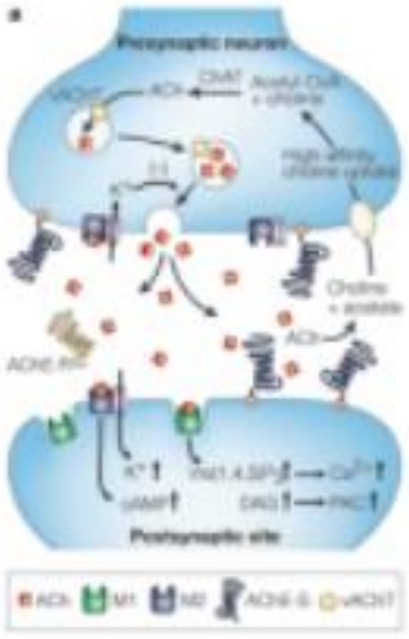
Principles of Drug Action in the Nervous System

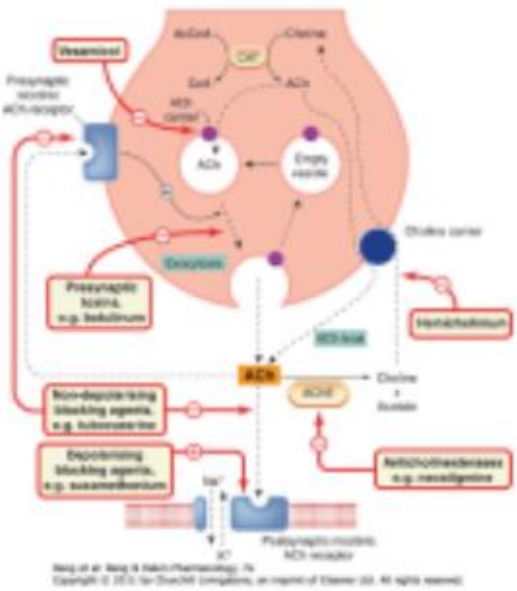
Lecture 3 – 8.8.17 – Cholinesterases and Inhibitors

Term	Definition
Cholinergic	Term for nerve ending or synapse in which ACh is the main N/T
ACh receptor	A receptor that binds and is activated by ACh and related drugs
Adrenergic	Term for nerve ending or synapse in which NAd is the main N/T
Adrenoceptor	A receptor that binds and is activated by NAd and related drugs
NMJ	Refers to synapse between somatic nerve and skeletal muscle
NEJ	Refers to synapse between autonomic nerve and smooth muscle
Postsynaptic	Receptor located on distal side of synapse, on effector cells
Presynaptic	Receptor located on nerve ending that modulates N/T release
Autoreceptors	Presynaptic receptor that is activated by the N/T that it modulates
Baroreceptor reflex	Homeostatic mechanism that tries to maintain blood pressure

Cholinergic Transmission

- Cholinergic transmission is the only means of neurotransmission whereby the neurotransmitter/ligand (ACh) is broken down extracellularly
- Decline in ACh function in CNS in patients with Alzheimer's disease
- ACh-mediated neurotransmission is fundamental in the functioning of the PNS and CNS
- Blockade is lethal, with a gradual loss of receptivity associated with cognitive, autonomic and neuromuscular dysfunction
- myasthenia gravis is an example of a related pathology, whereby nAChR are degraded (autoimmune) treated with AChE inhibitors





AChE Action

- AChE is the enzyme respomsible for ACh degradation in the synapse, inactivating ACh and thereby regulating its activity
- AChE is extracellularly attached to the membrane of the adjacent neurones as an insoluble tetramer
- ACh is involved in muscarinic cholinergic and nicotinic cholinergic synapse, reacting with M3 and nicotinic acetylcholine receptors respectively
- Inhibiting AChE results in an increase in ACh and thereby increase in its subsequent effects via excessive excitation of the postganglia and prevention of repolarisation at nicotinic cholinergic synapses = paralysis ie. depolarising block

AChE Structure

- Serine hydrolase; containing a catalytic site with histidine, serine and glutamate residue
- Contain a choline binding site, a peripheral site (for non-enzymic roles) and a catalytic site
- Reaction with ACh causes an acetyl-enzyme intermediate (short restoration time
- If other substrates are used, they intermediates are somewhat limiting and include carbamylation (medium restoration time) and phosphorylation (long restoration time/complete inhibition)

AChE Inhibitor Examples

Drug	Duration of action	Main site of action	Notes
Edrophonium	Short	NMJ	Diagnosis of myasthenia gravis
Neostigmine	Medium	NMJ	Treatment of myasthenia gravis and reversal of competitive nmj block
Tacrine	Medium	CNS	Alzheimer's disease: limited effectiveness
Malathion	Long	-	Insecticide + head lice (USA)
Sarin	Long	NMJ + P	Nerve gas: Tokyo 1995

Tacrine

- Synthesis by Dr Adrian Albert at USYD, and able to reverse anaesthetic-induced sleep, due to inhibition of cholinesterase activity
- Requirement of 4x daily dosage = risk of hepatotoxicity
- Help with people with alzheimers

Butyrylcholinesterase

- ACh can also be catalysed by a related less selective enzyme (BuChE)
- This is a soluble enzyme found in plasma
- Function still debated/not clear, but has importance as it metabolises suxamethonium (short acting neuromuscular junction, but if not BuChE in patient, then its long acting)

	Acetylcholinesterase (AChE)	Butyrylcholinesterase (BuChE)
Distribution	Limited: neuromuscular junction* and neuronal synapse**	Widespread: liver, skin, brain, GI smooth muscle; soluble form in plasma
Substrate specificity	Narrow: ACh and methacholine	Broad: BuCh, ACh, suxamethonium
Function	Hydrolyses and terminates action of ACh at synapse; also new roles	Physiological function unclear; keeps ACh levels low. Important role pharmacologically to metabolise some drugs (e.g. suxamethonium)

