Receptor pharmacology in neuroscience practice
Lecture 2: things that confound our newfound knowledge
Of pharmacology.....
A ligand cannot have an effect unless it occupies the receptor
agonist
antagonist
inverse agonist

The degree of occupancy is determined by **affinity** (and PK in vivo)

Once it has occupied a receptor, the magnitude of the effect is determined by **efficacy**

The **potency** of a ligand is therefore a function of **both** affinity and efficacy

- High affinity, low efficacy
- Low affinity, high efficacy

= Where potency doesn’t match affinity

With receptor classes with more than one member one also must be cognizant of **specificity**, especially when more than one member of the class is in your system
So what can confound our new knowledge of pharmacology?

1. Allosteric modulators

2. Receptor oligomerization

3. Receptor desensitization/downregulation

4. Biased agonism
1. Allosteric modulators: definition

A ligand that binds to a site other than that for the endogenous ligand

There are two kinds of allosteric modulators

A) positive modulators enhance the effects of agonists
   e.g. benzodiazepines and ethanol at the GABA-A receptor

B) negative modulators inhibit the effects of agonists
   e.g. strychnine at the Glycine receptor

There are two distinct ways in which an allosteric modulator can change the effects of an agonist
1. By changing affinity of the agonist

2. By changing the efficacy of the agonist
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Some receptors are obligate dimers/oligomers

All ion channels—subunit composition can dramatically change pharmacology
important for “plasticity” (LTP, LTD)
important for “selectivity”

Among GPCRs obligate dimers include mGluRs, GABA-B, and taste receptors

Mounting evidence that many GPCRs are dimers/oligomers,
both homomers and heteromers
The MOR crystallizes as a dimer

Manglik et al, 2012, Nature
1. Allosteric effects of one ligand on the others’ affinity
2. Generation of binding pockets with altered affinity
3. Heteromers that couple to different G proteins than their homomers
4. Receptor dimers with different desensitization/downregulation properties
So what can confound our new knowledge of pharmacology?

1. Allosteric modulators

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3. Receptor desensitization/downregulation

4. Biased agonism
Desensitization:

Receptors are present (and bind ligand) but no longer transduce signal to their downstream effector(s)

Downregulation:

Actual loss of receptor number

How would you distinguish between these possibilities? Will a functional dose response tell you?

Consider how the amount of “receptor reserve”, or “spare receptor” would influence your ability to detect either of these phenomena
Most GPCRs are desensitized (and endocytosed) after activation.

Most are also rapidly recycled/resensitized.

Some are not recycled/resensitized but instead are degraded/downregulated.
The mu opioid receptor resensitizes

On the other hand, D2 responses do not resensitize.
Endocytosis

Most GPCRs desensitized (and endocytosed) after activation

Most are also rapidly recycled/resensitized

Some are not recycled/resensitized but instead are degraded/downregulated

Different ligands have different intrinsic abilities to drive: desensitization, endocytosis, downregulation

Not always linearly related to efficacy at G protein
So what can confound our new knowledge of pharmacology?

1. Allosteric modulators
2. Receptor oligomerization
3. Receptor desensitization/downregulation
4. Biased agonism
A single ligand can be an agonist, partial agonist, antagonist or inverse agonist depending on the effector that is being measured.

**Functional Selectivity/Biased Agonism**

\[
L + R \xleftarrow{} LR^* \xrightarrow{} Effector 1 = Effector 2
\]

\[
L + R \xleftarrow{} LR^* \xrightarrow{\neq} G \text{ protein arrestin/endocytosis}
\]
The mu opioid receptor shows “biased agonism”!

How to distinguish “biased agonism” from partial agonism?

Keith et al, JBC 1996
How to distinguish “biased agonism” from partial agonism?
Does a change in bias matter?

What are the key caveats to these experiments?

Morphine  Methadone  Enkephalin  
All mu opioid receptor agonists for G protein  And all are analgesics

Analgesic Tolerance

Physical Dependence/ Withdrawal

Enkephalin (0.5 nmol)
Morphine (30 nmol)
The “cleanest” way to examine the importance of biased agonism is to selectively change only the receptor and only in response to the biased drug.
What else might have changed?
There is no difference in affinity or potency in wild type and mutant...
The mu opioid receptor shows biased agonism

Keith et al, JBC 1996

What are the functional consequences?

No treatment

Enkephalin

Morphine

“R”

“R∗”

“R∗∗”

L + R ↔ LR∗ → G protein arrestin/endocytosis
Does a change in bias matter?

WT + Morphine

mutant + Morphine

Analgesia --improved (morphine)
Tolerance --reduced
Dependence --reduced

Can we leverage this information to make better analgesics?

Opioid drugs with potency/efficacy/PK of morphine AND the ability to engage arrestin/promote receptor endocytosis will have excellent analgesic efficacy and reduced liability for the side effects of tolerance and dependence.

None of the current opioid drugs meet these criteria....
Can we use the dimeric nature of the MOR to alter morphine-induced endocytosis?
Methadone promotes morphine-induced endocytosis

He et al, Curr Biol 2005
Methadone, in trans, promotes morphine-induced endocytosis of MOR.

Occupancy of “all” protomers by agonist is required.
A sub-analgesic dose of Methadone prevents morphine tolerance

He et al, Curr. Biol 2005
Sub-analgesic methadone reduces morphine withdrawal

He et al, Curr. Biol 2005
When the bias is changed, morphine retains its “beneficial effects”: Analgesia
But not its “side effects”: Tolerance, Dependence

*Are there other new chemical entity opioids with the “right” profile?

The NCEs in development actually have a bias AGAINST arrestin recruitment
TRV130 is a G protein biased MOR selective agonist
TRV130 is a potent analgesic (more potent than morphine) with a similar affinity as morphine.

What does this tell us about the intrinsic efficacy of TRV130? What about receptor occupancy at therapeutically relevant doses?
TRV130 is a G protein biased MOR selective agonist with reduced gastrointestinal dysfunction.
...And reduced respiratory dysfunction at equi-analgesic doses

What is the difference in receptor occupancy under these conditions?
The best “ligand bias” may depend on the indication

For chronic pain, the primary liabilities are analgesic tolerance and dependence

For post surgical pain, the primary liabilities are constipation and respiratory suppression
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antagonist
inverse agonist

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With receptor classes with more than one member one also must be cognizant of **specificity**, especially when more than one member of the class is in your system

When in doubt, think about occupancy--who is bound, to what target(s)? Then hypothesize functional consequences

When there is more than one possibility, think about the experiment(s) you would do to discriminate between them.
Paper for Monday:

Fribourg et. al
Decoding the Signaling of a GPCR Heteromeric Complex Reveals a Unifying Mechanism of Action of Antipsychotic Drugs
Cell 147, 1011–1023, 2011

I’ll also post some papers related to this story, a review on biased agonism, and a recent paper from my lab that combines biased agonism, heteromerization and behavioral plasticity
The MOR shows agonist-specific endocytosis
Or “biased agonism” “functional selectivity”

No treatment

Enkephalin

Morphine

L + R $\leftrightarrow$ LR$^*$ $\not\rightarrow$ G protein arrestin/endocytosis

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Keith et al, JBC 1996