at the central nervous system (CNS). At the blood-brain barrier (BBB), several influx and efflux transporters, such as the ATP-binding cassette (ABC) P-glycoprotein (P-gp, ABCB1), Breast Cancer Resistance Protein (BCRP, ABCG2) and multidrug resistance-associated proteins (MRP, ABCC) transporters, and solute carrier transporters (SLC), are responsible for the transport of xenobiotics from the brain into the bloodstream or vice versa.

***Objective*:** ABC transporters export several clinically employed opioids, altering their neuropharmacokinetics and CNS effects. In this review, we explore the interactions between opioids and ABC transporters, and decipher the molecular mechanisms by which opioids can modify their expression at the BBB.

***Results*:** P-gp is largely implicated in the brain-to-blood efflux of opioids, namely morphine and oxycodone. Long-term exposure to morphine and oxycodone has proven to up-regulate the expression of ABC transporters, such as P-gp, BCRP and MRPs, at the BBB, which may lead to increased tolerance to the antinociceptive effects of such drugs. Recent studies uncover two mechanisms by which morphine may up-regulate P-gp and BCRP at the BBB: 1) *via* a glutamate, NMDA-receptor and COX-2 signaling cascade, and 2) *via* TLR4 activation, subsequent development of neuroinflammation, and activation of NF-κB, presumably *via* glial cells.

***Conclusion*:** The BBB-opioid interaction can culminate in bilateral consequences, since ABC transporters condition the brain disposition of opioids, while opioids also affect the expression of ABC transporters at the BBB, which may result in increased CNS drug pharmacoresistance.

ARTICLE HISTORY

Received: September 26, 2016
Revised: April 04, 2017
Accepted: April 25, 2017

DOI:
10.2174/1570159X15666170504095823

Keywords: Blood-brain barrier, opioids, ABC transporters, P-glycoprotein, neuroinflammation, TLR4.

1. INTRODUCTION

1.1. Opioids: General Considerations on Central Properties and Therapeutic Applications

Opioids represent a class of compounds endowed with potent analgesic properties, and thus are generally used to treat moderate to severe acute or chronic pain, and are among the most effective drugs available for pain management [1]. Morphine and codeine are the best-known opiate alkaloids, naturally occurring in opium. The semi-synthetic derivatives, oxycodone, hydrocodone, buprenorphine and heroin, and full synthetic opioids, such as tramadol and methadone, were developed given the interest of their pharmacological properties, and therapeutic value. However, their long-term use is generally compromised by the prevalence of undesirable side effects involving the central nervous system (CNS) and gastrointestinal system, with development of analgesic tolerance, physical dependence, and severe withdrawal symptoms when opioid administration is suspended [2]. The development of opioid addiction consists in craving accompanied by the development of tolerance and the resulting drug intake escalation to accomplish the original effect. The escalated drug use enhances the negative emotional symptoms of abstinence, which in turn results in profound dysphoria, irritability, sleep disturbances, anxiety and pain. Craving often precedes somatic signs of withdrawal, and it relates with obtaining the drug and anticipation of its rewarding effects, as well as with anticipation of the aversive effects of withdrawal. Consequently, it is expe-

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1875-6190/17 $58.00+.00 ©2017 Bentham Science Publishers

Current Neuropharmacology

rienced as a need to obtain and take the drug to avoid the severe dysphoria, discomfort, and psychic stress experienced during withdrawal and abstinence [3, 4].

The pharmacological properties of opioids result from their interaction with opioid receptors and their multiple subtypes [5-8]. Because opioid receptors are widely distributed throughout the entire CNS, opioids may exert their antinociceptive activity through different mechanisms [9]. To date, four major opioid receptors families have been identified and cloned, and each has unique central pharmacological actions: μ (mu, MOR), κ (kappa, KOR), δ (delta, DOR), and nociceptin or orphanin (NOP) receptor [10]. Central μ-opioid receptors are primarily responsible for the analgesic effects of opioids with less severe side effects, and thus remain the primary choice in the clinical setting. To date, there is scarce data on the expression of opioid receptors in brain endothelial cells that constitute the blood-brain barrier (BBB). Nevertheless, there is evidence of the presence of μ3-opioid receptors in human arterial and rat microvascular endothelial cells providing specific binding of morphine to endothelial cells [11]. Newborn piglet brain vascular endothelial cells exposed to either morphine or naloxone had led to the production of opposite effects on endothelin-1 levels [12], suggesting that these effects are mediated by opioid receptors. The presence of the NOP opioid receptor in the rat brain microvascular endothelium has also been identified [13].

More recently, opioids also evidenced to have direct affinity to pattern-recognition Toll-like receptors (TLRs), mainly TLR4 [14-17] along with its co-receptor myeloid differentiation factor-2 (MD-2), which modulate the opioid neuronal reinforcement and reward system. TLR4 is principally found in microglia [18], and its activation induces the production and release of pro-inflammatory cytokines, such as interleukin-1β (Il-1β) and tumor necrosis factor-α (TNF-α) [19]. Activation of the TLRs by opioid agonists also triggers opioid-specific inflammatory processes, through microglia [20, 21] and astrocyte activation [22, 23], suggesting that pro-inflammatory immune signaling *via* TLRs may represent a key role in opioid antinociception, tolerance and dependence phenomena [20, 24]. Blockade of TLRs, namely TLR2 and TLR4, showed to attenuate allodynia and hyperalgesia, and to improve opioid analgesia effectiveness [25, 26], which suggests that they may be a putative target for future pharmacological pain relief, especially when the effect of morphine is tolerated. While opioid-induced signaling has been argued to occur *via* glial TLRs activation [27], it is controversial whether brain endothelial cells also express these receptors [28-31]. Nevertheless, some studies show functional TLR expression in rat and human brain endothelial cells of the BBB, in particular TLR2, TLR3, TLR4 and TLR6 [30, 32]. Rat brain endothelial cells evidenced to express TLR4 mRNA, which showed to mediate lipopolysaccharide (LPS)-induced TNF-α mRNA up-regulation [32].

In order to exert their effects on either classic opioid or TLR receptors, opioids must cross the BBB to reach the CNS. Such opioid effects likely result in the modulation of neuronal, glial and neurovascular functioning. The consequences at the neurovascular level, in particular, may represent a change of the BBB properties, and thus of the pharmacokinetics and brain distribution of drugs.

2. NEUROPHARMACOKINETICS OF OPIOIDS: TRANSPORT AT THE BLOOD-BRAIN BARRIER

The BBB is the largest CNS interface for blood and brain exchange, and it exerts its barrier function at three different levels: (1) physical, through the extensive expression of tight junctions along adjacent endothelial cells, reducing the paracellular pathway, (2) transporter-dependent passage, due to the existence of specific transport mechanisms mediating solute flux, and (3) metabolic, given that enzymes present in the endothelium are capable of processing molecules in transit [33]. The neurovascular coupling at the BBB level allows the maintenance of brain homeostasis and protection of the CNS from toxins, pathogens, inflammation, injury, and disease, given the association of brain endothelial cells with various perivascular cells such as pericytes, microglia, astrocytes, and specialized extracellular compartments such as the endothelial glycocalyx and basal lamina [34]. In this way, the BBB plays a crucial role by exerting a bi-directional control over the passage of a large diversity of regulatory proteins, nutrients and electrolytes, as well as potential neurotoxins, maintaining a strict extracellular environment around synapses and axons.

At the BBB, several ATP-binding cassette (ABC) transporters participate in the efflux of xenobiotics and small compounds from the brain into the bloodstream, constituting key elements in controlling the brain penetration of many drugs and their CNS effects [33, 35, 36]. Their importance relies essentially on the following critical features: its localization in the luminal plasma membrane of brain parenchyma endothelial cells, the potent efflux against the substrate concentration gradient, and a very low substrate specificity, transporting a large number of structurally unrelated compounds [37]. To date, 49 genes encoding ABC transporters have been identified and divided into seven different subfamilies, A-G, based on their amino acid sequence similarities and evolutionary divergence [38, 39]. The ABCB, ABCC and ABCG subfamilies comprise the transporters involved in the cellular efflux of xenobiotics [40]. Knowing which ABC transporters are involved in the transport of opioids and their metabolites is clinically important to better understand the mechanisms responsible for inter-individual variability during pain treatment. P-glycoprotein (P-gp, ABCB1, MDR1), Breast Cancer Resistance Protein (BCRP, ABCG2), Multidrug Resistance-associated Proteins MRP4 (ABCC4) and MRP5 (ABCC5) have been identified as the main ABC transporters from B, G and C subfamilies at the luminal membrane of brain cortex endothelial cells of the human BBB [41-43]. Present evidence suggests P-gp contributes to the active brain-to-blood efflux of opioids at the BBB and, thus, to the development of a central tolerance. At a much lesser extent, other ABC transporters, such as BCRP and a few MRPs, were also reported to be implicated in brain transport of opioids. Table **1** summarizes a list of the ABC transporters and their respective opioid substrates described to present date.

2.1. P-glycoprotein

P-gp is considered one of the major constituents of the BBB in restricting the disposition of several opioids in the brain [44]. The interaction of P-gp with natural and synthetic

Table 1. List of known possible interactions up to recent days between several opioid derivatives and ABC transporters.

Interaction with P-gp	**Opioids**
Substrates	Morphine [46-49, 54, 55, 59], loperamide [87-91], desmethyl-loperamide [90, 93], norbuprenorphine [96, 98], buprenorphine [97], methadone [45, 55, 56, 109-114], oxycodone [101], meperidine [45, 56], fentanyl [55, 89, 103, 104], alfentanyl [108], tramadol [196], pentazocine [197], and deltorphin II, DPDPE, naltrindole, SNC 121, bremazocine and U-69593 [89]
Inhibitors	Methadone [45, 96, 109, 165], butorphanol [198]
Inducers	Morphine [150, 151, 159-162, 179], oxycodone [101, 163]
Interaction with MRPs	**Opioids**
Substrates	Morphine [127], morphine-3-glucuronide [126], morphine-6-glucuronide [126], oxymorphone [127]
Inducers	Morphine [151]

opioids was first reported in 1993 [45]. Since then, P-gp-mediated efflux of opioids at the BBB has been demonstrated using *in vitro* and *in vivo* approaches [46-52].

2.1.1. Morphine and Metabolites

Morphine represents the most studied interaction between opioids and ABC transporters [49, 53-55]. The first time morphine was identified to be a P-gp substrate was *in vitro* through the use of radiolabeled morphine across MDR cells [45, 56]. Morphine was found to be a weak P-gp substrate in Caco-2 [57], and L-MDR1 [58] cells, which showed an efflux:influx ratio of 1.5. *In vitro* studies on morphine transport in LLC-PK1 cells transfected with human or rodent P-gp confirmed the presence of P-gp-mediated transport of morphine, although marginal [49, 58]. Morphine was also shown to be a P-gp substrate *in vivo*, since P-gp knockout mice evidenced 1.7-fold higher brain levels of morphine than wild-type mice [49]. Subsequent *in vivo* studies in rodents showed that the inhibition of P-gp with either elacridar [46] or verapamil [59] resulted in significantly augmented antinociceptive effects following morphine administration. Recently, a similar study conducted in mice co-perfused with morphine and the P-gp inhibitor valspodar (PSC-833) resulted in a 1.5-fold increase of morphine transport rate across the BBB, accompanied by an increase in the effect and duration of the antinociceptive response [60]. Furthermore, morphine-induced hyperlocomotion was 2.6-times greater in valspodar-*versus* vehicle-pretreated animals [60]. Brain microdialysis and *in situ* brain perfusion studies performed using P-gp knockout mice also corroborated these data, showing that morphine is transported across the BBB by P-gp [50, 61]. Similarly, morphine showed greater antinociception in P-gp knockout mice than in wild-type mice, where knockout mice had an ED_{50} more than 2-fold inferior to that of wild-type mice [51, 55]. Groenendaal *et al*. evidenced that morphine brain distribution is essentially determined by 1) limited passive diffusion across the BBB, 2) active efflux at the BBB, which is reduced by 42% when P-gp is inhibited, and 3) low active uptake capacity [62], demonstrating that P-gp modulates the brain distribution of morphine within the biophase and so helps delay and decrease its CNS effect. It was also demonstrated that the variability of P-gp expression in the brain cortex of mice and the analgesic effect of morphine were correlated [63]. Furthermore, this study demonstrated a negative correlation between the expression of P-gp or its ATPase activity and the antinociceptive effect of morphine, suggesting that the inter-individual variability of the morphine effect could be due to a difference in the expression and/or function of P-gp in the brain [63].

The assessment of morphine efflux transport at the BBB and its CNS effects has also been conducted in humans. A study conducted in healthy human volunteer subjects, pretreated with quinidine, an antiarrhythmic drug that inhibits P-gp, showed that when morphine was given intravenously they did not experience enhanced CNS opioid effects [64], whereas when morphine was given orally, human volunteers showed sustained increased plasma concentrations in comparison to those only treated with morphine [54]. These results suggest that P-gp plays a role in orally administered morphine, probably by interfering with its intestinal absorption rather than by enhancing morphine BBB efflux. However, a clinical study also conducted in humans demonstrated that P-gp inhibition by valspodar did not significantly alter the pharmacokinetic and pharmacodynamic profile of morphine [65]. Inter-individual variability in the pain relief of patients with cancer has been associated with variants of the *ABCB1* gene that alter the expression and/or activity of P-gp [66, 67], evidencing the role of P-gp on the transport of morphine across the BBB. Pain relief variability in patients undergoing morphine therapy was shown to be significantly associated to the single nucleotide polymorphism (SNP) C3435T of *ABCB1* [66]. Homozygous TT patients demonstrated to have higher risk of persistent pain than wild-type CC patients [68].

Morphine extensive hepatic first-pass metabolism and systemic clearance [69, 70] produces morphine-3-glucuronide (M3G), its main metabolite (45-55%), and morphine-6-glucuronide (M6G) to a lesser extent (10-15%) [71]. M3G has limited affinity to opioid receptors with no analgesic activity [72] but is responsible for some morphine side effects such as opioid-induced hyperalgesia, *via* its interaction with TLR4 receptors [73, 74], whereas M6G demonstrated to greatly contribute to the analgesic effect of morphine, regardless the route of administration [75]. P-gp role on the brain-to-blood efflux of M6G seems controversial. *In vitro* studies first suggested that M6G is a substrate of P-gp [76, 77]. Also, P-gp inhibition with valspodar was shown to pro-

voke a 3-fold increase on the interstitial spinal cord levels of M6G and enhanced antinociception following M6G i.v. infusion in rats [78]. Still, further *in vivo* studies performed in mice do not provide evidence that P-gp is able to affect M6G transport [79, 80]. Furthermore, the pharmacokinetic and pharmacodynamic profile of M6G was not modified when P-gp was inhibited by the administration of valspodar in healthy human volunteers [65], and thus M6G is unlikely a P-gp substrate.

2.1.2. Heroin

Heroin has a higher pharmacological potency than morphine [81], which has long been attributed to its greater lipophilic properties, facilitating the passage through the BBB [82]. However, after systemic administration, heroin is quickly metabolized into 6-monoacetyl-morphine (6-MAM) and morphine [83, 84]. In fact, 6-MAM displays a higher affinity than morphine for μ-opioid receptors [82, 83], and 6-MAM brain concentrations proved to be higher than those of heroin following a s.c. injection of heroin into mice [85]. Consequently, 6-MAM is likely the metabolite responsible for the acute effects of heroin, which is consistent with its relatively easy BBB crossing from the blood to the brain, resulting in high 6-MAM brain levels [86]. A recent study conducted by Seleman *et al*. evidenced that 6-MAM readily cross the BBB, reaching the brain 35 times faster than morphine and 1.8 times slower than heroin, and that inhibition of P-gp did neither impact on the brain uptake of heroin and 6-MAM, nor affect the acute behavioral or transcriptional effects of heroin in mice [60]. Therefore, neither heroin nor its major metabolite 6-MAM are P-gp substrates.

