Aspects of Human Sensory and Metabolic Biochemistry

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Published: 8 February 2015

MODULE SYNOPSIS

This module introduces students to selected examples of inborn errors of metabolism, the, physiological consequences of those errors and their management. The biochemistry and physiology of visual transduction, olfaction and sensory biochemistry is covered. Aspects of central nervous system biochemistry such as the provision of energy for the brain, the metabolism of neurotransmitters and the role of neurotransmitters in controlling behaviour is also considered.

LEARNING OUTCOMES

At the end of this module students should be able to:
demonstrate a detailed knowledge of the biochemistry underlying the process of visual transduction,
critically appreciate the inter-relationship between sensory physiology and biochemistry,
demonstrate an understanding of the peculiarities of the central nervous system biochemistry,
demonstrate an understanding of the concept of inborn errors of metabolism and their consequences,
demonstrate an integrated knowledge of biochemical pathways and their relevance to human health.

Chapter 1 (Inborn errors of metabolism)

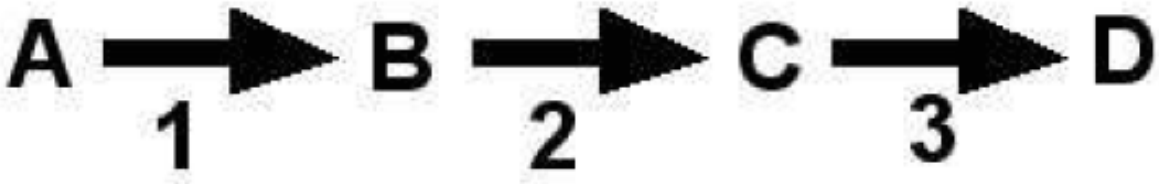
This chapter includes

- Inborn errors of metabolism
- tyrosinemias

Inborn Errors of Metabolism

We start of this module by looking at the inborn errors of metabolism also known as inherited metabolic diseases in congenital metabolic diseases. As the names suggest these are congenital conditions meaning that present from birth. They are genetic defects that result in an enzyme which is not functional. They may be partially functional as a result of this aberrant of the biochemistry, meaning that the normal metabolism that follows on from that enzyme tends to be affected. Depending upon on what the enzyme is and how its affected then the effects of the inborn error can be fairly minor and possible cause no problems what so ever on the other hand they could impact the life of the affect to a point where survival is unlikely. So to begin with lets look at what we mean by these inborn errors of metabolism.

If you have a metabolic pathway where A goes to B goes to C goes to D with enzymes 1,2 and 3 catalysing them.



Then you can sort of invasive whats going to happen. If you take away enzyme 2 then compound B is going to accumulate and so will compound A to some extent. Those things are going to build up in the body an then there may be consequences of that. Similarly Compounds C and D will become depleted so there are less of them in the body and that can have consequences. Sometimes things get a bit more complicated than that, if the enzyme is blocked then the same things build up but sometimes there are other pathways that metabolism the things that build up. So some enzymes get shuffled off in to another pathway and could lead to build ups in other pathways which can have consequences. The main consequences of damaged enzymes is the build up of some compounds upstream and the depletion of their compounds downstream. Some of the best understood inborn errors of metabolism are actually found in the pathways of amino acid metabolism, particularly the pathways by which the amino acids are broken down in the body. Some of the best known of these inborn errors tend to be amino acid catabolism the methods by which amino acids are broken down). Perhaps one of the best known of the lot is a condition known as **phenylketonuria**

Phenylketonuria

Phenylketonuria is caused by an *absence or deficiency of phenylalanine hydroxylase* or, more rarely, of its tetrahydrobiopterin cofactor. *Phenylalanine accumulates in all body fluids because it cannot be*

converted into tyrosine. Normally, three-quarters of the phenylalanine is converted into tyrosine, and the other quarter becomes incorporated into proteins. Because the major outflow pathway is blocked in **phenylketonuria**, the blood level of phenylalanine is typically at least 20-fold as high as in normal people. Minor fates of phenylalanine in normal people, such as the formation of phenylpyruvate, become major fates in phenylketonurics.

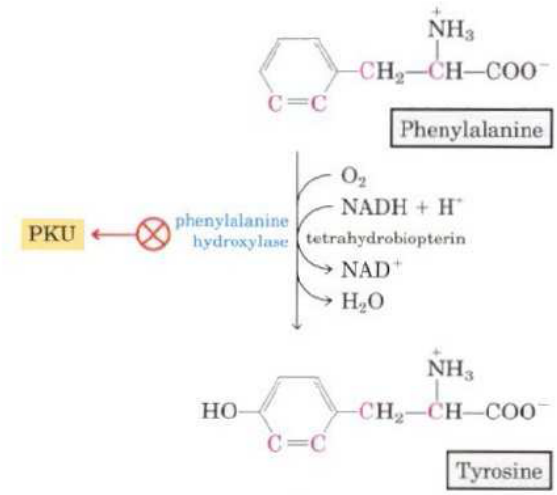
Indeed, the initial description of **phenylketonuria** in 1934 was made by observing the reaction of phenylpyruvate with $FeCl_3$, which turns the urine olive green. *Almost all untreated phenylketonurics are severely mentally retarded.* In fact, about 1% of patients in mental institutions have **phenylketonuria**. The brain weight of these people is below normal, myelination of their nerves is defective, and their reflexes are hyperactive. The life expectancy of untreated phenylketonurics is drastically shortened. Half are dead by age 20 and three-quarters by age 30. *The biochemical basis of their mental retardation is an enigma.*

Phenylketonurics appear normal at birth, but are severely defective by age 1 if untreated. The therapy for **phenylketonuria** is a *low phenylalanine diet.* The aim is to provide just enough phenylalanine to meet the needs for growth and replacement. Proteins that have a low content of phenylalanine, such as casein from milk, are hydrolyzed and phenylalanine is removed by adsorption. A low phenylalanine diet must be started very soon after birth to prevent irreversible brain damage. In one study, the average IQ of phenylketonurics treated within a few weeks after birth was 93; a control group treated starting at age 1 had an average IQ of 53.

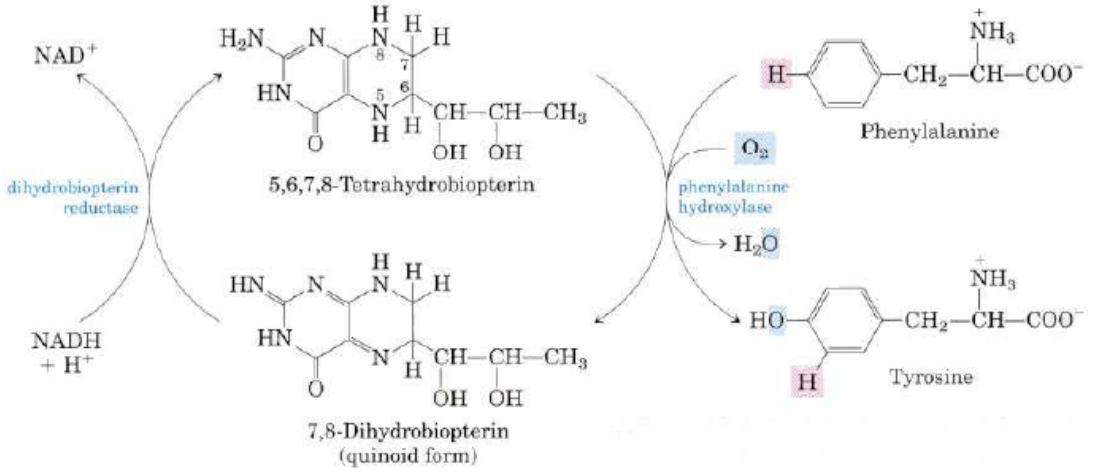
Early diagnosis of **phenylketonuria** is essential and has been accomplished by mass screening programs. The phenylalanine level in the blood is the preferred diagnostic criterion because it is more sensitive and reliable than the $FeCl_3$ test. Prenatal diagnosis of **phenylketonuria** with DNA probes has become feasible because the gene has been cloned and many mutations have been pinpointed to sites in the protein (see Figure 23.31). Interestingly, whereas some mutations affect the activity of the enzyme, others do not affect the activity itself but, instead, decrease the enzyme concentration. These mutations lead to degradation of the enzyme, at least in part by the ubiquitin-proteasome pathway.

The incidence of **phenylketonuria** is about 1 in 20,000 newborns. The disease is inherited in an *autosomal recessive* manner. Heterozygotes, who make up about 1.5% of a typical population, appear normal. Carriers of the **phenylketonuria** gene have a reduced level of phenylalanine hydroxylase, as indicated by an increased level of phenylalanine in the blood. However, this criterion is not absolute, because the blood levels of phenylalanine in carriers and normal people overlap to some extent. The measurement of the kinetics of the disappearance of intravenously administered phenylalanine is a more definitive test for the carrier state. It should be noted that a high blood level of phenylalanine in a pregnant woman can result in abnormal development of the fetus. This is a striking example of maternal-fetal relationships at the molecular level. Table 23.3 lists some other diseases of amino acid metabolism.

PKU is caused by an absence or defect in the enzyme Phenylalanine hydrolase normally metabolises the amino acid phenylalanine to tyrosine. If you lack that enzyme then you get the condition known as phenylketonuria (PKU). About three quarters of the phenylalanine normally ends up getting converted in to tyrosine and the other quarter ends up in proteins. People that suffer from PKU have a blood level of phenylalanine tends to be 20times higher than you would normally seen. So blocking of the pathway builds up Phenylalanine and tyrosine becomes depleted. The enzyme is quite a complicated enzyme in what it dose its one of the general class of enzymes known as mix function oxidases. They all catalyse this type of reaction. Basically its the

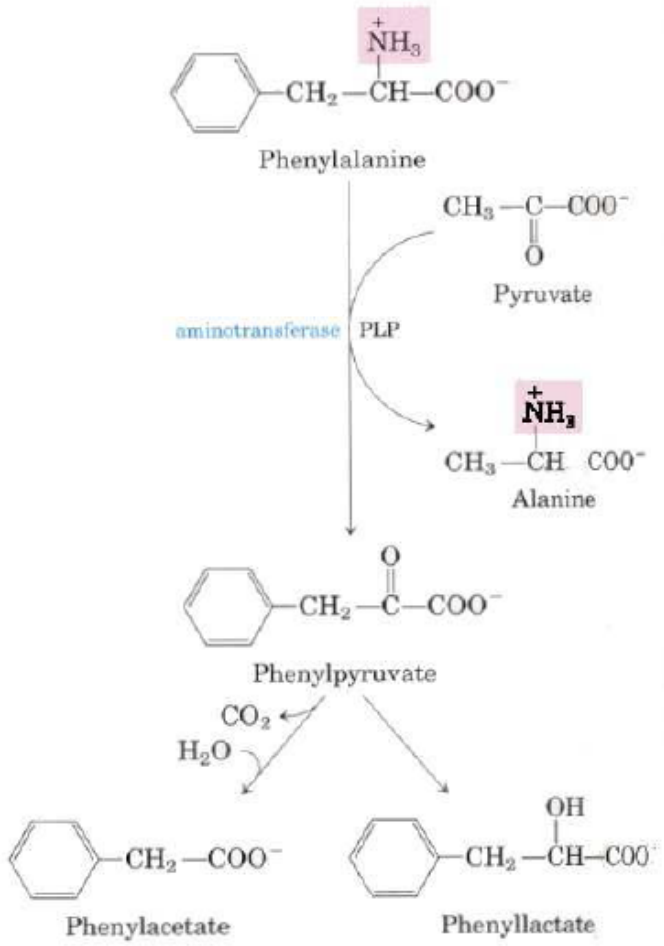


hydroxylation of the substrate that were doing by sticking hydroxyl group on the end. So the catalysing hydroxylation in the substrate by an oxygen atom derived from molecular oxygen and reduction of the other oxygen to water. So its a mixed function oxidase that chatelaines that kind of reaction is the hydroxylation of some sort of substrate and thats linked too the use of one oxygen going into the reaction and the presence of another oxygen in the water molecule. Now phylalanine hydroxyls requires another co-factor called tetrahydrobiopterin. Tetrahydrobiopterin is basically an electron carrier it carries electrons form the NADH through to the oxygen. Tetrahydrobiopterin actually links the NADH to the oxygen.



If we look at that stage of the pathway in a bit more detail this is whats actually going on. In the right side reaction is the phenylalanine hydroxylase reaction and the phenylalanine is converted to tyrosine with the hydroxylation happening on the left hand side of the molecuel. but this is where the tetrahydrobiopterin comes into play. Basically whats happening is tetrahydrobiopterin is being converted to 7, 8-Dihydrbiopterin by being oxidised. Then the dihydrobiopterin is converted back to tertahydrobiopterin by an enzyme called dihydrobiopterin reductase which is where the NADH comes in to the reaction. In this reaction there is something that presents asa little unusual in that there is a hydrogen atom with a pink background thats transferred directly from the C4 position to the C3. This is a rather peculiar chemistry reaction because your not simply putting a hydrogen onto the C4 hydrogen that hydrogen is actually moving and the -OH comes in as a new molecule which is a rather usual reaction thats being catalysed. We saw in the outset that the root cause of the phenylketonuria is a defect in the enzyme called phenylalanine hydroxlase but if you think about is if theres a problem with the reductase as well then it would stop the NADH reaction from occurring and would thus stop the activity of the hydroxlase. So we learn that phenylketonuria can occur from a defect in the hydroxylase but also form the dihyrodbiopterin reductase. So its a fairly simple reaction to generate tyrosine but if there are defects then you get a build up of phenylalanine in the blood but as well as the increased levels of phenylalanine you get in the blood. you also find you get increased levels of metabolites. When you get a build up of compounds in the body sometimes those compounds get shunted off in to other pathways where there normally not very reactive then other things start to build up as well. In this particular instance this is what tends to happen the phenylalanine can find its way through other relatively minor metabolic pathways which are then activated by the increased concentration of the phenylalanine and in one of those pathways the phenylalanine can undergo transamination with the amino transferase as seen here in the diagram below.