* includes neuroeffector
** autonomic ganglia and CNS

Summary

- Acetylcholinesterase (AChE) limits the activity of acetylcholine at synapses
- Butrylcholinesterase (BuChE) also breaks down ACh, but more specifically suxamethonium
- Inhibits of AChE (anticholineserases), therefore, enhance cholinergic neurotransmission
- Anticholinesterases are differentiated by their duration of action: short, medium and long lasting
- Those used clinically are reversible, and irreversible anticholinesterase are used as insectesides and nerve gases

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FROM LAST SEM;

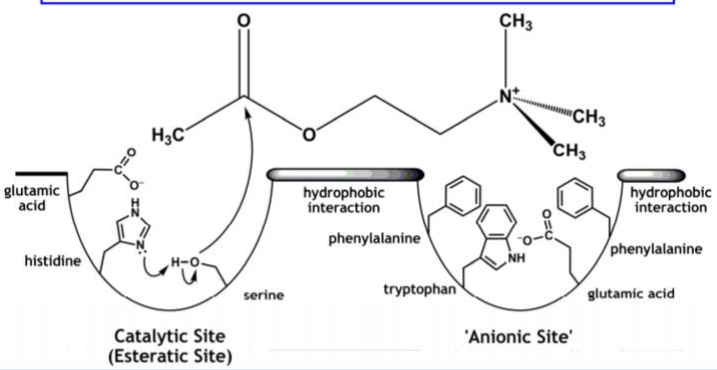
Cholinesterases

AChE – acetylcholine, in RBCs, cholinergic fibres and muscle

➔ Measured via acetylthiocholine, making thiocholine which can optically active when combing with Ellman's reagent = quantify presence

- Substrates include ACh and methacholine (not carbachol, benzoylcholine or suxamethonium

ACh binds to the enzyme via two major subsites, the 'esteratic' site and the peripheral anionic site (PAS).



'Anionic Site': a cation-π (pi) interaction between quaternary N^+ ion and pi-cloud of aromatic amino acid (Phe, Trp) residues.

BChE (pseudo cholinesterase) – butyrul, liver, skin, brain GI, smooth muscle, plasma

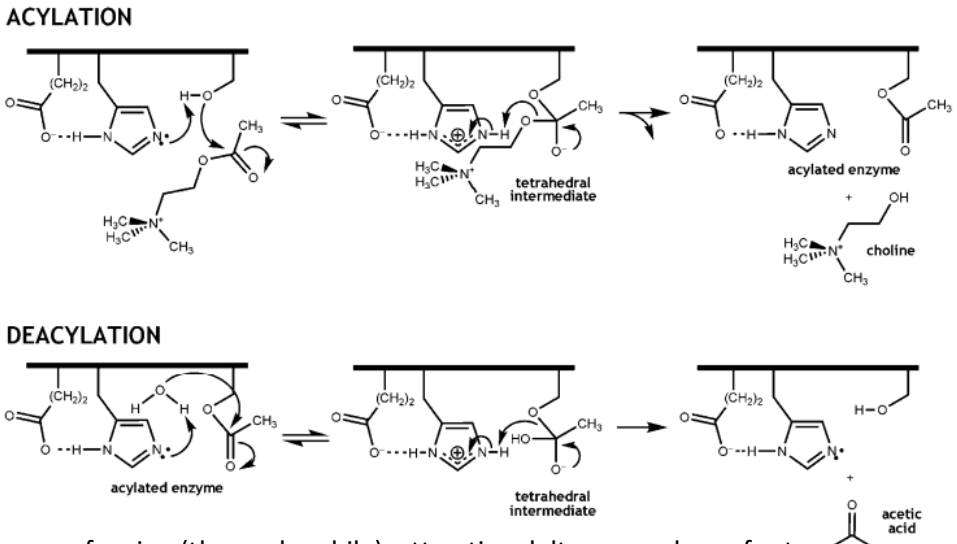
- Substrates are butyrylcholine, ACh, benzoylcholine, suxamethonium (not carbachol or methacholine)

Catalytic Triad

- Glutamate, histidine and serine

AChE Mechanism

Nucleophile = donates electron



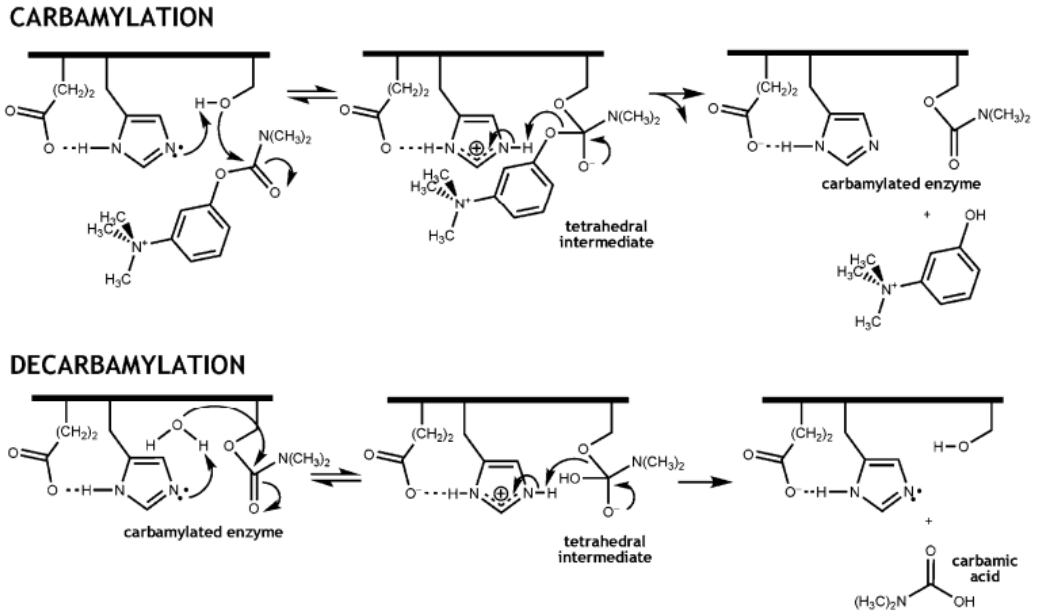
Acylation – lower pKa value of hydroxyl group of serine (the nucleophile), attracting delta +ve carbon of ester causing rearrangement and release of choline, as acetyl remains on O of serine (instead of previous H that removed via low pKa) therefore enzyme is 'acetylated'

