Neuropharmacology

CNS disorders are a major cause of morbidity but are mostly not well understood which impacts on availability of successful drug treatments.

The BBB (tight junctions between endothelia, thick basement membrane and astrocyte foot-processes) provides a significant physical and molecular barrier to substances seeking to enter the brain via the circulation.

There are >100 neurotransmitter/neuromodulator substances that activate / modulate / inhibit CNS neurotransmission (including neuropeptides, nucleotides, gases and lipids).

Most drugs acting on the CNS modulate synaptic communication in some way

GLUTAMATE

Main excitatory neurotransmitter in the CNS. Can be synthesised by 1) from GABA by GABA-transaminase, 2) by glutamate dehydrogenase and 3) from glutamine by glutaminase.

Glutamate enters vesicles via vesicular glutamate transporters (VGLUT).

Transmission is terminated by re-uptake – mostly into glial cells (excitatory amino acid transporters – EAATs). Uptake requires influx of 3 Na and 1 H, and efflux of 1 K. Following uptake, glutamate converted to glutamine (glutamine synthase)

Receptors

3 x inotropic – AMPA, NMDA, Kainate
8 x metabotropic – mGluR 1-8

AMPA – cation channels (Na and K). Tetrameric. 4 glutamate molecules required for activation. Activation of AMPA receptors causes depolarisation and mediate the majority of fast excitatory neurotransmission.

Kainate – tetrameric, activated by glutamate, kainate and domoate. Cation influx causing depolarisation – and rapid desensitisation of the channel. ACET is an antagonist with no clinical use.

NMDA – tetrameric (7 potential subunits) – usually 2 x GluN1 and either 2 x GluN2, or 1 x GluN2 and 1 x GluN3. Glutamate binds GluN2 but for activation requires co-agonist glycine or D-serine (binding to N1 and N3 subunits). Additionally, at rest a Mg ion blocks the pore, requiring a depolarisation to displace this, before the NMDA channel can become fully activated. Channels are permeable to Ca and have slower kinetics than AMPA/Kainate receptors.

Activation of NMDA receptors requires more significant glutamate release (such as that from multiple action potentials) to permit adequate depolarisation to displace the Mg ions

before the NMDA receptor can be opened and therefore has a more regulatory role and is important in long term potentiation. Other substances can modulate this receptor: protons (+), spermine (-) and Zn (-)

Ketamine – NMDA blocker – used in anesthesia
Mematine – NMDA blocker – used in alzheimers disease

STROKE

Stroke is caused by disruption to blood supply to the brain. 3rd cause of death in the UK. 85% are ischaemic caused by thromboembolic obstruction. 15% caused by haemorrhage. Cells close to occlusion (umbra) die rapidly. Surrounding cells (penumbra) are ischemic and can survive with rapid reperfusion.

Thrombolysis – Alteplase – tissue plasminogen activator, is a first line treatment for patients with ISCHAEMIC stroke with drug administered within 4.5 hours – has significant impact on functional improvement.

Neuronal death is due to excitotoxicity (and acidotoxicity). Ischaemia leads to depletion of ATP, therefore failure of Na-K ATPase, leading to depolarisation and excessive glutamate release. This permits AMPA and then NMDA receptor activation, leading to increased Ca levels intracellularly. Via multiple pathways this leads to cellular damage, including mitochondrial dysfunction and reactive oxygen species development. Unfortunately, no treatments aimed at excitotoxicity have clinical benefit (NMDA antagonists, Ca antagonists, ROS scavengers).

GABA

Gamma-aminobutyric acid (GABA) a γ amino acid is the main inhibitory neurotransmitter in the CNS.

GABA is formed from glutamate by glutamic acid decarboxylase. Transported into vesicles by VGAT. After release GABA is taken up by GABA transporters (GAT1-3) into predominantly neuronal cells.

GABA activates both ionotropic and metabotropic receptors, $GABA_A$ and $GABA_B$ respectively.

$GABA_A$ receptors are ionotropic pentameric receptors (numerous combinations of subunits). Cl channels – leading to hyperpolarisation. Have multiple modulators including neurosteroids, benzodiazepines and barbiturates

Bicuculline – $GABA_A$ antagonist – causes epileptic seizures in animals

Barbiturates (e.g. Thiopental) – positive allosteric modulator – cause unconsciousness used in anaesthesia and as anxiolytics and anticonvulsants

Benzodiazepines - $GABA_A$ modulator - bind to the α/γ interface to enhance GABA function by allosterically increasing the affinity for GABA and are used as sedatives, anxiolytics and anti-convulsants. Some have metabolites that are also $GABA_A$ modulators. Different α subunits underlie these different effects

α1 = anticonvulsant, sedative/hypnotic and addictive effects
α2 = anxiolytic effects,
α2, α3- and α5 = muscle relaxation
α1 and α5 = amnesic effects

Diazepam – $t_{1/2}$ >20 hours, long duration of activity. Used for anxiety. Develop tolerance overtime. Side effects: drowsiness, amnesia, impaired motor coordination and nausea

Lorazepam – $t_{1/2}$ 8-12 hours – less tolerance. Used as hypnotic

Abrupt cessation of benzodiazepines causes a rebound effect with heightened anxiety, and thus withdrawal should be gradual.