This transformation involves private and then you get the formation of alanine and this generates in turn this compound called phenylpyruvate. High concentrations of phenylalanine cause the activation of this aminotransferase and cause the transfer group from pyruvate to alanine and generating this compound phenylpruvate. Its actually the accumulation of that compound as well as the phenylalanine that gives the name phenylketonuria because the phenylpruvate is a ket acid. These compounds then start to show up in the urine and in fact most of the excess phenylalanine formed in the body isn't entreated as phenylalanine directly it tends to be converted to things like phenylpuruvate and then is excreted. The phenylpyruvate can its self undergo further metabolism to get these compounds called phenyl acetate and phenyl lactate. The build up then of phylalanine tends to creat the build up of other things in different pathways one of which is the decarboxylation that forms phenyl acetate or it can be reduced to form the phenyl lactate but then these things again end up in the urine. phenyl acetate in aprticulartend to cause a characteristic smell in the urine sort of like a mousy smell that you get from people affected by this sort of thing. This traditionally was used in the ways phenylketonuria was detected in new born babies where experienced midwifes could detect the smell of this substance and sujested the condition of phenylketonuria. The premise of this stuff as well the phenylpyruvate also the basis of one of the classic tests for the disease if you mix ferric chloride with a sample of urine from an affected child then it reacts with the phenylpyrivate and gives a characteristicc olive green colouration and that was the classic way in which phenylketonuria was diagnosed. Today its a bit more sophisticated about it and basically all new born children are subject to a blogod test for these sorts of things where a pin prick of the heel is done.



Phenylketonuria then is amongst the first of the metabolic diseases to be recognised its quite a classic one in that sense. It was discovered back in 1934 when people first recognised what it was. Phenylketonurics appear normal at birth and gain thats not surprising when you have an unborn child ands its metabolism is basically is covered by its mothers metabolism so the child with such a defect is building up phenyalanine then the mothers metabolism takes that away and the unborn child is fine. Its only when the child is born and this system is taken away that the independence takes over and these problems begin to arise. So if we look at pphenylketonurics and the appear to be normal at birth but if the condition is left untreated they become severely mentally retarded and there IQ drops off quite dramatically especially if you define the brain weight it is below normal and they have smallish brains. The melioration of there nerves is defective and this accounts for the low brain weight. The myelin sheath is often defective in phenylketonurics and there reflects are quite hyperactive and they tend to be so to jittery. The life expectancy of an untreated phenylketonuric is also dramatically reduced. Typically half of them will die by the age of 20 and the remains half by the age of 30. Exactly why this condition should lead to metal retardation is unclear. People don't understand why this build up of phenylalanine can

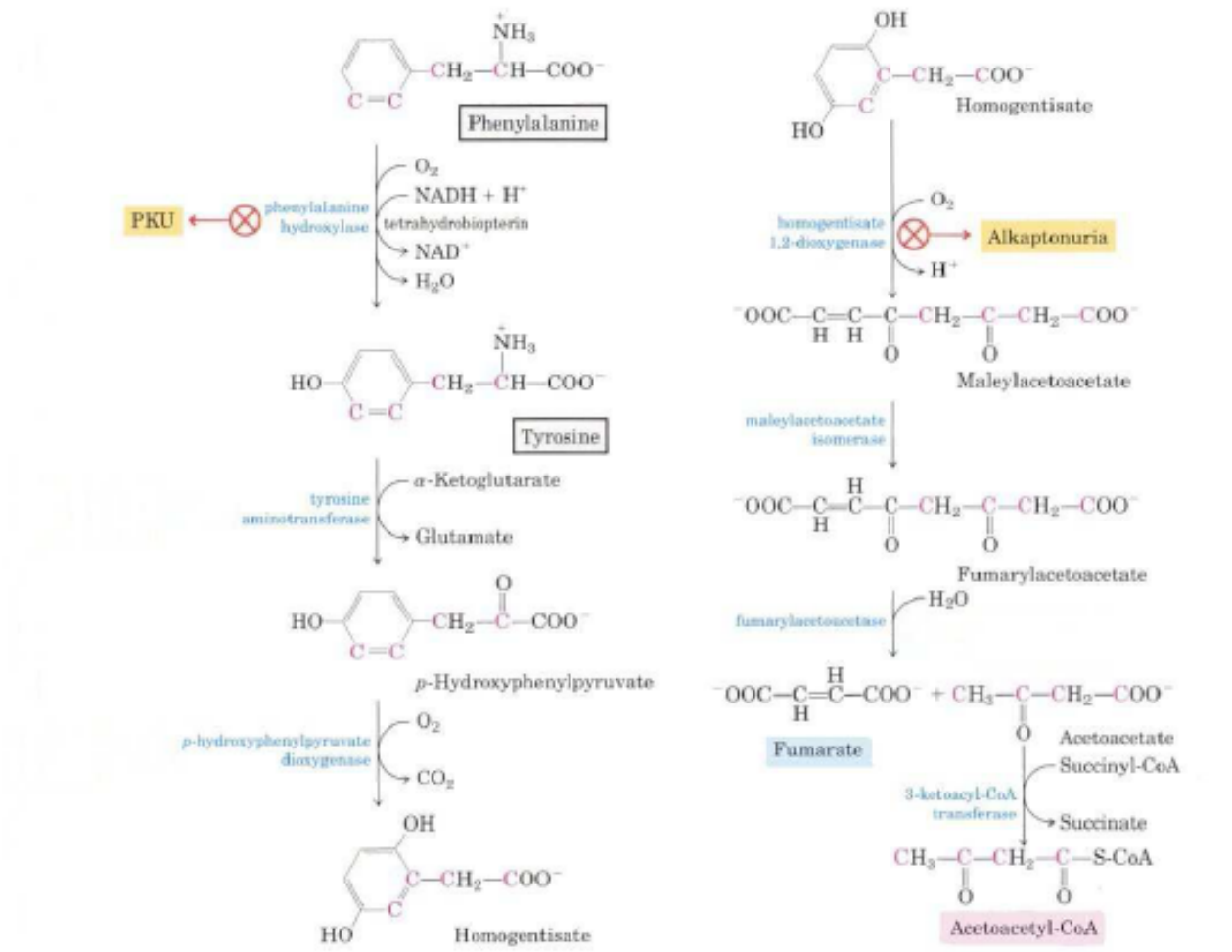
cause metal retardation. one of the ideas is that the high concentrations of phenylalanine compete with other amino acids to get into the brain and it may affect the transport of other amino cids in to the brain as well. This affect then in turn affects the development of the brian.

Its also been sujested that theres an enzyme called pyruvate carboxylase thats one of the important enzymes in the brain. This enzyme is inhibited by high concentrations of pheylpyruvic acid. So this may be why people with this condition have a metal retardation. Anothe rpaculiar aspect of the condition as well is that because the normal product produces from phenylalanine is tyrosine you have lower levels of tyrosine in the body and tyrosine is the precursor of a number of sin pigments so you tend to find that due to the lack of tyros there is a lack of pigment in the skin and phenylketonurics tend to be quite fair skinned with quite a pale complexion. There are then a number of spin off effects from having this defect as well. So its a fairly sever condition but if its recognised early in infancyy then it can be treated the metal retardation we see can be largely prevented by rigid dietary control. The condition is obviously caused by a build up of phenylalanine so if you can stop the person ingesting phenylalanine then you can aleviate some of the conditions. The diet of these people has to be controled to supply just enough phenylalanine and tyrosine for normal development in the needs of protein synthesis but not enough that you get a build up of the stuff and you get problems. Basically what people do is they are given a protein free diet but the proteins they are alone to have are ones that are naturally low phenylalanine are caciun for example form cows milk for example has a naturally low presence of phenylalanine and those sorts of proteins are taken hydrolysed and then the excess phylalenine removed. these people can be given these protein supplements that are low in phenylalenin and this helps prevent some of the worst affects of the disease.

We have a couple of figures here that give us an idea of the benefits of this where the IQ of phenylketonurics given a low phenylalanine diet form within a few weeks of birth was about 93 so not a lot lower than the average of 100. However another group who were not treated with a low phenylalanine diet until hey were about the average age of 1 have an average IQ of 53. So you can see the first year was enough to cause the decrease in IQ because they didn't have this low phenylalanine diet from an early age. Its also interesting in this respect actually that theres another compound that we find quite widely in the food myth days called aspartame. Aspartame is an artificial sweetner and it crops up in quite a lot of fizzy drinks and stuff like that but aspartame is actually a dipeptide of aspartate of phenylalanine. So although its a sweetener its not actual a sugar its not a carbohydrate its actually a dipeptide and it forms from aspartate and phenylalanine and of course because of that its a source of phenylalanine in the diet. Therefor phenylketonurics have to avoid the food stuffs that have been arfcially sweetened with things like aspartame as well.

So this is how you classically treat phenylketonuria but we noticed ealrer on that theres another form of phenylketonuria cause by a defect in the enzyme called the reductase which is the enzyme that regenerated the tetrahydrobiopterin co factor. Treatment of these types of phenylketonurics is a bit more complex because this simple dietary restriction is not enough. Yes you need to restrict the intake of phylalanine but you have to some how have to overcome the defect in the dihyrdobiopterin reductase. Basically tetrahydrobiopterin is required for the formation of another number of compounds as well. Theres a compound called L-Dopa which is par to the metabolic pathways in dopamine metabolisem and that compound is need for the synthesis of dopamine and also 5-hydroxytryptophan. This is also another neurotransmitter we will see later on. Because of the defect in that enzyme “dihyrobiopterin reductase” these other compoundds are also reduced. So basically these things have to be supplemented int eh diet as wellwhere they need to be given to the people suffering from this disease. You may say why not give them dihydrobiopterin and you cant simply do that because its quite unstable and also it docent get into the brain if given int he diet.

So how common is it? Well the incidente of phenylketonuria is about 1 in 20,000 newborns. Its inherited in an autosomal recessive fashion as to say the affected gene that located on chromosome 12 it is a recessive trait where you need to have two parents come together and about 1/4 of there children will be affected. Its recommend that the heterozygouse individual, so those who have a single defective gene, are representative of about 1.5% of the population who are supposedly carriers of the gene for phenylketonuria. however the heterozygotes appear normal. Carriers of the defective gene tend to have slightly higher concentrations of phenylalanien in trebled but not enough to cause problems. Unless your tested for it you wouldn't know you've got it.

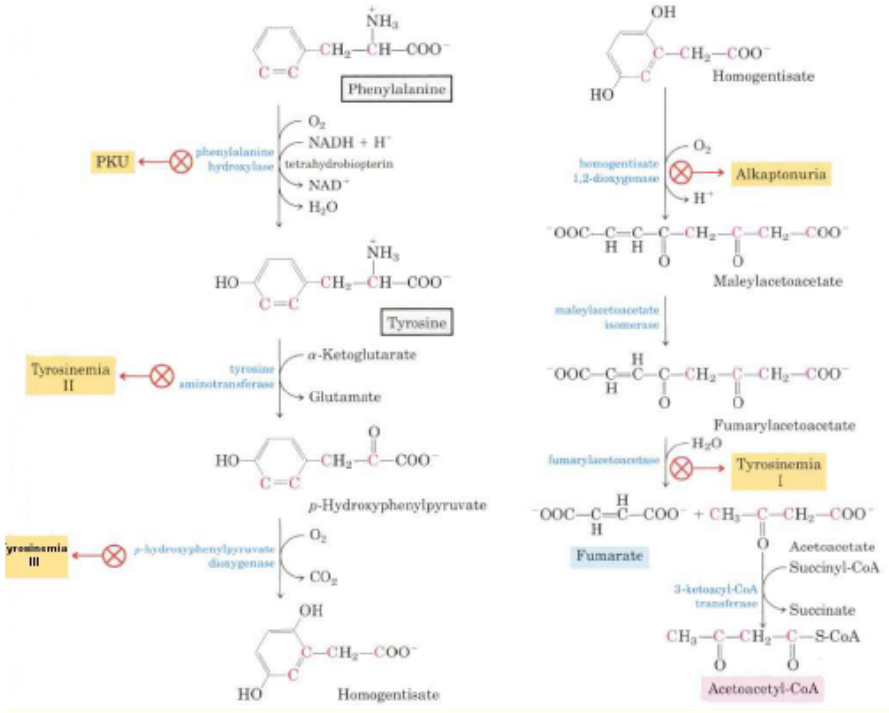


Tyrosinemias

If we return to the metabolic pathway that follows on form that defective enzyme then what you see initially is the phenylalanine hydroxyls which when defective gives rise to PKU but you might say what about these other enzymes. Well there can be defects in these further enzymes and this one called homogentisate 1,2-dioxygenase is another enzyme that can be defective in this pathway and if you suffer from a defect in that particular enzyme then it gives rise to a condition called Alkapeonuria seen with the shaded yellow background. Alkapeonuria has certain historic importance in that its the first condition that was discovered to be genetic in origin. Theres a chap called Archibald Garrod who recognised, way back in 1902, that alkaptonuria is transmitted as a single reserve medialian trait. as a sort of a genetic disorder that was alter tracted down to that particular enzyme. This was the first time a condition had been traced

down to a genetic condition so its historic in that sense. Alkapeonuria is actually a less serious condition than phenylketonuria and it produces relatively few ill effects from that enzyme. Although saying that people with alkaptonuria are prone to developing arthritis in there later years. As you might predict form the pathway the first word alkaptonuria produced quite high levels of homogentisate that tends to build up. This compound then homogentisate spills over into the urine and is secreated. One of the peculiar things about homogenitsate is that it turns black on standing so the homogentisate in the urine tends to darken. This was then discovered that homogenitsate was oxidised and polymerised to a pigmented substance. This also explains the alternative name for the disease for alkatonuria which is black urine disease. The indcidence rate of alkaptonuria is around 1 in 250,000. The defective gene has been localised to chromosome number 3. So again its quite well understood what the defect is there. children born with that particular condition again are largely asymptomatic they dont have and particular deficiencies or defects apart form this black coloured urine. which is fairly obvious in the nappies of these children. normally it progressess to couse pain in the spine, knees, hips and other various joints and this is because of a prevalence of arthritis later in life. Some times this pain could lead to the need for a replacement joint so its not a life threateningg condition it can be quite debilitating. The problems seem to be caused by the homogentisic acid attaching itself to the collagen in the joints. We know that collagens important for the material that forms these kinds of tendons and stuff in the joints. That stuff seems to attach to the collagen and thats what causes some of the problems of the arthritis. Becausee it causes this kind of dark pigment as well then it leaves the joints with quite a dark colour called orchronosis. as seen form the pictures tot he right. this is the term given to this collagen that has had this pigmentation forming. This happens in a number of ways patients have these dark spots forming in the eyes where there is a blue black discolouration the ears can start to darken. You can get deposits int he hands and discolouration and even more remarkablele are these joints like the knee and elbow joints where you can see quite clearly the discolouration and pigmentation. This is caused by the attachment of homogeniseic acids to the collagen. So thats another one of the conditions that we can get by the defects in metabolism but what about the others. So we have have looked at phenylketonuria and alkocaptanuria but we have other enzymes as well and they also can suffer form defects and collectively these conditions are known as tyroinemias. So the classic one is called PKU and the alkatonuria are the two most common ones but there are others and they tend to be called tyrosenimai 1, 2 and 3 depending on which of the other enzymes are defective. These other tyrosinamias are much rarer and less common conditions.

