Interaction of Morphine, Fentanyl, Sufentanil, Alfentanil, and Loperamide with the Efflux Drug Transporter P-glycoprotein

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Background: The efflux transporter P-glycoprotein, a member of the adenosine triphosphate–binding cassette superfamily, is a major determinant of the pharmacokinetics and pharmacodynamics of the opioid loperamide, a well-recognized antidiarrheal agent. Animal studies indicate that P-glycoprotein limits morphine entry into the brain. In this study, the authors examined whether other opioids of importance to anesthesiologists such as fentanyl, sufentanil, and alfentanil, and also morphine-6-glucuronide and morphine-3-glucuronide, are P-glycoprotein substrates and whether, in turn, these opioids act also as P-glycoprotein inhibitors.

Methods: The transcellular movement of the various opioids, including loperamide and morphine, was assessed in L-MDR1 (expressing P-glycoprotein) and LLC-PK1 cell monolayers (P-glycoprotein expression absent). A preferential basal-to-apical versus apical-to-basal transport in the L-MDR cells but not the LLC-PK1 cells is seen for P-glycoprotein substrates. In addition, the effect of the various opioids on the transcellular movement of the prototypical P-glycoprotein substrate digoxin was examined in Caco-2 cell monolayers. IC50 values were calculated according to the Hill equation.

Results: Loperamide was a substrate showing high dependence on P-glycoprotein in that basal-apical transport was nearly 10-fold greater than in the apical-basal direction in L-MDR1 cells. Morphine also showed a basal-to-apical gradient in the L-MDR1 cell monolayer, indicating that it too is a P-glycoprotein substrate, but with less dependence than loperamide in that only 1.5-fold greater basal-apical directional transport was observed. Fentanyl, sufentanil, and alfentanil did not behave as P-glycoprotein substrates, whereas the morphine glucuronides did not cross the cell monolayers at all, whether P-glycoprotein was present or not. Loperamide, sufentanil, fentanyl, and alfentanil inhibited P-glycoprotein-mediated digoxin transport in Caco-2 cells with IC50 values of 2.5, 4.5, 6.5, and 112 µM, respectively. Morphine and its glucuronides (20 µM) did not inhibit digoxin (5 µM) transport in Caco-2 cells, and therefore IC50 values were not determined.

Conclusions: Opioids have a wide spectrum of P-glycoprotein activity, acting as both substrates and inhibitors, which might contribute to their varying central nervous system–related effects.

THE ability of drugs to cross the blood–brain barrier has long been recognized to be predicated by molecular weight, degree of ionization, protein binding, and lipid solubility. However, more recently, investigators have demonstrated that the membrane-bound drug transporter P-glycoprotein is capable of actively pumping a variety of drugs out of the central nervous system (CNS) and is an important component of the blood–brain barrier.1,2 P-glycoprotein is the protein encoded by the multidrug resistance gene (MDR1), so named because of its role in determining resistance to cancer chemotherapeutic agents.3,4 Chronic anticancer therapy often induces P-glycoprotein expression in cancer cells and increases cellular efflux of such agents, resulting in decreased intracellular concentrations, reduced therapeutic effect, and resistance to anticancer treatment, i.e., multidrug resistance. Blockade of the pump by inhibition with specific inhibitors (e.g., PSC-833) and widely used drugs, including cyclosporin, quinidine, and verapamil, has been used as a strategy to reverse multidrug resistance.5–7 Blockade of P-glycoprotein allows enhanced CNS entry of some drugs,8–12 offering new possibilities to explain CNS-related adverse effects during the administration of drugs that are substrates of P-glycoprotein and, furthermore, to manipulate the CNS entry of drugs whose target is located in the brain, an area of great interest to anesthesiologists.13

P-glycoprotein is a member of the adenosine triphosphate–binding cassette superfamily of cellular efflux drug transporters, which is expressed not only in the capillary endothelium of the blood–brain barrier, but also in many other cell membranes, such as intestinal enterocytes and biliary and renal epithelial cells. It is of interest that as long ago as 1987, the P-glycoprotein substrate and inhibitor cyclosporin was shown to increase fentanyl-induced analgesia in mice.14 More recently, morphine has been shown to increase analgesia in P-glycoprotein knockout mice compared with wild-type mice.15 Thus, P-glycoprotein may limit morphine entry into the brain. As new drugs are introduced into clinical practice, it will be important to assess whether they are P-glycoprotein substrates or inhibitors to assess their potential for drug interaction. Interindividual variability in P-glycoprotein activity is now recognized, which may at least partially depend on genetic polymorphism. Homozygosity for an allele associated with deficient P-glycoprotein activity occurs in 2% of whites.16

