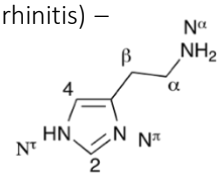
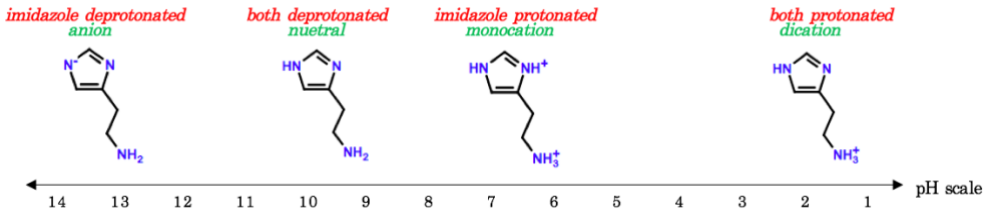
PHAR 3817 – Respiratory

Histamine and Antihistamines

- Histamine (2-4-imidazoylethylaine):
  - Discovered in 1910 –suggested to mediate symptoms of allergy (e.g. wheal, flare, rhinitis) – now known that it doesn't account for *all* symptoms of allergy
  - Formed by decarboxylation reaction of histidine via histidine decarboxylase
  - Secreted from mast cells and some CNS neurons



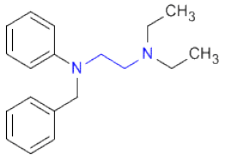
- Allergy = hypersensitivity reaction of antibody class IgE. Allergens bind to IgE, activating mast cells or basophils which release large amounts of histamine.
- Histamine causes inflammation by increasing vasodilation and capillary permeability, smooth muscle contraction, mucus secretion and parasympathetic nerve stimulation
  - Inflammatory responses range from runny nose to anaphylactic shock
- Histamine targets:
  - H_1 receptors: in smooth muscle of intestine, bronchi and blood vessels. Also found unevenly distributed in the brain. Effects of these receptors are blocked by H_1 antagonists.
  - H_2 receptors: mainly in gastric parietal cells (control release of gastric acid) and uterus
  - H_3 receptors: discovered in the CNS when histamine was shown to inhibit its own synthesis and release; probably via presynaptic auto receptors. Histamine also shown to modulate release of other neurotransmitters (e.g. Ach, dopamine, serotonin).
    - Predominantly present in basal ganglia, hippocampus and cortical areas
  - H_4 receptors: widely expressed in components of the immune systems – spleen, thymus and leukocytes. May benefit allergic conditions or treatment of autoimmune diseases (e.g. rheumatoid arthritis).
- Histamine contains a basic imidazole group and a basic amine group, hence exists as a mixture of different ionic and uncharged tautomeric species depending on pH condition
  - At acidic (low) pH, both groups are protonated and the molecule is a di-cation
  - Mono-cationic conjugate species: as pH is increased, the proton from the imidazole ring is lost and an equilibrium is formed. The alkyl ring is protonated and the imidazole ring is neutral. This is the species present at physiological pH (tele-tautomer predominates).
    - Proton must be on the tele nitrogen for transfer and activation of the receptor to occur
  - As pH is further increased, a proton is lost from the alkyl amine and the neutral species is formed, with potential equilibrium existing as well
  - At very basic (high) pH, the imidazole ring is deprotonated and the molecule becomes negatively charged



- Histamine receptors are G-protein-coupled receptors with 7 transmembrane domains. They are histamine inverse agonists with an extracellular and intracellular terminal.
  - Generally, there is an equilibrium between active and inactive receptors
  - The agonist (histamine) shifts equilibrium towards the active state causing the effects of histamine
  - An inverse agonist (antihistamine) shifts equilibrium to the inactive state, meaning histamine will not be able to bind/activate the receptor as easily
- Binding/activation of histamine:
  - Ion-ion bond between positively charged alkyl amine group in histidine and Asp in receptor's TM3 region
  - Imidazole ring approaches TM5 forming H-bonds, VDW and ion-dipole bonds in the region