Tyrosinemai 1 seen on the right of the digram is perhaps the best charecterised of the three conditions or the more common of the three and patients with this particular condition have a defect in the enzyme Fumarylactoseacetase and



they have a blockage in this step of the pathway leading to the build up of fumarylactoseacetate patients with this condition have a slightly strange cabbage type smell around them caused by the build up of these compounds that tends to manifest themselves in a variety of ways. However this smell is the least of the worries with this condition. Its quite a serious condition and typically people who have this condition tend to suffer from liver disease as well as there kidneys and they typically don't survive much more than a year after birth so its quite a severe condition. Basically if born with this condition you will suffer liver failure in the first few years of life and they also have the increased propensity to develop hepatic carcinoma (Cancer of the liver) caused by this build up.

Tyrosinemia I
(Hereditary Infantile Tyrosinemia)

Characterised by a rapid onset of liver failure in first few months of life.

Symptoms of the disease believed to be caused by the build-up of succinylacetone formed by the decarboxylation of succinylacetoacetate, which is itself formed from the catabolic intermediate fumarylacetoacetate.

Treatment options are limited but consist of;

1) Dietary intervention to limit the intake of phenylalanine and tyrosine.

2) Metabolic inhibition of the proximal tyrosine pathway using the drug, 2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3-cyclohexanedione (NTBC or Nitisinone). This compound inhibits the enzyme, 4-hydroxyphenylpyruvate dioxygenase so blocking the pathway at the step before the production of homogentisate.

3) Liver transplantation.

People don't really know what the biochemical basis of this was until the 1970's until they discovered that people suffering from this condition had a build up of something called succinylacetone in the urine. so where has this succinylacetone come form? Well it turns out that succinylacetone is a decarboxylation of the succinylacetoacetate. This in itself is derived from fumarylacetoacetate. So you start off with fumaryacetoacetate which forms succinylacetoacetate and that in turn is converted to succinylacetone and thats what ends up in the urine. Many of the symptoms of that disease the liver cancer and kidney fauiler and so on are believed to be caused by the succinylacetone which is a toxic compound that causes liver and kidney damage. It has been shown that succinylacetone is actually a mitochondrial toxin that affects quite a few metabolic pathways and processes. It affects things like membrane transport from the kidney it affect substrate phosphrilation in the kerbs cycle. So the succinylacetone seems to be the culprit in many of the symptoms of the disease. The condition itself is rarer than the others like PKU. Typically 1 in 100,000 to 1 in 120,000 children may be born with this condition. Its inherited as autosomal

recessive mendilian trait. We say these are the general figures but there are certain populations int he world where these incidences ar much higher and particularly in populations that are a little bit inbred. For example we are told that this particular condition tryosenemia 1 we get much higher incidentce in places like Norway and Finland where the incidence rate is around 1 in 60,000. So you say that you do sometimes find these increased incidence in certain population in the world. Apparently the highest incidence occurs in a country need quebec where about 1 in 2,000 live births is affected by this tyrosinemia 1. so again this is remainsnt of populations that don't mix much. It has been predicted that about 1 in 25 in this region of quebec is a carrier of this condition. So in the wider world its not a very common disease but in certain populations its much higher. The defective gene for this condition has been localized to chromosome 50 and there are about 30 different mutations that characterise the disease. That again leads onto another factor that leads on from this where we tend to think affected gene affected enzyme and then disease. Of course however though within the gene there can be different genetic mutations and the enzymes thats produced ay have some function so in some individuals you can have an almost normal functiuon of the enzyme where as other individuals have a more sever form of the enzyme and you can hence have a spread of severities. So even within these conditions there can be variation of the disease and this severity depends on the defect you have and which protein it affects.

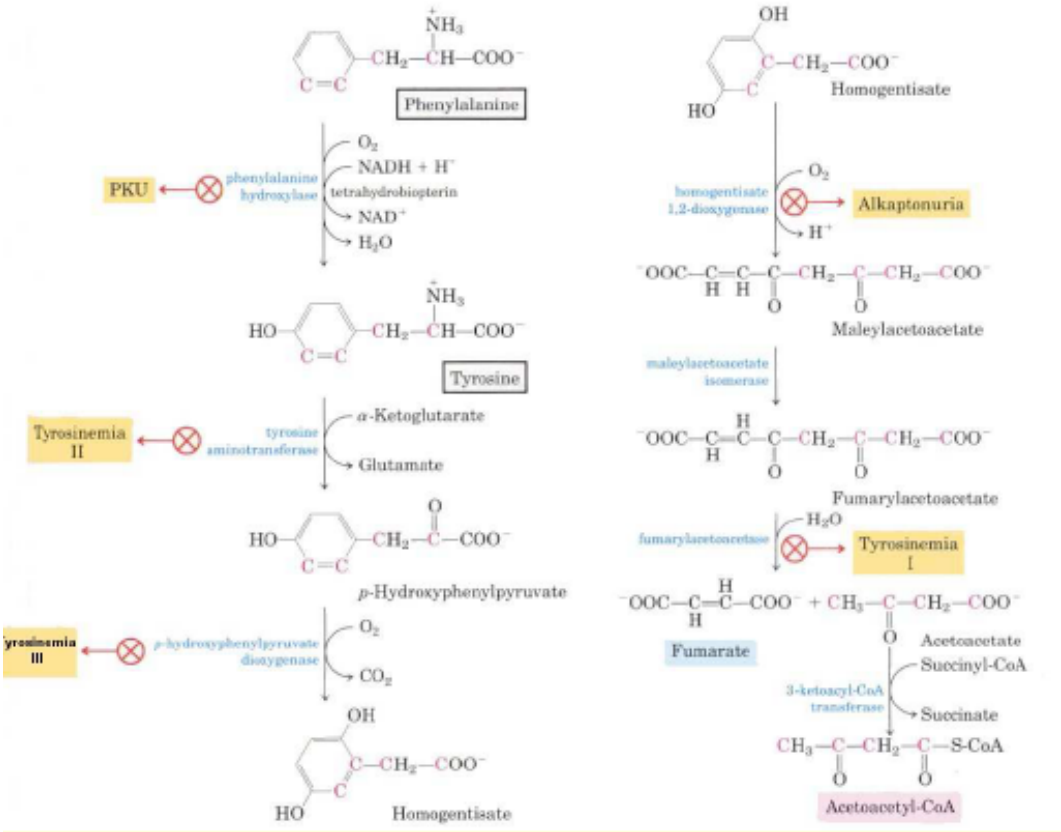
Ok so how do you treat tyrosenamia 1 well here are three stachatory structures avalable. the first is a dietary restriction by the limiting of pehnlyalenin and tyrosine in the diet. There are however other things to be done as well. That treatment alone is quite affective and in limiting the progression of the disease but its not a cure. and the survival rate for people receiving the dietary treatment are varying quite a lot but the 20, 30 and up to 70% of normal life expectancy. If people wer to just do the dietary restriction alone if there diagnosed in less than two months of age then the treatment is about a 29% survival rate. If they are diagnosed 2-6 months then there is a 74% survival and if there dietary treatment happens after 6 months then there survival is about 96%. It seems counterintuitive but the people that are diagnosed early have a more severe form of the disease. so if symptoms occur before 6 months then the diagnosis is quicker and dietary intervention is occurring sooner to control the condition.

The secondary strategy is metabolic inhibition of the tyrosine pathway using this beatutifully named drug called 2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3-cyclohexanedione (NTBC or Nitisinone) and this compound inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase so blocking the pathway at the step before the production of homogentisate. So its basically a metabolic inhibitor that blocks the pathway and stops it progeressing down to where the structure compounds are formed and this has proved quite affective at alleviating some of the conditions. There are some mixed sort of results form it but it seems to be the case that if the child is treated before the disease symptoms unfold then it can be quite benificial in slowing the progress of the disease.

Finally liver transplant has been used because the succinylacetone is a toxin thats affecting the liver and causing liver damage one. this has shown to be quite a resonable method outcome but then there are obvious complications of doing a liver transplant the mortality rate is about 10-20% for liver transplants anyway. Then also of course once someone has had a liver transplant then they will s[end a lifetime on immunosuppressant drugs.

A few of the final conditions we will touch on briefly are the other two which are tryosenemia 1 and 2 and were not going to say mucha bout these because they are quite rare conditions. Tyrosenimia 2 has another name and its called Richner-Hanhart syndrome and basically its caused by a defect in that tyrosine aminotransferase gene as seen in the diagram below. The defect is located on chromosome 16. its very rare it has a frequency of less than 1 in 1,000,000. most of the cases that have been reported have

developed from what are called consanguineous (denoting people coming form the same ancestor) relationships. This is seen in closed communities where people tend to marry another individual that have children with people closely related to them. Typically its cousins and things like that. This was first described in 1973 and it tends to be more prevalent in the Mediterranean populations. There are other groups of individuals that tend to be affected by it but most common in Mediterranean populations. these people tend to suffer from these blisters or ulcers on the palms and the feet. these are called Keratotic lesions. People tend to have these legions appearing also in the corner of the eye. which of course can be very painful and it can sometimes be called occulocutaniouse tyrosenimia



Individuals that suffer from that also tend to have a metal retardation where some of them, not all, but some of them suffere from behavioural problems as well where they self injure themselves. they tend to believe that these legions are quite painful and removing them may elevate some of the pain. The legions are actually said to occur from the deposition of tyrosine in the cells. As you may expect the biochemical features of the disease tend to be an elevated level of tyrosine in the blood and also tyrosine turning up in the urine as well. How is it treated? again there is a low protein (low tyrosien and phenyalanie diet) and that tends to elevate some of the symptoms of the disease.

The final type the tyrosenmia 3 again is extremely rare that has a defect in the enzyme p-hydorxyphenylpyruvate dioxygenase. Only a few patients world wide have been described with it and the frequency is less than 1 in 1,000,000. Its inherited as an autosomal recessive trait like all the others and the affected gene for this disease has been linked to chromosome 12. That particular condition prsents with a wide range of effects some of them quite mild but some more severe. typically things like metal retardation and also you get a condition known as ataxia (loss of bodily control and movements). Again this is treated by a dietary restriction of phenylalanine and tyrosine in the diet.

Chapter 2 (Inborn errors of metabolism)

This chapter includes

- Albinism
- Oculocutaneous
- Branched chain errors of metabolism
- The urea cycle

Albinism

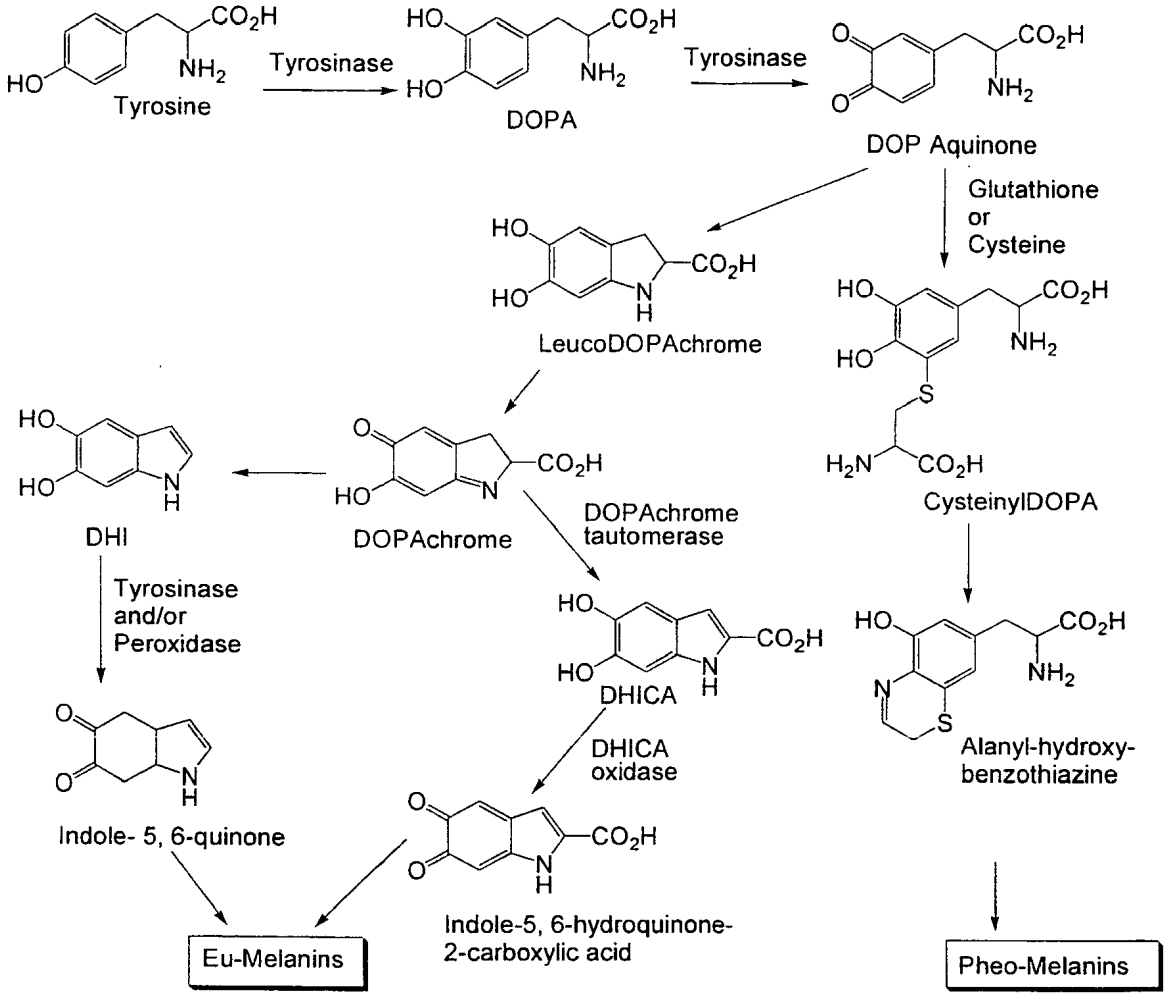


The term albiunism comes from the latin Albert which means white. There are in fact many forms of albinism this is just one type and the amount of pigment you actually see in the eyes can vary quite a lot. this pink eye appearance is one sort of it. Most individuals that suffere from albinism actually have some pigment in there eyes where there is a reddish or violet colour in there eyes. Most of them in fact have blue eyes so this is actually one extreme form of it and some albinos have brown or hazel coloured eyes.