2.1.3. Loperamide

Loperamide, a potent opioid receptor agonist, was one of the first drugs that illustrated the impact of P-gp on the bioavailability of CNS-acting drugs. In fact, this opioid has only an antidiarrheal effect and shows lack of CNS effects in P-gp-competent animals, due to its poor absorption in the intestine and limited BBB penetration. P-gp-deficient mice exhibited increased loperamide brain accumulation and central opioid-like effects, in comparison to wild-type animals [87]. Furthermore, when loperamide was co-administered with quinidine, a P-gp inhibitor, it produced respiratory depression due to enhanced CNS exposure [88]. The influence of P-gp on the brain efflux of loperamide was further confirmed by *in situ* brain perfusion in mice [89]. Despite its favorable physicochemical properties and a chemical structure similar those of other opioids, P-gp greatly limits the brain distribution of loperamide, which allows it to act selectively on opioid receptors of the intestinal tract, conferring ideal antidiarrheal properties with no CNS effects in normal clinical practice. Radiolabelled ^{11}C-loperamide has been used as a positron emission tomography (PET) probe to evaluate the role of P-gp transport activity at the BBB [90, 91]. P-gp inhibition by tariquidar resulted in a 3-fold increase in brain radioactivity after an i.v. injection of ^{11}C-loperamide in monkeys [92]. ^{11}C-N-desmethyl-loperamide has been used as a substitute of ^{11}C-loperamide in the study of the function of P-gp at the human BBB by PET, since loperamide is rapidly metabolized *in vivo*, and this metabolite is also an avid P-gp substrate [90, 93].

2.1.4. Buprenorphine

Buprenorphine, widely used as a maintenance treatment for opioid addiction in combination with the opioid receptor antagonist naloxone, is extensively and rapidly metabolized in humans to its active metabolite norbuprenorphine [94]. Buprenorphine was not shown to be a P-gp substrate in either MDCKII-MDR1 or Caco-2 cells, nor in an *in vivo* transport study using P-gp-deficient mice [95, 96]. Nevertheless, a study conducted in rats demonstrated that P-gp inhibitors, such as cyclosporine A, quinidine and verapamil, enhanced the apparent brain uptake of buprenorphine by 1.5-fold, and thus suggesting P-gp-mediated efflux of buprenorphine at the BBB [97]. Unlike buprenorphine, norbuprenorphine has been demonstrated to be a gold-standard P-gp substrate in a bidirectional transport assay conducted in MDCKII-MDR1 cells [96]. This was further confirmed in mice, where P-gp demonstrated to play a protective role against the buprenorphine-related respiratory effects, since either valspodar administration or P-gp knockout mice resulted in decreased norbuprenorphine efflux at the BBB, accompanied by an exacerbation of buprenorphine and norbuprenorphine-respiratory effects [98].

2.1.5. Oxycodone

Oxycodone is an opioid agonist also used to treat moderate to severe pain, with a pain-relieving effect similar to that of morphine [99]. Regarding its interaction with P-gp, *in vivo* studies conducted in rats co-treated with oxycodone and the P-gp inhibitor valspodar indicated that it did not alter its plasma pharmacokinetics, total brain concentrations, nor its antinociceptive effect, suggesting that it may not represent a P-gp substrate [100]. Still, a study using the Caco-2 cell line where P-gp was inhibited using verapamil suggested that this opioid worked as a P-gp substrate [101]. Also, it showed that the brain concentrations of oxycodone were significantly higher in P-gp knockout mice than in control mice [101], indicating that oxycodone is a substrate of both mice and human P-gp. Thus, the interaction of oxycodone as a P-gp substrate remains a matter of debate.

2.1.6. Meperidine and Fentanyl Derivatives

Meperidine and fentanyl are strong, short acting μ-opioid agonist analgesics, while the fentanyl derivatives, alfentanyl and sufentanyl, with shorter half-lives and potency, are more suitable for anesthesia and rapid adjustment of response during i.v. infusion. Meperidine was shown to be a P-gp substrate *in vitro* [45, 56], but studies performed using P-gp knockout mice did not reveal greater antinociceptive effects of meperidine compared to wild-type animals [55, 89, 102], indicating that it does not represent a P-gp substrate *in vivo*. Increased antinociception was registered in P-gp-deficient mice following the administration of fentanyl [55]. The analgesic effects of fentanyl were also increased when mice were co-treated with the P-gp inhibitors cyclosporine and valspodar [103, 104]. In line with these data, *in situ* brain perfusion studies indicated P-gp-mediated BBB efflux of fentanyl in mice [89]. Clinical studies revealed that the plasma concentrations of oral fentanyl increased when co-administered with quinidine [105], which should interfere with intestinal P-gp or other quinidine-sensitive metabolizing

enzymes and/or transporters of fentanyl. Still, controversial data has been generated regarding the role of P-gp in the transport of fentanyl, since some studies have reported that fentanyl does not behave as a P-gp substrate [58, 106]. In another study, the *ABCB1* SNPs 2677G>T/A and 3435C>T did not show to contribute to the variability of the analgesic response to fentanyl [107]. On the other hand, alfentanyl and sufentanyl have not been identified to be P-gp substrates in *in vitro* studies. P-gp inhibitor elacridar did not affect the transport of any of these fentanyl derivatives when using MDCKII-MDR1 cells or Caco-2 cells [58]. Nevertheless, *in vivo* studies conducted in P-gp knockout mice identified an influence of P-gp on the alfentanyl brain concentrations and its antinociceptive effects [108].

2.1.7. Methadone, Naloxone and Naltrexone

Likewise, the determination of P-gp substrate activity of methadone has been studied using *in vitro* and *in vivo* studies, but their interaction remains controversial. *In vitro* studies using intestinal [109, 110], placental [111] and BBB [45, 56] models demonstrated that methadone is a substrate of P-gp. Corroborating such results, increased antinociception was registered in mice lacking P-gp and in rats treated with a P-gp inhibitor, following the administration of methadone [55, 112, 113]. Its transport by P-gp in rodents is significantly stereo-selective, favoring the transport of the (+)S-enantiomer [112], but this lacked confirmation in human MDR1 cells [96]. P-gp-mediated efflux showed to significantly limit methadone absorption at the human intestinal barrier [114], which may allow inter-individual differences in the amount of P-gp throughout the intestinal epithelia to influence the bioavailability of methadone taken orally. Also, the SNP P-gp variant c.1236C>T [115], but not the c.3435C>T variant [115, 116], was shown to be linked with a higher methadone dose requirement to prevent withdrawal symptoms and relapse in heroin dependent patients, suggesting that specific ABCB1 variants may have clinical relevance by influencing the methadone absorption and distribution. A study conducted in healthy volunteers also demonstrated that cyclosporine A, an inhibitor of P-gp, significantly alters the pharmacokinetics of methadone given orally and intravenously, but it failed to improve its clinical effects (such as miosis) and overall pharmacodynamics [117], unlike what was previously registered for morphine [118]. This suggests that there is little to no role for P-gp in mediating methadone brain access and pharmacodynamics in humans. Such contrasting results between rodent and human models regarding the effect of lack or inhibition of P-gp on the methadone-related clinical effects may result from the inter-individual variability, but also from the now well-recognized interspecies difference in the expression of ABC transporters at the BBB, as it is the case of P-gp [119].

In what concerns opioid antagonists and their interaction with P-gp, naloxone and naltrexone have been reported to not act as P-gp substrates [120, 121]. Whereas naltrexone is mostly given orally, naloxone is administered intravenously or nasally due to its poor oral bioavailability. However, P-gp is not responsible for this different oral bioavailability between the two opioid antagonists, since naloxone did not show to be a P-gp substrate neither when using Caco-2 cells [122] nor MDCK-MDR1 cells [120]. Structure-activity relationship studies later corroborated these findings [123].

2.2. ABCC Transporters

The ABCC (MRP) subfamily of ABC transporters, known to be implicated in the transport of endogenous compounds such as inflammatory mediators like prostaglandins and leukotrienes [124], is also associated with drug resistance and with a wide spectrum of substrates [40, 125]. This subfamily has also been implicated in the brain efflux clearance of opioids. It was demonstrated that MRP3 transports M3G and M6G *in vitro*, and that Mrp3-deficient mice were unable to export M3G from the liver into the bloodstream, resulting in a major alteration in the pharmacokinetics of morphine and M3G [126]. Lack of Mrp3-driven efflux decreased their plasma concentration, and indirectly affected morphine brain concentrations and antinociceptive effects. More recently, it was revealed that Mrp1 also has a role in the brain disposition of morphine and oxymorphone, given that its antisense down-regulation or *Mrp1* knockout, in rat and mouse models respectively, resulted in decreased brain-to-blood transport when injected i.c.v., accompanied by an increased systemic potency of both drugs [127]. Given the evident expression of this transporter at the choroid plexus [128, 129], but not at the BBB [42, 130, 131], loss of Mrp1 expression in choroid plexus may decrease the efflux of morphine and oxymorphone from the CSF into the bloodstream, enhancing their levels in the brain and thus their analgesic activity.

Nonetheless, the role of ABC transporters in the alteration of the pharmacokinetics and pharmacodynamics of the listed opioids presented as a result of studies conducted in preclinical species should be carefully interpreted and not easily extrapolated to humans. In fact, a significant interspecies difference among small and larger species has been observed between the levels of expression of the two major drug efflux transporters, P-gp and BCRP: P-gp is the more abundant of the two transporters in the rodent BBB, whereas BCRP is more abundant than P-gp in the monkey and human BBB [119]. Therefore, whenever the transport of P-gp is affected in rodents, it is expected to observe greater consequences on the pharmacokinetics and pharmacodynamics of P-gp-transported opioids than in humans.

2.3. Influx Transport of Opioids at the BBB

As previously mentioned, morphine, the leader molecule among opioids for the treatment of both severe acute and chronic pain management, is well absorbed at the gastrointestinal tract [71], but suffers extensive hepatic first-pass metabolism into morphine glucuronides [69, 70]. Since, M3G does not exhibit analgesic properties, pain-relieving effects are mainly due to the interaction of both morphine and M6G with centrally-located μ-opioid receptors. Morphine and M6G can be distributed within the brain cerebrospinal, intra- and extracellular fluids by diffusion or by several transporters located at the different brain interfaces. Among these transporters are the probenecid-sensitive transporters [132, 133], contributing to the neuropharmacokinetics and CNS effects of opioids. M6G has been shown to have

a potent analgesic activity, with less marked respiratory depressive effects than morphine. However, following systemic administration, M6G analgesic power is markedly lower than morphine [134], presumably due to its considerably lower brain penetration as a consequence of its higher molecular weight and hydrophilicity [135-139]. Nevertheless, M6G uptake transport at the BBB has been suggested to occur but at low rate, presumably *via* Glut-1 and a digoxin-sensitive transporter, such as the organic anion-transporting polypeptide 2 (Oatp2) [77, 80].

Oxycodone exhibits a similar analgesic potency to that of morphine [99], despite its lower affinity to opioid receptors [140]. This is due to a carrier-mediated influx of oxycodone at the BBB since its BBB influx clearance is close to brain blood flow, regardless its relatively low lipophilicity [141]. Consequently, oxycodone unbound concentrations proved to be 3 times higher in the brain than in the blood, following a 120 min constant i.v. infusion in rats [141]. A competitive inhibition between opioids, like oxycodone and diphenhydramine, pyrilamine, or clonidine, has been identified at the BBB both *in vitro* and *in vivo*, suggesting that these compounds share the same uptake transporter, presumably a proton-coupled antiporter for organic cations that has not yet been molecularly identified but functionally described to transport these compounds [142-145]. Some results based on trans-stimulation experiments conducted in mice suggested that similarly to oxycodone, heroin, tramadol and methadone, but not morphine, could also be substrates of this drug/proton-coupled antiporter [146].

Therefore, the polarized expression of numerous transporters, both of influx or efflux, at the BBB level determines the brain entrance and distribution of opioids and their therapeutic effects, and contributes to the understanding of the pharmacokinetic-pharmacodynamic relationships of opioids. Consequently, any modulation of the expression and/or activity of these transporters will have an impact on the effect of opioids actively transported at the BBB. In this review, we will give particular emphasis to how opioids can modulate the efflux transport carried by ABC transporters at the BBB.

3. INFLUENCE OF OPIOIDS ON THE BBB

The pharmacokinetics of opioids can be modified by the inhibition and/or induction of different transporters and drug-metabolizing enzymes that usually play a role in the disposition of these compounds, many of them being present at the BBB. The fact that opioids may exert an influence on the expression of proteins specifically present at the BBB is of great interest, given existing data suggesting a possible correlation between the BBB modulation and the development of tolerance to opioids.

Data regarding the influence of opioids on the BBB is still limited. However, some studies showed that morphine is able to modify certain features of the BBB, especially its permeability characteristics. A significant increase of the brain concentration of sodium fluorescein, used as a permeability marker, was shown following an acute s.c. or i.c.v. morphine administration in mice [147]. This increased permeability was inhibited by the administration of naloxone, suggesting that the μ-opioid receptors are involved in the morphine-mediated modifications of the BBB permeability. More recently, it the effect of a morphine chronic treatment on the BBB permeability to large molecule tracers, such as Evans blue, albumin and radioiodine, was investigated in several rat brain regions [148]. Morphine was given *via* i.p. daily for 12 days at a dose of 10mg/kg, and such treatment caused no significant BBB opening on the 12th day of morphine exposure. On the other hand, spontaneous withdrawal of morphine on day 1 after treatment suspension resulted in profound stress symptoms, which were much more intense on the second day of morphine withdrawal. During morphine withdrawal, a marked increase in the BBB permeability to these protein tracers was seen in several regions of the brain, and this was more evident in the second day of morphine withdrawal [148]. These findings suggest that morphine, its associated withdrawal syndrome, and the subsequently provoked stress are capable to modify BBB integrity.

Another study demonstrated that an acute morphine injection did not alter blood-to-brain transport of IL-1α, IL-2 or TNF-α, whereas both spontaneous and precipitated morphine withdrawal following a chronic morphine treatment had increased blood-to-brain transport of IL-2, with no change in blood-to-brain transport of IL-1α or TNF-α [149]. The permeability of the BBB to IL-2 is usually dominated by brain-to-blood efflux, with only limited blood-to-brain transport. With this study it was found that chronic morphine and withdrawal from morphine did not alter brain-to-blood efflux, but induced a novel saturable blood-to-brain transport system [149].

Treatment of a primary culture of human brain microvessel endothelial cells with both morphine and the HIV-1 tat viral protein induced the release of pro-inflammatory cytokines, which can trigger common intracellular pathways to regulate tight junction protein expression, and, consequently, the structural and the functional integrity of the BBB [150]. Treatment of these cells with a combination of morphine and tat resulted in a significant decrease in the tight junction protein *ZO-1* and *occludin* gene expression in comparison to untreated control cells. Morphine alone and in combination with tat also increased the junctional adhesion molecule JAM-2 gene expression.

A study conducted in rats involving a chronic 5-day treatment with morphine registered an impact on the expression of several genes encoding for proteins critically involved in the maintenance of BBB properties, such as junction proteins, influx and efflux transporters [151]. The expression of *occludin* registered a slight increase (1.23-fold induction) in isolated brain microvessels of morphine-treated rats in comparison to control rats [151]. However, the induction of this gene did not seem to have an effect on BBB tightness, as morphine-treated and saline-treated animals did not register any difference in terms of the brain accumulation of radiolabeled sucrose, which was used as a marker of BBB integrity [151].

3.1. Focus on the Regulatory Role of Opioids on the Expression of ABC Transporters

Among the several mechanisms that have been proposed to be implicated in the development of tolerance to opioid

analgesia, the alteration of the brain-to-blood efflux of opioids, namely the up-regulation of some ABC transporters has been an emerging one. The expression and/or activity of ABC transporters can be inhibited or induced by other drugs and/or xenobiotics that are often co-administered with opioids, resulting in an alteration in the CNS-driven effects of opioids. There is current evidence that several ligand-activated receptors such as the pregnane X receptor (PXR) [152, 153], constitutive androstane receptor (CAR) [154], aryl hydrocarbon receptor (AhR) [155], vitamin D receptor (VDR) [156] and glucocorticoid receptor (GR) [157], to which many highly prescribed drugs, dietary constituents, and nutraceuticals are ligands, are able to increase P-gp expression at the BBB. The expression of MRPs at the BBB has also been demonstrated to be target of modulation in the context of different neurological disorders and pathological conditions [158].

