2. Targets for drug action

Estimate of the number of drug targets based on an analysis of the entries in the DrugBank (www.drugbank.ca)

1542* unique drugs, **1214** of which have known protein targets *(Rask-Andersen et al., Nat. Rev. Drug Disc. 10, pp 579, 2011)*
Of these, **192** drugs have non-human targets (bacteria, etc.)
If drugs with non-therapeutic targets are removed from this list, we end up with **989 drugs** acting on **435 therapeutic effect-mediating targets**

* There are in fact about 21'000 drug products, if different drugs having the same active principle, or different forms, are counted.

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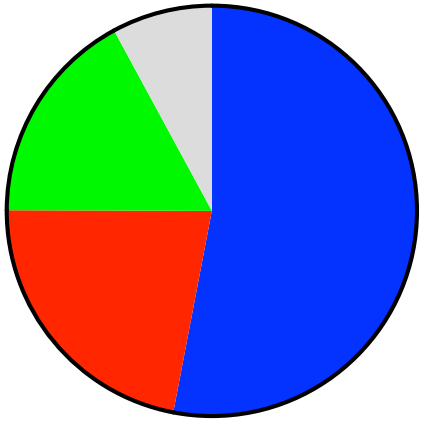
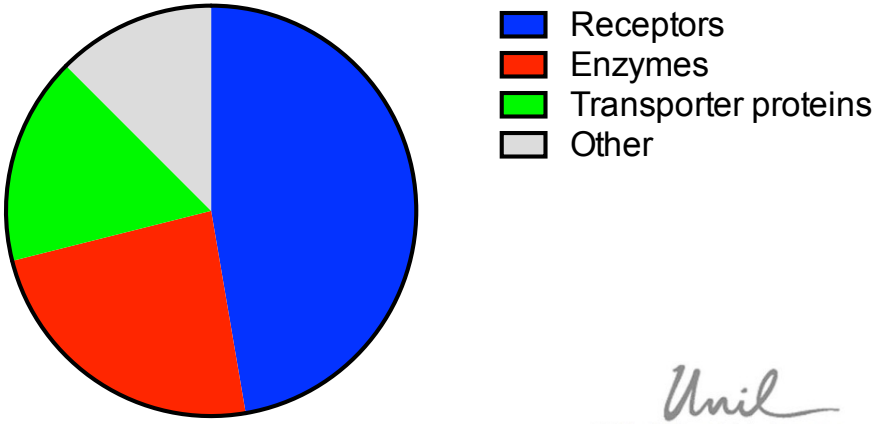
Top 10 drug indications	Number
Antihypertensive agents	108
Antineoplastic agents	91
Anti-inflammatory agents	66
Hypnotics and sedatives	42
Anti-allergic agents	39
Anticonvulsants	37
Anti-arrythmia agents	37
Antipsychotic agents	37
Antidepressants	32
Analgesics	30

Target class	Number
Receptors	193
Enzymes	124
Transporters	67
Other	51

Number of drugs



Number of targets



Based on Rask-Andersen et al., Nat. Rev. Drug Disc. 10, pp 579, 2011

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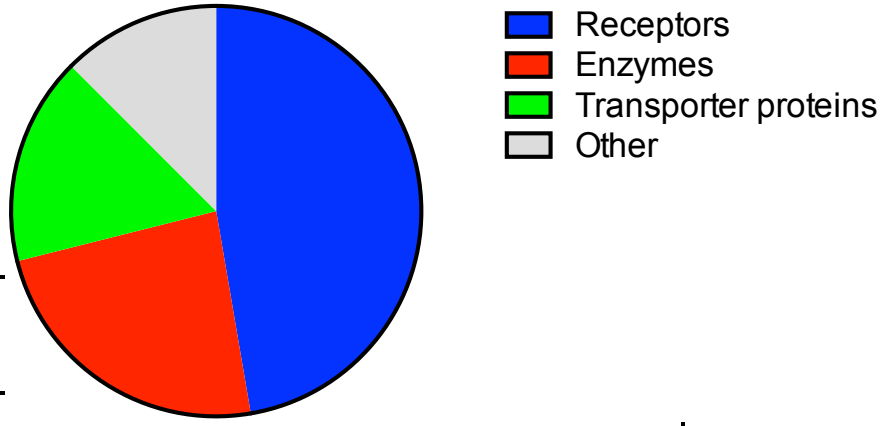
Targets for drug action



Drug targets

Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	*- adrenergic receptors* *- opioid receptors*
2.1.2. ligand-gated ion channels	*- $GABA_A$ receptors*
2.1.3. kinase-linked receptors	*- insulin receptor*
Intracellular receptors	
2.1.4. nuclear receptors	*- pregnane X receptor*
2.2. Other targets/approaches	
2.2.1. enzymes	*- dihydrofolate reductase* *- tyrosine kinases* *- angiotensin-converting enzyme* *- COX inhibitors (in pain chapter)*
2.2.2. ion channels and transporters	*- voltage-gated Na channels*
2.2.3. protein therapeutics	*- GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	*- Nusinersen* *- Tisagenlecleucel /Axicabtagene ciloleucel*

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2. Targets for drug action

2.1. Receptors (for physiological ligands) as drug targets

quick information about receptors:

-iuphar site: www.guidetopharmacology.org

-sites for expression of proteins:

general: http://biogps.org

brain: http://portal.brain-map.org/

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2. Targets for drug action
2.1. Receptors (for physiological ligands) as drug targets
2.1.1. G protein-coupled receptors (GPCRs)

examples of drug targets:

- muscarinic acetylcholine receptors
- **adrenoceptors**
- opioid receptors
- dopamin receptors
- serotonin receptors
- cannabinoid receptors
- vasopressin receptors

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Receptor-mediated activation of a G protein and the resultant effector interaction

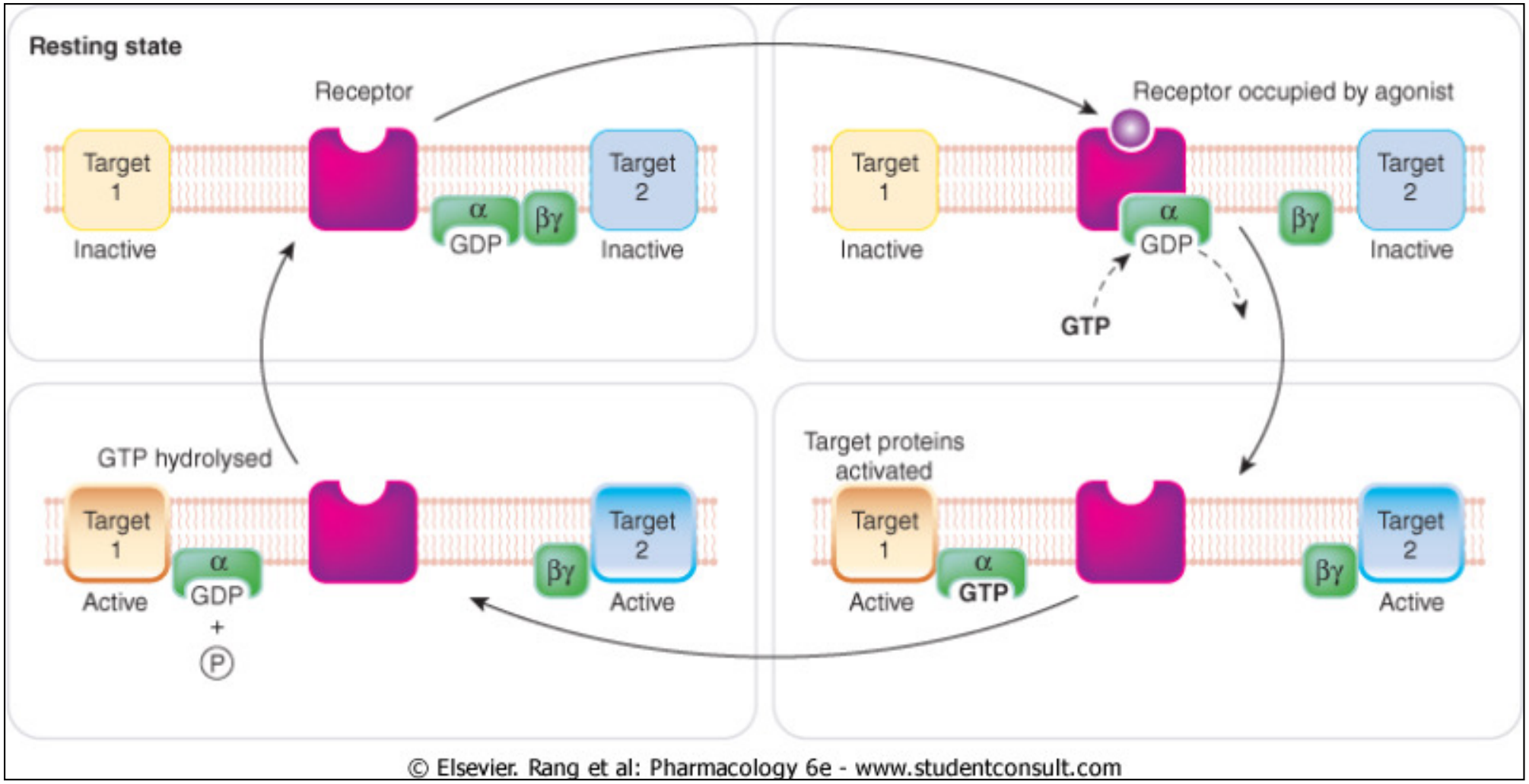

Figure 3-8 **The function of the G-protein.** The G-protein consists of three subunits (α, β, γ), which are anchored to the membrane through attached lipid residues. Coupling of the α subunit to an agonist-occupied receptor causes the bound GDP to exchange with intracellular GTP; the α-GTP complex then dissociates from the receptor and from the βγ complex, and interacts with a target protein (target 1, which may be an enzyme, such as adenylate cyclase, or an ion channel). The βγ complex may also activate a target protein (target 2). The GTPase activity of the α subunit is increased when the target protein is bound, leading to hydrolysis of to GDP, whereupon the α subunit reunites with βγ.

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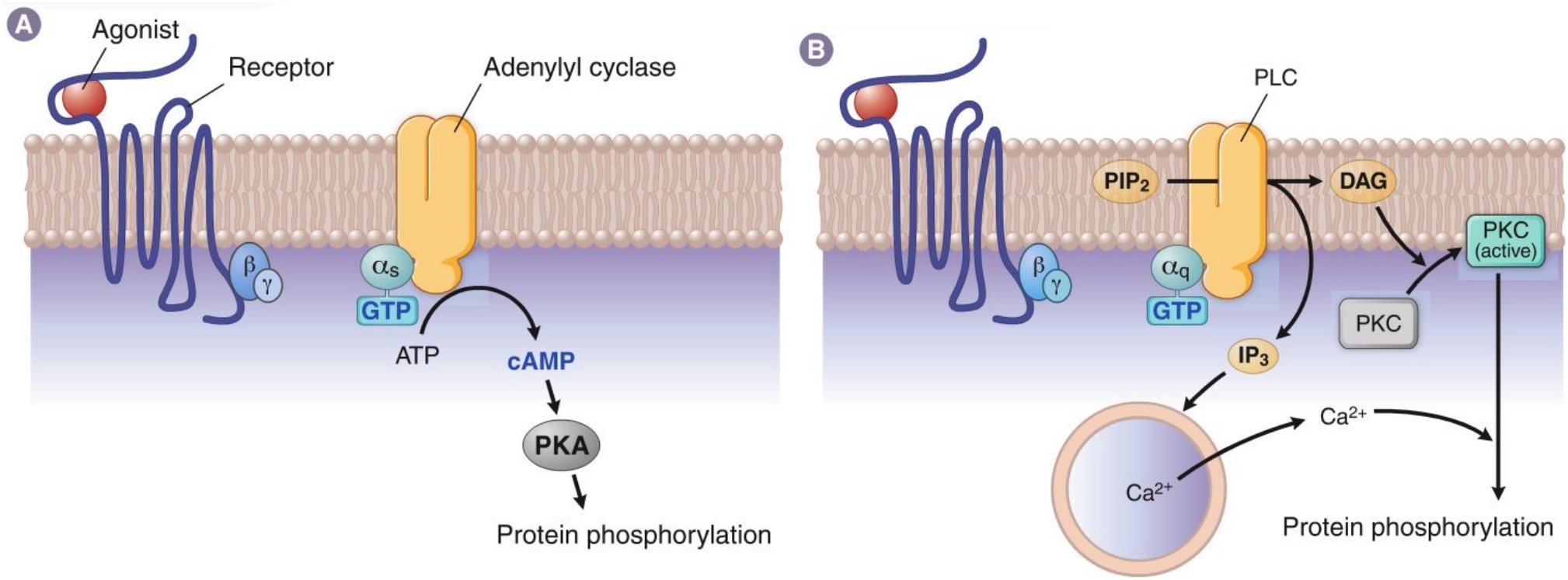
GPCR signaling pathways



G protein	Actions
$G\alpha_s$ (G stimulatory)	Activates adenylyl cyclase , activates Ca^{2+} channels
$G\alpha_i$ (G inhibitory)	Inhibits adenylyl cyclase, activates K^+ channels
$G\alpha_o$	Inhibits Ca^{2+} channels
$G\alpha_q$	Activates phospholipase C
$G\beta\gamma$	Act on ion channels and other targets

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Desensitization of GPCRs

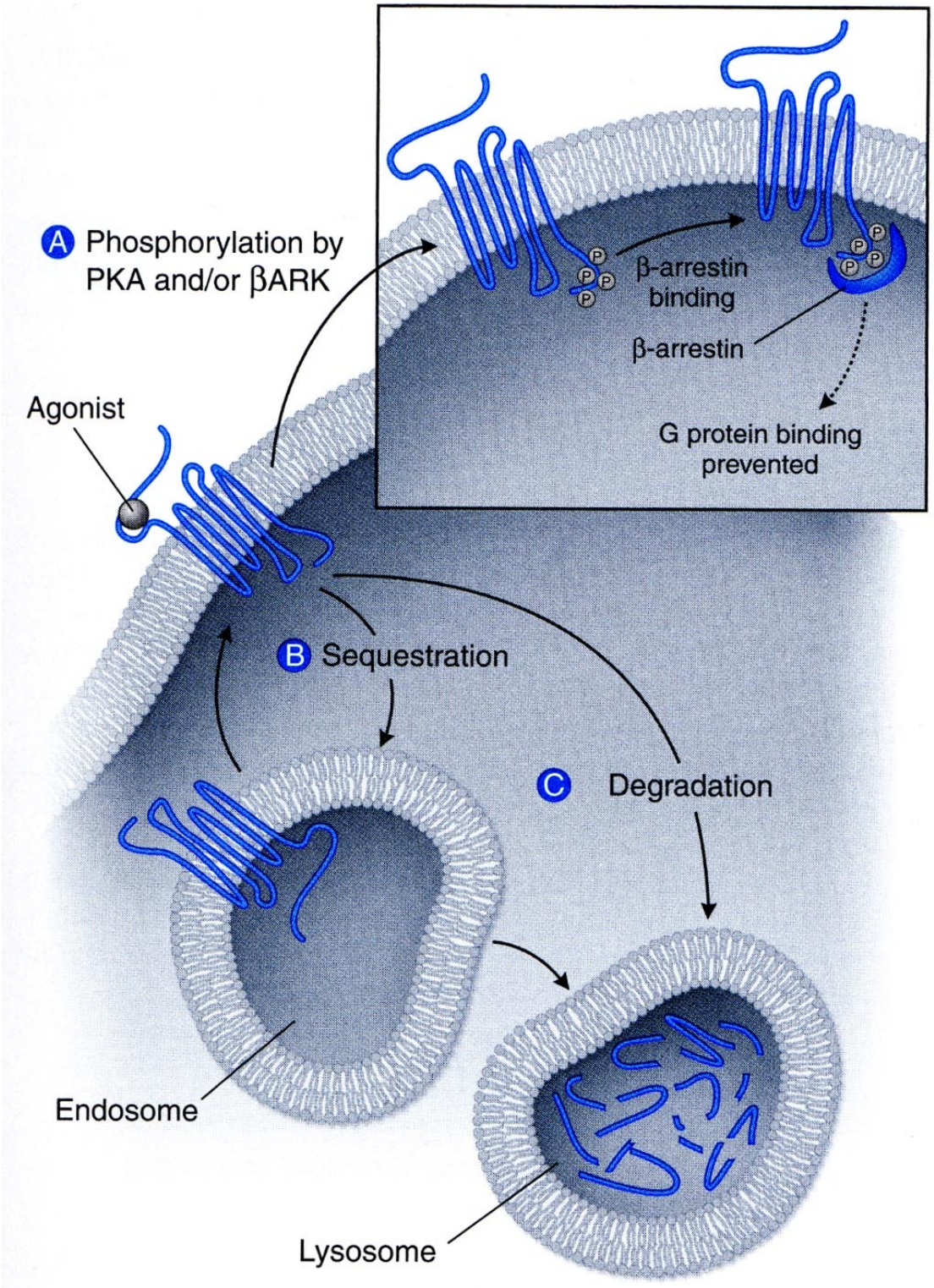

Figure 1-10. β-Adrenergic Receptor Regulation. Agonist-bound β-adrenergic receptors activate G proteins, which then stimulate adenylyl cyclase activity. **A.** Repeated or persistent stimulation of the receptor by agonist results in phosphorylation of amino acids at the C-terminus of the receptor by protein kinase A (PKA) and/or β-adrenergic receptor kinase (βARK). β-Arrestin then binds to the phosphorylated domain of the receptor and blocks G_s binding, thereby decreasing adenylyl cyclase (effector) activity. **B.** Binding of β-arrestin also leads to receptor sequestration into endosomal compartments, effectively neutralizing β-adrenergic receptor signaling activity. The receptor can then be recycled and reinserted into the plasma membrane. **C.** Prolonged receptor occupation by agonist can lead to receptor down-regulation and eventual receptor degradation. Cells can also reduce the number of surface receptors by inhibiting the transcription or translation of the gene coding for the receptor (not shown).

The mechanism described is called homologous (or agonist-specific) desensitization. In addition, heterologous (cross-)desensitisation occurs as a result of phosphorylation of one type of receptor as a result of activation of kinases by another.

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Desensitization due to changes in receptors

A: Receptors linked to ion channels: time-course of milliseconds, due to conformational change

B: G-Protein-coupled receptors: time-course of minutes, due to phosphorylation, interfering with coupling to 2nd messenger cascades

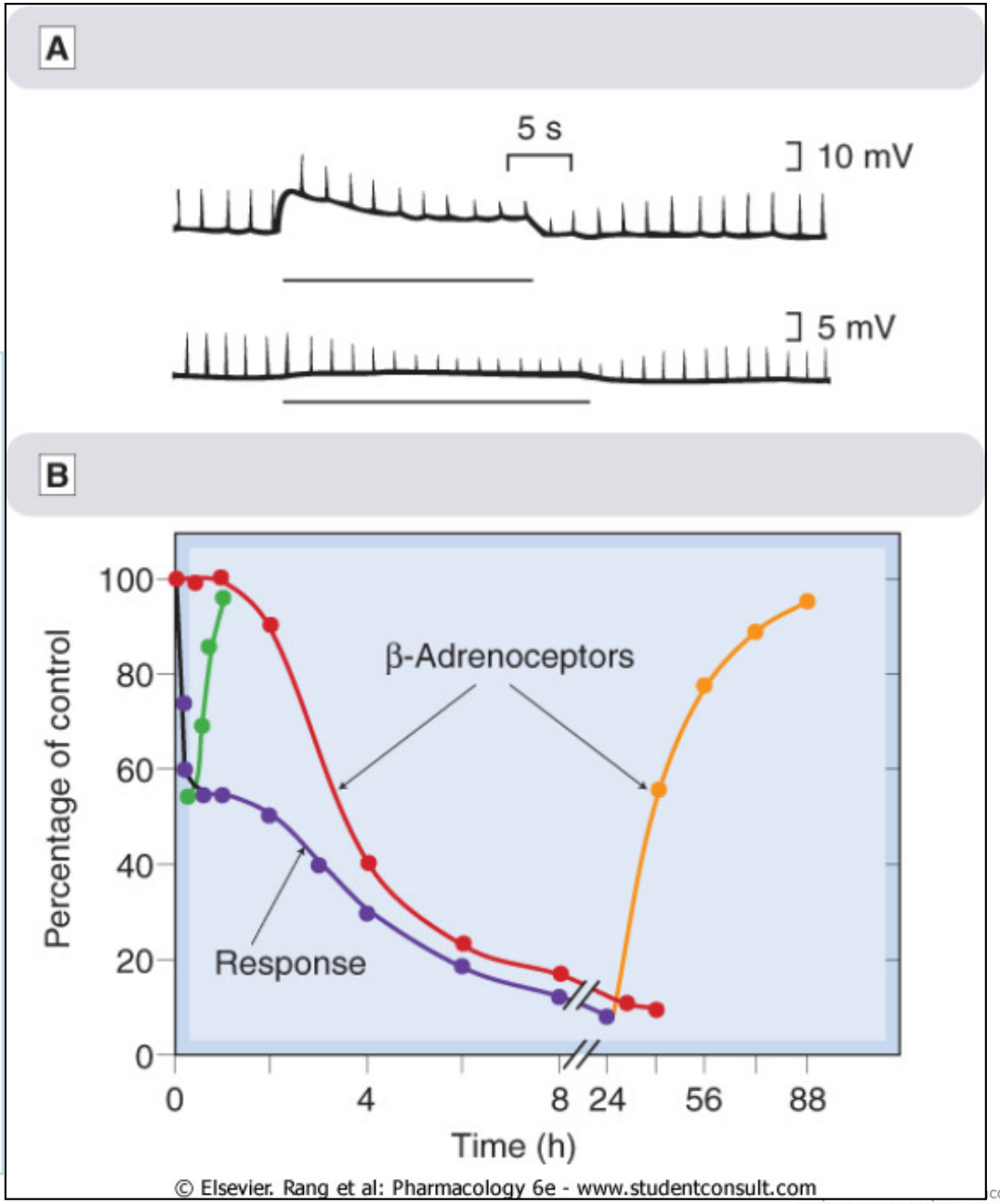
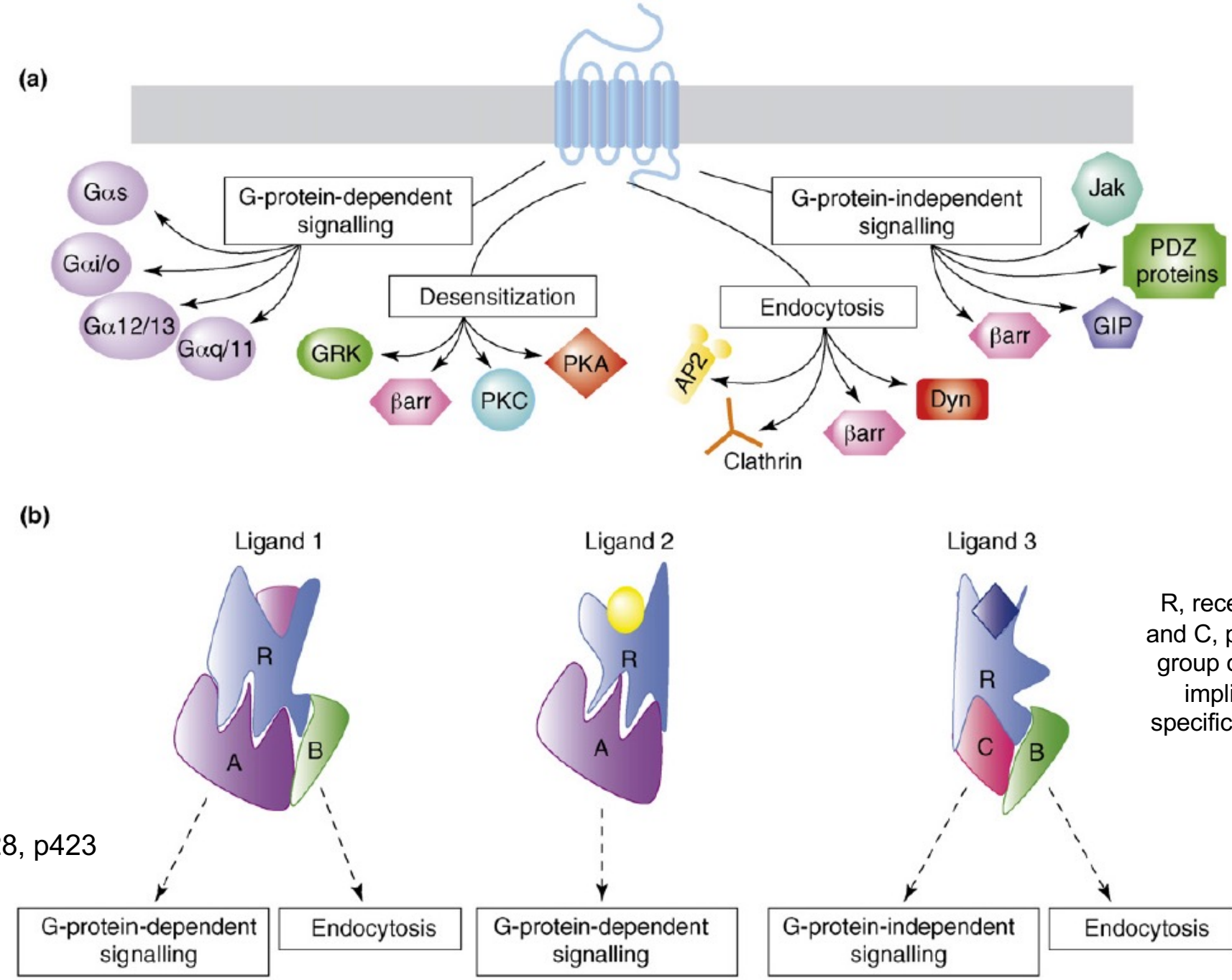


Figure 2-11 Two kinds of receptor desensitisation. Acetylcholine (ACh) at the frog motor endplate. Brief depolarisations (upward deflections) are produced by short pulses of ACh delivered from a micropipette. A long pulse (horizontal line) causes the response to decline with a time course of about 20 seconds, owing to desensitisation, and it recovers with a similar time course. B, β-Adrenoceptors of rat glioma cells in tissue culture. Isoprenaline (1μmol/l) was added at time zero, and the adenylate cyclase response and β-adrenoceptor density measured at intervals. During the early uncoupling phase, the response (blue line) declines with no change in receptor density (red line). Later, the response declines further concomitantly with disappearance of receptors from the membrane by internalisation. The green and orange lines show the recovery of the response and receptor density after the isoprenaline is washed out during the early or late phase. (From: (A) Katz B, Thesleff S 1957 J Physiol 138: 63; (B) Perkins J P 1981 Trends Pharmacol Sci 2: 326.)