Deacylation – water is involved here, acting as the nucleophile and precipitating acetic acid, re- forming the serine with OH

Therefore an inhibitor must prevent one of these processes →

Anti(acetyl)cholinesterases

- Eg. Physostigmine (serine) is a reversible anti-ChE
- Acts by forming a carbamylated form of th enzyme (rather than an acylated formed as a result of the native substrate

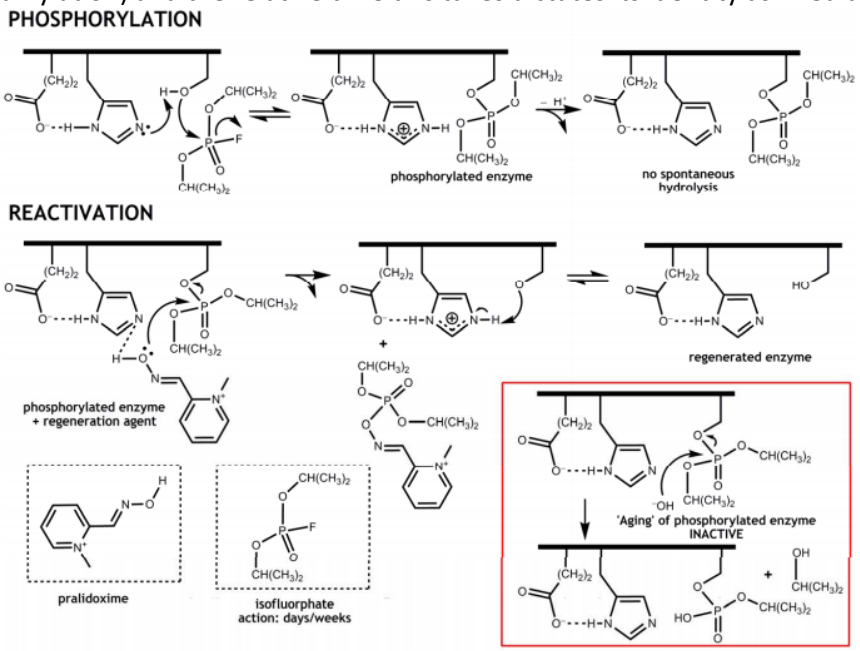


- Ester group is still attacked, but contains N(CH3)2 instead of CH3 as on ACh
- This results in greater difficulty in acting the double bond O2, due to the positively charged N
- It can still by hydrolysed (decarbamylation) and the relative time this takes dictates its identity as 'medium' or 'long' acting (irreversible)

Short – edrophonium (MG)

Medium – neostigmine, physostigmine (MG and glaucoma)

Long – ecothiopate (eye drops, for glaucoma, has systemic effects), malathion (pesticides)



Anti-Alzhiemer Drugs

- Only two drugs approved in US; tacrine, donepezil → both AChEI
- Cholinergic hypothesis explains treatment of alzheimers, whereby a symptom involves impaired cholinergic transmission → inhibitors reverse deficits in levels of ACh
- Also suggested AChE accelerates aggregation of amyloid-B-peptides

- **Anti-ChE has 3 main types, including short (slower acetylation/deacetylation), medium (N, carbamylate/decarbamylate), long acting (P, irreversible phosphorylation)**
- **Act at the neuromuscular junction, autonomic nervous system (mainly parasympathetic) and central nervous system to increase ACh concentration**
- **Used clinically in reversing anaesthesia, myasthenia gravis, glaucoma, Alzhemier's disease and can be used as an insecticide and in chemical warfare (nerve gas)**
- **Antidote for poisoning of Anti-ChE atropine (muscarinic antagonist) and pralidoxime (reactivates enzyme)**

Principles of Drug Action in the Nervous System

Lecture 4 – 10.8.17 – AChE Pesticide Poisoning

- Organophosphorus compounds are a potent pesticide, develop in WW1
- Tabun (GA) also used as pesticide, but not in warfare
- Less stable in the environment than organochlorines
- Used as nerve agents with a spectrum of toxicity based on inhibition of esterase enzymes in the body
- Oxygen saturation low because lungs are full of secretions
- Vomiting/diahhreoa due to excessive gut secretion
- Excess ACh action on sympathetic preganglia causes release of adrenaline at post ganglia
- Hence blood vessels constrict and increased heart rate & sweating (ACh pre and post ganglia)

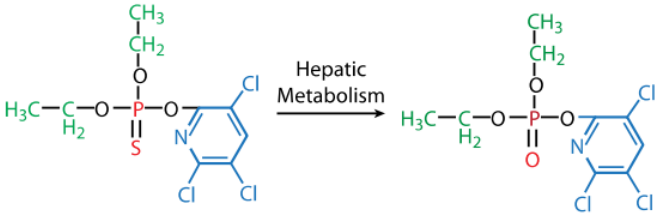
Spectrum of severity/symptoms is the result of;

Rate of binding – inherent reversibility/irreversibility (ie. short, medium and long acting) of different AChEi

Dosage – a huge dosage is essentially irreversible even if type of AChEi is inherently reversible in nature

Distribution – whether it can make it through the blood brain barrier and actually effect the CNS (eg. Negostigmine used in treatment of myasthenia gravis, not lipid soluble so cant cross BBB)

Onset – timing of effect, similar to distribution, as the result of the specific AChEi and differences in its metabolism (between compound/between people), for example;