There are two main types of albinism that we find in humans there is one thats called an ocular cutaneous form. Coulee pertains to the eyes and cutaniouse to the skin its thus a pigmentation that affects the eyes and the skin. Theres also a less common form thats just known as ocular which simply affects the eyes People with albuinism always have problems with there vision and this is the main way its usually characterised. We tend to think of it as an absence of any pigmentation in the skin but in fact all people that suffer from albinism suffer from poor vision. Basically it results from abnormal development of the retina but the retina and the rental pathways that link the eyes to the brian are affected in albinos so they tend to have poor vision as a result of that. Its the presence of those visual effects that gives the basis for diagnosing albinism rather than pigmentation. Its also worth remembering before we move on that albinism is something that affects many other animals and not just ourselves. There have been a number of well documented cases one the years such as Koala bear with no pigment and moose and even white squirrels. And it not simple one condition either there are different forms of albiunism and these different forms can manifest themselves in different ways. Some forms of albuinism can actually manifest themselves as a temperature sensitive enzyme. That actual result sin the formation of a pigment the enzyme is temperature sensitive and the classic example of that is the siamese cat. This cat has a the characteristic pattern of pigmentation and it results form the fact that the enzyme responsible for the dark pigment is only found in the cooler parts of the cats body. Or more acutely its only active in the cooler parts

of the cats body. It has a genetic defect that makes that temperature dependence sensitive. Therefore the cooler parts like the face, ears, tail and feet appear to have an active enzyme where the enzyme works and the pigmentation occurs. However in the warmer parts like in the trunk of the cat the cats bodies too warm and the pigmentation dosent form and thats why siamese cats have a a characteristic pattern of pigmentation. Another form of it occurs in pie ball horses in the pie ball horse there is a charecteristic patterning of this white and black regions on its body. This is actually caused by a mutation in the horse embryo and the albino effect only affects those parts of the body where those enzymes have become defective. Now what you find in these organisms is in the embryo itself if the genetic defect arises when the embryo is quite small then the areas of whit eon the body are quite large if the defeect occurs when the embryo is larger then the areas of white patterning tend to be smaller on the horse. So thats piebaldsim where there is an albino pigmentation effect. this is a genetic defect in the enzyme responsible for generating pigment. in order to understand albinism though in these different forms we

SCHEME 1



need to look a little bit at the biochemistry underlying the development of the pigmentation. The pathways that give rise to pigments in the skin are shown on this particular pathway here. Its actually a simple little pathway. The skin coloration observed int he individual is actually due to pigments in the skin called melanins. so the distribution and type of pigments that we see are the final skin colouration that we see. the two principle forms ar the black brown melanine known as Eu-Malanins and the red melanins known as the pheo-Melanines. So theres two branches from this pathway that give rise to these black and

red pigments. The final skin colouration we see in an individual is largely affected by the levels of these melanins in the skin. So those compounds are synthesised from tyrosine up the top. They are produced in special pigment producing cells known as melanocytes and it was originally thought that the pathway would involve just one enzyme called tyrosinase but in fact we now know that that same enyme has a number of effects throughout the pathway. The initial reaction is the hydroxylation fo the tyrosine to the compound DOPA so thats the enzyme tyrosinase there that works tyrosine and converts it to the compound DOPA and that s the first stage of the reaction. This is a peroxidese reaction to do that thing there. That DOPA is then itself worked on by the same enzyme (the tyrosinase) to form the compound DOP Aquinone. The tyrosinase enzyme there is actually carrying out a second oxidation so this is a per oxidation to form DOPA and there is a second reaction with oxidation to form DOP Aquinone. DOP Aquinone can then go down one of two pathways here and a lot of the stages in these pathways are non enzymatic and they seem to occur naturally in the body without the involvement of an enzyme. They go through a number of stages here but tyrosinase comes up once more in the conversion of this dihydroxyindol (DHI) to form the compound indol-5,6-quinone so its the same enzyme there that you see in the first two reactions that catalzye the tyrosne. Its then one enzyme but it works in a number of stages of the pathway and this particular pathway can be inhibited by tyrosine itself so it seems to be involved in the way the activity of the pathway is operating. The other brach on the right involves the incorporation of cysteine to give cystinalDOPA which then causes it to go down the pathway ending up in the red melanins. So its a fail simple little pathway with two end products.

Oculocutaneous

As well as the enzyme tyrosinase there are actually two other enzymes that eveovled and partculalry int he production of the Eu-Melanins there not actually shown oaths slide but there called tyrosinase related protein or TRP1. there is then a TRP2 as well. So these are two other protiens that are involved in this formation of the Eu melanins here.

Disease	Incidence
Oculocutaneous Type 1	1 in 40,000
Oculocutaneous Type 2	1 in 15,000
Oculocutaneous Type 3	Unknown
Ocular Albinism	1 in 50,000
Chediak-Higashi syndrome (CHS)	Very rare
Hermansky-Pudlak syndrome (HPS)	Rare, except in Puerto Rico where the frequency is 1 in 1,800

both of these things are coded for on chromosome number 9. The individuals pigmentation is coven rend by the relative amounts of these two proteins here and also from the distribution what are called the melanocytees which produce these pigments themselves. Now we might imagine that one form of albuinism is associated with a lack of the enzyme called tyrosinase which is an obvious place to start. There are other sorts as well. In the little table below there is a summary of the different sorts of albuinisms that we see. the sort that is describede from the lack of the tyrosinase enzyme is whats known as oculocutaneous type 1. Individuals with that defect tend to present with an absence of any pigmentation in the skin. These are the people we think of with the fair skin, the white hair and the lack of vision those people people are also referred to as oculocutanious type 1 A so those ones with the classic form of albuinism are type 1 A. You cn also get varying degrees of severity in these kinds of conditions depending on which mutation you actually have and how damaged the tyrosinese enzyme actually is.

There is another form known as oculocutanious type 1B so its the same basic defect but not so sever and in the type1B form these people tend to form some pigmention so they have some colouration in the skin and the hair is not completely white. Theres also anuther form known as oculocutaneous type 1 TS for temperature sensitive and you can imagine that this is a bit like the siames cat where these people have a form of the enzyme which is still functional but is temperature sensitive so these people tend to form some pigment in the cooler parts of the body but not in the trunk and warmer parts. All forms of this oculocutaneouse type 1 though, all tend to present with some charecterisitc type features they tend to have photophobia where they are light sensitive so they are not particularly fond of things likee strong sunlight. They have visual problems where there vision is affected quite notably and they also tend to suffer from something called nystagmus (rapidly involuntary eye movement) which is distinguished as uncontrolled moment of the eyes typicaly the eyes tend to flick form side to side in an uncontrolled fashion. People with the oculocutanioise type 1 tend to suffer from that. Most of those sort of effects on the eyes and vision tend to result form a misrouting of the optic fiber which are the fibers that go from the eye to the brain. This is a problem is a developmental issue that preensts as a symptoom of the defect and causes the visual probelms. These types of albuinism though are not associated with increased mortality where we saw there was a much redusced life expectancy with those contitions with these peopel tend to live a normal sort of life span.

TYPE 2

The most common form of albuinism is oculocutaneouse type 2 which affects thousands of individiuals and this type arrises from a mutation of a gene coding for a protein known as the P protein so its not actually a lack of that tyrosine enzyme or a defect in it . This is actually cause by a defe int he gene coding for a protein known a s the P protein. The function of that protein in melanins styless is not well understood and this is where it starts to get a little bit more complicated. It seems to be involved more with the transport of tyrosine needed for melanine production so rather than an actual defect in the biochemical pathway itself its a defect in this so called P ration and that protein seems to be involved in the transport of tyrosine into the melanocytes. So you get a loss of pigmentation but its not a direct defect of the pathway itself. People with this type of albunism tend not to have a compleat absence of the pigment. So they tend to have some kind of pigment but its reduced compared to normal.

TYPE 3

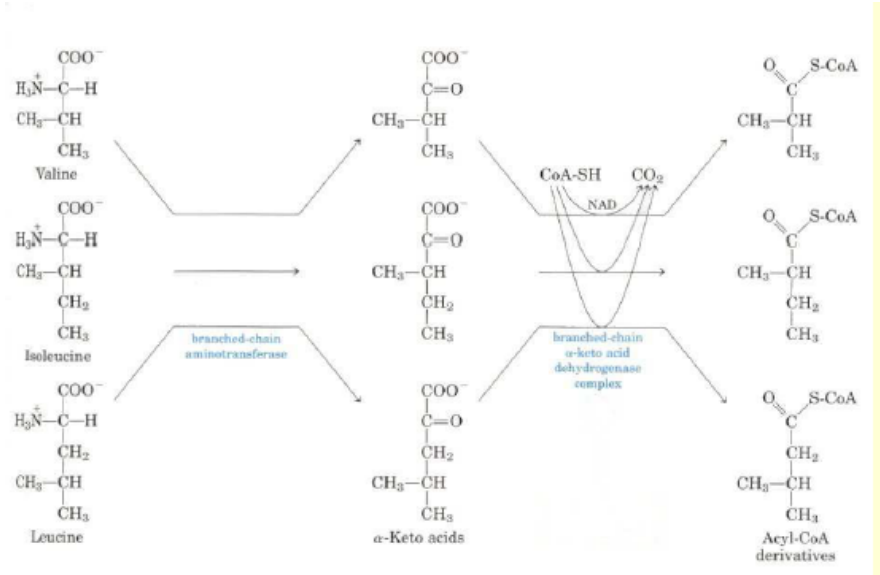
The third type Occulocutaniouse type 3and this actually arises from a defect in the TRP1 gene. You might say why dose a disturbance in malling biochemistry result in problems in a visual process. Its not very well understood but it is known that melanin has a role in the development of the visual processing system in the body if you have a problem with your melanin metabolism then it done t nessiarily mean you have a problem with your skin and hair colouration. It dose actually cause problems in the developing visual transduction pathways. We have summarised a couple of the effects of that are well known. the first one is this so called **Decussation** which is a crossing of the optic nerve fibers. You may know that in normal individuals the nerve fibers stay on the same side of the brain and only a few cross over. Co if you have the fibers form the back of your eyes going to one half of your brain and going to the other half as well and the the other eye the same. As we say normally a little crossing over is normal and this helps in determining things like binocular visiona nd depth perception. In people with albuinism if too many fo the fibers cross over and that gives them problems with depth perception and part of the problem they have with vision. We also see this **Foveal Hyperplasia** which affects the fovea which is part of the retina responsiblee for detiailed vision. It dosent develop correctly in albuinins an this would be another factor that affects vision. Finally they tend to observe more ocular starlight where light entering the eye. So light tends to dazzle them and thats what gives rise to the photophobia a bit. The light entering the eye tends to be more scattered so they get dazzled more easily than others do. These are all developing conditions associated with melanin in the nervous system.

All of those types of lbuinism are inherited in an autosomal recessive fashion. Theres no real treatment for this either and we cant cure it so basically these people have to use quite broad spectrum sunscreens to prevent there skin getting sunburn as there quite perceptive to UV damage to the skin if there exposed to the sunlight. As for visual impairment theres not a lot that can be done heer either apart from prescribing things like glasses and contact lenses.

We mentioned two other types in the table and these are on there for completeness sakes they may tend to pop up in conversations about albunism but these are more complex conditions they are not directly related to errors in the pigmentation pathways. There are other more general problems that impinge upon the development of the pigmentation for example this chediak-Higashi syndrome is extremely rare condition and its actually a childhhod autosomal condition that affects a number of systems int he body. As well ads the lack of pigmentation people with that condition tend to suffer prolonged bleeding times if there cut, there quite easily bruised and they can suffere from recurring infections so its a whole spectrum of conditions that are associated with that disease. Its nothing to do with the pathways of melanine biosynthesis it actually seems to be an immunodeficiency problem as there immune system manifests its self in a varietyy of ways. simeraly with the Hermansky-Pudlak syndrome (HPS) its a relatively rare condition. It is autosomal resessive again where there is increased bleeding time as well as the lack of pigmentation, This seems to have is origins in the paired formation with the lysozyme which are organelles. They seem to have a defect int here lysozyme production and that manifests its self in a varietyy of ways. So we can say that these two seems to be rather different to the others as there more complex. Its worth pointing out as well as last lecture we were talking about different populations as well where people have i higher incidence ethan normal. This is an extremely rare condition world wide apart from puerto rico where the incidence is much higher than the general population with an incidence rate of about 1 in 1800.