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Loperamide is a widely used antidiarrheal agent that, although a potent opioid in vitro, produces only gastrointestinal opioid effects and lacks CNS effects. This apparent tissue selectivity is probably a result of loperamide being a P-glycoprotein substrate, so that P-glycoprotein in the CNS effectively prevents access of loperamide to the CNS. In support of this hypothesis is the finding that in mice with the MDR1 gene disruption, brain loperamide concentrations were eightfold higher than those observed in normal mice, and lethal opioid effects were produced. In humans, the coadministration of loperamide with the P-glycoprotein inhibitor quinidine results in pharmacodynamic respiratory changes, indicating increased drug delivery to the brain by P-glycoprotein inhibition, whereas inhibition of P-glycoprotein in mice results in loperamide producing analgesic effects.

Because penetration of the blood–brain barrier is pivotal for CNS opioid effects, we examined whether the potent anesthetic opioids fentanyl, alfentanil, sufentanil, and, for comparison, morphine and its metabolites morphine-6-glucuronide (M6G) and morphine-3 glucuronide (M3G) are indeed substrates or inhibitors for P-glycoprotein in vitro, by determining the drug’s transport in cell lines lacking and expressing P-glycoprotein.

Methods

Materials
Loperamide, morphine, M6G, M3G, digoxin (a P-glycoprotein substrate), and bovine serum albumin were purchased from Sigma Chemical (St. Louis, MO). Fentanyl, sufentanil, and alfentanil were purchased from US Pharmacopeial Convention Inc. (Rockville, MD). Tritiated morphine and tritiated digoxin were purchased from NEN (Boston, MA), whereas tritiated loperamide, fentanyl, alfentanil, and sufentanil were a gift from Janssen Pharmaceuticals (Beerse, Belgium). All tissue culture media and reagents (Gibco-BRL, Gaithersburg, MD), tissue culture plastics, and tissue culture inserts (Nunc) were supplied by Life Technologies Ltd. (Paisley, Scotland).

Cultured LLC-PK1, L-MDR1, and Caco-2 Cells
LLC-PK1 is a porcine kidney–derived cell line lacking P-glycoprotein expression, whereas L-MDR1 cells are LLC-PK1 cells transfected with the human MDR1 gene, expressing human P-glycoprotein. Caco-2 cells are a human colon carcinoma cell line that constitutively expresses P-glycoprotein. L-MDR1 and LLC-PK1 cells were provided by Drs. A. H. Schinkel (Division of Experimental Therapy, Netherlands Cancer Institute, Amsterdam, the Netherlands) and E. G. Schuetz (Department of Pharmaceutical Sciences, St. Jude’s Children’s Research Hospital, Memphis, TN), and Caco-2 cells were from Dr. R. J. Coffey (Division of Gastroenterology, Vanderbilt University, Nashville, TN). Cells were plated on Transwell™ filters (Costar Corporation, Cambridge, MA) and grown under identical conditions as described previously. Approximately 1–2 h before the start of the transport experiments, the medium in each compartment was replaced with a serum free medium (Optimem, Gibco BRL). Then, the medium in each compartment was replaced with 700 μl serum free medium (Optimem), with or without drug (radiolabeled or unlabeled). The amount of the drug appearing in the opposite compartment after 1, 2, 3, and 4 h was measured in 25-μl aliquots taken from each compartment. Figure 1A shows a schematic of these cells when grown using a Transwell™ format in which expression of P-glycoprotein is localized to the apical cell membrane domain. Thus, the transcellular movement of P-glycoprotein drug substrates are enhanced in the basal-to-apical versus apical-to-basal direction, as compared with LLC-PK1 cells, which lack P-glycoprotein (fig. 1B). Therefore, the difference in the
basal-to-apical net transport of a compound between L-MDR1 and LLC-PK I cells is a measure of the contribution of P-glycoprotein activity to transcellular movement. The larger this difference is, the more important P-glycoprotein activity is to transcellular movement. It should be noted that the term “P-glycoprotein affinity” is used here not to describe transport kinetics, but to convey extent of observed transport.