Flumazenil - benzodiazepine antagonist, used to treat benzodiazepine overdose

Zolpiderm – not benzodiazepine by structure, but bind benzodiazepine binding site. selective for the α1 $GABA_A$ subunit and so is sedative. Has $t_{1/2}$ 2 hours and is less addictive.

$GABA_B$ receptors are Gi/o-coupled GPCRs that are expressed both pre- and postsynaptically (reduce neurotransmitter release and decrease excitability respectively).

Baclofen - $GABA_B$ agonist – spinal cord action predominantly, treatment of spasticity associated with multiple sclerosis or spinal injury

Sleep disorders

Narcolepsy and insomnia – can be treated cognitive behavioural therapy and a variety of medications:

benzodiazepines (although many side effects)
Zolpiderm.
Histaminergic H_1 receptor antagonists that cross the BBB can also be used – e.g clozapine or diphenhydramine.
Trazodone – Antidepressant; promotes sleep by antagonising the action of wake-promoting serotonin neurons. Blocks 5-HT_2 receptors.
Ramelteon – melatonin MT_1 and MT_2 receptor agonist.

NORADRENALINE

Activation of noradrenaline neurons induces alertness and vigilance and is importance for maintaining arousal and attention. They are involved in: sleep-wake cycle, sensory stimuli, central control of BP, mood.

Amphetamine - enhances wake-promoting action of noradrenergic neurons by displacing vesicular NA/DA (VMAT and NET substrate)

EPILEPSY

Seizures that result from aberrant, high frequency neuronal discharges in the brain. Two types of epileptic seizures: generalised involves the whole brain and partial (also referred to as focal) involves just part of it. Thought there is a paroxysmal depolarising shift generating a string of action potentials at the focus. Variety of drug targets:

Na channel blockers
Carbamazepine - side effects including drowsiness and ataxia, it also induces hepatic enzymes, which can affect the metabolism of other drugs
Phenytoin - 80-90% bound to plasma albumin, which is affected by other drugs. Hepatic metabolism shows saturation impacting pharmacokinetics. Side effects: rashes, ataxia and headache. High plasma concentration can cause seizures.
Lamotrigine
Lacosamide - enhances slow inactivation, involving structural rearrangement

Enhanced GABA function
Diazepam – see above
Phenobarbitol – see above
Vigabatrin - irreversibly inhibits GABA transaminase leading to an increase in brain [GABA] and increased GABA release – used for refractory cases
Tiagabine - inhibits GAT1 leading to increased extracellular [GABA]

Calcium channel blockers - inhibition leads to reduced Ca2+ influx and decreased neurotransmitter release
Valproate - blocks T-type Ca channels making it efficacious in treating absence seizures, also weakly inhibits GABA transaminase. Side effect – hair thinning and hepatic toxicity
Ethosuximide – similar to above
Gabapentin - binds to Ca channel accessory subunit α2δ1, reduces Ca channel plasma membrane expression thus reducing Ca2+ influx

Others
Topiramate – Na channel, Ca channel and AMPA receptor block, plus $GABA_A$ facilitation
Perampanel - non-competitive AMPA receptor antagonist
Zonisamide - blocks Na and Ca channels, possible enhancement of $GABA_A$ function

SEROTONIN

5-HT is expressed at high levels in platelets, intestinal enterochromaffin cells and serotonergic neurones of the enteric and CNS

5-HT is synthesised from tryptophan by tryptophan hydroxylase and L-aromatic acid decarboxylase (DOPA decarboxylase)

Once synthesised, 5-HT is pumped into vesicles by the vesicular monoamine transporter (VMAT), which also transports dopamine

Multiple 5-HT receptors – all metabotropic except 5-HT_3 (a ligand gated ion channel)

5-HT is inactivated through neuronal reuptake via SERT, a 5-HT specific transporter. Following uptake, degradation is a 2-step process: oxidative deamination via monoamine oxidase followed by oxidation to produce 5-hydroxyindoleacetic acid (5- HIAA – excreted in the urine)

5-HT has roles in: sleep/wakefulness, appetite, sensory transmission, hallucinations, mood

ANXIETY

= same set of physiological and behavioural events take place as in fight or flight, but in an anticipatory manner without there being any actual stimulus. Can be generalised anxiety disorder, phobias, panic disorder and obsessive-compulsive disorder.

