Nonclinical Pharmacology and Toxicology Considerations Regarding Opioid Comparisons and Risk Assessments (Basic Opioid Pharmacology 101)

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Objectives

• Provide a quick overview and history of opioid pharmacology (refresher)

• Describe the challenges with methods to compare opioid potency, from a basic science and nonclinical perspective

• Compare data from binding affinities with a toxicological endpoint to illustrate challenges of potency estimates

• Identify the challenges for translation of animal potency studies to humans
A Very Brief History of Opioid Pharmacology

• No one knows who first cultivated the opium poppy (4200 BC – large numbers of poppy seed capsules found in burial sites in Spain)

• Sumerians possibly as far back as between 3400 BC called opium “gil” (joy) and the poppy “hul gil” (plant of joy).

• Note on terminology:
  – **Opiates** are drugs derived from opium (morphine, codeine and semisynthetics)
  – **Opioids** are all agonists and antagonists (more inclusive as it includes synthetics)

https://en.wikipedia.org/wiki/Opium
https://study.com/academy/answer/sumeria-was-located-in-an-area-known-as.html
https://www.deamuseum.org/ccp/opium/history.html
https://en.wikipedia.org/wiki/Opium
A Very Brief History of Opioid Pharmacology

• Sometime between 1803 and 1805 morphine was first extracted from opium resin by Friedrich Sertturner, a German pharmacist.

• The concept of opioid receptors was first proposed by Beckett and Casy (1954) based on rigid chemical structural requirements for activity.

• Opioid receptors were first demonstrated in 1973 using radioligand binding assays:
  – Candace Pert & Solomon Snyder, 1973
  – Eric Simon, Jacob Hiller, and Irit Edelman, 1973
  – Lars Terenius, 1973
Discovery of Opioid Receptors

Opioid Receptor Subtypes (all coded by one gene)

- **Delta** (δ, DOP, or formerly OP1)
  - Two variants based on receptor binding studies (d1 and d2)
- **Kappa** (κ, KOP, or formerly OP2)
  - Three variants based on receptor binding studies (k1, k2, and k3)
- **Mu** (μ, MOP, or formerly OP3)
  - Three variants based on receptor binding studies (m1, m2, and m3)
- **Nociceptin/Orphanin FQ** Receptor (ORL-1, NOP, or formerly OP4)
  - not naloxone sensitive

Source: Laurence L. Brunton, Randa Hilal-Dandan, Bjørn C. Knollmann: Goodman & Gilman’s: The Pharmacological Basis of Therapeutics, Thirteenth Edition. Copyright © McGraw-Hill Education. All rights reserved.

Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman’s: The Pharmacological Basis of Therapeutics, 13e
Opioid Receptor Signal Transduction

- Mu, delta, and kappa receptors couple to pertussis toxin-sensitive, Gᵢ/Gₒ proteins.
- On receptor activation, the Gᵢ/Gₒ coupling results in a number of intracellular events that are mediated by α and βγ subunits of these G proteins, including the following:
  - Inhibition of adenylyl cyclase activity (decreases cAMP and PKA activation)
  - Reduced opening of voltage-gated Ca²⁺ channels (reduces neurotransmitter release from presynaptic terminals)
  - Stimulation of K⁺ current through several channels (hyperpolarization of neurons)
  - Activation of PKC and PLCβ
  - Can be phosphorylated for β-arrestin interactions

Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman’s: The Pharmacological Basis of Therapeutics, 13e

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Receptor Desensitization and Drug Tolerance

• **Desensitization** – usually refers to the molecular changes at level of receptor signaling that result in progressive reduction of signal transduction after receptor activation
  – Rapid desensitization (seconds to minutes)
  – Short term tolerance (minutes to tens of minutes)
  – Long term tolerance (greater than 1 day)

• **Molecular Mechanisms are Complicated**
  – E.g., phosphorylation following activation, endocytosis, resensitization, recycling
  – Homologous and Heterologous Desensitization