LLC-PK1 and L-MDR1 cells were cultured in M199 medium (Gibco BRL) supplemented with 50 μg/ml streptomycin and 10% (vol/vol) fetal bovine serum (Gibco BRL) at 37°C in the presence of 5% CO2; cells were subcultured after trypsinization every 6–7 days. Caco-2 cells were grown with Dulbecco modified eagle’s medium (high glucose) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U penicillin/ml, 100 μg/ml streptomycin, and 1% nonessential amino acids (Gibco BRL), and incubated at 37°C in 5% CO2. LLC-PK1 and L-MDR1 cells were plated at a density of 4 × 105 cells/12-mm well on porous (3.0 μm) polycarbonate membrane filters (Transwell™), whereas Caco-2 cells were plated at a density of 1 × 105 cells/12-mm well on 0.4-μm polycarbonate membrane filters (Transwell™). Cells were supplemented with fresh media every 2 days and used in the transport studies on the fourth day after plating. Transepithelial resistance was measured in each well using a Millicell ERS ohmmeter (Millipore, Bedford, MA); wells registering a resistance of 200 Ω or greater, after correcting for the resistance obtained in control blank wells, were used in the transport experiments. When using inulin as a marker of paracellular leak in the same cell lines, we did not see any significant inulin leak at the resistances observed.

Approximately 1–2 h before the start of the transport experiments, the medium in each compartment was replaced with a serum free medium (OptiMEM). Then, the medium in each compartment was replaced with 700 μl serum free medium (OptiMEM) with or without 5 μM drug (radiolabeled or unlabeled). The amount of the drug appearing in the opposite compartment after 1, 2, 3, and 4 h was measured in 25-μl aliquots taken from each compartment and expressed as percentage of amount originally added to the apical and basal site, respectively.

As previously described, inhibition of P-glycoprotein–mediated transport in Caco-2 cells was determined in a similar manner after the addition of the putative inhibitor to both the apical and basal compartments and using radiolabeled digoxin (5 μM) as the P-glycoprotein substrate. Complete inhibition of P-glycoprotein–mediated transport would be expected to result in the loss of digoxin’s basal-to-apical (B→A) versus apical-to-basal (A→B) transport difference. Accordingly, percentage inhibition was estimated by the following equation:

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\text{Degree of inhibition (\%) = } (1 - \frac{(i_{B-A} - i_{A-B})}{(a_{B-A} - a_{A-B})}) \times 100, \quad (1)
\]

where i and a are the percentages of digoxin transport in the presence and absence of the putative inhibitor, according to the direction of transport. Values estimated at each time point were averaged because digoxin transport appeared to be linear with respect to time. These studies were repeated at multiple inhibition concentrations. The calculated percentages of inhibition were fitted using the Hill equation, and the IC50 values were determined. To minimize the effects of interday variability in the transport capacity, control digoxin transport in the absence of any inhibitor (two wells per plate) were included on every plate (12 wells). Data shown represent results obtained from studies conducted on at least three preparations on different days.

In the experiments, radioactivity was counted using a liquid scintillation counter (Model 1219, Rackbeta™; LKB Instruments, Inc., Gaithersburg, MD). M3G and M6G were measured by high-performance liquid chromatography as described previously.

**Results**

**Identification as P-glycoprotein Substrates**

Transport characteristics of loperamide, morphine, fentanyl, sufentanil, and alfentanil in L-MDR1 cells versus LLC-PK1 cells are illustrated in figure 2. The preferential basal-to-apical transport of loperamide in the L-MDR1 (a cell line that overexpresses P-glycoprotein) cells, as compared with the parallel LLC-PK1 cells lacking P-glycoprotein, demonstrated loperamide to be a highly dependent P-glycoprotein substrate. The basal-to-apical versus apical-to-basal transport in the L-MDR1 cell line was much less for morphine and absent for fentanyl, sufentanil, and alfentanil, indicating that, although morphine is a relatively poor P-glycoprotein substrate if compared with loperamide, the other opioids do not appear to be P-glycoprotein substrates in vitro. For the morphine metabolites M3G and M6G, the amount of transepithelial movement in either direction was less than 1% of the added concentration after 4 h of incubation. This finding indicates that both glucuronides did not permeate the cell membrane to any extent.