Branched chain errors of metabolism

not all of the inborn errors of metabolism affect the dramatic amino acids there are defects in amino acid metabolism that affect other ares. Its quite a serious condition which is called maple syrup urine disease. this actually affects the catabolism of the brach chain amino acids like valine, isoleucine and lucine. These are the brach chain amino acids and this disease comes from a defect in the enzyme branched-chain aminotransferase. the usual name of the disease "maple syrup urine disease" results from the characteristic small of the urine which dose smell like maple syrup quite strongly. now the branch chain amino acids are a little unusual in the way the way are broken down. most amino acids are broken don in the liver, these are not these tend to be broken down in other tissues. Places like the muscle, adipose tissue, kidneys and the brain. There oxidised as fuel stuffs so



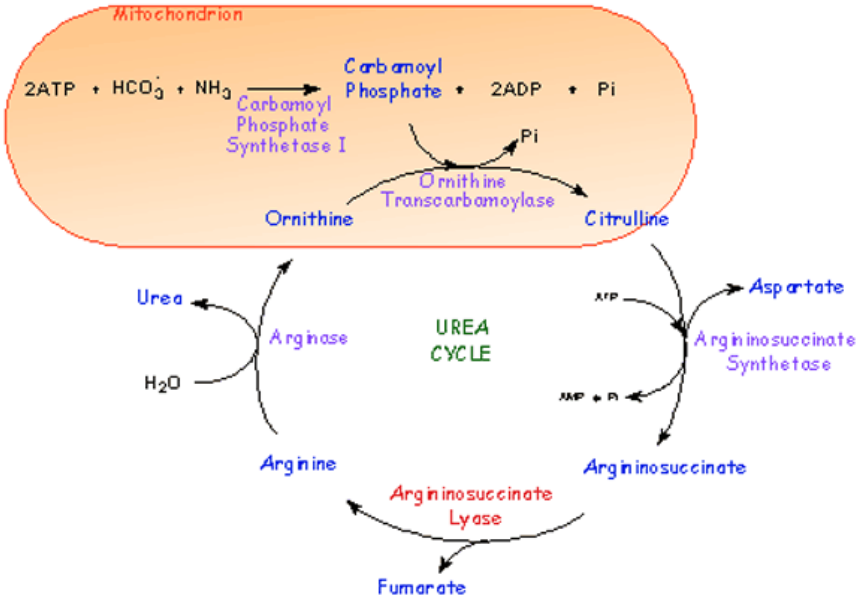
these are the amino acids we can use as fuel, the pathway in which there broken down is shown in the diagram here and those tissue, the extra hepatic tissues like the muscle contain this amminotransferase which takes off the amino groups and leaves us with the alpha-keto acids arriving from the three amino acids seen on the left. Those alpha-ketoacids are then inters acted on by the branched chain alpha-ket dehydrogenase complex converting them to there CoA derivatives and releasingg CO2 in the process so thats a normal pathway of there catabolism. The enzyme dehydrogenase is analogous to too other important complexes that we see in the body. The pyruvate dehydrogenase complex enzyme is similar which normally catalyses the pyruvate to acetyl-CoA. Now also the alpha ket glutamate dehydrogenase complex (the one that converts alpha ket glutamate to succinyl-CoA in the TCA cycle).So we are just seeing that the comparison between this branched chain alpha-ket dehydrogenase is similar to two other dehydrogenase. There quite complex and we should say that the hdehydrogenase complx there are a number of proteins and a number of Co-Factos involved in the reaction. some have been shown on the diagram but there are more. so that particular enzyme actually has 5 co-factors associated with it. There is thiamine-pyrophosphate, FAD, NAD, lipoate (lipoic acid) and CoA so there are five other co-factors for the functioning of that particular complex there. now if this complx is defective or absent in some way then there eis a second part where that reaction cant occur and as we saw before eis you get a build up of these three alpha ket acids which then spill over into the bloodd stream and the urine and such. Its the presence of those ket acids that gives the urine its characteristic maple syrup smell. In particular the one thats serve form isoleucine. Despite its innocuous sounding name maple syrup urine disease is actually a severe condition it leads too an abnormal development of the brain so these people become physically and mentally retarded and usually die in infancy. so the most sever form of the disease is quite a nasty condition. As we saw with PKU and things like that yesterdayay effected infants appear quite normal at birth because of the maternal metabolism would have protected the child until it was born. Once its born it becomes independent so the problems then tend to show up. Soi although the infants appear normal at birth normally within 4-5 days after birth they tend to become a bit lathargic and loose interest in feeding and develop some form of ketosis which is a build up of ketone bodies. in the blood. Again left untreated that disease can lead on too seishures where these ifs start to have fits. They eventually fall into a coma and perhaps due to a swelling of the brain means they would die. So ye its quite a serious condition.

How do you treat it? basically by limiting the intake of the branch chain fatty acids to the minimum thats required for growth then you can limit the effects of the condition. Maple sryup urine disease is actually very rare in the general population where eis occurs in about 1 in 180,000 newborns so quite rare in the general population. As we saw yesterday there are certain ethnic groups or groups of the population where the occurrence can be quite high and this one is slightly unusual in that it tends to be as high as about 1 in 200 or there abouts in the amish community. Some may be familiar with the amish community who ar eh old meningitis that are a religious order that lives in Pennsylvania in the states. They shun a lot of the trappings of modern life and tend not to use motor vehicles and use farmingg as a way of life. because there in a closed community almost they tend to inter marry in that community and have children and these kinds of conditions tend to become more pronounced it inserted into the community gene pool. The amish community thus tend to suffer from maple syrup urine disease. As ypuve seen before there are a number of variants of this, it done arise because of one single genetic defect there are a number of defects you can get and depending on which one you've got the severity of the condition can vary quite a lot. The classic disorder as described by absence or completely non functional is the most serious form of the condition. There is a less sever intermediate form that we tend to sometimes see. It manifests its self in childhood wher there is a growth or development slowing so typically if children would not suffer hugely but there is a losing down of there growth. where they are a little bit behind in some ways. Children that affected with a milder form of the condition often do quite well if the intake of these things is restricted so they can cope with it. There is also a mutation that occurs in the

enzyme there which can be overcome by giving large quantities of one of the co-factors thiamine-pyrophosphate. There seems to be a form of the condition in which the enzyme is there but it requires larger than normal amounts of thiamine-pyrophosphate so again if people have that bparticular variety then treating them with vitamin B1 then you can over come the condition. Patients that have that kind of thiamine sensitive or intermittent form of the disease are often quite normal between attacks but if something happens to stresss there bodies then they tend to develop the condition. So if you have something like a febrile (having or showing signs of a fever) illness then often that trips the body over and it cant cope and the disease starts to show itself . So things like infections or febrile illnesses that stress the body tends to bring out the disease in people otherwise there OK. Theres not one simple genetic defect to say that causes the disease. Because its such a compllex system there are many things or many potensial casesing the reactivity of the enzyme. The various genes have been located to chromosome 1, 6,7 and 19 so there are a variety of genetic defects that have been associated with that.

The urea cycle

So thats maple syrup urine disease which is another example of a metabolic disorder that comes from the brake down of branched chain amino acids that can lead to the various disease. There are others and in particular conditions that effect the urea cycle and how they impact on us. now one of the consequences of an amino acid metabolism is of course that you generate ammonia or more accurately ammonium ions so your metabolising proteins or ions where theres an NH3 group thats going to come off somewhere. Theres an ammonia being generated. This is a feature of most organisms to take excess portion into there diet. now your probably aware that those excess amino acids in our bodies cannot be stored and so what tends to happen is those excess amino acids in the diet are brocken down and diaminated the carbon skeletons can be used as a fuel source but the ammonia bit or th NH3 bit has to be gotten rid of. Ammonia is quite toxic in some ways as it has to be detoxified or recreated from the body as quickly as its produced really. Now for aquatic organisms this is not a problem as there swimming around in a vast excess of water. and ammonia can simply dissolve in that water and flat away so the excess nitrogen or ammonia just feeds strait into the water. However terrestrial animals have ot conserve water as we cannot afford to loose water in this way so we tend to convert our ammonia into a form that can be excreted in a small amount of water. birds terestrial animals and insects and such tend to convert there ammonia in to uric acid which is then extracted. Of course though most animals excreatet the excess of there ammonia and nitrogen in the form of urea. So thats what were gonna look at here. Now urea is synthesised exclusively in the liver. Your liver is where you make urea and then its transported through the blood stream to the kidneys where its excreted in the urine. Now theres a cyclic pathway involved in the production of urea and it was discovered by Krebs. This is the sam block who figured out the TCA cycle. He actually discovered three cycles but this is the first one he discovered about 3 years before he discovered the TCA cycle. This is it summariesd here. The



cycle actually starts inside the mitochondiran which is what this bit is here but all of the three remaining steps take place int he cytosol. Just to run thorough it in outline then. We start of at the top where we have the ammonia which is being generated froth amino acids and we have got too get rid of it. The ammonia is combined with carbon dioxide so its carbonated to form the compound carboxyl phosphate. That reaction is catalysed by a enzyme carbomoyl phosphate synthetase 1. This is because there is also a carboxyl phosphate synthetase 2. The second form isn't actually involved in the forming of urea. the second form is involved in pyrimidine biosynthesis and metabolism. The carbomyl phosphate itself then ends up in the urea cycle proper at this stage here where it undergoes 1 of 4 enzymaticc steps. Firstly the carbomoyl phosphate is operated on by the enzyme ornithine transcarbomolayse to form this compound citraline. The citraline then passes out of the mitochondria and into the cytosol and we now have a second amino groups coming in form aspartate so this bit here is also form the mitochondria and we are getting aspartate formed and that amino group comes into play as well and the enzyme responsible for this stage is the arginosuccinate synthetase. The compound thats formed is called arginosuccinate and the reaction involves aTP and pyrolytic cleavage. the arginosuccinate now with two nitrogens in it (one form the aspiratete and one form the citraline) the arginosuccinate is then cleaved by a molecule called arginosuccinase (seen in the diagram as arginosuccinate lyase) to form fumarate and arginine. the Arginine contains two carbons. The fumarate ends up back in the mitochondria and the TCA cycle. Then the arginine is then finally cleaved by the arginase enzyme to give off urea and regenerate ornithine which can then go back into the cycle. So the two nitrogens you loose in the urea comes from the citriline (that got its nitrogen form the ammonia) and aspartate. and this is the urea cycle. This synthesis of urea is the major root by which we remove ammonia from our bodies. theres no other way you can do it really. So any blockage or defect in those enzyme in the urea cycle has quite sever consequences. So you can make urea or excreta the nitrogen very well.
Now not surprisingly and defectin the urea system tends to lead to a elevated level of ammonia or ammonium ions i the body. That has a name is called hyperammonemia. Nowif you have an elevated level of ammonia in the blood it tends to form lethargy (you slow down and dont feel very well) Higher concentrations lead to things like vomiting and untreated leads on to convulsions and fits. typically if left untreated you will develop irreversable brain damage and a coma and death. So you cant tolerate high levels of ammonia circulating in your blood for anylenght of time. Its not actually understood why high levels of ammonium in the blood cause those particular problems. but one possibility is that it dose tend to lead to the increased concentration of glutamine in the brain. The levels of Glutamine tend to go up in the CNS and in the brain itself. That causes osmotic effect in the brain that causes it too smell and that could account for some of the problems we tend to see. Other tissues in the body can swell up but the brian cant because its in a hard bone casing and the pressure can lead to unwanted pressure and burtsing of cells. So thats one reason why ammonia can cause brain damage.

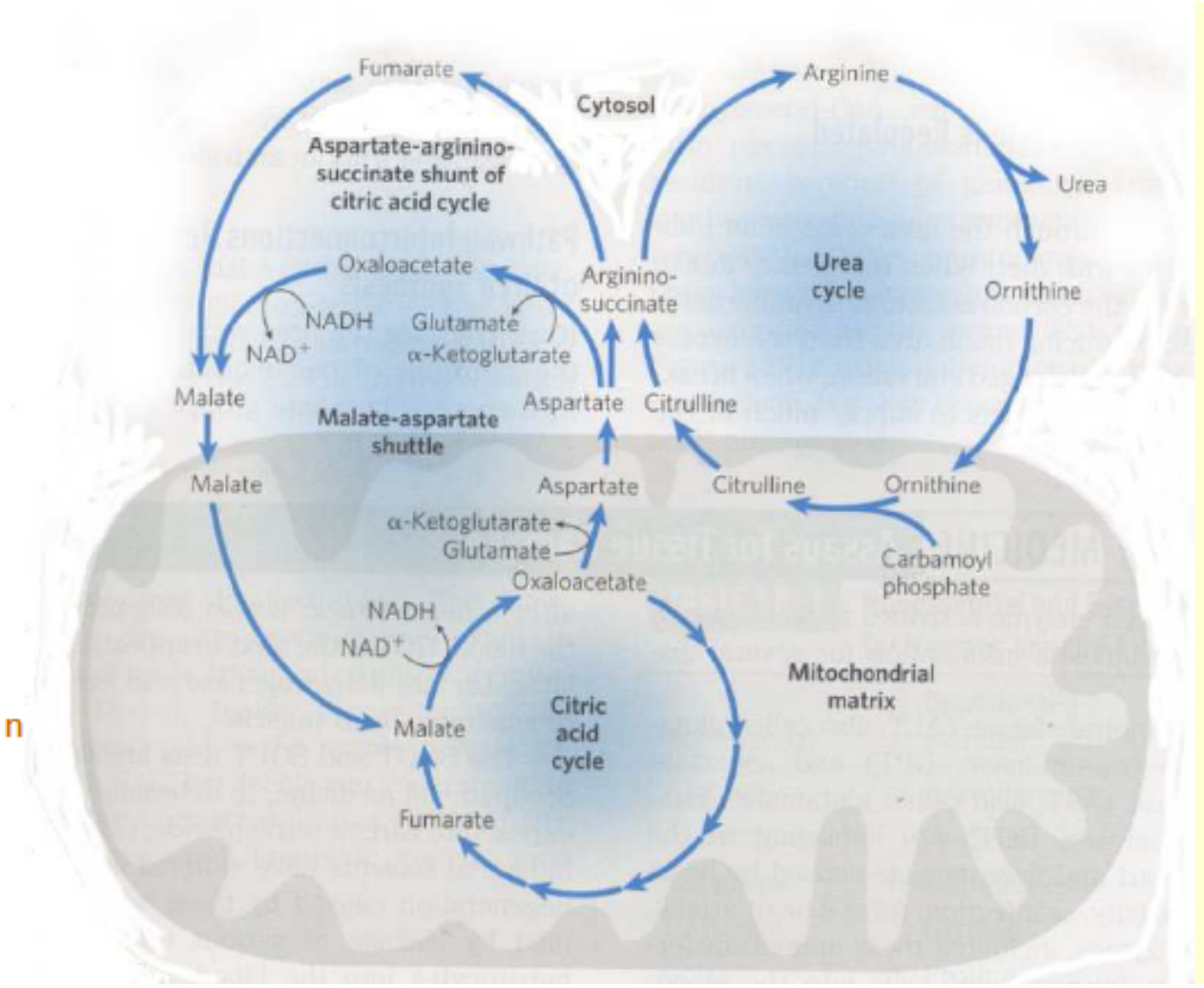
Chapter 3 (Inborn errors of metabolism)