GPCR signaling not only via G proteins



R, receptor; A, B and C, proteins or group of proteins implicated in a specific signalling pathway

TiPs 28, p423

Some recent developments in GPCR research

- GPCR desensitization: involves receptor phosphorylation and internalization. Phosphorylation allows arrestins to bind, leading to a block of the interaction with G proteins, and to internalization
- GPCR dimerization. GPCRs can function as monomers, but also as heterodimers. Examples are $GABA_B$ receptors and Opioid receptors
- Constitutively active receptors. There is evidence that GPCRs can be active in the absence of agonist (β-adrenergic receptors, H3 receptors)
- The cellular effects of different ligands are qualitatively different, implying the existence of more than one active (R*) state.
- G protein-independent signaling, for example via arrestins to the MAP kinase pathway

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Example: adrenergic receptors

Physiology of the autonomic nervous system

- The autonomic system controls smooth muscle (visceral and vascular), exocrine (and some endocrine) secretions, rate and force of the heart, and certain metabolic processes (e.g. glucose utilisation).

- Sympathetic and parasympathetic systems have opposing actions in some situations (e.g. control of heart rate, gastrointestinal smooth muscle), but not in others (e.g. salivary glands, ciliary muscle).

- Sympathetic activity increases in stress ('fight or flight' response), whereas parasympathetic activity predominates during satiation and repose. Both systems exert a continuous physiological control of specific organs under normal conditions, when the body is at neither extreme.

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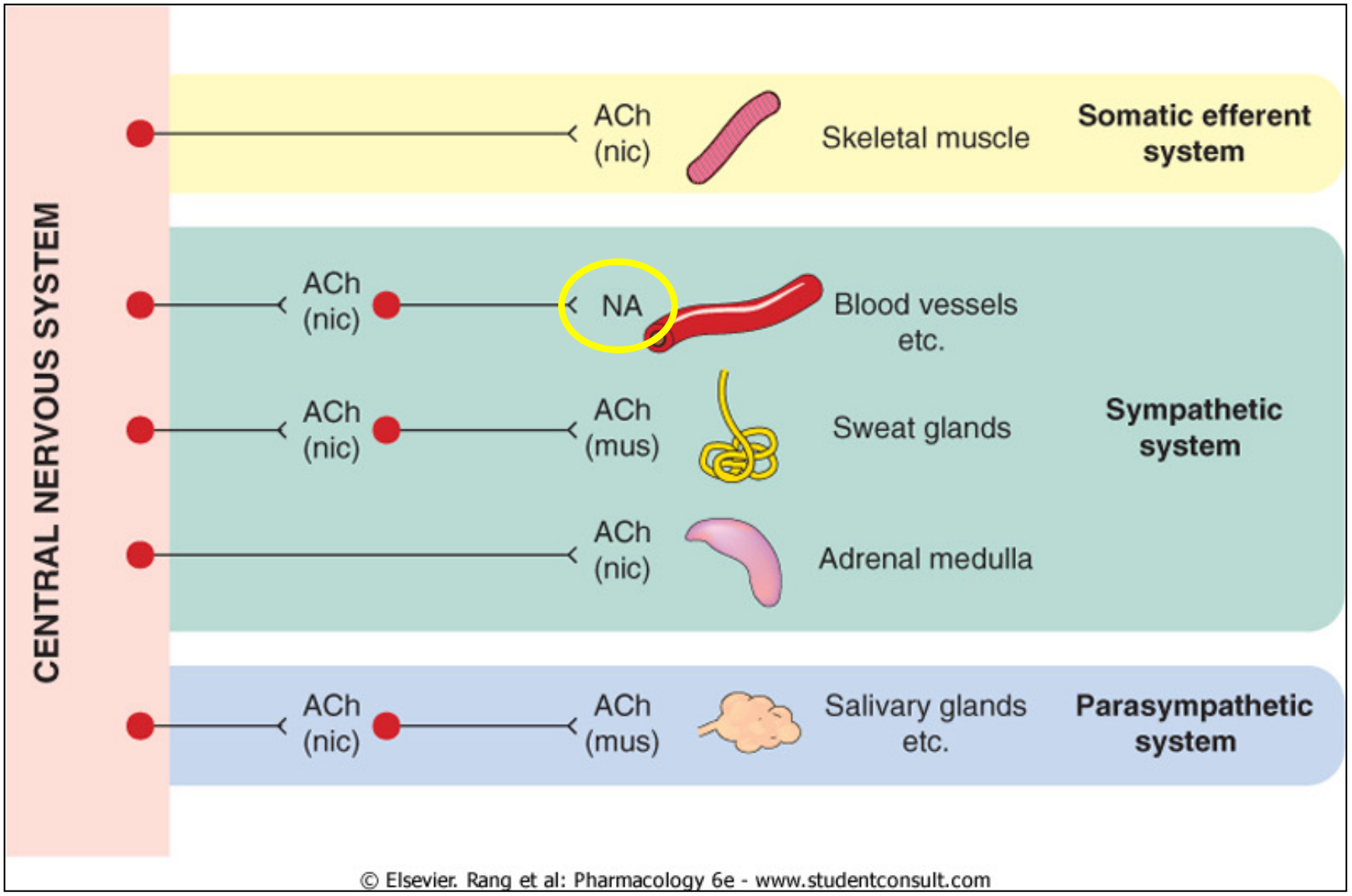
Neurotransmitters in the peripheral nervous system



ACh → nicotinic ACh rec. (Channel)

ACh → muscarinic ACh rec. (GPCR)

NA → Adrenergic receptors (GPCRs)

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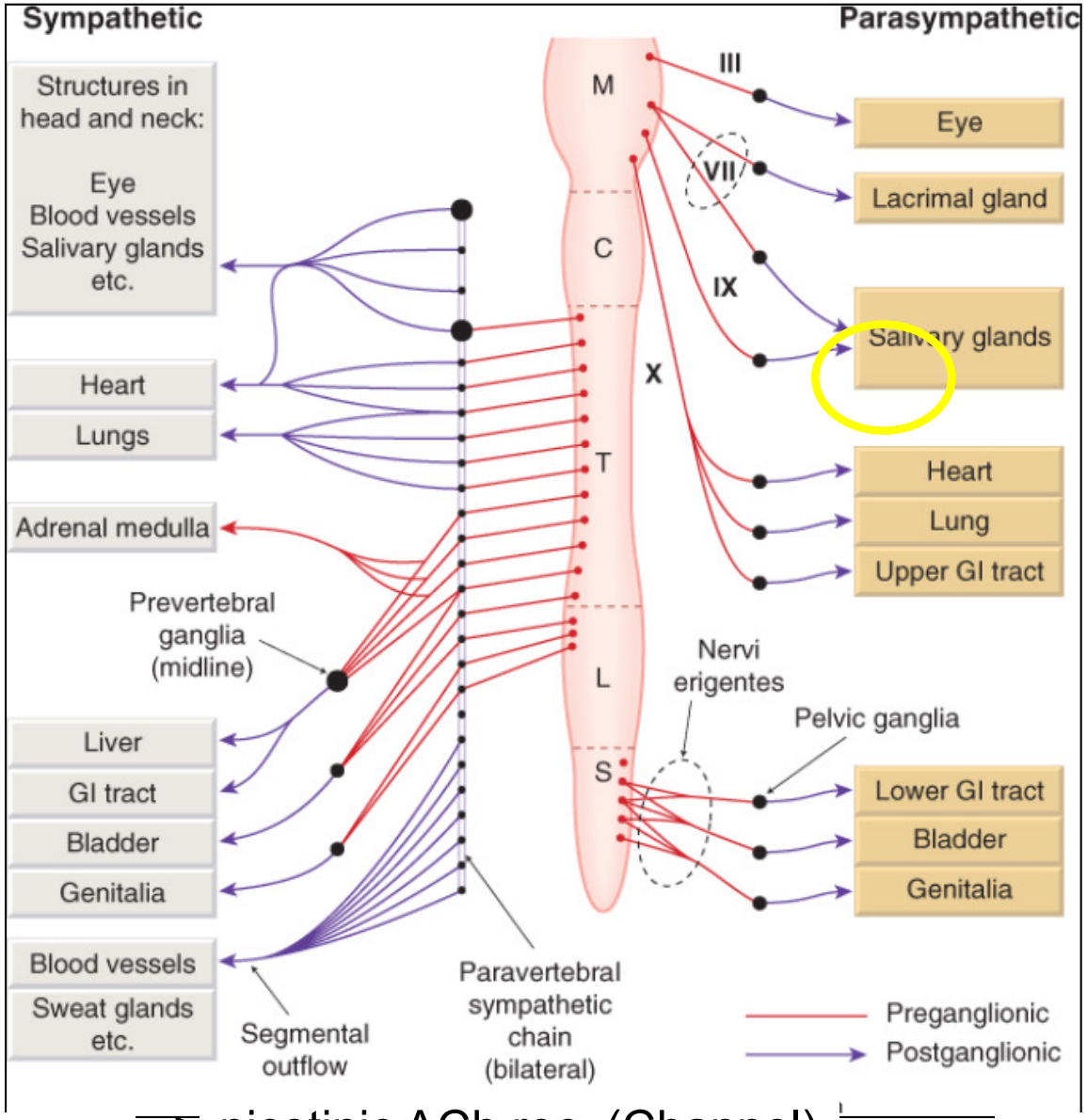
Neurotransmitters in the peripheral nervous system



ACh → nicotinic ACh rec. (Channel)

→ muscarinic ACh rec. (GPCR)

NA → Adrenergic receptors (GPCRs)

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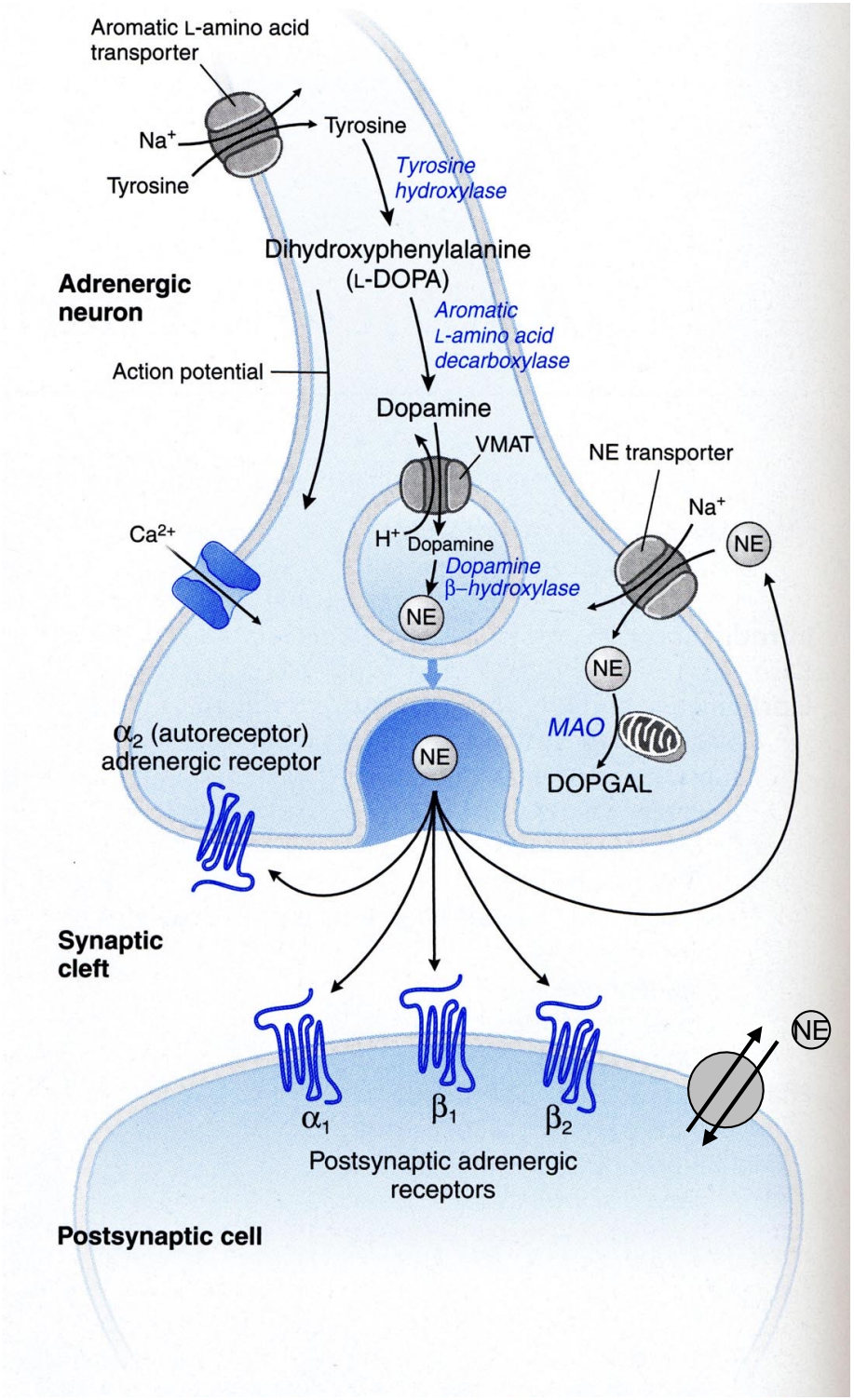
Localization of adrenergic receptors and downstream signaling



receptor subtype	post-/pre-synaptic	G-protein	signaling mechanisms
$\alpha 1$	post	$G_{q/11}$	↑ IP_3, DAG
$\alpha 2$	pre	G_i	↓cAMP
$\beta 1,2$	post	G_s	↑cAMP

Figure 8-1. Catecholamine Synthesis, Storage, Release, and Re-uptake Pathways. The endogenous catecholamines dopamine, norepinephrine and epinephrine are all synthesized from tyrosine. The rate-limiting step in catecholamine synthesis, the oxidation of cytoplasmic tyrosine to dihydroxyphenylalanine (L-DOPA), is catalyzed by the enzyme tyrosine hydroxylase. Aromatic L-amino acid decarboxylase then converts L-DOPA to dopamine. Vesicular monoamine transporter (VMAT) translocates dopamine (and other monoamines) into synaptic vesicles. In adrenergic neurons, intravesicular dopamine-β–hydroxylase converts dopamine to norepinephrine (NE). Norepinephrine is then stored in the vesicle until release. In adrenal medullary cells, norepinephrine returns to the cytosol, where phenylethanolamine N-methyltransferase (PNMT) converts norepinephrine to epinephrine. The epinephrine is then transported back into the vesicle for storage (not shown). α-Methyltyrosine inhibits tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis (not shown). Released norepinephrine can stimulate postsynaptic α_1-, β_1- or β_2-adrenergic receptors, or presynaptic α_2-adrenergic autoreceptors. Released norepinephrine can also be taken up into presynaptic terminals by the selective NE transporter. NE in the cytoplasm of the presynaptic neuron can be further taken up into synaptic vesicles by VMAT (not shown) or degraded to DOPGAL by mitochondrion-associated monoamine oxidase (MAO).

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Main effects of receptor activation:

- α_1-receptors: vasoconstriction, relaxation of gastrointestinal smooth muscle; salivary secretion and hepatic glycogenolysis
- α_2-receptors: inhibition of transmitter release (including noradrenaline and acetylcholine release from autonomic nerves); platelet aggregation, contraction of vascular smooth muscle, inhibition of insulin release
- β_1-receptors: increased cardiac rate and force
- β_2-receptors: bronchodilatation, vasodilatation, relaxation of visceral smooth muscle, hepatic glycogenolysis and muscle tremor
- β_3-receptors (expressed in adipose tissue and the detrusor muscle of the bladder): lipolysis, relaxation of detrusor muscle

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physiological agonists at adrenergic receptors: catecholamines

- **Noradrenaline (Norepinephrine):** a transmitter released by sympathetic nerve terminals. Has actions at α1 and β1 receptors, but relatively little action at β2 receptors
- **Adrenaline (epinephrine),** a hormone secreted by the adrenal medulla. E. stimulates both α and β receptors. At low concentrations predominantly β1 and β2 effects, at high concentrations the α1 effects predominate.
- **Dopamine** is a prominent CNS neurotransmitter. Systemic administration has few CNS effects because it does not readily cross the blood-brain barrier.

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Adrenoceptor agonists: specificity and clinical applications

Noradrenaline and **adrenaline** show relatively little receptor selectivity. Adrenaline is used in cardiac arrest and in anaphylactic shock

Selective α_1 agonists include **phenylephrine** and **oxymetazoline**. *α1 agonists are used for nasal decongestion (nasal sprays)*

Selective α_2 agonists include **clonidine** and **α-methylnoradrenaline**. They cause a fall in blood pressure, partly by inhibition of noradrenaline release and partly by a central action. *They are used to lower blood pressure and intraocular pressure and to reduce the frequency of migraine attacks.*

Selective β_1 agonists include **dobutamine.** *Dobutamine is used in cardiogenic shock.*

Selective β_2 agonists include **salbutamol, terbutaline** and **salmeterol**, *used mainly for their bronchodilator action in asthma.*

Selective β_3 agonist: **mirabegron**, *to treat symptoms of overactive bladder (relaxation of the smooth muscle of the bladder)*

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Adrenoceptor antagonists

- Selective **α_1 antagonists** (e.g. **prazosin, doxazosin, terazosin**) are used to treat hypertension. Postural hypotension is an unwanted effect. Another application of α1 antagonists is to treat the symptoms of benign prostatic hyperplasia. Drugs used for this indication are **tamsulosin and doxazosin.** They have some selectivity for the bladder. They induce relaxation of the smooth muscle of the bladder and prostate; they inhibit prostate hypertrophy.

β-adrenergic receptor antagonists

- Non-selective between β_1 and β_2 adrenoceptors: **propranolol**, **alprenolol**, **oxprenolol**.
- β_1-selective: **atenolol** , nebivolol.
- **Alprenolol** and **oxprenolol** have partial agonist activity.
- Cardiovascular uses:
  - *angina pectoris*
  - *cardiac dysrhythmias*
  - *anxiety, tremor*
  - *hypertension*
-
- Important hazards are bronchoconstriction, bradycardia and cardiac failure (possibly less with partial agonists).
- Side effects include cold extremities, insomnia, depression, fatigue.
- Some show rapid first-pass metabolism, hence poor bioavailability.

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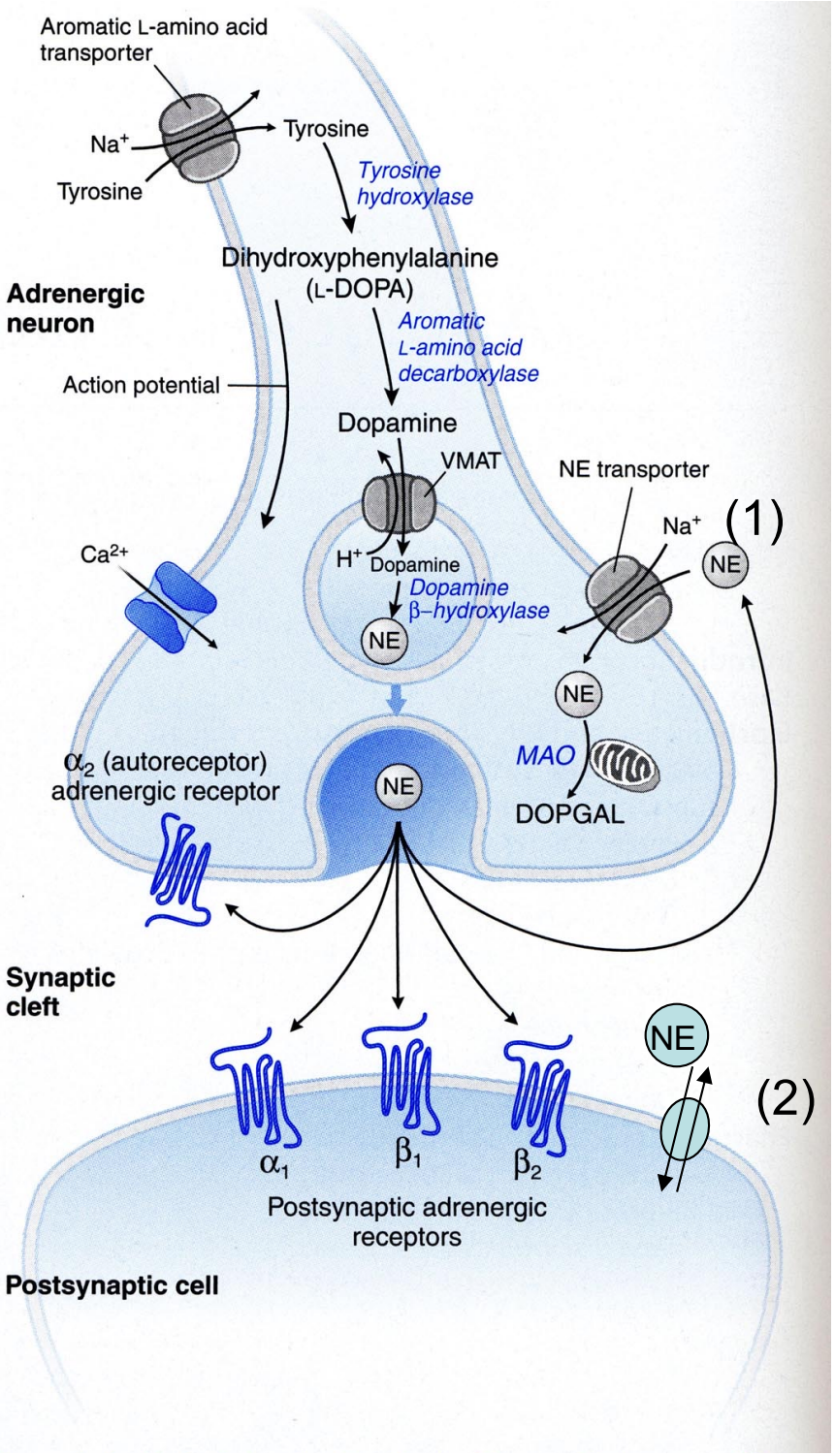

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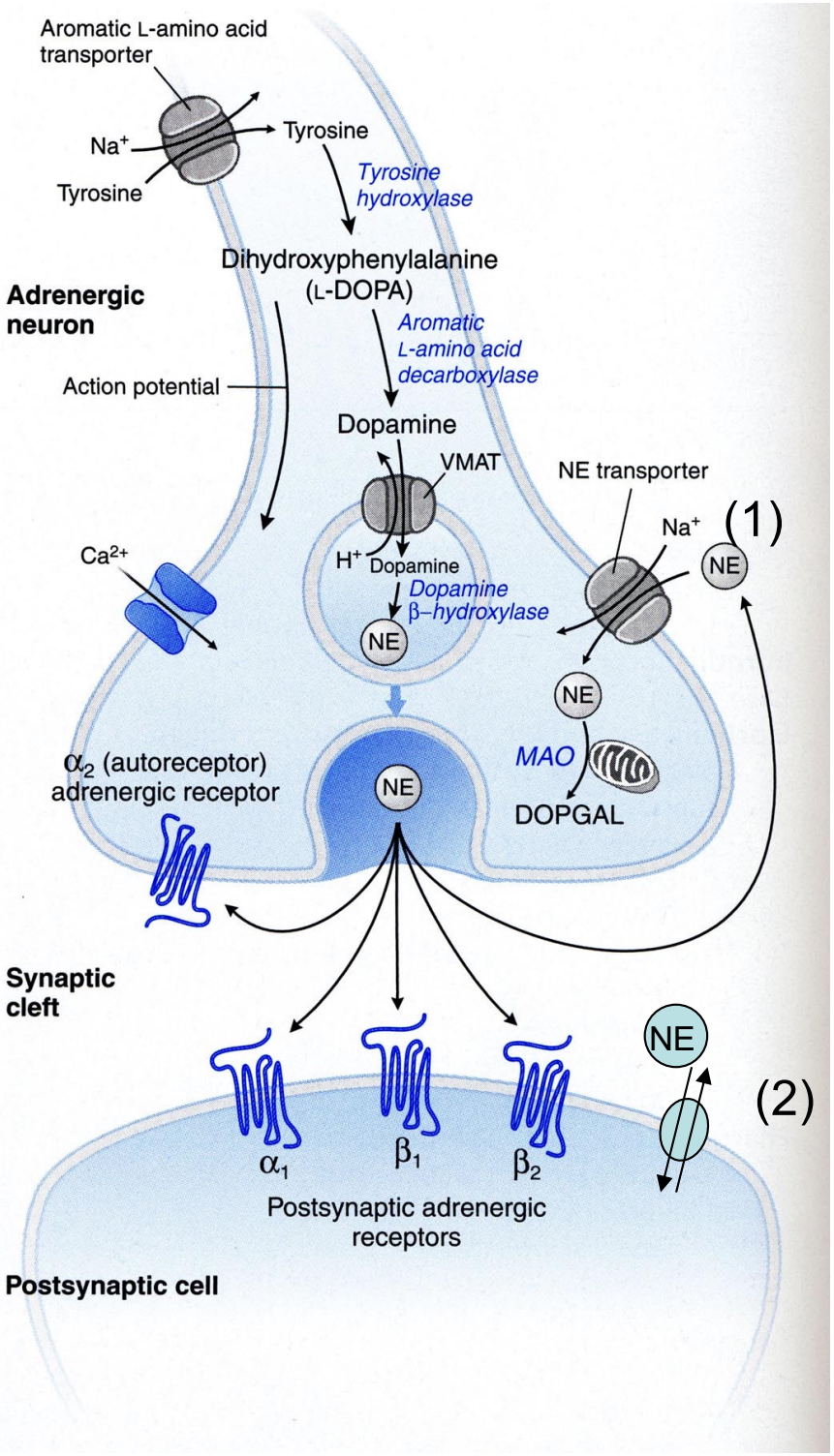
the numbers identify the two types of transmitter uptake systems, uptake 1, present in the neuronal membrane, and uptake 2, present in the non-neuronal cell (smooth muscle, cardiac muscle, endothelium)



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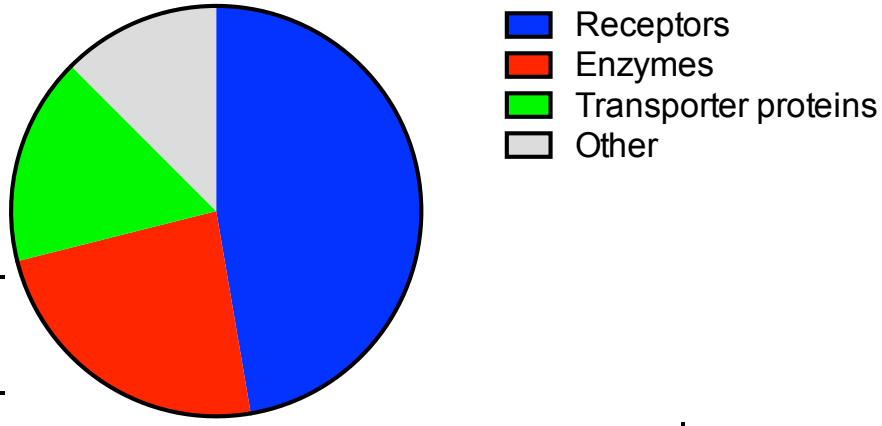
Targets for drug action



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Pain pharmacology

Pain pharmacology: definitions

Definition of pain: An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (international association for the study of pain).

→Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life.

Other definitions:

Noxious Stimulus
A noxious stimulus is one which is damaging to normal tissues.

Nociceptor
A receptor preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged.

Allodynia
Pain due to a stimulus which does not normally provoke pain.

Hyperalgesia
An increased response to a stimulus which is normally painful

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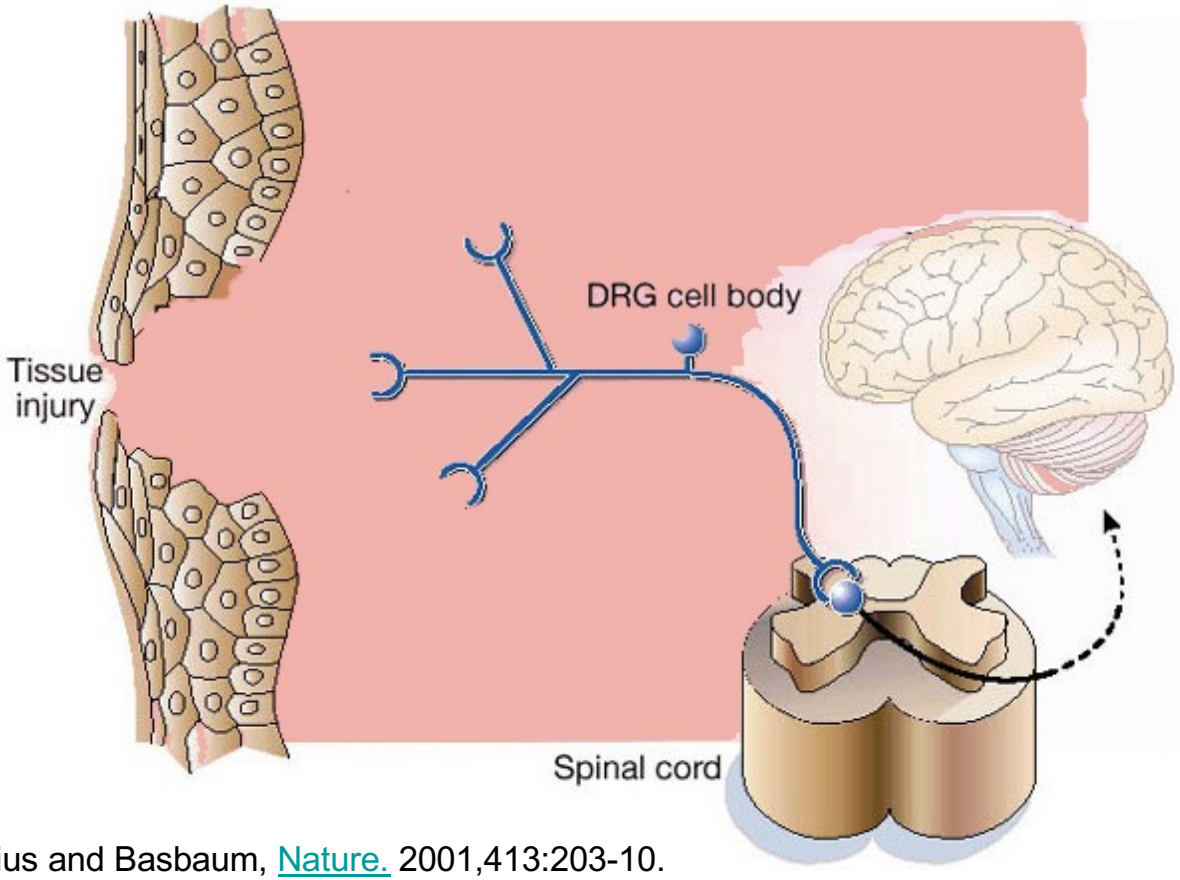
The 4 steps in pain sensation:

Transduction: activation of the peripheral nerve terminal by noxious stimuli (thermal, mechanical or chemical stimuli) → depolarization of the nerve ending

Conduction: travelling of the signal from the nerve terminal to the CNS

Transmission/Modulation: transmission of the signal in the dorsal horn of the spinal cord; integration and relay of the information by a complex neuronal network in the spinal cord and the brain

Perception: final step, subjective



Adapted from Julius and Basbaum, Nature. 2001,413:203-10.

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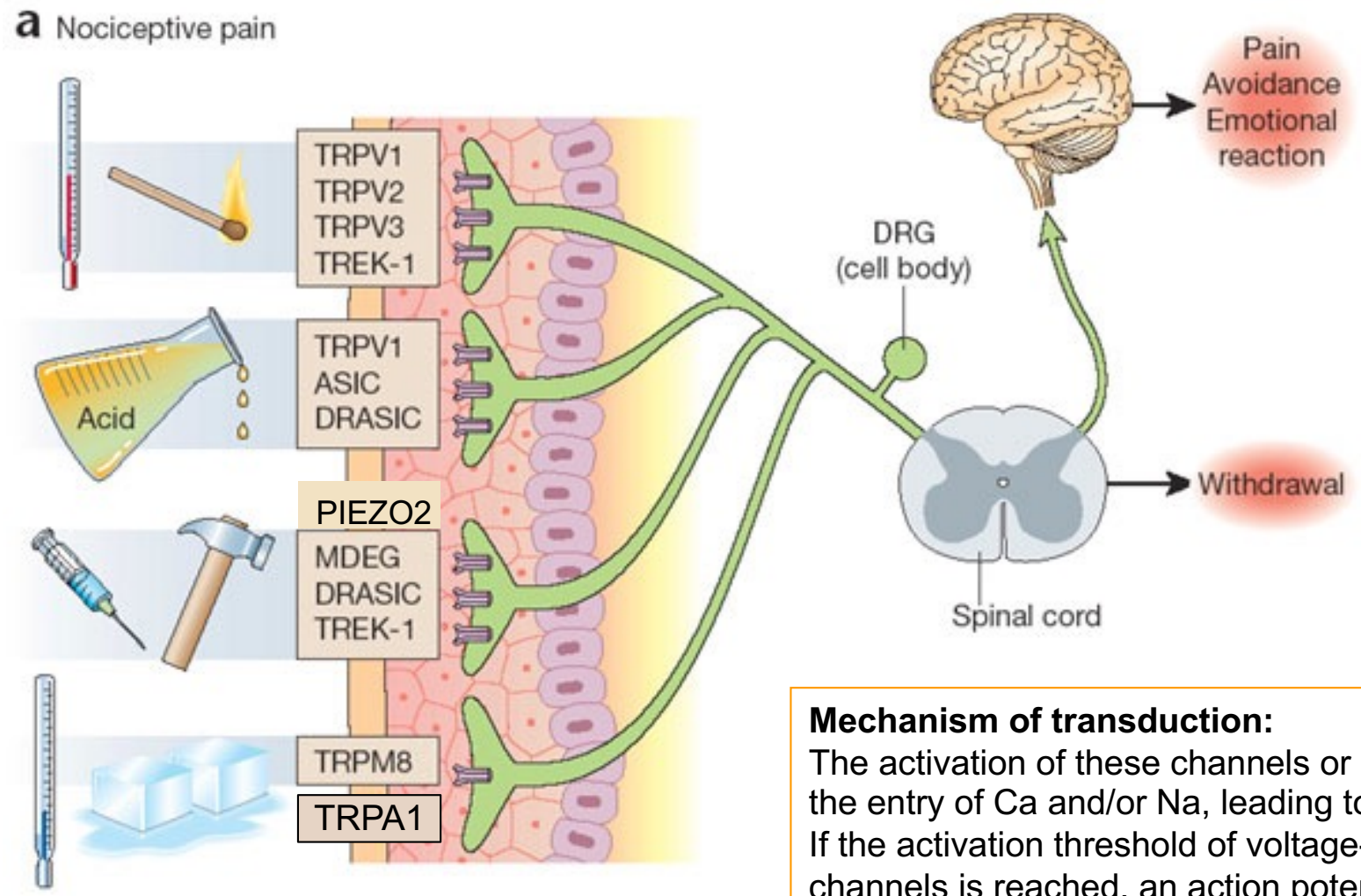

Mechanism of transduction:
The activation of these channels or receptors allows the entry of Ca and/or Na, leading to a depolarization. If the activation threshold of voltage-gated Na channels is reached, an action potential is induced.

(**a**) Noxious stimuli are transduced into electrical activity at the peripheral terminals of unmyelinated C-fiber and thinly myelinated A -fiber nociceptors by specific receptors or ion channels sensitive to heat, mechanical stimuli, protons and cold. This activity is conducted to the spinal cord and, after transmission in central pathways, to the cortex, where the sensation of pain is experienced. (Scholz and Woolf, Nature Neuroscience 2002)

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Trp channels

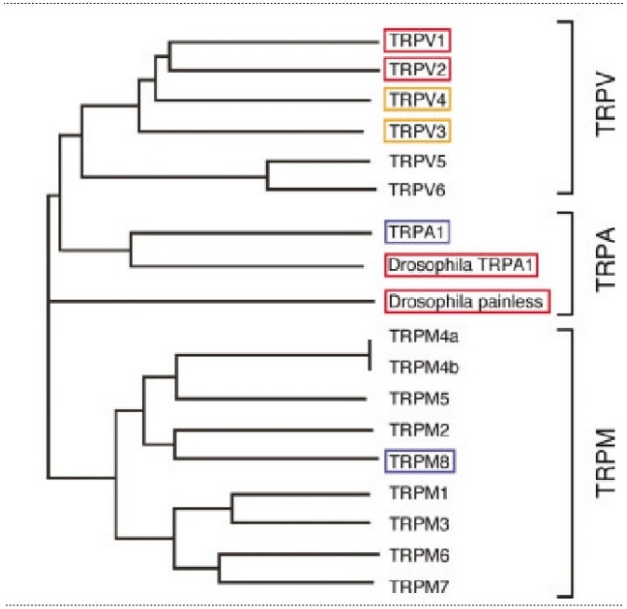
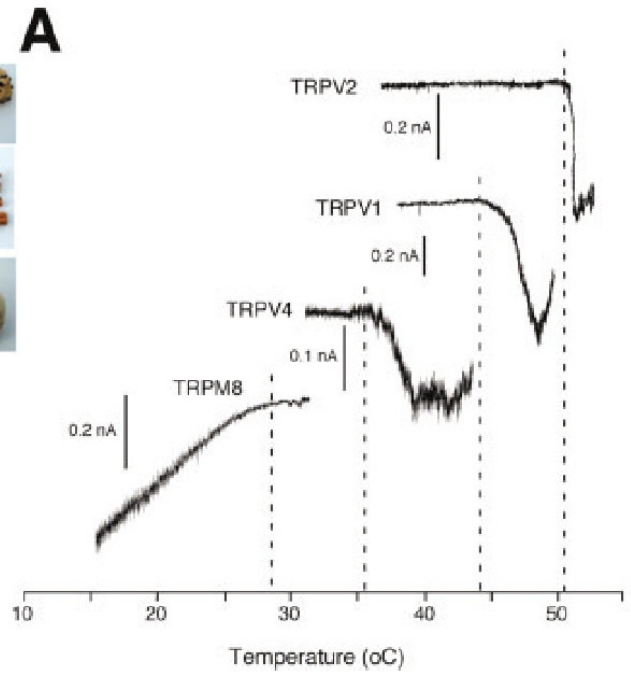
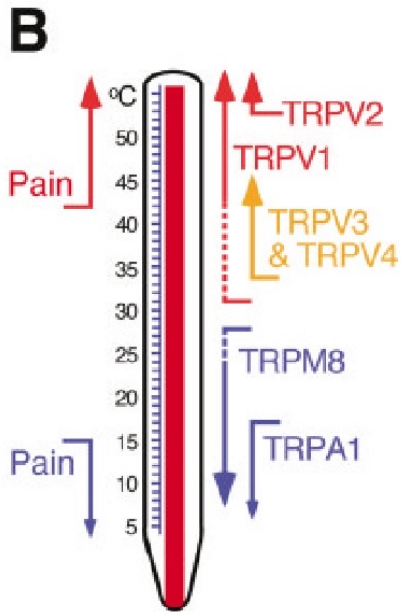


Figure 1 Phylogenetic relationship among the mammalian TRPV, TRPM, and TRPA channels with two *Drosophila* TRPA channels. Red, orange, and blue squares indicate channels activated by high heat, warm, and cold stimuli, respectively.





movie TRPA1 ko

Figure 2 (A) Temperature response pro.les of heat- or cold-induced activation of TRPV1, TRPV2, TRPV4, and TRPM8 at a holding potential of 60 mV in HEK293 cells expressing those
channels. Dotted lines indicate the threshold temperatures for activation. (B) Temperatures causing
pain and activating six TRP channels. Dotted lines indicate that threshold temperatures for activation
of TRPV1 and TRPM8 are not .xed but changeable in the presence of other stimuli (see text).

Thermosensation and Pain
Makoto Tominaga Michael J. Caterina
J Neurobiol. 2004 Oct;61(1):3-12

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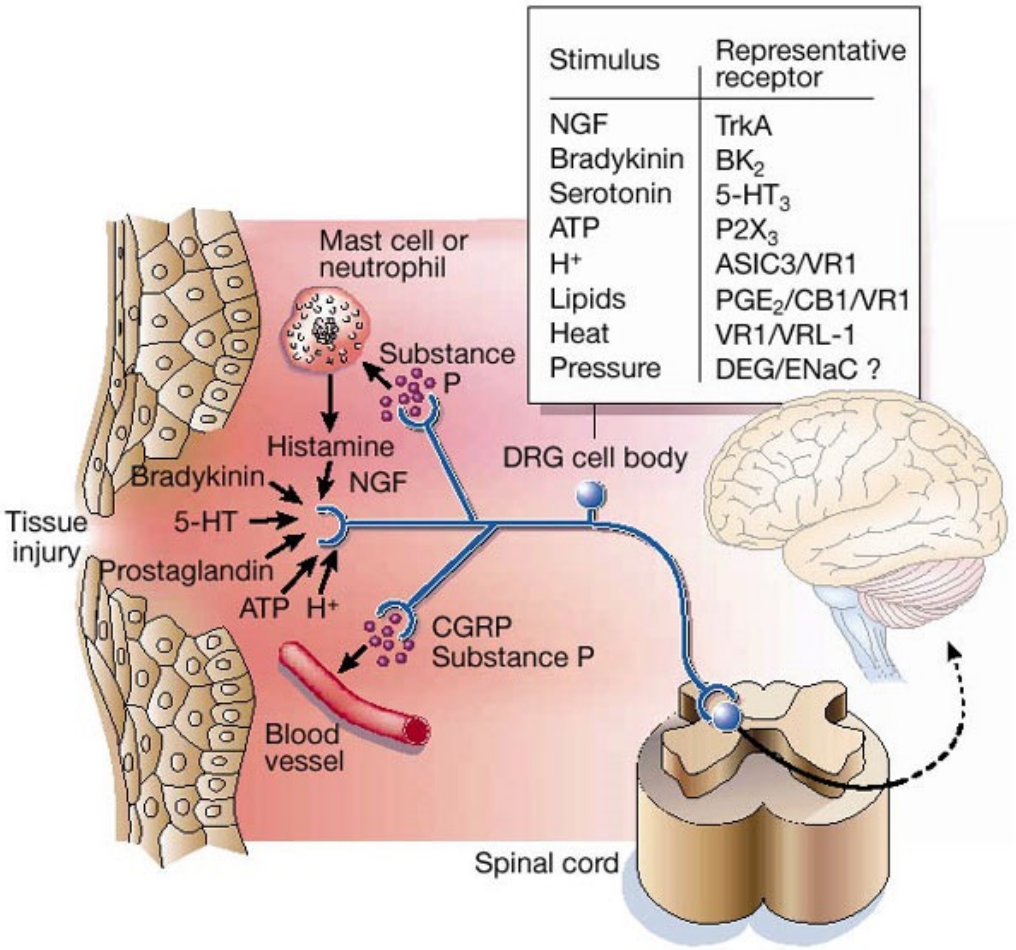
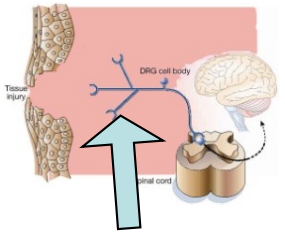
sensitizing agents: « chemical transducers that make pain worse»

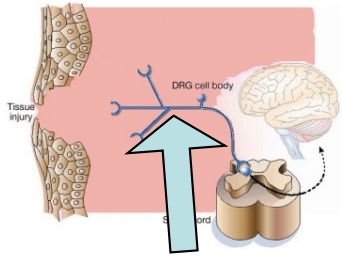




Some of the main components of the 'inflammatory soup' are shown, including peptides (bradykinin), lipids (prostaglandins), neurotransmitters (serotonin (5-HT) and ATP) and neurotrophins (NGF). The acidic nature of the inflammatory soup is also indicated. Each of these factors sensitize (lower the threshold) or excite the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons. Examples of these factors and representative molecular targets are indicated in the box. Activation of the nociceptor not only transmits afferent messages to the spinal cord dorsal horn (and from there to the brain), but also initiates the process of neurogenic inflammation. This is an efferent function of the nociceptor whereby release of neurotransmitters, notably substance P and calcitonin gene related peptide (CGRP), from the peripheral terminal induces vasodilation and plasma extravasation (leakage of proteins and fluid from postcapillary venules), as well as activation of many non-neuronal cells, including mast cells and neutrophils. These cells in turn contribute additional elements to the inflammatory soup. Figure adapted from refs 75,76.

Julius and Basbaum, Nature. 2001,413:203-10.

Peripheral Sensitization (→ leading to increased pain)



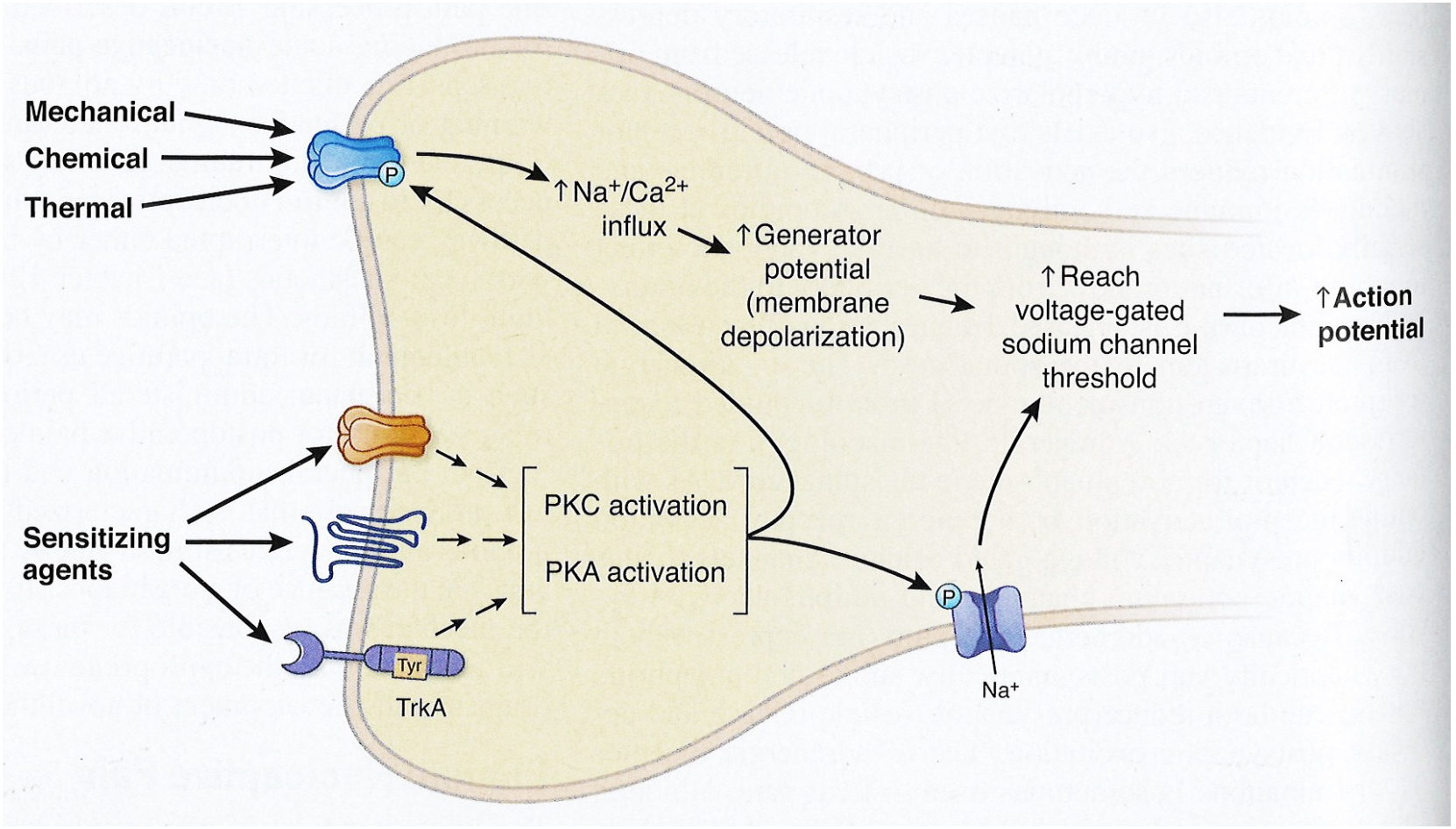


Figure 15-5. Peripheral Sensitization. Peripherally released sensitizing agents activate signal transduction that can increase sensitivity of the peripheral nerve terminal. Mechanisms mediating increased sensitivity include: (1) enhancement of ion influx in response to a noxious stimulus, and (2) reduction of the activation threshold of the voltage-sensitive sodium channels responsible for initiating and propagating action potentials. In the example shown, a sensitizing agent activates its G protein-coupled receptor. This receptor initiates two parallel signaling cascades. One branch activates the phospholipase C (PLC) pathway, which results in increased release of calcium from intracellular stores and in activation of protein kinase C (PKC). Both of these effects increase the ion influx in response to a nociceptive stimulus. The second branch of the signaling cascade activates adenylyl cyclase (AC), leading to increased formation of cAMP, activation of protein kinase A (PKA), and ion channel phosphorylation. Both signaling cascades serve to increase the likelihood of action potential initiation and propagation.

The 4 steps in pain sensation:

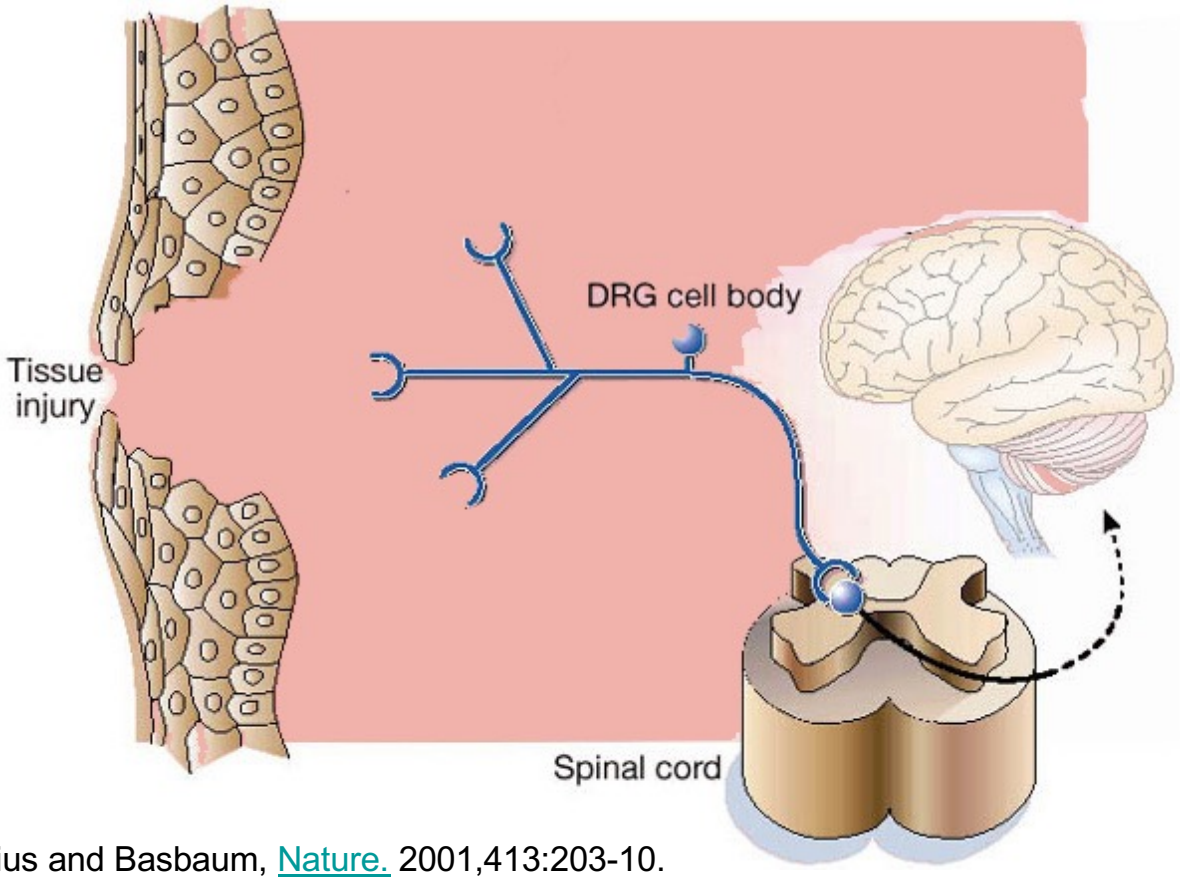
Transduction: activation of the peripheral nerve terminal by noxious stimuli (thermal, mechanical or chemical stimuli) → depolarization of the nerve ending

Conduction: travelling of the signal from the nerve terminal to the CNS

Transmission/Modulation: transmission of the signal in the dorsal horn of the spinal cord; integration and relay of the information by a complex neuronal network in the spinal cord and the brain

Perception: final step, subjective

…..how can we test pain sensation (e.g. in order to evaluate the efficacy of drugs)?