**Identification as P-glycoprotein Inhibitors**

Loperamide, fentanyl, and sufentanil showed inhibition of P-glycoprotein activity (> 50%) at a concentration of 20 μM (fig. 3). The IC50 value was 2.5 μM for loperamide, 4.5 μM for sufentanil, 6.5 μM for fentanyl, and 112 μM for alfentanil (fig. 3). Morphine and its glucuronides (M3G and M6G) did not inhibit P-glycoprotein at a concentration of 20 μM (figs. 2 and 3). Therefore, the determination of their IC50 values was not
pursued. Figure 4 shows the inhibitory effect of loperamide, sufentanil, fentanyl, and alfentanil on P-glycoprotein–mediated digoxin transport.

Discussion

The membrane-bound transport protein, P-glycoprotein, in the blood–brain barrier has been shown to affect brain uptake or penetration of a number of drugs in a mouse model, including ondansetron and loperamide.10 Studies in the MDR knockout mouse, in which P-glycoprotein is not expressed, have shown that loperamide administration produces profound, even lethal, CNS effects in contrast to the lack of CNS effects in the wild-type mouse.10 Thus, we were interested as to whether other opioids used in anesthetic practice are also substrates or inhibitors of the P-glycoprotein transporter. The characterization of drugs as P-glycoprotein substrates or P-glycoprotein inhibitors, respectively, has gained significant interest because P-glycoprotein activity may affect the kinetics and subsequently the dynamics of its substrates. Importantly, P-glycoprotein activity may differ widely among individuals. Recently, a functionally relevant polymorphism of P-glycoprotein was described, emphasizing the clinical relevance of P-glycoprotein activity. Subjects homozygous for the C3435T mutation had higher digoxin plasma concentrations,16 indicating improved digoxin absorption as a result of reduced P-glycoprotein function in the intestinal enterocytes. Second, there is serious potential for drug interactions with P-glycoprotein that will assume considerable clinical relevance as specific and potent P-glycoprotein inhibitors are used for anticancer drug therapy. Such drugs are currently in phase III development. Many patients scheduled for anesthesia and surgery are likely to be taking these new potent P-glycoprotein inhibitors in the future.

In the current study, we examined loperamide, morphine and its glucuronides, as well as fentanyl, sufentanil, and alfentanil as both potential P-glycoprotein substrates and inhibitors. The use of the same in vitro system allowed the comparison of P-glycoprotein dependence between opioids. Although loperamide was found to be a high dependent P-glycoprotein substrate, in

Fig. 2. Transepithelial transport across L-MDR1 (top) and LLC-PK1 (bottom) cell culture monolayers: (A and B) [3H]-loperamide (5 μM), (C and D) [3H]-morphine (5 μM), (E and F) [3H]-sufentanil (5 μM), (G and H) [3H]-fentanyl (5 μM), and (I and J) [3H]-alfentanil (5 μM). Translocation from basal to apical compartments is indicated by triangles and solid line; translocation from apical to basal compartments is indicated by squares and dotted line. Data are mean ± SD from three or more experiments.
keeping with previous work, this was not the case for all opioids. P-glycoprotein expression did not influence the transcellular movement of fentanyl, sufentanil, or alfentanil and had only a relatively small effect on morphine. Thus, morphine is indeed a P-glycoprotein substrate but with less dependence than loperamide, a well-recognized substrate for P-glycoprotein. Thus, the relative dependence of opioids for P-glycoprotein is an important determinant of opioid action. At one extreme is loperamide, which is used for the treatment of diarrhea because its effects are normally localized to the gut with no CNS effects, yet it is a potent opioid. The explanation for this apparent contradiction is that loperamide is a high-affinity P-glycoprotein substrate that is so effectively pumped out of the CNS by P-glycoprotein that pharmacologically effective concentrations are not achieved in the brain. However, when P-glycoprotein is lacking, as in the P-glycoprotein knockout mouse, or when P-glycoprotein is inhibited in humans, loperamide has CNS effects. Morphine, an opioid drug with CNS effects, has been shown to produce greater analgesia in the P-glycoprotein knockout mice than in wild-type mice, suggesting that P-glycoprotein also limits morphine-induced analgesia in vivo. Because morphine has

Fig. 3. Transepithelial transport of [3H]-digoxin (5 μM) across a Caco-2 cell culture monolayer in the absence (control, A) or presence of various opioids (20 μM, B–H). Translocation from basal to apical compartments is indicated by triangles and solid line; translocation from apical to basal compartments is indicated by the squares and dotted line. Data are mean ± SD from three or more experiments.
CNS effects in humans and loperamide do not; it is unlikely that morphine disposition is critically dependent on P-glycoprotein. It is of interest that M6G-induced analgesia was not greater in the mice lacking P-glycoprotein, in keeping with our results. Thus, individual variability in P-glycoprotein function (whether genetic or caused by drug interaction) could potentially play a role in the extent of opioid analgesia achieved in humans.