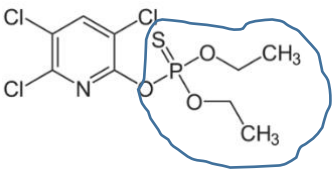


Chloropyrifos | Chloropyrifos-oxon

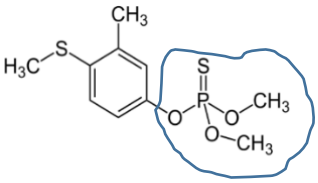
- This explains the metabolism of the pro poison chloropyrifos (thion /w S) to an active 'oxon' /w O

Offset

- duration of effects, for example carbamate goes quickly and once its gone, so are symptoms, whereas if there's a phosphate linked to the enzyme, it doesn't come off, and more AChE must be made
- Differences in OP structure; **diethyl/dimethyl**, result in differences in binding to the enzyme

Diethyl = O-CH_2-CH_3 

Chlorpyrifos

Dimethyl = O-CH_3 

Fenthion

- Offset determined by the binding of the circled groups ie. diethyl vs. dimethyl
- Spontaneous reactivation refers to the unbinding of the compound for AChE to regenerate form
- Aging refers to when one of either the methyl/ethyl break off enabling ACh to bind irreversibly, destroying enzyme all together
- dimethyl spontaneously reactivate better than, but age more rapidly
- diethyl spontaneously reactivate slowly, and age slowly
- other organophosphates, which have variation in reversibility of binding and can age within minutes
- some can be neuropathic, damaging nerves, whereas some don't

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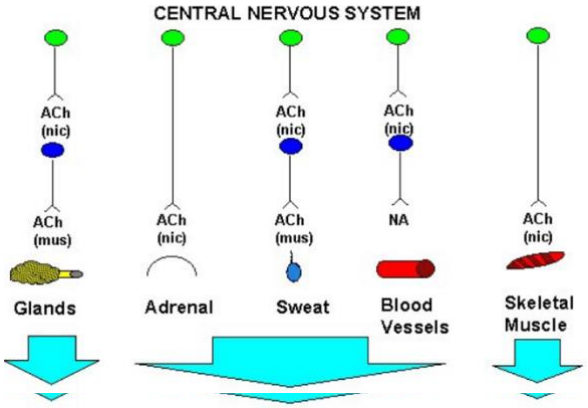
Toxicity

- there is a huge variety, due to the above reasons, but most pertain to the following scaffold

Acute cholinergic crisis

- within seconds/hours of exposure, and is the main cause of death
- this responds well to atropine (ie. muscarinic inhibitor, so is linked to parasympathetic effects)
- also high heartbeat due to ACh (pre ganglia) → NAd (post ganglia) which is sympathetic



Intermediate Syndrome

- 1-4 days post exposure, lasting a further 5-18 days
- Muscle weakness and respiratory failure, the result of depolarising block
- No treatment really (?oximes?)

OP induced delayed polyneuropathy

- 1-3 weeks post exposure where all nerves die, due to excessive action on skeletal muscle at NMJ

Treatment

- drugs must target processes relevant to effects and symptoms, without being a detriment to the patient
- atropine is effective to prevents excess glands secretion in both lungs (causes low oxygen saturation) and gut (causing vomiting and diarrhoea) + stopping excessive sweating = cumulative risk of dehydration reduced
- hard to deal with the blocking nicotinic (due to adrenal gland, we need adrenaline/don't want to paralyse us at NMJ), or noradrenaline (need blood vessels to constrict etc)

Oximes

- is able to reactivate AChE by removing the OP binded to the enzyme
- Diethyl reactivates better than dimethyls in presence of oximes, due to diethyl having slower aging therefore less likely to be past point of no return (dimethyl fast reactivate alone, and fast age, opposite to diethyl)
- Pralidoxime is an example of an oxime reactivation acetylcholinesterase enzyme
- the esterase enzymes that the antidote works on are just biomarkers, and are affected by oximes, however patients still died, implicating that they AChE and BuChE shouldn't be targets, and rather a dose related epiphenomenon

Q&A

- Muscle fasciculations in the setting of anticholinesterase poisoning indicate; neuromuscular junction nicotinic receptor stimulation
- Peripheral muscarinic receptor stimulation will be antagonised by atropine
- Monocrotophos and malathion are both organophosphorus pesticides, the first being banned in Australia and the second used in nit shampoo – why might monocrotophos be >100 times more toxic (per g) than malathion? Malathion requires metabolic conversion to be active, and hence can be used in shampoo, bypassing metabolism. This means it is a thion (S), yet to be converted into an oxon (O)
- Both organophosphorus and carbamate compounds inhibit acetylcholinesterase. What is the most important difference? The reversible action of carbamate
- What is the explanation for why oximes (pralidoxime) reverse AChE binding by diethyl-OPs but not some other types? Diethyl OP 'age' very slowly and therefore are unlikely to be at the point of no return.
- What is the best explanation for why Ops sometimes cause intermediate syndrome but carbamates do not? Because OP cause prolonged AChE inhibition due to not being reversible

Principles of Drug Action in the Nervous System

Lecture 5 – 15.8.17 – Neurotransmitters Part 1 (Monoamines)

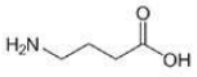
- Neurotransmitters are chemicals that transmit information to control behaviour of cells at a short distance
- Chemical messenger involved in communication between neurons specifically
- Substance in synthesised in the neuron, present in terminals and released when neurons fire
- There is an enzyme or uptake mechanism present in synapse to destroy/remove substance, thereby inactivating it and preventing effects

Classical Neurotransmitters

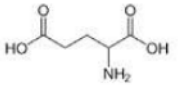
- Acetylcholine

Monoamines (Part 1)
- **Serotonin (5-HT)**
- **Noradrenaline**
- **Dopamine**
- Adrenaline

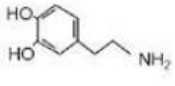
Amino Acids (Part 2)
- **GABA**
- **Glycine**
- **Glutamate**
- Histamine