Adapted from Julius and Basbaum, Nature. 2001,413:203-10.

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nature Vol 444|14 December 2006|doi:10.1038/nature05413

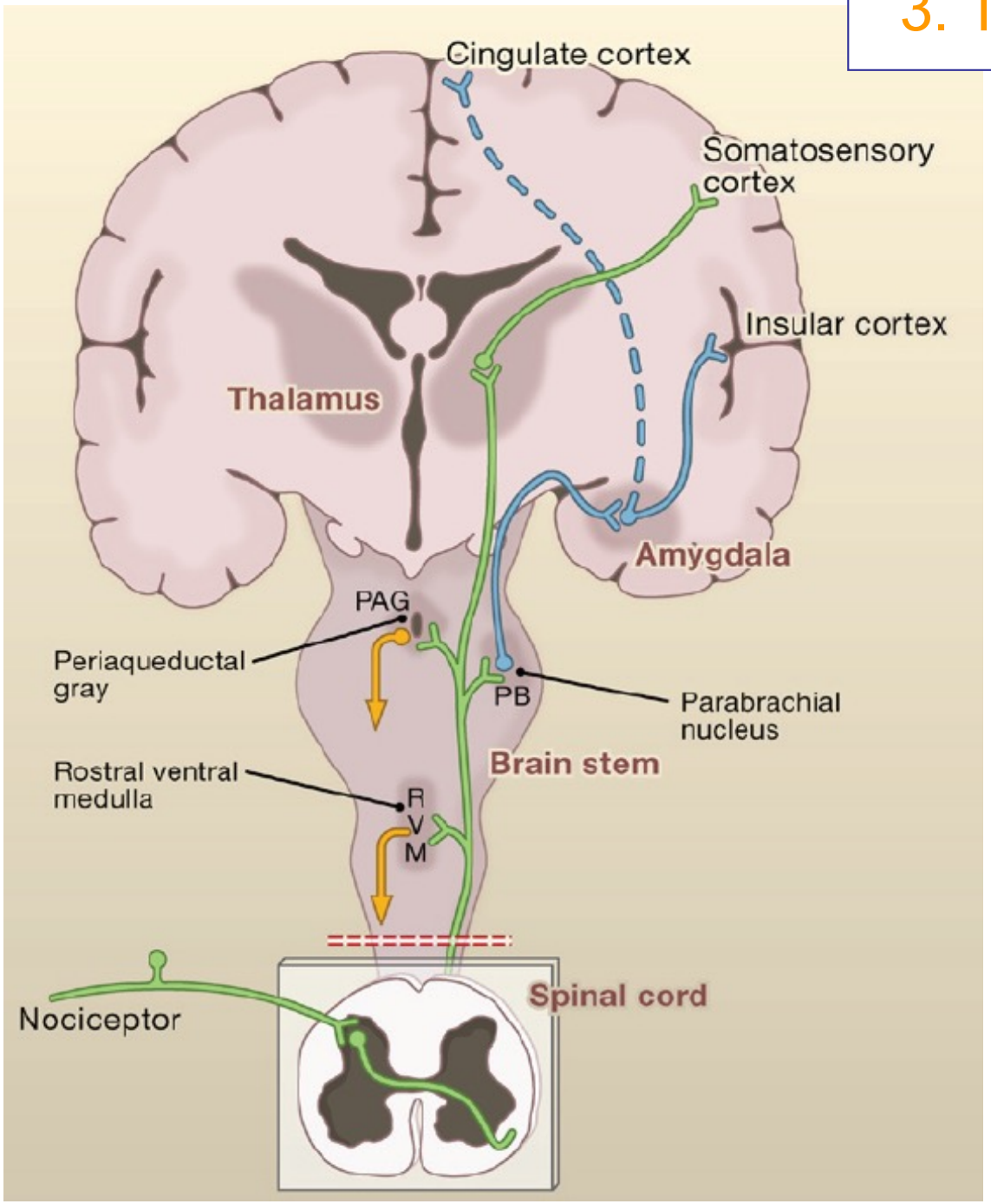
An *SCN9A* channelopathy causes congenital inability to experience pain

James J. Cox[1]*, Frank Reimann[2]*, Adeline K. Nicholas[1], Gemma Thornton[1], Emma Roberts[3], Kelly Springell[3], Gulshan Karbani[4], Hussain Jafri[5], Jovaria Mannan[6], Yasmin Raashid[7], Lihadh Al-Gazali[8], Henan Hamamy[9], Enza Maria Valente[10], Shaun Gorman[11], Richard Williams[12], Duncan P. McHale[12], John N. Wood[13], Fiona M. Gribble[2] & C. Geoffrey Woods[1]

Absence of pain phenotype

The index case for the present study was a ten-year-old child, well known to the medical service after regularly performing 'street theatre'. He placed knives through his arms and walked on burning coals, but experienced no pain. He died before being seen on his fourteenth birthday, after jumping off a house roof. Subsequently, we studied three further consanguineous families in which there were individuals with similar histories of a lack of pain appreciation, each originating from northern Pakistan and part of the Qureshi birdari/clan (Fig. 1). All six affected individuals had never felt any pain, at any time, in any part of their body. Even as babies they had shown no evidence of pain appreciation. None knew what pain felt like, although the older individuals realized what actions should elicit pain (including acting as if in pain after football tackles). All had injuries to their lips (some requiring later plastic surgery) and/or tongue (with loss of the distal third in two cases), caused by biting themselves in the first 4 yr of life. All had frequent bruises and cuts, and most had suffered fractures or osteomyelitis, which were only diagnosed in retrospect because of painless limping or lack of use of a limb. The

3. Transmission/Modulation



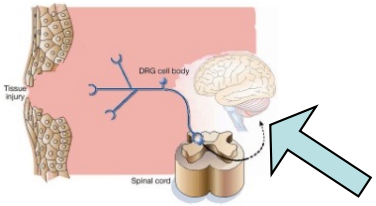


Figure 15-10. Summary of the Sites of Action of the Major Drug Classes Used for Pain Management. Analgesics target various steps in pain perception, from the initiation of a pain stimulus to the central perception of that pain. Nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors modulate the initial membrane depolarization (signal transduction) in response to a peripheral stimulus. Sodium channel blockers decrease action potential conduction in nociceptive fibers. Opioids, antidepressants, NSAIDs, anticonvulsants, and α_2-adrenergic agonists all modulate transmission of pain sensation in the spinal cord by decreasing the signal relayed from peripheral to central pain pathways. Opioids also modulate the central perception of painful stimuli. The multiple sites of action of analgesics allow a combination drug approach to be used in pain management. For example, moderate pain is often treated with combinations of opioids and NSAIDs. Because these drugs have different mechanisms and sites of action, the combination of the drugs is more effective than either drug alone.

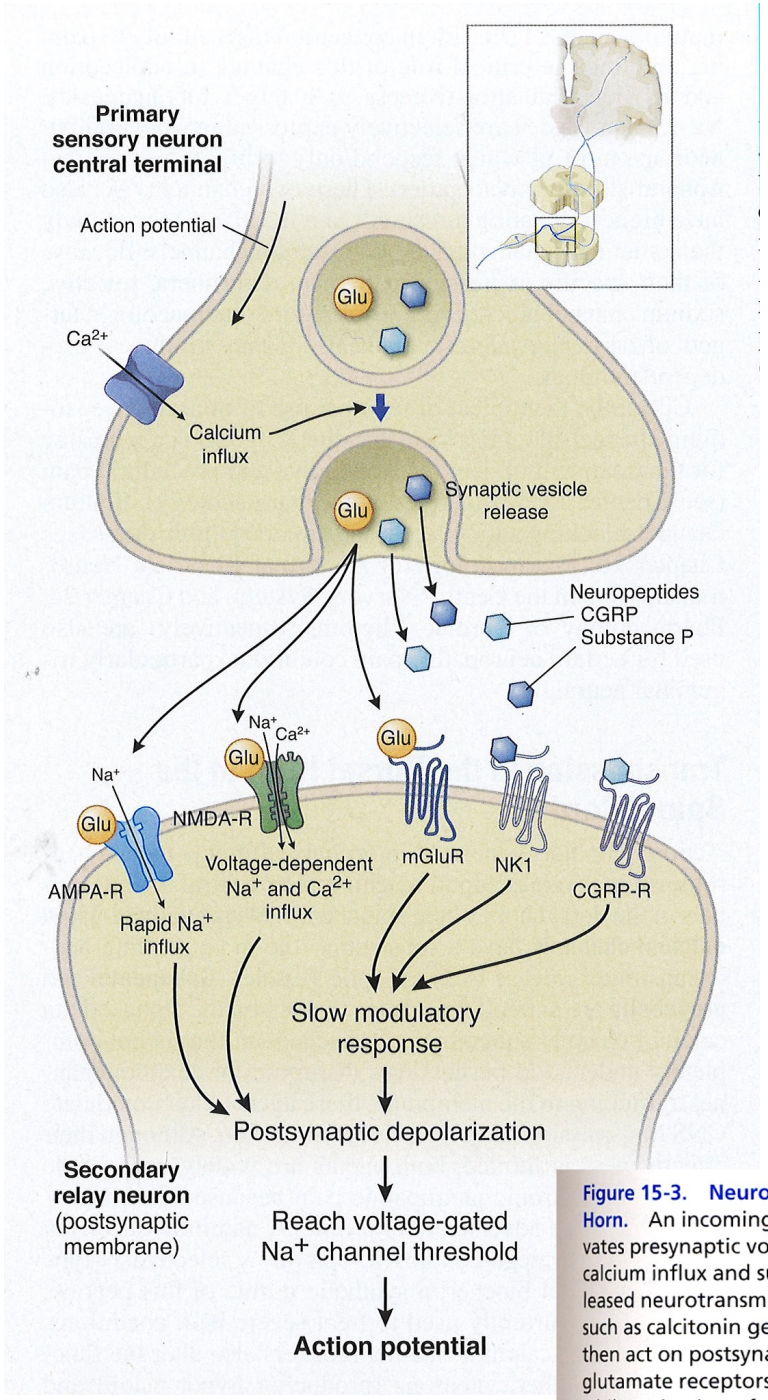
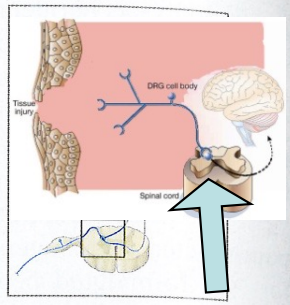
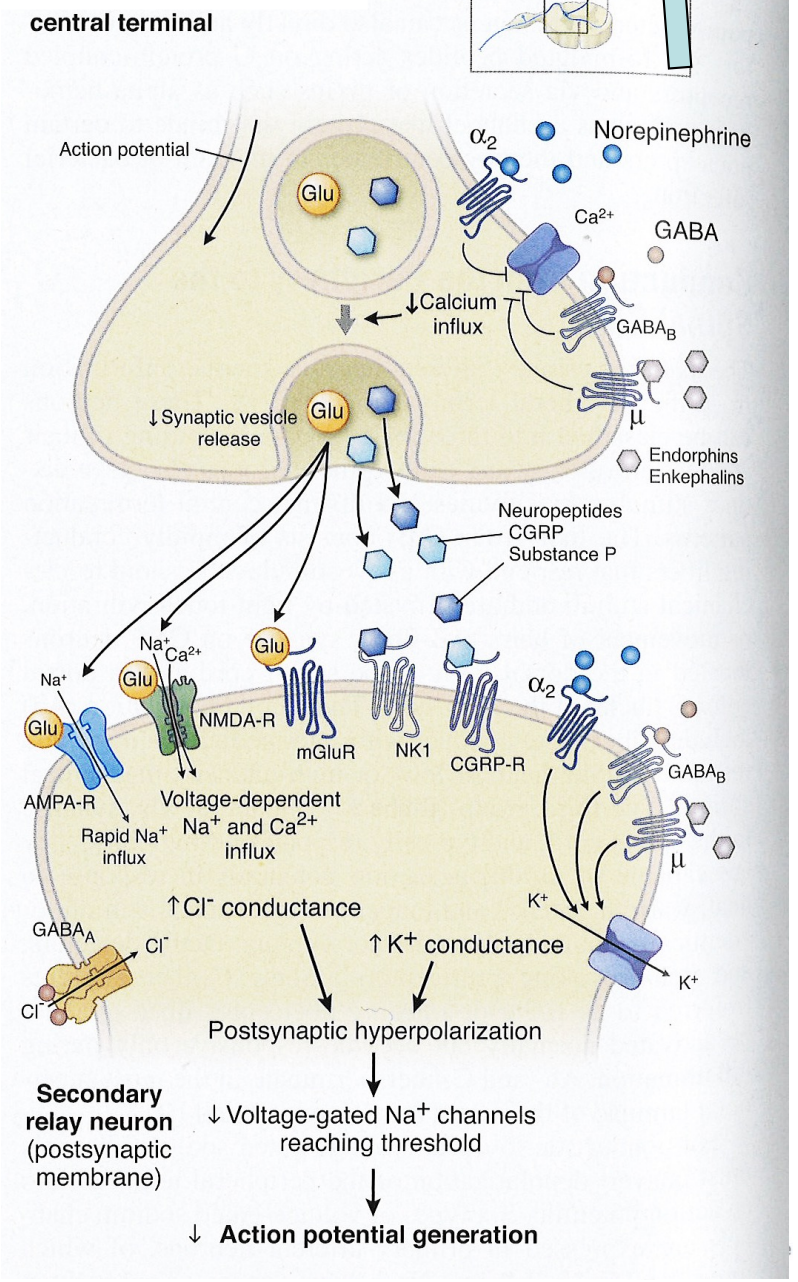


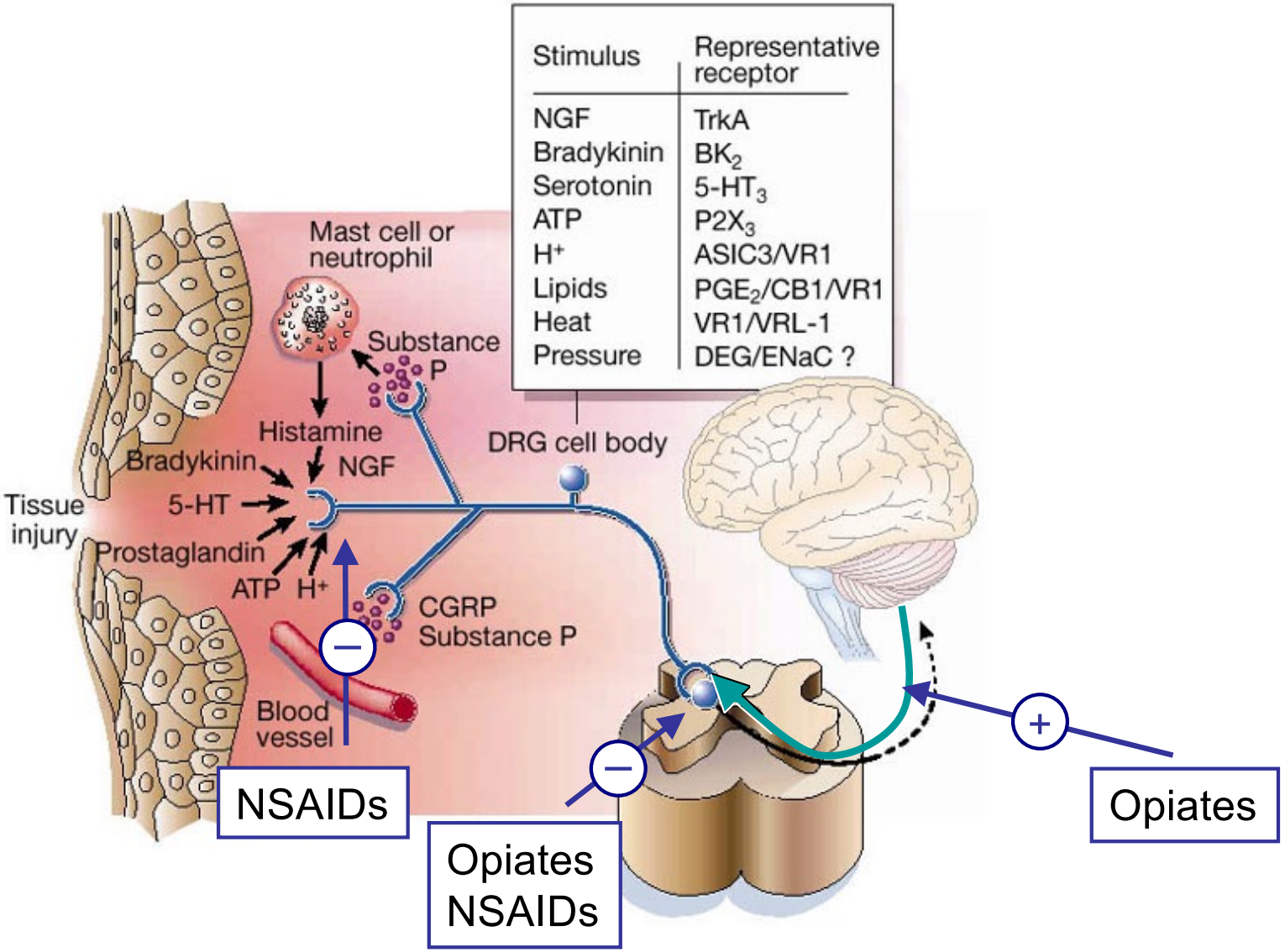
Figure 15-3. Neurotransmission in the Spinal Cord Dorsal Horn. An incoming action potential from the periphery activates presynaptic voltage-sensitive calcium channels, leading to calcium influx and subsequent synaptic vesicle release. The released neurotransmitters (i.e., glutamate and neuropeptides, such as calcitonin gene-related peptide [CGRP] and substance P) then act on postsynaptic receptors. Stimulation of ionotropic glutamate receptors leads to fast postsynaptic depolarization, while activation of other modulatory receptors mediates slower depolarization. Postsynaptic depolarization, if sufficient, leads to action potential production (signal generation) in the secondary relay neuron.

Figure 15-4. Inhibitory Regulation of Neurotransmission. Norepinephrine, GABA, and opioids, released by descending and/or local-circuit inhibitory neurons, act both presynaptically and postsynaptically to inhibit neurotransmission. Presynaptic inhibition is mediated through reduced activity of voltage-sensitive calcium channels, whereas postsynaptic inhibition is mediated primarily by enhanced chloride influx and potassium efflux.





1. Sites of action of analgesics



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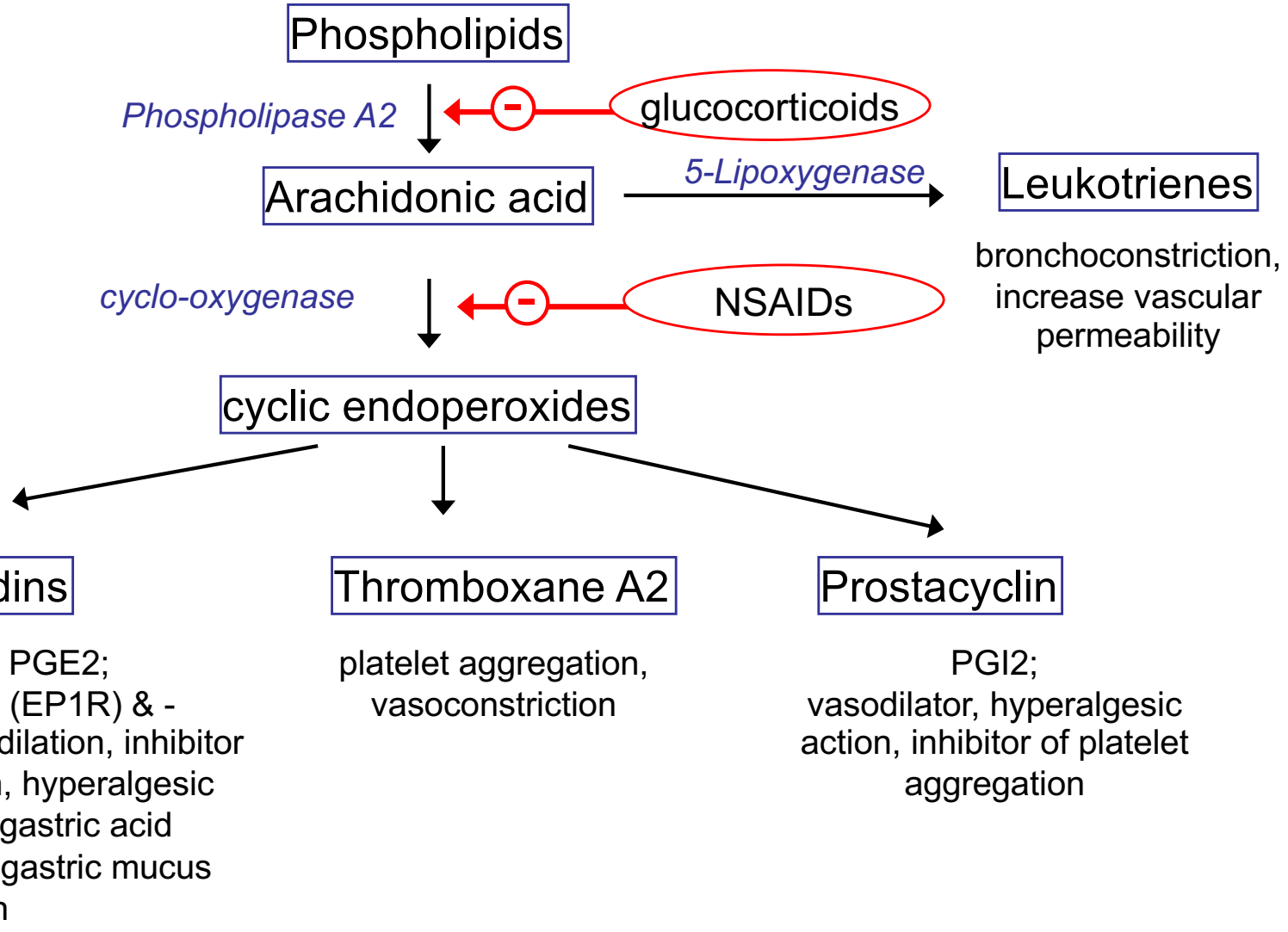
Mechanism of action of NSAIDs



2 cyclo-oxygenase (COX) enzymes exist:

COX-1: constitutively expressed, ubiquitous, protection and maintenance functions

COX-2: inducible expression, in inflamed and activated tissues, produces the inflammatory mediators

Actions of NSAIDs

The NSAIDs have three major pharmacologically desirable actions, stemming from the suppression of prostanoid synthesis in inflammatory cells through inhibition of the cyclo-oxygenase (COX)-2 isoform of the arachidonic acid COX. They are as follow.

- *An anti-inflammatory action*: the decrease in prostaglandin E_2 and prostacyclin reduces vasodilation and, indirectly, oedema.
- *An analgesic effect*: decreased prostaglandin generation means less sensitisation of nociceptive nerve endings to inflammatory mediators such as bradykinin and 5-hydroxytryptamine. At the dorsal horn of the spinal cord it means less prostaglandins that act as pain-producing neuromodulators (for NSAIDs that cross the blood-brain barrier). Relief of headache is probably a result of decreased prostaglandin-mediated vasodilatation.
- *An antipyretic effect*: interleukin-1 releases prostaglandins in the central nervous system, where they elevate the hypothalamic set point for temperature control, thus causing fever. NSAIDs prevent this.
- Some important examples are aspirin℞, ibuprofen℞, naproxen℞, indometacin, piroxicam℞ and paracetamol. Newer agents with more selective inhibition of COX-2 (and thus fewer adverse effects on the gastrointestinal tract) include celecoxib℞ and etoricoxib.

unwanted effects:

- *gastrointestinal effects: nausea, vomiting, gastric discomfort, bleeding or ulcers*
- *adverse cardiovascular effects, may be related to inhibition of COX-2 in the kidney or elsewhere, leading to hypertension*
- *Increased risk of cardiovascular death*
- *bronchospasms in "aspirin-sensitive" asthma*
- *reversible renal insufficiency (inhibition of prostaglandin-mediated vasodilatation in kidney)*
- *all NSAIDs except COX-2 inhibitors prevent platelet aggregation and therefore may prolong bleeding*

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Some important NSAIDs

Substance	brand name	analgesic/antipyretic effect	anti-inflammatory effect	Remarks
acetyl salicylic acid	Aspirin	x x	 x	- dosage ≤ 2-3 g /day - dosage ≥ 3-4 g /day
ibuprofen diclofenac mefenamate	Brufen Voltaren Ponstan	x	x	NSAIDs
paracetamol	Panadol	x		
celecoxib	Celebrex	x	x	COX-2 specific inhibitor

Mechanism of action:
Acetylsalicylic acid covalently acetylates the active site of the enzyme; most other NSAIDs are reversible competitive inhibitors; the exact mechanism of action of paracetamol is not known

Paracetamol differs from the NSAIDs by inhibiting central, but not peripheral COX. Due to this, paracetamol has no anti-inflammatory action, no effect on platelet aggregation and does not induce the unwanted gastrointestinal effects

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2. Opioid receptor agonists

- The opiates act in a system with endogenous ligands (opioid peptides: *enkephalins, β-endorphin and dynorphins*) and receptors (opioid receptors)

- This system interacts at several levels with pain sensation

- Opioid receptors are coupled to protein G; three types of opioid receptors are known, μ, δ and κ (the new terminology is MOPr, DOPr and KOPr).