Yet, the expression and activity of ABC transporters can be modulated by the opioid itself. There are a few studies that evidenced that morphine is able to influence the expression and activity of ABC transporters. In 2000, a chronic administration of morphine in rats resulted in decreased antinociceptive response, which was accompanied by a 2-fold increase in P-gp protein expression in whole brain homogenates [159]. Rats subjected to a 5-day treatment with morphine received morphine at 10mg/kg in the first 2 days, and 20mg/kg in the last 3 days of treatment, which resulted in a decrease of the antinociceptive effect of morphine. It was hypothesized that the registered P-gp up-regulation was an important cause for the enhanced morphine efflux from the brain, and, thus, reducing its pharmacological activity and contributing to the development of morphine tolerance [159]. A similar study was later conducted in 2003 by Zong and Pollack, who demonstrated that mice treated with morphine for 3 days showed a 1.4-fold increase in the P-gp expression in comparison to control mice [160]. In 2004, a study explored the effect of short-term (72 h) morphine exposure on the P-gp expression of several human cancer cell lines and of the mouse fibroblast NIH-3T3 cell line, as well as its effects on cellular resistance to the known P-gp substrates, vinblastine and colchicine [161]. This study has shown that the P-gp regulation may be cell-type dependent, as the morphine treatment induced P-gp expression in the mouse fibroblast cell line, but not in any of the tested human cancer cell lines. *ABCB1* gene expression was also reported to increase in human brain microvessel endothelial cells when exposed to either morphine (100 nM) or the HIV-1 tat protein, either alone or in combination [150].

A chronic treatment with morphine for 5 days was able to increase the mRNA levels of several ABC efflux transporters, namely *Mdr1a*, *Bcrp* but also of *Mrp1*, but not those of *Mrp2*, *Mrp3*, *Mrp4* or *Mrp5*, in rat brain microvessels [151]. However, no change was registered following this protocol on the P-gp protein expression or functional activity, assessed by western blot and *in situ* brain perfusion, respectively. Still, chronic treatment with morphine showed to induce the expression of P-gp in the brain cortex and hippocampus, as evidenced at both mRNA and protein level [151]. These findings are in agreement with earlier results presented by Aquilante and colleagues [159]. One should note that these results were obtained from morphine-treated rats sacrificed 12h after the last dose of morphine. More recently, we evidenced that a 5-day subchronic morphine regimen was able to up-regulate not only P-gp, but also Bcrp 12h to 24h after the last dose of morphine, an effect that was not registered at earlier time-points of animal sacrifice (6h, 9h) [162]. This was confirmed at both mRNA and protein level. The peak of *Abcb1a* and *Abcg2* mRNA induction was registered 24h after the last dose of morphine, with a 1.4-fold and a 2.4-fold increase, respectively. Similarly, P-gp and Bcrp protein levels were 1.5-fold and 1.6-fold increased 24h after the last dose of morphine [162]. Such up-regulation was, therefore, not registered during the treatment nor when the last dose of morphine was administered, but only several hours after. On the other hand, neither a single dose of morphine given in rats, nor the direct exposure of the hCMEC/D3 human brain endothelial cell line to morphine were able to modify the mRNA levels of *Abcb1a/ABCB1* or *Abcg2/ABCG2* [162]. This suggests that changes in the expression of these ABC transporters are more likely linked to a prolonged exposure to morphine, which may involve other cells of the neurovascular unit other than only endothelial cells, in a cell-cell interaction complex mechanism. Since morphine is a substrate of P-gp in both rodents and humans [47, 51, 63] and its chronic exposure up-regulates P-gp, morphine may thus play a role in its own brain-to-blood active efflux at the BBB and potentiate its long-term poor brain penetration. However, it should not account for the development of tolerance that occurs in the course of its chronic treatment since P-gp and Bcrp up-regulation was only observed once the morphine treatment was suspended. Furthermore, given that morphine is often co-administered with several anticancer drugs, the majority being substrates of both P-gp and BCRP, the modulation of such ABC transporters at the BBB triggered by morphine may be hypothesized to modify the efflux, distribution and efficacy of these chemotherapeutic agents and, thus, cancer therapy.

Morphine is not the only opioid that has shown to modulate the expression of ABC transporters, namely that of P-gp. Oxycodone has also demonstrated to induce P-gp up-regulation, upon a chronic regimen where rats developed tolerance to oxycodone [101]. In this study, it was shown that oxycodone altered paclitaxel tissue distribution, and thus its pharmacokinetic parameters through an increase in P-gp expression, since this anti-cancer drug is also P-gp substrate [101]. Furthermore, chronic exposure to oxycodone not only showed to up-regulate P-gp, but also Bcrp at the rat BBB, affecting the brain-to-blood Bcrp-mediated transport of its substrates [163]. Oxymatrine, in turn, showed to abolish the morphine-induced up-regulation of P-gp at the BBB [164], which attenuated the development of tolerance to morphine. Therefore, upon chronic administration, both morphine and oxycodone showed to up-regulate P-gp and BCRP at the BBB, which can have an impact not only on the brain concentrations of both opioids, exacerbating tolerance and compromising their central analgesic effects, but also on the brain concentrations of the remarkable number of P-gp and BCRP substrates that constitute CNS-acting drugs, and thus contribute to pharmacoresistance.

On the other hand, *in vitro* studies indicate that methadone may exert P-gp inhibitory properties. The first demon-

stration of the interaction of methadone with P-gp activity was evidenced through the decrease of transport of vinblastine in chinese hamster ovary P-gp-expressing cells [45]. This was later corroborated in Caco-2 cells and in HEK293-MDR1 transfected cells, where methadone contributed to the inhibition of the P-gp-mediated transport of rhodamine123 and calcein-AM [96, 109]. Conversely, methadone was not shown to affect the transport activity of BCRP *in vitro* [96]. More recently, in Flp-In™-293 cells stably transfected with various genotypes of human P-gp, methadone showed to be a non-competitive inhibitor of human wild-type P-gp, as well as the variant-type human P-gp, under methadone therapeutic range [165]. Nonetheless, this P-gp inhibition effect remains to be demonstrated *in vivo*.

Table 2. Experimental models exposed to different opioid treatments and consequent effects on the expression levels of ABC transporters in the brain.

Experimental Model	Opioid	Treatment	Washout	Methodologies	Effect on ABC Transporters	Refs.
Adult Sprague Dawley rats, whole brain homogenates	Morphine	10-20 mg/kg s.c., 2x/day, 5 days	12h	WB	2-fold P-gp up-regulation	[159]
Adult CF-1 mice, whole brain homogenates	Morphine	10-30 mg/kg s.c., 3x/day, 3 days	24h	WB	1.4-fold P-gp up-regulation	[160]
Mouse fibroblast NIH-3T3 cell line	Morphine	72h incubation	-	WB	P-gp up-regulation	[161]
Primary human brain microvessel endothelial cells	Morphine	48h incubation, 100 nM	-	RT-PCR	1.4-fold *ABCB1* up-regulation	[150]
Adult Sprague Dawley rats, brain microvessels, whole brain cortex, and hippocampus	Morphine	10-40 mg/kg i.p., 2x/day, 5 days	12h	RT-PCR, WB	1.38-fold, 1.44-fold and 1.58-fold up-regulation of *Abcb1a Abcg2* and *Abcc1* in brain microvessels 1.4-fold and 1.8-fold P-gp up-regulation in whole brain cortex and hippocampus, respectively	[151]
Adult Sprague Dawley rats, brain microvessels	Morphine	10-40 mg/kg i.p., 2x/day, 5 days	24h	RT-PCR, WB	1.4-fold and 2.4-fold *Abcb1a* and *Abcg2* up-regulation 1.5- and 1.6-fold P-gp and Bcrp up-regulation	[162]
Adult Sprague Dawley rats, brain microvessels	Morphine	10 or 40 mg/kg i.p.	3h, 6h or 24h	RT-PCR	No effect in *Abcb1a* nor *Abcg2* levels	[162]
Adult Sprague Dawley rats, brain microvessels	Morphine	10-40 mg/kg i.p., 2x/day, 5 days, naloxone 1 mg/kg s.c.	12h	RT-PCR, WB, UPLC-MS/MS	1.4-fold and 2.5-fold *Abcb1a* and *Abcg2* up-regulation No effect on P-gp and Bcrp protein levels	[179]
Adult Sprague Dawley rats, brain microvessels	Morphine	10 mg/kg s.c., 2x/day, 5 + 3 days, naloxone 1 mg/kg s.c.	4h	RT-PCR, WB, UPLC-MS/MS	1.4-fold and 2.3-fold *Abcb1a* and *Abcg2* up-regulation No effect on P-gp and Bcrp protein levels	[179]
Adult Sprague Dawley rats, brain microvessels	Morphine	0.65 $mg.kg^{-1}.h^{-1}$ i.v. infusion, 5 days	None	WB, UPLC-MS/MS	No effect on P-gp and Bcrp protein levels	[179]
Human brain microvessel endothelial cell line hCMEC/D3	Morphine	24h incubation, 0.01, 0.1, 1, or 10 μM	-	RT-PCR	No effect in *ABCB1* nor *ABCG2* levels	[162]
Adult Sprague Dawley rats, several organs	Oxycodone	5 mg/kg i.p., 2x/day, 8 days	Presumably 12h	WB	2.0-fold, 4.0-fold, 1.6-fold, and 1.3-fold P-gp up-regulation in intestine, liver, kidney, and brain homogenates, respectively	[101]
Adult Sprague Dawley rats, whole brain homogenates	Oxycodone	15 mg/kg i.p., 2x/day, 8 days	12h	RT-PCR, WB	2.1-fold *Abcg2* up-regulation 1.8-fold Bcrp up-regulation	[163]

Even though the MRP transporter sub-family has been linked to the transport of opioids, notably in the transport of the glucuronide metabolites of morphine and oxymorphone, little evidence of the influence of opioid exposure on the modulation of their expression at the BBB level is available. Still, as previously mentioned, morphine given for 5 days produced an increase of the *Mrp1* mRNA levels in rat brain microvessels [151], evidencing once again an effect of a substrate-transporter interaction on the regulation of ABC transporters. The consequences of opioid exposure on the levels of ABC transporters at the BBB known up to date are summarized in Table **2**.

3.2. Mechanism of Regulation of ABC Transporters at the BBB by Morphine

Many studies have identified some of the signaling pathways and regulatory networks that control the expression of ABC transporters, and several of them have been identified at the BBB, particularly for P-gp. Such studies evidenced that ABC transporter expression and activity at the BBB can be altered by multiple factors, including disease, stress, diet, therapy and toxicant exposure [166, 167]. Yet, until very recently, the mechanism by which a chronic morphine treatment can lead to P-gp and Bcrp up-regulation remained unexplored and unknown.

Morphine withdrawal syndrome should be considered as a potential cause for P-gp and Bcrp regulation following a subchronic morphine treatment given that the up-regulation of these transporters was not registered in the course of the treatment with morphine, but only 12-24h after the last administration of morphine [162]. Different studies have demonstrated that morphine withdrawal symptoms start to appear as early as 12h, and evolve for the following 24-72h after the last dose of morphine [168, 169]. Spontaneous and naloxone-precipitated morphine withdrawal showed to provoke an important increase on the extracellular glutamate levels in different brain regions in both rats and mice [170-172]. In turn, high levels of glutamate (50-100 μM) have shown to up-regulate both P-gp expression and activity in rodent brain microvessels through action on the NMDA receptor, and subsequent COX-2 activity [173-175]. When animals were exposed to either an NMDA receptor antagonist, MK-801, or to a COX-2 inhibitor, meloxicam, following a subchronic morphine protocol, P-gp and Bcrp up-regulation at the rat BBB was no longer seen, 24h after the last dose of morphine [162]. These results suggest that glutamate activity on NMDA receptors and COX-2 are implicated in the morphine-dependent P-gp and Bcrp up-regulation. Furthermore, an up-regulation of MRP expression and function at the BBB have been described in the context of epilepsy [176], such as MRP1 and MRP2, and similarly a glutamate-induced NMDA receptor/COX-2 signaling pathway was evidenced to contribute to this regulation [177, 178]. Therefore, even though the mechanisms of an eventual up-regulation of Mrp1 expression following a subchronic morphine treatment [151] have not been explored so far, there is supporting evidence that a similar signaling pathway involving glutamate and NMDA receptors could be implicated, as for P-gp and BCRP.

Thus, the development of a morphine withdrawal is more likely to drive the modulation of the expression of P-gp, Bcrp and Mrp at the rat BBB, rather than a direct effect of morphine exposure. In line with this hypothesis, the effect of a naloxone-precipitated withdrawal on the P-gp and Bcrp contents at the rat BBB was investigated, following escalating or chronic morphine dose regimens, where it showed to increase *Mdr1a* and *Bcrp* mRNA levels by 1.4-fold and 2.4-fold, respectively [179]. Such results are of similar magnitude to the previously obtained, during a spontaneous morphine withdrawal [162]. However, P-gp and Bcrp up-regulation was not confirmed at protein level [179]. P-gp or Bcrp transport activities at the rat BBB, analyzed by *in situ* brain perfusion, also remained unchanged, corroborating such findings.

Still, these studies evidence a potential role of morphine withdrawal, with the subsequent glutamate release, on the up-regulation of ABC transporters at the BBB. It is known that there are several signaling pathways implicated in the cellular regulation of P-gp and BCRP at the BBB [39]. Thus, P-gp and Bcrp up-regulation following a subchronic morphine treatment may result from a sum of multiple mechanisms and signaling pathways implicated in the modulation of both transporters at the BBB. The administration of LPS, for example, has shown to trigger cytokine-induced inflammation and alter P-gp function in the brain, in both rats and mice [180, 181]. This is likely mediated by TLR4 receptors, since their activation leads to downstream production and release of pro-inflammatory cytokines like TNF-α and Il-1β, which in turn are among the most important inflammatory mediators that participate in the regulation of P-gp and Bcrp at the BBB [32, 182, 183]. In rat brain capillaries, a long-term (> 6h) TNF-α exposure activates TNF-R1 and induces a series of cascade events that culminate in the activation of the transcription factor NF-κB, and subsequent increase of the expression and activity of P-gp [184, 185]. On the other hand, short-term exposure of isolated rat brain capillaries (1h) to TNF-α decreased P-gp activity without affecting its expression, while expression and activity of Bcrp and Mrp1 remained unchanged [186, 187]. Thus, P-gp transport activity is reduced after short exposure to TNF-α but increased with longer exposure times, indicating a complex, time-dependent regulatory mechanism [39, 187]. Accordingly, a recent study evidenced that seizure-induced P-gp overexpression occurs, at least partially, *via* activation of TLR4 and downstream activation of NF-κB in mouse brain microvascular endothelial cells [188]. Up-regulation of P-gp involving the signaling agents LPS, TNF-α, TLR4 and NF-κB has been also described in other cell types [189-192], suggesting that TLR4 contributes to the modulation of P-gp expression and function. Since morphine has proven to trigger proinflammatory responses in TLR4-expressing non-neuronal cells, such as brain endothelial cells, *via* its interaction with TLR4 receptors [17], one may hypothesize that P-gp and Bcrp up-regulation in brain microvessel endothelial cells following a subchronic exposure to morphine also partly occurs as a result of the subsequent TLR4 activation and locally-induced inflammation.

