No Pain, Big Gain:
Truncated G-protein Coupled Receptors and New Targets for Opiate Action

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3-Iodobenzoyl-6β-naltrexamide (IBNtxA)

- Potent Analgesic (10-fold more potent than morphine)
- Active in a triple E1-MOR-1/DOR-1/KOR-1 KO mouse
- Inactive in an exon 11 MOR-1 KO mouse
- Reversed by the opioid antagonist levallorphan
- Not sensitive to selective traditional opioid antagonists
- No respiratory depression
- No physical dependence
- No rewarding behavior in conditioned place preference
- No cross tolerance to morphine
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**Spared Nerve Injury**

Baseline vs. D7 Post-SNI withdrawal threshold over time:

- **Saline**
- **IBNtxA**

Data from Mogil and Weiskopf
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* *p<0.001
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Crystal Structure of the mouse mu opioid receptor

*Binding pocket: TM 3, 5, 6, 7*

*Note: The N- & C-termini have been truncated*

PMID: 22437502
3’- Alternative splicing of selected full length MOR-1 variants

Mouse *Oprm1* gene

Exon 1

mMOR-1
mMOR-1A
mMOR-1B1
mMOR-1B2
mMOR-1B3
mMOR-1B4
mMOR-1B5
mMOR-1C
mMOR-1D
mMOR-1E
mMOR-1F

These exons encode all 7TM domains and the binding pocket

These exons encode the variable intracellular C-terminus

LENLEAETAPLP
VRSL
KIDLF
KLMMWRAMPT
FKRHLAIMLSL
DN
TSRLQ
AHQKPQECCLK
CRCLSLTILVIC
LHFQHQFFIMI
KKNVS
CV
PTLAVSVAQIFT
GYPSPT7VEKP
CKSCMDRGMR
NLLPDGPQRGE
SGEQGLGR
RNEEPS
KKLDSQIYGC
VQHPV
APCACEVPANR
GQTKASDLLDEL
LETGSHQADAE
TNPGPYESKGC
AEPLAISLVPLY
**125I-BNtxA Binding in KO Mice**

- High affinity binding in triple KO mice
- High affinity binding in WT mice with blockade of traditional opioid receptors
- Loss of binding in E11 and E2 KO mice
- The target lacks MOR-1 exon 1 but contains E11 and E2

![Diagram showing MOR-1 splice variants and binding affinity](attachment:image.png)
Schematic of MOR-1 splicing in the mouse

Oprm1

- mMOR-1
- mMOR-1A
- mMOR-1B1
- mMOR-1B2
- mMOR-1B3
- mMOR-1B4
- mMOR-1B5
- mMOR-1C
- mMOR-1D
- mMOR-1E
- mMOR-1F
- mMOR-1H
- mMOR-1i
- mMOR-1J
- mMOR-1o
- mMOR-1P
- mMOR-1G
- mMOR-1K
- mMOR-1L
- mMOR-1M
- mMOR-1N
- mMOR-1Q
- mMOR-1R
- mMOR-1S
- mMOR-1T

Exon 11 12 1 13 14 2 3 15 5 4 10 6 7 8 9

7TM

6TM (lacks TM1/exon 1)

1TM (only has TM1)
### Selectivity of $^{125\text{I}}$-IBNtxA Binding in triple KO mouse brains

<table>
<thead>
<tr>
<th>Inactive Drugs ((K_i &gt; 1000) nM)</th>
<th>Drug</th>
<th>(K_i) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mu selective</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAMGO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxymorphone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxycodone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morpine-glucuronide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\beta)-Endorphin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meperidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Kappa(_1) selective</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U50,488H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DynorphinA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha)-Neoendorphin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Delta selective</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enkephalin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DADLE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPDPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNC80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Antagonists**                  |      |             |
| \(\beta\)-FNA                   |      | 36          |
| Naloxonone                       |      | 52          |
| Naltrexone                       |      | 21          |
| Diprenorphine                    |      | 2.2         |
| Levallophan                      |      | 0.34        |

| **Benzomorphans**                |      |             |
| Ketocyclazocine                  |      | 0.04        |
| (-)-SKF10,047                    |      | 14          |
| (-)-Ethylketocyclazocine         |      | 0.21        |
| Cyclazocine                      |      | 1.8         |

| **Kappa\(_3\)**                 |      |             |
| NalBzoH                          |      | 0.6         |
| Nalorphine                       |      | 3.7         |
| Levorphanol*                     |      | 8.8         |
| Buprenorphine*                   |      | 1.8         |
| Nalbuphine*                      |      | 3.5         |
| Butorphanol*                     |      | 2.9         |

*Used clinically as analgesics*
## Analgesia in Exon 11 MOR-1 KO

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED$_{50}$ (mg/kg)</th>
<th>Shift</th>
<th>$^{125}$I-BNtxA Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT C57</td>
<td>Exon 11 MOR-1 KO</td>
<td>Triple KO Ki (nM)</td>
</tr>
<tr>
<td>Morphine*</td>
<td>1.6</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>IBNtxA</td>
<td>0.53</td>
<td>&gt; 20</td>
<td>&gt;35</td>
</tr>
<tr>
<td>NalBzoH</td>
<td>22</td>
<td>&gt;100</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>41.8</td>
<td>&gt;200</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Ketocyclazocine</td>
<td>4.2</td>
<td>50</td>
<td>12</td>
</tr>
<tr>
<td>Levorphanol *</td>
<td>5</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>Butorphanol *</td>
<td>12.4</td>
<td>200</td>
<td>16</td>
</tr>
<tr>
<td>Buprenorphine*</td>
<td>0.2</td>
<td>&gt;10</td>
<td>&gt;50</td>
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</tbody>
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$^{125}$I-BNtxA Binding to MOR-1G / ORL$_1$ dimers

<table>
<thead>
<tr>
<th>Transfection</th>
<th>$^{125}$I-BNtxA Binding</th>
<th>Structure</th>
</tr>
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<tbody>
<tr>
<td>MOR-1G alone</td>
<td>None</td>
<td>6 TM</td>
</tr>
<tr>
<td>ORL$_1$ alone</td>
<td>None</td>
<td>7 TM</td>
</tr>
<tr>
<td>MOR-1G + ORL$_1$</td>
<td>$K_D$ 0.19 nM</td>
<td>Heterodimer</td>
</tr>
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</table>

![Graph showing the binding of IBNtxA to different transfections]
Opioid receptor diversity

Opioid receptor actions are complex, both at the pharmacological and molecular levels.

Receptor diversity can be achieved both by alternative splicing and dimerization.

Alternative splicing of the C-terminal of the full length variants may impact the composition of the receptor complex, its localization within the cell and within the brain and thereby define their functions.

When splice variants contain identical binding pockets, selectivity may be achieved by varying the intrinsic activity/efficacy of a drug at the target rather than by affinity.

Truncated variants can modulate full length MOR-1 variants or generate novel receptor targets through heterodimerization.

The molecular mechanisms of opioid receptor diversity may be revealing a generalized approach for generating receptor diversity among G-protein coupled receptors.
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