• **Drug Tolerance** – loss of responsiveness to an agonist after continued exposure (without specifying cellular or molecular mechanism)

Opioid Receptor Trafficking

• MOR and DOR undergo rapid agonist-mediated internalization
  – MOR recycle to membrane after internalization
    • May be different for different ligands
      – Etorphine and Enkephalins rapid internalization
      – Morphine has been reported to not cause internalization
  – DOR are degraded after internalization
  – KOR do not internalize
• Different ligands may result in different receptor trafficking and physiological responses

Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e
“Biased” Ligands

- Data suggest some ligands produce unbalanced activation of G proteins vs β-arrestin
- The differential intracellular signaling effects may alter the physiological responses, possibly leading to options to increase efficacy and reduce adverse effects and different rates of desensitization

Fig. 3. Ligand bias at MOR. The intrinsic efficacies (operational model) of a range of structurally dissimilar MOR agonists to activate \[^{35}\text{S}]\text{GTP}\gamma\text{S}\) binding and arrestin recruitment was determined and the bias factor (\(\beta\)) calculated according to the method of Rajagopal et al. (2011). Reproduced from Rivero et al. (2012).
Examples of the Potential Impact of Genetics

• Single Nucleotide Polymorphisms (SNPs)
  – RS1799971 SNP changes an adenine (A) to guanine (G) at Position 118 in OPRM1 gene (codes for mu opioid receptor)
  – Present in 15-30% Europeans, 40-50% Asians, 1-3% Latinos and African Americans
  – Results in change of the amino acid at Position 40 from asparagine to aspartate
  – Removes potential asparagine-linked glycosylation which can alter MOR affinity for different ligands, signal transduction, and half-life of the receptor.
  – Adds methylation site which can reduce MOR mRNA

• Epigenetic Modifications
  – Differential methylation of OPRM1 promotor linked to a variety of physiological responses (e.g., alcohol dependence, opioid dependence, pain responses, neuropathic pain conditions, Alzheimer’s disease)

• Splice Variants
  – 7-TM vs 6-TM splice variants of MOR may have differential effects on efficacy and adverse effects

Reviewed by: Cuitavi et al. (2021) Trends in Biochemical Sciences 46(4):315-328
Opioid Receptors Can Dimerize

- There is evidence for both homodimers and heterodimers
  - Can impact ligand binding, intracellular signaling, and receptor trafficking/desensitization
  - Could contribute to the ultimate diversity of pharmacological properties of the individual receptors

Can also dimerize with other nonopioid GPCRs
Pharmacodynamics of Opioid Receptors

Mu (MOR)
- Analgesia
- Physical dependence
- Respiratory depression
- Miosis
- Euphoria
- Reduced GI motility

Delta (DOR)
- Analgesia
- Antidepressant effects
- Convulsant effects
- Physical dependence
- Modulation of MOR-mediated respiratory depression

Kappa (KOR)
- Analgesia
- Anticonvulsant effects
- Depression
- Dissociative/hallucinogenic effects
- Diuresis
- Miosis
- Neuroprotection
- Sedation
- Stress

## Selectivity of Common Opioid Analgesic Ligands

<table>
<thead>
<tr>
<th>Opioid Ligand</th>
<th>Mu</th>
<th>Delta</th>
<th>Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methadone</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>P</td>
<td></td>
<td>--</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>P</td>
<td></td>
<td>+++</td>
</tr>
</tbody>
</table>

+ = Agonist activity  
- = Antagonist activity  
P = Partial agonist activity  

In potency: + < ++ < +++

Source: Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman’s: The Pharmacological Basis of Therapeutics, 13e  
Receptor Binding Assays Measure Affinity

