Receptor and blood-brain barrier characterization of opioid peptides in drug research & early development

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Drug Quality and Registration (DruQuaR) group
“Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. (The International Association for the Study of Pain)”

Three classes of pain:

- **Nociceptive**
- **Neuropathic**
- **Inflammatory**

! Opioid peptides are key role players in modulation of pain!

Endogenous + externally administered + other systems (e.g. CB)

Localization of opioid receptor expression

**MOR:** $\mu_1$, $\mu_2$, $\mu_3$

**KOR:** $\kappa_1$, $\kappa_2$

**DOR:** $\delta_1$, $\delta_2$

**NOR:** ORL$_1$

**OPIOID PEPTIDES**

**WIDE RANGE OF PHARMACOLOGICAL RESPONSES:**
- Pain and analgesia
- Stress and anxiety
- Tolerance and dependence
- Learning and memory
- Eating and drinking
- Endocrinology
- Mental illness and mood
- Neurological disorders
- Neurophysiology
- Gastrointestinal, renal and hepatic functions
- Cardiovascular
- Immunological

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Pain process:

- Pain perception
- Pain modulation:
  - Peripheral pain control
  - Central modulation of pain

OPIOIDS AND ITS (GPC) RECEPTORS

Role of opioid peptides in pain modulation:

Molecular recognition of opioid ligand-receptor interactions: In silico research.

MESSAGE-ADDRESS CONCEPT

<table>
<thead>
<tr>
<th>Receptor</th>
<th>MOR</th>
<th>KOR</th>
<th>DOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM3</td>
<td>Asp$^{147}$ Tyr$^{148}$</td>
<td>Asp$^{138}$ Tyr$^{139}$</td>
<td>Asp$^{128}$ Tyr$^{129}$</td>
</tr>
<tr>
<td>TM5</td>
<td>Lys$^{233}$ Phe$^{237}$</td>
<td>Lys$^{227}$ Phe$^{231}$</td>
<td>Lys$^{214}$ Phe$^{218}$</td>
</tr>
<tr>
<td>TM6</td>
<td>Val$^{300}$ Ala$^{304}$ His$^{297}$ Lys$^{303}$</td>
<td>Ile$^{294}$ Ala$^{298}$ His$^{291}$ Glu$^{297}$</td>
<td>Val$^{281}$ Thr$^{285}$ His$^{278}$ Trp$^{284}$</td>
</tr>
<tr>
<td>TM7</td>
<td>Trp$^{318}$ Gln$^{314}$</td>
<td>Tyr$^{312}$ Leu$^{309}$</td>
<td>Leu$^{300}$ Val$^{296}$</td>
</tr>
</tbody>
</table>

ADDRESS determines selectivity

MOR: polar interaction
KOR: salt bridge interaction
DOR: hydrophobic interaction

Molecular recognition of opioid ligand-receptor interactions: *In silico* research.

**Endomorphin-1: Tyr-Pro-Trp-Phe-NH$_2$**

- **Salt bridge:** Hydrogen-bond and electrostatic interaction
- **Hydrophobic pocket:** π-π interaction

<table>
<thead>
<tr>
<th>Opioid peptide</th>
<th>Message</th>
<th>Address</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endomorphin-1</td>
<td>Tyr</td>
<td>Pro Trp Phe-NH$_2$</td>
</tr>
<tr>
<td>Dynorphin(1-8)</td>
<td>Tyr</td>
<td>Gly-Gly Phe Leu-Arg-Arg-Ile</td>
</tr>
<tr>
<td>Leu-enkephalin</td>
<td>Tyr</td>
<td>Gly-Gly Phe Leu-NH$_2$</td>
</tr>
</tbody>
</table>

Two types of endogenous peptides – message sequence:

1. **Tyr-Gly-Gly-Phe**
   - Enkephalins, endorphins, dynorphins

2. **Tyr-Pro-Phe/Trp**
   - Endomorphins

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Structure-activity/selectivity relationship for opioid receptors.