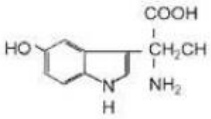
GABA



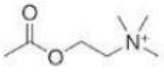
glutamate



catecholamine



indoleamine



acetylcholine

Categorisation

Chemical classes (amino acids vs. catecholamines)

- Quaternary amines (acetylcholine)
- Monoamines; catecholamines (noradrenaline, adrenaline, dopamine), indoleamines (serotonin), histamine
- Amino acid transmitters; glutamate, GABA (glutamate derivative), glycine, aspartate

Inhibitory vs. excitatory

Excitation – influx of Na+ ions causing depolarisation (more positive than resting) and thus ensuring neurostranmission pathway happens

Inhibition – influx of Cl- ions (and efflux of K+) causing hyperpolarisation (more negative than resting) and thereby preventing neurotransmission

INHIBITORY	EXCITATORY
• GABA	• Glutamate
• Glycine (strychnine-sensitive)	• Glycine (NMDA co-agonist)
• Dopamine (D_2)	• Dopamine (D_1)
• Serotonin (5-HT_1)	• Serotonin
• Acetylcholine (M_2)	• Acetylcholine (M_1)
• Histamine (H_2)	• Histamine (H_1, H_3)
• Noradrenaline (α_2)	• Noradrenaline (α_1, β)

- The type of receptor the neurotransmitter acts on dictates the resulting response

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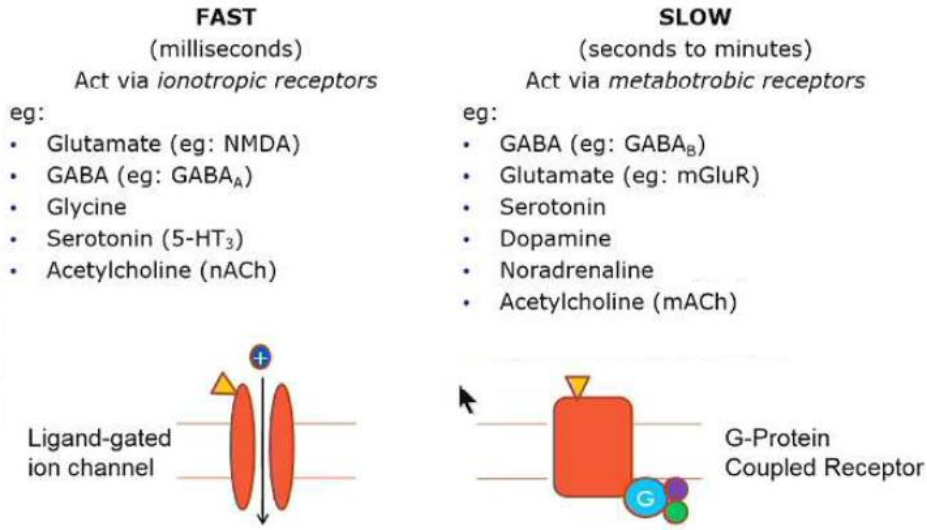
Fast vs slow (milliseconds vs seconds to minutes)

- Also determined by the characteristic of receptors
- Ionotropic receptors MEDIATE transmission
- Metabotropic receptors MODULATE transmission



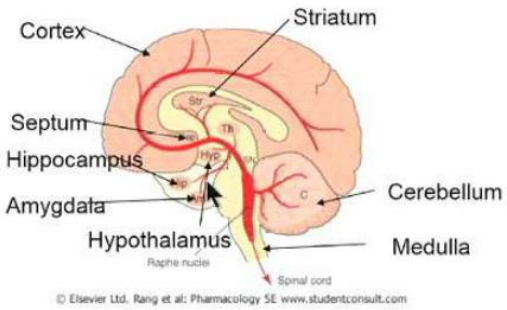
5-Hydroxytryptamine (Serotonin – 5HT)

- ~1% total body 5-HT in CNS

7 main receptor types;

- 1 ionotropic (LGIC): $5\text{-}HT_3$
- 6 metabotropic (GPCR): $5\text{-}HT_{1\ (6\ subtypes)}$, $5\text{-}HT_{2\ (3\ ubstypes)}$, $5\text{-}HT_4$, $5\text{-}HT_5$, $5\text{-}HT_6$, $5\text{-}HT_7$

- 1 inhibitory: $5\text{-}HT_{1\ (6\ subtypes)}$
- 6 excitatory: $5\text{-}HT_{2\ (3\ subtypeS)}$, $5\text{-}HT_3$, $5\text{-}HT_4$, $5\text{-}HT_5$, $5\text{-}HT_6$, $5\text{-}HT_7$



Pathways (serotoninenergic)

- Cell bodies grouped in raphe nuclei in pons and upper medulla
- Project to cortex, limbic system and hypothalamus
- Project to cerebellum, medulla and spinal cord

Major functions

- Sleep, wakefulness, feeding, control of sensory transmission
- mood, sexual behaviour, vomit reflex, nociception
- regulation of body temperate & blood pressure, vasoconstriction/vasodilation

Therapeutic Targets

- $5\text{-}HT_3$ antagonists (eg. Ondansetron) used to treat vomiting
- $5\text{-}HT_1$ agonists (eg. Sumatriptain) used to treat migraine via vasodilation
- 5-HT transport blockers aka selective serotonin reuptake inhibitors (eg. Fluoxetine, paroxetine) used to treat depression

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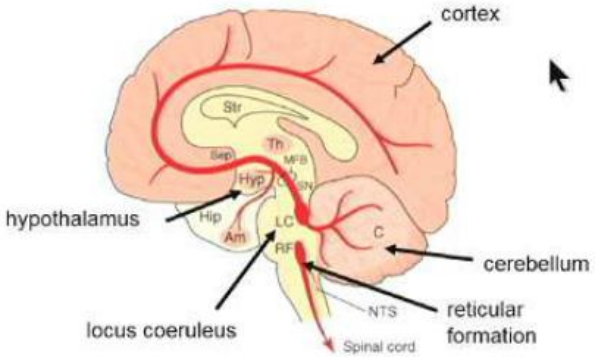
Noradrenaline

