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Opioids and Efflux Transporters. 1. P-Glycoprotein Substrate Activity of N-Substituted Analogs of Meperidine.

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Abstract

P-Glycoprotein (P-gp) is an efflux transporter which is up-regulated at the blood brain barrier in both morphine and oxycodone tolerant rats. Numerous studies have shown that many clinically employed opioid analgesics are substrates for P-gp, suggesting that up-regulation of P-gp may contribute to the development of central tolerance to opioids. The studies herein focus on the development of SAR for P-gp substrate activity in the meperidine series of compounds, and show that a meperidine analog of greater potency, *N*-phenylbutyl-*N*-normeperidine, has low activity as a P-gp substrate and has the potential to be utilized as a tool to study the contribution of P-gp on the development of central tolerance to opioids.

The development of improved agents for the treatment of chronic pain remains an important goal in public health¹. The vast majority of currently employed agents for the treatment of severe chronic pain are opioid analgesics, which act as agonists at mu opioid receptors in the CNS.² Unfortunately, clinically employed opioid analgesics suffer from the development of tolerance, necessitating escalating doses to maintain the patient in a pain-free state³, thereby leading to escalated side-effects such as constipation^{4,5}. Numerous mechanisms at the receptor and cellular level have been indicated in the development of tolerance to mu opioid receptor agonists⁶, but recent reports have suggested that efflux transporters at the blood-brain barrier (BBB) may also contribute towards the development of central tolerance^{7,8}. P-glycoprotein (P-gp) is an efflux transporter which is located in numerous tissues⁹, and its function at the BBB is to actively remove xenobiotics from the CNS⁹. Two commonly employed opioid analgesics, morphine (1) and oxycodone (2) (Figure 1), are known substrates for this transporter and rats tolerant to both morphine⁸ and oxycodone⁷ show up-regulation in P-gp level at the BBB. Thus, on chronic administration, the up-regulated P-gp would be expected to result in lower brain concentrations of opioid thereby exacerbating tolerance to the central analgesic effects. P-gp knockout animals are available and offer a useful model to study the effects of P-gp on opioids¹⁰, but an alternative approach in wild-type animals is the development of mu opioid receptor agonists which are not substrates for P-gp. These compounds would allow a full investigation of the contribution of up-regulated P-gp to opioid tolerance as full cross-tolerance between morphine and the opioid with no P-gp substrate activity would not be anticipated to occur, and also potentially be developed into opioid analgesics with lower degrees of tolerance.

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The μ opioid analgesic meperidine (3) has been shown to possess low activity as a P-gp substrate¹¹, but only moderate antinociceptive activity *in vivo*^{12, 13}, thus initial investigations described herein are focused on delineating the structure-activity relationships (SAR) of the N-substituent in this class for low P-gp substrate activity, while increasing μ opioid potency based on known SAR for potency in this series¹³.

A range of previously reported and novel N-substituted analogs of meperidine were prepared from nitrile 4 (obtained from Sigma-Aldrich, Inc), via alkylation with alkyl halides in DMF in the presence of K_2CO_3 , followed by hydrolysis of the nitrile to the ethyl esters (5–14) through treatment with H_2SO_4 and EtOH¹⁴ (Scheme 1). The alkyl substituents were chosen based on known active meperidine analogs and also following a series as previously described for other classes of opioids¹⁵, and include arylalkyl, alkyl, and branched alkyl groups (Table 1).

All esters were converted to oxalate salts (Scheme 1). Drug stimulated P-gp ATPase activity was estimated by Pgp-Glo assay system²⁰ (Promega, Madison, WI) and the results were shown in Figure 2. This method relies on the ATP dependence of the light-generating reaction of firefly luciferase where ATP consumption is detected as a decrease in luminescence, the greater the decrease in signal the higher the P-gp activity. Sodium orthovanadate was used as a P-gp ATPase inhibitor, whereas verapamil was used as a positive control. Test compounds (all tested at 200 μ M) which are significantly lower than the control (NT) are P-gp substrates, whereas test compounds significantly higher than the NT are P-gp inhibitors. Compounds which are not significantly different from the NT are neither P-gp substrates nor inhibitors.

The P-gp substrate activity of the esters showed differences depending on the nature of the N-substituent. Most analogs were substrates for P-gp like verapamil, with the exception of meperidine itself and *N*-phenylbutyl-*N*-normeperidine (7). These results show a distinct SAR for P-gp substrate activity in this series as *N*-phenylalkyl analogs of shorter length (phenethyl (5), phenylpropyl (6)) were substrates.

Previous studies have shown that 7 has twice the antinociceptive activity as meperidine¹³. Thus 7 has the profile of low P-gp substrate activity and greater potency than meperidine required for use as a tool to study the contribution of P-gp upregulation on the development of opioid tolerance and cross tolerance between opioids with P-gp substrate activity and opioids that are not P-gp substrates. Studies on this compound are currently underway, and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Morphine (1) and oxycodone (2)

Figure 2.

Results of compounds and standards in the Pgp-Glo assay system; all compounds assayed at 200 μ M. Data are represented as mean $\pm$ SEM (n=4). * indicates significant difference from the control at $p < 0.05$ as indicated by t-test.

Reagents and conditions: a-RX, K_2CO_3 , DMF;
b- H_2SO_4 , EtOH, reflux

Scheme 1.

Table 1

Compounds prepared, salt form, yield, and melting points. Citations reference previously known compounds.

R	Ester	Salt, Yield, mp
CH ₃	3 ¹⁴	Oxalate, 7%, 190–192°C
(CH ₂) ₂ (C ₆ H ₅)	5 ¹⁶	Oxalate, 33%, 205–20°C
(CH ₂) ₃ (C ₆ H ₅)	6 ¹⁷	Oxalate, 14%, 225°C
(CH ₂) ₄ (C ₆ H ₅)	7 ¹⁷	Oxalate, 25%, 170°C
CH ₂ (C ₆ H ₅)	8 ¹⁴	Oxalate, 46%, 204–205°C
CH ₂ CH=CH ₂	9 ¹⁸	Oxalate, 40%, 213–214°C
(CH ₂) ₂ CH ₃	10 ¹⁹	Oxalate, 57%, 215–216°C
CH ₂ CH=CHCH ₃	11	Oxalate, 67%, 173–177°C
(CH ₂) ₃ CH ₃	12 ¹⁹	Oxalate, 24%, 190–192°C
CH ₂ C(CH ₃)=CH ₂	13	Oxalate, 35%, 180–181°C
CH ₂ CH(CH ₃) ₂	14	Oxalate, 55%, 165–167°C