1. Structural characteristics determine activity
   - Carboxamide C-Terminal (for μ)
   - Acid C-Terminal (for δ)
   - δ-Selectivity Pocket
   - μ-Selective Pocket
   - Dual μ-agonist/δ-antagonist profile of TIPP-NH₂

2. Interplay with other receptors
   - Binding to other receptors, e.g. TLR4/MD2
     - Docking of remifentanyl and its metabolite
     - Heterodimerization:
       - Change the endocytosis/recycling kinetics receptor
       - Allosterically enhancement of ligand binding capacities of the other partner
       - Functional modulation signaling pathways

3. Bioavailability of peptide therapeutics
   - Vasopressin
   - Desmopressin
     - enhanced membrane permeation and enzymatic stability

**In vitro** opioid receptor binding assays: peptide purity level.

- 46 peptides from one supplier (PPR)
- CoA specification ≥ 90 % HPLC purity
- Observation: 30% of the peptides < 90 %

**Also:** 1 peptide from ≠ suppliers: problematic!

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Developments</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLB</td>
<td>No immobilization</td>
<td>Labelling</td>
<td>New RLB method to measure the concentration of non-radiolabeled drugs in the brain (ex vivo)</td>
<td>Investigation supraspinal cross-talk between MOR and DOR: enhanced sensitivity to antinociception induced by MOR agonists</td>
</tr>
<tr>
<td>NMR</td>
<td>Specific binding</td>
<td>Structure determination at atomic resolution</td>
<td>Characterization of stability of backbone hydrogen (using a newly developed pressure cell)</td>
<td>Binding characterization of linear, cyclic monomer and dimer of enkephalin</td>
</tr>
<tr>
<td>ITC</td>
<td>Label-free and no immobilization</td>
<td>Relatively poor sensitivity</td>
<td>Nano ITC, e.g. analysis of Binding Organic Compounds to Nanoparticles by Isothermal Titration Calorimetry</td>
<td>Complexation of the opioid peptide tynorphin and dipeptidyl peptidase III (ΔS dominated process)</td>
</tr>
<tr>
<td>SPR</td>
<td>Real time</td>
<td>Upper limit for protein size</td>
<td>Requires relatively high [sample]</td>
<td>Real-time kinetic analysis of $K_D$-value for label-free therapeutic antibody on native cell surface antigens</td>
</tr>
<tr>
<td>SAW</td>
<td>Label-free</td>
<td>Non-specific binding</td>
<td>Immobilization</td>
<td>DOR membrane preparations</td>
</tr>
</tbody>
</table>

**Advantages**
- No immobilization
- Specific binding

**Disadvantages**
- Labelling
- Relatively poor sensitivity
- Upper limit for protein size

**Developments**
- New RLB method to measure the concentration of non-radiolabeled drugs in the brain (ex vivo)
- Characterization of stability of backbone hydrogen (using a newly developed pressure cell)
- Nano ITC, e.g. analysis of Binding Organic Compounds to Nanoparticles by Isothermal Titration Calorimetry

**Application**
- Investigation supraspinal cross-talk between MOR and DOR: enhanced sensitivity to antinociception induced by MOR agonists
- Binding characterization of linear, cyclic monomer and dimer of enkephalin
- Complexation of the opioid peptide tynorphin and dipeptidyl peptidase III (ΔS dominated process)
Tissue organ bath (TOB) experiments: “Golden standard”

- **Isolated tissues for opioid activity:**
  - Mouse vas deferens ($\delta \gg \mu > \kappa$)
  - Guinea pig ileum ($\mu > \kappa >> \delta$)

- **Advantages:**
  - Relatively small amount of test material
  - Tissue responses (incl. adsorption, penetration, metabolization)
  - Identification receptor target using antagonists

- **Disadvantages:**
  - Non-specific binding
  - Adsorption to glass

- **Advances – mimicking in-vivo functionality:**
  - Bi-ventricular working heart system
  - Whole lung decellularization/recellularization chamber for regenerative medicine
Impurity profile of peptides

Crude purity

> 95 % purity

Guinea pig ileum

Stability profile of peptides.

<table>
<thead>
<tr>
<th>Specification</th>
<th>SBO121</th>
<th>SBO215_7</th>
<th>SBO397</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide purity</td>
<td>100 %</td>
<td>97.4 %</td>
<td>100 %</td>
</tr>
<tr>
<td>n_{impurity peaks}</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Level max impurity</td>
<td>N/A</td>
<td>2.6 %</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Reference sample</th>
<th>Test sample</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery main peak</td>
<td>61.3 %</td>
<td>26.3 %</td>
<td>92.3 %</td>
</tr>
<tr>
<td>n_{impurity peaks}</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Level max impurity</td>
<td>N/A</td>
<td>71.4 %</td>
<td>N/A</td>
</tr>
<tr>
<td>Mass balance</td>
<td>61.3 %</td>
<td>99.7 %</td>
<td>92.3 %</td>
</tr>
<tr>
<td>Conclusion</td>
<td>Adsorption</td>
<td>Degradation</td>
<td>Stable</td>
</tr>
</tbody>
</table>

N/A: not applicable, as only one peak is observed due to the main peak present
Test sample: 10 min exposure to tissue organ bath with isolated guinea pig ileum

QUALITY OF PEPTIDES IS AN IMPORTANT ASPECT TO AVOID FALSE FUNCTIONALITY CONCLUSIONS

Pain management within the CNS: Opioid peptides should cross the BBB.

