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Nonclinical Pharmacology and Toxicology Considerations Regarding Opioid Comparisons and Risk Assessments (Basic Opioid Pharmacology 101)

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Disclaimer

This Presentation Reflects the Views of the Authors and Should Not Be Construed to Represent FDA's Views or Policies

Objectives

- Provide a quick overview and history of opioid pharmacology (refresher)
- Describe the challenges with methods to compare opioid potency, from a basic science and nonclinical perspective
- Compare data from binding affinities with a toxicological endpoint to illustrate challenges of potency estimates
- Identify the challenges for translation of animal potency studies to humans

A Very Brief History of Opioid Pharmacology

- No one knows who first cultivated the opium poppy (4200 BC – large numbers of poppy seed capsules found in burial sites in Spain)
- Sumerians possibly as far back as between 3400 BC called opium “gil” (joy) and the poppy “hul gil” (plant of joy).
- Note on terminology:
  - **Opiates** are drugs derived from opium (morphine, codeine and semisynthetics)
  - **Opioids** are all agonists and antagonists (more inclusive as it includes synthetics)

https://study.com/academy/answer/sumeria-was-located-in-an-area-known-as.html

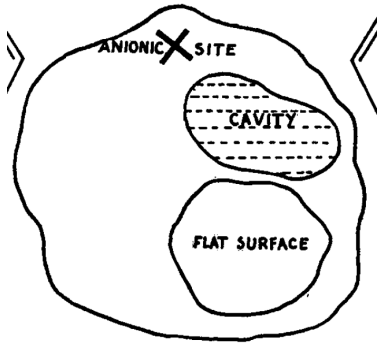
https://www.deamuseum.org/ccp/opium/history.html

https://en.wikipedia.org/wiki/Opium

A Very Brief History of Opioid Pharmacology

- Sometime between 1803 and 1805 **morphine** was first extracted from opium resin by Friedrich Serturner, a German pharmacist
- The **concept of opioid receptors** was first proposed by Beckett and Casy (1954) based on rigid chemical structural requirements for activity
- **Opioid receptors** were first demonstrated in 1973 using radioligand binding assays
  - Candace Pert & Solomon Snyder, 1973
  - Eric Simon, Jacob Hiller, and Irit Edelman, 1973
  - Lars Terenius, 1973

https://asmalldoseoftoxicology.squarespace.com/serturner/

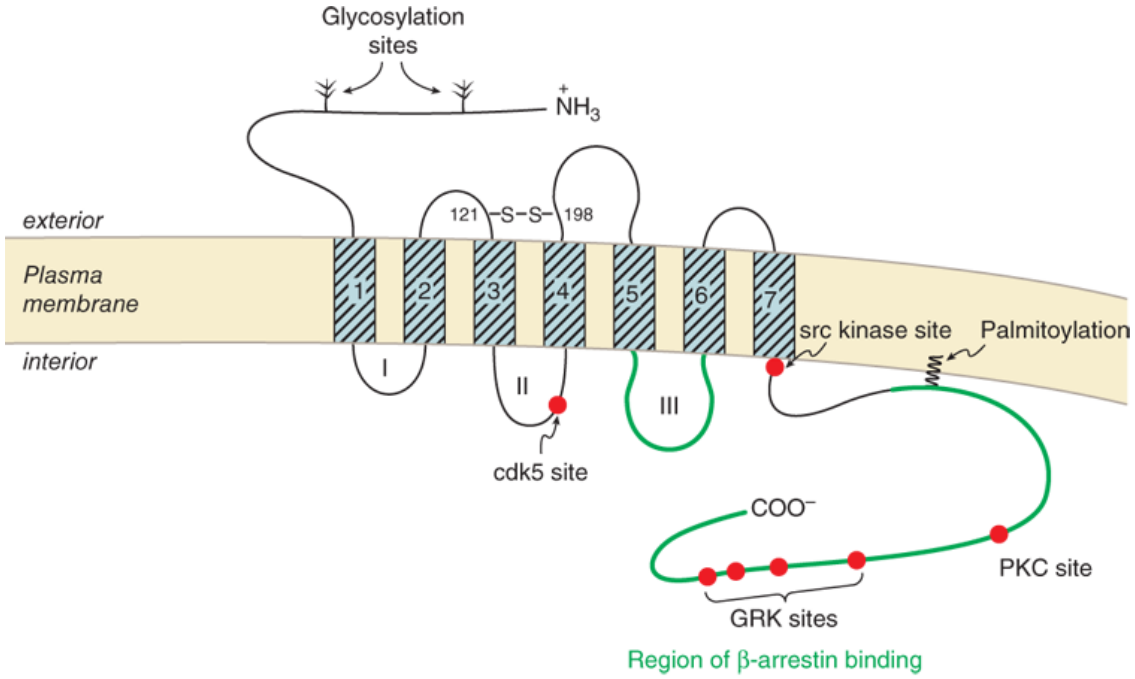


(XIV) Receptor surface

Discovery of Opioid Receptors

Opioid Receptor Subtypes (all coded by one gene)

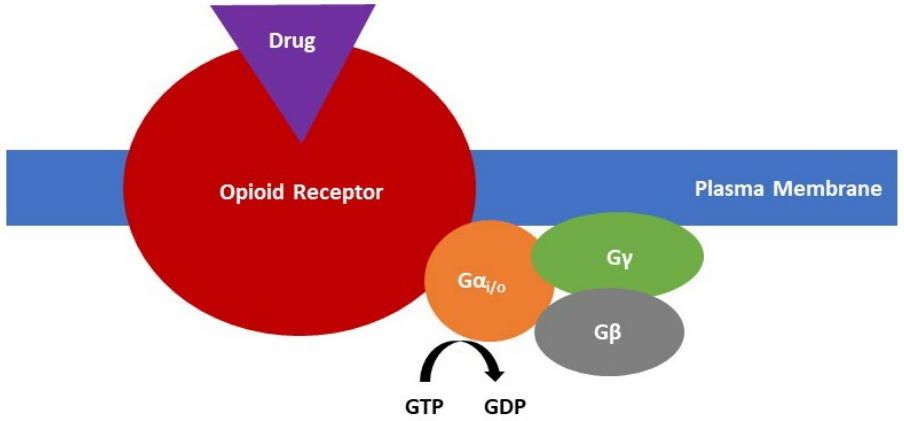
- **Delta** (δ, DOP, or formerly OP1)
  - Two variants based on receptor binding studies (d1 and d2)
- **Kappa** (κ, KOP, or formerly OP2)
  - Three variants based on receptor binding studies (k1, k2, and k3)
- **Mu** (μ, MOP, or formerly OP3)
  - Three variants based on receptor binding studies (m1, m2, and m3)
- **Nociceptin/Orphanin FQ** Receptor (ORL-1, NOP, or formerly OP4)
  - not naloxone sensitive



Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e

Opioid Receptor Signal Transduction

- Mu, delta, and kappa receptors couple to pertussis toxin-sensitive, G_i/G_o proteins
- On receptor activation, the G_i/G_o coupling results in a number of intracellular events that are mediated by α and βγ subunits of these G proteins, including the following:
  - Inhibition of adenylyl cyclase activity (decreases cAMP and PKA activation)
  - Reduced opening of voltage-gated Ca^{2+} channels (reduces neurotransmitter release from presynaptic terminals)
  - Stimulation of K^+ current through several channels (hyperpolarization of neurons)
  - Activation of PKC and PLCβ
  - Can be phosphorylated for β-arrestin interactions



Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e

Receptor Desensitization and Drug Tolerance

- Desensitization – usually refers to the molecular changes at level of receptor signaling that result in progressive reduction of signal transduction after receptor activation
  - Rapid desensitization (seconds to minutes)
  - Short term tolerance (minutes to tens of minutes)
  - Long term tolerance (greater than 1 day)
- Molecular Mechanisms are Complicated
  - E.g., phosphorylation following activation, endocytosis, resensitization, recycling
  - Homologous and Heterologous Desensitization
- Drug Tolerance – loss of responsiveness to an agonist after continued exposure (without specifying cellular or molecular mechanism)

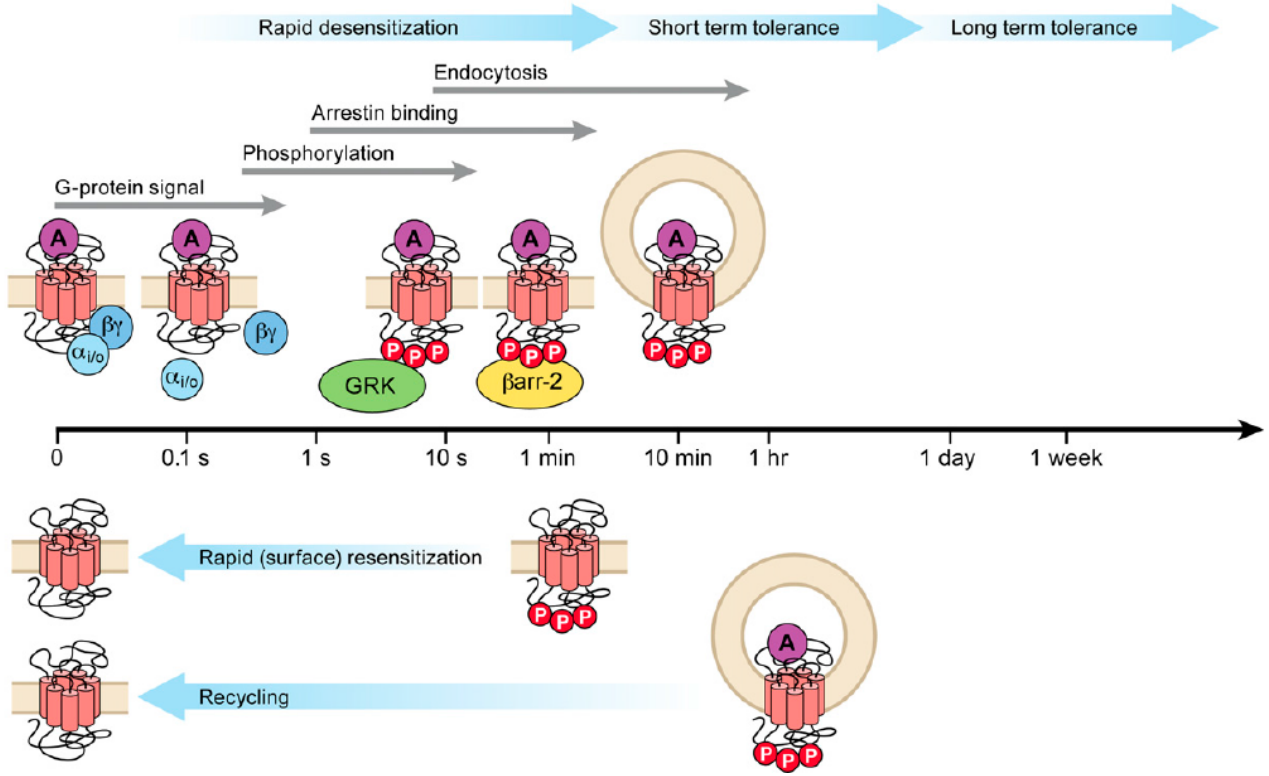


Image Source: Williams et al. (2013) Pharmacological Reviews 65:223-254

Opioid Receptor Trafficking

- MOR and DOR undergo rapid agonist-mediated internalization
  - MOR recycle to membrane after internalization
    - May be different for different ligands
      - Etorphine and Enkephalins rapid internalization
      - Morphine has been reported to not cause internalization
  - DOR are degraded after internalization
  - KOR do not internalize
- Different ligands may result in different receptor trafficking and physiological responses

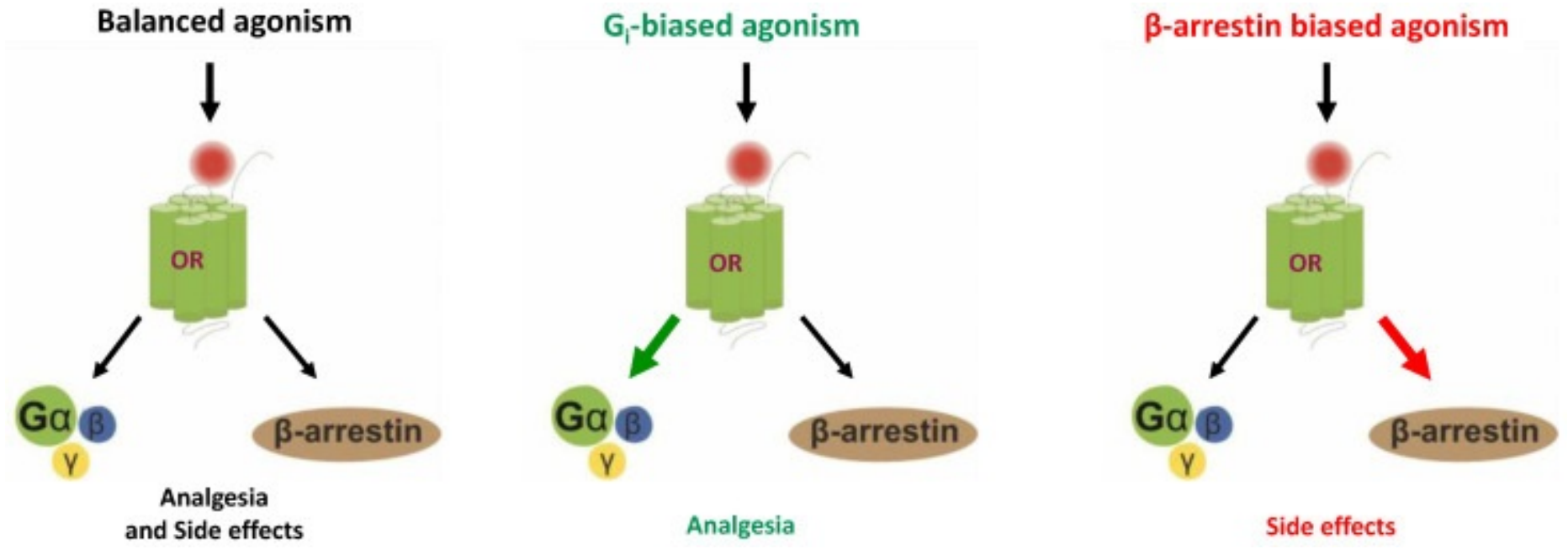
Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e

Response		Morphine	Etorphine
G protein activation		+ + +	+ + +
MOR phosphorylation		+	+ + +
β-arrestin recruitment	β-arrestin 1	–	+ + +
	β-arrestin 2	+	+ + +
MOR internalization		+/–	+ + +
PKC_ε activation		+ + +	–
MOR desensitization** (assessed as Ca^{2+}release)		+ + +	–
ERK 1/2 activation		+ + +	+ + +

* Responses assembled from literature data, mostly from cultured cell systems. See papers by Raehal et al. (2011) and Zheng et al. (2011).
** Result depends on response measured.