- Acts on the sympathetic nervous system effecting all post ganglia efferent signals except in sweat glands and adrenal medulla
- In the CNS, receptors include a1-2 and b1 (not so much b2)



Pathways (Noradrenergic)

- Cell bodies locus coeruleus (in pons)
- Project widely throughout the brain; cortex, amygdala, hypothalamus, cerebellum and spinal cord

Major Functions

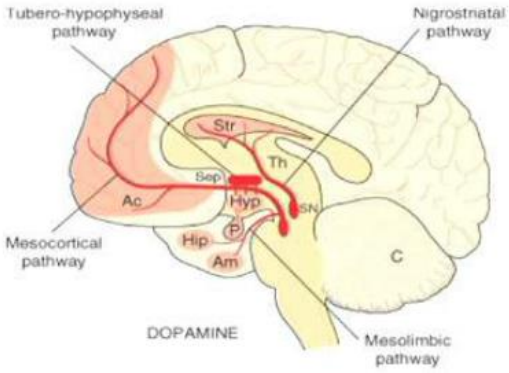
- Arousal, vigilance
- Mood, reward
- Blood pressure regulation and analgesia

Therapeutic Targets

- Tricyclic antidepressants (eg. Amitriptyline, imipramine) block NA reuptake to increase action of NA in the synapse
- Clonidine (a2-adrenoreceptor partial agonist) used to treat hypertension

Dopamine

- Most predominant in CNS (whereas noradrenaline prominent in PNS)



Pathways

- Nigrostriatal pathway (substantia nigra to striatum) → important in motor control
- Mesolimbic/mesocortical pathway (ventral tegmental area to limbic system and cortex) → regulation of mood
- Tuberohypophyseal pathway (Hypothalamus to pituitary stalk) → regulates hormone production

Major Functions

- Hormone regulation, movement, reward, emesis (vomiting)

Therapeutic Targets

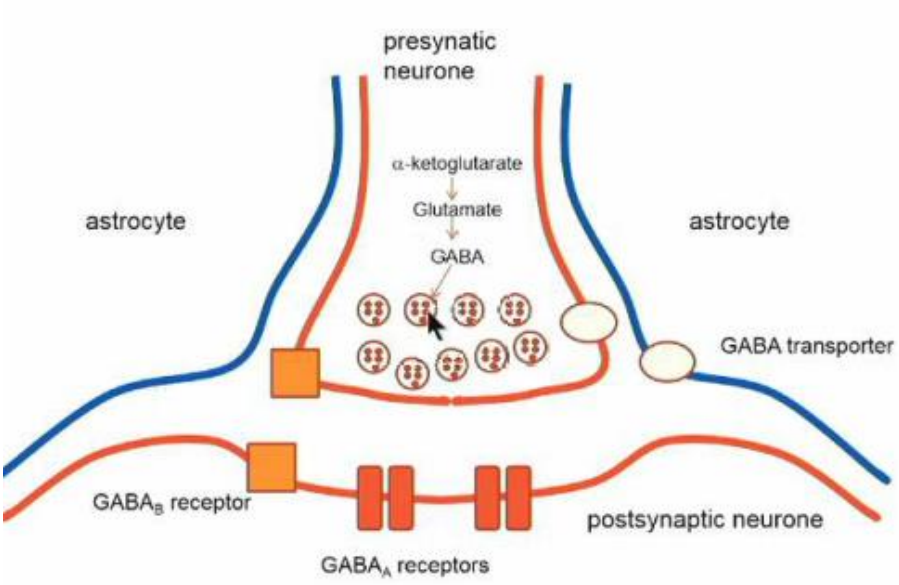
- Antipsychotic drugs (eg. Haloperidol, olanzapine) are D_2 antagonists
- L-DOPA given in parkinson's disease, a pre-cursor to DA, to increase levels of DA = increase nigrostriatal pathway

Principles of Drug Action in the Nervous System

Lecture 6 – 17.8.17 – Neurotransmitters Part 2 (Amino Acids)

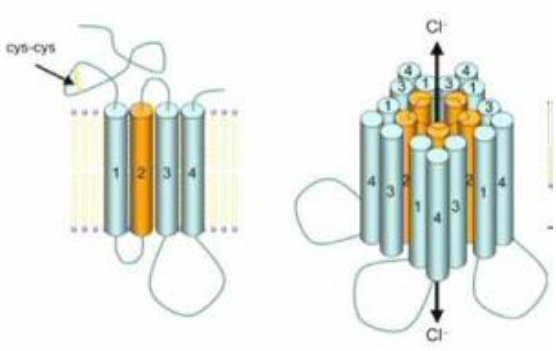
GABA

- Highly abundant in a wide variety of neurons in the CNS
- Activation of GABA receptors usually inhibits neurone activity
- Enhancement of GABA neurotransmission can have therapeutic benefits in epilepsy, huntington disease, tardive dyskinesia, alcoholism and addiction, insomnia, anxiety
- This can be achieved by both GABA agonists and glutamate antagonists



- Concentration of GABA in vesicles ~10-100mM
- $GABA_A$ are ionotropic (ligand gated), so are FAST
- $GABA_B$ are metabotropic, so are slower
- GABA diffuses out to GABA transporters, repackaged into vesicles if through synapse

$GABA_A$ Receptors



- 5 subunits, with each subunit exhibiting the same structure /w 4 transmembrane domain and an extra/intracellular domain
- the extracellular domain has a cys-cys loop, which is a defining feature of the $GABA_A$ receptor
- the pore is the result of the five 2nd transmembrane proteins
- GABA distorts the protein, allowing conformational shift = open

Drug Modulation

- Bicuculline = antagonist
- Benzodiazepines (diazepam, temazepam) is anti-anxiety/sedative/anti-convulsant
- Barbituates
- Ethanol
- Neurosteroids