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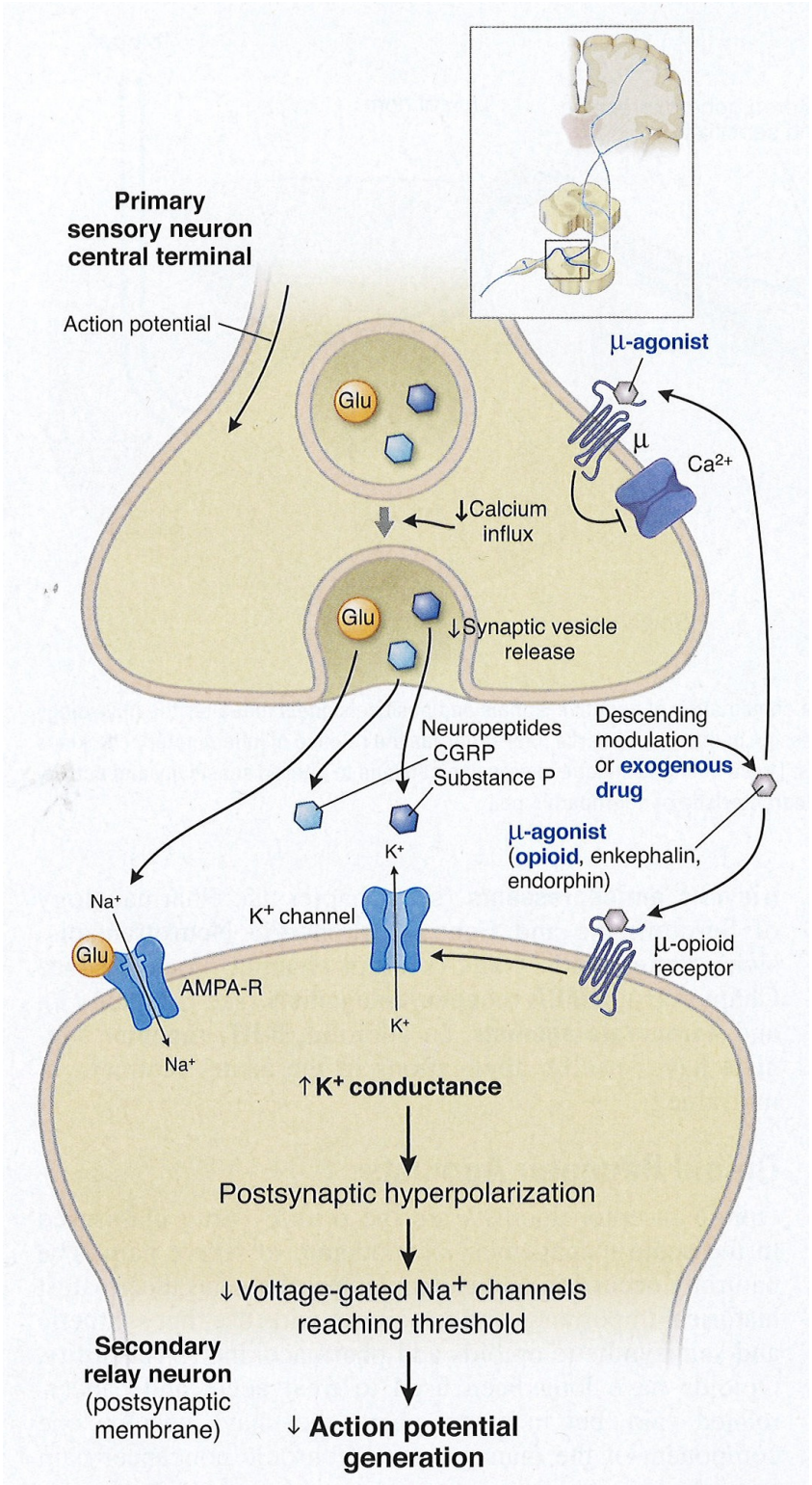

All opioid receptors are linked through G proteins to inhibition of adenylate cyclase. They also facilitate opening of potassium channels (causing hyperpolarisation), and inhibit opening of calcium channels (inhibiting transmitter release). These membrane effects are not linked to the decrease in cAMP formation.

<u>signal transduction mechanism:</u>

- Gi/o → cAMP modulation
- Gi/o → K channel opening
- Go → Ca channel closing

FIGURE 18-8. Mechanism of action of μ-opioid receptor agonists in the spinal cord. Activation of both presynaptic and postsynaptic μ-opioid receptors by descending and local circuit inhibitory neurons inhibits central relaying of nociceptive stimuli. In the presynaptic terminal, μ-opioid receptor activation decreases Ca^{2+} influx in response to an incoming action potential. Postsynaptic μ-opioid receptor activation increases K^+ conductance and thereby decreases the postsynaptic response to excitatory neurotransmission.

Actions of morphine

The most important pharmacological effects are on the CNS and the gastrointestinal tract:

- CNS effects
  - Analgesia
  - Hyperalgesia (prevalence estimated >5%, different from tolerance)
  - Euphoria and sedation
  - Respiratory depression (most dangerous side effect)
  - Suppression of cough
  - Nausea and vomiting (in up to 40% of patients)
  - Pupillary constriction
- Effects on the gastrointestinal tract
  - Reduced gastrointestinal motility, causing severe constipation
- Other actions
  - Histamine release, causing bronchoconstriction and hypotension, or local effects (itching).
- Development of tolerance and physical dependence can be a problem in the therapy
- Morphine derivatives (agonists) that don't reach the CNS in therapeutic doses are used as anti-diarrhea drugs: Loperamid (Imodium®) and Diphenoxylate (Reasec®)
- μ-opioid receptor antagonists that do not cross the blood-brain barrier, such as methylnaltrexone bromide, alvimopan, naloxegol and naldemedine can be used in combination with opioid agonists to block unwanted peripheral effects, most notably reduced gastrointestinal motility, nausea and vomiting.

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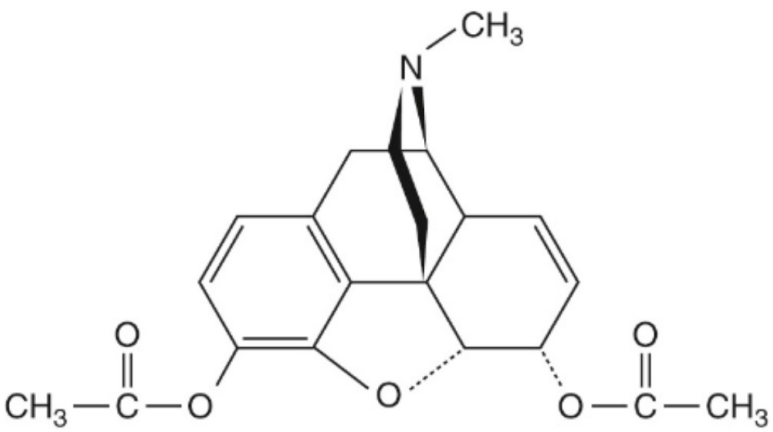
Related drugs

- Diamorphine (Heroin, 3,6-diacetylmorphine): conversion to 6-mono-acetylmorphine and morphine; due to its greater lipid solubility it crosses the blood-brain barrier more rapidly than morphine

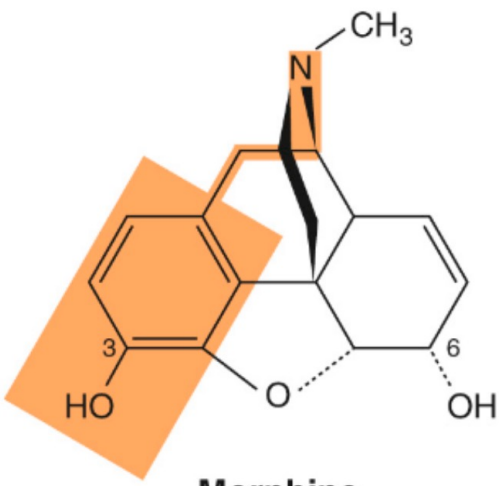
- Codeine (3-methoxymorphine): more reliably absorbed, but has only 20% or less of the analgesic power of morphine

- Fentanyl and derivatives: highly potent, with more rapid onset and shorter duration of action than morphine

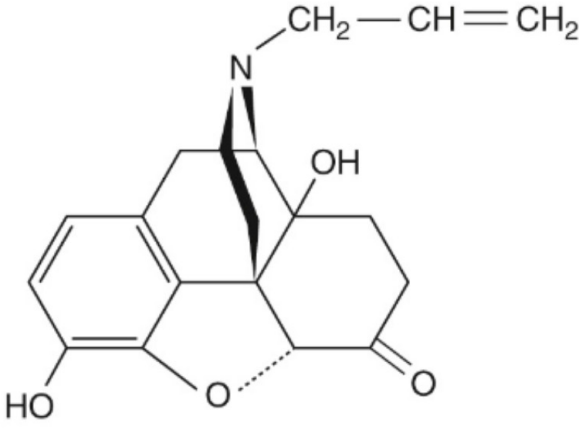
- Methadone: orally active and pharmacologically similar to Morphine. Big difference in pharmacokinetics ($t_{1/2}$ of Methadone is 27h, that of Morphine 2h)



Heroin

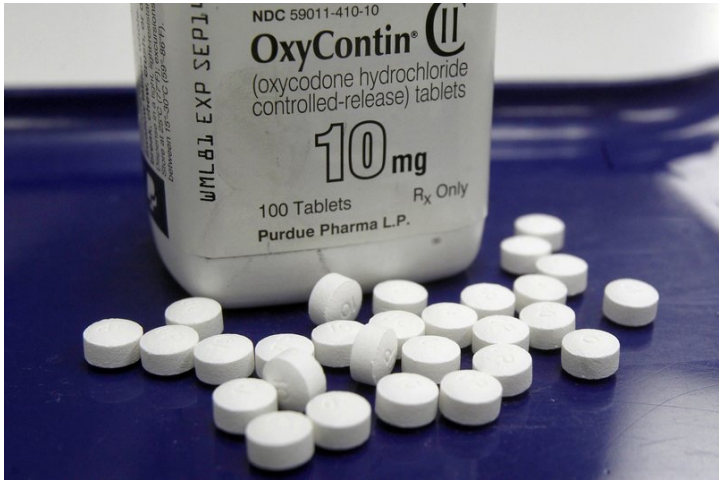


Morphine



Naloxone

Opioid addiction

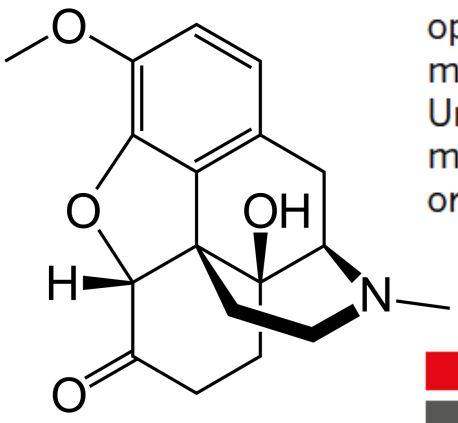


The New York Times

Purdue Pharma Pleads Guilty to Criminal Charges for Opioid Sales

The Justice Department announced an $8 billion settlement with the company. Members of the Sackler family will pay $225 million in civil penalties but criminal investigations continue.

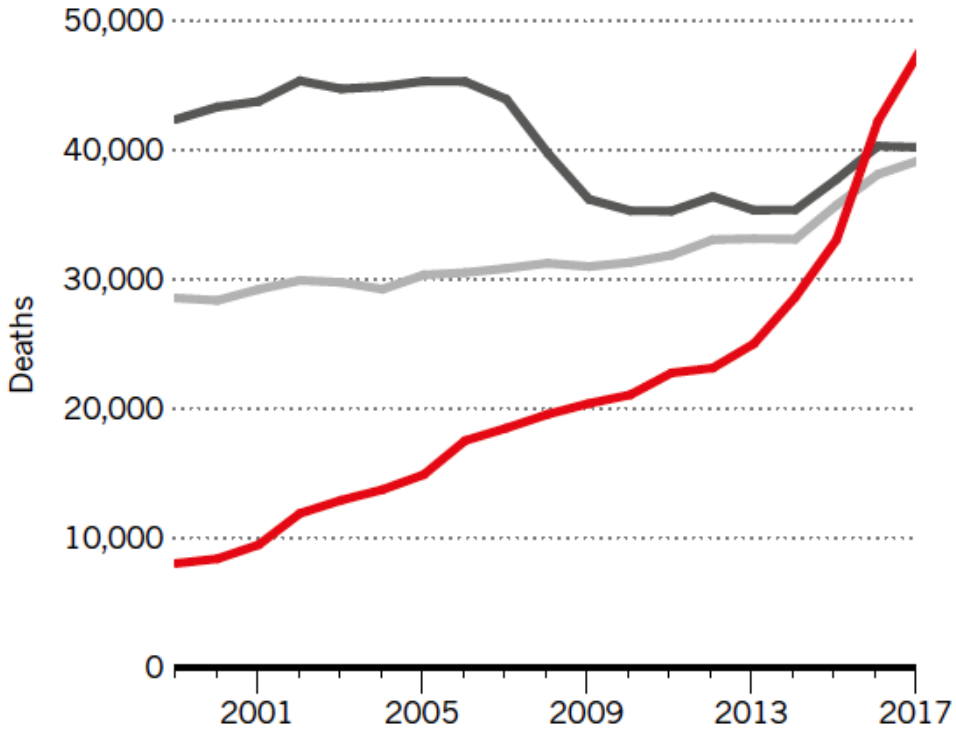
(October 2021)



(Oxycodone)

Rising death toll

Fatalities involving opioids now account for more deaths in the United States than motor-vehicle accidents or firearms incidents.



(in USA)

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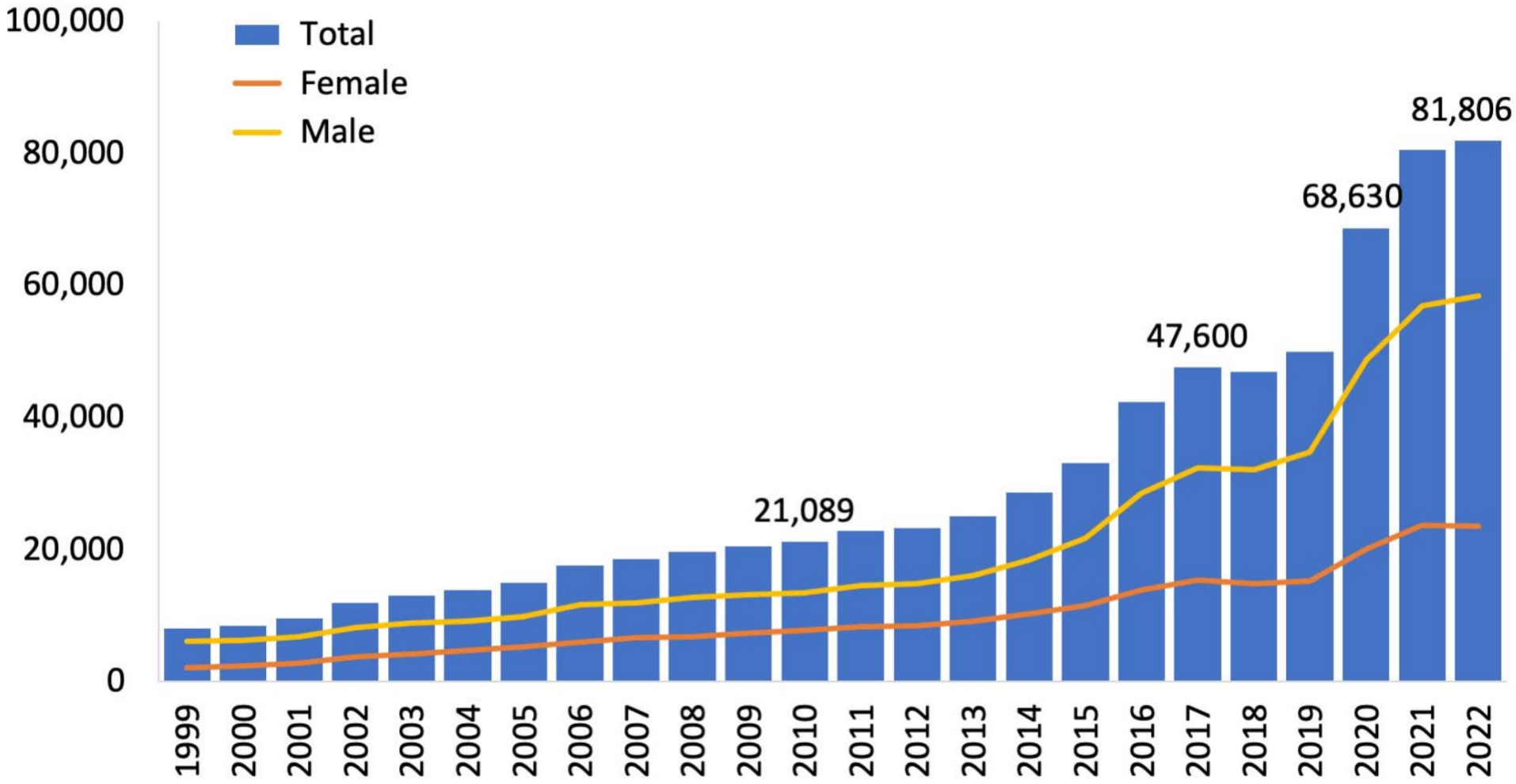
Figure 3. U.S. Overdose Deaths Involving Any Opioid* by Sex, 1999-2022

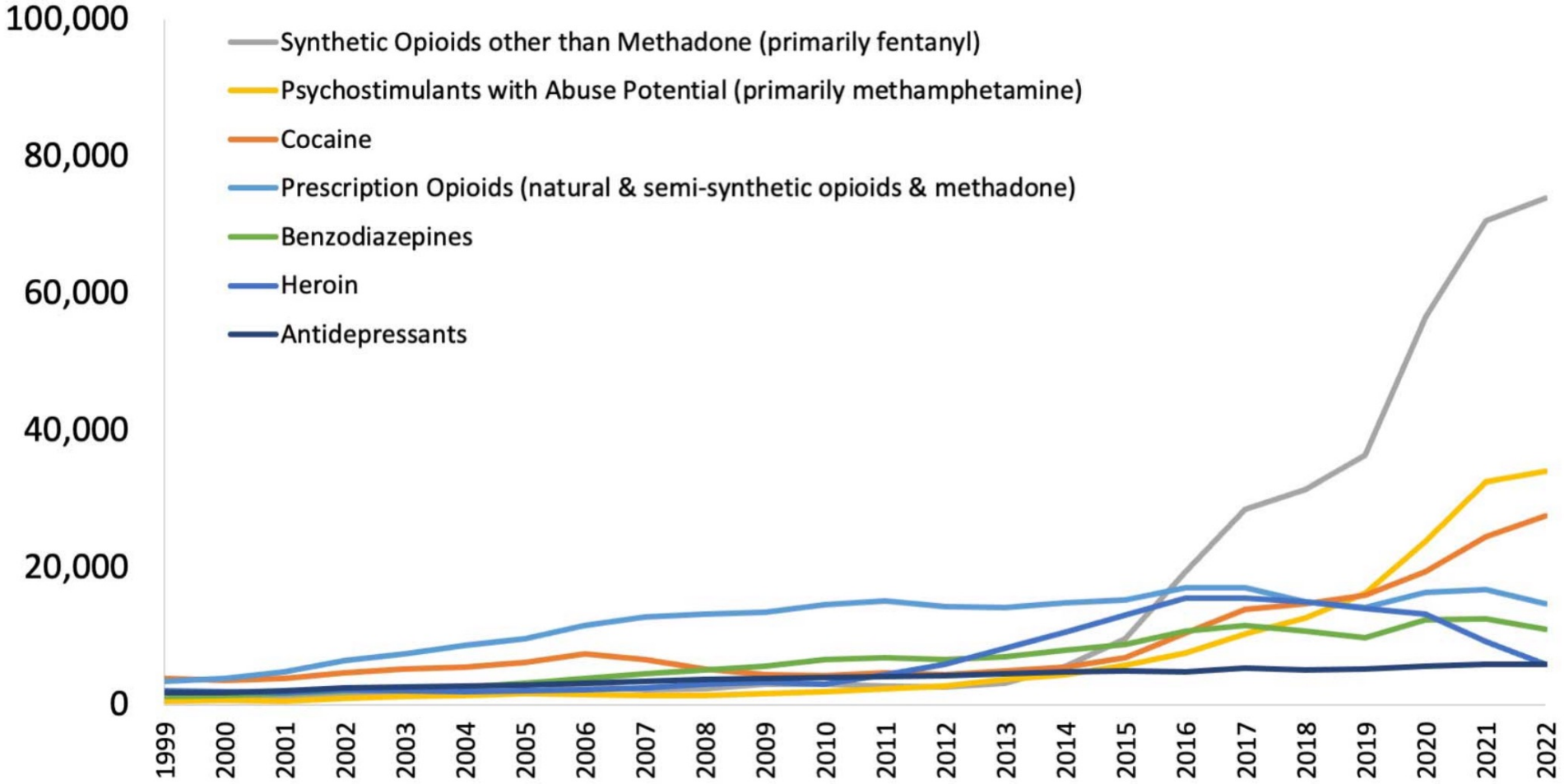


*Among deaths with drug overdose as the underlying cause, the "any opioid" subcategory was determined by the following ICD-10 multiple cause-of-death codes: natural and semi-synthetic opioids (T40.2), methadone (T40.3), other synthetic opioids (other than methadone) (T40.4), or heroin (T40.1). Source: Centers for Disease Control and Prevention, National Center for Health Statistics. Multiple Cause of Death 1999-2022 on CDC WONDER Online Database, released 4/2024.

(in USA)

https://nida.nih.gov/research-topics/trends-statistics/overdose-death-rates

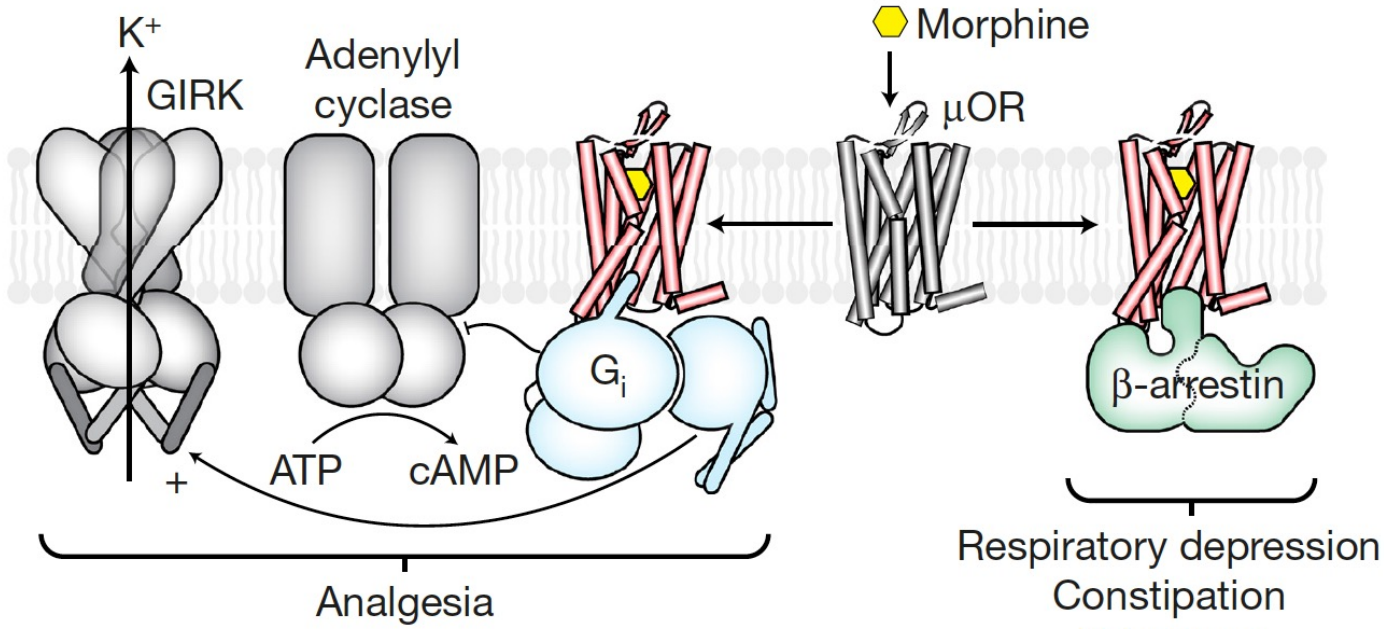
Figure 2. U.S. Overdose Deaths*, Select Drugs or Drug Categories, 1999-2022

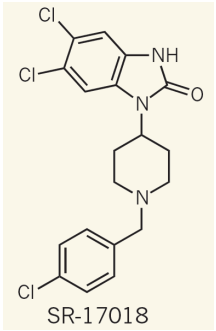


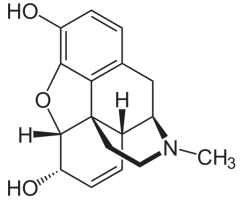
*Includes deaths with underlying causes of unintentional drug poisoning (X40–X44), suicide drug poisoning (X60–X64), homicide drug poisoning (X85), or drug poisoning of undetermined intent (Y10–Y14), as coded in the International Classification of Diseases, 10th Revision. Source: Centers for Disease Control and Prevention, National Center for Health Statistics. Multiple Cause of Death 1999-2022 on CDC WONDER Online Database, released 4/2024.

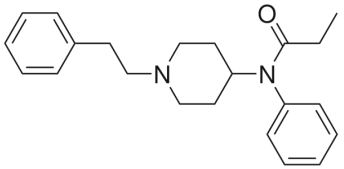
(in USA)

Different downstream signaling pathways mediate analgesia and respiratory depression of μ opioid receptor agonists









Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics

Cell *171*, 1165–1175, November 16, 2017 © 2017 Elsevier Inc.