C. *Biased agonism: disparate effects of two MOR agonists*.*

"Biased" Ligands



- Data suggest some ligands produce unbalanced activation of G proteins vs β-arrestin
- The differential intracellular signaling effects may alter the physiological responses, possibly leading to options to increase efficacy and reduce adverse effects and different rates of desensitization

Image Source: Faouzi et al. (2020) Biased Opioid Ligands. Molecules 25(18):4257

Ligand Bias at MOR

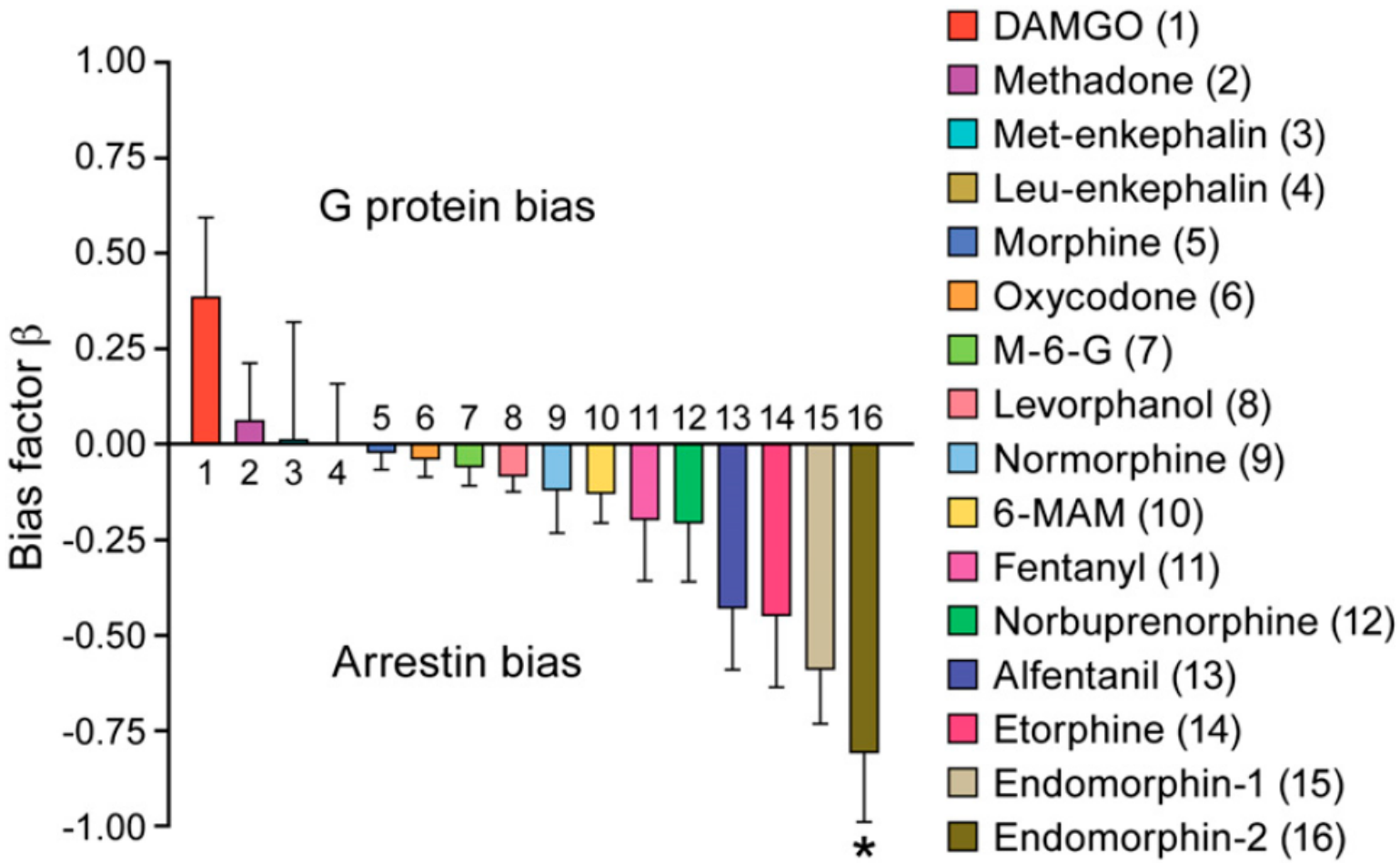


Fig. 3. Ligand bias at MOR. The intrinsic efficacies (operational model) of a range of structurally dissimilar MOR agonists to activate [^{35}S]GTPγS binding and arrestin recruitment was determined and the bias factor (β) calculated according to the method of Rajagopal et al. (2011). Reproduced from Rivero et al. (2012).

Image Source: Williams et al. 2013 Pharmacological Reviews 65:223-254

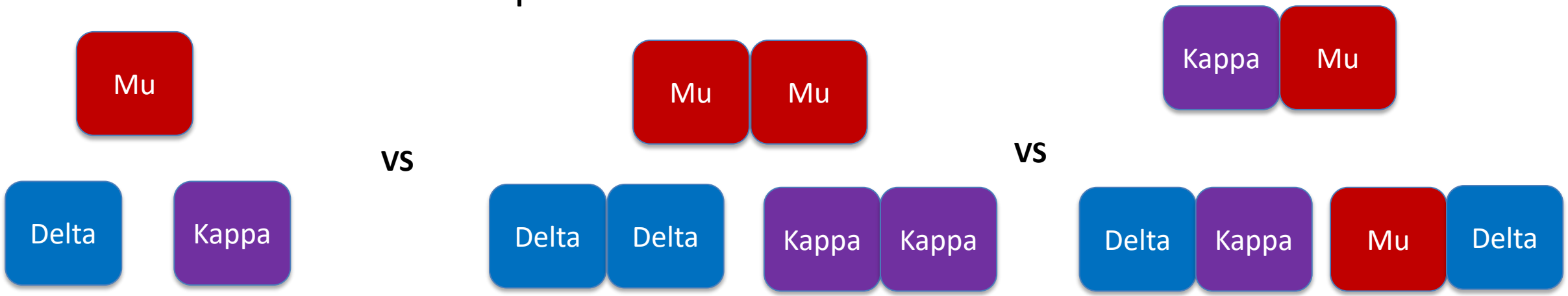
Examples of the Potential Impact of Genetics

- Single Nucleotide Polymorphisms (SNPs)
  - RS1799971 SNP changes an adenine (A) to guanine (G) at Position 118 in OPRM1 gene (codes for mu opioid receptor)
  - Present in 15-30% Europeans, 40-50% Asians, 1-3% Latinos and African Americans
  - Results in change of the amino acid at Position 40 from asparagine to aspartate
  - Removes potential asparagine-linked glycosylation which can alter MOR affinity for different ligands, signal transduction, and half-life of the receptor.
  - Adds methylation site which can reduce MOR mRNA
- Epigenetic Modifications
  - Differential methylation of OPRM1 promotor linked to a variety of physiological responses (e.g., alcohol dependence, opioid dependence, pain responses, neuropathic pain conditions, Alzheimer's disease)
- Splice Variants
  - 7-TM vs 6-TM splice variants of MOR may have differential effects on efficacy and adverse effects

Reviewed by: Cuitavi et al. (2021) Trends in Biochemical Sciences 46(4):315-328

Opioid Receptors Can Dimerize

- There is evidence for both homodimers and heterodimers
  - Can impact ligand binding, intracellular signaling, and receptor trafficking/desensitization
  - Could contribute to the ultimate diversity of pharmacological properties of the individual receptors



Can also dimerize with other nonopioid GPCRs

Pharmacodynamics of Opioid Receptors

Mu (MOR)

- Analgesia
- Physical dependence
- Respiratory depression
- Miosis
- Euphoria
- Reduced GI motility

Delta (DOR)

- Analgesia
- Antidepressant effects
- Convulsant effects
- Physical dependence
- Modulation of MOR-mediated respiratory depression

Kappa (KOR)

- Analgesia
- Anticonvulsant effects
- Depression
- Dissociative/hallucinogenic effects
- Diuresis
- Miosis
- Neuroprotection
- Sedation
- Stress

Trescot *et al*. Pain Physician. 2008; 11:S133-153. Pathan & Williams. Br J Pain. 2012; 6:11-16.