For anesthetic opioids, uptake transporters may also be important in determining drug entry into the brain, and it is of interest to note that Henthorn et al. demonstrated that, although fentanyl is a substrate of P-glycoprotein in primary cultured bovine brain microvascular endothelial cell monolayers, there may be an as-yet-unidentified active fentanyl uptake transporter that promotes the inward transport of fentanyl. Henthorn et al. tested whether fentanyl might be a substrate for P-glycoprotein; release of tritiated fentanyl or rhodamine 123 (a recognized substrate of P-glycoprotein) previously loaded in the brain endothelial cell monolayers was studied in the presence or absence of either fentanyl or verapamil. Verapamil is a known nonspecific weak competitive inhibitor of P-glycoprotein. Both fentanyl and verapamil decreased release of rhodamine 123 from the endothelial cell monolayers. The investigators concluded, in contrast to our results, that fentanyl is a substrate for P-glycoprotein in their system. However, verapamil is neither a potent nor specific P-glycoprotein inhibitor. Release of $^3$H-fentanyl was significantly increased when incubated with either unlabeled fentanyl or verapamil. The results of Henthorn et al. suggest that any active P-glycoprotein efflux of fentanyl in these cells is overshadowed by an active inward transport process, and the investigators postulated that this active inward transport is mediated by an as-yet-unidentified transporter. The additional involvement of the cellular uptake transporter complicated the unequivocal assessment of fentanyl as a P-glycoprotein substrate in this system. However, it is of interest that the study by Henthorn et al. also showed that fentanyl may act as a P-glycoprotein inhibitor, confirming our findings. Thompson et al. using standard hot-plate methodology as an assessment of opioid-induced analgesia, demonstrated that morphine-induced analgesia in mice lacking P-glycoprotein is both increased and prolonged compared with in mice that possess normal P-glycoprotein function. Our group also recently showed that loperamide shows marked analgesic effects in mice either lacking P-glycoprotein or when P-glycoprotein is inhibited. Detailed pharmacokinetic studies were not performed in the study by Thompson et al. for fentanyl. Cyclosporin, a nonspecific P-glycoprotein inhibitor, markedly increased analgesia in wild-type mice but had no effect in knockout mice. These studies in mice in vivo would also suggest that morphine is a P-glycoprotein substrate, in keeping with our results. A potent, more specific P-glycoprotein inhibitor, GF120918, enhanced antinociception and elevated systemic M3G concentrations in rats but did not affect the kinetics of the parent compound when rats were given a single intravenous dose of morphine. In humans, the P-glycoprotein inhibitor PSC833 did not result in increase in plasma concentration of morphine, whereas the coadministration of morphine and PSC833 did not alter morphine pharmacodynamics in a clinically significant fashion, when assessed by carbon dioxide tension, reaction time, and blood pressure changes. Thus, morphine and fentanyl may have complex pharmacokinetic and pharmacodynamic effects in vivo related to a combination of many factors, including drug interactions, formation of metabolites, and passage across cell membranes throughout the body dependent on whether they act as either substrates or inhibitors of both efflux and uptake transporters. Comparative in vitro assessment in cell lines such as we describe allows a separation of activity as either P-glycoprotein substrates or inhibitors and is a first approach to providing a mechanistic explanation for observed effects in vivo.