MOR main target pain management: Pharmacokinetic properties

**B BB penetration mechanisms:**
- Influx ($K_{in}$, PS, P)
- Efflux ($K_{out}$, $t_{1/2 \text{ brain}}$)

**Brain distribution mechanisms:**
- Metabolic clearance ($t_{1/2 \text{ brain}}$)
- Plasma protein binding (Free vs. bound)
- Non specific binding
- Clearance to ECF and CSF

**BBB kinetics vs. central nervous system functionality:** BBB$^{+/−}$ vs. CNS$^{+/−}$

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Penetration =
Permeation (rate): $P_{\text{app}}, K_{\text{in}}$
Distribution (extent): $B/P$, $K_p$

1 technique: ≠ responses
1 response: ≠ techniques

Brainpeps database: [www.brainpeps.ugent.be](http://www.brainpeps.ugent.be)

Brainpeps® is a resource of blood-brain barrier peptides, developed by the University. Based upon your input of physicochemical properties and receptor properties, the blood-brain barrier peptide database is automatically correlated to each other.

Reference: [BibTeX]

Citation
If you are using or downloading data from the database, please cite "Van Dorpe S., Bronselaer A., Nielandt J., Stalmans S., Wynendaele E., Audenaert K., Van De Wiele C., Burvenich C., Peremans K., Hoehnou H., De Tré G., De Spiegeleer B. Brainpeps: The blood-brain barrier peptide database. Brain Structure and Function, 217 (3), 687-718".

General Information
- Data submission to Brainpeps
- Contact us

Brainpeps database: www.brainpeps.ugent.be

Drugability of opioid peptides: one global score which includes different characteristics.

Drugability reflected in a desirability value:

- **Receptor affinity:** $K_D$
- **Metabolic stability:** plasma vs. brain
- **BBB behavior:** influx vs. efflux

\[
D = \sqrt[n]{\prod_{i=1}^{n} d(Y)^{p_i}}
\]

\[
d(Y) = \frac{Y_i - 0.9Y_{\min}}{1.1Y_{\max} - 0.9Y_{\min}}
\]

<table>
<thead>
<tr>
<th>Peptide</th>
<th>D-value</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB Aba</td>
<td>0.956</td>
<td>1</td>
</tr>
<tr>
<td>P41</td>
<td>0.929</td>
<td>2</td>
</tr>
<tr>
<td>P42</td>
<td>0.867</td>
<td>3</td>
</tr>
<tr>
<td>Dermorphin</td>
<td>0.711</td>
<td>4</td>
</tr>
<tr>
<td>EM-1</td>
<td>0.566</td>
<td>5</td>
</tr>
<tr>
<td>EM-2</td>
<td>0.551</td>
<td>6</td>
</tr>
<tr>
<td>TAPP</td>
<td>0.549</td>
<td>7</td>
</tr>
<tr>
<td>DAMGO</td>
<td>0.486</td>
<td>8</td>
</tr>
<tr>
<td>CTOP</td>
<td>0.448</td>
<td>9</td>
</tr>
<tr>
<td>TAPS</td>
<td>0.422</td>
<td>10</td>
</tr>
<tr>
<td>P43</td>
<td>0.224</td>
<td>11</td>
</tr>
<tr>
<td>CTAP</td>
<td>0.214</td>
<td>12</td>
</tr>
</tbody>
</table>

Determination of the overall antinociceptive activity of opioid peptides.

Tail-flick

Hot plate test

1. **STATUS PEPTIDES (2010)**

- **334 therapeutic peptides**
- **Development** 40%
- **Discontinued** 43%
- **Withdrawn** 2%
- **Approved** 15%

**Challenge for opioid peptides:**
analgesic activity lacking the dependence liability
2. TO STORE IN THE HIPPOCAMPUS (long-term memory)

- Dissection of pharmacological roles and interplay of “systems” (pain-immune)

- Receptor affinity and selectivity (biological activity): message-address related, as well as metabolic stability and BBB behavior => DRUGABILITY

- Quality and stability of peptides is an important aspect to avoid false functionality conclusions in opioid-peptide research
THANKS FOR YOUR ATTENTION

Hey! We want in!

I am sorry, you cannot enter the brain!

To the brain

http://faculty.washington.edu/chudler/cart.html