

AUTONOMIC NERVOUS SYSTEM

Sympathetic system originates from the sympathetic ganglia that are derived from *thoracolumbar* segments (T1 – L2/3). System primarily active in times of stress

Parasympathetic system originates from sacral spinal segments, as well as the medulla, as cranial nerves III, VII, IX, X. Primarily active in times of satiation.

Both generally provide tonic stimulation which means that modulation of *levels* of stimulation occurs, rather than 'switching' on and off.

Transmitters

- Preganglionic neurones: cholinergic – nAChR
- Post-ganglionic parasympathetic neurones: cholinergic – mAChR (and VIP, substance P, NO, cholecystokinin)
- Post-ganglionic sympathetic neurones: noradrenergic with ATP (except to sweat glands which are cholinergic, and innervation of adrenal gland medulla: adrenaline)

Transmitters are released as discrete multimolecular packets. There is a releasable, and a reserve, pool of synaptic vesicles.

Synaptic vesicle cycle

Uptake of neurotransmitter into vesicle (ATP dependent – proton anti-porter) → vesicles move to active zone via Kinesin motor protein → docking at the active zone → ATP dependent pre-fusion/priming → Ca triggered fusion.

Snares – Synaptobrevin (*vesicle* – v-SNARE) and syntaxin and SNAP-25 (plasma membrane *target* t-SNARE) donate α -helices to the **CORE COMPLEX** that forms at docking, leading to formation of leucine zipper.

Upon fusion α -SNAP directs the ATPase NSF to disassemble the complex.

Calcium level detected by synaptotagmin (homology to PKC, bind Ca with low affinity)
Synapsins – link vesicles to cytoskeleton (phosphorylation leads to disassociation and this permits vesicles to traffic to active zone for release)

- *Botulinum toxins* (preferentially affecting cholinergic neurones) and *tetanus toxin* act to degrade these proteins to prevent vesicle release.

Synaptic plasticity – the amount of neurotransmitter released with each action potential is not constant.

- *Facilitation* – Ca is not removed between pulses so builds up
- *Depression* – neurotransmitter runs out as release > replenishment
- *Long term potentiation* – long lasting increase in neurotransmitter release following repetitive stimulation of a synapse.

CHOLINERGIC TRANSMISSION IN THE ANS

Processing of ACh at the synapse

- Na⁺/choline secondary active transport delivering choline into the presynaptic neurone. Rate limiting step. *HEMICHOLINIUM inhibits*
- Choline-acetyl transferase synthesises ACh synthesis from choline and acetyl coenzyme A.
- ACh/H⁺ antiporter pumps ACh into the vesicle. [VAcHT] *VESAMICOL blocks*
- Release of vesicles from active zone. *BOTULINUM TOXIN's and TETANUS TOXIN inhibits*
- ACh degradation in synapse by cholinesterases. AChE is specific, Butyrylcholinesterase has a broader specificity. AChE – 3 tetramers joined by a collagenous tail – 12 active sites. *ANTICHOLINESTERASES inhibit*

Ganglionic transmission

- Nicotinic AChR → fast excitatory polarising potential.
- Receptors are (α3)₂(β4)₃
- Trimetophan *sensitive*, α-bungarotoxin *insensitive* – differentiating between nmj nAChR.

Blocking of ganglionic transmission:

- Inhibit ACh synthesis/release
- Competitive antagonism e.g. *trimetophan*
- Channel pore blocker (use dependency) e.g. *hexamethonium*
- *Depolarising block* – caused by sustained presence of agonist. Phase I block due to inactivation of Na channels (closed but unopenable state). Phase II block is due to a desensitisation due to signalling cascades modulating the response of the receptor to the agonist – note postganglionic neurone can be directly stimulated electrically. *decamethonium* and *suxamethonium/succinylcholine*
- *Non depolarising block* – competitive antagonists of nmj nicotinic receptor. *atracuronium* and *pancuronium*. Block can be reversed by acetylcholinesterase inhibitors.