It can be hypothesized that naloxone when used to precipitate opioid withdrawal interacted with non-opioid receptors, such as the TLRs [179]. The opioid antagonist (-)-naloxone and the non-opioid (+)-naloxone have been dem-

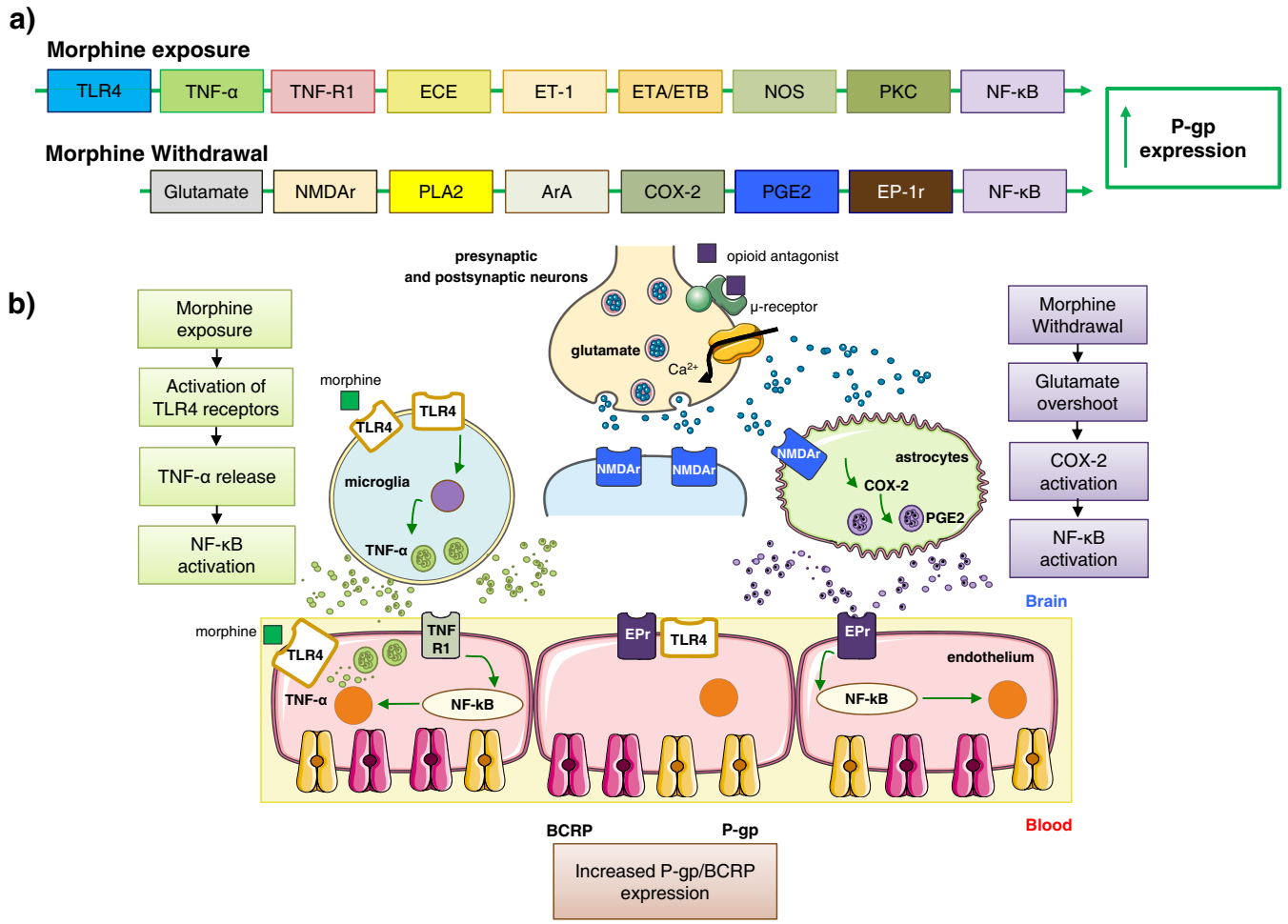

Fig. (1). Hypothetical regulation of P-gp and Bcrp expressions at the BBB following subchronic exposure to morphine, and presumably implicated signaling pathways. a) Implicated factors of signaling cascades activated during morphine-induced neuroinflammation or morphine withdrawal-provoked glutamate overshoot. **b)** Schematic representation of the implicated signaling pathways and BBB cellular environment that conduct to the regulation of P-gp and Bcrp expressions at the BBB following a subchronic exposure to morphine. Brain inflammation occurs in several conditions such as stroke, multiple sclerosis, Alzheimer's and Parkinson's diseases, and during morphine chronic exposure [17, 158]. Morphine activates TLR4 receptors with subsequent release of pro-inflammatory cytokines [17], such as TNF-α, which influences P-gp and Bcrp expressions at the BBB *via* a signaling pathway involving TNF-α receptor 1 (TNF-R1), endothelin-converting enzyme (ECE), endothelin-1 (ET-1), endothelin A and B receptors (ETA/B), nitric oxide synthase (NOS), protein kinase C (PKC), and nuclear factor-κB (NF-κB), as previously described [37, 158, 184]. During either morphine withdrawal or in the epileptic brain, high glutamate levels are found in the brain extracellular fluid [170-172, 194], which induce overexpression of P-gp and Bcrp at the BBB in endothelial cells by an NMDA receptor and COX-2-dependent mechanism [162, 174, 175, 179, 195].

onstrated to inhibit TLR4 signaling [15, 193], and naloxone has been used as a TLR4 inhibitor [190]. Thus, given the evidence that TLRs participate in the modulation of P-gp expression, and that TLR are expressed at the rat and human BBB [30, 32], in our previous study naloxone probably hampered P-gp and Bcrp up-regulation following subchronic morphine treatment [179].

On the other hand, opioid chronic exposure and its withdrawal produce an activation of COX-2 in the brain, given its implication in the up-regulation of P-gp, BCRP and MRP1 [151, 162, 175, 178]. Therefore, in addition to the fact that opioid agonists bind and activate TLRs, COX-2 activation may be another important consequence of chronic opioid administration, contributing to the triggering of inflammatory processes that limit opioid analgesic activity. Also, given that MRPs are known to transport both opioid and inflammatory mediators, such as eicosanoids, and that morphine may account for their modulation, this further suggests a close correlation between opioid exposure, the development of neuroinflammation, and the likely consequences on the expression of ABC transporters at the BBB.

CONCLUSION

In conclusion, it is clear nowadays that ABC transporters present at the BBB, and mainly P-gp, have an important impact on the pharmacokinetics of opioids, their brain disposition and, consequently, on their CNS effects. In turn, opioids are also capable to have an effect on BBB properties and to

promote neuroinflammation by a TLR-dependent pathway, which may involve mainly glial cells but also brain capillary endothelial cells. Such brain-related opioid effects likely have consequences particularly at the level of the expression of ABC transporters at the BBB. Therefore, opioid-induced up-regulation of ABC transporters, namely that of P-gp, may contribute to the development of tolerance to the antinociceptive effects of these drugs, as well as it may render more difficult the brain penetration of drugs which have a central effect and are also substrates of these transporters, such as some antidepressants, antipsychotics, anti-epileptic and brain anti-cancer drugs. Even though the mechanisms are still not fully understood, the mechanisms by which morphine and opioid withdrawal, following a chronic exposure may up-regulate the expression of P-gp and BCRP at the BBB can be summarized (Fig. **1**) presently in two essential signaling pathways: a first one involving glutamate release, NMDA-receptor and COX-2 activation, and a second one signaling through TLR4 activation and subsequent development of neuroinflammation and activation of NF-κB.

LIST OF ABBREVIATIONS

ABC	=	ATP-binding cassette
BBB	=	blood-brain barrier
BCRP	=	breast cancer resistance protein
CNS	=	central nervous system
LPS	=	lipopolysaccharide
M3G	=	morphine-3-glucoronide
M6G	=	morphine-6-glucoronide
MRP	=	multidrug resistance-associated protein
P-gp	=	P-glycoprotein
SNP	=	single nucleotide polymorphism
TLR	=	toll-like receptor
TNF-α	=	tumor necrosis factor-α
6-MAM	=	6-monoacetyl-morphine

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This study was granted by Catarina Chaves' PhD grant provided by Fundação para a Ciência e Tecnologia (FCT), reference number SFRH/BD/79196/2011. Catarina Chaves acknowledges FCT for her PhD grant.

REFERENCES

[1] Kalso, E.; Edwards, J.E.; Moore, R.A.; McQuay, H.J. Opioids in chronic non-cancer pain: systematic review of efficacy and safety. *Pain,* **2004**, *112*(3), 372-380. [http://dx.doi.org/10.1016/j.pain.2004.09.019] [PMID: 15561393]

[2] Ballantyne, J.C.; Mao, J. Opioid therapy for chronic pain. *N. Engl. J. Med.,* **2003**, *349*(20), 1943-1953. [http://dx.doi.org/10.1056/NEJMra025411] [PMID: 14614170]

[3] Koob, G.F.; Le Moal, M. Addiction and the brain antireward system. *Annu. Rev. Psychol.,* **2008**, *59*, 29-53. [http://dx.doi.org/10.1146/annurev.psych.59.103006.093548] [PMID: 18154498]

[4] Shurman, J.; Koob, G.F.; Gutstein, H.B. Opioids, pain, the brain, and hyperkatifeia: a framework for the rational use of opioids for pain. *Pain Med.,* **2010**, *11*(7), 1092-1098. [http://dx.doi.org/10.1111/j.1526-4637.2010.00881.x] [PMID: 20545871]

[5] Pert, C.B.; Snyder, S.H. Opiate receptor: demonstration in nervous tissue. *Science,* **1973**, *179*(4077), 1011-1014. [http://dx.doi.org/10.1126/science.179.4077.1011] [PMID: 4687585]

[6] Simon, E.J. In search of the opiate receptor. *Am. J. Med. Sci.,* **1973**, *266*(3), 160-168. [http://dx.doi.org/10.1097/00000441-197309000-00001] [PMID: 4583407]

[7] Terenius, L. Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. *Acta Pharmacol. Toxicol. (Copenh.),* **1973**, *33*(5), 377-384. [PMID: 4801083]

[8] Martin, W.R.; Eades, C.G.; Thompson, J.A.; Huppler, R.E.; Gilbert, P.E. The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. Exp. Ther.,* **1976**, *197*(3), 517-532. [PMID: 945347]

[9] Ossipov, M.H.; Lai, J.; King, T.; Vanderah, T.W.; Malan, T.P., Jr; Hruby, V.J.; Porreca, F. Antinociceptive and nociceptive actions of opioids. *J. Neurobiol.,* **2004**, *61*(1), 126-148. [http://dx.doi.org/10.1002/neu.20091] [PMID: 15362157]

[10] Pasternak, G.W. Multiple opiate receptors: déjà vu all over again. *Neuropharmacology,* **2004**, *47*(Suppl. 1), 312-323. [http://dx.doi.org/10.1016/j.neuropharm.2004.07.004] [PMID: 15464147]

[11] Stefano, G.B.; Hartman, A.; Bilfinger, T.V.; Magazine, H.I.; Liu, Y.; Casares, F.; Goligorsky, M.S. Presence of the mu3 opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. *J. Biol. Chem.,* **1995**, *270*(51), 30290-30293. [http://dx.doi.org/10.1074/jbc.270.51.30290] [PMID: 8530450]

[12] Van Woerkom, R.; Beharry, K.D.; Modanlou, H.D.; Parker, J.; Rajan, V.; Akmal, Y.; Aranda, J.V. Influence of morphine and naloxone on endothelin and its receptors in newborn piglet brain vascular endothelial cells: clinical implications in neonatal care. *Pediatr. Res.,* **2004**, *55*(1), 147-151. [http://dx.doi.org/10.1203/01.PDR.0000100756.32861.60] [PMID: 14605251]

[13] Granata, F.; Potenza, R.L.; Fiori, A.; Strom, R.; Caronti, B.; Molinari, P.; Donsante, S.; Citro, G.; Iacovelli, L.; De Blasi, A.; Ngomba, R.T.; Palladini, G.; Passarelli, F. Expression of OP4 (ORL1, NOP1) receptors in vascular endothelium. *Eur. J. Pharmacol.,* **2003**, *482*(1-3), 17-23. [http://dx.doi.org/10.1016/j.ejphar.2003.09.049] [PMID: 14660000]

[14] Hutchinson, M.R.; Lewis, S.S.; Coats, B.D.; Rezvani, N.; Zhang, Y.; Wieseler, J.L.; Somogyi, A.A.; Yin, H.; Maier, S.F.; Rice, K.C.; Watkins, L.R. Possible involvement of toll-like receptor 4/myeloid differentiation factor-2 activity of opioid inactive isomers causes spinal proinflammation and related behavioral consequences. *Neuroscience,* **2010**, *167*(3), 880-893. [http://dx.doi.org/10.1016/j.neuroscience.2010.02.011] [PMID: 20178837]

[15] Hutchinson, M.R.; Zhang, Y.; Shridhar, M.; Evans, J.H.; Buchanan, M.M.; Zhao, T.X.; Slivka, P.F.; Coats, B.D.; Rezvani, N.; Wieseler, J.; Hughes, T.S.; Landgraf, K.E.; Chan, S.; Fong, S.; Phipps, S.; Falke, J.J.; Leinwand, L.A.; Maier, S.F.; Yin, H.; Rice, K.C.; Watkins, L.R. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. *Brain Behav. Immun.,* **2010**, *24*(1), 83-95. [http://dx.doi.org/10.1016/j.bbi.2009.08.004] [PMID: 19679181]

[16] Hutchinson, M.R.; Northcutt, A.L.; Hiranita, T.; Wang, X.; Lewis, S.S.; Thomas, J.; van Steeg, K.; Kopajtic, T.A.; Loram, L.C.; Sfregola, C.; Galer, E.; Miles, N.E.; Bland, S.T.; Amat, J.; Rozeske, R.R.; Maslanik, T.; Chapman, T.R.; Strand, K.A.; Fleshner, M.; Bachtell, R.K.; Somogyi, A.A.; Yin, H.; Katz, J.L.; Rice, K.C.; Maier, S.F.; Watkins, L.R. Opioid activation of toll-like receptor 4 contributes to drug reinforcement. *J. Neurosci.,* **2012**, *32*(33), 11187-11200. [http://dx.doi.org/10.1523/JNEUROSCI.0684-12.2012] [PMID: 22895704]

[17] Wang, X.; Loram, L.C.; Ramos, K.; de Jesus, A.J.; Thomas, J.; Cheng, K.; Reddy, A.; Somogyi, A.A.; Hutchinson, M.R.; Watkins, L.R.; Yin, H. Morphine activates neuroinflammation in a manner parallel to endotoxin. *Proc. Natl. Acad. Sci. USA,* **2012**, *109*(16), 6325-6330. [http://dx.doi.org/10.1073/pnas.1200130109] [PMID: 22474354]

[18] Bsibsi, M.; Ravid, R.; Gveric, D.; van Noort, J.M. Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol.,* **2002**, *61*(11), 1013-1021. [http://dx.doi.org/10.1093/jnen/61.11.1013] [PMID: 12430718]

[19] Dauphinee, S.M.; Karsan, A. Lipopolysaccharide signaling in endothelial cells. *Lab. Invest.,* **2006**, *86*(1), 9-22. [http://dx.doi.org/10.1038/labinvest.3700366] [PMID: 16357866]

[20] Zhang, Y.; Li, H.; Li, Y.; Sun, X.; Zhu, M.; Hanley, G.; Lesage, G.; Yin, D. Essential role of toll-like receptor 2 in morphine-induced microglia activation in mice. *Neurosci. Lett.,* **2011**, *489*(1), 43-47. [http://dx.doi.org/10.1016/j.neulet.2010.11.063] [PMID: 21130144]

[21] Cui, Y.; Liao, X.X.; Liu, W.; Guo, R.X.; Wu, Z.Z.; Zhao, C.M.; Chen, P.X.; Feng, J.Q. A novel role of minocycline: attenuating morphine antinociceptive tolerance by inhibition of p38 MAPK in the activated spinal microglia. *Brain Behav. Immun.,* **2008**, *22*(1), 114-123. [http://dx.doi.org/10.1016/j.bbi.2007.07.014] [PMID: 17919885]

[22] Narita, M.; Miyatake, M.; Narita, M.; Shibasaki, M.; Shindo, K.; Nakamura, A.; Kuzumaki, N.; Nagumo, Y.; Suzuki, T. The involvement of glial cells in the development of morphine tolerance. *Neurosci. Res.,* **2001**, *39*(3), 281-6.

[23] Song, P.; Zhao, Z.Q. The involvement of glial cells in the development of morphine tolerance. *Neurosci. Res.,* **2001**, *39*(3), 281-286. [http://dx.doi.org/10.1016/S0168-0102(00)00226-1] [PMID: 11248367]

[24] Eidson, L.N.; Murphy, A.Z. Blockade of Toll-like receptor 4 attenuates morphine tolerance and facilitates the pain relieving properties of morphine. *J. Neurosci.,* **2013**, *33*(40), 15952-15963. [http://dx.doi.org/10.1523/JNEUROSCI.1609-13.2013] [PMID: 24089500]

[25] Jurga, A.M.; Rojewska, E.; Piotrowska, A.; Makuch, W.; Pilat, D.; Przewlocka, B.; Mika, J. Blockade of toll-like receptors (TLR2, TLR4) attenuates pain and potentiates buprenorphine analgesia in a rat neuropathic pain model. *Neural Plast.,* **2016**, *2016*, 5238730. [http://dx.doi.org/10.1155/2016/5238730] [PMID: 26962463]

[26] Lewis, S.S.; Loram, L.C.; Hutchinson, M.R.; Li, C.M.; Zhang, Y.; Maier, S.F.; Huang, Y.; Rice, K.C.; Watkins, L.R. Watkins, (+)-naloxone, an opioid-inactive toll-like receptor 4 signal-ing inhibitor, reverses multiple models of chronic neuropathic pain in rats. *J. Pain,* **2012**, *13*(5), 498-506.