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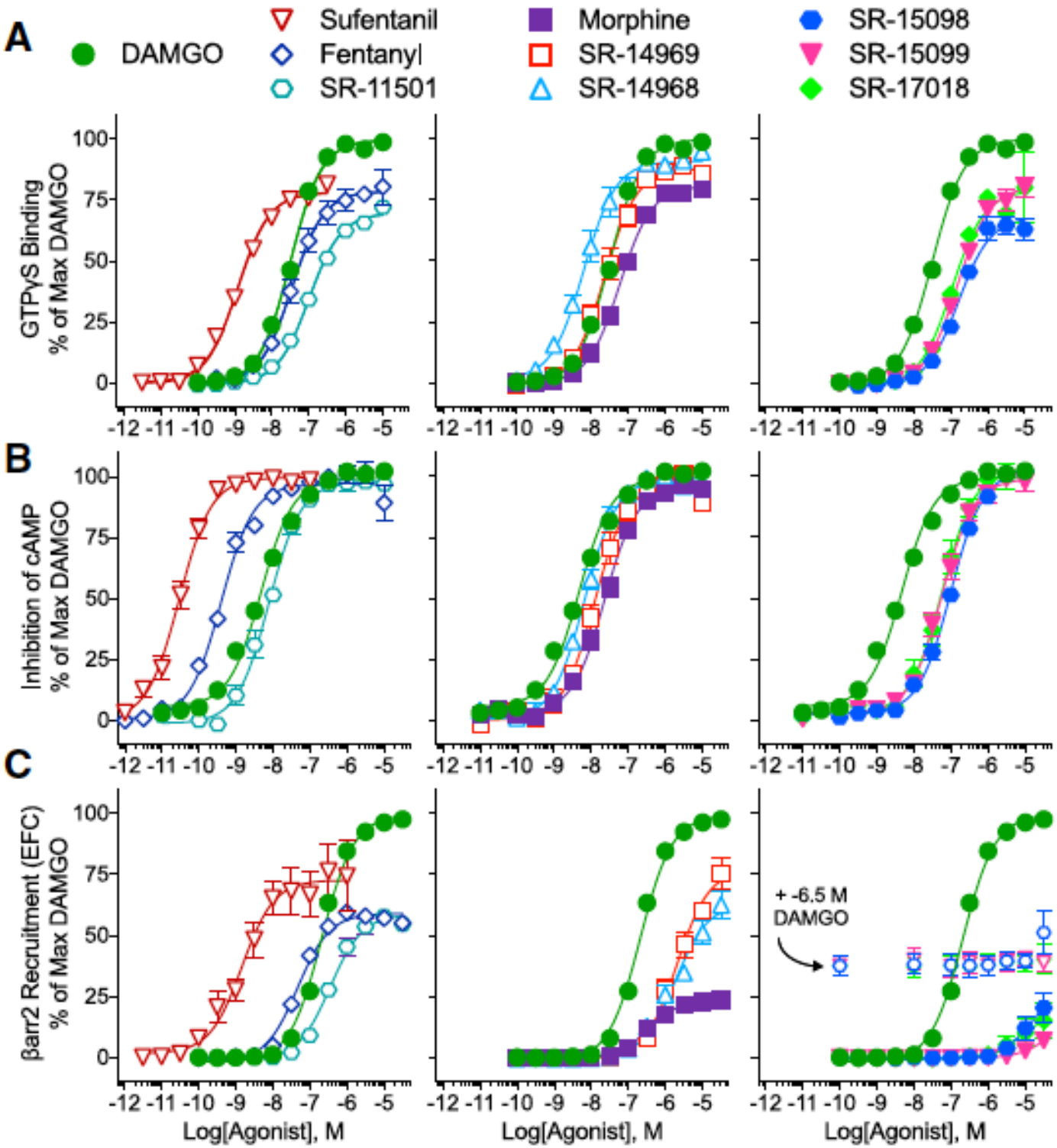

Figure 1. SR Compounds Are Potent Activators of GTPγS Binding but Have Differential βARRESTIN2 Signaling Profiles at the Human MOR

(A–C) Cell-based assays assessing (A) stimulation of GTPγS binding in membranes, (B) inhibition of forskolin-stimulated cAMP accumulation in CHO-hMOR cells, and (C) stimulation of βarrestin2 recruitment in the U2OS-βarrestin-hMOR-PathHunter via the EFC assay. For SR-15098, SR-15099, and SR-17018, βarrestin2 EFC-concentration response curves were also performed in the presence of $10^{-6.5}$ M DAMGO (open symbols) to test for partial agonism. For all three assays, the data were normalized to the % maximal response for DAMGO and are presented as mean ± SEM of three or more assays run in duplicate or triplicate.

(D and E) The $\Delta\Delta\text{Log}(\tau/K_A)$ bias values with 95% CI for the (D) human MOR and (E) mouse MOR. The G protein signaling was determined by either the GTPγS binding assay in CHO-hMOR or CHO-mMOR cells or mouse brainstem or by inhibition of forskolin-stimulated cAMP in CHO-hMOR cells. βarrestin2 recruitment to the MOR was determined by the EFC assay in U2OS-βarrestin-hMOR-PathHunter cells for the human receptor and by the βarrestin2-imaging-based assay using the U2OS-βarrestin2-GFP-mMOR cell line for the mouse receptor. In all assays, DAMGO served as the reference agonist.

See also Table 2 for the $\text{Log}(\tau/K_A)$ and $\Delta\Delta\text{Log}(\tau/K_A)$ values with statistical comparison and Figure S3 for the concentration response curves for the mouse MOR assays (cells and brainstem).

Biased agonism at the opioid receptors: compounds

Oliceridine/TRV130
Showed in initial studies a favorable profile, and safe side effects profile.
Approved by the FDA in 2020 (not available in CH)

PZM21
Very interesting profile in initial studies; several follow-up studies showed however respiratory depression and other side effects.

Mitragynine and 7-OH mitragynine (plant alkaloid and derivative)
Shows also biased agonism towards G_i; may still induce addiction.

Piperidine benzimidazoles (including SR-17018 of the previous slide)
Good G_i-β-arrestin selectivity. Follow-up studies showed that small changes in the molecule affect the selectivity. Development still ongoing.

The concept of the biased agonism and the role of β-arrestin in Morphin side effects is still under discussion, and more studies are needed for its validation.

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3. Approaches for the treatment of neuropathic pain

- Chronic pain
  - Affects 20-30% in the general population, for 60% of them during more than 5 years

- Empirical classification:
  - Neuropathic pain syndromes, related to peripheral or central nervous system injuries
  - Dysfunctional or primary pain, including various chronic pain syndromes, such as irritable bowel syndrome, fibromyalgia, etc.

- Pain relief generally only partial, and achieved in < 50% of patients

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Neuropathic pain

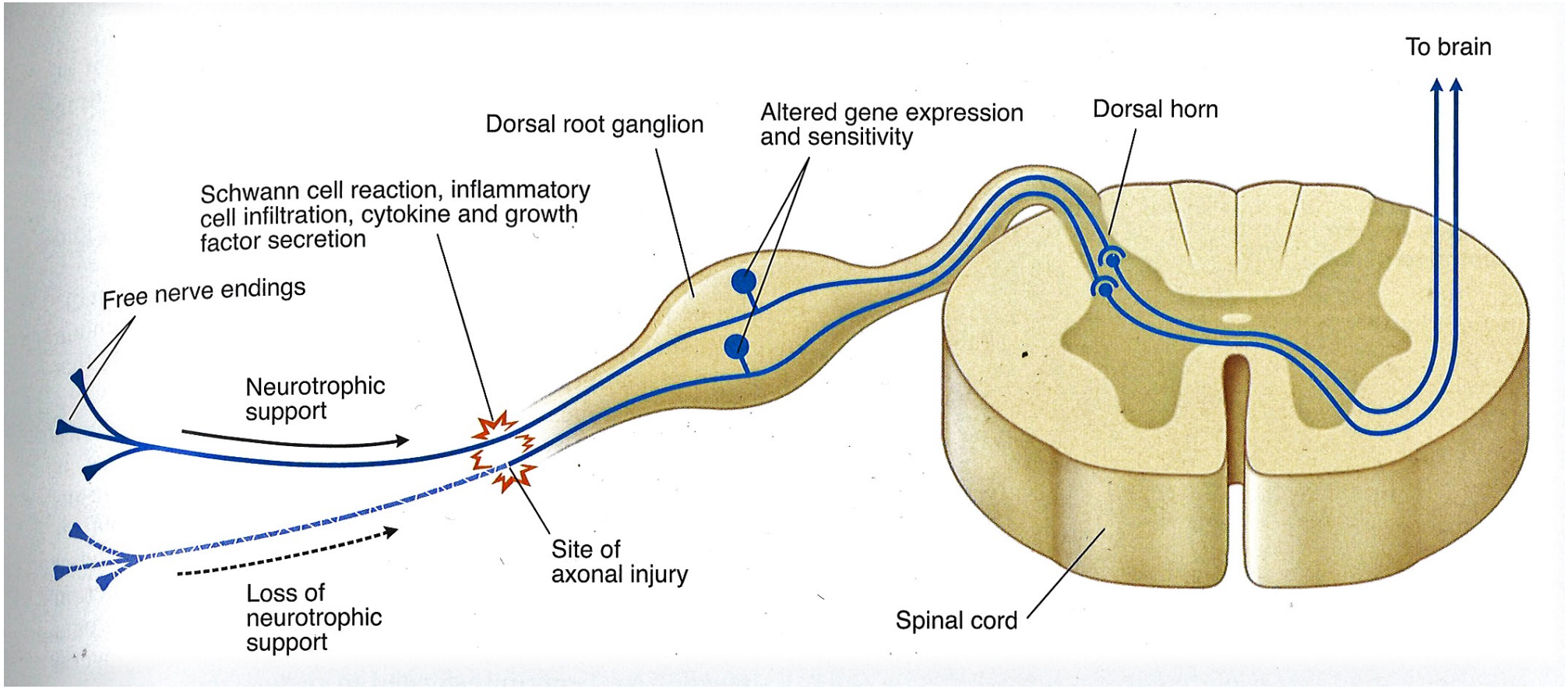

Figure 15-7. Schematization of Neuropathic Pain. Nerve injury results in a combination of negative signals and positive signals that alter the physiology of the nociceptive system. The loss of neurotrophic support alters gene expression in the injured nerve fiber, whereas the release of inflammatory cytokines alters gene expression in both the injured and adjacent uninjured nerve fibers. These changes in gene expression can lead to altered sensitivity and activity of nociceptive fibers, and, thus, to the continued perception of injury that is characteristic of neuropathic pain.

Drugs for the treatment of neuropathic pain

Drug	Drug class	Mechanism of action	Remarks
Gabapentin, Pregabalin	Anti-epileptic drugs	Reduce trafficking of voltage-dependent calcium channels --> attenuation of neuronal hyperexcitability	Pregabalin has a more predictable bioavailability than Gabapentin
Amitriptyline, Duloxetine	Antidepressants	Reduction of central sensitization by modulation of the descending control systems	Effect independent of antidepressant effect
Lidocaine	Local anesthetic drug	Voltage-gated Na-channel inhibition	Topical application
Ziconotide	Peptide inhibitor	Voltage-gated calcium channel inhibition	Narrow therapeutic window

Other drugs that may be used: NMDA receptor antagonists, α2-adrenergic agonists

Different new approaches in development:

- Gene delivery to DRGs (ex. Nav1.3 shRNA)
- Stem cell therapy (ex. Secretion of anti-inflammatory and analgesic cytokines)

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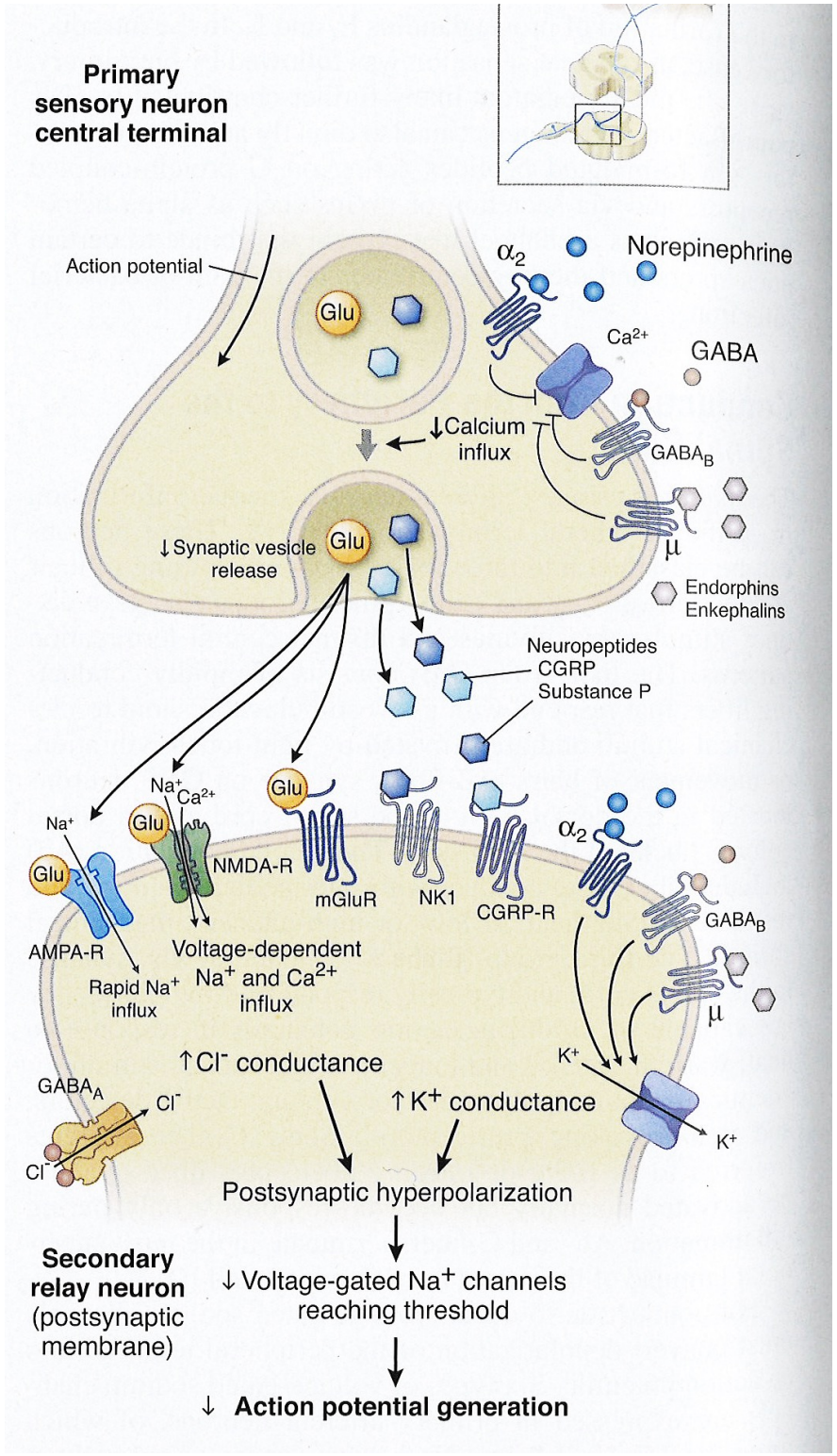

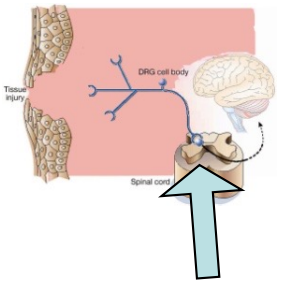

Figure 15-4. Inhibitory Regulation of Neurotransmission. Norepinephrine, GABA, and opioids, released by descending and/or local-circuit inhibitory neurons, act both presynaptically and postsynaptically to inhibit neurotransmission. Presynaptic inhibition is mediated through reduced activity of voltage-sensitive calcium channels, whereas postsynaptic inhibition is mediated primarily by enhanced chloride influx and potassium efflux.

Questions NSAIDs

Mrs. D., a 57-year-old Caucasian woman, goes to her physician because of joint pain and chronic fatigue. Her history reveals general joint stiffness and pain, especially in the early morning, and pain in the left metatarsophalangeal joint of 3 weeks' duration. Mrs. D. is advised to take ibuprofen as needed, and this medication provides relief of her pain for some time.

Questions

1. How did ibuprofen control Mrs. D.'s symptoms of joint stiffness and pain?
2. How do nonsteroidal anti-inflammatory drugs (NSAIDs) contribute to gastric irritation and bleeding?
3. Aspirin is the first and oldest of the NSAIDs. In which way does aspirin differ from the other NSAIDs?
4. What is the difference between COX-1 and COX-2?

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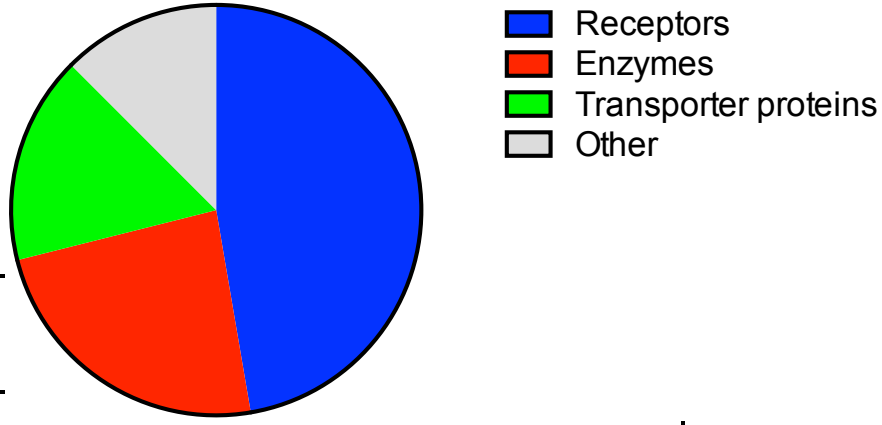
Targets for drug action



Drug targets

Target	*examples*
2.1. Receptors for physiological ligands	
Transmembrane receptors	
2.1.1. G-protein-coupled receptors	*- adrenergic receptors* *- opioid receptors*
2.1.2. ligand-gated ion channels	*- $GABA_A$ receptors*
2.1.3. kinase-linked receptors	*- insulin receptor*
Intracellular receptors	
2.1.4. nuclear receptors	*- pregnane X receptor*
2.2. Other targets/approaches	
2.2.1. enzymes	*- dihydrofolate reductase* *- tyrosine kinases* *- angiotensin-converting enzyme* *- COX inhibitors (in pain chapter)*
2.2.2. ion channels and transporters	*- voltage-gated Na channels*
2.2.3. protein therapeutics	*- GLP-1 receptor agonists* *-TNF-α monoclonal antibodies (e.g. infliximab)*
2.2.4. gene therapy	*- Nusinersen* *- Tisagenlecleucel /Axicabtagene ciloleucel*

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2.1.2. ligand-gated ion channels

- $GABA_A$ receptors
- Glycine receptors
- Nicotinic Acetylcholine receptors
- Ionotropic glutamate receptors

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$GABA_A$ receptors

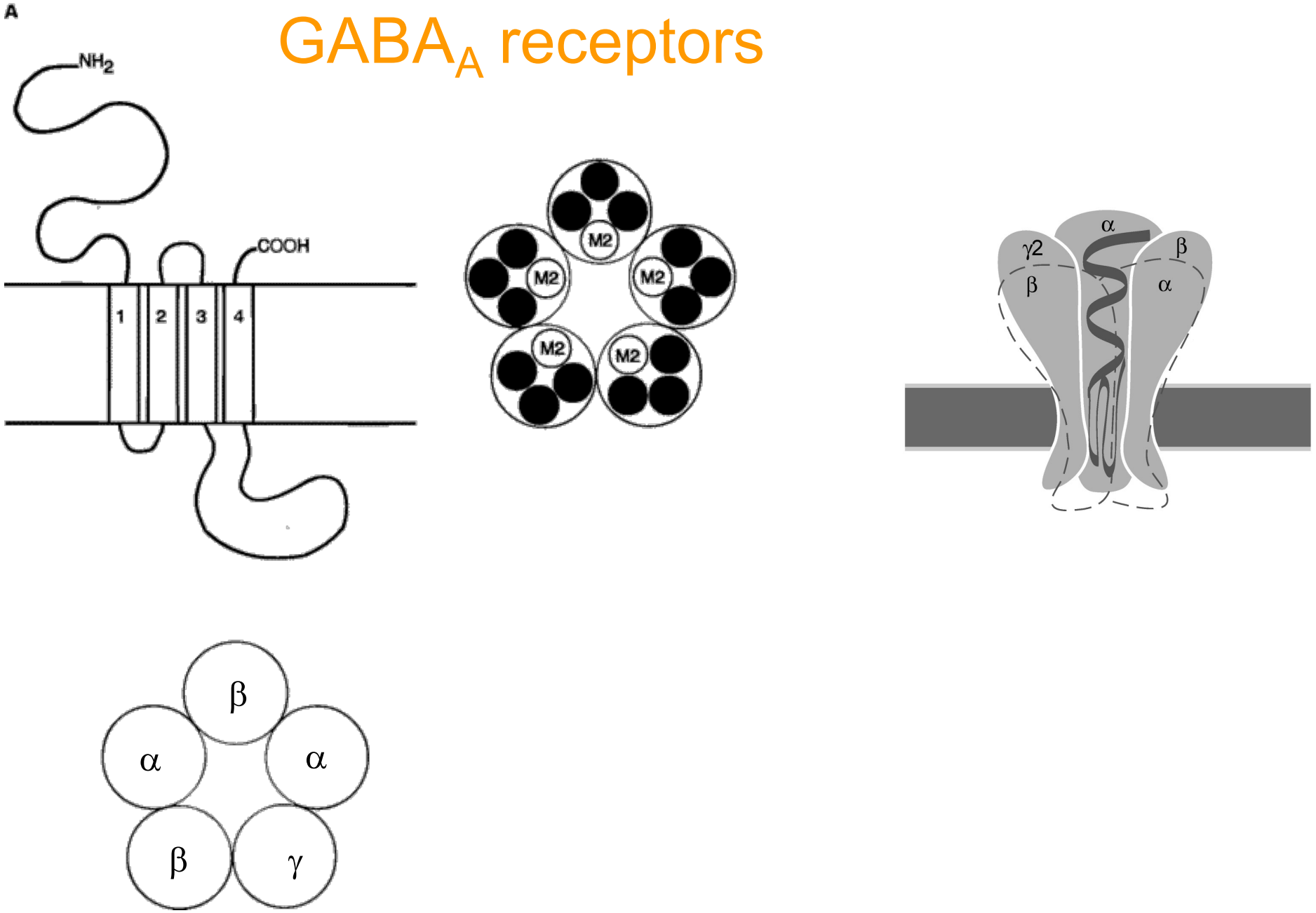
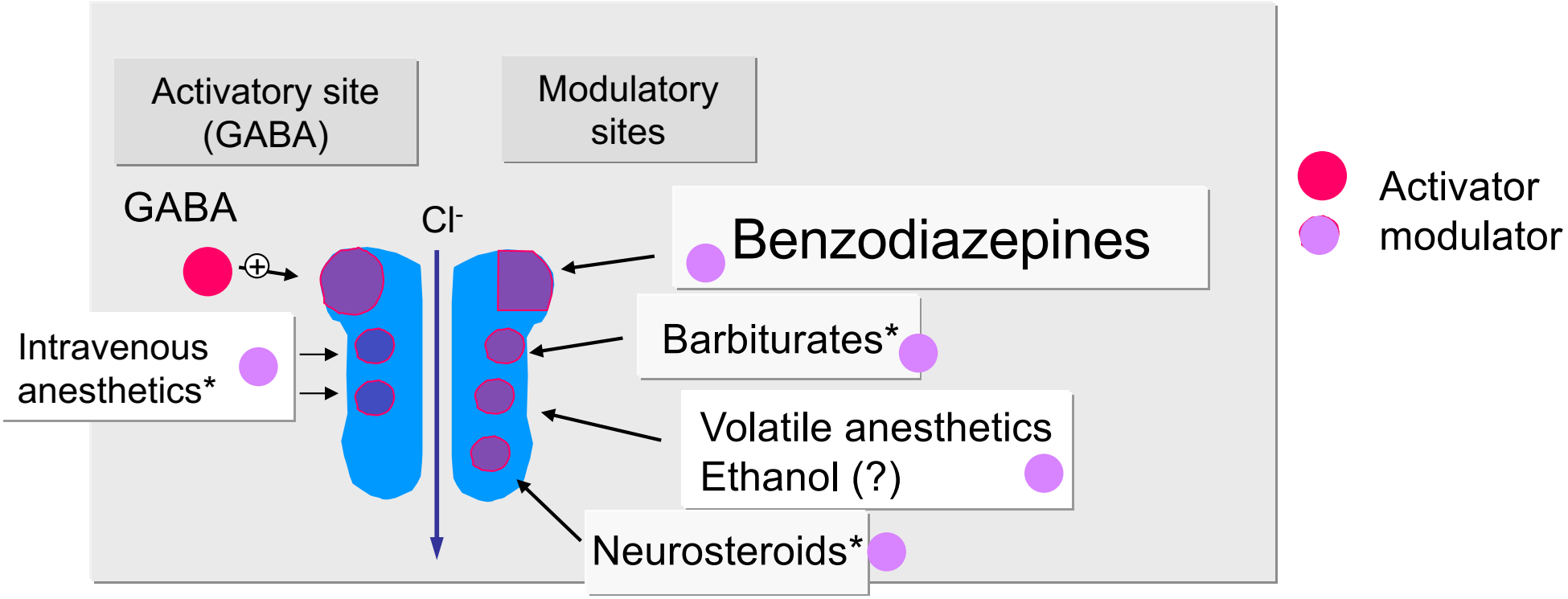


Figure 1 Representations of GABA-activated ligand gated ion channels: A, general structure of one of the five protein subunits showing four membrane spanning regions; B, pentameric arrangement of the protein subunits showing the second membrane spanning region lining the pore of the ion channel; C, heteromeric makeup of a GABAA receptor with two α1, two β2, and one γ2 protein subunits; and D, homomeric makeup of a GABAC receptor with five 1 protein subunits. Chebib and Johnston, J. Med. Chem. 43, 1427

$GABA_A$ receptor modulators



- Several general anesthetics increase the sensitivity of $GABA_A$ receptors for GABA (inhalation anesthetics: *halotane, isoflurane*; intravenous anesthetic agents: *propofol, barbiturates, etomidate*). Some neurosteroids have similar effects.