This chapter includes

- A
- O
- B
- T

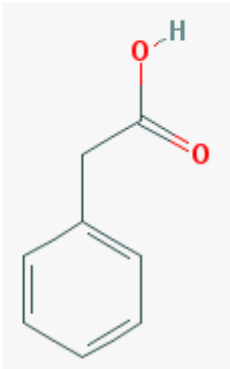
A

Last lecture we were looking at the urea cycle and the effects of excess ammonium in the blood. So this is a completely different condition where we are looking a the treatments of Treatment Of Carbamoyl



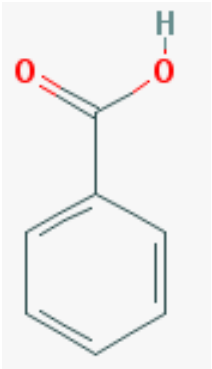
Phosphate Synthetase And Ornithine Transcarbamoylase Deficiencies which are the last two enzymes in the urea cycle. These defective enzymes can lead to problems with nitrogen excretion. A deficiency of either of those two enzymes can lead to hyperammonemia due to the reduced ability to excreta ammonia and carboxyl phosphate sythetase defeicintcy is actually quite a rare condition. Again it can affect individuals at different stages of life. The form that affects newborns is generally very sever and will quite quickly lead to death if left untreated. Some individuals have a milder form of the condition. Where they have some function in the enzyme and they are able to live through to middle age before diagnosis. Quite often what you find with those individuals is theres a background level of hypoammonemia but its

just not being picked up and that in turn can lead to brian damage if its not picked up. So although people may not find the condition fatal in the early stages of life it dose cause brian damage if lef untreated.
Now carboxyl phosphate synthetase actually has two enzymes that carry out this particular function. There are two carboxyl phosphate sythetase enzymes known as CPS1 and CPS2. The first one is exclusively mitochondrial and its the one that is responsible for this condition if its defective so a lack of CPS1 a mitochondrial forming enzyme is the root cause of the urea cycle defects. Its actually extremely abundant enzyme CPS1. Its reckoned to account for about 20% of the protein in the mitochondria. The CPS2 form on the other hand is a cytosolic form and this form of the enzyme (The CPS2 form) is used in the denovo synthesis of the pyrimidines. So the CPS1 form is a mitochondrial form used in the urea cycle and the CPS2 form is a cytosolic form of the enzyme that carries out the same reaction but its involved in the synthesis of the pyrimidines. Like most of the inborn errors of metabolism the condition where there is a lack of CPS1 is an inherited disease that is autosomal recessive. In this case the localised gene is found on chromosome 2. Ornithine transcarbomylase deficiency is the most common deficiency of the urea cycle defects. It occurs with an incidence with about 1 in 80,000 and its also unusual amongst these in born errors of metabolism in that its inherited as an X linked trait, now we say all the others are inherited as autosomal recessive fashions where as this one is an X linked trait. Thats to say the gene for the CPS2 is carried on the X chromosome so the condition is typically seen only in males that have a single X chromosome as we only have one X chromasomes. Heterozygous females carriers of course have two X chromosomes that can receive a normal copy of the gene and an abnormal copy. They tend to show an abnormal complex range of the condition. Most are Asymptomatic and dont have any problams but some females to tend to show some evidence of the condition and some of them will show a mental retardation and hyperammonemia but its a more variable picture in females. Its also the case that the ornithine transcarbomylase enzyme is quite highly variable. There are about 300 different mutations that have been identified in the gene encoding fo that protein and that again accounts for huge variability. It accounts for why some people are serveer effects and why some people can live quite happily with it.
The treatment of carboxyl phosphate synthaetase and ornithine transcrabomylase deficiencies is a little bit differ tot what we saw earlier. We cant bypass those two steps of the cycle by providing things like arginine so its not easily bypassed but the diet and things. If you boost the sythesis of citraline or arginosuccinate then it dosent bypass the required steps in the cycle so you cant do anything about it.



Phenylacetate

The treatment process for this really works on the fact that in those two conditions the excess nitrogen that builds up in the body, a lot of it ends up in two compounds such as glycine and glutamine so the body dose try to do something with the excess nitrogen and as we say a lot ends up in amino acids. So the challenge really is to try and persuade the body to get rid of the excess glycine and glutamine. We can do this in a couple of ways obviously we can restrict the dietary intake of protein but you can also supplement the diet with two other compounds. You can give people benzoic acid or benzoate and phenyl acetate. Well basically what happens is if you take benzoic acid in the diet then it will convert to benzoyl-CoA and the this can form an adduct with the lysine to form the compound hippurate and this is excreted form the body. So some of your excess nitrogen is building up is ending up in glycine but by giving benzoate it is converted to a benzoyl-CoA which can bind with glycine to form hippurat and then its excreated. Similarly with phenyl acetate that will also be converted to phenylacetate-CoA whitecap bind with glutamine to form phenylacetylglutamine. This tinkering with the biochemistry can be seen in the diagram below. Tinkering with the biochemistry means that some of the nitrogen is not finding its way into urea but it is finding its way into both glycine and lcutamine and as a result of that by supplementng the diet we can form hippurate or phenylacetylglutamine and then there excreated from the body as excess nitrogen. Here you

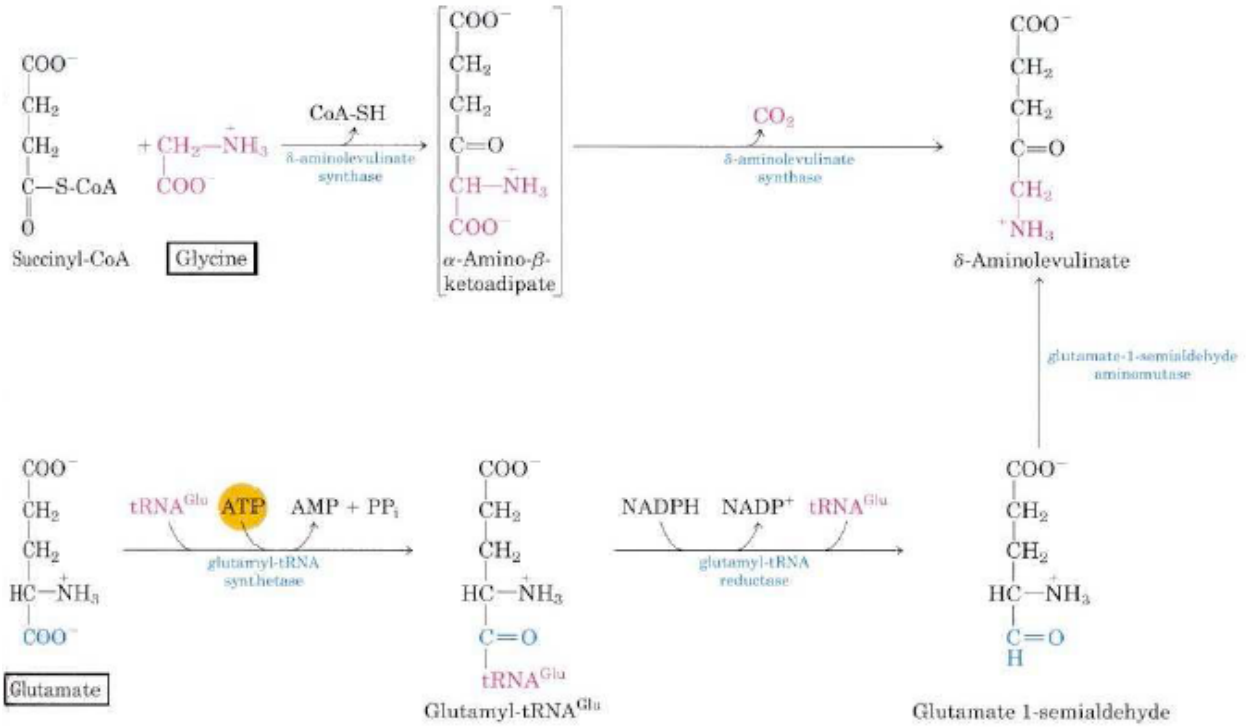


Benzoic acid

activate two latent biochemical pathways in order to bypass the genetic defect. These pathways would obviouslyy not have been particularly important in a normal individuale as benzoic acid and pheylacetate are not widely found int he diet. Curiously as a side effect this used to be a parquetalcal we did with the students years that was stopped for health and safety reasons. These compounds would form a sludge in orange juice and you could drink. Then if you collect the urine from the student over a period of a few yours you could test them for the formation of hippuric acids in the urine quite easily.

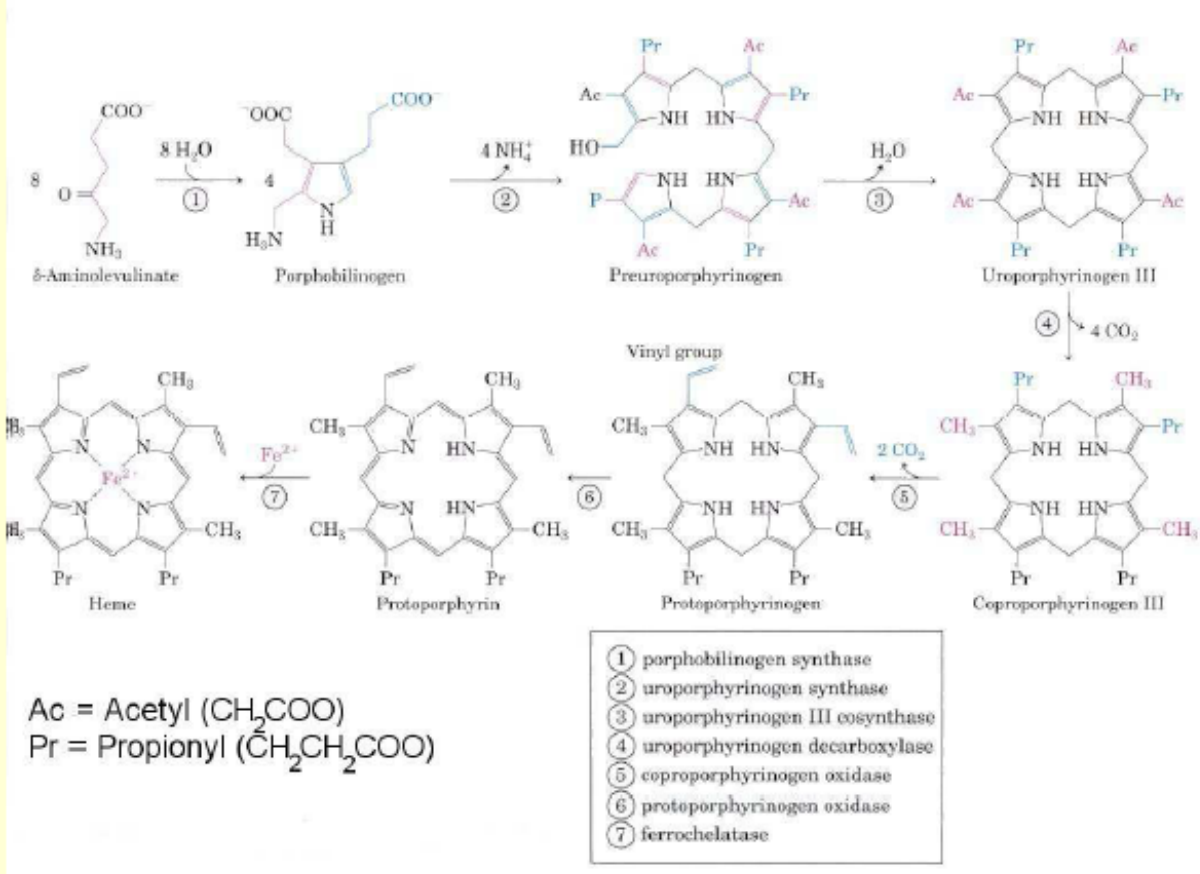
So thats all we are really going to discuss about the amino acid inborn errors of metabolism.

What were going to to now then in the second part orf the lecture is too move onto a whole new set of conditions of inborn errors of metabolism and these are called the porphyria. Basically these are formed by defects in him metabolism. There quite interesting actually and we will see as we go along. To understand how these conditions affect us and what the underlying causes are, then we first need to consider the synthesis of the porphyrines from there precursor glycine. Now the first stage in the synthesis of the porphyrins and there precursor glycine. Now the first stage in the synthesis of the porthrines and the heams is the combination of glycine with -CoA to form this compound (delta-aminolevulinate. Porphorines are the central molecules to haemoglobin and chlorophyll and such and there quite complex molecules but like a lot of the synthesis in biochemical things there actually form form quite simple precursor molecules just built up together. The porthorines start off life s glycine and there converted under the action of Delta-aminolevullinate synthase firstly to the first intermediate seen in the diagram above (delta-amino-betaketoadipate). This bottom part of the pathway here is just in there for completeness sack really. Animals make it the way on top but plants and bacteria tend to make delta-aminolevulinate and this is what were interested in. So we have made delta-aminolevulinate so how do we make porphyrins from it? well the synthesis of heam is shown in the little slide below
It looks quite complicated but its not. We have the delta-aminolevulinate in the top left corner and we get two molecules of that coming together to form the compound porphodilinogen. This then consists



