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Uniform assessment and ranking of opioid Mu receptor binding constants for selected opioid drugs ☆

Donna A. Volpe [a,*], Grainne A. McMahon Tobin [a], R. Daniel Mellon [b], Aspandiar G. Katki [a], Robert J. Parker [a], Thomas Colatsky [a], Timothy J. Kropp [c], S. Leigh Verbois [c]

[a] Laboratory of Clinical Pharmacology, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, USA
[b] Division of Anesthesia and Analgesia Products, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, USA
[c] Division of Drug Oncology Products, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, USA

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ABSTRACT

The safe disposal of unused opioid drugs is an area of regulatory concern. While toilet flushing is recommended for some drugs to prevent accidental exposure, there is a need for data that can support a more consistent disposal policy based on an assessment of relative risk. For drugs acting at the Mu-opioid receptor (MOR), published measurements of binding affinity (K_i) are incomplete and inconsistent due to differences in methodology and assay system, leading to a wide range of values for the same drug thus precluding a simple and meaningful relative ranking of drug potency. Experiments were conducted to obtain K_i's for 19 approved opioid drugs using a single binding assay in a cell membrane preparation expressing recombinant human MOR. The K_i values obtained ranged from 0.1380 (sufentanil) to 12.486 μM (tramadol). The drugs were separated into three categories based upon their K_i values: $K_i > 100$ nM (tramadol, codeine, meperidine, propoxyphene and pentazocine), $K_i = 1-100$ nM (hydrocodone, oxycodone, diphenoxylate, alfentanil, methadone, nalbuphine, fentanyl and morphine) and $K_i < 1$ nM (butorphanol, levorphanol, oxymorphone, hydromorphone, buprenorphine and sufentanil). These data add to the understanding of the pharmacology of opioid drugs and support the development of a more consistent labeling policies regarding safe disposal.

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1. Introduction

When patients have extra prescription drug products remaining at the end of a treatment regimen, there are questions regarding their proper disposal. The Food and Drug Administration (FDA) recommends that patients seeking to dispose of unneeded drugs follow recommendations in the Federal Guidelines: Proper Disposal of Prescription Drug (Office of National Drug Control Policy, 2009). While these guidelines recommend disposing of medicines in the household waste and community take back programs for the vast majority of drug products, toilet flushing is recommended as a means of disposal for a limited number of products, some of which contain opioid drugs (FDA, 2010). This method renders the opioid drug product immediately and permanently unavailable for accidental exposures, thus eliminating the risk of overdose and death from severe respiratory depression. However, the practice of toilet flushing as a disposal method has become a subject of debate due to public health concerns about pharmaceuticals in the water and the environment (Boleda et al., 2009; Postigo et al., 2008; Zuccato et al., 2008). Alternative methods for disposal of these substances that prevent accidental exposures would be welcome, such as drug take-back programs for opioid drugs.

With any drug, potential benefits are balanced against observed risks that must be determined prior to drug approval and also evaluated post-marketing. Additional information collected in post-marketing can be used to develop strategies that are needed to mitigate risks and ensure that the benefit of approved drugs continue to outweigh the known risk. Since there is extensive interest in encouraging the appropriate use of opioid drugs to treat pain

Abbreviations: MOR, Mu opioid receptor; DOR, delta opioid receptor; KOR, kappa opioid receptor; DAMGO, (D-Ala2, *N*-MePhe4, Gly-ol)-enkephalin; GPCR, G-protein coupled receptors; FDA, Food and Drug Administration; K_i, equilibrium dissociation constant for the test compound; K_d, equilibrium dissociation constant for the ligand; B_{max}, total number of receptors in the membranes; IC_{50}, drug concentration resulting in 50% of the maximal radioligand binding to receptor; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane-sulfonic acid; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; PEI, polyethyleneimine; IM, intramuscular; logP, octanol:water partition coefficient; GTPγS, guanosine-5′-*O*-[γ-thio(triphosphate)].

☆ *Disclaimer:* The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

* Corresponding author. Fax: +1 301 796 9818.
E-mail address: donna.volpe@fda.hhs.gov (D.A. Volpe).

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and in minimizing their misuse and abuse, the FDA continues to work to understand their pharmacology as well as their patterns of use.

Opioid drugs elicit their pharmacological effects through activation of one or more membrane-bound receptors that are part of the G coupled-protein receptor (GPCR) family. Opioid receptors have been classified as μ (MOR), κ (KOR), δ (DOR), and nociceptin (Waldhoer et al., 2004). Mu opioid receptors are responsible for supraspinal analgesia, respiratory depression, euphoria, sedation, decreased gastrointestinal motility, and physical dependence (Waldhoer et al., 2004; Gutstein and Akil, 2006; Trescott et al., 2008). The majority of the clinical opioid analgesic and anesthetic drugs have significant agonist activity at the MOR.

Competitive receptor binding studies provide a means of measuring the interaction between a given drug and its receptor (Leslie, 1987; Trescott et al., 2008). Determinations of receptor binding affinities for different families of GPCRs are subject to significant variability across laboratories and model systems. The differences in K_i values (equilibrium dissociation constant) are due to the ligand selectivity, species/strain, tissue or cell source for the receptor, and assay methodology (e.g., pre-incubation, ligand and drug concentration) (de Jong et al., 2005; Leslie, 1987; Simantov et al., 1976; Thomasy et al., 2007; Robson et al., 1985; Selley et al., 2003; Nielsen et al., 2007; Titeler et al., 1989; Yoburn et al., 1991). As a result, available data sets are incomplete and often inconsistent due to differences in receptor source and analytical methods, which confounds comparisons of relative binding affinities within this pharmacologic class. A compendium of uniformly derived binding constants for drugs interacting with the MOR would be considered an important contribution to the basic understanding of the comparative pharmacology of this important GPCR family.