Selectivity of Common Opioid Analgesic Ligands

Opioid Ligand	Mu	Delta	Kappa
Morphine	+++		+
Hydromorphone	+++		+
Fentanyl	+++		
Methadone	+++		
Buprenorphine	P		--
Butorphanol	P		+++

+ = Agonist activity

- = Antagonist activity

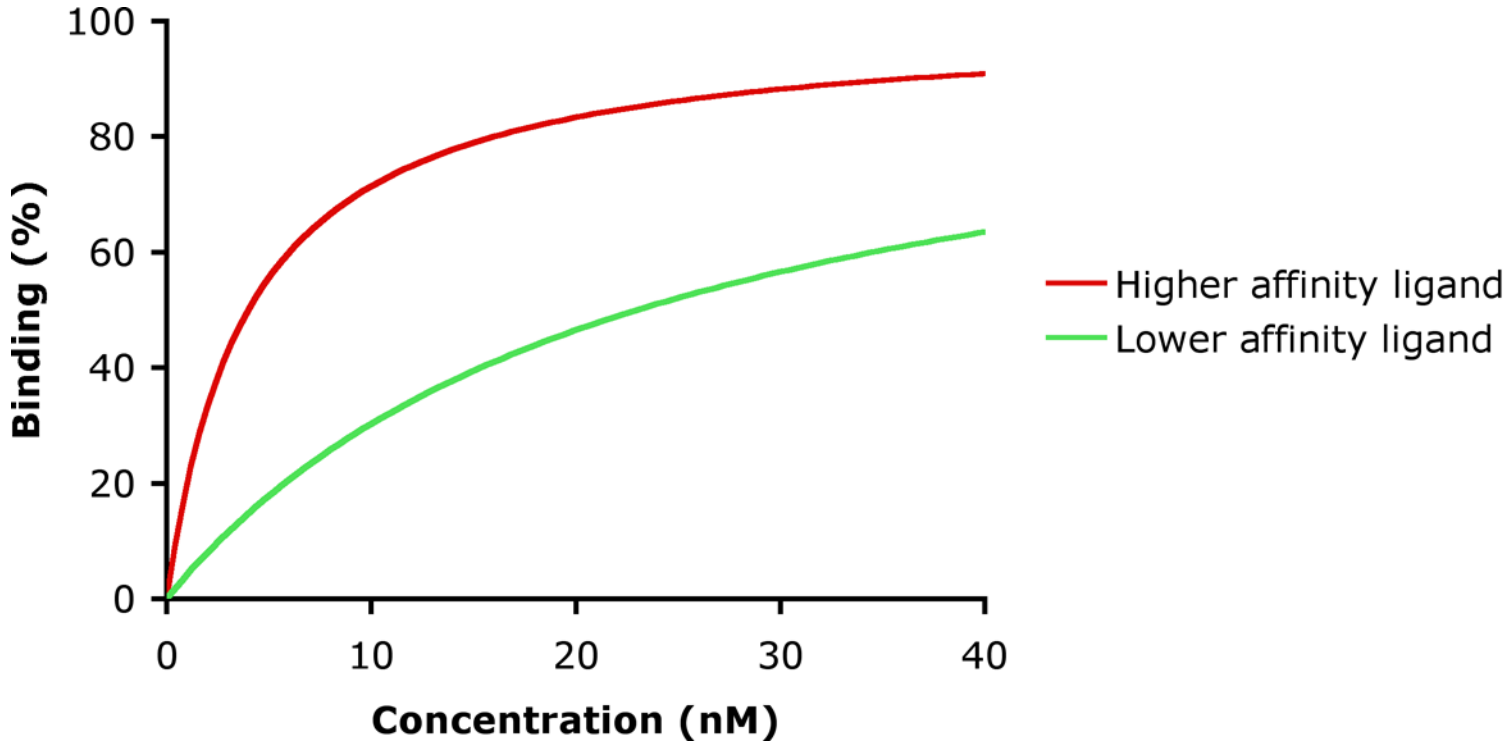
P = Partial agonist activity

In potency: + < ++ < +++

Source: Yaksh and Wallace 2017 Chapter 20: Opioids, Analgesia, and Pain Management in Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e
Derived from Raynor K et al. Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. Mol Pharmacol, 1994;45:330–334.

Receptor Binding Assays Measure Affinity

- Radioligand (*e.g.*, [^{3}H]-Naltrexone, [^{3}H]-DAMGO) binds to receptors in tissue or membrane sample
- Increasing concentrations of radioligand eventually saturate the binding sites

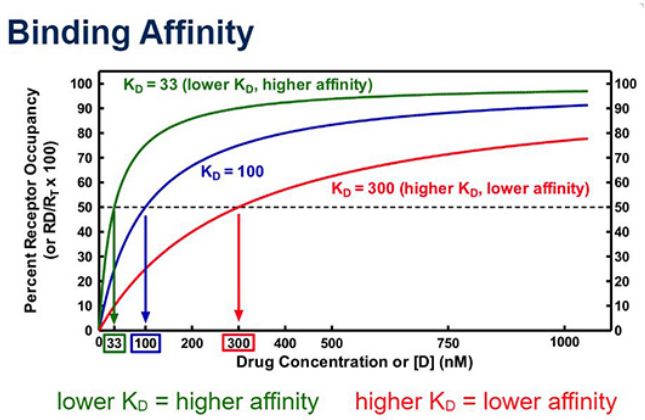


https://en.wikipedia.org/wiki/Ligand_(biochemistry)

Comparison of Binding Affinities

Direct Binding Affinity

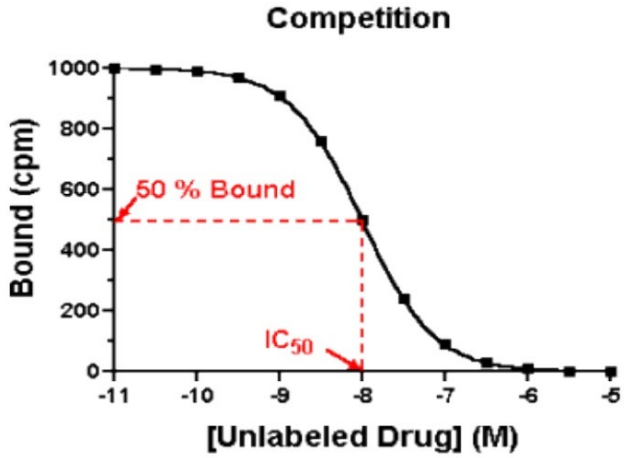
- K_D = dissociation constant
- Binding of a radioligand to a receptor