• Radioligand (e.g., $[^3]H$-Naltrexone, $[^3]H$-DAMGO) binds to receptors in tissue or membrane sample
• Increasing concentrations of radioligand eventually saturate the binding sites

https://en.wikipedia.org/wiki/Ligand_(biochemistry)
Comparison of Binding Affinities

Direct Binding Affinity
- $K_D = \text{dissociation constant}$
- Binding of a radioligand to a receptor

Indirect Binding Affinity
- $K_i = \text{Inhibition constant}$
- Displacement of a radioligand from the receptor by increasing concentrations of an unlabeled compound

Credit: University of Nebraska Medical Center eLearning
https://www.unmc.edu/elearning/egallery/receptor-binding/
Developed by Cassandra Moshfegh, Sarah Schlichte, and Dr. Myron Toews
Receptor Binding Affinity

![Graph of receptor binding affinity with different types of ligands: receptor saturation (Y), full agonist, partial agonist, neutral antagonist, inverse agonist.](http://watcut.uwaterloo.ca/webnotes/Pharmacology/Pharmacodynamics.html)
Goal: Compare FDA-approved opioid analgesic drug affinities to the mu opioid receptor as a surrogate for opioid potency

- Concern at the time was to determine what drugs may be more dangerous than others to warrant disposal via flushing rather than other means of disposal that could result in diversion or inadvertent exposures.
- Review of literature resulted in wide range of values reported for MOR
  - Due to differences in: radioligands used, definition of nonspecific binding, laboratory methods, tissue sources, species tested, etc.
Literature MOR $K_i$ Values

- Range of $K_i$ values for drugs as much as 10- to 100,000-fold different
- Variability due to:
  - radioligand
  - tissue source
  - animal species and strain
  - assay methodology

Literature DOR and KOR $K_i$ Values

Variability in literature $K_i$ values for DOR and KOR as seen with MOR
Receptor Binding Assay

- Determination of binding affinities ($K_i$)
- Membranes expressing recombinant human mu-opioid receptor
- Single standardized assay
- Uniform experimental conditions with $[^3H]DAMGO$
- Test set: 19 FDA approved opioid drugs
- Reference standard: Naloxone

MOR Binding Curves for Opioids

Challenge: We do not have uniform data for $\delta$ or $\kappa$ opioid receptor binding

<table>
<thead>
<tr>
<th>Drug</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sufentanil</td>
<td>0.138</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>0.2157</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>0.3654</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>0.4055</td>
</tr>
<tr>
<td>Levorphanol</td>
<td>0.4194</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>0.7622</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.168</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>1.346</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>2.118</td>
</tr>
<tr>
<td>Methadone</td>
<td>3.378</td>
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<tr>
<td>Alfentanil</td>
<td>7.391</td>
</tr>
<tr>
<td>Diphenoxylate</td>
<td>12.37</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>25.87</td>
</tr>
<tr>
<td>Hydrocodone</td>
<td>41.58</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>117.8</td>
</tr>
<tr>
<td>Meperidine</td>
<td>120.2</td>
</tr>
<tr>
<td>Codeine</td>
<td>450.1</td>
</tr>
<tr>
<td>Tramadol</td>
<td>12486</td>
</tr>
</tbody>
</table>