[27] Hutchinson, M.R.; Shavit, Y.; Grace, P.M.; Rice, K.C.; Maier, S.F.; Watkins, L.R. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacol. Rev.,* **2011**, *63*(3), 772-810. [http://dx.doi.org/10.1124/pr.110.004135] [PMID: 21752874]

[28] Singh, A.K.; Jiang, Y. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? *Toxicology,* **2004**, *201*(1-3), 197-207. [http://dx.doi.org/10.1016/j.tox.2004.04.015] [PMID: 15297033]

[29] Shih, R.H.; Yang, C.M. Induction of heme oxygenase-1 attenuates lipopolysaccharide-induced cyclooxygenase-2 expression in mouse brain endothelial cells. *J. Neuroinflamm.,* **2010**, *7*, 86. [http://dx.doi.org/10.1186/1742-2094-7-86] [PMID: 21118574]

[30] Nagyoszi, P.; Wilhelm, I.; Farkas, A.E.; Fazakas, C.; Dung, N.T.; Haskó, J.; Krizbai, I.A. Expression and regulation of toll-like receptors in cerebral endothelial cells. *Neurochem. Int.,* **2010**, *57*(5), 556-564. [http://dx.doi.org/10.1016/j.neuint.2010.07.002] [PMID: 20637248]

[31] Verma, S.; Nakaoke, R.; Dohgu, S.; Banks, W.A. Release of cytokines by brain endothelial cells: A polarized response to lipopolysaccharide. *Brain Behav. Immun.,* **2006**, *20*(5), 449-455. [http://dx.doi.org/10.1016/j.bbi.2005.10.005] [PMID: 16309883]

[32] Grace, P.M.; Ramos, K.M.; Rodgers, K.M.; Wang, X.; Hutchinson, M.R.; Lewis, M.T.; Morgan, K.N.; Kroll, J.L.; Taylor, F.R.; Strand, K.A.; Zhang, Y.; Berkelhammer, D.; Huey, M.G.; Greene, L.I.; Cochran, T.A.; Yin, H.; Barth, D.S.; Johnson, K.W.; Rice, K.C.; Maier, S.F.; Watkins, L.R. Activation of adult rat CNS endothelial cells by opioid-induced toll-like receptor 4 (TLR4) signaling induces proinflammatory, biochemical, morphological, and behavioral sequelae. *Neuroscience,* **2014**, *280*, 299-317. [http://dx.doi.org/10.1016/j.neuroscience.2014.09.020] [PMID: 25241065]

[33] Abbott, N.J.; Patabendige, A.A.; Dolman, D.E.; Yusof, S.R.; Begley, D.J. Structure and function of the blood-brain barrier. *Neurobiol. Dis.,* **2010**, *37*(1), 13-25. [http://dx.doi.org/10.1016/j.nbd.2009.07.030] [PMID: 19664713]

[34] Stanimirovic, D.B.; Friedman, A. Pathophysiology of the neurovascular unit: disease cause or consequence? *J. Cereb. Blood Flow Metab.,* **2012**, *32*(7), 1207-1221. [http://dx.doi.org/10.1038/jcbfm.2012.25] [PMID: 22395208]

[35] Begley, D.J. ABC transporters and the blood-brain barrier. *Curr. Pharm. Des.,* **2004**, *10*(12), 1295-1312. [http://dx.doi.org/10.2174/1381612043384844] [PMID: 15134482]

[36] Scherrmann, J.M. Expression and function of multidrug resistance transporters at the blood-brain barriers. *Expert Opin. Drug Metab. Toxicol.,* **2005**, *1*(2), 233-246. [http://dx.doi.org/10.1517/17425255.1.2.233] [PMID: 16922639]

[37] Hartz, A.M.; Bauer, B. Regulation of ABC transporters at the blood-brain barrier: new targets for CNS therapy. *Mol. Interv.,* **2010**, *10*(5), 293-304. [http://dx.doi.org/10.1124/mi.10.5.6] [PMID: 21045243]

[38] Moitra, K.; Dean, M. Evolution of ABC transporters by gene duplication and their role in human disease. *Biol. Chem.,* **2011**, *392*(1-2), 29-37. [http://dx.doi.org/10.1515/bc.2011.006] [PMID: 21194360]

[39] Miller, D.S. Regulation of ABC transporters at the blood-brain barrier. *Clin. Pharmacol. Ther.,* **2015**, *97*(4), 395-403. [http://dx.doi.org/10.1002/cpt.64] [PMID: 25670036]

[40] Chaves, C.; Shawahna, R.; Jacob, A.; Scherrmann, J.M.; Declèves, X. Human ABC transporters at blood-CNS interfaces as determinants of CNS drug penetration. *Curr. Pharm. Des.,* **2014**, *20*(10), 1450-1462. [http://dx.doi.org/10.2174/13816128113199990466] [PMID: 23789951]

[41] Dauchy, S.; Dutheil, F.; Weaver, R.J.; Chassoux, F.; Daumas-Duport, C.; Couraud, P.O.; Scherrmann, J.M.; De Waziers, I.; Declèves, X. ABC transporters, cytochromes P450 and their main transcription factors: expression at the human blood-brain barrier. *J. Neurochem.,* **2008**, *107*(6), 1518-1528. [http://dx.doi.org/10.1111/j.1471-4159.2008.05720.x] [PMID: 19094056]

[42] Shawahna, R.; Uchida, Y.; Declèves, X.; Ohtsuki, S.; Yousif, S.; Dauchy, S.; Jacob, A.; Chassoux, F.; Daumas-Duport, C.; Couraud, P.O.; Terasaki, T.; Scherrmann, J.M. Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Mol. Pharm.,* **2011**, *8*(4), 1332-1341. [http://dx.doi.org/10.1021/mp200129p] [PMID: 21707071]

[43] Uchida, Y.; Ohtsuki, S.; Katsukura, Y.; Ikeda, C.; Suzuki, T.; Kamiie, J.; Terasaki, T. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J. Neurochem.,* **2011**, *117*(2), 333-345. [http://dx.doi.org/10.1111/j.1471-4159.2011.07208.x] [PMID: 21291474]

[44] Tournier, N.; Declèves, X.; Saubaméa, B.; Scherrmann, J.M.; Cisternino, S. Opioid transport by ATP-binding cassette transporters at the blood-brain barrier: implications for neuropsychopharmacology. *Curr. Pharm. Des.,* **2011**, *17*(26), 2829-2842. [http://dx.doi.org/10.2174/138161211797440203] [PMID: 21827411]

[45] Callaghan, R.; Riordan, J.R. Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells. *J. Biol. Chem.,* **1993**, *268*(21), 16059-16064. [PMID: 8101846]

[46] Letrent, S.P.; Pollack, G.M.; Brouwer, K.R.; Brouwer, K.L. Effect of GF120918, a potent P-glycoprotein inhibitor, on morphine pharmacokinetics and pharmacodynamics in the rat. *Pharm. Res.,* **1998**, *15*(4), 599-605. [http://dx.doi.org/10.1023/A:1011938112599] [PMID: 9587957]

[47] Letrent, S.P.; Pollack, G.M.; Brouwer, K.R.; Brouwer, K.L. 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.,* **1999**, *27*(7), 827-834. [PMID: 10383928]

[48] Letrent, S.P.; Polli, J.W.; Humphreys, J.E.; Pollack, G.M.; Brouwer, K.R.; Brouwer, K.L. P-glycoprotein-mediated transport of morphine in brain capillary endothelial cells. *Biochem. Pharmacol.,* **1999**, *58*(6), 951-957. [http://dx.doi.org/10.1016/S0006-2952(99)00180-X] [PMID: 10509747]

[49] Schinkel, A.H.; Wagenaar, E.; van Deemter, L.; Mol, C.A.; Borst, P. Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J. Clin. Invest.,* **1995**, *96*(4), 1698-1705. [http://dx.doi.org/10.1172/JCI118214] [PMID: 7560060]

[50] Xie, R.; Hammarlund-Udenaes, M.; de Boer, A.G.; de Lange, E.C. The role of P-glycoprotein in blood-brain barrier transport of morphine: transcortical microdialysis studies in MDR1A (-/-) and mdr1a (+/+) mice. *Br. J. Pharmacol.,* **1999**, *128*(3), 563-568. [http://dx.doi.org/10.1038/sj.bjp.0702804] [PMID: 10516633]

[51] Zong, J.; Pollack, G.M. Morphine antinociception is enhanced in MDR1A gene-deficient mice. *Pharm. Res.,* **2000**, *17*(6), 749-753. [http://dx.doi.org/10.1023/A:1007546719287] [PMID: 10955852]

[52] Mercer, S.L.; Coop, A. Opioid analgesics and P-glycoprotein efflux transporters: a potential systems-level contribution to analgesic tolerance. *Curr. Top. Med. Chem.,* **2011**, *11*(9), 1157-1164. [http://dx.doi.org/10.2174/156802611795371288] [PMID: 21050174]

[53] Ederoth, P.; Tunblad, K.; Bouw, R.; Lundberg, C.J.; Ungerstedt, U.; Nordström, C.H.; Hammarlund-Udenaes, M. Blood-brain barrier transport of morphine in patients with severe brain trauma. *Br. J. Clin. Pharmacol.,* **2004**, *57*(4), 427-435. [http://dx.doi.org/10.1046/j.1365-2125.2003.02032.x] [PMID: 15025740]

[54] Kharasch, E.D.; Hoffer, C.; Whittington, D.; Sheffels, P. Role of P-glycoprotein in the intestinal absorption and clinical effects of morphine. *Clin. Pharmacol. Ther.,* **2003**, *74*(6), 543-554. [http://dx.doi.org/10.1016/j.clpt.2003.08.011] [PMID: 14663457]

[55] Thompson, S.J.; Koszdin, K.; Bernards, C.M. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *Anesthesiology,* **2000**, *92*(5), 1392-1399. [http://dx.doi.org/10.1097/00000542-200005000-00030] [PMID: 10781286]

[56] Huwyler, J.; Drewe, J.; Gutmann, H.; Thöle, M.; Fricker, G. Modulation of morphine-6-glucuronide penetration into the brain by P-glycoprotein. *Int. J. Clin. Pharmacol. Ther.,* **1998**, *36*(2), 69-70. [PMID: 9520146]

[57] Crowe, A. The influence of P-glycoprotein on morphine transport in Caco-2 cells. Comparison with paclitaxel. *Eur. J. Pharmacol.,* **2002**, *440*(1), 7-16. [http://dx.doi.org/10.1016/S0014-2999(02)01366-3] [PMID: 11959083]

[58] Wandel, C.; Kim, R.; Wood, M.; Wood, A. Interaction of morphine, fentanyl, sufentanil, alfentanil, and loperamide with the efflux drug transporter P-glycoprotein. *Anesthesiology,* **2002**, *96*(4), 913-920. [http://dx.doi.org/10.1097/00000542-200204000-00019] [PMID: 11964599]

[59] Shimizu, N.; Kishioka, S.; Maeda, T.; Fukazawa, Y.; Dake, Y.; Yamamoto, C.; Ozaki, M.; Yamamoto, H. Involvement of peripheral mechanism in the verapamil-induced potentiation of morphine analgesia in mice. *J. Pharmacol. Sci.,* **2004**, *95*(4), 452-457. [http://dx.doi.org/10.1254/jphs.FP0040252] [PMID: 15297768]

[60] Seleman, M.; Chapy, H.; Cisternino, S.; Courtin, C.; Smirnova, M.; Schlatter, J.; Chiadmi, F.; Scherrmann, J.M.; Noble, F.; Marie-Claire, C. 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. *Psychopharmacology (Berl.),* **2014**, *231*(16), 3139-3149. [http://dx.doi.org/10.1007/s00213-014-3490-9] [PMID: 24705903]

[61] Cisternino, S.; Rousselle, C.; Dagenais, C.; Scherrmann, J.M. Screening of multidrug-resistance sensitive drugs by in situ brain perfusion in P-glycoprotein-deficient mice. *Pharm. Res.,* **2001**, *18*(2), 183-190. [http://dx.doi.org/10.1023/A:1011080418027] [PMID: 11405289]

[62] Groenendaal, D.; Freijer, J.; de Mik, D.; Bouw, M.R.; Danhof, M.; de Lange, E.C. Population pharmacokinetic modelling of non-linear brain distribution of morphine: influence of active saturable influx and P-glycoprotein mediated efflux. *Br. J. Pharmacol.,* **2007**, *151*(5), 701-712. [http://dx.doi.org/10.1038/sj.bjp.0707257] [PMID: 17471182]

[63] Hamabe, W.; Maeda, T.; Kiguchi, N.; Yamamoto, C.; Tokuyama, S.; Kishioka, S. Negative relationship between morphine analgesia and P-glycoprotein expression levels in the brain. *J. Pharmacol. Sci.,* **2007**, *105*(4), 353-360. [http://dx.doi.org/10.1254/jphs.FP0071287] [PMID: 18071274]

[64] Skarke, C.; Jarrar, M.; Erb, K.; Schmidt, H.; Geisslinger, G.; Lötsch, J. Respiratory and miotic effects of morphine in healthy volunteers when P-glycoprotein is blocked by quinidine. *Clin. Pharmacol. Ther.,* **2003**, *74*(4), 303-311. [http://dx.doi.org/10.1016/S0009-9236(03)00220-0] [PMID: 14534517]

[65] Drewe, J.; Ball, H.A.; Beglinger, C.; Peng, B.; Kemmler, A.; Schächinger, H.; Haefeli, W.E. Effect of P-glycoprotein modulation on the clinical pharmacokinetics and adverse effects of morphine. *Br. J. Clin. Pharmacol.,* **2000**, *50*(3), 237-246. [http://dx.doi.org/10.1046/j.1365-2125.2000.00226.x] [PMID: 10971308]

[66] Campa, D.; Gioia, A.; Tomei, A.; Poli, P.; Barale, R. Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief. *Clin. Pharmacol. Ther.,* **2008**, *83*(4), 559-566. [http://dx.doi.org/10.1038/sj.clpt.6100385] [PMID: 17898703]

[67] Lötsch, J.; Klepstad, P.; Doehring, A.; Dale, O. A GTP cyclohydrolase 1 genetic variant delays cancer pain. *Pain,* **2010**, *148*(1), 103-106. [http://dx.doi.org/10.1016/j.pain.2009.10.021] [PMID: 19959292]

[68] Sia, A.T.; Sng, B.L.; Lim, E.C.; Law, H.; Tan, E.C. The influence of ATP-binding cassette sub-family B member -1 (ABCB1) genetic polymorphisms on acute and chronic pain after intrathecal morphine for caesarean section: a prospective cohort study. *Int. J. Obstet. Anesth.,* **2010**, *19*(3), 254-260. [http://dx.doi.org/10.1016/j.ijoa.2010.03.001] [PMID: 20627697]

[69] Hanks, G.W.; Conno, F.; Cherny, N.; Hanna, M.; Kalso, E.; McQuay, H.J.; Mercadante, S.; Meynadier, J.; Poulain, P.; Ripamonti, C.; Radbruch, L.; Casas, J.R.; Sawe, J.; Twycross, R.G.; Ventafridda, V. Morphine and alternative opioids in cancer pain: the EAPC recommendations. *Br. J. Cancer,* **2001**, *84*(5), 587-593. [http://dx.doi.org/10.1054/bjoc.2001.1680] [PMID: 11237376]

[70] Glare, P.A.; Walsh, T.D. Clinical pharmacokinetics of morphine. *Ther. Drug Monit.,* **1991**, *13*(1), 1-23. [http://dx.doi.org/10.1097/00007691-199101000-00001] [PMID: 2057987]

[71] Christrup, L.L. Morphine metabolites. *Acta Anaesthesiol. Scand.,* **1997**, *41*(1 Pt 2), 116-122. [http://dx.doi.org/10.1111/j.1399-6576.1997.tb04625.x] [PMID: 9061094]

[72] Smith, M.T. Neuroexcitatory effects of morphine and hydromorphone: evidence implicating the 3-glucuronide metabolites. *Clin. Exp. Pharmacol. Physiol.,* **2000**, *27*(7), 524-528. [http://dx.doi.org/10.1046/j.1440-1681.2000.03290.x] [PMID: 10874511]

