Other Neurotransmitter/Receptors: Neuroimmune implications

Opioid, Purinergic & Toll-like Receptors
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Neurochemistry and Neuropharmacology
(BioMed 532) Fall 2014 (3 credits)
Course Director: Kevin Caldwell
Tuesdays and Thursdays 10:00 – 11:30 A. M.
BMSB 243
Lecture Objectives

I. Opioids
   A. Pain inhibition
   B. Endogenous opioids
   C. Opioid receptors & morphine actions

II. Purinergic Receptors
    A. Classification
    B. Regulation of extracellular ATP
    C. Cellular localization

III. Toll-like Receptors
    A. Receptor structure
    B. Adaptor proteins and signaling pathways
    C. Ligands
Drugstore Analgesics (Pain relievers)

Aspirin: Known for centuries; originally isolated from *spiria ulmaris* (from which *Aspirin* comes from); Bayer figured out how to make in the mid-1800’s; action: Prostaglandin synthesis inhibitor

Ibuprofen & Nalproxen (propionic acid derivatives) act similarly to prevent prostaglandins – differ in their biodistribution, i.e. what tissues they concentrate in, how they are broken down and the side effects
Local Anesthetics

Cocaine: The first local anesthetic discovered

Procaine: first (1905) synthetic cocaine substitute; lidocaine, novacaine etc followed. The “-caine” ending refers to their similarity to cocaine.

Mechanism of Action: Block nerve conduction by binding to a specific receptor site within the sodium channel, thereby physically blocking it (blockade of NaV channels)
Morphine

Opium ("juice"): >4,000 yr old; 20+ pain suppressive compounds including morphine

Morphine: named for Greek god of dreams; synthesized in 1805; legal in the US until early 1900’s (major constituent of patent medicine in old wild west times)

Addiction liability: search for non-addicting alternatives - found Heroin, Methadone & Naloxone but no non-addictive agonist
Endogenous opioid peptides and opioid receptors

<table>
<thead>
<tr>
<th>Major Groups of Endogenous Opioid Peptides</th>
<th>Opioid Receptor (Highest affinity)</th>
<th>CNS Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endorphins</td>
<td>µ (mu)</td>
<td>Brain, brainstem (PAG, medulla), spinal cord</td>
</tr>
<tr>
<td>Enkephalins</td>
<td>δ (delta)</td>
<td>brainstem (PAG, medulla), spinal cord</td>
</tr>
<tr>
<td>Dynorphins</td>
<td>κ (kappa)</td>
<td>brainstem (PAG, medulla), spinal cord</td>
</tr>
</tbody>
</table>

Mu opioid receptor distribution
How might one activate the brain’s morphine-like transmitter system without giving morphine?

Peri-Aqueductal Gray (PAG): First site of electrical stimulation induced analgesia (Reynolds, 1968)
Descending modulation of Pain

Stimulation of the PAG neurons:
  a. Axons from PAG descend to neurons in the Ventral Medial Medulla (a.k.a Raphe Nuclei)
  b. Ventral Medial Medulla axons descend to the spinal cord & inhibit pain in 3 ways:

1. **Excite** lamina II neurons
2. Directly **inhibit** postsynaptic 2\textsuperscript{nd} order lamina I & V neurons
3. Presynaptic **inhibition** of A\textgreek{d} & C nociceptors
The job of many Lamina II Neurons is to **inhibit** 2\textsuperscript{nd} order pain projection neurons within the spinal cord.

Inhibitory lamina II interneurons become activated:

a. Inhibit pain neurons in the **spinal cord dorsal horn**.

b. Shown here, A\(\delta\) and C fibers relay their information to nociceptive neurons in spinal lamina I and V.

c. Lamina I and wide dynamic range neurons in lamina V are inhibited by lamina II neurons.