Selective serotonin reuptake inhibitors (SSRI) – e.g. sertraline
Buspirone - partial agonist of 5-HT_{1A} receptors (inhibitory auto-receptors). Can initially increase anxiety
Benzodiazepines
Propranolol – can be used to reduce the sympathetic symptoms

DEPRESSION

The monoamine theory is a key theory underlying depression biogenesis, many drugs used to treat depression modulate levels of the monoamine neurotransmitters 5-HT and NA. Discovered because it was observed drugs affecting NA and 5-HT caused/alleviated symptoms of depression. E.g Reserpine and α-methyltyrosine decreased mood and clorgyline increased mood. Note, all drug treatments take several weeks to have effect, and not all drugs work in all patients.

Moclobemide – MAO-A inhibitor. One of the first drugs used. Increases the cytoplasmic concentration of 5- HT/NA and spontaneous leak into the synapse. Many side effects. If given with SSRI can cause serotonin syndrome.

Imiprimaine and amitryptiline (tricyclic antidepressants) - inhibit NET and SERT, i.e. NA and 5-HT reuptake. NET inhibition is thought to relieve the biological symptoms of depression, whereas SERT inhibition relieves the emotional symptoms. Affect other receptors so many side effects, e.g H_1 and ACh. Metabolised by CYP_{450} enzymes, so metabolism can be affected by other drugs. Toxic in overdose

Fluoxetine and citalopram (SSRI) – more selective than above. Fewer side effects. Safe in overdose. First line treatment for depression.

Duloxetine and venlafaxine (SNRI) - relatively non-selective between inhibition of NET and SERT. Few side effects, but duloxetine can cause hepatotoxicity.

Mirtazipine - combined antagonist properties at presynaptic α2 adrenoceptors and 5-$HT_{2A/C}$ and 5-HT_3. Fewer side effects than SSRI, but due to H_1 blockade, does cause some sedation

Agomelatine - used to treat severe depression and it is thought to work by correcting disturbances in circadian rhythm that commonly occur in depression.

Bipolar disorder – periods of depression and periods of euphoria/mania.

Lithium – effective at treating mania. Enters cells through Na channels. Inhibits inositol monophosphatase toning down G_q signalling pathways. Narrow therapeutic window (drug monitoring required). Main toxicity effects being: nausea/vomiting, polyuria, thyroid enlargement/hyperthyroidism and neurological effects.

Lamotrigine – as above – can also be used for bipolar disorder.

DOPAMINE

Important neurotransmitter, changes in its function are associated with a variety of brain disorders, such as Parkinson's disease and schizophrenia.

Synthesis as for NA – but cells lack dopamine β-hydroxylase. Taken up into vesicle via VMAT. After release, reuptake via the DA transporter, DAT. Breakdown of DA happens in presynaptic terminals via MAO and COMT.

Receptors are all GPCRs: D_1-like (Gs-coupled, D_1 and D_5) and D_2-like (Gi/o-coupled, D_{2-4})

Functionally, the dopaminergic pathways are involved in motor control (nigrostriatal), emotion/behaviour (mesocortical and mesolimbic) and endocrine regulation (tuberohypophyseal)

Bromocriptine – D_2 receptor agonist – supresses prolactin secretion by pituitary tumours
Domperidone - D_2 receptor antagonist - chemoreceptor trigger zone receptors, Antiemetic
Methylphenidate – inhibit reuptake transported- DAT - treatment attention deficit hyperactivity disorder (ADHD)

SCHIZOPHRENIA

Condition where patients exhibit 3 characteristics: positive symptoms (e.g. delusions and hallucinations), negative symptoms (e.g. alogia, anhedonia, asociality and avolition) and cognitive symptoms.

It is unclear what causes schizophrenia. Some genetic predisposition. Genes e.g. neuregulin-1, dysbindin, DISC-1. There are environmental influences thought to be important: maternal virus infections, substance abuse, such as cannabis in adolescence/early adulthood.

Some evidence supporting misregulation of DA function came from work showing that amphetamine can induce behaviour in humans similar to that seen in acute schizophrenia. Also, levodopa and DA receptor agonists like bromocriptine can be hallucinogenic, and cause stereotyped behaviour. Thought that Dopaminergic activity is overactive in the mesolimbic pathway to produce positive symptoms, and deceased mesocortical DA activity may underlie negative symptoms

5-HT hypothesis - Many antipsychotics have additional actions on 5-HT receptors (5-HT_{2A} and 5-HT_{1A}), suggesting that blockade of dopaminergic **and** serotonergic transmission is important. Also NMDA receptor antagonists like ketamine exhibit the 3 sets of symptoms (positive, negative and cognitive) observed in schizophrenia, suggesting glutamate may also be dysregulated.