The objective of this study was to generate a single, well controlled set of MOR binding data for currently prescribed opioid drugs using a single competitive receptor binding assay in a cell membrane preparation expressing recombinant human MOR. The opioids tested included MOR agonists (alfentanil, codeine, diphenoxylate, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine, methadone, morphine, oxycodone, oxymorphone, propoxyphene, sufentanil and tramadol) and mixed agonists–antagonists (buprenorphine, butorphanol, nalbuphine, pentazocine). Naloxone, a MOR antagonist, served to monitor assay quality and reproducibility for the radioligand, DAMGO ([D-Ala2, *N*-MePhe4, Gly-ol]-enkephalin), which was chosen as it is a stable synthetic opioid peptide agonist with high MOR specificity and is routinely used in MOR binding studies.

2. Methods

2.1. Materials

Trizma-HCl, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethane-sulfonic acid (HEPES), dimethyl sulfoxide (DMSO), magnesium chloride, calcium chloride, bovine serum albumin (BSA), and polyethyleneimine (PEI) were purchased from Sigma Chemical Company (St. Louis, MO). The opioid drugs, DAMGO and naloxone were from Sigma, USP (Rockville, MD), RBI (St. Louis, MO) or Fluka (St. Louis, MO). Tramadol metabolites ±M1, +M1 and −M1 were from Toronto Research Chemicals (North York, Ontario, Canada). [^{3}H]-DAMGO was from Perkin Elmer (Waltham, MA). The Chemiscreen™ membrane preparation (Millipore, Billerica, MA) contained a full length OPRM1 cDNA encoding the human MOR in an adherent Chem-5 cell line. In order to avoid the adverse effect of freezing and thawing, the membranes were thawed and aliquoted into single use preparations and stored at −80 °C. Corning 3641 non-binding polystyrene 96-well plates (Corning, NY) and MultiScreen® GF/C 96-well plates with glass fiber filters (Millipore) were used in the binding assays. For measuring the bound radioligand, scintillation cocktail (Complete Counting Cocktail 3a70B™, Research Products International, Mount Prospect, IL) and glass vials (Wheaton Science Products, Millville, NJ) were utilized.

2.2. Drug stock solutions

All drugs were prepared as 10, 100 or 1000 mM stock solutions depending upon final concentrations in the competitive assays (Table 1). Drugs were resuspended at the required concentration in purified distilled water (Barnstead NANOpure, Dubuque, IA), except for those resuspended in DMSO (codeine, buprenorphine, diphenoxylate, oxymorphone and pentazocine) or methanol (butorphanol, ±*O*-desmethyltramadol (±M1), and its enantiomers +M1, and −M1).

2.3. Binding assay

The Chemiscreen™ MOR membrane preparations (Millipore, 2008) were rapidly thawed and diluted in binding buffer (50 mM HEPES, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, pH 7.4) to a concentration of 0.1 mg/mL. The radioligand and unlabeled compounds were diluted in binding buffer to achieve the desired final concentration in each well. The assays were performed in microtiter plates with 40 μL of binding buffer or unlabeled ligand, 10 μL of radioligand, and 50 μL of diluted membranes with three wells per group. The plates were then incubated at room temperature for various time points. The binding incubation was terminated by the addition of 100 μL cold binding buffer to each well. The glass fiber filter plates were presoaked for 30–45 min with 0.33% PEI buffer. The PEI solution was removed from the filter plate with a vacuum manifold (Millipore) and the filters washed with 200 μL priming buffer (50 mM HEPES, 0.5% BSA, pH 7.4) per well. The binding reaction was transferred to the filter plate and washed with 200 μL washing buffer (50 mM HEPES with 500 mM NaCl and 0.1% BSA, pH 7.4). The plate was dried and the filters removed in a cell harvester and punch assembly (MultiScreen® HTS, Millipore) for analysis in a scintillation counter (Beckman Coulter, Fullerton, CA).

2.4. Competition assays

For the competitive binding experiments, assays were conducted as above with 2 nM (^{3}H)-DAMGO and an incubation time of 2 h. The unlabeled opioid drugs were added at one third-log increments with 5 log separation between highest and lowest concentrations (Table 1). Naloxone inhibition of (^{3}H)-DAMGO binding was evaluated (0.01–1000 nM) in the same plate in separate wells to monitor assay quality and reproducibility.

Table 1

Assay concentration (nM)	Drug stock	Drugs
0.001–100	10 mM	Butorphanol, levorphanol, sufentanil
0.01–1000	10 mM	Buprenorphine, fentanyl, hydromorphone, methadone, morphine, nalbuphine, oxymorphone, ±M1, +M1
0.1–10,000	10 mM	Alfentanil, diphenoxylate
1–100,000	10 mM	Hydrocodone, oxycodone, pentazocine, propoxyphene
10–1000,000	1000 mM	Codeine, meperidine
100–10,000,000	1000 mM	Tramadol, −M1

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