--> termed "positive allosteric modulators" (PAMs)

-*, *act as potentiators at low, and as activators at high concentrations*

-Some of the general anesthetics act in addition on other targets, such as glycine receptors (as activators) and neuronal nicotinic Acetylcholine receptors (as inhibitors)

- Other targets of certain inhalation anesthetics are the NMDA type glutamate channels and two-pore domain K channels

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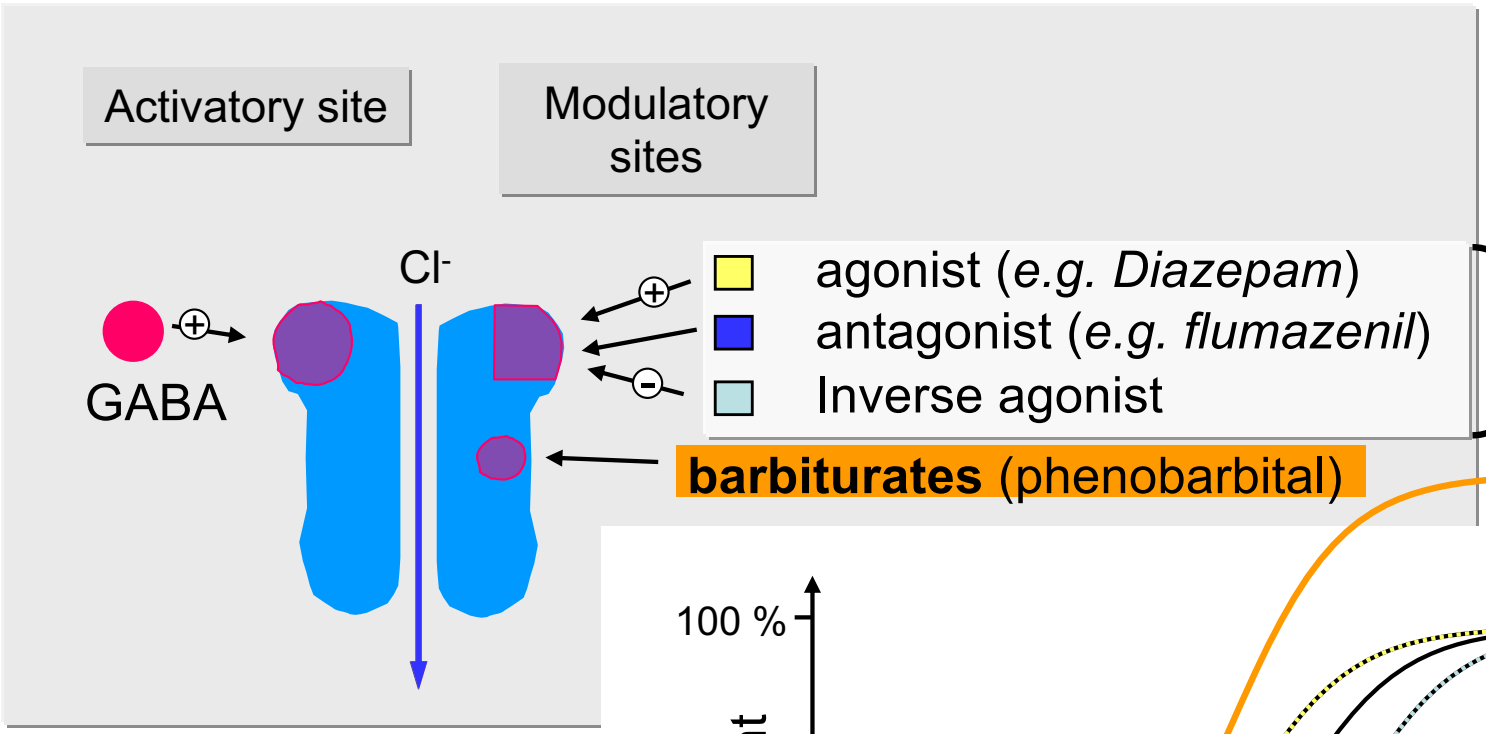
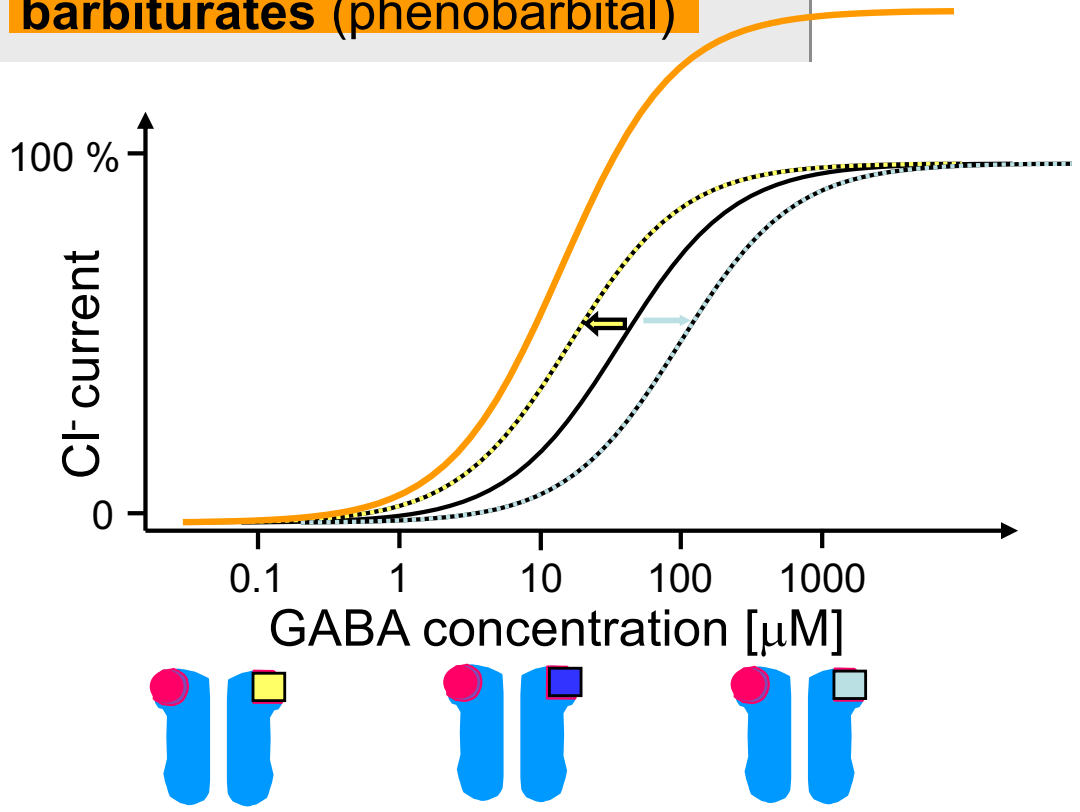
Mechanisms of action of drugs at the receptor





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Clinical uses

Common to all benzodiazepines:

The pharmacological effects:

- anxiolytic effect
- sedation
  - hypnotic effect
  - amnesia
- myorelaxation (inhibition of muscle tone)
- anticonvulsive effect

pharmacodynamics:

The efficacy is similar between benzodiazepines; differences in the *potency* are compensated by dosage

Differences

pharmacokinetics

- half-life (from < 6h to >48 h, due to differences in metabolism and excretion)

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Unwanted effects

- sedation

- Respiratory depression (high therapeutic index)

Strongly increased if combined with other depressors of the CNS (alcohol, opiates)

- Anterograde amnesia

- tolerance (mainly for sedation, less for anti-epileptic effect)

- Physical dependence: adverse physical symptoms when drug is withdrawn (anxiety, insomnia, convulsions)

conclusions for the therapy:

for therapies of limited duration

gradual reduction of the dose at the end of the therapy

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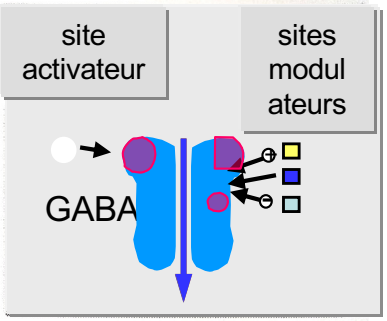
«Les anxiolytiques ont détruit ma vie»

24 heures, 2022

Un Vaudois raconte «l'enfer» de son sevrage aux benzodiazépines. Il se bat pour sensibiliser aux dangers d'un usage prolongé de ces médicaments.

Dave Catillaz n'a plus pris de benzodiazépines depuis Nouvel-An 2019. Son parcours de sevrage aura duré six ans. «Un enfer.»

Exercise GABAA receptor agonists/antagonists



Experiment

interpretation

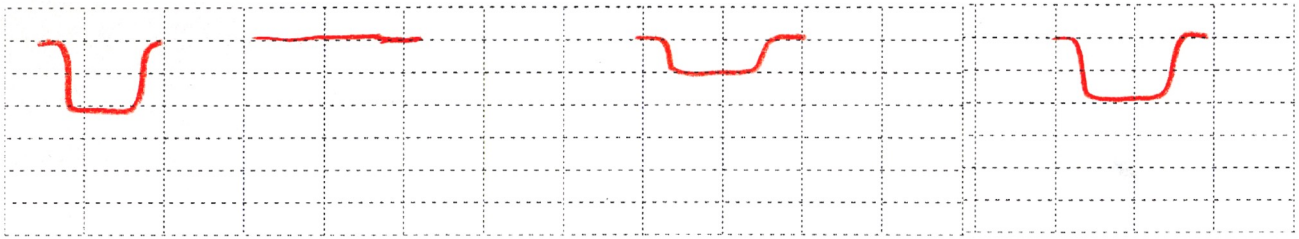
(agonist, etc; activator/modulator site)

GABA

Substance D

GABA + substance D

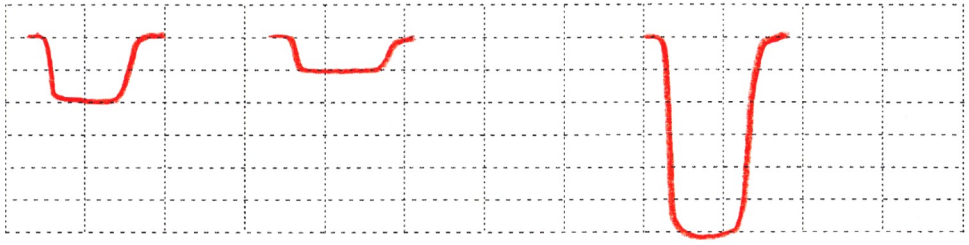
GABA + Flumazenil + substance D



GABA

Substance E

GABA + substance E

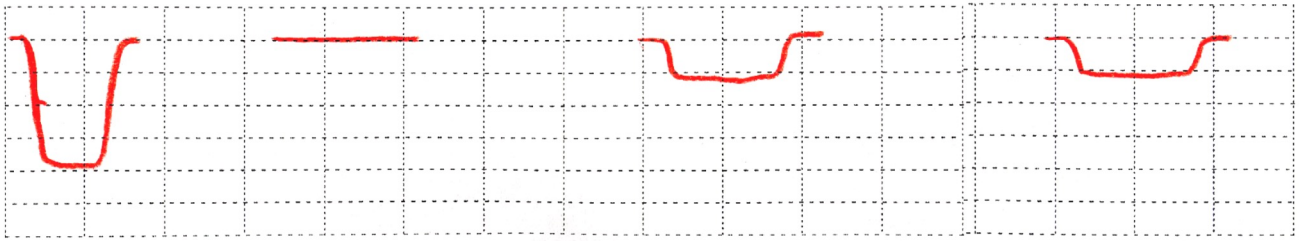


GABA

Substance F

GABA + substance F

GABA + Flumazenil + substance F

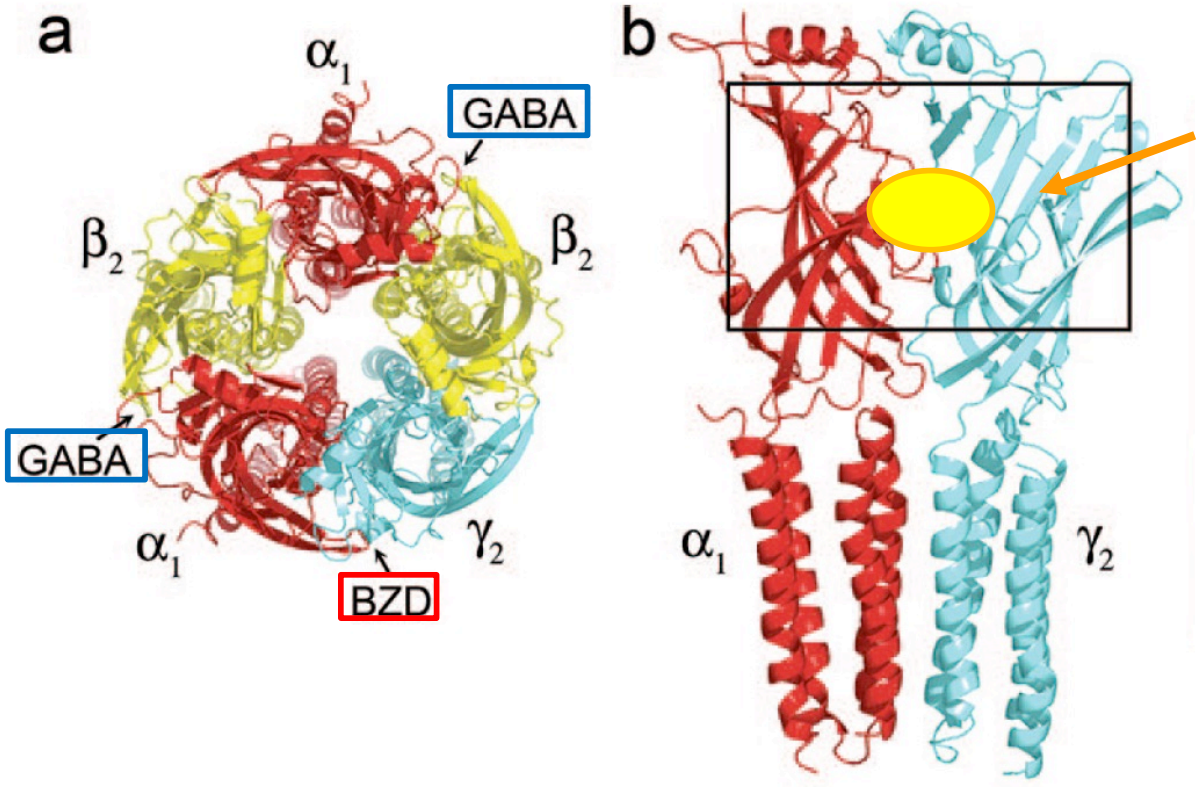


example

Localization of modulator binding sites

The main benzodiazepine binding site is located at the extracellular α–γ subunit interface

Benzodiazepines bind to the interface between α and γ subunits, in the extracellular part



Benzodiazepine binding site (*structural data + confirmed by mutagenesis*)

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$GABA_A$ receptor subunit composition and effect of benzodiazepines

Number of different receptor subtypes:
α: 1-6
β: 1-3
γ: 1-3
(δ, ε, Φ, π, ρ 1-3)

"Rules"
- The pentamer contains 2 α subunits, 2 β subunits, and one additional subunit
- The presence of a γ2 in the pentamer is required for the binding of benzodiazepines
- Pentamers with an α4 or an α6 subunit are not sensitive to the majority of benzodiazepines in use

Subtype	Frequency	
α1β2γ2	60%	synaptic
α2βnγ2	15-20%	synaptic
α3βnγ2	10-15%	synaptic
α4βnδ/γ	10%	extrasynaptic

Table from Vinkers and Olivier, Adv Pharmacol Sci 2012, ID 416864

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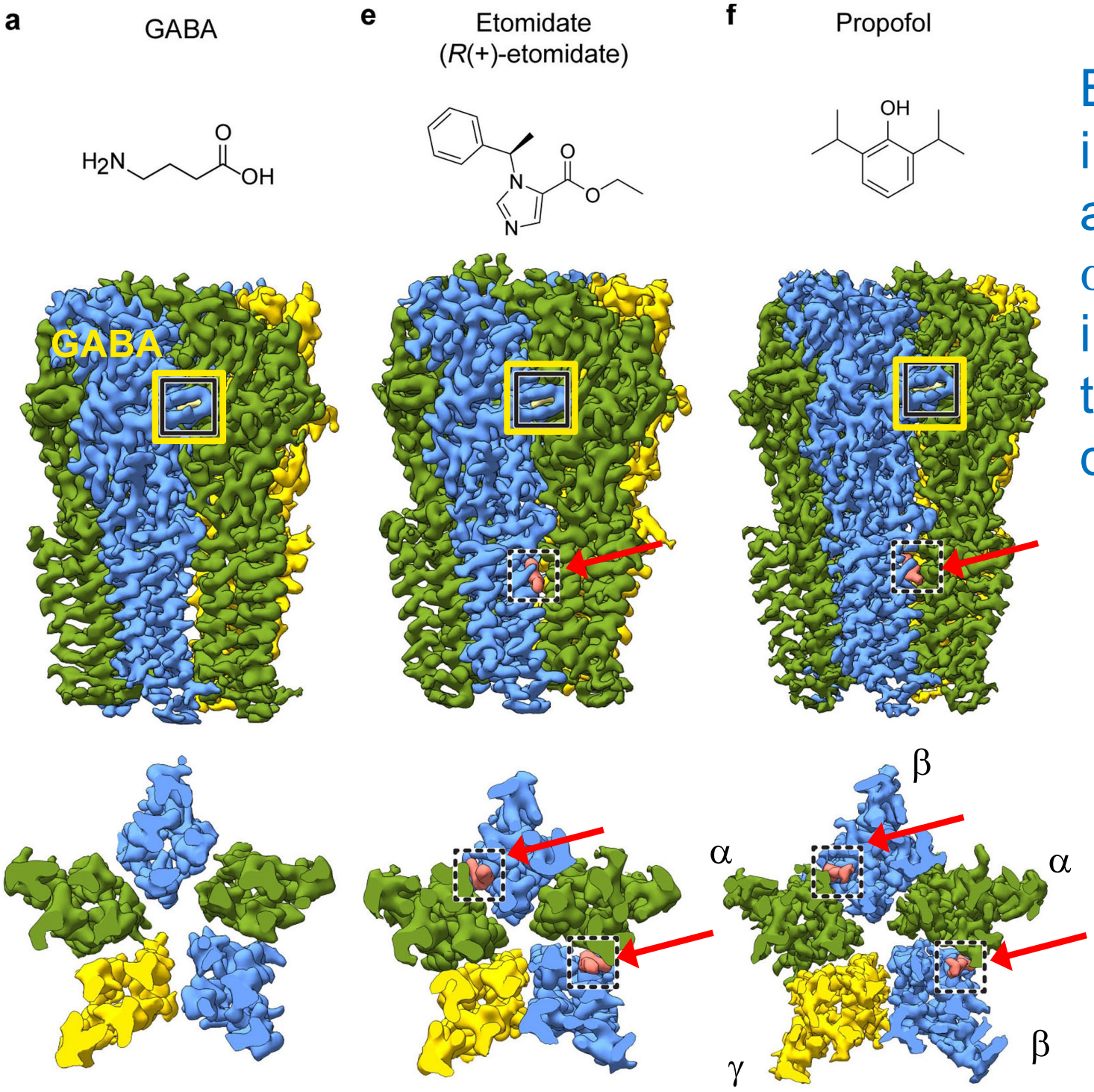

Binding sites of intravenous anesthetics are at α–β subunit interfaces in the transmembrane domain.

Etomidate and propofol potentiate (and activate at higher concentrations) the $GABA_A$ receptors

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Neurosteroids

Neurosteroids acting on $GABA_A$ receptors

- Local generation of endogenous neurosteroids
- Typical molecules: allopregnanolone, allotetrahydrodeoxycorticosterone, androstanediol
- Regulation of anxiety, stress, reproductive and sexual behaviors
- Part of their effect is mediated by direct binding to $GABA_A$ receptors

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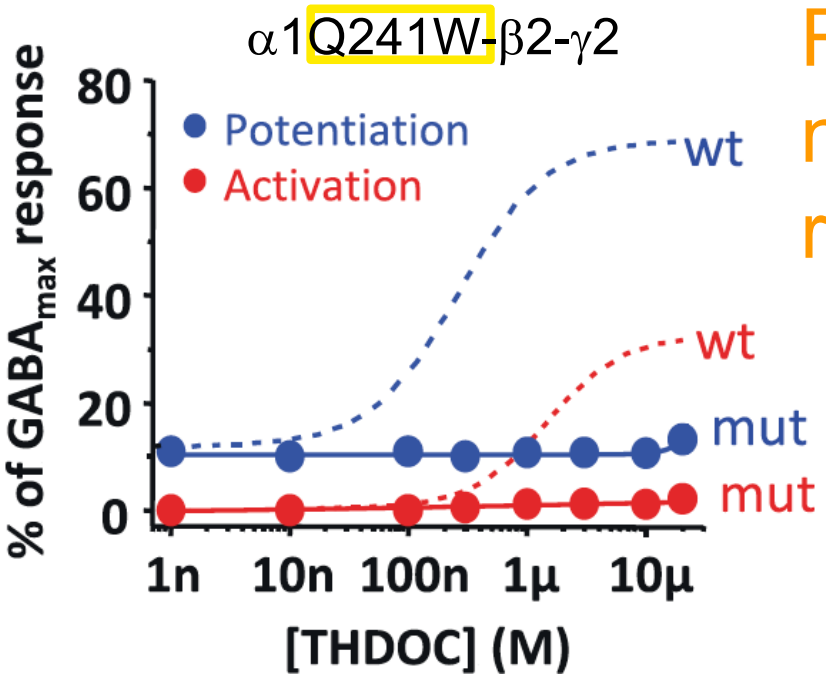
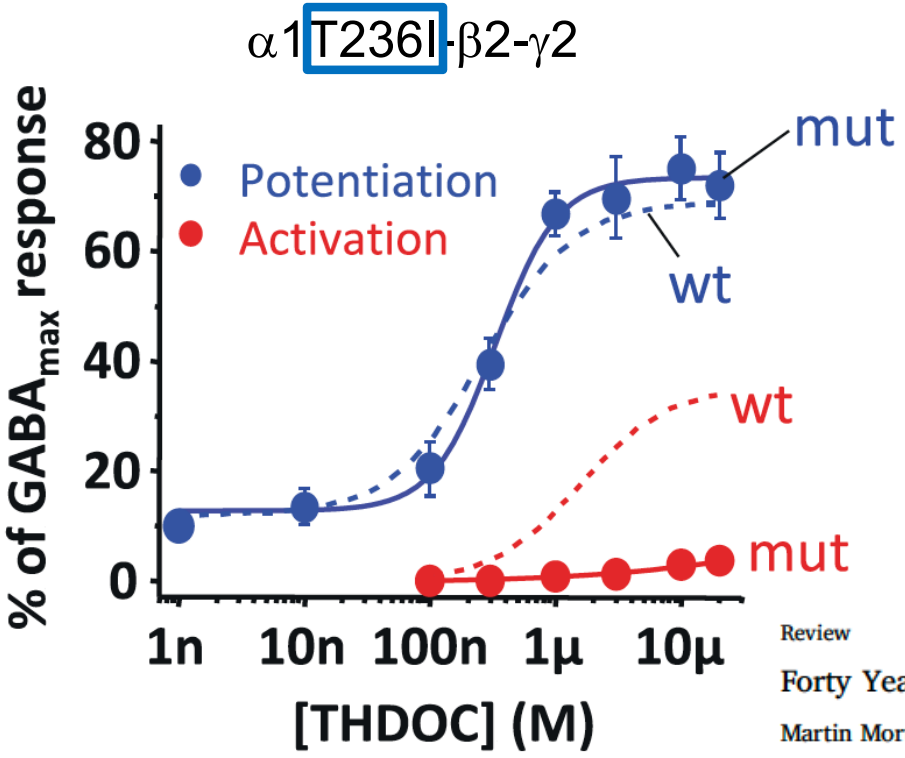
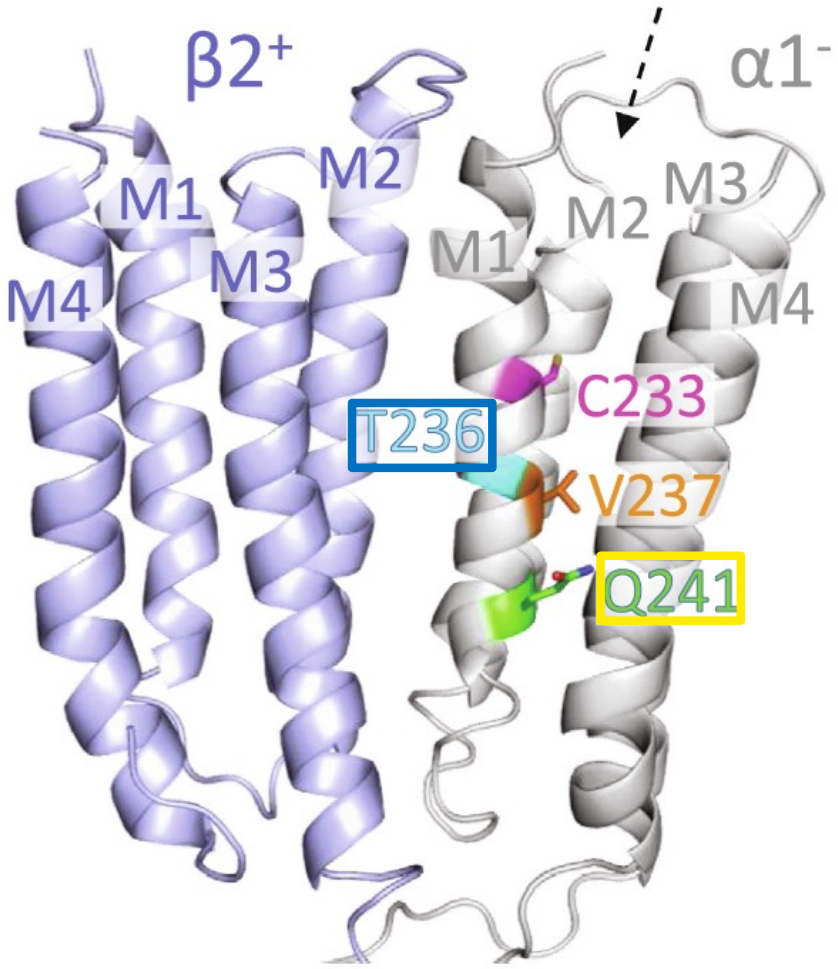
Residues important for neurosteroid effects on $GABA_A$ receptors