Enhance the function of GABA

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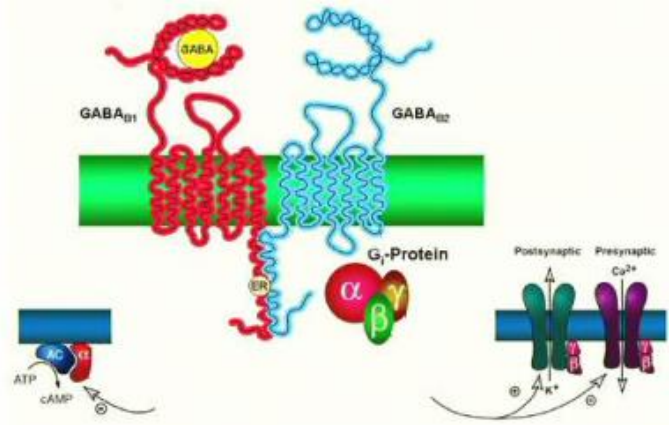
- General anaesthetics

$GABA_B$ Receptors



- Insensitive to bicuculline
- Must form a dimer, ie. two indibividual substrates to come together for effect to result (GPCR)
- GABA binds to $GABA_{B1}$ subunit to link with $GABA_{B2}$ which is connected to the G-protein
- Works via opening of K+ efflux channels = repolarisation & inhibition of Ca2+ influx

Drug Modulation

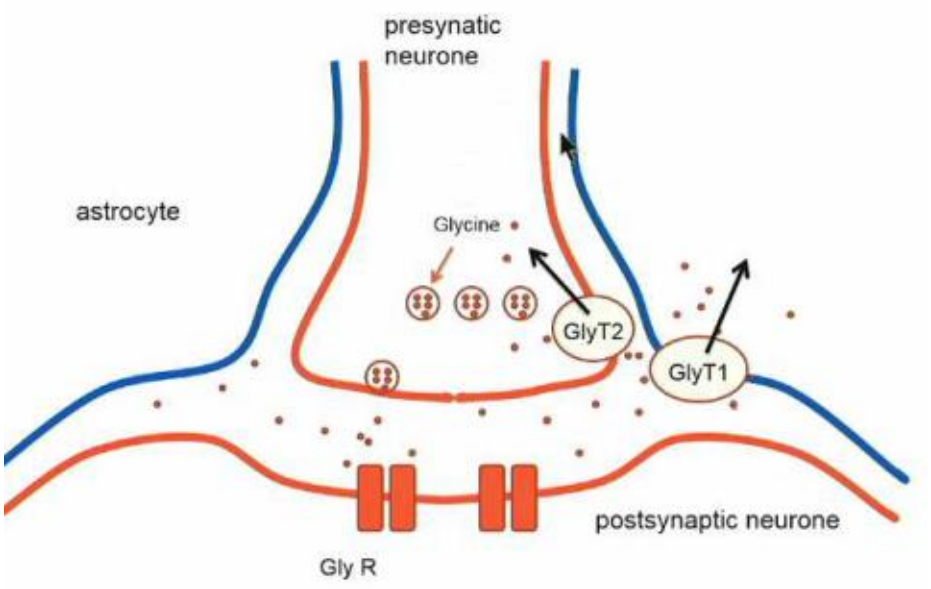
- Bacoflen = $GABA_B$ agonist, used as muscle relaxant

GABA Transporters

- GABA transporters require co-transport of Na+ and Cl- ions
- Closely related to Dopmaine Transporter, Serotonin transpoter, Noradrenaline transporter, Glycine Transporters (2)
- There are 4 subtypes of GABA transporters GAT1-4 → reflects diversity of the types of GABA signalling throughout CNS
- Expressed in neurons and glial cells throughout the CNS

Glycine

- Smallest amino acid with multiple roles
- Aids protein synthesis and amino acid metabolism
- Precursor for synthesis of many biomoleculars
- Inhibitory amino acid neurotransmitter
- No GPCR receptors relevant to Gly
- Also a very similar structure to $GABA_A$



Drug Modulation

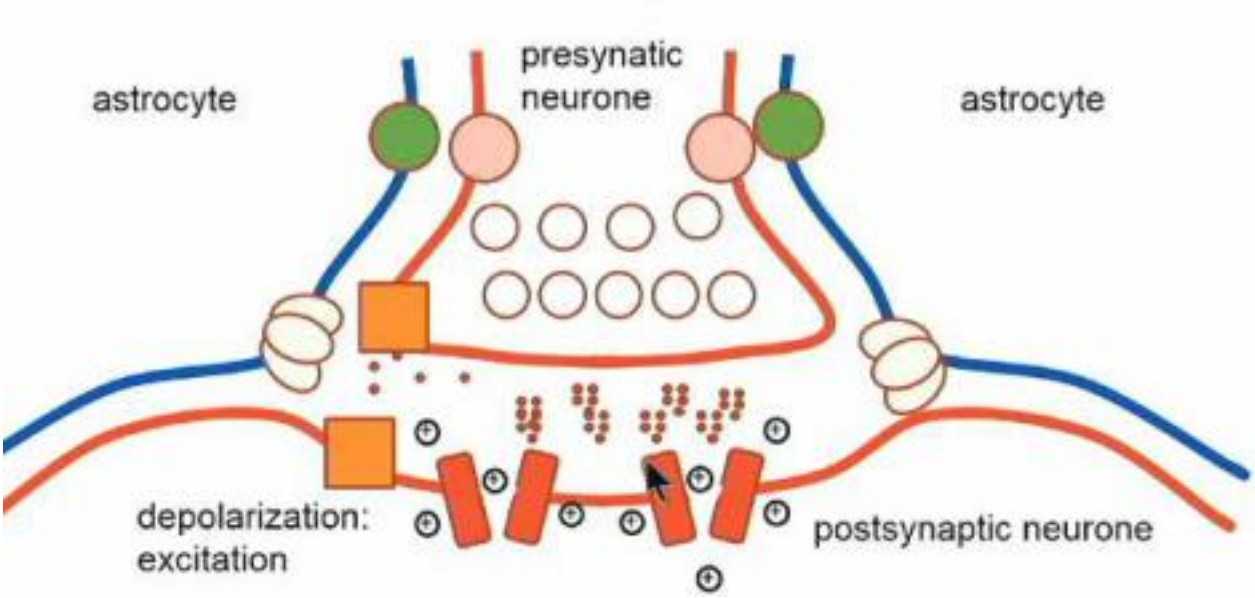
- Strychnine, a GlyR antagonist, overlapping binding site with glycine
- Causes convulsions and muscle rigidity
- Has been used in very low doses as a stimulant in horses, but mostly thought of as a poison