Credit: University of Nebraska Medical Center eLearning
https://www.unmc.edu/elearning/egallery/receptor-binding/
Developed by Cassandra Moshfegh, Sarah Schlichte, and Dr. Myron Toews

Indirect Binding Affinity

- K_i = Inhibition constant
- Displacement of a radioligand from the receptor by increasing concentrations of an unlabeled compound



Receptor Binding Affinity

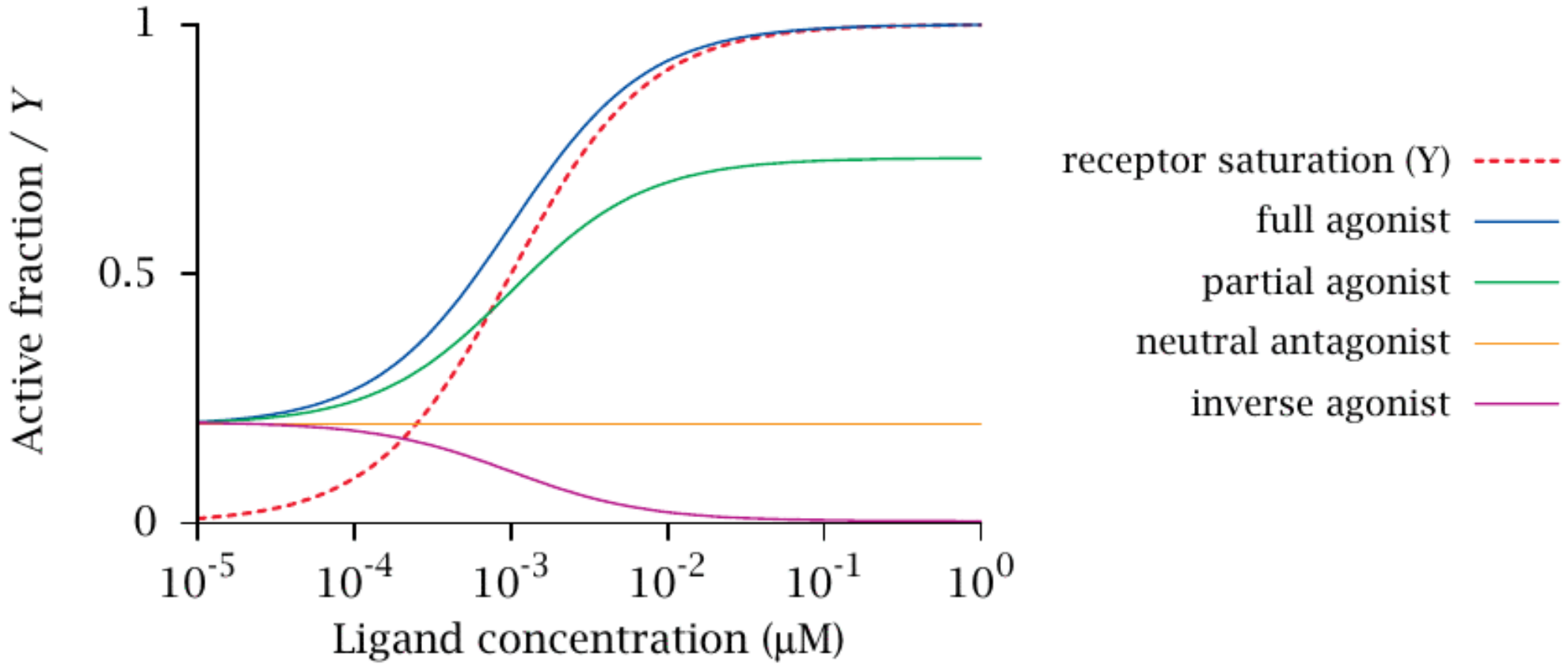


Image Source: http://watcut.uwaterloo.ca/webnotes/Pharmacology/Pharmacodynamics.html

Uniform assessment and ranking of opioid Mu receptor binding constants for selected opioid drugs ☆

Donna A. Volpe [a,*], Grainne A. McMahon Tobin [a], R. Daniel Mellon [b], Aspandiar G. Katki [a], Robert J. Parker [a], Thomas Colatsky [a], Timothy J. Kropp [c], S. Leigh Verbois [c]

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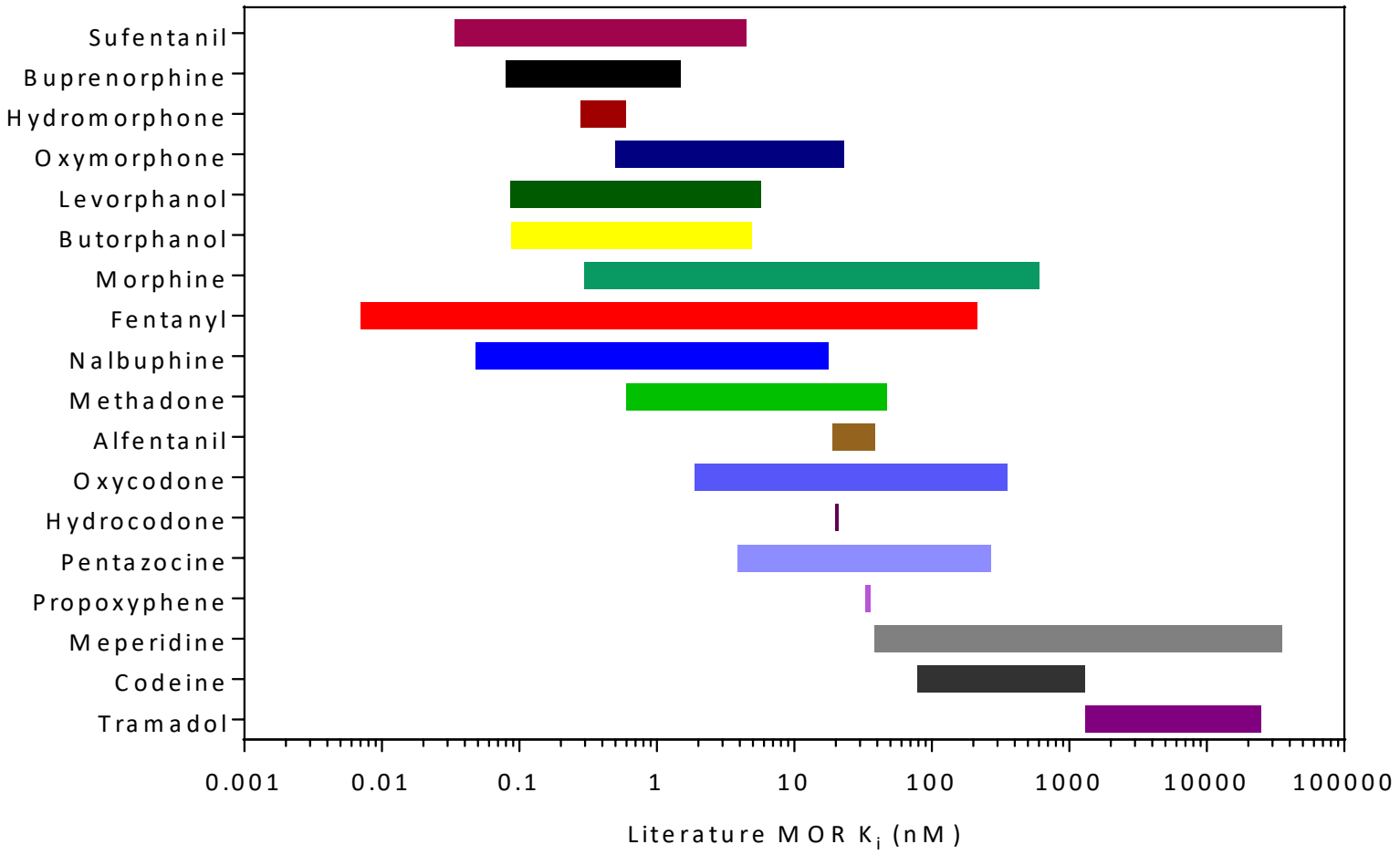
FDA

Goal: Compare FDA-approved opioid analgesic drug affinities to the mu opioid receptor as a surrogate for opioid potency

- Concern at the time was to determine what drugs may be more dangerous than others to warrant disposal via flushing rather than other means of disposal that could result in diversion or inadvertent exposures.
- Review of literature resulted in wide range of values reported for MOR
  - Due to differences in: radioligands used, definition of nonspecific binding, laboratory methods, tissue sources, species tested, *etc*.