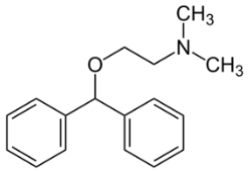
First generation anti-histamines

- Ethylene diamines: first class to be discovered, significant CNS and GI effects
  - Mepyramine - available as sedative and topical antihistamine
  - General structure:



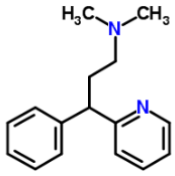
* 2 aromatic rings
* tertiary amine (terminal)
* aliphatic N

- Amino alkyl ethers:
    - Diphenhydramine maleate: anticholinergic, sedative, causes low GI irritation
    - Dimenhydrinate: travel-sickness medication, mixture of two drugs –diphenhydramine and 8-chlorotheophylline
    - Doxylamine succinate: very potent OTC sedative with potent anticholinergic effects
    - Clemastine: anti-pruritic and anti-cholinergic
    - General structure:



* 2 aromatic rings
* tertiary amine (terminal)
* aliphatic O

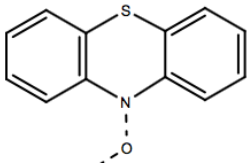
- Amino propyl compounds: mainly used in cold & flu remedies; less sedation and low GI irritation but high incidence of CNS stimulation
    - Include: pheniramine, brompheniramine and chlorpheniramine
    - H_1 anti-histamines with an unsaturated side-chain e.g. triprolidine
    - Basic cyclic chains – antazoline
    - General structure:



* 2 aromatic rings
* tertiary amine (terminal)

→ Unsubstituted pyridine ring of Chlorpheniramine binds to the hydrophobic site on the H_1 receptor. Chemically, the p-chlorophenyl ring cannot bind to the H_1 receptor hydrophobic site as it is sterically restricted and cannot be accessed by a ring with a large substituent such as chlorine.

- Tricyclic antihistamines: structurally related to tricyclic antidepressants and have similar side effects (include cholinergic effects) and sedative effects
    - Two aromatic rings joined together by a six-membered ring that contains sulphur and nitrogen within it
    - Tertiary alkyl amine is protonated at physiological pH
    - Promethazine: anti-cholinergic, sedative and anti-pruritic
        - In potent tricyclic systems, rings A and C are not in the same plane (B ring has a boat shape)
    - Alimemazine/trimeprazine: antipruritic (for eczema/poison ivy), sedative and anti-emetic (motion sickness). Available as syrup, often used with babies and children as a sleep aid.
    - Cyproheptadine: used in allergy, migraine prophylaxis and appetite stimulation (esp. in animals) – antihistamine, anticholinergic and anti-serotonergic
    - Azatadine: anti-pruritic (eczema/poison ivy) and sedative. One aromatic ring in structure contains a N.
    - General structure:



* 2 aromatic rings joined by a six-membered ring
* not on the same plane

- Summary of side effects:

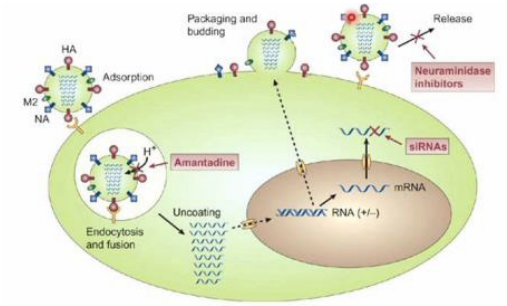
Antihistamine Class	Anticholinergic effects	Sedation	Gastrointestinal	CNS effects
Ethylene diamines	++	+++	++	++
Aminoalkyl ethers	++++	+++	-	+++
Aminopropyl	++	++	-	+++
Tricyclic	+++	+++	-	+++