Postganglionic transmission: muscarinic AChR

- **M1** – G_{q/11} – gastric acid secretion and neurone excitability (since PIP2 is a cofactor for M channel (K) and PLC degrades this)

- **M2** – $G_{i/o}$ – reduces rate (not force) of heart activity. α_i decreases PKA inhibiting Ca channels, and β_y activates GIRK (GPCR activated inward rectifier K channel)
- **M3** – $G_{q/11}$ – secretions, contractions of visceral tissues.

Agonists:

ACh, Metacholine (m>n), carbachol (n>m) bethanecol (m), pilocarpine (m)

Cevimeline – selective M_3 agonist

Methacholine + enantiomer has most affinity and is broken down by AChE.

The problem with the muscarinic (m) agonists is that they are not specific for particular receptor and thus there are a range of effects observed e.g. heart slows, smooth muscle contraction, secretions increase. The same is true of antagonists.

Glaucoma – increase intraocular pressure. Drainage impeded by a dilated pupil. Contraction with muscarinic agonists (or adrenergic antagonists) reverses the effect, e.g. pilocarpine

Antagonists

Atropine (CNS excitement), Hyoscine (CNS depression),

Pirenzepine (M_2)

Darifenacin (M_3) mediating bladder constriction in urinary incontinence

Benzilycholine mustard – irreversible antagonist

Again, wide range effects due to lack of selectivity – decreased secretions, increased heart rate, relaxed smooth muscle and motility.

Anticholinesterases

AChE are serine hydrolases that can cleave >10,000 molecules s^{-1} active site⁻¹

Similar effects to excessive stimulation, can even see depolarising block.

Enzyme has both esteratic (important serine residue 203 and histidine 447) and anionic sites (glutamate residue 334)

- *Short action* – edrophonium – binds via ionic bond to the anionic site, readily reversible [diagnosis of myasthenia gravis]
- *Medium action* – neostigmine – binds both sites forming carbamoyl esters. The carbamoylated enzyme takes ‘minutes’ to hydrolyse
- *Long action* – organophosphorous compounds such as dyflos, malathione and ecothiopate. Phosphorylate enzyme which is effectively irreversible and new enzyme synthesis is required. *Pralidoxime* – strong nucleophile can reactivate enzyme inhibited with dyflos before covalent bond ‘ages’

Myasthenia Gravis – Autoimmune disease characterised by the presence of auto-antibodies targeted against the nmj nicotinic receptor, leading to their destruction. This leads to fatigueability – muscle weakness with use. Diagnosed with edrophonium, treated with neostigmine,

ADRENERGIC TRANSMISSION

Catecholamines – e.g. noradrenaline, dopamine, 5-HT, adrenaline.

Biosynthesis

Tyrosine → (*tyrosine hydroxylase*) → DOPA → (*DOPA decarboxylase*) → Dopamine → (*Dopamine β hydroxylase*) → Noradrenaline → (*phenylethanolamine N methyl transferase*) → Adrenaline.

Tyrosine hydroxylase is rate limiting. End product inhibition regulates, as does phosphorylation (reduces sensitivity to EPI).

- α-methyltyrosine – inhibits tyrosine hydroxylase
- carbidopa – inhibits DOPA decarboxylase (used with L-DOPA in parkinsons as peripheral DDC inhibitor)
- disulfiram - dopamine β hydroxylase inhibition (used in alcohol dependency due to inhibiting alcohol dehydrogenase)

- methyldopa – processed to α-methynoradrenaline – *false transmitter* presynaptic α2 inhibitory receptor activity > α1
- reserpine – blocks accumulation of NA in vesicles and leads to depletion of stores (VMAT)

Degradation

There is no enzymatic degradation in the synapse as with ACh. Uptake plays an important role in terminating response

UPTAKE 1: Taken up by sympathetic neurone, where 50% is recycled. (NET) High affinity, low capacity, sodium dependent transport. Blocked by cocaine and imipramine

UPTAKE 2: Circulating catecholamines taken up by various non neuronal tissues Low affinity, high capacity. (OCT3, ENT). Blocked by normetanephrine, corticosteroids and phenoxybenzamine

There is consequent degradation in the neurone:

Monoamine oxidase (MAO) – associated with mitochondrial membrane. Acts to convert monoamine to an aldehyde and consequent reactions convert to carboxylic acids. Enzyme also acts on dopamine and 5-HT.

