Opioids and the future of pain therapy: Hope is on the horizon

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Once dismissed as an impossibility, approaches are arising to develop mu opioids lacking many of the adverse effects of current agents, yielding safer and more efficacious compounds.
The essence of opioid therapy is selectivity

Selectivity can be achieved by:

Route/site of administration
  Topical, epidural/intrathecal
  Peripherally restricted (agonist or antagonists)

Functional bias at the receptor
  (Biased Signaling)

Allosteric modulation of transduction
  (PAM)

Alternative receptor targets
  (Receptor subtypes)
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Biased Signaling

- Opioid receptors are members of the GPCR family
- Humans have approximately 1000 different GPCR genes (~50% taste/smell)
- 40% of all drugs target GPCR’s
- Full length variants have the traditional 7 transmembrane (TM) domains
- Interact with G-proteins and with β–arrestin

The ratio of G-protein/β-arrestin provides a measure of ‘bias’
**β-Arrestin2 knockout mice**

- **Enhance morphine analgesia**
- **Diminish morphine respiratory depression**
- **Diminish morphine tolerance**

**Graphs and Data**

1. **Enhance morphine analgesia**
   - Graph showing % MPE with bars for WT and βarr2-KO groups.
   - Summary in *Science 286:2495, 1999*.

2. **Diminish morphine respiratory depression**
   - Graph showing breathing frequency (breaths/min) with data points for WT and βarr2-KO.
   - Summary in *JPET 314:1195, 2005*.

3. **Diminish morphine tolerance**
   - Graph showing % MPE over time (no. of days).

**References**

- *Nature 408:720, 2000*
- *JPET 314:1195, 2005*
- *Science 286:2495, 1999*
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Positive Allosteric Modulators

• No activity alone

• Potentiate the activity of orthosteric agonists
  • Enhance the actions of physiologically released endogenous ligand
  • Requires appropriate release of endogenous ligand
  • Advantage of use with exogenous agonists not clear

Crystal Structure of the mouse mu opioid receptor

Cross section

Top

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Splicing of the *Oprm1* gene

Full Length 7TM variants
- Exon 1 promoter
- 3’ splicing - unique sequences
- Lengths vary from 1 – 88 aa
  - Mice have 24
  - Humans have 12
  - Rats have 13

1TM variants
- Exon 1 promoter
- 3’ splicing - unique sequences
  - Mice have 5 (1-127 aa after exon 1)
  - Humans have 4 (1-90 aa after exon 1)
  - Rats have 2 (1 - 127 aa after exon 1)

6TM Exon 11 associated variants
- Exon 11 promoter
- 5’ splicing eliminates exon 1
- Unique exon 11 sequence (~25 aa) before exon 2 in most variants
- 3’ splicing - unique sequences
  - Mice have 5
  - Humans have 3
  - Rats have 2
Role of C-terminus on morphine tolerance in B6 mice

Exon 7 variants facilitate morphine tolerance

Exon 4 variants lower morphine tolerance

J Clin Inv 127:1561, 2017
Influence of 7TM variant 3’ splicing on biased signaling

35S-GTPγS binding and β-arrestin-2 bias was calculated for each drug and for each variant and normalized to DAMGO for each variant (top) or normalized to the specific drug and compared across the variants (bottom).

<table>
<thead>
<tr>
<th></th>
<th>MOR-1</th>
<th>MOR-1A</th>
<th>MOR-1B1</th>
<th>MOR-1E</th>
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</table>
Mu opioid analgesia

![Diagram showing the potency of different morphine derivatives (MOR-1, MOR-1A, MOR-1B1, MOR-1C, MOR-1D) for mu opioid analgesia.药物1 (Drug 1) has the highest potency among the listed compounds.]
Knockout models of the mu opioid receptor can be used to genetically define the roles of different set of variants in a drug's activity.
### Sensitivity of mu opioids to loss of 6TM variants

<table>
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<tr>
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<th>ED$_{50}$ (mg/kg, s.c.)</th>
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<tr>
<td></td>
<td>WT</td>
<td>Exon 11 KO</td>
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<tr>
<td>Methadone</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Fentanyl</td>
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<td>3.2</td>
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<tr>
<td>Levorphanol</td>
<td>5</td>
<td>30</td>
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<td>Butorphanol</td>
<td>12.4</td>
<td>200</td>
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<tr>
<td>Buprenorphine</td>
<td>0.2</td>
<td>&gt;10</td>
</tr>
<tr>
<td>IBNtxA</td>
<td>0.53</td>
<td>&gt; 20</td>
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Knockout models indicate:
- Morphine and methadone analgesia are independent of 6TM
- IBNtxA analgesia is independent of 7TM
- Other drugs involve both 6TM and 7TM for analgesia
IBNtxA Analgesia

**Majumdar et al., PNAS 108: 19776, 2011**

**IBNtxA analgesia**

- Independent of traditional 7TM mu, delta and kappa receptors
- Totally dependent upon 6TM exon 11-associated variants
- Is more effective in neuropathic and inflammatory than thermal pain models

<table>
<thead>
<tr>
<th>Assay</th>
<th>$ED_{50}$ mg/kg</th>
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<td>Tail-Withdrawal (49°C)</td>
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<td>Hot-Plate (54°C)</td>
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<tr>
<td>Paw-Withdrawal (15%)</td>
<td>0.27</td>
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<tr>
<td>Formalin (late phase)</td>
<td>0.32</td>
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<td>Complete Feund’s Adjuvant (mechanical allodynia)</td>
<td>0.35</td>
</tr>
<tr>
<td>Spared Nerve Injury (mechanical allodynia)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

**Weiskopf et al., Pain 155:2063, 2014**
Lentivirus/mMOR-1G vector restores expression in the Dorsal Horn of Spinal Core.

Lentivirus/mMOR-1G vector restores only IBNtxA analgesia.

*J Clin Invest 125:2626, 2015*
IBNtxA Effects on Respiratory Rate
# Pharmacological Profiles of 7TM and 6TM Compounds

<table>
<thead>
<tr>
<th></th>
<th>Morphine (7TM)</th>
<th>IBNtxA (6TM)</th>
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<tr>
<td>Analgesia</td>
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<td>++++</td>
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<tr>
<td>Thermal</td>
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<td>Inflammatory</td>
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<tr>
<td>Neuropathic</td>
<td>+</td>
<td>++++</td>
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<tr>
<td>Respiratory depression</td>
<td>++++</td>
<td>-</td>
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<td>Constipation</td>
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<td>+</td>
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<td>Sedation</td>
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<td>Reward</td>
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<td>-</td>
</tr>
<tr>
<td>Physical Dependence</td>
<td>++++</td>
<td>-</td>
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<tr>
<td>Straub tail</td>
<td>++</td>
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</table>
Summary

Cloning the opioid receptors has permitted the transition to a molecular classification of receptors and their subtypes.

The mu opioid receptor gene *Oprm1* undergoes extensive splicing to generate three major classes of variants:

- Full length 7TM variants
- Truncated 6TM variants
- Truncated 1TM variants

Mu opioids can be classified into three categories:

- Dependent upon E1, but not E11 variants  
  *Morphine, Methadone*
- Dependent upon E11, but not E1 variants  
  *IBNtxA*
- Dependent upon both E1 and E11 variants  
  *Buprenorphine, fentanyl, M6G, levorphanol*
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