Glycine Transporters

- Glycine transporters are closely related to GABA transporters
- Na+ and Cl- coupled transporters
- Two subtypes of Glycine transpoters
- GlyT1 expressed in astrocytes
- GlyT2 expressed in presynaptic neurones

Glutamate

- Is one of the 20 standard amino acids used to make proteins
- Is highly abundant in the brain and involved in many things
- Aids in protein synthesis, part of multiple metabolic pathways
- Excitatory neurotransmitter



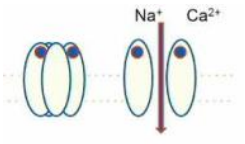
- Presynaptic neurone has 100mM → synaptic cleft at 10mM → 10nM via transporters… into predominantly astrocytes (only some presynaptic)
- Reacts with ionotropic glutamate receptors to cause influx of positive ions, depolarising cell
- Once taken up by astrocyte, is converted into glutamine (improves retention and maintains gradients), and deposited back into synapse for reconversion
- Powerful transporters because important to be fast ie. one transmission is quickly stopped ensuring both dynamic and efficient signalling

Glutamate Receptors

Fast neurotransmissions

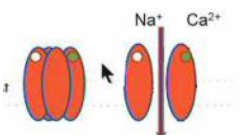
- Ionotropic glutamate receptors (iGIuRs) classified based on the actions of selected agonists
- Tetrameric protein

AMPA and Kainate Receptors



- Agonists include glutamate, AMPA and kainite, and require 2
- Enables sodium and calcium influx

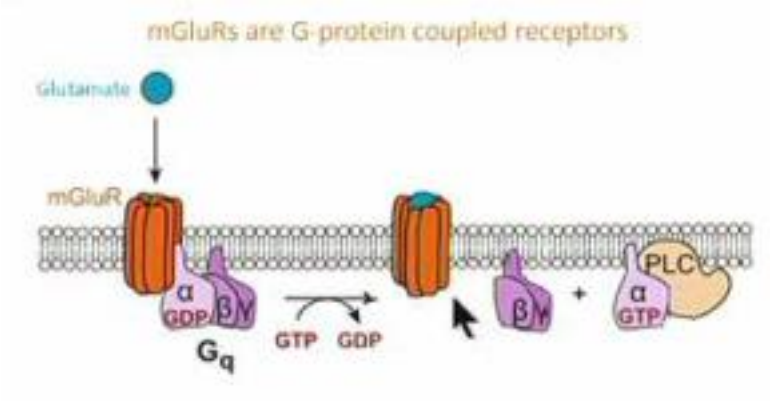
NMDA Receptors



- Agonists – glutamate, N-methyl-D-aspartate

- Also require glycine or D-serine as a co-agonist

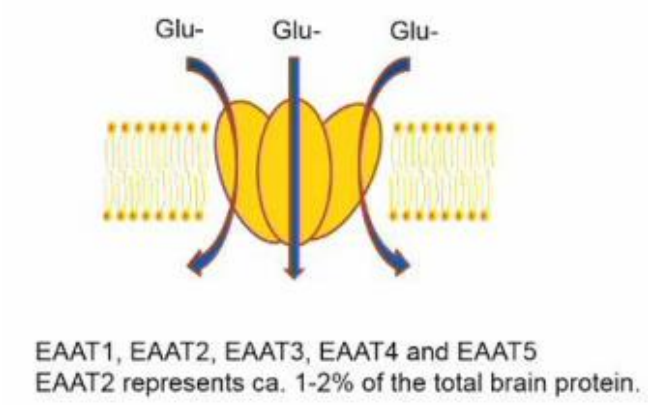
Slow Neurotransmission



- Metabotropic receptors (mGluRs)
- 7 different subtypes exist
- has a large extracellular domain, closing around the substrate causing conformational changes and thereby activation
- b/y can inactivate K+ channels = excitation

GLUTAMATE RECEPTORS NOT GOOD TARGET 4 DRUGS

Glutamate transporters



- involved in termination of neurotransmission
- also termed excitatory amino acid transporters and are considerably more powerful
- structurally unrelated to other transporters – are trimers, and each one of the subunits can function independently = efficient capture of glutamate
- glutamate transporter coupled to the co-transport of Na+ ions (tendency to go in) and the counter-transport of K+ ions (tendency to go out)
- enables conc. change from 10mM → 10nM
- greater presence/density than glutamate receptors, as it so important to finely control glutamate concentrations

Problems

Excitotoxicity – the result of uncontrolled glutamate action whereby excessive/prolonged activation of receptors via glutamate will kill neurons

- this occurs during periods of reduced energy supply to the brain (eg. Hypoxia, stroke, neurodegenerative conditions like parkinsons disease and alzheimers)
- this prevents control of glutamate actively via glycolysis (ATPase Na+/K+ pump stops)
- causes excessive activation of glutamate receptors
- fucks with recycling glutamate and presynaptic calcium entry
- all leads to cell death via excessive activation of NMDA receptors particularly :--(
- activation of NMDA = influx of large amounts of Ca2+ and Na+ triggering apoptosis
- ionic imbalance also fucks with this, dragging water molecules into the cell causing necrosis

Monosodium Glutamate (MSG)

- used as a flavour enhancers, due to metabotropic glutamate receptors found on tongue
- used as neurotransmitter
- possibility of adverse reactions