[73] Lewis, S.S.; Hutchinson, M.R.; Rezvani, N.; Loram, L.C.; Zhang, Y.; Maier, S.F.; Rice, K.C.; Watkins, L.R. Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement *via* toll-like receptor 4/MD-2 and interleukin-1beta. *Neuroscience,* **2010**, *165*(2), 569-583. [http://dx.doi.org/10.1016/j.neuroscience.2009.10.011] [PMID: 19833175]

[74] Due, M.R.; Piekarz, A.D.; Wilson, N.; Feldman, P.; Ripsch, M.S.; Chavez, S.; Yin, H.; Khanna, R.; White, F.A. Neuroexcitatory effects of morphine-3-glucuronide are dependent on Toll-like receptor 4 signaling. *J. Neuroinflammation,* **2012**, *9*, 200. [http://dx.doi.org/10.1186/1742-2094-9-200] [PMID: 22898544]

[75] Klimas, R.; Mikus, G. Morphine-6-glucuronide is responsible for the analgesic effect after morphine administration: a quantitative review of morphine, morphine-6-glucuronide, and morphine-3-glucuronide. *Br. J. Anaesth.,* **2014**, *113*(6), 935-944. [http://dx.doi.org/10.1093/bja/aeu186] [PMID: 24985077]

[76] Huwyler, J.; Drewe, J.; Klusemann, C.; Fricker, G. Evidence for P-glycoprotein-modulated penetration of morphine-6-glucuronide into brain capillary endothelium. *Br. J. Pharmacol.,* **1996**, *118*(8), 1879-1885. [http://dx.doi.org/10.1111/j.1476-5381.1996.tb15619.x] [PMID: 8864518]

[77] Sattari, M.; Routledge, P.; Mashayekhi, S. The influence of active transport systems on morphine -6-glucuronide transport in MDCKII and MDCK-PGP cells. *Daru,* **2011**, *19*(6), 412-416. [PMID: 23008686]

[78] Lötsch, J.; Schmidt, R.; Vetter, G.; Schmidt, H.; Niederberger, E.; Geisslinger, G.; Tegeder, I. Increased CNS uptake and enhanced antinociception of morphine-6-glucuronide in rats after inhibition

of P-glycoprotein. *J. Neurochem.,* **2002**, *83*(2), 241-248. [http://dx.doi.org/10.1046/j.1471-4159.2002.01177.x] [PMID: 12423235]

[79] Lötsch, J.; Tegeder, I.; Angst, M.S.; Geisslinger, G. Antinociceptive effects of morphine-6-glucuronide in homozygous MDR1a P-glycoprotein knockout and in wildtype mice in the hotplate test. *Life Sci.,* **2000**, *66*(24), 2393-2403. [http://dx.doi.org/10.1016/S0024-3205(00)00569-5] [PMID: 10864101]

[80] Bourasset, F.; Cisternino, S.; Temsamani, J.; Scherrmann, J.M. Evidence for an active transport of morphine-6-beta-d-glucuronide but not P-glycoprotein-mediated at the blood-brain barrier. *J. Neurochem.,* **2003**, *86*(6), 1564-1567. [http://dx.doi.org/10.1046/j.1471-4159.2003.01990.x] [PMID: 12950465]

[81] Bardo, M.T.; Rowlett, J.K.; Harris, M.J. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci. Biobehav. Rev.,* **1995**, *19*(1), 39-51. [http://dx.doi.org/10.1016/0149-7634(94)00021-R] [PMID: 7770196]

[82] Oldendorf, W.H.; Hyman, S.; Braun, L.; Oldendorf, S.Z. Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. *Science,* **1972**, *178*(4064), 984-986. [http://dx.doi.org/10.1126/science.178.4064.984] [PMID: 5084666]

[83] Selley, D.E.; Cao, C.C.; Sexton, T.; Schwegel, J.A.; Martin, T.J.; Childers, S.R. mu Opioid receptor-mediated G-protein activation by heroin metabolites: evidence for greater efficacy of 6-monoacetylmorphine compared with morphine. *Biochem. Pharmacol.,* **2001**, *62*(4), 447-455. [http://dx.doi.org/10.1016/S0006-2952(01)00689-X] [PMID: 11448454]

[84] Watson, B.; Meng, F.; Akil, H. A chimeric analysis of the opioid receptor domains critical for the binding selectivity of mu opioid ligands. *Neurobiol. Dis.,* **1996**, *3*(1), 87-96. [http://dx.doi.org/10.1006/nbdi.1996.0009] [PMID: 9173916]

[85] Oldendorf, W.H. Some relationships between addiction and drug delivery to the brain. *NIDA Res. Monogr.,* **1992**, *120*, 13-25. [PMID: 1501682]

[86] Boix, F.; Andersen, J.M.; Mørland, J. Pharmacokinetic modeling of subcutaneous heroin and its metabolites in blood and brain of mice. *Addict. Biol.,* **2013**, *18*(1), 1-7. [http://dx.doi.org/10.1111/j.1369-1600.2010.00298.x] [PMID: 21481103]

[87] Schinkel, A.H.; Wagenaar, E.; Mol, C.A.; van Deemter, L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J. Clin. Invest.,* **1996**, *97*(11), 2517-2524. [http://dx.doi.org/10.1172/JCI118699] [PMID: 8647944]

[88] Sadeque, A.J.; Wandel, C.; He, H.; Shah, S.; Wood, A.J. Increased drug delivery to the brain by P-glycoprotein inhibition. *Clin. Pharmacol. Ther.,* **2000**, *68*(3), 231-237. [http://dx.doi.org/10.1067/mcp.2000.109156] [PMID: 11014404]

[89] Dagenais, C.; Graff, C.L.; Pollack, G.M. Variable modulation of opioid brain uptake by P-glycoprotein in mice. *Biochem. Pharmacol.,* **2004**, *67*(2), 269-276. [http://dx.doi.org/10.1016/j.bcp.2003.08.027] [PMID: 14698039]

[90] Kreisl, W.C.; Liow, J.S.; Kimura, N.; Seneca, N.; Zoghbi, S.S.; Morse, C.L.; Herscovitch, P.; Pike, V.W.; Innis, R.B. P-glycoprotein function at the blood-brain barrier in humans can be quantified with the substrate radiotracer 11C-N-desmethyl-loperamide. *J. Nucl. Med. Techhnol.,* **2010**, *51*(4), 559-66.

[91] Tournier, N.; Valette, H.; Peyronneau, M.A.; Saba, W.; Goutal, S.; Kuhnast, B.; Dolle, F.; Scherrmann, J.M.; Cisternino, S.; Bottlaender, M. Transport of selected PET radiotracers by human P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2): an *in vitro* screening. *J. Nucl. Med. Techhnol.,* **2011**, *52*(3), 415-23.

[92] Ryu, Y.H.; Liow, J.S.; Zoghbi, S.; Fujita, M.; Collins, J.; Tipre, D.; Sangare, J.; Hong, J.; Pike, V.W.; Innis, R.B. Disulfiram inhibits defluorination of (18)F-FCWAY, reduces bone radioactivity, and enhances visualization of radioligand binding to serotonin 5-HT1A receptors in human brain. *J. Nucl. Med.,* **2007**, *48*(7), 1154-1161. [http://dx.doi.org/10.2967/jnumed.107.039933] [PMID: 17574977]

[93] Kannan, P.; Brimacombe, K.R.; Zoghbi, S.S.; Liow, J.S.; Morse, C.; Taku, A.K.; Pike, V.W.; Halldin, C.; Innis, R.B.; Gottesman, M.M.; Hall, M.D. N-desmethyl-loperamide is selective for P-glycoprotein among three ATP-binding cassette transporters at the blood-brain barrier. *Drug Metab. Dispos.,* **2010**, *38*(6), 917-922. [http://dx.doi.org/10.1124/dmd.109.031161] [PMID: 20212014]

[94] Kuhlman, J.J., Jr; Levine, B.; Johnson, R.E.; Fudala, P.J.; Cone, E.J. Relationship of plasma buprenorphine and norbuprenorphine to withdrawal symptoms during dose induction, maintenance and withdrawal from sublingual buprenorphine. *Addiction,* **1998**, *93*(4), 549-559. [http://dx.doi.org/10.1046/j.1360-0443.1998.93454910.x] [PMID: 9684393]

[95] Hassan, H.E.; Myers, A.L.; Coop, A.; Eddington, N.D. Differential involvement of P-glycoprotein (ABCB1) in permeability, tissue distribution, and antinociceptive activity of methadone, buprenorphine, and diprenorphine: *in vitro* and *in vivo* evaluation. *J. Pharm. Sci.,* **2009**, *98*(12), 4928-4940. [http://dx.doi.org/10.1002/jps.21770] [PMID: 19370547]

[96] Tournier, N.; Chevillard, L.; Megarbane, B.; Pirnay, S.; Scherrmann, J.M.; Decleves, X. Decleves, Interaction of drugs of abuse and maintenance treat-ments with human P-glycoprotein (ABCB1) and breast cancer re-sistance protein (ABCG2). *Int. J. Neurosychopharmacol.,* **2010**, *13*(7), 905-15.

[97] Suzuki, T.; Zaima, C.; Moriki, Y.; Fukami, T.; Tomono, K. P-glycoprotein mediates brain-to-blood efflux transport of buprenorphine across the blood-brain barrier. *J. Drug Target.,* **2007**, *15*(1), 67-74. [http://dx.doi.org/10.1080/10611860601141606] [PMID: 17365275]

[98] Alhaddad, H.; Cisternino, S.; Declèves, X.; Tournier, N.; Schlatter, J.; Chiadmi, F.; Risède, P.; Smirnova, M.; Besengez, C.; Scherrmann, J.M.; Baud, F.J.; Mégarbane, B. Respiratory toxicity of buprenorphine results from the blockage of P-glycoprotein-mediated efflux of norbuprenorphine at the blood-brain barrier in mice. *Crit. Care Med.,* **2012**, *40*(12), 3215-3223. [http://dx.doi.org/10.1097/CCM.0b013e318265680a] [PMID: 22975888]

[99] Silvasti, M.; Rosenberg, P.; Seppälä, T.; Svartling, N.; Pitkänen, M. Comparison of analgesic efficacy of oxycodone and morphine in postoperative intravenous patient-controlled analgesia. *Acta Anaesthesiol. Scand.,* **1998**, *42*(5), 576-580. [http://dx.doi.org/10.1111/j.1399-6576.1998.tb05169.x] [PMID: 9605375]

[100] Boström, E.; Simonsson, U.S.; Hammarlund-Udenaes, M. Oxycodone pharmacokinetics and pharmacodynamics in the rat in the presence of the P-glycoprotein inhibitor PSC833. *J. Pharm. Sci.,* **2005**, *94*(5), 1060-1066. [http://dx.doi.org/10.1002/jps.20327] [PMID: 15799017]

[101] Hassan, H.E.; Myers, A.L.; Lee, I.J.; Coop, A.; Eddington, N.D. Oxycodone induces overexpression of P-glycoprotein (ABCB1) and affects paclitaxel's tissue distribution in Sprague Dawley rats. *J. Pharm. Sci.,* **2007**, *96*(9), 2494-2506. [http://dx.doi.org/10.1002/jps.20893] [PMID: 17593551]

[102] Hamabe, W.; Maeda, T.; Fukazawa, Y.; Kumamoto, K.; Shang, L.Q.; Yamamoto, A.; Yamamoto, C.; Tokuyama, S.; Kishioka, S. P-glycoprotein ATPase activating effect of opioid analgesics and their P-glycoprotein-dependent antinociception in mice. *Pharmacol. Biochem. Behav.,* **2006**, *85*(3), 629-636. [http://dx.doi.org/10.1016/j.pbb.2006.10.018] [PMID: 17134744]

[103] Cirella, V.N.; Pantuck, C.B.; Lee, Y.J.; Pantuck, E.J. Effects of cyclosporine on anesthetic action. *Anesth. Analg.,* **1987**, *66*(8), 703-706. [http://dx.doi.org/10.1213/00000539-198708000-00001] [PMID: 3605689]

[104] Mayer, U.; Wagenaar, E.; Dorobek, B.; Beijnen, J.H.; Borst, P.; Schinkel, A.H. Full blockade of intestinal P-glycoprotein and extensive inhibition of blood-brain barrier P-glycoprotein by oral treatment of mice with PSC833. *J. Clin. Invest.,* **1997**, *100*(10), 2430-2436. [http://dx.doi.org/10.1172/JCI119784] [PMID: 9366556]

[105] Kharasch, E.D.; Hoffer, C.; Altuntas, T.G.; Whittington, D. Quinidine as a probe for the role of p-glycoprotein in the intestinal absorption and clinical effects of fentanyl. *J. Clin. Pharmacol.,* **2004**, *44*(3), 224-233. [http://dx.doi.org/10.1177/0091270003262075] [PMID: 14973303]

[106] Groenendaal, D.; Freijer, J.; Rosier, A.; de Mik, D.; Nicholls, G.; Hersey, A.; Ayrton, A.D.; Danhof, M.; de Lange, E.C. Pharmacokinetic/pharmacodynamic modelling of the EEG effects of opioids: the role of complex biophase distribution kinetics. *Eur. J.*

Pharm. Sci., **2008**, *34*(2-3), 149-163. [http://dx.doi.org/10.1016/j.ejps.2008.03.003] [PMID: 18467078]

[107] Kim, K.M.; Kim, H.S.; Lim, S.H.; Cheong, S.H.; Choi, E.J.; Kang, H.; Choi, H.R.; Jeon, J.W.; Yon, J.H.; Oh, M.; Shin, J.G. Effects of genetic polymorphisms of OPRM1, ABCB1, CYP3A4/5 on post-operative fentanyl consumption in Korean gynecologic patients. *Int. J. Clin. Pharmacol. Ther.,* **2013**, *51*(5), 383-392. [http://dx.doi.org/10.5414/CP201824] [PMID: 23557865]

[108] Kalvass, J.C.; Olson, E.R.; Pollack, G.M. Pharmacokinetics and pharmacodynamics of alfentanil in P-glycoprotein-competent and P-glycoprotein-deficient mice: P-glycoprotein efflux alters alfentanil brain disposition and antinociception. *Drug Metab. Dispos.,* **2007**, *35*(3), 455-459. [http://dx.doi.org/10.1124/dmd.106.011445] [PMID: 17178769]

[109] Störmer, E.; Perloff, M.D.; von Moltke, L.L.; Greenblatt, D.J. Methadone inhibits rhodamine123 transport in Caco-2 cells. *Drug Metab. Dispos.,* **2001**, *29*(7), 954-956. [PMID: 11408360]

[110] Bouër, R.; Barthe, L.; Philibert, C.; Tournaire, C.; Woodley, J.; Houin, G. The roles of P-glycoprotein and intracellular metabolism in the intestinal absorption of methadone: *in vitro* studies using the rat everted intestinal sac. *Fundam. Clin. Pharmacol.,* **1999**, *13*(4), 494-500. [http://dx.doi.org/10.1111/j.1472-8206.1999.tb00009.x] [PMID: 10456292]

[111] Nanovskaya, T.; Nekhayeva, I.; Karunaratne, N.; Audus, K.; Hankins, G.D.; Ahmed, M.S. Role of P-glycoprotein in transplacental transfer of methadone. *Biochem. Pharmacol.,* **2005**, *69*(12), 1869-1878. [http://dx.doi.org/10.1016/j.bcp.2005.03.030] [PMID: 15876424]

[112] Wang, J.S.; Ruan, Y.; Taylor, R.M.; Donovan, J.L.; Markowitz, J.S.; DeVane, C.L. Brain penetration of methadone (R)- and (S)-enantiomers is greatly increased by P-glycoprotein deficiency in the blood-brain barrier of Abcb1a gene knockout mice. *Psychopharmacology (Berl.),* **2004**, *173*(1-2), 132-138. [http://dx.doi.org/10.1007/s00213-003-1718-1] [PMID: 14712343]

[113] Rodriguez, M.; Ortega, I.; Soengas, I.; Suarez, E.; Lukas, J.C.; Calvo, R. Effect of P-glycoprotein inhibition on methadone analgesia and brain distribution in the rat. *J. Pharm. Pharmacol.,* **2004**, *56*(3), 367-374. [http://dx.doi.org/10.1211/0022357022782] [PMID: 15025862]

[114] Kharasch, E.D.; Hoffer, C.; Whittington, D. The effect of quinidine, used as a probe for the involvement of P-glycoprotein, on the intestinal absorption and pharmacodynamics of methadone. *Br. J. Clin. Pharmacol.,* **2004**, *57*(5), 600-610. [http://dx.doi.org/10.1111/j.1365-2125.2003.02053.x] [PMID: 15089813]