Spinal cord circuitry provides a good example. **Descending** fibers can inhibit incoming pain signals at the level of the spinal cord.
Opioids like morphine act on opioid receptors in the brainstem and spinal to create pain inhibition (analgesia)

- Activation of PAG, which projects to and activates the Ventral Medial Medulla (a.k.a Raphe Nuclei)
- Ventral Medial Medulla axons descend to the spinal cord & inhibit pain in 3 ways:
Opioid Receptor: G\textsubscript{i} Protein Function

A. Basal Conditions

- Basal conditions
- GTPase activity
- GTP binding
- GTPase activity
- Net Inhibition

B. Initial Opioid Receptor Activation

- Initial opioid receptor activation
- Functionally active

C. GTP binding

- GTP binding
- Functionally active

D. = Net Inhibition

E. GTPase activity

- GTPase activity
- GTP
- GDP

Opioid receptors alter 2 general types of effector proteins:
1. Ion channels
2. Enzymes that regulate the generation of 2\textsuperscript{nd} messengers

Morphine induces α-1 subunit dissociation from the β-γ subunits. β-γ bind presynaptic voltage-gated Ca\textsuperscript{2+} channels. Function is inhibited and transmitter release is inhibited.
Molecular Adaptations Following Opioid Exposure

Initial Opioid binding

- AC VIII
- AC I
- α
- βγ
- GTP
- K+
- VGCC
- GIRK

- cAMP
- PKA
- Catalytic
- Type II Reg Unit

- Regulation of Numerous Cellular processes
- Reduced neurotransmitter release

VGCC: Voltage gated Ca^{2+} channels
GIRK: G protein dependent
Inwardly rectifying K+ channels

Altered gene expression
Nucleus

CREB
Opioids like morphine act on opioid receptors in the brainstem and spinal to create pain inhibition (analgesia).

But, how does morphine cause activation when it activates an inhibitory G protein?
GABAergic tonic inhibition is lifted (Disinhibition)

On cells

Decreased excitation of pain facilitatory LI and LII neurons

Increased excitation of inhibitory LII interneurons (majority)

Off cells

Direct opioid inhibition

opioid

On cells

Mu opioid receptor

GABAergic neurons

Off cells

GABAergic neurons

PAG

Pons

(Locus ceruleus)

Raphe Nuclei

a.k.a. VMM

(in Medulla)

Spinal Cord
Morphine or Opioid Pharmakokinetics

- 1\textsuperscript{st} pass elimination upon oral administration.

  Parenteral route: elsewhere other than mouth or alimentary canal; e.g. i.m., i.v., sub. Q., intrathecal.

**Morphine**: high 1\textsuperscript{st} pass elimination, so taking orally will result in lower efficacy.

Some synthetics opioid analgesics have low 1\textsuperscript{st} pass elimination, so oral route remains effective; e.g. methadone
Morphine Metabolism

- Converted to polar metabolites; glucuronic acid. Excreted by the kidneys.
- Example: Morphine conjugates to morphine-3-glucuronide = active metabolite.
- Also, conjugates to morphine-6-glucuronide = active metabolite.
- M3G & M6G poorly cross BBB, but at high doses, can have CNS effects.
- Patients with renal (kidney) failure cannot filter blood - this leads to high circulating levels.
- M3G = neuroexcitatory properties (seizures) via GABA/Gly system.
- M6G = enhanced opioid actions.
- Hydromorphone is metabolized similarly (e.g. H3G).

Opioid Classifications:
- Phenanthrene
- Phenylheptylamine
- Phenylpiperidine
- Morphanins
- Benzomorphan
Glial & Neuronal Purinergic Receptor Functions

Nucleotides – particularly ATP – are well known for their function as a universal energy currency.
P1 Purinergic Receptor

- A nucleotide receptor activated by adenosine, which is a metabolite of ATP.
- Is a G protein receptor activated by adenosine
- 4 different subtype receptors widely expressed on neurons, astrocytes, oligodendrocytes & microglia:
  - $A_1$
  - $A_{2A}$
  - $A_{2B}$
  - $A_3$

Adenosine is generated from extracellular ATP through the actions of the ectonucleoside, CD39. Conversion of ATP or ADP to AMP.
AMP is then enzymatically converted to Adenosine via the ectonucleoside, CD73.
Adenosine signaling is terminated by the uptake of extracellular adenosine toward the intracellular compartment through equilibrative nucleoside transporters.
Then, intracellular adenosine is metabolized to inosine by the enzyme, adenosine deaminase.
P2 receptors are activated by ATP, ADP &/or UTP.
Two major subdivisions: P2Y (metabotropic) & P2X (ionotropic)
All 7 P2X receptor subtypes are expressed on neurons and astrocytes
All are involved in either: (1) fast synaptic transmission, (2) synaptic plasticity, or (3) neuronal-glial signaling