Antiviral Therapy

- There are two main types of viruses:
  - **DNA-containing viruses** such as herpes virus (shingles, chicken pox), papovavirus (human warts) and poxvirus (smallpox, cowpox)
  - **RNA-containing viruses** such as orthomyxovirus (influenza A, B, C), togavirus (rubella), paramyxovirus (mumps, measles) and retrovirus (HIV)
- Typical infectious cycle of a virus:
  1. Attachment
  2. Penetration
     - Viral envelope contains glycoproteins distinct for different viruses –aids in attachment and penetration
  3. Uncoating –elimination of material (viral information) contained in the virus
  4. Replication
  5. Assembly –formation of new virion
  6. Release –elimination of virion from cell into system
- Antiviral agents/drugs affect:
  - Inhibition or interference of viral attachment to host cell
  - Inhibition of virus associated enzymes
  - Inhibition of transcription/translation processes
  - Interference with viral regulatory proteins
  - Interference with glycosylation, phosphorylation, sulfation etc.
  - Interference with assembly of viral proteins
  - Interference with release of virus from cell surface membrane
- **Endemic** – virus is around all the time (e.g. flu virus) but has **epidemics** (peaks). **Pandemic** – world-wide crisis (no immunity against new strains).
  - Reason for new strains: mutations in surface glycoproteins that change the structure either slightly (drift) or completely (shift)
  - These glycoproteins are hemagglutinin molecules in influenza

<u>Influenza</u>

- Influenza A virus replication:



  - Because it is an RNA virus, it can attach itself to cells in the respiratory tract (via adsorption). Once attached, the virus undergoes endocytosis/fusion due to pH changes in the environment.
  - RNA content (information) is then released into the cytoplasm of the affected cell and enters the nucleus, where it is converted from ssRNA to dsRNA (this can form proteins)
  - Once proteins are formed, information travels to the surface of the cell (budding) and is then released into the system

  * Neuraminidase inhibitors inhibit the release of the new virus
- A series of sugar molecules is present on the surface of influenza A virus –terminal sugar group is sialic acid. Neuraminidase is an enzyme that catalytically cleaves the glycosidic bonds connected to the terminal sialic acid which weakens the membrane and allows release. Hence cleavage facilitates the virus exiting the host cell.
  - Studies show that when virions are released, sialic acid is bound to the NA of the virion
- In the active site of the enzyme, an intermediate/transition state is formed known as DANA that is converted to sialic acid following hydrolysation
  - DANA is a six-membered ring sugar group
  - Contains a carboxylic acid group that forms a salt bridge with 3 Arg groups in active site to stabilise
  - Glycerol group bound in a site surrounded with arginine and glutamate
  - Acetamide group forms a bond with an arginine
  - Hydroxyl group forms ionic bond with aspartate and glutamine

- The first neuraminidase inhibitor –Zanamivir (Relenza), was developed using an understanding of the structure and binding properties of DANA
    - Developed by replacing the 4-OH of DANA –binding to NA increases with 4-amino or 4-guanidine groups
    - 4-amino group binds to Glu 119 via salt bridge
    - 4-guanidino group binds as a salt bridge to Glu 119 and via charge-charge interaction with Glu 227
    - It is an inhaler; inactive if given orally
    - Zanamivir resistance has been rarely reported to date –may be due to similarity to sialic acid (original molecule), hence key amino acids that are vital for binding are unlikely to mutate (some reports of resistance in strains of avian flu H7N9)
- Oseltamivir phosphate (Tamiflu) is another neuraminidase inhibitor administered as a prodrug. X-ray crystallographic studies of NA revealed an additional binding site; a hydrophobic region (containing: Glu, Ala, Arg, Iso) that binds to the C6 hydrophobic group
    - Glycerol is replaced with a 3-pentyloxy side chain
    - Needs to be hydrolysed to the active form –none of the metabolic products are active
    - Oseltamivir resistant strains of H1N1 and H2N3 exist; large number of patients might become oseltamivir-resistant as a result of oseltamivir use. This occurs due to change/mutation in enzymes from year to year –hence, molecules will bind differently.
- RWJ-270201 is a NA inhibitor in Phase III of clinical trials. Contains a cyclopentane core structure (5-membered ring). It is active against all known influenza A and B strains both in vitro and in vivo.
    - The 5-membered ring structure is positioned differently to 6-membered rings of other inhibitors, however:
        - The 4 key functional groups have similar active site interactions
        - Potency is therefore dependant on positioning of functional groups and not the ring
    - Superimposition of 5-membered compound against DANA shows the same substituents as DANA and sialic acid; however, the arrangements are different
        - Potency is comparable to DANA against influenza A