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Opioids and Efflux Transporters. Part 3: P-Glycoprotein Substrate Activity of 3-Hydroxyl Addition to Meperidine Analogs

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Abstract

Numerous studies have shown that many clinically employed opioid analgesics are substrates for P-glycoprotein (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 opioids. Addition of a 3-OH to meperidine and the ketone analog of meperidine yielding bemidone and ketobemidone, respectively, significantly increased P-gp substrate affinity. The results of this study have implications in the development of novel analgesics to be utilized as tools to study the contribution of P-gp on the development of central tolerance to opioids.

Introduction

There is a growing body of evidence that suggests efflux transporters, specifically P-glycoprotein (P-gp), may play a role in the development of opioid related central tolerance and constipation.^{1–6} Recent studies have shown that opioids are substrates for P-gp, although to differing extents,⁷ and P-gp is up-regulated at the blood-brain barrier (BBB) of morphine³ (1) and oxycodone² (2) (Figure 1) tolerant rats. Upon 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 animals⁸ are available and offer a useful model to study the effects of P-gp on opioids, however an alternative approach in wild-type animals is the development of mu opioid receptor agonists which are not P-gp substrates. 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 lacking P-gp substrate activity would not be anticipated to occur. Additionally, opioids lacking P-gp substrate activity may potentially be developed into analgesics with lower degrees of tolerance.

Meperidine (3), a moderately potent mu opioid analgesic,^{9, 10} has been reported to possess low P-gp substrate activity⁷. Therefore, our investigations are focused on delineating the structure-activity relationship (SAR) for the addition of a *m*-OH, while increasing mu opioid potency based on known SAR for this series.¹⁰

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Results and Discussion

The compounds synthesized are readily known in the literature as mu opioid analgesics;¹⁰ however, the syntheses described here are novel approaches. Meperidine (**3**) was prepared from nitrile **4** (obtained from Sigma-Aldrich, Inc.), via alkylation with MeI in DMF in the presence of K₂CO₃, followed by aqueous NH₄Cl hydrolysis of the *N*-methyl nitrile **5** to the ethyl ester through treatment with H₂SO₄ and EtOH. Treatment of **5** with EtMgBr, via a Grignard reaction,¹¹ produced the ketone meperidine analog **6** (Scheme 1).

Bemidone **9** was prepared from the condensation of mechloroethamine hydrochloride and 3-methoxyphenyl-acetonitrile (both reagents obtained from Sigma-Aldrich, Inc.) with NaH and NaOH to yield **7**. *O*-dealkylation of **7** was performed with BBr₃ and NH₄OH,¹² converting the methoxy group to a phenol **8**, followed by nitrile hydrolysis to give the *m*-OH ethyl ester as previously described.⁴ Treatment of **7** with an EtMgBr Grignard reagent,¹¹ followed by aqueous NH₄Cl hydrolysis produced **10**, which then underwent treatment with BBr₃ to produce ketobemidone (**11**) (Scheme 2).

All compounds were converted to their respective salts (see Table 1). Drug stimulated P-gp ATPase activity was estimated using the Pgp-Glo assay system¹³ (Promega, Madison, WI) and the results are shown in Table 1. Briefly, 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. All test compounds were analyzed at 200 μM and fold stimulation values were calculated using Equation 1. Fold stimulation values greater than 2.0 indicate a P-gp substrate.¹⁴

$$\text{Fold stimulation by a test compound} = \frac{\text{Test compound stimulated P-gp activity}}{\text{Basal P-gp activity}} \quad \text{Equation 1}$$

The addition of a *m*-OH into the phenyl ring significantly increases the P-gp fold stimulation of meperidine analogs. Meperidine itself has a P-gp fold stimulation value of 1.78 and increases to 2.64 with the *m*-OH addition (bemidone, **9**). Whereas the ketone analog **6**, with a P-gp fold stimulation value of 1.37, increases to 4.89 with the *m*-OH addition (ketobemidone, **11**). Thus, the addition of a *m*-OH increases the P-gp substrate activity of these meperidine analogs, which are members of the 4-phenylpiperidine class of opioids.

The hydroxylated meperidine analogs were initially pursued to investigate the relationship between P-gp and increased opioid potency. Interestingly, these results are consistent with previous studies in our laboratory which showed that removal of the 3- and 6-OH from morphine resulted in decreased P-gp substrate activity,⁵ as morphine is a P-gp substrate.⁷ These studies attest that the *m*-OH substituent increases P-gp substrate activity across the phenylpiperidine and morphinan classes of opioids. Furthermore, the development of opioids lacking P-gp substrate activity should not possess a *m*-OH substituent. The interaction between opioids and P-gp is currently under investigation and these results aid in further SAR development. The ultimate goal is to develop a potent opioid with low P-gp substrate activity for use as a tool to study the contribution of P-gp up-regulation to the development of opioid tolerance and cross-tolerance between opioids with P-gp substrate activity and those without.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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

Scheme 1.

Reagents and conditions: (a) MeI, K₂CO₃, DMF; (b) H₂SO₄, EtOH, reflux; (c) EtMgBr, NH₄Cl hydrolysis.

Scheme 2.

Reagents and conditions: (a) NaH, NaOH; (b) BBr₃, NH₄OH; (c) H₂SO₄, EtOH; (d) EtMgBr, NH₄Cl hydrolysis.

Table 1

Fold stimulation values of test compounds prepared, salt form, yield, and melting point.

Compound	Name	Salt	Yield (%)	m.p. (°C)	Fold Stimulation ± SEM
3	Non Treated (control)				1.00
6	Meperidine	oxalate	7	190–192	1.78 ± 0.39 *
9	Ketone analog	citrate	56	170–171	1.37 ± 0.19 *
11	Bemidone	oxalate	36	200–202	2.64 ± 0.82 *
	Ketobemidone	oxalate	51	233–235	4.89 ± 1.94 *

All compounds assayed at 200 μM. Data are represented as fold stimulation ± SEM (*n* = 3).

* Indicates significant difference (*p* < 0.05) from control (non-treated) as determined from *t*-test. All compounds gave satisfactory CHN (± 0.4%) and spectral analysis (see Supporting Information).