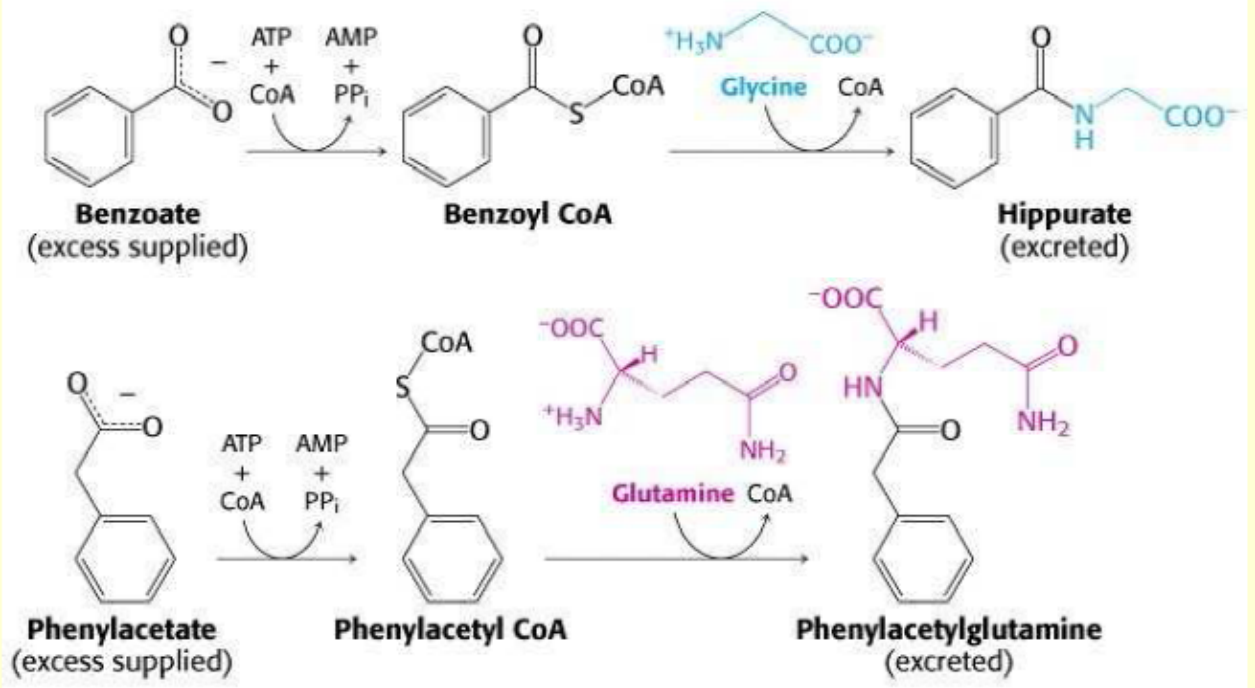
taught in aheads to tail for to form preuroporphrynogen and the enzyme is uroporphrynogen synthase. This compound you see isn't actually a closed ring structure yet its still open but there is a second

enzyme that comes int play called uroporphrynogen III cosynthase and thats where water is released and the ring is closed to form the compound uroporphrynogen III. Now its l=slightly odd tha the uroporphrynogen synthase can actually compleat that closing of the ring without the cosynthase. It becomes better understood when we look at defects of this but if you lack the cosynthase the synthase itself can actually close the ring and form the uroporphrynogen. Instead though what it forms is called uroporphrynogen I not uroporphrynogen III and the difference between the I and the III is in the layout of the ring structure yourll notice if you look at that ring structure III its asymmetric. If you look at it across that axis there, You have a Pr group and switched with the acetyl in the top right ring making it an asymmetric compound. So the molecule is asymmetric and thats brought about by the cosynthase. If you leave the synthase alone to close the ring what you end up with is a symmetric structure where the ring has not been flipped. The symmetric is not actually any use to us so we need the cosynthase to form the asymmetric structure. That molecule is actually a key intermediate in the formation of molecules like vitamin B12 and chlorophyll as well. The remains stages of the pathway along here basically involve alterations of those side chains that are there and to the degree of saturation within the ring structures themsleves. The next stage then is a decarboxylation reaction using enzyme 4 the uroporphrynogen

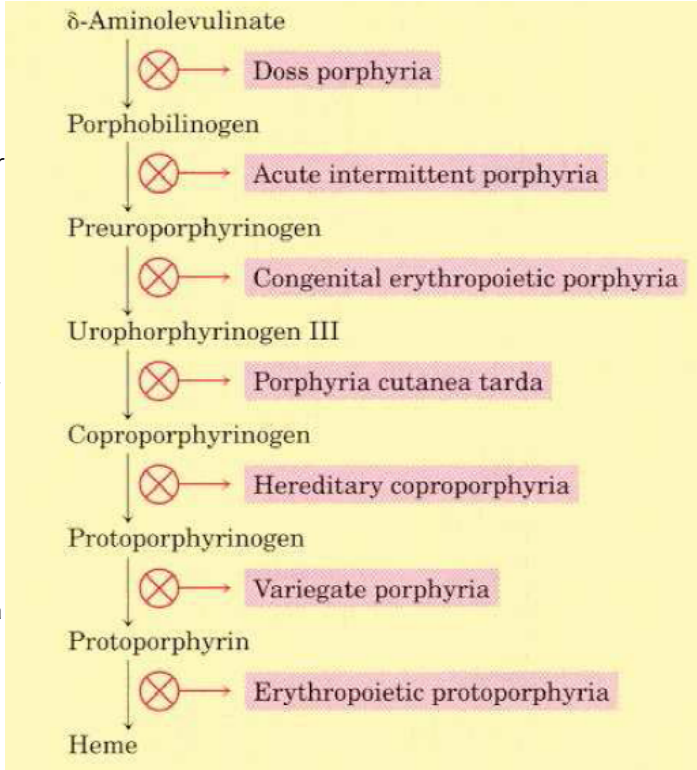


decarboxolayse where you loos four carbon dioxides and your left with coropophyrinogen III so basically what you have done is convert them acetyl groups to methyl groups.

Theres then two more reactions catalysed by 5) coropophyrinogen oxidase and 6) proroporphyrinogen oxidease. Basically what these do is carry out further decarboxylations converting those propyOnyl groups to a vinyl group and then theres also a desaturation where you can see in the properly groups there is a double bond to form the compound protoporphyrinogen and then in the final stag of the process catalysed by enzyme 7 ferrochelatase which is the enzyme that produces the iron atom to produce our final heam structure. At that stage the heam can combine with a whole bunch of proteins to from a range of molecules like myoglobin haemoglobin and various cytochromes. Now all of the enzymes



involved in that pathway can potentially be defective and theres a whole family of conditions. Some of those medical conditions as shown int he next slide they are collectively known as the porphyrias. At the top we see our delta-aminolevulinate and we see if there is a defect in the first enzyme then we get whats called doss porphyria named after a man called doss who first discovered it. Here you get an acute intermittent porphyria which is a congenital eurythropoetic porphyria. Then ther is porphyria cuteness trade herediatary coprophyria, variegate porphyria and Erythropoietic protopophyria. So there is a whole family of porphyrias that results form defects in those various enemies and each of them show some degree of relation to the urea cycle but there also some subtle differences between them.



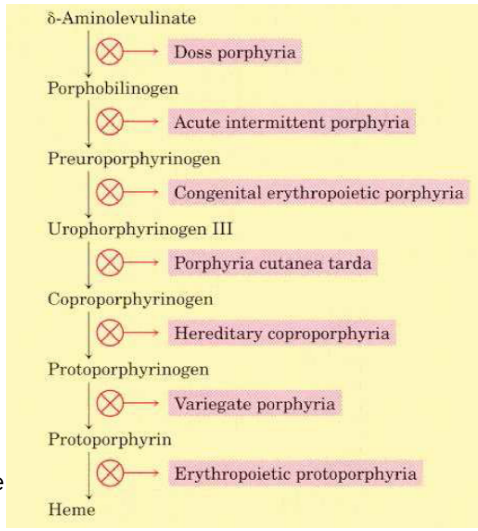
Chapter 4 (Inborn errors of metabolism)

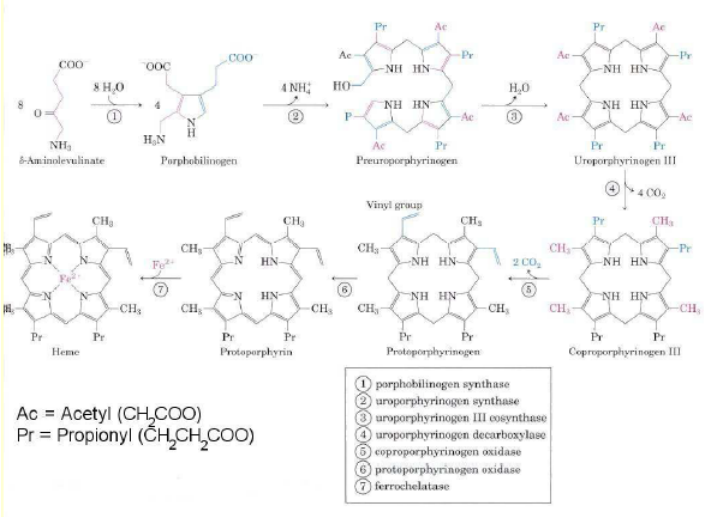
This chapter includes

- The porphyrias

The porphyrias

So we saw last time that the production of haemoglobin is done with the starting material aminolevulinate going through 7 step to make heam which is incorporated into heamoglobin. The names of the enzymes used in these steps are seen on the right here and we can see how defects in these enzymes can lead to different inborn errors of metabolism. We saw last time that these diseases are collectively called the porphyries. We can see from the first slide that the first few stages involve the use of linear compound. The third compound known as preuroorphyrinogen has faded round but it hasn't actually closed up yet. and then she we get to the final stages of the pathway the ring becomes closed and you get a cyclic structure. This process has consequences actually because when the ring becomes closed the structure is able to absorbe light energy. The linear forms tend not to for any great extent but once its closed it absorbs light. This then tend to mean that disorders that affect the latter stages of the process tend to cause photosensitivity in individuals. The defects that have effect prior to closing the ring tend not to have photosensitivity. So we have a number of conditions collectively known as the porphyries and we start off with the first one there is called Doss porphyria





Doss porphyrias

Doss porhyria tend sto be canused by deficiency by the first enzyme called porphobilinogen synthase. The defective enzyme has been localised to chromosome number 9 but doss porphyria is actually an extremely rare condition. There are only a handful of cases that have been reported world wide. Its quite an unusual rare condition. It gets its unusual name as well from professor Landfrey Doss who is the guy who first described it. Because of that condition you tend to get a build up of delta-aminolevulinate as you might expect which tends to spill over into the urine and plasma and things like that. Patients often have quite sever attacks or episodes from thiss but as we said before they don't have a photosensitivity that you get with the latter defects. Typically the condition is notice in children and young adults and people like that and the disease is inherited in an autosomal recessive fashion. Interestingly enough actually poisoning with lead causes a very similar condition which is called plumbo porphyria. Lead is actually quite a potent inhibitor or porphobilinogen synthase. So lead can inhibit that enzyme and not surprisingly the condition cause by lead poisoning is very much like this porphyria but its called plumbo porphyria caused by this lead poisoning. It works basically like that because that porphobilinogen

synthase has a zinc as a cofactor and the lead can displace the zinc on the enzyme and therey inhibits it. Thinnest addition down the tree is then is acute intemritant porphyria.

Acute intermittent porphyria

This is caused by the next enzyme down the chain (2) known as uroporphyinogen synthase this is the second most common form of the porphyries typically an instance of one in five individuals in 100,000 so as things go its relatively common. As you see before with some other conditions there are descreat populations that tend to suffer from much higher instances of it and in this case the incidence is much higher in northern Sweden where the incidence is about 6-100 cases in 100,000. In this case the enzyme has been localised to chromosome number 11. Now most affected individuals with this particular disorder are heterozygotes and typically asymptomatic. This is because the single functioning allele or gene is able to provide a sufficient amounts of the enzyme under normal surcustances. If a person is stressed or under particular circumstances there is an impaired ability to manufacture that enzyme starts to show through and the condition then starts to merge. Critters induces of attacks are chemicals or situations that induce heam or cytochromes p450 synthesis.

Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme cofactor and, therefore, are hemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorized as P450-containing systems. The term *P450* is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with CO.

So if this pathway is kicked into action by some environmental condition then it tends to manifest its weekness basically. A coupe of examples include alcohol as its good at tipping people ove rat edge. Fasting so anything involving the give and take tends to affect it as well. Some people treated wit oestrogen again sometimes push people over the edge and cause a condition to come forward. Typically the attacks that people suffer ein this stye of pophria manifest themselves as quite sever abdominal pains they also have some neurological dysfunctions like lins and needles and things like that. So there are some neurological effects and some abnomial pains but as we noted before as this agin occurs in a enzyme that is used before the ring has been closed then typically these people dont suffer from skin problems and there is no typical hypersensitivity. We should actually point out that this one is unusual as its inherited as an autosomal dominant trait. And this is why most of the individuals tend to be heterozygouse and still show evidence of the condition. There is an interesting side to this actually where a particle type of condition where king george the III basically is suspected to have suffered with this condition. He took some form of ailment for this that tend to impart his judgement at times. King George the III was the monarch that during the time of the american revolution and people people said he was a well accomplished man at the time but he had these intermittent periods of madness wher ehe would do some sillythings. Popel, also tend to thing that america might have gone down a different pathway if it were not for the actions of king George. So the whole world would have been slightly different had he not suffered that. If he did indeed suffer from that. Its an interesting though anyway. How do you treat it? well you treat this particular condition by administering a high carbohydrate diet and in sever cases they can give people intrevenouse glucose. You might say whats the rational behind that well the rational is that glucose is an inhibitor of delta-aminolevulinic acid synthase which is the enzyme that makes delta-aminolevulinate and so there for by giving a high glucose diet you can actually inhibit the formation of the starting materials and therey alleviate some of the conditions. So thats the acute intermittent form so ned we will look at the congenital erythropoietic porphyria.

congenital erythropoietic porphyria

This is also known as gunther disease this again is one of the rare conditions and is one of the less common forms of porphyria there is about 200 cases that have been reported worldwide so as these things go it is extremely rare. It results form a defect in the cosynthase enzyme (3). And if we remember from the last lectures that this enzyme (2) is capable of closing the raing but what we get is a symmetric product. Now you remember the proper product is asymmetric with the different groups ing of the acetile and Pr groups. If the cosynthase is not present or dosent function that is formed but it is formed in a symmetrical form which is not active. This results in the Pr and Ac groups not being inverted. So this si a different condition formed from a different construct of the molecuel. The ocsynthase is actually coded for on chromosome number 10 and this one is inherited in an autosomal recessive pattern. It is characterised by an accumulation of the symmetrical form of the Uropophryinogen that doesn't actually do anything. Now there are problems but not unusual problems associated with this accumilation, As we saw before these enzymes closed the ring and caused an absorbance of light energy so typiclally with these conditions tend to be light sensitive. In some ways thats the least of there problems. They have skin that is actually sensitive to light and they blister very easily. But these compounds are covered whilst this ring is closed there is a delocalised electrons which means that the absorption bands of these molecules cause them to become coloured in the visible range. They are red coloured and they become diposited in certain tissues of the body. Typically places like the teeth. these compounds also flours as well so you can have red florescent teeth. Another problems is that the erythrocytes that have been formed from this starting material tend to be destroyed prematurely and they are brocken down. This then makes affected individuals anaemic so they become a bit pale skinned. Because of the light sensitivity these people tend not to go out into sunlight or during daylight hours.