Drugs that are not themselves substrates for P-glycoprotein but may nevertheless inhibit P-glycoprotein are a potential source of important drug interactions. A drug that inhibits P-glycoprotein but is itself not a P-glycoprotein substrate will allow the increased penetration into the CNS of concomitant drugs that are P-glycoprotein substrates such as loperamide, digoxin, cyclosporin, and human immunodeficiency virus protease inhibitors. Thus, P-glycoprotein has the potential to affect drug pharmacokinetics broadly; it may not only control drug

Fig. 4. Inhibitory effects of varying concentrations of loperamide (open squares), sufentanil (filled circles), fentanyl (open circles), and alfentanil (filled triangles) on P-glycoprotein-mediated $^{[3]}$H-digoxin (5 $\mu$M) transport across a Caco-2 cell culture monolayer. Data are mean ± SD from three or more experiments. The units on the x-axis for inhibitor concentration are micromolars.
distribution to tissues such as the brain and testes, but also drug elimination by the kidney and biliary tract and bioavailability \emph{via} the gastrointestinal tract. P-glycoprotein is also expressed in the human placenta and may protect the fetus from drug effects.\textsuperscript{26,27} Pharmacokinetic drug interactions occur when the administration of one drug affects the pharmacokinetics of a concomitantly administered drug, resulting in either decreased or increased pharmacologic effect, often because of inhibition of specific drug metabolizing enzymes such as cytochrome P450 3A.\textsuperscript{28} Well-recognized substrates of cytochrome P450 3A include alfentanil, nifedipine, and cyclosporin. It is now recognized that inhibition of P-glycoprotein might also result in severe drug interactions, with the simultaneous dual inhibition of P-glycoprotein and cytochrome P450 3A producing profound increases in plasma drug concentration and potential increased drug entry in the CNS.

The IC\textsubscript{40} values measured for loperamide, sufentanil, fentanyl, and alfentanil were 2.5, 4.5, 6.5, and 112 \textmu M, respectively. These IC\textsubscript{40} values for the anesthetic opioids are higher than the respective therapeutic plasma concentrations usually obtained in clinical practice. When used in high dosage for cardiac surgery, plasma concentrations of fentanyl and sufentanil are in the region of 50 ng/ml (\emph{i.e.,} < 0.2 \textmu M)\textsuperscript{29,30} and 5.0 ng/ml (\emph{i.e.,} < 0.02 \textmu M) respectively,\textsuperscript{29–31} much lower than their respective IC\textsubscript{40} values for P-glycoprotein inhibition defined in the current study. Similarly, therapeutic plasma concentrations of alfentanil are lower than the IC\textsubscript{40} value of 112 \textmu M.\textsuperscript{32} Thus, the P-glycoprotein inhibition demonstrated \emph{in vitro} would be unlikely to result in significant interactions in clinical practice. It may be argued that, in accordance with their high volumes of distribution, the plasma concentrations of fentanyl and sufentanil do not necessarily represent tissue concentrations, \emph{e.g.,} fentanyl concentrations in the brain, kidneys, and liver, all major sites of P-glycoprotein expression, are, respectively, twofold, threefold, and fourfold higher than in plasma.\textsuperscript{33} However, even these tissue concentrations would be expected to be substantially lower than the IC\textsubscript{40} values for P-glycoprotein inhibition found in this study.

The importance of P-glycoprotein in anesthetic practice is a function of its presence in the blood–brain barrier, gastrointestinal tract, placenta, and other membrane systems in the body. This transport system can be inhibited by drugs such as quinidine and verapamil and recently developed more potent and specific inhibitors.\textsuperscript{20} Ondansetron, a powerful antiemetic agent used in anesthetic practice with no major CNS effects, is also a P-glycoprotein substrate\textsuperscript{10} and is in widespread use. The potential exists for drug interactions to result during the perioperative period because of P-glycoprotein inhibition for other drugs. Cyclosporin and verapamil are both inhibitors and substrates for P-glycoprotein, digoxin, loperamide, vincristine, and dexamethasone are substrates for P-glycoprotein, whereas quinidine, LY335959, and ketoconazole are inhibitors but not substrates for P-glycoprotein. If we can control transporter-mediated drug access to its site of action, then it is exciting to speculate that we can change the way we administer intravenous drugs by manipulation of drug entry in or out of the CNS.

In summary, we conclude that, in contrast to loperamide, opioids in widespread clinical use as intravenous anesthetic agents or adjuvants, such as fentanyl, sufentanil, and alfentanil, are \emph{not} \emph{in vitro} substrates of the cellular efflux transporter P-glycoprotein. Morphine is a P-glycoprotein substrate with clearly less clinical relevance for P-glycoprotein than loperamide. Inhibitory effects of the opioids fentanyl, sufentanil, and alfentanil on P-glycoprotein activity \emph{in vitro} are reached only with relatively high concentrations.

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