[MAO-A – NA, 5-HT, dopamine; MAO-B – dopamine]

Catechol – O – methyl transferase (COMT) – methylation of catechol, able to act on both catecholamines themselves as well as the deaminated products.

These processes generate intermediates that can be excreted such as VMA and MOPEG.

- Clorgyline: MAO-A inhibitor (depression)
- Selegiline: MAO-B inhibitor (Parkinsons)
- Tranylcypromine – non selective reversible MAO inhibitor
- These MAO inhibitors act to increase cytoplasmic concentration which leads to reversal of transporter processes
- Entacapone: COMT inhibitor
- Amphetamine: indirectly acting sympathomimetic amines. Taken up via uptake 1, enter vesicles displacing NA, also inhibits MAO leading to increased release of NA
- Guanethidine: competes with NA for uptake 1, displaces NA from vesicles. But chronic use leads to NA depletion leading to neurone blocking and inhibits vesicle fusion.

Adrenergic receptors

α_1	$G_{q/11}$	NA>A	Vasoconstriction, secretion, GI relaxation (I_{KCa})
α_2	$G_{i/o}$	A>NA	Inhibition VGCC \rightarrow inhibit transmitter release
β_1	G_s	A=NA	Activate VGCC, I_f . Increase rate + force heart
β_2	G_s	A>NA	MLCK inhibition of broncho/vasodilatation etc.

	Agonist	Antagonist
α_1	Phenylephrine	Prazosin/tamsulosin
α_2	Clonidine/Xylazine	Yohimbine/Idazoxan
β_1	Dobutamine	Atenolol
β_2	Salbutamol	Butaxamine

Labetalol – α_1 α_2 β_1 blocker – used for hypertension in pregnancy

Mirabegron – β_3 agonist – relaxes bladder muscle for treating overactive bladder

Ergot alkaloid – ergotamine – partial agonist at α receptors, used in migraine – probably working via 5-HT receptors.

NON ADRENERGIC – NON CHOLINERGIC TRANSMISSION

It has become more accepted over recent years that other substances are important neurotransmitters in the ANS

Cotransmission – many neurones contain and release >1 neurotransmitter, each of which interacts with specific receptors and produces effects

SYMPATHETIC: NA, ATP, Neuropeptide Y

PARASYMPATHETIC: NA, NO, Vasoactive interstitial peptide

There is often differential release depending on impulse pattern.

Purinergic transmission

Adenosine and AMP/ADP/ATP.

P1 receptors: Adenosine>AMP>ADP>ATP (affinity)

P2 receptors: ATP>ADP>AMP>adenosine

P2X receptors – LGIC – ATP

P2Y receptors – GPCR – ATP and ADP

P1 receptor family – GPCR – adenosine (much work on this 'neuromodulator')

Dipyridamole: potentiates adenosine by blocking transport into cells which inactivates adenosine.

Neuropeptides

Large secretory vesicles. Not concentrated in the active zone (usually excluded), hence requirement for generalised increase in Ca in neurone terminal, i.e. high stimulation, in order that the Ca concentration away from the active zone rises above threshold for exocytosis. Exocytosis doesn't occur at the active zone.

Terminate response by diffusion, rather than degradation, and so these neuromodulators often give rise to prolonged responses. These are usually 'neuromodulators' rather than neurotransmitters. Unlike neurotransmitter 1:1 transmission, peptides can diffuse widely and exert effects on neighbouring neurones also.

Often produced as 'preprohormones'. 'pre' sequence directs peptide to ER.

Peptides are poor drugs:

- poorly absorbed orally
- short duration of activity due to rapid degradation *in vivo*
- fails to cross the BBB

Much work is concentrating on developing non-peptide antagonists for peptide receptors.

Nitric oxide

A gas, not stored in vesicles. (Endothelial derived relaxing factor)
Soluble, able to cross membranes.

