Amino acids as drug targets.

Learning Objectives:

☐ To appreciate how many amino acids are modified to produce hormones and neurotransmitters
☐ To give examples; catecholamines, thyroxin, serotonin, histamine, GABA
☐ To gain some understanding of drug target strategies; inhibitors of reuptake and inactivation, receptor antagonists/agonists, fluorine substitutions, suicide inhibitors

In the first lecture in this series I would like to start by considering the monomers of those wonderful information containing biopolymers, DNA, RNA and protein, the nucleic acids and amino acids. We will focus on these from the viewpoint of drug design rather than straight biochemistry. In particular I would like to start with some famous drug targets which are derivatives of amino acids. These candidates are adrenalin or epinephrine, noradrenalin/norepinephrine, serotonin, GABA or γ amino butyrate, thyroxin and histamine.

From a pharmacological point of view you don’t get more dramatic than adrenalin. Let’s consider its structure and how it is made. Adrenalin is one of a number of catecholamines made from tyrosine. This family also includes such famous members as L-Dopa, dopamine and noradrenalin (norepinephrin). The best part of this scheme is they are all on the same pathway; each is a step in the synthesis of adrenalin from tyrosine. This pathway occurs both in sympathetic neurons and the adrenal glands and the products act as neurotransmitters and hormones respectively. The two pools are kept independent thanks to the blood brain barrier which prevents all but the most hydrophobic molecules from crossing to the brain.

You will recall (I am sure) that tyrosine is the amino acid with the aromatic side chain and the –OH?? In case it has slipped your mind since MBLG1001 here it is:
The tyrosine is hydroxylated by, surprisingly, tyrosine hydroxylase, which is by the way, the rate limiting step. The product, L-Dopa (dihydroxyphenylalanine) is then decarboxylated to produce Dopamine, hydroxylated to give noradrenalin and methylated to give adrenalin. The whole big happy catecholamine family are there in one pathway. Each of these family members is important to the pharmacist: L-Dopa or levadopa is the most commonly prescribed treatment for Parkinson’s disease. Dopamine and Noradrenalin are neurotransmitters and adrenalin is everything!

Adrenalin and noradrenalin, both produced in the adrenal gland (adrenal medulla which is considered to be an extension of the sympathetic nervous system) are stored in granules and released into the circulation under the action of various stimuli from the sympathetic nervous system (flight or fight) where they bind to a class of receptors. These adrenergic receptors are glycoproteins which span the plasma membrane are known as G-coupled proteins and, once a ligand is bound, elicit all sorts of responses. Many of the responses are very short term and involve activating pre-existing enzymes. There are 4 adrenergic receptors classified as alpha and beta with subclasses (α1, α2, β1 and β2) and these are located in different tissues in mammals. These receptors respond differently to catecholamines. The alpha receptors stimulate smooth muscle contraction in peripheral organs via inhibition of adenyl cyclase whereas the beta receptors activate adenyl cyclase to mobilise fuels, relax smooth muscles of the bronchi and blood vessels supplying skeletal muscles and increase heart rate. The end result of these actions is to mobilise and shunt energy reserves to where they are most needed, prepare for action!

Many types of drugs are designed to block or activate one or more of these receptors (the receptors themselves have been identified by their response to various agonists and antagonists). Because it is a vasoconstrictor and increases heart rate adrenalin is most often used in cardiac arrest and anaphylactic reactions (also it has immunosuppressant properties). The bronchodilator properties make it useful in treatment of asthma.
Noradrenalin is a neurotransmitter uniquely found between the junctions of sympathetic neurons and smooth muscle cells. Noradrenalin is also involved in synaptic responses and it is here that the plot thickens. Decreased levels of noradrenalin in the brain are associated with some forms of clinical depression and many antidepressant drugs are designed to increase the levels in the brain; either by inhibiting its inactivation or its reuptake. Briefly noradrenalin is inactivated by 2 processes, catechol-O-methyl transferases (COMTs) which methylate one of the –OHs on the aromatic ring, or by monamine oxidases (MAOs) which oxidatively deaminate replacing the amino group with a double bonded O producing an aldehyde. The COMTs work to knock off the noradrenalin in the synaptic cleft while the MAOs, which live on the outer surface of mitochondia in the neurons, will inactivate the noradrenalin once it has been taken back up by the neurons. Many antidepressant medications act as inhibitors of these enzymes. The MAO inhibitors include tranylcypromine (Parnate) and Nardil. Another class of antidepressants, tricyclics of which desipramine is an example, work to inhibit the reuptake of noradrenalin and other neurotransmitters from the synapse cavity, hence you get prolonged stimulation of the receptors on the post synaptic neuron. This is one of the sites of action of cocaine also.