Regulatory Toxicology and Pharmacology 59 (2011) 385–390

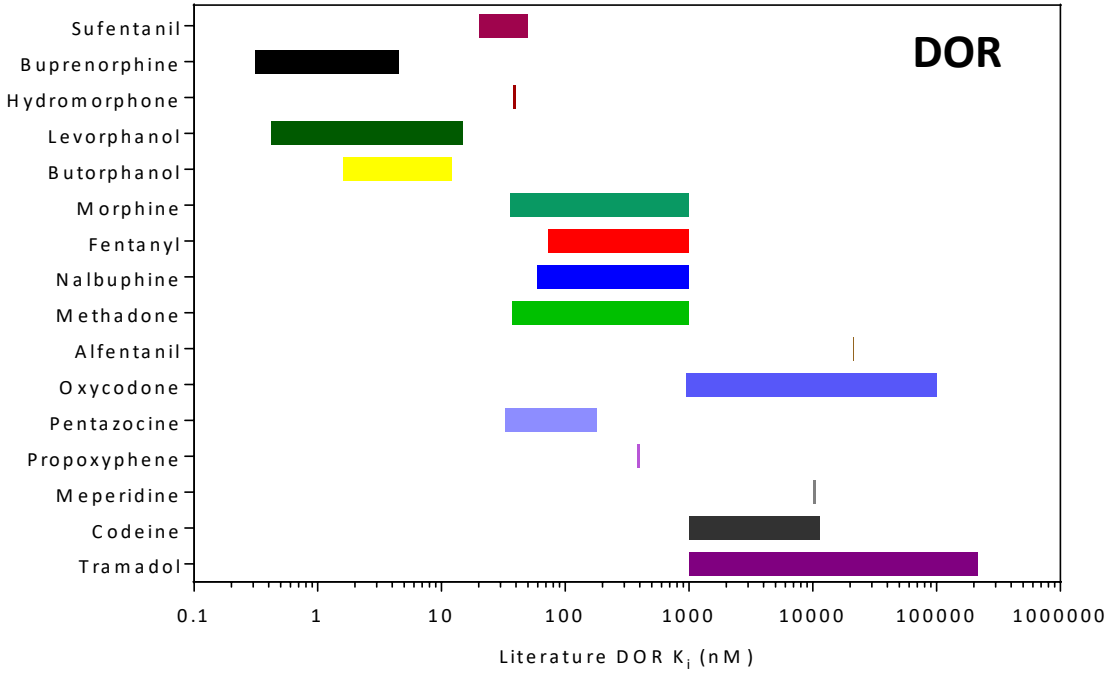
Literature MOR K_i Values

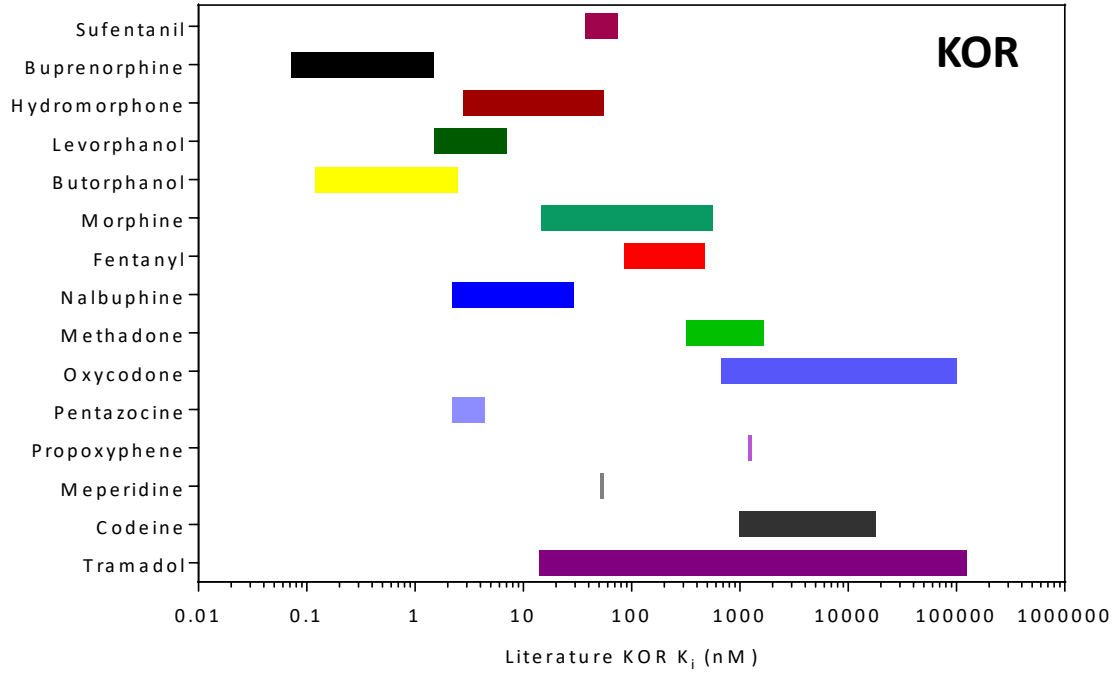


- Range of K_i values for drugs as much as 10- to 100,000-fold different
- Variability due to:
  - radioligand
  - tissue source
  - animal species and strain
  - assay methodology

Volpe *et al*. Reg Toxicol Pharmacol. 2011; 59:385-390.