L-arginine → citrulline + NO

Catalysed by nitric oxide synthase (3 isotypes: 'i' inducible (macrophages), 'n' neuronal and 'e' endothelia and platelets)

NO can diffuse into smooth muscle where it can interact with the haem moiety of soluble guanylyl cyclase: GTP → cGMP. This can then activate PKG leading to muscular relaxation.

[cGMP can be degraded by Type 5 phosphodiesterases. Inhibitor of this is *Sildenafil (Viagra)*.]

NO is inactivated by combination with haem of Hb or oxidation to nitrite and nitrates excreted in urine.

Overproduction of NO can be toxic and can cause neurodegeneration.

Various L-arginine analogues have been generated to inhibit NOS, e.g. L-NMMA, L-NOARG, 7-NI (n-NOS) and L-NIO (i-NOS)

MCQ – True/False – Negatively marked

1. *Intro to ANS*

- a) Sympathetic system involves cranial nerve input
- b) Synaptotagmin is part of the 'core complex'
- c) Tetanus toxin disrupts formation of the core complex

2. *Cholinergic transmission*

- a) Transport of acetyl groups into neurone is the rate limiting step of ACh synthesis
- b) Ganglionic nAChR is sensitive to α -bungarotoxin
- c) M2 muscarinic receptor is coupled to G_s
- d) A muscarinic agonist could lead to raised secretions
- e) Glaucoma is caused by increased intraocular pressure
- f) AChE are serine hydrolases
- g) Irreversible anticholinesterases form carbamoyl-esters with AChE
- h) Myasthenia Gravis is an autoimmune disease

3. *Adrenergic transmission*

- a) Noradrenaline is a catecholamine
- b) Tyrosine hydroxylase is the rate limiting enzyme in biosynthesis of NA
- c) There is no enzyme able to degrade NA in the synapse
- d) Uptake 2 is the primary mechanism employed at the sympathetic synapse
- e) COMT is associated with mitochondria
- f) α_2 receptors are coupled to $G_{i/o}$
- g) β_2 receptors inhibit myosin light chain kinase

4. *NANC transmission*

- a) Neuropeptides are released at the active zone
- b) High stimulation is required to release the neuropeptides
- c) L-citrulline \rightarrow L-arginine + NO

MCQ – True/False – Negatively marked

1. Intro to ANS

- d) Sympathetic system involves cranial nerve input **F**
- e) Synaptotagmin is part of the 'core complex' **F – Calcium sensor**
- f) Tetanus toxin disrupts formation of the core complex **T**

2. Cholinergic transmission

- i) Transport of acetyl groups into neurone is the rate limiting step of ACh synthesis **F – CHOLINE UPTAKE IS LIMITING**
- j) Ganglionic nAChR is sensitive to α -bungarotoxin **F - INSENSITIVE**
- k) M2 muscarinic receptor is coupled to G_s **F – Gi/o**
- l) A muscarinic agonist could lead to raised secretions **T**
- m) Glaucoma is caused by increased intraocular pressure **T**
- n) AChE are serine hydrolases **T**
- o) Irreversible anticholinesterases form carbamoyl-esters with AChE **F**
PHOSPHORYLATE
- p) Myasthenia Gravis is an autoimmune disease **T**

3. Adrenergic transmission

- h) Noradrenaline is a catecholamine **T**
- i) Tyrosine hydroxylase is the rate limiting enzyme in biosynthesis of NA **T**
- j) There is no enzyme able to degrade NA in the synapse **T**
- k) Uptake 2 is the primary mechanism employed at the sympathetic synapse **F**
UPTAKE 1
- l) COMT is associated with mitochondria **F - MAO IS**
- m) α_2 receptors are coupled to $G_{i/o}$ **T**
- n) β_2 receptors inhibit myosin light chain kinase **T**

4. NANC transmission

- d) Neuropeptides are released at the active zone **F**
- e) High stimulation is required to release the neuropeptides **T**
- f) L-citrulline \rightarrow L arginine + NO **F ARGININE \rightarrow CITRULLINE**