Antipsychotic drugs

> ***Typical*** – first generation (cause psychomotor slowing and emotional quietening, mesolimbic D_2 receptor antagonism). Chlorpromazine and haloperidol.
>
> Many side effects due to binding D2 receptors elsewhere;
>
> - D_2 mesocortical -> exacerbation of negative symptoms
> - D_2 in striatum -> extrapyramidal symptoms (acute dystonia and tardive dyskinesia)
> - D_2 tuberoinfundibular -> prolactin secretion
>
> Also, bind histamine H_1 receptors, muscarinic M_1 receptors and α_1 adrenoceptors
>
> ***Atypical*** – second generation. D_2 and 5-HT_{2A}receptor antagonists (decreased propensity to cause extrapyramidal motor side effects due to rapid dissociation kinetics - hypothesised that rapidly dissociating drugs may enable some dopaminergic signalling). Clozapine (side effect: agranulocytosis). Aripriprazole (partial agonists at Gi/o coupled 5-HT_{1A} pre-synaptic autoreceptors which reduce 5-HT release)

PARKINSONS DISEASE

Features: bradykinesia, rigidity and 'pill rolling' tremor. Significant reduction in DA in the substantia nigra and degeneration of dopaminergic terminals in the corpus striatum. Several theories to explain loss of DA neurones. Symptoms only appear once DA content has fallen to <40% normal levels.

DA promotes activity: too much dopaminergic activity causes hyperactivity (as is associated with stimulants such as amphetamine), too little dopaminergic activity can cause reduction in activity (as occurs in Parkinsons). The loss of nigrostriatal dopaminergic neurones results in decreased activation of D_1 (Gq) and D_2 (Gi) receptors of the direct and indirect pathways respectively: stimulation of the direct pathway decreases and inhibition of the indirect pathway is diminished.

All the drugs used to treat Parkinsons do not affect the underlying neurodegeneration, but rather aim to counteract the DA deficiency:

Levodopa (aka L-DOPA) - converted to DA by DOPA decarboxylase; co-administration of carbidopa, a peripherally acting DOPA decarboxylase inhibitor, reduces the overall dose needed and lessens side effects. Less effective with disease progression. Reduces rigidity and bradykinesia with 20% gaining normal motor function. Unwanted effects include dyskinesia (involuntary movements) – thought to be due to short $t_{1/2}$ leading to fluctuating concentrations - bradykinesia and rigidity can suddenly worsen and then improve, an 'on-off' effect.

To stabilise plasma concentrations COMT inhibitor, such as entacapone (peripherally restricted) or tolcapone (centrally penetrant), can be used, this prevents the breakdown in the periphery of levopoda to 3-methoxydopa and in the brain of DA to 3-methoxytyrosine.

Ropinirole - $D_{2/3}$ selective agonist and does not show the same fluctuation in efficacy

Selegiline - selective MAO-B inhibitor, therefore protecting DA from degradation. Often used with levodopa to improve therapy

Safinamide - inhibits both MAO-B (decreased degradation), as well as inhibiting DAT (decreased reuptake)

ALZHEIMERS DISEASE

Degenerative condition increasing in incidence with age. Cognitive symptoms including memory loss and difficulties with thinking and problem solving. Prevalence closely associated with ageing, i.e. 5% at age 65, >90% aged 95. Disease mainly affects the hippocampus and basal forebrain, and it is the loss of cholinergic neurones that is thought to underlie the cognitive deficits.

Two key characteristics of AD brains are extracellular amyloid plaques comprised of β-amyloid (Aβ) protein and neurofibrillary tangles consisting of the microtubule associated protein Tau in a phosphorylated form.

APP and presenilin mutations cause Aβ42 overproduction, mutations in ApoE4, a lipid transport protein that acts to clear Aβ oligomers, are also associated with rare forms of AD, most likely due to poorer clearance of aggregated Aβ.

Donepezil, galantamine and rivastigmine - anticholinesterases that are approved for early-mid stage Alzheimer's disease. These show selectivity for CNS AChE, but still causes some side effects associated with enhanced ACh signalling in the periphery.

Memantine - a weak NMDA receptor antagonist that produces a modest improvement in cognitive function in mild to moderate AD, although neuroprotection (i.e. through preventing NMDA receptor-mediated excitotoxicity) does not appear to underlie the effect.

Aducanumab - monoclonal antibody, developed to specifically target Aβ oligomers and aggregates. Phase 1B study reported 1yr treatment, dose-related decrease in amyloid plaque density + slowed cognitive decline – may bind Aβ + recruit microglia phagocytosis of Aβ – trials ongoing.