[115] Levran, O.; O'Hara, K.; Peles, E.; Li, D.; Barral, S.; Ray, B.; Borg, L.; Ott, J.; Adelson, M.; Kreek, M.J. ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. *Hum. Mol. Genet.,* **2008**, *17*(14), 2219-2227. [http://dx.doi.org/10.1093/hmg/ddn122] [PMID: 18424454]

[116] Mouly, S.; Bloch, V.; Peoc'h, K.; Houze, P.; Labat, L.; Ksouda, K.; Simoneau, G.; Declèves, X.; Bergmann, J.F.; Scherrmann, J.M.; Laplanche, J.L.; Lepine, J.P.; Vorspan, F. Methadone dose in heroin-dependent patients: role of clinical factors, comedications, genetic polymorphisms and enzyme activity. *Br. J. Clin. Pharmacol.,* **2015**, *79*(6), 967-977. [http://dx.doi.org/10.1111/bcp.12576] [PMID: 25556837]

[117] Meissner, K.; Blood, J.; Francis, A.M.; Yermolenka, V.; Kharasch, E.D. Cyclosporine-inhibitable cerebral drug transport does not influence clinical methadone pharmacodynamics. *Anesthesiology,* **2014**, *121*(6), 1281-1291. [http://dx.doi.org/10.1097/ALN.0000000000000391] [PMID: 25072223]

[118] Meissner, K.; Avram, M.J.; Yermolenka, V.; Francis, A.M.; Blood, J.; Kharasch, E.D. Cyclosporine-inhibitable blood-brain barrier drug transport influences clinical morphine pharmacodynamics. *Anesthesiology,* **2013**, *119*(4), 941-953. [http://dx.doi.org/10.1097/ALN.0b013e3182a05bd3] [PMID: 23851346]

[119] Ball, K.; Bouzom, F.; Scherrmann, J.M.; Walther, B.; Declèves, X. Physiologically based pharmacokinetic modelling of drug penetration across the blood-brain barrier--towards a mechanistic IVIVE-based approach. *AAPS J.,* **2013**, *15*(4), 913-932. [http://dx.doi.org/10.1208/s12248-013-9496-0] [PMID: 23784110]

[120] Mahar D.K.M.; Humphreys, J.E.; Webster, L.O.; Wring, S.A.; Shampine, L.J.; Serabjit-Singh, C.J.; Adkison, K.K.; Polli, J.W. Passive permeability and P-glycoprotein-mediated efflux differentiate central nervous system (CNS) and non-CNS marketed drugs. *J. Pharmacol. Exp. Ther.,* **2002**, *303*(3), 1029-1037. [http://dx.doi.org/10.1124/jpet.102.039255] [PMID: 12438524]

[121] Callaghan, R.; Riordan, J.R. Opiates inhibit ion conductances elicited by cell swelling and cAMP in cultured cells. *Eur. J. Pharmacol.,* **1995**, *291*(2), 183-189. [http://dx.doi.org/10.1016/0922-4106(95)90141-8] [PMID: 8566169]

[122] Kanaan, M.; Daali, Y.; Dayer, P.; Desmeules, J. P-glycoprotein is not involved in the differential oral potency of naloxone and naltrexone. *Fundam. Clin. Pharmacol.,* **2009**, *23*(5), 543-548. [http://dx.doi.org/10.1111/j.1472-8206.2009.00724.x] [PMID: 19656202]

[123] Metcalf, M.D.; Rosicky, A.D.; Hassan, H.E.; Eddington, N.D.; Coop, A.; Cunningham, C.W.; Mercer, S.L. Opioids and efflux transporters. Part 4: influence of N-substitution on P-glycoprotein substrate activity of noroxymorphone analogues. *Bioorg. Med. Chem. Lett.,* **2014**, *24*(15), 3592-3595. [http://dx.doi.org/10.1016/j.bmcl.2014.05.033] [PMID: 24915880]

[124] Sharom, F.J. ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics,* **2008**, *9*(1), 105-127. [http://dx.doi.org/10.2217/14622416.9.1.105] [PMID: 18154452]

[125] Cole, S.P.; Deeley, R.G. Multidrug resistance mediated by the ATP-binding cassette transporter protein MRP. *BioEssays,* **1998**, *20*(11), 931-940. [http://dx.doi.org/10.1002/(SICI)1521-1878(199811)20:11<931::AID-BIES8>3.0.CO;2-J] [PMID: 9872059]

[126] Zelcer, N.; van de Wetering, K.; Hillebrand, M.; Sarton, E.; Kuil, A.; Wielinga, P.R.; Tephly, T.; Dahan, A.; Beijnen, J.H.; Borst, P. Mice lacking multidrug resistance protein 3 show altered morphine pharmacokinetics and morphine-6-glucuronide antinociception. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*(20), 7274-7279. [http://dx.doi.org/10.1073/pnas.0502530102] [PMID: 15886284]

[127] Su, W.; Pasternak, G.W. The role of multidrug resistance-associated protein in the blood-brain barrier and opioid analgesia. *Synapse,* **2013**, *67*(9), 609-619. [http://dx.doi.org/10.1002/syn.21667] [PMID: 23508590]

[128] Aronica, E.; Gorter, J.A.; Jansen, G.H.; van Veelen, C.W.; van Rijen, P.C.; Leenstra, S.; Ramkema, M.; Scheffer, G.L.; Scheper, R.J.; Troost, D. Expression and cellular distribution of multidrug transporter proteins in two major causes of medically intractable epilepsy: focal cortical dysplasia and glioneuronal tumors. *Neuroscience,* **2003**, *118*(2), 417-429. [http://dx.doi.org/10.1016/S0306-4522(02)00992-2] [PMID: 12699778]

[129] Rao, V.V.; Dahlheimer, J.L.; Bardgett, M.E.; Snyder, A.Z.; Finch, R.A.; Sartorelli, A.C.; Piwnica-Worms, D. Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. *Proc. Natl. Acad. Sci. USA,* **1999**, *96*(7), 3900-3905. [http://dx.doi.org/10.1073/pnas.96.7.3900] [PMID: 10097135]

[130] Aronica, E.; Gorter, J.A.; Ramkema, M.; Redeker, S.; Ozbas-Gerçeker, F.; van Vliet, E.A.; Scheffer, G.L.; Scheper, R.J.; van der Valk, P.; Baayen, J.C.; Troost, D. Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. *Epilepsia,* **2004**, *45*(5), 441-451. [http://dx.doi.org/10.1111/j.0013-9580.2004.57703.x] [PMID: 15101825]

[131] Bronger, H.; König, J.; Kopplow, K.; Steiner, H.H.; Ahmadi, R.; Herold-Mende, C.; Keppler, D.; Nies, A.T. ABCC drug efflux pumps and organic anion uptake transporters in human gliomas and the blood-tumor barrier. *Cancer Res.,* **2005**, *65*(24), 11419-11428. [http://dx.doi.org/10.1158/0008-5472.CAN-05-1271] [PMID: 16357150]

[132] Bourasset, F.; Scherrmann, J.M. Carrier-mediated processes at several rat brain interfaces determine the neuropharmacokinetics of morphine and morphine-6-beta-D-glucuronide. *Life Sci.,* **2006**, *78*(20), 2302-2314. [http://dx.doi.org/10.1016/j.lfs.2005.09.043] [PMID: 16309712]

[133] Tunblad, K.; Jonsson, E.N.; Hammarlund-Udenaes, M. Morphine blood-brain barrier transport is influenced by probenecid co-

administration. *Pharm. Res.,* **2003**, *20*(4), 618-623. [http://dx.doi.org/10.1023/A:1023250900462] [PMID: 12739770]

[134] De Gregori, S.; De Gregori, M.; Ranzani, G.N.; Allegri, M.; Minella, C.; Regazzi, M. Morphine metabolism, transport and brain disposition. *Metab. Brain Dis.,* **2012**, *27*(1), 1-5. [http://dx.doi.org/10.1007/s11011-011-9274-6] [PMID: 22193538]

[135] Yoshimura, H.; Ida, S.; Oguri, K.; Tsukamoto, H. Biochemical basis for analgesic activity of morphine-6-glucuronide. I. Penetration of morphine-6-glucuronide in the brain of rats. *Biochem. Pharmacol.,* **1973**, *22*(12), 1423-1430. [http://dx.doi.org/10.1016/0006-2952(73)90320-1] [PMID: 4732523]

[136] Bickel, U.; Schumacher, O.P.; Kang, Y.S.; Voigt, K. Poor permeability of morphine 3-glucuronide and morphine 6-glucuronide through the blood-brain barrier in the rat. *J. Pharmacol. Exp. Ther.,* **1996**, *278*(1), 107-113. [PMID: 8764341]

[137] Stain-Texier, F.; Boschi, G.; Sandouk, P.; Scherrmann, J.M. Elevated concentrations of morphine 6-beta-D-glucuronide in brain extracellular fluid despite low blood-brain barrier permeability. *Br. J. Pharmacol.,* **1999**, *128*(4), 917-924. [http://dx.doi.org/10.1038/sj.bjp.0702873] [PMID: 10556926]

[138] Bouw, M.R.; Xie, R.; Tunblad, K.; Hammarlund-Udenaes, M. Blood-brain barrier transport and brain distribution of morphine-6-glucuronide in relation to the antinociceptive effect in rats--pharmacokinetic/pharmacodynamic modelling. *Br. J. Pharmacol.,* **2001**, *134*(8), 1796-1804. [http://dx.doi.org/10.1038/sj.bjp.0704406] [PMID: 11739257]

[139] Wu, D.; Kang, Y.S.; Bickel, U.; Pardridge, W.M. Blood-brain barrier permeability to morphine-6-glucuronide is markedly reduced compared with morphine. *Drug Metab. Dispos.,* **1997**, *25*(6), 768-771. [PMID: 9193881]

[140] Peckham, E.M.; Traynor, J.R. Comparison of the antinociceptive response to morphine and morphine-like compounds in male and female Sprague-Dawley rats. *J. Pharmacol. Exp. Ther.,* **2006**, *316*(3), 1195-1201. [http://dx.doi.org/10.1124/jpet.105.094276] [PMID: 16291875]

[141] Boström, E.; Simonsson, U.S.; Hammarlund-Udenaes, M. *In vivo* blood-brain barrier transport of oxycodone in the rat: indications for active influx and implications for pharmacokinetics/pharmacodynamics. *Drug Metab. Dispos.,* **2006**, *34*(9), 1624-1631. [http://dx.doi.org/10.1124/dmd.106.009746] [PMID: 16763013]

[142] André, P.; Debray, M.; Scherrmann, J.M.; Cisternino, S. Clonidine transport at the mouse blood-brain barrier by a new H^+ antiporter that interacts with addictive drugs. *J. Cereb. Blood Flow Metab.,* **2009**, *29*(7), 1293-1304. [http://dx.doi.org/10.1038/jcbfm.2009.54] [PMID: 19458607]

[143] Chapy, H.; Goracci, L.; Vayer, P.; Parmentier, Y.; Carrupt, P.A.; Declèves, X.; Scherrmann, J.M.; Cisternino, S.; Cruciani, G. Pharmacophore-based discovery of inhibitors of a novel drug/proton antiporter in human brain endothelial hCMEC/D3 cell line. *Br. J. Pharmacol.,* **2015**, *172*(20), 4888-4904. [http://dx.doi.org/10.1111/bph.13258] [PMID: 26220580]

[144] Gharavi, R.; Hedrich, W.; Wang, H.; Hassan, H.E. Transporter-mediated disposition of opioids: Implications for clinical drug interactions. *Pharm. Res.,* **2015**, *32*(8), 2477-2502. [PMID: 25972096]

[145] Okura, T.; Hattori, A.; Takano, Y.; Sato, T.; Hammarlund-Udenaes, M.; Terasaki, T.; Deguchi, Y. Involvement of the pyrilamine transporter, a putative organic cation transporter, in blood-brain barrier transport of oxycodone. *Drug Metab. Dispos.,* **2008**, *36*(10), 2005-2013. [http://dx.doi.org/10.1124/dmd.108.022087] [PMID: 18606742]

[146] Chapy, H.; André, P.; Declèves, X.; Scherrmann, J.M.; Cisternino, S. A polyspecific drug/proton antiporter mediates diphenhydramine and clonidine transport at the mouse blood-retinal barrier. *Br. J. Pharmacol.,* **2015**, *172*(19), 4714-4725. [http://dx.doi.org/10.1111/bph.13246] [PMID: 26177775]

[147] Baba, M.; Oishi, R.; Saeki, K. Enhancement of blood-brain barrier permeability to sodium fluorescein by stimulation of mu opioid receptors in mice. *Naunyn Schmiedebergs Arch. Pharmacol.,* **1988**, *337*(4), 423-428. [http://dx.doi.org/10.1007/BF00169534] [PMID: 2841613]

[148] Sharma, H.S.; Ali, S.F. Alterations in blood-brain barrier function by morphine and methamphetamine. *Ann. N. Y. Acad. Sci.,* **2006**, *1074*, 198-224. [http://dx.doi.org/10.1196/annals.1369.020] [PMID: 17105918]

[149] Lynch, J.L.; Banks, W.A. Opiate modulation of IL-1alpha, IL-2, and TNF-alpha transport across the blood-brain barrier. *Brain Behav. Immun.,* **2008**, *22*(7), 1096-1102. [http://dx.doi.org/10.1016/j.bbi.2008.04.004] [PMID: 18502094]

[150] Mahajan, S.D.; Aalinkeel, R.; Sykes, D.E.; Reynolds, J.L.; Bindukumar, B.; Fernandez, S.F.; Chawda, R.; Shanahan, T.C.; Schwartz, S.A. Tight junction regulation by morphine and HIV-1 tat modulates blood-brain barrier permeability. *J. Clin. Immunol.,* **2008**, *28*(5), 528-541. [http://dx.doi.org/10.1007/s10875-008-9208-1] [PMID: 18574677]

[151] Yousif, S.; Saubaméa, B.; Cisternino, S.; Marie-Claire, C.; Dauchy, S.; Scherrmann, J.M.; Declèves, X. Effect of chronic exposure to morphine on the rat blood-brain barrier: focus on the P-glycoprotein. *J. Neurochem.,* **2008**, *107*(3), 647-657. [http://dx.doi.org/10.1111/j.1471-4159.2008.05647.x] [PMID: 18761714]

[152] Bauer, B.; Yang, X.; Hartz, A.M.; Olson, E.R.; Zhao, R.; Kalvass, J.C.; Pollack, G.M.; Miller, D.S. *In vivo* activation of human pregnane X receptor tightens the blood-brain barrier to methadone through P-glycoprotein up-regulation. *Mol. Pharmacol.,* **2006**, *70*(4), 1212-1219. [http://dx.doi.org/10.1124/mol.106.023796] [PMID: 16837625]

[153] Chan, G.N.; Saldivia, V.; Yang, Y.; Pang, H.; de Lannoy, I.; Bendayan, R. *In vivo* induction of P-glycoprotein expression at the mouse blood-brain barrier: an intracerebral microdialysis study. *J. Neurochem.,* **2013**, *127*(3), 342-352. [http://dx.doi.org/10.1111/jnc.12344] [PMID: 23777437]

[154] Wang, X.; Sykes, D.B.; Miller, D.S. Constitutive androstane receptor-mediated up-regulation of ATP-driven xenobiotic efflux transporters at the blood-brain barrier. *Mol. Pharmacol.,* **2010**, *78*(3), 376-383. [http://dx.doi.org/10.1124/mol.110.063685] [PMID: 20547735]

[155] Wang, X.; Hawkins, B.T.; Miller, D.S. Aryl hydrocarbon receptor-mediated up-regulation of ATP-driven xenobiotic efflux transporters at the blood-brain barrier. *FASEB J.,* **2011**, *25*(2), 644-652. [http://dx.doi.org/10.1096/fj.10-169227] [PMID: 21048045]

[156] Durk, M.R.; Chan, G.N.; Campos, C.R.; Peart, J.C.; Chow, E.C.; Lee, E.; Cannon, R.E.; Bendayan, R.; Miller, D.S.; Pang, K.S. 1α,25-Dihydroxyvitamin D3-liganded vitamin D receptor increases expression and transport activity of P-glycoprotein in isolated rat brain capillaries and human and rat brain microvessel endothelial cells. *J. Neurochem.,* **2012**, *123*(6), 944-953. [http://dx.doi.org/10.1111/jnc.12041] [PMID: 23035695]