More About ATP

• In neurons, released by presynaptic terminals and post-synaptic membranes
• Glia, like astrocytes, contain the machinery necessary to release ATP
• Can contribute to CNS pathology (hypoxia from stroke). E.g. cell dies and generates millimolar ATP concentration in the extracellular milieu.
• Ultimately activates either protective or harmful mechanisms
Regulation of Extracellular ATP by Ectonucleotidases

- 4 main groups:
  - 1. Ectonucleoside triphosphate diphosphohydrolases (NTPDases)
  - 2. Ecto-5’-nucleotidase (CD73)
  - 3. Ectonucleotide pyrophosphatase/phosphodiesterases
  - 4. Alkaline phosphatases

The NTPDases represent a family of ubiquitously expressed membrane-bound enzymes

Pause to Consider: the complementary roles of P1 and P2 receptors may have in the context of these ectonucleotidases

- NTPDase1, which is expressed in many tissue systems, catalyzes the conversion of ATP (and ADP) down to AMP. Without ATP available, the P2 purinergic receptors wont activate.
- a different ectonuclease, NTPDases CD73, catalyzes the conversion of AMP to adenosine, so extracellular ATP and P2R activation is fully terminated.

- It turns out, there is good evidence that adenosine-purinergic P1 receptor signaling dampens acute inflammation and further tissue injury, which can oppose the inflammatory functions that are set into motion by P2 receptors.

Yes, P2 receptors are strongly implicated in inflammation …
P2Y & P2X Receptors Mediate Inflammatory or Wound Healing Processes

# P2Y

<table>
<thead>
<tr>
<th>Glia (Astrocytes, Microglia, Oligos)</th>
<th>Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2Y1 (ADP) Gq proteins – PLC (less so on microglia)</td>
<td>*P2Y1 (ADP) Gq proteins - PLC</td>
</tr>
<tr>
<td>P2Y2 (ATP/UTP) Gq proteins - PLC</td>
<td>P2Y2 (ATP/UTP) Gq proteins - PLC (Schwann cells, Oligos, Astocytes)</td>
</tr>
<tr>
<td>*P2Y4 (ATP/UTP) Gq proteins – PLC</td>
<td>P2Y4 (ATP/UTP) Gq proteins - PLC</td>
</tr>
<tr>
<td>P2Y7 *Microglia</td>
<td></td>
</tr>
<tr>
<td>P2Y6 (UDP) Gq proteins - PLC</td>
<td>P2Y6 (UDP) Gq proteins - PLC</td>
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<tr>
<td>P2Y11 (ATP) (Oligo.)</td>
<td>P2Y11 (ATP)</td>
</tr>
<tr>
<td>P2Y12 (ADP) *Microglia; Gi proteins – reduced AC &amp; cAMP</td>
<td>P2Y12 (ADP) Gi proteins – reduced AC &amp; cAMP</td>
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<tr>
<td>P2Y13 (ADP) Gi proteins – reduced AC &amp; cAMP</td>
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<tr>
<td>P2Y14 (UDP-sugars); Gi proteins – reduced AC &amp; cAMP</td>
<td>P2Y14 (UDP-sugars) Gi proteins – reduced AC &amp; cAMP</td>
</tr>
</tbody>
</table>

* Predominantly expressed
<table>
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<th>Glia</th>
<th>Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2X1 Astrocytes f.</td>
<td></td>
</tr>
<tr>
<td>P2X2 Astrocytes</td>
<td>P2X2: in cortex, hippo., cerebellum, spinal cord</td>
</tr>
<tr>
<td>P2X3 Astrocytes</td>
<td>P2X3 in DRG and spinal cord</td>
</tr>
<tr>
<td>P2X4 Astrocytes, *Microglia</td>
<td>P2X4: in cortex, hippo., cerebellum, spinal cord</td>
</tr>
<tr>
<td>P2X5 Astrocytes f.</td>
<td></td>
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<tr>
<td>P2Y6</td>
<td></td>
</tr>
<tr>
<td>P2X7 Astrocytes f. *Microglia</td>
<td>P2X7: presynaptic terminals, influence neuron activity</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>P2Y12</td>
<td></td>
</tr>
<tr>
<td>P2Y13 (ADP) G1 proteins – reduced AC &amp; cAMP</td>
<td></td>
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<tr>
<td>P2Y14 (UDP-sugars)</td>
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</table>

* Predominantly expressed
The Coordinated Action of Glial & Neuronal Purinergic Receptors in the CNS

TLR Receptors
Emphasis on TLR4
TLR Structure

Ectodomain

Important for PAMP recognition

TIR is about 160 amino acids long
Cytosolic region – activation of downstream signaling pathways