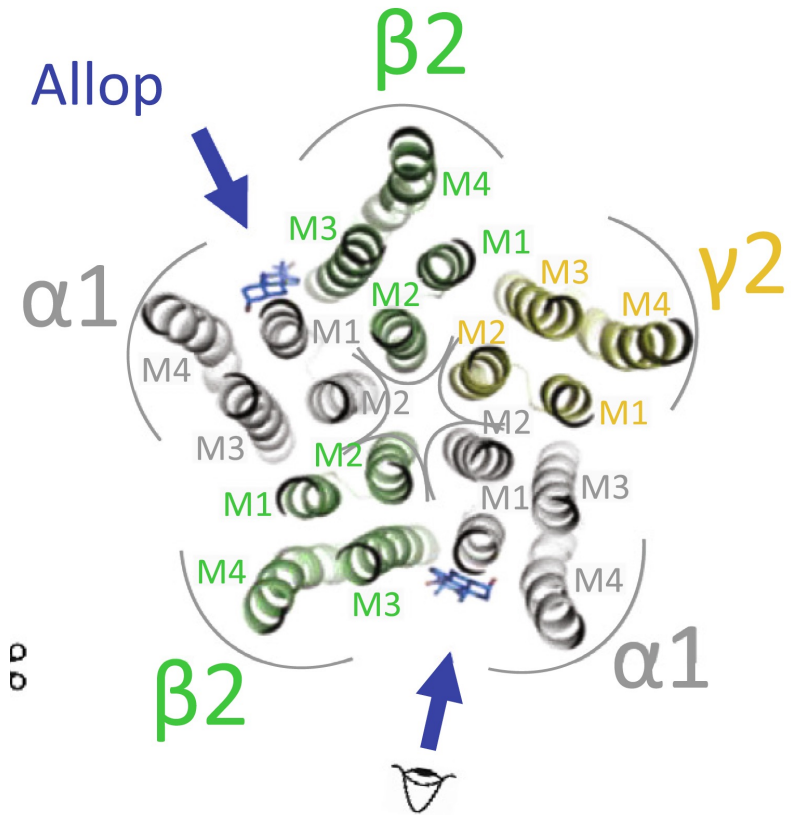
Review

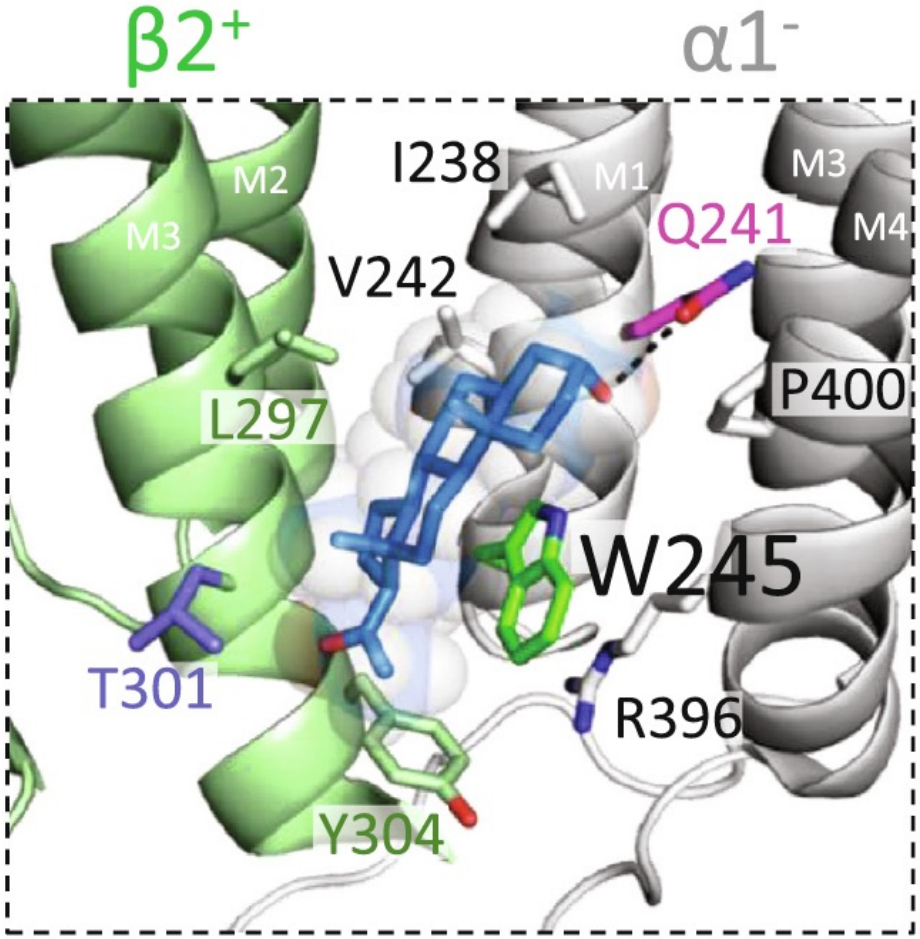
Forty Years Searching for Neurosteroid Binding Sites on $GABA_A$ Receptors

Martin Mortensen, Damian P. Bright, Juliane Fagotti, Valentina Dorovykh, Barbora Cerna, Trevor G. Smart*

University College London, Dept Neuroscience, Physiology & Pharmacology, Gower Street, London WC1E 6BT, United Kingdom

Neurosteroids bind at α–β subunit interfaces at the bottom of the transmembrane domains

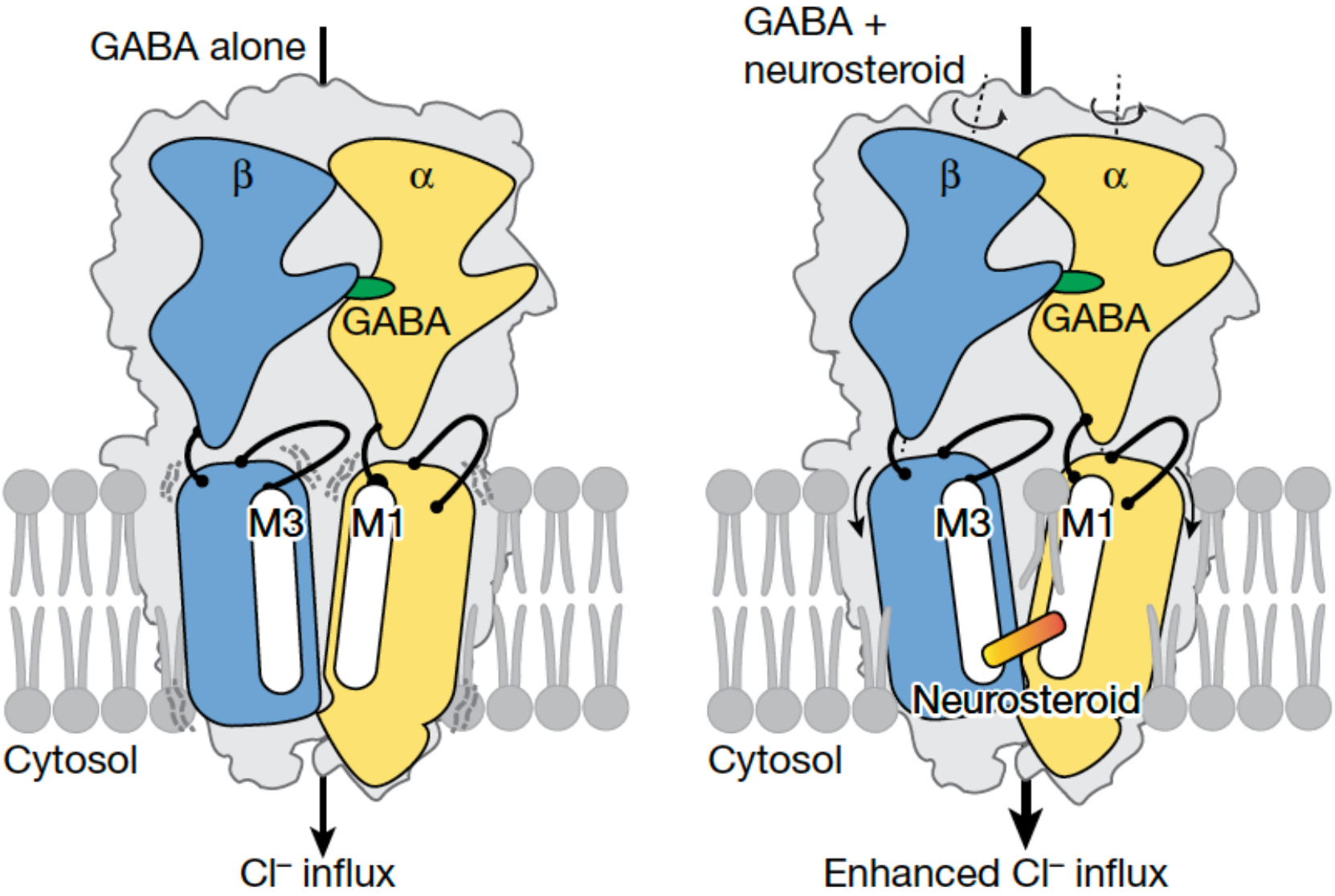




High resolution side view of allopregnanolone (blue) bound to the PAM neurosteroid site in the β2+- α1- interface.

https://doi.org/10.1038/s41586-023-06556-w

Mechanism of the neurosteroid effect



Mechanism of neurosteroid potentiation. GABA interacts with the receptor at the ECD β+/α– pocket, inducing anticlockwise rotations of the ECD that reorganize the TMD for Cl^- ion conduction. Neurosteroids bind to the receptor at the TMD β+/α– pocket, causing additional anticlockwise ECD rotation and acting as a diagonal brace to stabilize the TMD open-channel conformation. https://doi.org/10.1038/s41586-023-06556-w

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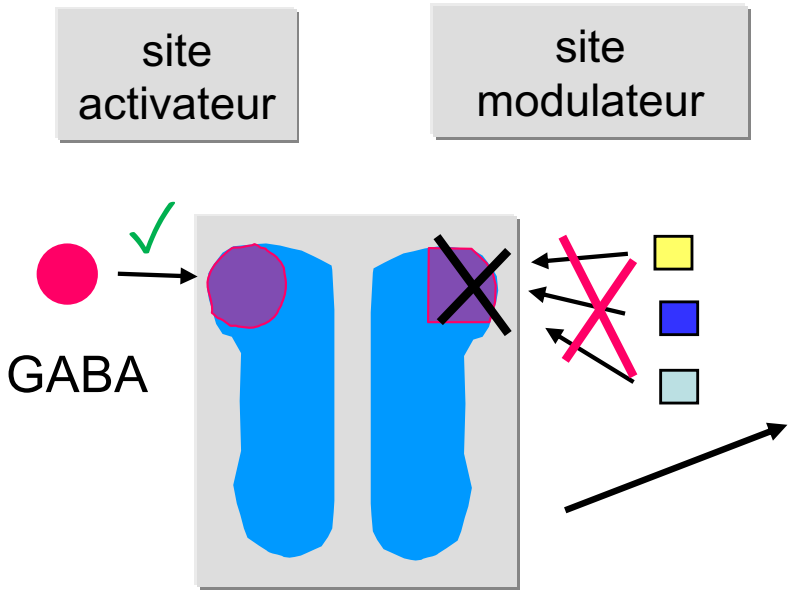
Do different $GABA_A$ receptor subtypes have distinct roles?

The group of Mohler at the University of Zürich introduced point mutations in the benzodiazepine binding site of specific α subunits, to test this hypothesis :



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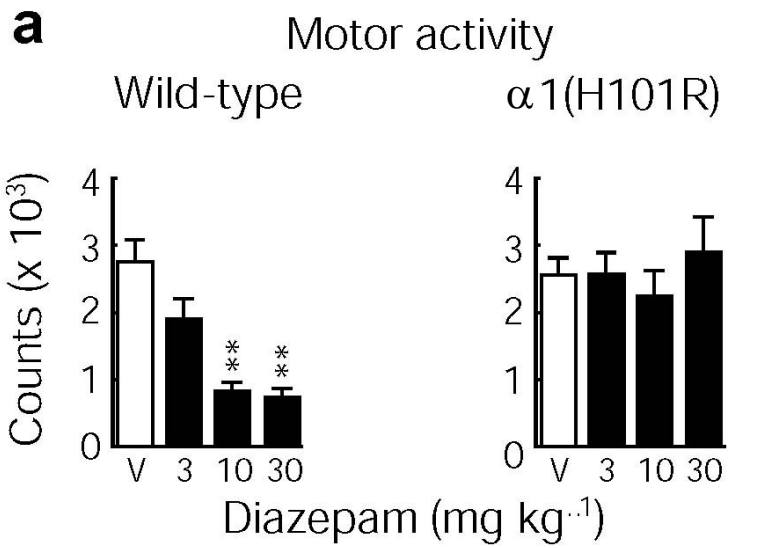
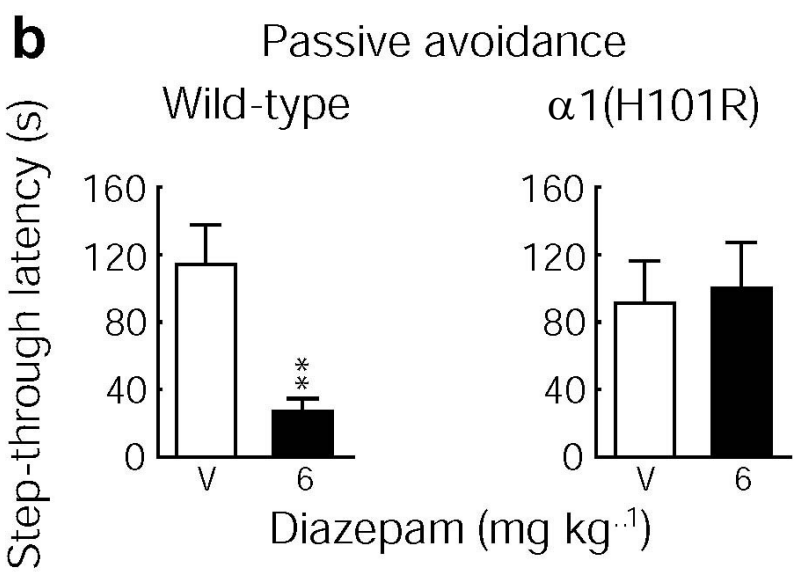
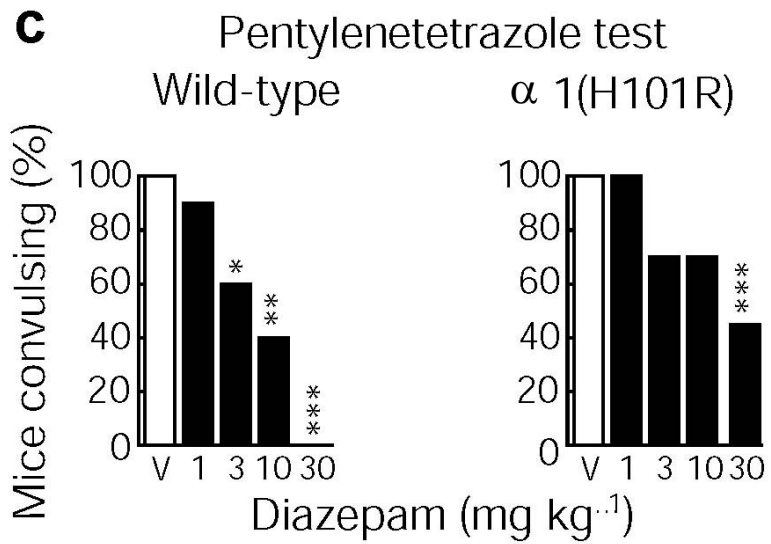
Testing drug-induced sedation (a), amnesia (b) and anticonvulsant activity (c)





b) 2 chambers, one dark, one well lit. Once the mouse enters the dark chamber, it receives an electrical shock. One day later this procedure is repeated and the latency to entering the dark chamber is measured.



(pentylenetetrazole induit des convulsions)

Figure 3 Behavioural assessment of drug-induced sedation, amnesia and anticonvulsant activity in wild-type and α1(H101R) mice. a, Diazepam induced a dose-dependent decrease in motor activity in wild-type but not in α1(H101R) mice (n = 16 per group). b, In a passive avoidance paradigm, diazepam shortened the latency to re-enter the dark compartment 24 h after training in wild-type mice but not in α1(H101R) mice (n = 10 per group). c, Partial anticonvulsant activity of diazepam against pentylenetetrazole (120 mg kg^{-1} i.p.) in α1(H101R) compared with wild-type mice (n = 10–11 per group). All results are given as means ± s.e.m. Asterisk, $P < 0.05$; double asterisks, $P < 0.01$; triple asterisks, $P < 0.001$. Diazepam was administered p.o.; V, vehicle.

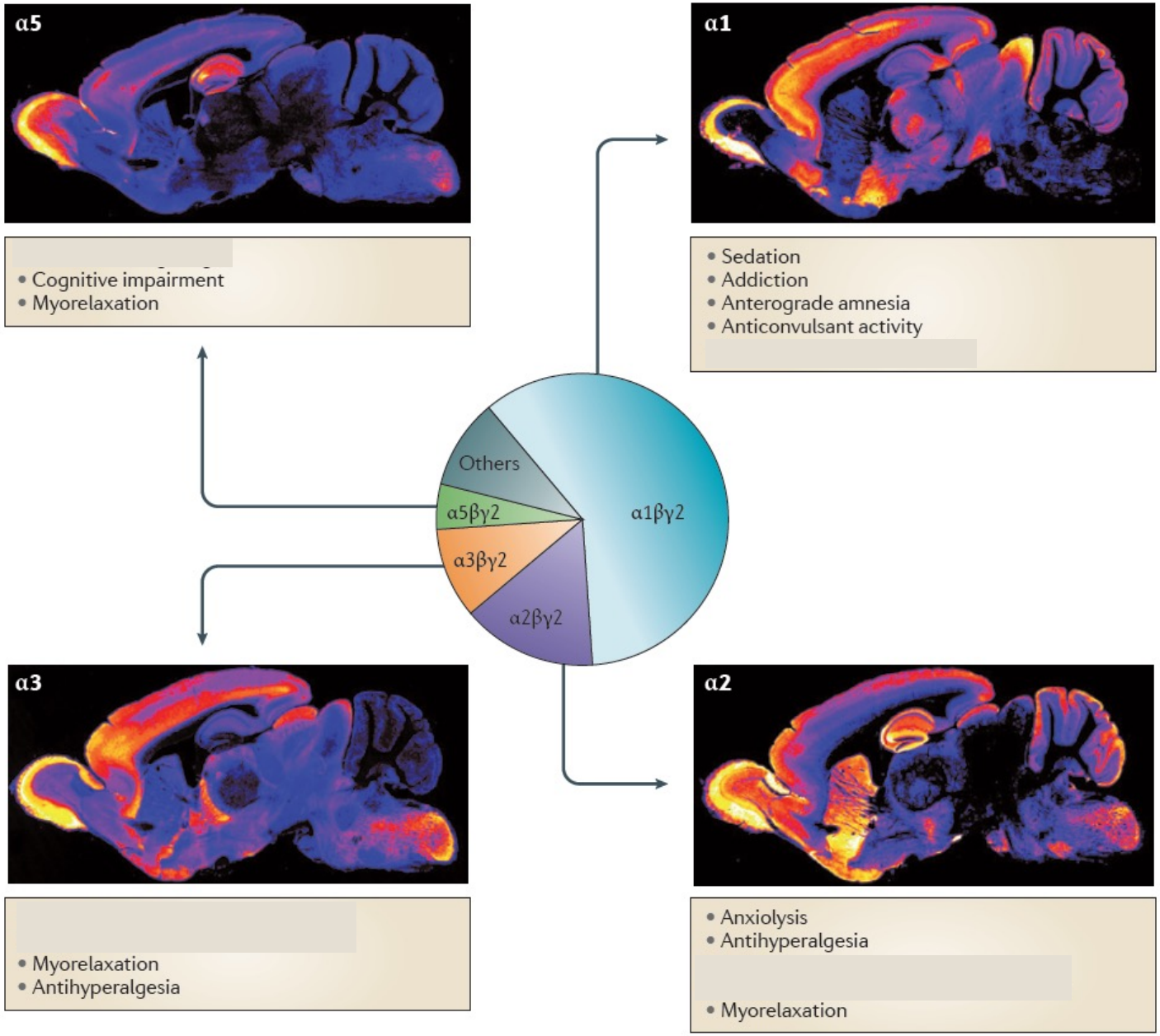


Figure 1 | **Pharmacological effects and distribution of $GABA_A$ receptor α subunits in the mouse brain.** The pie chart represents the approximate abundance of the $GABA_A$ (γ-aminobutyric acid, type A) receptor subtypes that are known to exist *in vivo*. α1 is expressed in cortex, thalamus, pallidum and hippocampus. α2 is expressed in hippocampus, cortex, striatum and nucleus accumbens (not shown). α3 is expressed in the cortex and the reticular nucleus of the thalamus, and α5 is expressed in the hippocampus and in deep layers of the cortex. The antihyperalgesic actions are mediated by spinal $GABA_A$ receptors. Data are from REFS 42,106–108. Immunohistochemical images are reproduced, with permission, from REF. 109 © (2001) Elsevier.

From: Rudolph and Knoflach, Nat Rev Drug Discovery 10, 685 (2011)

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$GABA_A$ receptor subtype-specific ligands

- **α1**-selective ligands: the « Z compounds », Zaleplon, Zolpidem, Eszopiclone are on the market, used as hypnotics

- **α2/α3**- selective ligands would be of clinical interest
- Many such candidate drugs were studied
- Currently however none is on the market, due to problems unrelated to their main effect, observed in clinical trials
- Several interesting new such compounds are currently in the pipeline of development

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KRM-II-81, a promising α2/α3-selective positive allosteric modulator (PAM) in pre-clinical studies

Table 2
Potency and efficacy of KRM-II-81 and the structural analog, MP-III-080, compared to diazepam and PF-06372865 (CVL-865).[1]

Compounds	$GABA_A$-α1		$GABA_A$-α2		$GABA_A$-α3		$GABA_A$-α4		$GABA_A$-α5		$GABA_A$-α6		References
	Potency (nM)	Efficacy (%)	Potency (nM)	Efficacy (%)	Potency (nM)	Efficacy (%)	Potency (nM)	Efficacy (%)	Potency (nM)	Efficacy (%)	Potency (nM)	Efficacy (%)	
KRM-II-81 (9)[2]	1730.0	115.6	101.9	252.2	60.9	262.3	>30,000	97.4	192.6	114.1	>30,000	98.8	Lewter et al., 2017
KRM-III-80 (7)[2]	241.3	115.0	102.1	178.0	102.0	208.6	ND	ND	61.3	173.6	ND	ND	Witkin et al., 2017
Diazepam	18.1	256.2	17.6	286.5	19.6	272.9	>30,000	100.3	11.1	211.2	>30,000	100.4	Poe et al., 2016, Witkin et al., 2017
PF-06372865[3]	0.2	121.0	2.9	234.0	1.1	192.0	>19,900	ND	18.0	191.0	>19,900	ND	Owen et al., 2019

ND: no data exist for these diazepam insensitive $GABA_A$ receptor configurations.
[1] Data are from different sources and sometimes from different methods as described in the references.
[2] Compound number in parentheses are compound designations in Poe et al. (2016) and in Witkin et al. (2017).
[3] Potency data are affinity values.

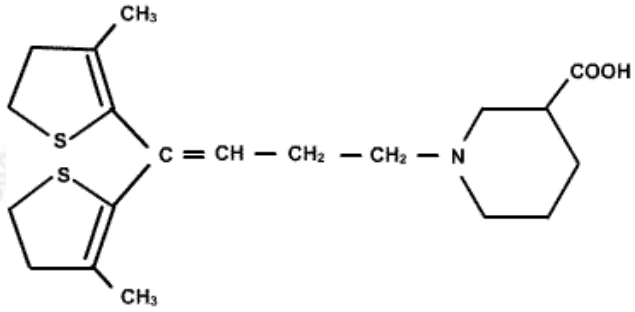
J Pharmacol Exp Ther. 2024 Nov 19;391(3):389-398. doi: 10.1124/jpet.123.002070.

Antinociceptive Effects of α 2/ α 3-Subtype-Selective $GABA_A$ Receptor Positive Allosteric Modulators KRM-II-81 and NS16085 in Male Rats: Behavioral Specificity

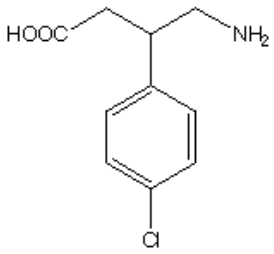
Lakeisha A Lewter [1], Kristen Woodhouse [1], V V N Phani Babu Tiruveedhula [1], James M Cook [1], Jun-Xu Li [2]

This study demonstrates that α2/α3 subtype-selective $GABA_A$ PAMs KRM-II-81 and NS16085 produce selective antinociceptive effects devoid of sedation, myorelaxation, and cognitive impairment in two rat models of persistent pain.

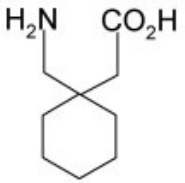
GABA



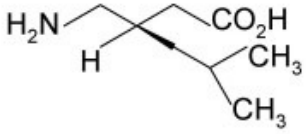
tiagabin



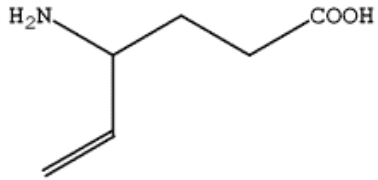
baclofen



Gabapentin



Pregabalin



Vigabatrin

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