Literature DOR and KOR K_i Values

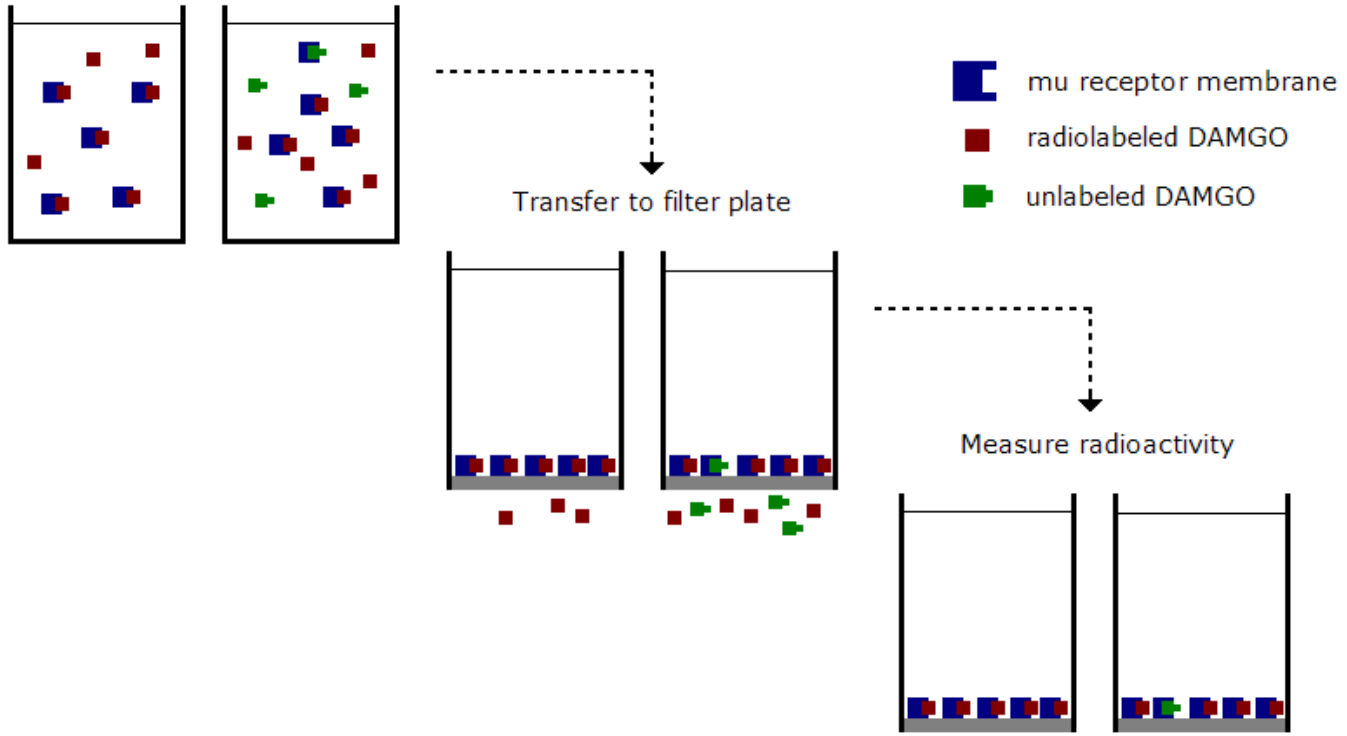




Variability in literature K_i values for DOR and KOR as seen with MOR

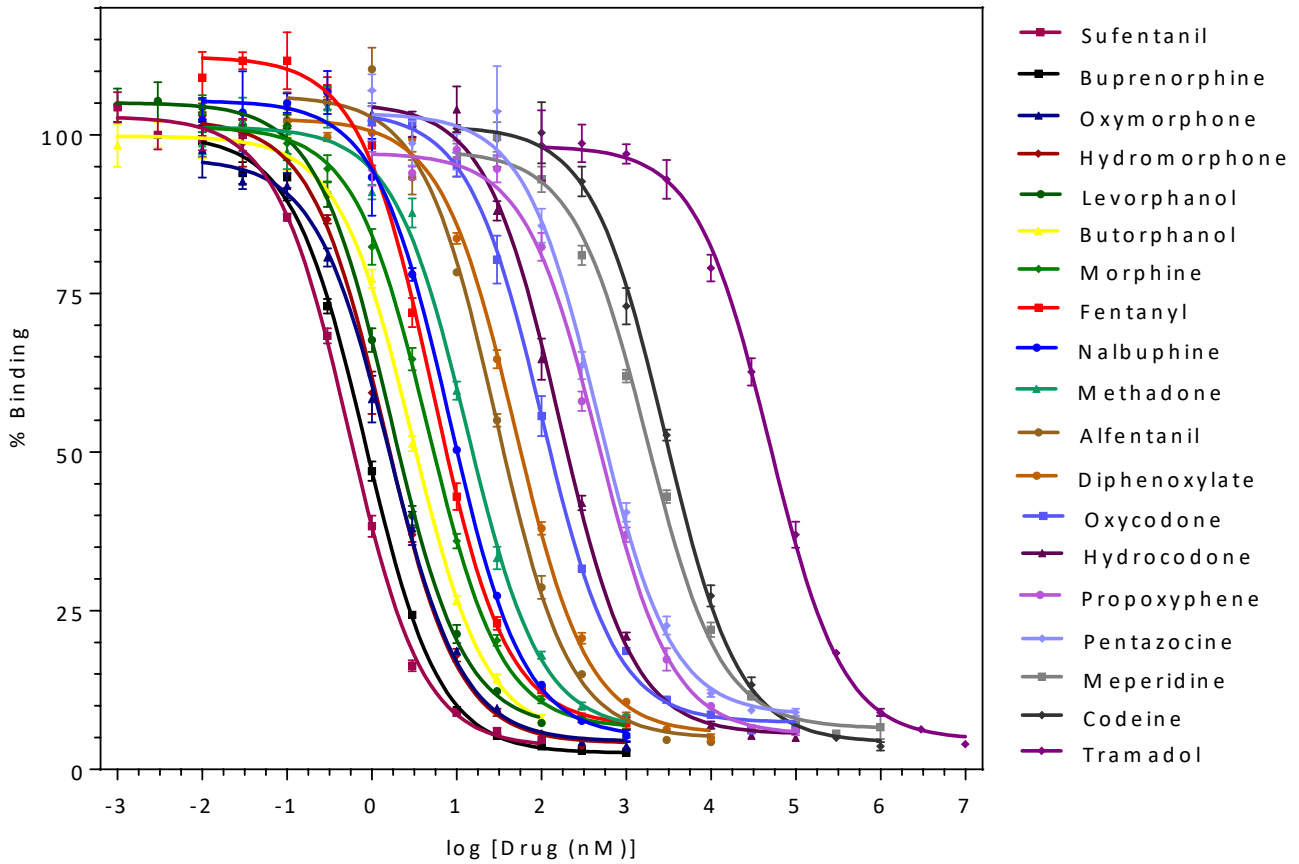
Receptor Binding Assay

- Determination of binding affinities (K_i)
- Membranes expressing recombinant human mu-opioid receptor
- Single standardized assay
- Uniform experimental conditions with [^{3}H]DAMGO
- Test set: 19 FDA approved opioid drugs
- Reference standard: Naloxone



Volpe *et al*. Reg Toxicol Pharmacol. 2011; 59:385-390.

MOR Binding Curves for Opioids



Volpe *et al*. Reg Toxicol Pharmacol. 2011; 59:385-390.

Drug	K_i (nM)
Sufentanil	0.138
Buprenorphine	0.2157
Hydromorphone	0.3654
Oxymorphone	0.4055
Levorphanol	0.4194
Butorphanol	0.7622
Morphine	1.168
Fentanyl	1.346
Nalbuphine	2.118
Methadone	3.378
Alfentanil	7.391
Diphenoxylate	12.37
Oxycodone	25.87
Hydrocodone	41.58
Pentazocine	117.8
Propoxyphene	120.2
Meperidine	450.1
Codeine	734.2
Tramadol	12486

Challenge: We do not have uniform data for δ or κ opioid receptor binding

Overdose Risk (LD_{50})

Opioid	Rat Oral LD_{50} (mg/kg)	MOR K_i (nM)	Octanol:Water Partition Coefficient*	Comment
Fentanyl Citrate	18	1.346	860:1	Highly lipophilic
Methadone HCl	30	3.378		
Tramadol HCl	228	12486	1.35:1	MOR agonist (M1) and SNRI
Butorphanol tartrate	315	0.7622		
Hydrocodone bitartrate	375	41.58		
Codeine sulfate	430	734.2		
Morphine sulfate	461	1.168	1.42:1	
Buprenorphine HCl	> 1000	0.2157		Partial agonist at MOR
Oxycodone HCl	No data	25.87	0.7:1	
Hydromorphone HCl	No data	0.3654		
Oxymorphone HCl	No data	0.4055	0.98:1	