## Overdose Risk (LD₅₀)

<table>
<thead>
<tr>
<th>Opioid</th>
<th>Rat Oral LD₅₀ (mg/kg)</th>
<th>MOR Ki (nM)</th>
<th>Octanol:Water Partition Coefficient*</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl Citrate</td>
<td>18</td>
<td>1.346</td>
<td>860:1</td>
<td>Highly lipophilic</td>
</tr>
<tr>
<td>Methadone HCl</td>
<td>30</td>
<td>3.378</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tramadol HCl</td>
<td>228</td>
<td>12486</td>
<td>1.35:1</td>
<td>MOR agonist (M1) and SNRI</td>
</tr>
<tr>
<td>Butorphanol tartrate</td>
<td>315</td>
<td>0.7622</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocodone bitartrate</td>
<td>375</td>
<td>41.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codeine sulfate</td>
<td>430</td>
<td>734.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Morphine sulfate</strong></td>
<td><strong>461</strong></td>
<td><strong>1.168</strong></td>
<td><strong>1.42:1</strong></td>
<td></td>
</tr>
<tr>
<td>Buprenorphine HCl</td>
<td>&gt; 1000</td>
<td>0.2157</td>
<td></td>
<td>Partial agonist at MOR</td>
</tr>
<tr>
<td>Oxycodone HCl</td>
<td>No data</td>
<td>25.87</td>
<td>0.7:1</td>
<td></td>
</tr>
<tr>
<td>Hydromorphone HCl</td>
<td>No data</td>
<td>0.3654</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxymorphone HCl</td>
<td>No data</td>
<td>0.4055</td>
<td>0.98:1</td>
<td></td>
</tr>
</tbody>
</table>

*Data Source: Merck Index*
Measures of Opioid Potency

**In Vitro Assays**
- Receptor binding affinity
- G protein-coupled activation ([\(^{35}\)S]GTP\(\gamma\)S)
- Inhibition of adenylyl cyclase
- Calcium flux/signaling
- cAMP inhibition

**Animal Models**
- Tail-flick anti-nociception assay
- Knock-out rodent models

*Challenge: We do not have uniform data for clinically relevant opioids on these endpoints*
Animal to Human Comparison

**Human**
- Analgesia
  - “insensitivity to pain without loss of consciousness” (Merriam Webster)

**Animal**
- Antinociception
  - “the action or process of blocking the detection of a painful or injurious stimulus by sensory neurons” (Merriam Webster)

There is both a sensory and emotional response to pain. We can measure sensory response but have no idea of emotional response.

http://clipart-library.com

Before Tail Flick

After Tail Flick

https://commons.wikimedia.org/wiki/File:Tail_Flick_Test_Apparatus.jpg

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Animal Models Are Evolving

Gonzalez-Cano et al. (2020) Neuroscience & Biobehavioral Reviews 113: 238-261

https://www.sciencedirect.com/science/article/abs/pii/S0149763419309753#fig0045
Strengths and Limitations of Nonclinical Assays

• *In vitro* assays:
  – Focus on one or a few endpoints (*e.g.*, opioid receptor binding, signal transduction cascade)
  – Interlaboratory variability due to differential methods employed (generally lack uniform assessments in single model)

• *In vivo* animal studies:
  – Species and strain differences
  – Differences in drug metabolism and transport compared to humans
  – Translational challenges (*e.g.*, analgesia vs antinociception)
Developing an Algorithm?
Some Factors Contributing to Pharmacodynamic Variability

**Drug/Drug Product Factors**
- Selectivity and impact of receptor dimerization and splice variants
- Dosage form/route of administration
- Relative bioavailability
- Lipophilicity (distribution)
- Affinity
- Avidity
- Potency
- Rate/mechanism of receptor desensitization
- Protein binding

**Individual Patient Factors**
- Age
- Sex
- Body mass index
- Kidney function
- Hepatic function
- Level of tolerance
- Concomitant medications and supplements
- Underlying disorders
- Genetics (receptors, enzymes, transporters)
Some Final Thoughts

- Opioid pharmacology is incredibly old, yet there is still a great deal unknown.
- Basic science and nonclinical studies contribute to the foundation of our knowledge.
- Cross-study comparisons of data in published literature are extremely challenging given variabilities in laboratories and models used (e.g., species, tissues, ligands), uniform assessments are required.
- Cannot look at any one endpoint to predict cross opioid comparisons – need to consider the relative contribution of the many variables that impact outcome to develop an ideal algorithm.
- Nonclinical studies inform on specific differences between opioids in a highly controlled setting, but the results require testing in the clinical setting given the variabilities in humans and PK/PD contributing factors.

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