From a pharmaceutical point of view the intermediates further down the pathway; Dopamine and L-Dopa, are also fascinating for it is these neurotransmitters and their respective neurons that feature in Parkinson’s disease and schizophrenia. Parkinson’s disease is characterised by degeneration of dopaminergic neurons (pre-synaptic neurons that produce and release dopamine) while the psychotic symptoms of schizophrenia result from excess dopamine production and/or heightened receptor sensitivity. These dopamine releasing and responding neurons have a fairly specific location in 2 regions of the brain stem. The common treatment for Parkinson’s disease is L-Dopa, marketed as levadopa. This works to elevate dopamine levels. Essentially L-Dopa is administered because it is can cross the blood brain barrier where dopamine can’t. Once in the CNS it is rapidly converted to dopamine and thus relieves many of the Parkinsonian symptoms. It is often administered with a peripheral decarboxylase inhibitor to stop the conversion of Dopa to Dopamine before it gets to the blood brain barrier.

The rate limiting enzyme, tyrosine hydroxylase, sounds an interesting target. Alternative splicing produces 3 transcripts hence 3 isoforms; a, b and c. They vary in exon 1, isoform a being the longest. However the major and immediate regulation of adrenalin and its other family members is much more focused on release and inactivation. It really is beyond the scope of this lecture to go further into the whole story but rest assured you will revisit it later in far more detail.

The reaction catalysed by tyrosine hydroxylase is tricky and not commonly performed in life. It requires incorporating a –OH into an aromatic ring, which is a very tight-knit little community of atoms that is hard to break into. The reaction requires O₂ and biopterin (structures all shown in slides). One of the O atoms from the O₂ molecule goes to form the –OH and the other goes onto the biopterin. The biopterin is in its reduced form when it reacts and it donates the 2 Hs. The-OH on the biopterin then leaves as water.
Another notable example of this type of reaction, which I feel is my duty to tell you about is the formation of tyrosine from phenylalanine. Phenylalanine has an aromatic ring in its sidechain but not –OH. The enzyme phenylalanine hydroxylase breaks into the ring and puts the –OH in by the same reaction. This means that, although tyrosine is vital to our health it is not an essential amino acid (meaning we have to eat it to survive). We can make it from phenylalanine and this is the way we dispose of excess phenylalanine. A famous genetic disorder, phenylketonuria (PKU) is the result of a mutation in phenylalanine hydroxylase. The excess Phe is shunted off to a minor deamination, producing phenylpyruvate. This would be fine except phenylpyruvate causes severe mental retardation when developing brains are exposed to too much of it. The disorder must be picked very soon after birth so babies can be put onto a low Phe diet. The screening test carried out in Australia, the Guthrie’s test, is carried out before the baby goes home from hospital. Sufferers cannot drink diet Coke or any foods containing aspartame (NutraSweet) which is a dipeptide of aspartate and phenylalanine.

While we are on tyrosine (and I have the template in Chemdraw) let’s consider the other tyrosine derivative, thyroxine and its related hormone triiodothyronine (one less iodine), the hormones produced by the thyroid gland. This actually takes 2 tyrosines to make and is iodinated in 3 or 4 spots (T3 and T4). The hormones are synthesised on a protein, thyroglobulin, where some 20% of the tyrosines are iodinated and 2 will oxidatively couple. The thyroglobulin is digested in the lysosome yielding 5 to 6 T3/T4 molecules. This proteolytic digestion is stimulated by hormonal stimulation of the thyroid by TSH (thyroid stimulating hormone). Hypothyroidism and hyperthyroidism are common conditions; hypo producing lethargy, obesity and cold skin, while hyper has the opposite presentation. Low iodine in the diet, often resulting from low iodine in the soil leads to hypothyroidism and a goiter (enlarged thyroid
gland). Thyroid hormone is essential to growing mammals and a deficiency during development leads to cretinism.

![Thyroid hormone structure](image1)

Serotonin is a derivative of tryptophan, one of the more ugly amino acids to draw. Serotonin is a neurotransmitter and low levels of it have also been linked to depression. Prozac acts to inhibit the degradation of serotonin.

![Tryptophan and Serotonin structures](image2)

Another important amino acid derivative from a pharmacists point of view is histamine. The name would imply some relationship to histidine, actually a decarboxylation of histidine. Histamine is produced by mast cells as the allergic response and most allergy treatments contain antihistamines. Histamine is also a neurotransmitter, involved in sleep regulation and the control of acid secretions in the stomach. No wonder antihistamines make you drowsy.
Neurotransmitters are always going to be important in drug design because many of the disorders we face have a neuro component. Apart from the obvious acetyl choline there are an ever growing list of neurotransmitters (this is a field that is just getting bigger). One candidate that has been proven and has been around for a long time is GABA or γ amino butyrate. GABA is synthesised from the decarboxylation of glutamate and is the major inhibitory neurotransmitter. Some 30% synapses release GABA.