*Data Source: Merck Index

Measures of Opioid Potency

In Vitro Assays

- Receptor binding affinity
- G protein-coupled activation ([^{35}S]GTPγS)
- Inhibition of adenylyl cyclase
- Calcium flux/signaling
- cAMP inhibition

Animal Models

- Tail-flick anti-nociception assay
- Knock-out rodent models

Challenge: We do not have uniform data for clinically relevant opioids on these endpoints

Animal to Human Comparison

Human

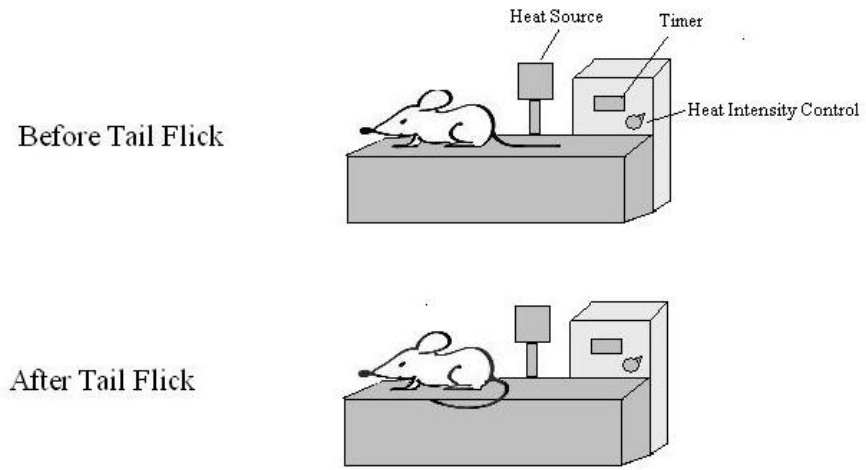
- Analgesia
  - "insensitivity to pain without loss of consciousness" (Merriam Webster)

There is both a sensory and emotional response to pain

http://clipart-library.com

Animal

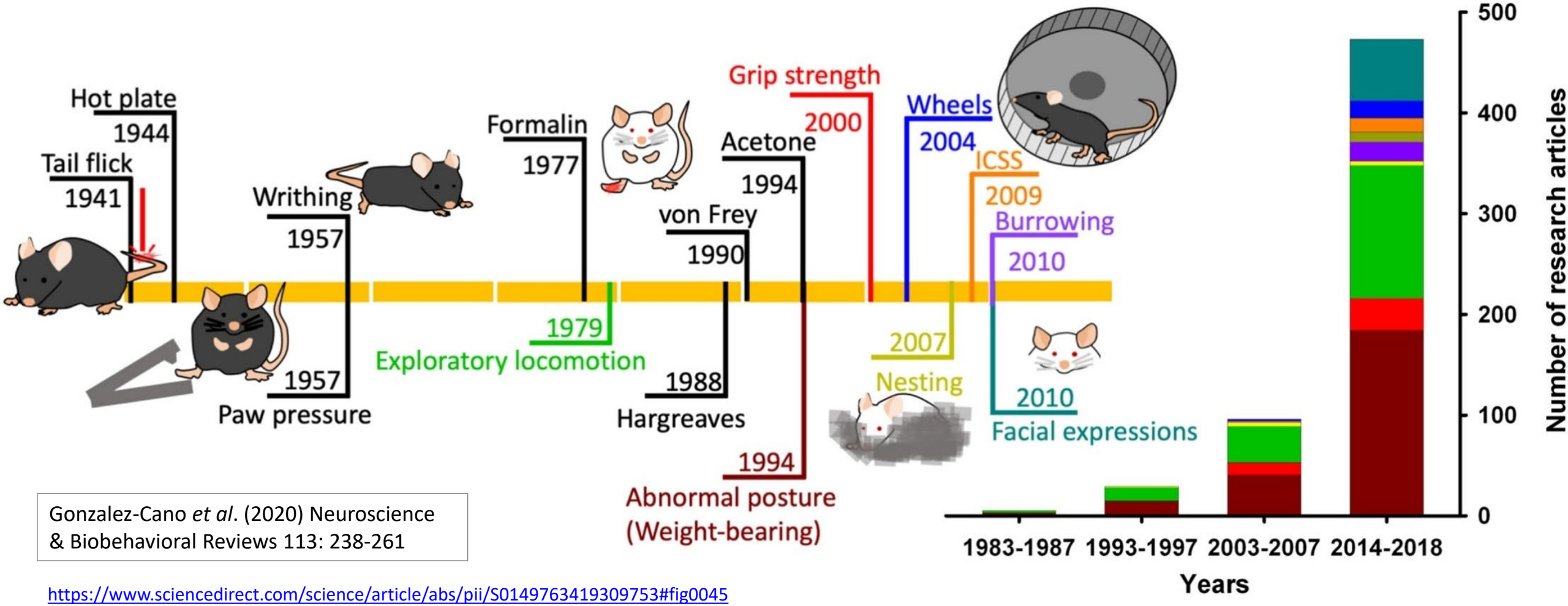
- Antinociception
  - "the action or process of blocking the detection of a painful or injurious stimulus by sensory neurons" (Merriam Webster)



We can measure sensory response but have no idea of emotional response

https://commons.wikimedia.org/wiki/File:Tail_Flick_Test_Apparatus.jpg

Animal Models Are Evolving



Gonzalez-Cano *et al*. (2020) Neuroscience & Biobehavioral Reviews 113: 238-261

https://www.sciencedirect.com/science/article/abs/pii/S0149763419309753#fig0045

Strengths and Limitations of Nonclinical Assays

- *In vitro* assays:
  - Focus on one or a few endpoints (*e.g.*, opioid receptor binding, signal transduction cascade)
  - Interlaboratory variability due to differential methods employed (generally lack uniform assessments in single model)
- *In vivo* animal studies:
  - Species and strain differences
  - Differences in drug metabolism and transport compared to humans
  - Translational challenges (*e.g.*, analgesia vs antinociception)

Developing an Algorithm?

Some Factors Contributing to Pharmacodynamic Variability

Drug/Drug Product Factors

- Selectivity and impact of receptor dimerization and splice variants
- Dosage form/route of administration
- Relative bioavailability
- Lipophilicity (distribution)
- Affinity
- Avidity
- Potency
- Rate/mechanism of receptor desensitization
- Protein binding

Individual Patient Factors

- Age
- Sex
- Body mass index
- Kidney function
- Hepatic function
- Level of tolerance
- Concomitant medications and supplements
- Underlying disorders
- Genetics (receptors, enzymes, transporters)

Some Final Thoughts

- Opioid pharmacology is incredibly old, yet there is still a great deal unknown
- Basic science and nonclinical studies contribute to the foundation of our knowledge
- Cross-study comparisons of data in published literature are extremely challenging given variabilities in laboratories and models used (*e.g.*, species, tissues, ligands), uniform assessments are required
- Cannot look at any one endpoint to predict cross opioid comparisons – need to consider the relative contribution of the many variables that impact outcome to develop an ideal algorithm
- Nonclinical studies inform on specific differences between opioids in a highly controlled setting, but the results require testing in the clinical setting given the variabilities in humans and PK/PD contributing factors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