TLRs: 1, 2, 4, 5, & 6 at plasma membrane
TLRs 3, 7, 8, & 9 at intracellular acidic endosomes
A single pathogen can activate multiple TLRs. Ex: Salmonella.
Salmonella contains lipoproteins, LPS, flagellins and CpG unmethylated DNA

FOUR MAJOR ADAPTER PROTEINS
1. MyD88: myeloid differentiation primary response gene 88
2. MAL: MyD88 adapter like. = TIRAP: TIR domain-containing adapter protein
3. TRIF: TIR domain-containing adapter protein inducing IFN-β. = TICAM1: TIR domain-containing adapter molecule 1
4. TRAM: TRIF-related adapter molecule. = TICAM2: TIR domain-containing adapter molecule 2

<table>
<thead>
<tr>
<th>PRR</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Borrelia burgdorferi, neisseria, lipoproteins (mycobacteria)</td>
</tr>
<tr>
<td>TLR2</td>
<td>Trypanosomes, mycoplasma, borrelia, listeria, HSV, zymosan (yeast), lipoteichoic acid peptidoglycan (Gram +), lipoproteins (mycobacteria), Lipopolysaccharide (LPS) (Gram-), glycolipids, HSP 70,</td>
</tr>
<tr>
<td>TLR3</td>
<td>Viral double-stranded RNA, Poly I:C</td>
</tr>
<tr>
<td>TLR4</td>
<td>Plant product taxol, mycobacteria, respiratory syncytal virus, fibronogen, bacterial LPS (Gram-), HSP90, HSP 70, HSP 60</td>
</tr>
<tr>
<td>TLR5</td>
<td>Bacterial flagellins</td>
</tr>
<tr>
<td>TLR6</td>
<td>Zymosan (fungi), lipopeptides (mycoplasma), lipotechoic acid, diacyl lipopeptides</td>
</tr>
<tr>
<td>TLR7</td>
<td>Single-stranded RNA, imidazole quinolines e.i.: Imiquimod, guanosine nucleotides</td>
</tr>
<tr>
<td>TLR8</td>
<td>Single-stranded RNA, imidazole quinolines e.i.: Imiquimod, guanosine nucleotides</td>
</tr>
<tr>
<td>TLR9</td>
<td>Bacterial DNA, viral DNA, CpG oligonucleotides (synthetic)</td>
</tr>
<tr>
<td>TLR10 (mouse)</td>
<td>Unknown ??</td>
</tr>
<tr>
<td>TLR11 (mouse)</td>
<td>Bacterial components from uropathogenic bacteria</td>
</tr>
<tr>
<td>TLR12 (mouse)</td>
<td>Unknown ??</td>
</tr>
<tr>
<td>TLR13 (mouse)</td>
<td>Unknown ??</td>
</tr>
<tr>
<td>NOD1 &amp; NOD2</td>
<td>Bacterial peptidoglycans</td>
</tr>
<tr>
<td>Macrophage mannose receptor</td>
<td>Sulfated sugars, mannose, fucose,, galactose modified polysaccharides and proteins</td>
</tr>
<tr>
<td>Type 3 complement receptors</td>
<td>Zymosan cell wall particles, beta-glucan</td>
</tr>
</tbody>
</table>
TLR4 Pathway

**Cellular Expression:**
1. Microglia
2. Astrocytes
3. Neurons

**Activated by:**
1. Cell stress (HSP)
2. Damage
3. Death

What might their role be in Neurodegeneration?