[157] Narang, V.S.; Fraga, C.; Kumar, N.; Shen, J.; Throm, S.; Stewart, C.F.; Waters, C.M. Dexamethasone increases expression and activity of multidrug resistance transporters at the rat blood-brain barrier. *Am. J. Physiol. Cell Physiol.,* **2008**, *295*(2), C440-C450. [http://dx.doi.org/10.1152/ajpcell.00491.2007] [PMID: 18524938]

[158] Qosa, H.; Miller, D.S.; Pasinelli, P.; Trotti, D. Regulation of ABC efflux transporters at blood-brain barrier in health and neurological disorders. *Brain Res.,* **2015**, *1628*(Pt B), 298-316. [http://dx.doi.org/10.1016/j.brainres.2015.07.005] [PMID: 26187753]

[159] Aquilante, C.L.; Letrent, S.P.; Pollack, G.M.; Brouwer, K.L. Increased brain P-glycoprotein in morphine tolerant rats. *Life Sci.,* **2000**, *66*(4), PL47-PL51. [PMID: 10665989]

[160] Zong, J.; Pollack, G.M. Modulation of P-glycoprotein transport activity in the mouse blood-brain barrier by rifampin. *J. Pharmacol. Exp. Ther.,* **2003**, *306*(2), 556-562. [http://dx.doi.org/10.1124/jpet.103.049452] [PMID: 12721332]

[161] Pajic, M.; Bebawy, M.; Hoskins, J.M.; Roufogalis, B.D.; Rivory, L.P. Effect of short-term morphine exposure on P-glycoprotein expression and activity in cancer cell lines. *Oncol. Rep.,* **2004**, *11*(5), 1091-1095. [PMID: 15069552]

[162] Yousif, S.; Chaves, C.; Potin, S.; Margaill, I.; Scherrmann, J.M.; Declèves, X. Induction of P-glycoprotein and Bcrp at the rat blood-brain barrier following a subchronic morphine treatment is mediated through NMDA/COX-2 activation. *J. Neurochem.,* **2012**, *123*(4), 491-503. [http://dx.doi.org/10.1111/j.1471-4159.2012.07890.x] [PMID: 22845665]

[163] Hassan, H.E.; Myers, A.L.; Lee, I.J.; Chen, H.; Coop, A.; Eddington, N.D. Regulation of gene expression in brain tissues of rats re-

peatedly treated by the highly abused opioid agonist, oxycodone: microarray profiling and gene mapping analysis. *Drug Metab. Dispos.,* **2010**, *38*(1), 157-167. [http://dx.doi.org/10.1124/dmd.109.029199] [PMID: 19786507]

[164] Li, Y.; Yue, H.; Xing, Y.; Sun, H.; Pan, Z.; Xie, G. Oxymatrine inhibits development of morphine-induced tolerance associated with decreased expression of P-glycoprotein in rats. *Integr. Cancer Ther.,* **2010**, *9*(2), 213-218. [http://dx.doi.org/10.1177/1534735410369671] [PMID: 20587445]

[165] Hung, C.C.; Chiou, M.H.; Teng, Y.N.; Hsieh, Y.W.; Huang, C.L.; Lane, H.Y. Functional impact of ABCB1 variants on interactions between P-glycoprotein and methadone. *PLoS One,* **2013**, *8*(3), e59419. [http://dx.doi.org/10.1371/journal.pone.0059419] [PMID: 23527191]

[166] Miller, D.S. Regulation of P-glycoprotein and other ABC drug transporters at the blood-brain barrier. *Trends Pharmacol. Sci.,* **2010**, *31*(6), 246-254. [http://dx.doi.org/10.1016/j.tips.2010.03.003] [PMID: 20417575]

[167] Miller, D.S. ABC transporter regulation by signaling at the blood-brain barrier: relevance to pharmacology. *Adv. Pharmacol.,* **2014**, *71*, 1-24. [http://dx.doi.org/10.1016/bs.apha.2014.06.008] [PMID: 25307212]

[168] Desjardins, S.; Belkai, E.; Crete, D.; Cordonnier, L.; Scherrmann, J.M.; Noble, F.; Marie-Claire, C. Effects of chronic morphine and morphine withdrawal on gene expression in rat peripheral blood mononuclear cells. *Neuropharmacology,* **2008**, *55*(8), 1347-1354. [http://dx.doi.org/10.1016/j.neuropharm.2008.08.027] [PMID: 18801381]

[169] Sharma, H.S.; Lundstedt, T.; Boman, A.; Lek, P.; Seifert, E.; Wiklund, L.; Ali, S.F. A potent serotonin-modulating compound AP-267 attenuates morphine withdrawal-induced blood-brain barrier dysfunction in rats. *Ann. N. Y. Acad. Sci.,* **2006**, *1074*, 482-496. [http://dx.doi.org/10.1196/annals.1369.049] [PMID: 17105947]

[170] Zhang, T.; Feng, Y.; Rockhold, R.W.; Ho, I.K. Naloxone-precipitated morphine withdrawal increases pontine glutamate levels in the rat. *Life Sci.,* **1994**, *55*(2), PL25-PL31. [http://dx.doi.org/10.1016/0024-3205(94)90108-2] [PMID: 7912397]

[171] Guo, M.; Xu, N.J.; Li, Y.T.; Yang, J.Y.; Wu, C.F.; Pei, G. Morphine modulates glutamate release in the hippocampal CA1 area in mice. *Neurosci. Lett.,* **2005**, *381*(1-2), 12-15. [http://dx.doi.org/10.1016/j.neulet.2005.01.071] [PMID: 15882781]

[172] Sepúlveda, J.; Oliva, P.; Contreras, E. Neurochemical changes of the extracellular concentrations of glutamate and aspartate in the nucleus accumbens of rats after chronic administration of morphine. *Eur. J. Pharmacol.,* **2004**, *483*(2-3), 249-258. [http://dx.doi.org/10.1016/j.ejphar.2003.10.037] [PMID: 14729114]

[173] Zhu, H.J.; Liu, G.Q. Glutamate up-regulates P-glycoprotein expression in rat brain microvessel endothelial cells by an NMDA receptor-mediated mechanism. *Life Sci.,* **2004**, *75*(11), 1313-1322. [http://dx.doi.org/10.1016/j.lfs.2004.02.027] [PMID: 15234189]

[174] Bauer, B.; Hartz, A.M.; Pekcec, A.; Toellner, K.; Miller, D.S.; Potschka, H. Seizure-induced up-regulation of P-glycoprotein at the blood-brain barrier through glutamate and cyclooxygenase-2 signaling. *Mol. Pharmacol.,* **2008**, *73*(5), 1444-1453. [http://dx.doi.org/10.1124/mol.107.041210] [PMID: 18094072]

[175] Zibell, G.; Unkrüer, B.; Pekcec, A.; Hartz, A.M.; Bauer, B.; Miller, D.S.; Potschka, H. Prevention of seizure-induced up-regulation of endothelial P-glycoprotein by COX-2 inhibition. *Neuropharmacology,* **2009**, *56*(5), 849-855. [http://dx.doi.org/10.1016/j.neuropharm.2009.01.009] [PMID: 19371577]

[176] Yao, D.; Liu, L.; Jin, S.; Li, J.; Liu, X.D. Overexpression of multidrug resistance-associated protein 2 in the brain of pentylenetetrazole-kindled rats. *Neuroscience,* **2012**, *227*, 283-292. [http://dx.doi.org/10.1016/j.neuroscience.2012.10.006] [PMID: 23059797]

[177] Hao, Y.; Wu, X.; Xu, L.; Guan, Y.; Hong, Z. MK-801 prevents overexpression of multidrug resistance protein 2 after status epilepticus. *Neurol. Res.,* **2012**, *34*(5), 430-438. [http://dx.doi.org/10.1179/1743132811Y.0000000055] [PMID: 22643638]

[178] Luna-Munguia, H.; Salvamoser, J.D.; Pascher, B.; Pieper, T.; Getzinger, T.; Kudernatsch, M.; Kluger, G.; Potschka, H. Glutamate-mediated upregulation of the multidrug resistance protein 2 in porcine and human brain capillaries. *J. Pharmacol. Exp. Ther.,* **2015**, *352*(2), 368-378. [http://dx.doi.org/10.1124/jpet.114.218180] [PMID: 25503388]

[179] Chaves, C.; Gomez-Zepeda, D.; Auvity, S.; Menet, M.C.; Crete, D.; Labat, L.; Remiao, F.; Cisternino, S.; Decleves, X. Effect of subchronic intravenous morphine infusion and naloxone-precipitated morphine withdrawal on P-gp and Bcrp at the rat blood-brain barrier. *J. Pharm. Sci.,* **2015**. [PMID: 26554626]

[180] Goralski, K.B.; Hartmann, G.; Piquette-Miller, M.; Renton, K.W. Downregulation of mdr1a expression in the brain and liver during CNS inflammation alters the *in vivo* disposition of digoxin. *Br. J. Pharmacol.,* **2003**, *139*(1), 35-48. [http://dx.doi.org/10.1038/sj.bjp.0705227] [PMID: 12746221]

[181] Salkeni, M.A.; Lynch, J.L.; Otamis-Price, T.; Banks, W.A. Lipopolysaccharide impairs blood-brain barrier P-glycoprotein function in mice through prostaglandin- and nitric oxide-independent pathways. *J. Neuroimmune Pharmacol.,* **2009**, *4*(2), 276-82.

[182] Hartz, A.M.; Bauer, B.; Block, M.L.; Hong, J.S.; Miller, D.S. Diesel exhaust particles induce oxidative stress, proinflammatory signaling, and P-glycoprotein up-regulation at the blood-brain barrier. *FASEB J.,* **2008**, *22*(8), 2723-2733. [http://dx.doi.org/10.1096/fj.08-106997] [PMID: 18474546]

[183] Poller, B.; Drewe, J.; Krähenbühl, S.; Huwyler, J.; Gutmann, H. Regulation of BCRP (ABCG2) and P-glycoprotein (ABCB1) by cytokines in a model of the human blood-brain barrier. *Cell. Mol. Neurobiol.,* **2010**, *30*(1), 63-70. [http://dx.doi.org/10.1007/s10571-009-9431-1] [PMID: 19629677]

[184] Bauer, B.; Hartz, A.M.; Miller, D.S. Tumor necrosis factor alpha and endothelin-1 increase P-glycoprotein expression and transport activity at the blood-brain barrier. *Mol. Pharmacol.,* **2007**, *71*(3), 667-675. [http://dx.doi.org/10.1124/mol.106.029512] [PMID: 17132686]

[185] Rigor, R.R.; Hawkins, B.T.; Miller, D.S. Activation of PKC isoform beta(I) at the blood-brain barrier rapidly decreases P-glycoprotein activity and enhances drug delivery to the brain. *J. Cereb. Blood Flow Metab.,* **2010**, *30*(7), 1373-1383. [http://dx.doi.org/10.1038/jcbfm.2010.21] [PMID: 20197783]

[186] Hartz, A.M.; Bauer, B.; Fricker, G.; Miller, D.S. Rapid regulation of P-glycoprotein at the blood-brain barrier by endothelin-1. *Mol. Pharmacol.,* **2004**, *66*(3), 387-394. [http://dx.doi.org/10.1124/mol.104.001503] [PMID: 15322229]

[187] Hartz, A.M.; Bauer, B.; Fricker, G.; Miller, D.S. Rapid modulation of P-glycoprotein-mediated transport at the blood-brain barrier by tumor necrosis factor-alpha and lipopolysaccharide. *Mol. Pharmacol.,* **2006**, *69*(2), 462-470. [http://dx.doi.org/10.1124/mol.105.017954] [PMID: 16278373]

[188] Chen, Y.; Huang, X.J.; Yu, N.; Xie, Y.; Zhang, K.; Wen, F.; Liu, H.; Di, Q. HMGB1 contributes to the expression of P-glycoprotein in mouse epileptic brain through toll-like receptor 4 and receptor for advanced glycation end products. *PLoS One,* **2015**, *10*(10), e0140918. [http://dx.doi.org/10.1371/journal.pone.0140918] [PMID: 26485677]

[189] Heemskerk, S.; Peters, J.G.; Louisse, J.; Sagar, S.; Russel, F.G.; Masereeuw, R. Regulation of P-glycoprotein in renal proximal tubule epithelial cells by LPS and TNF-alpha. *J. Biomed. Biotechnol.,* **2010**, *2010*, 525180. [http://dx.doi.org/10.1155/2010/525180] [PMID: 20300455]

[190] Grybauskas, A.; Koga, T.; Kuprys, P.V.; Nolan, M.; McCarty, R.; Walker, L.; Green, K.A.; Norkett, W.M.; Yue, B.Y.; Knepper, P.A. ABCB1 transporter and Toll-like receptor 4 in trabecular meshwork cells. *Mol. Vis.,* **2015**, *21*, 201-212. [PMID: 25802484]

[191] Xu, F.; Wang, F.; Yang, T.; Sheng, Y.; Zhong, T.; Chen, Y. Differential drug resistance acquisition to doxorubicin and paclitaxel in breast cancer cells. *Cancer Cell Int.,* **2014**, *14*(1), 142. [http://dx.doi.org/10.1186/s12935-014-0142-4] [PMID: 25550688]

[192] Mishra, J.; Zhang, Q.; Rosson, J.L.; Moran, J.; Dopp, J.M.; Neudeck, B.L. Lipopolysaccharide increases cell surface P-glycoprotein that exhibits diminished activity in intestinal epithelial cells. *Drug Metab. Dispos.,* **2008**, *36*(10), 2145-2149. [http://dx.doi.org/10.1124/dmd.108.022632] [PMID: 18687802]

[193] Hutchinson, M.R.; Zhang, Y.; Brown, K.; Coats, B.D.; Shridhar, M.; Sholar, P.W.; Patel, S.J.; Crysdale, N.Y.; Harrison, J.A.; Maier, S.F.; Rice, K.C.; Watkins, L.R. Non-stereoselective reversal of neuropathic pain by naloxone and naltrexone: involvement of toll-like receptor 4 (TLR4). *Eur. J. Neurosci.,* **2008**, *28*(1), 20-29. [http://dx.doi.org/10.1111/j.1460-9568.2008.06321.x] [PMID: 18662331]

[194] Bankstahl, J.P.; Hoffmann, K.; Bethmann, K.; Löscher, W. Glutamate is critically involved in seizure-induced overexpression of P-glycoprotein in the brain. *Neuropharmacology,* **2008**, *54*(6), 1006-1016. [http://dx.doi.org/10.1016/j.neuropharm.2008.02.008] [PMID: 18394657]

[195] Pekcec, A.; Unkrüer, B.; Schlichtiger, J.; Soerensen, J.; Hartz, A.M.; Bauer, B.; van Vliet, E.A.; Gorter, J.A.; Potschka, H. Targeting prostaglandin E2 EP1 receptors prevents seizure-associated P-glycoprotein up-regulation. *J. Pharmacol. Exp. Ther.,* **2009**, *330*(3), 939-947. [http://dx.doi.org/10.1124/jpet.109.152520] [PMID: 19494186]

[196] Slanar, O.; Nobilis, M.; Kvétina, J.; Matousková, O.; Idle, J.R.; Perlík, F. Pharmacokinetics of tramadol is affected by MDR1 polymorphism C3435T. *Eur. J. Clin. Pharmacol.,* **2007**, *63*(4), 419-421. [http://dx.doi.org/10.1007/s00228-006-0255-3] [PMID: 17265061]

[197] Moriki, Y.; Suzuki, T.; Fukami, T.; Hanano, M.; Tomono, K.; Watanabe, J. Involvement of P-glycoprotein in blood-brain barrier transport of pentazocine in rats using brain uptake index method. *Biol. Pharm. Bull.,* **2004**, *27*(6), 932-935. [http://dx.doi.org/10.1248/bpb.27.932] [PMID: 15187451]

[198] Wen, J.; Zhang, T.; Shan, Z.M.; Qi, M.Y.; Xiu, H.H.; Liu, L.; Wu, S.Z.; Jia, Z.; Xu, K.Q. Butorphanol, a synthetic opioid, sensitizes ABCB1-mediated multidrug resistance *via* inhibition of the efflux function of ABCB1 in leukemia cells. *Oncol. Rep.,* **2015**, *34*(2), 755-762. [http://dx.doi.org/10.3892/or.2015.4052] [PMID: 26062728]