TRIF-dependent signaling

Results from TLR3 & TLR4 activation only.
TLR4 in the CNS

- Observed in microglia, astrocytes, endothelial cells and neurons
- Role in regulating cellular development, differentiation, survival; e.g. neuroprotective in stroke and Alzheimer’s
- HSP released from damaged cells (neurons & glia) trigger TLR4 activation
- Similar to the response to primary (first time exposure), LPS TLR4-induced HSP60 causes proinflammatory microglial responses. Neurons further produce HSP60 creating a feed-forward cycle. HSPs are endogenous danger signals.
<table>
<thead>
<tr>
<th>Putative TLR4 Interactor</th>
<th>Explanation</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopolysaccharide (LPS) and LPS derivatives</td>
<td>Outer cell wall component of gram-negative bacteria; potent initiator of TLR4 signaling; LPS structure varies with bacterial species</td>
<td>Structure–activity relationship of LPS and TLR4 (Park et al. 2008), of LPS (Rietschel et al. 1994)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Polyphenol found in the plant Curcuma longa. Inhibits TLR4 by binding MD-2</td>
<td>(Youn et al. 2006)</td>
</tr>
<tr>
<td>Cinemaldehyde (3-phenyl-2-propanoal)</td>
<td>Anti-inflammatory, inhibits ligand-induced TLR4 oligomerization and downstream signaling</td>
<td>(Youn et al. 2006)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Appears to redistribute TLR4 complexes on the cellular membrane by preventing receptor association and/or dimerization in the lipid raft</td>
<td>(Blanco et al. 2005, 2008; Szabo et al. 2007; Fernandez-Lizarbe et al. 2008)</td>
</tr>
<tr>
<td>E5564 (citrinin)</td>
<td>LPS analogue clinically tested for sepsis; inhibits TLR4 signaling</td>
<td>(Rossignol et al. 2004; Yamada et al. 2005; Kim et al. 2007)</td>
</tr>
<tr>
<td>Opioids</td>
<td>Both opioid stereoisomers alter downstream TLR4 signaling. Opioid agonists (e.g. morphine) have different effects than antagonists (e.g. naloxone)</td>
<td>(Liu et al. 2000; Hutchinson et al. 2007, 2009b; Juni et al. 2007)</td>
</tr>
<tr>
<td>TAK-242 (Ethyl(6R)-6-[N-(2-chloro-4-fluorophenyl) sulfamoyl]cyclohex-1-eno-1-carboxylate)</td>
<td>Clinically tested cyclohexene derivative, selectively inhibits intracellular signaling by TLR4</td>
<td>(Li et al. 2006; Sha et al. 2007; Takashima et al. 2009)</td>
</tr>
<tr>
<td>Paziflur (Taxol)</td>
<td>Widely used cancer therapeutic, reported to inhibit MD-2, thereby knocking down TLR4 activity which was found to correlate with drug efficacy</td>
<td>(Wang et al. 2009)</td>
</tr>
<tr>
<td>Rosmarin (trans-3,5,4-trihydroxystilbene)</td>
<td>Antioxidant reported to inhibit TLR4 signaling; found in the skin of grapes, it is known for anti-inflammatory and anti-carcinogenic effects</td>
<td>(Youn et al. 2006; Yusuf et al. 2009)</td>
</tr>
<tr>
<td>Statins</td>
<td>Statin drugs influence TLR4-mediated cytokine expression through a Rho-protein feedback mechanism</td>
<td>(Konot et al. 2008)</td>
</tr>
<tr>
<td>Amyloid-β 42 peptide</td>
<td>The peptide hallmark of Alzheimer's pathogenesis, appears to activate TLR4 directly and also through signals from damaged neurons (e.g. 4-hydroxynonenal)</td>
<td>(Liu et al. 2002b; Tang et al. 2006; Balisteri et al. 2007, 2009)</td>
</tr>
<tr>
<td>Extracellular matrix proteins</td>
<td>Negatively charged glycoproteins are reported to activate TLR4 signaling</td>
<td>(Schaefer et al. 2005; Smiley et al. 2001; Okamura et al. 2001; Midwood et al. 2006)</td>
</tr>
<tr>
<td>Fatty acids*</td>
<td>Fatty acids are reported to regulate TLR4 receptor dimerization and recruitment into lipid rafts</td>
<td>(Weatherill et al. 2005; Wong et al. 2009)</td>
</tr>
<tr>
<td>Heat-shock proteins (HSP) 60, 70, 90</td>
<td>Released from dead or dying cells. HSP60 mediates neurodegeneration via TLR4 (Lehnardt et al. 2008). HSP90 may influence TLR4 pain amplification (Hutchinson et al. 2009b). LPS contamination is a common problem in HSP studies</td>
<td>HSP60 (Lehnardt et al. 2008); HSPs 70, 90 (Traintallou and Traintallou 2004; Hutchinson et al. 2009b). Contamination, (Tsan and Gao 2004b)</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>Heparin sulfate and endogenous hyaluronic acid fragmentation products may activate dendritic cells and macrophages through TLR4</td>
<td>(Termeer et al. 2002)</td>
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</table>
This completes ‘Other Neuropeptides & Non-classical CNS Receptor Systems: Potential for Neuroimmune Regulation Part I