You can see where this is leading where you have people who have got red stained teeth, red urine, they oddest come out during the day light and there every pale. This is believed to be the origins of the so called vampire legend that we got in eastern europe. It is believed to be from that medical origin of pophyria. The other thing actually about individuals with this disorder is that thy tend to show hypertricosis which means an excessive growth of hair typically on the hands extremities which ties into he werewolves legends as well. So affected individuals that suffer from this disorder have been painted with a horrible brush over the years. Theres not much to offer in the way of treatment for this disorder you cant cure it. patients tend to stay out of the sun because of th blistering.

Porphyria Cutanea Tarda

The acute photosensitivity and blistering makes it clear in the next condition called porphyria cuteness tarda suffered the same but worse consequence where eoften th hands are covered in blisters and so on which is caused by the deposition of the heams molecules in the hands and skin. They absorbe light energy and by doing so they creat free radicals and stuff which damages the skin. That particular condition porphyria cuteness tarda is caused by a defect in the next enzyme in our system (4) that converts the /uroporphyinogen III in to Coporphyrinogen III with the decarboxylating enzyme. This particular enzyme or gene is localised to chromosome number 1. It gets its usual name as you may expect the porphyria bit but the cutania bit relates to the skin the tarda means delayed and this relates to the fact that as people get older they get this symptoms of this disease. Its the most common form of the disease and particularly as you see form between 1-25 to 1-50,000 which is relatively common for this condition. It also tends to form in a sporadic form as well. Some people tend to not to inherit it in the same way they tend to have a susceptibility to the condition and again we see that they are ok for the most circumstances but something happens in there life and something comes along that triggered the affects of the condition. We tend to see this triggere as an external trigger something like alcohol. Its

actually believed that 20% of the cases of cutting tarda are familial so as to say they are inherited or passed on through families. The other 80% seem to be acquired in some way and are in susceptible individuals. The most common acquired form is known as sporadic cutanea trade. Basically what seems to be the problem with these individuals is they have a normal europorphinogen decarboxylase enzyme but they seem to have other genetically determined sensitivities that tend to inhibit that enzyme. So as the enzyme appears to be normal there are other things that inhibit its correct function. Again how do you treat these people. Its quite difficult, they need to be kept out of strong sunlight because they will get the blistering effect on the skin. Its useful to mention at this point that conventional sunscreens are not any good. these conventional sunscreens are used to block UV radiation but these people with these cuteness compounds absorb light in the visible wavelengths. They tend to have to use quite rich barrier conditions to prevent sunlight reaching the skin.

Hereditary coproporphyria

This is unusual in that its an autosomal dominant trait so traditional its a dominant trait and it results from defects in the coproporphynogen oxidase enzyme (5) and the gene for that is located on chromosome number 3. Its sort of takes the conversion of copropophinogen into protoporphyrinogen and typically when its non functional then you tend to get a build up of the substances that come before it. It dose cause some light sensitivity but strange enough not as much as some of the other porphinogens so not as much light sensitivity. However it dose tend to cause some neurological damage to the nerves and so on. People tend to also have some phyciatric problems for this condition as well. Which is a bit differne tin that respect Its quite a rare condition again its 20 times less common that the acute intermittent form typically 1 in 4 cases in 1000,000 so quite rare form of th porphyria.

Variegate porphyria

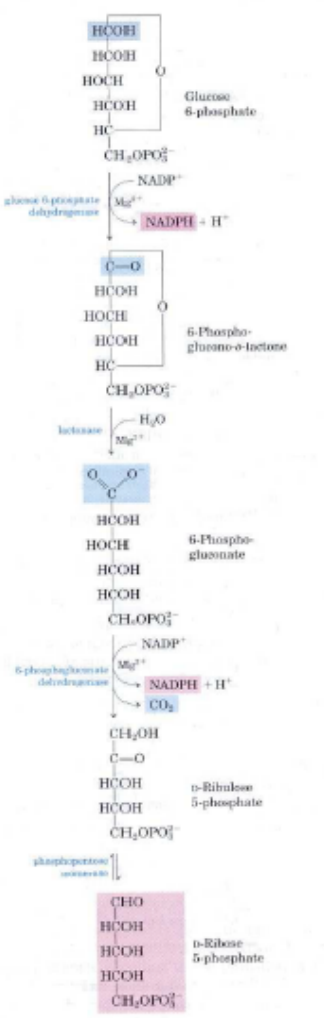
The next one down the train is a condition that results from a defect int he enzyme (6) or the protoporphyrinogen oxidase. IT is believed again to be localised to chromasome 1 again. Again people with this disorder tend not to show any manifested symptoms for th time but when they do experience an attack with this condition it tends not to be abdominal pain, vomiting and the light sensitivity. Again an acute attack is triggered by something int he enviroment sometimes a medication or something different that there not normally exposed to and sets off the conditon. Its inherited as an autosomal dominant trait and again that reflect the fact that if people ar eheterozygouse they have one functioning copy of the gene and they are therefore ok under normal circumstance but then a factor tips them over the edge and then you get the onset of the condition its lf. Variegate porphyria is an uncommon disease worldwide but it is quite an unusual condition accept in south africa. It tends to be quite common amongst the africana population of south africa. History shows that the african populations are manily dutch immigrants of africa and the incidente of this disease amoungst the africanas is about 1 in 300 so although its very rare worldwide amoungst the africans is much more common. because its so rare people have been able to trace back the condition to a single paire or single couple of dutch immigrants that arrived in 1680 in south africa who married, had children and all the people that subsequently suffer from that condition can trace there ancestry back to that single couple back in 1680.

Erythropoietic protoporphyria

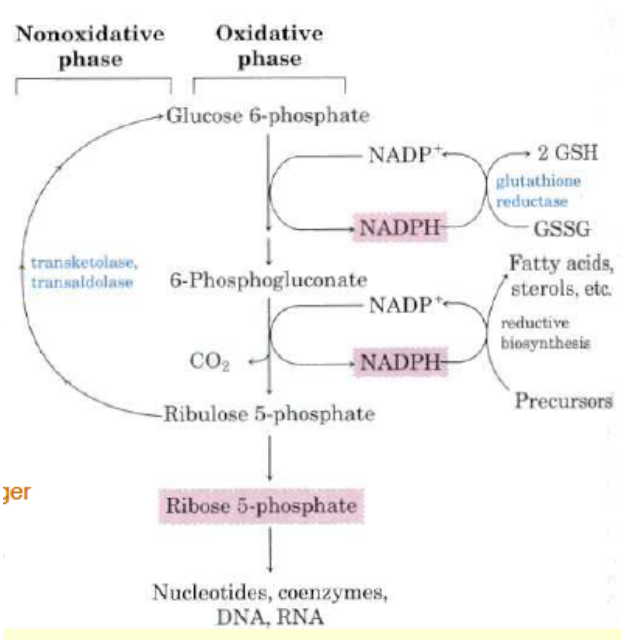
This is the final condition of the porphyrias and it is affected by the final enzyme this one number (7) the ferrochelatase enzyme which is the one that sticks the iron atom into the molecule. This is localised to chromasome number 18. Typically a defect means you get a build up of the precursor compounds. They have a distinctive photosensitivty because again we have these things that are absorbing light but they

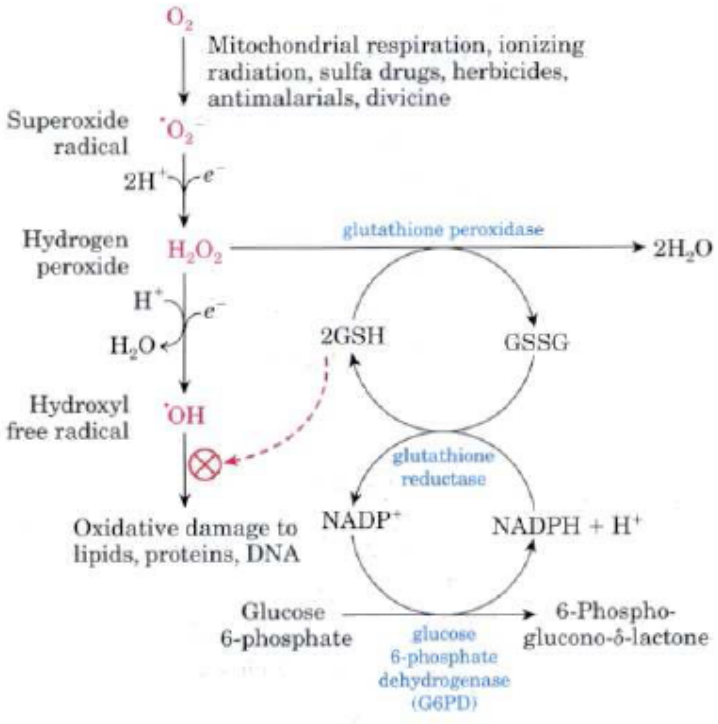
tend to be photosensitive. They also tend to have liver dissease as well. A lot of the heam metabolism takes place ein the liver so when theres something wrong with the heam metabolism it dose tend to affect the liver. The photosensitivity in this case tends to cause things like itching and burning sensation. The isidance of this particular condition is estimated to be somewhere between 1-75,000 to 1-200,000. Its quite a wide variety but because these things are so rare one aditional case of it will cause a big difference eto the incidence of it. There is a huge variability and severity in that condition and that seems to arise form the fact that theres is not just one mutation in the gene there is least 78 different mutation that have been found int he gene that encodes this protein. So this probably epxlains why you get this huge variety of situations with the condition. As we say the inheritance pattern with this one is a little bit complicated it is often referred to as an autosomal recessive condition but its is also treffered to as semi dominant sometimes or as sudo dominant trait so it has quite a couplex pattern of inheritance that dosnet fall nicley into tone o f those ctagories. IT appears to be the case that perhaps there is more than one gene involved actually forming this deases. How d you manage it, well basically you stay out of bright light or they get the photosensitive effects.

We are now going to discuss one final condition that is a bit different ot these ones but is a little more interstig and perhaps are more relevant one. The last form of inborn error of metabolism were going to talk about is the condition known as **fauvism.** Favism arises because of a deficiency in the enzyme that causes the glucose 6 phosphate dehydrogenase. It gets the name fauvism form the fact that affected individuals suffer a reaction to eating broad beans. This is the latin name which is fava. It contains a compound which can trigger this condition. Now the disease itself is charecterised by a haemolytic anaemia. Where you have splitting of the red blood cellsand that in turn causes anemia. So the red blood cells tend to brake down and cause anaemia. So the symptoms people tend to suffer form are those typical of anaemia. they are fatigued , breathless and this sort of thing in addition to this occurs the red blood cells are braking down and releasing there haemoglobin and so that increased turnover of heamoglobin can lead to jaundice because 8the liver is trying to clear the heamoglobin and that can inhibit live function and causes jaundice. Patients that tend to suffer from fauvism tend to be male. This is mainly because this has an X linked pattern of inheritance. Females can sometimes be affected by it though. Now most people that suffer form G6PD deficiency then to be asymptomatic. They tend not to have any problems under normal circumstances and its only in times of stress of exposed to certain chemicals that the condition tends to show itself. One we have said is eating broad beans but it can also arise after taking certain drugs or other recreational substance but there are a number of things i n the environment that if your exposed to can push people over the edge and we will see why in a minute. Heaming broad beans can cause haemolytic anomie we ned to understand alittle more about whats going on in the pathway here. As we said before ether faulty enzyme is the G-6-PD and it is the first enzyme in the pentose phosphate pathway. the PPP is a pathway that converts glucose 6-phosphat all the way through to form ribose phostphat at then end which will be used in the synthesis of DNA and RNA wher eye need pentose phosphatete sugars. But the problem is not with the substance sin the PPP pathway the issue arises because the PPP pathwayy dose something else it producess NADPH in tow of its teps. Its that which is keyto understanding the condition of fauvism. If we look in more detail at the PPP pathway we see that only a small part of it used to generate 5 carbon sugars for nucleic acids. The bulk of the rubies e5 phosphate that generated by this pathway is recycled back to glucose 6



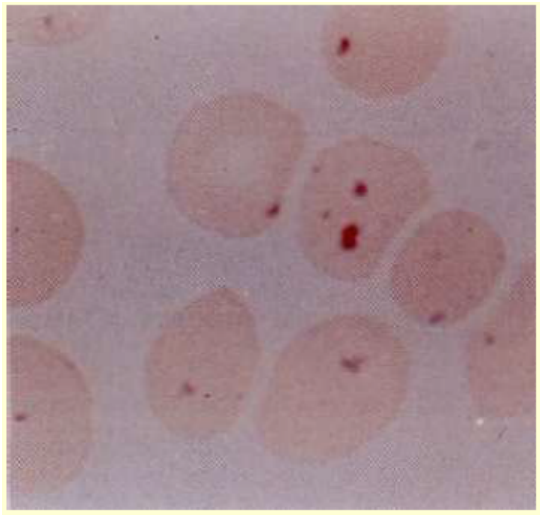
phosphate. We can see the NADPH being generate as it runs through that stage of the pathway. Its not simply making 5 carbon sugars its also making NADPH intact actually some six molecules of the ribulose phosphate are circulated back to make five of the 6 carbon ones. This thing continues to runso that after about six cycles through it the carbon that was originaly int he glucose ends up in the carbon dioxide. Now we knoww the NADPH is an important reductant that is used in a number of other chemical processes one of the main ones of course is the synthesis of things like fatty acids and sterols and such. It has another role as it also reduces glutothione and it is reduced by glutathione reductase using NADPH as a reductant. Now Glutathione is important in the detoxification of the free radicals in the cell so where using a little bit away form the glucose-6-phosphate deficiency. Which causes a defence of the NADPH thats affecting the reductase enzyme and thats leading to reduced glutathione. We are getting close ot where the problem now lies. The diagram on the right shows at the bottom our G6P pathway. It is producing NADPH. This reduces the gluatatione here by the action of the reductase. The reduced glutathione then is used by the enzyme glutathione peroxidase to deal with things like hydrogen peroxidas. These peroxidase comes form the respiration processes of the mitochondira. We have ionising radiation, there are sulphur drugs. Herbicides which also produces free radicals and such. this one here adidasine is a compound that is present in broad beans. The body emtabolisses it and you produce a free radical as a result. they often end up producing hydrogen proxyde. This then is then quite damaging and is a powerul oxidant and the body needs to get rid of it quickly. This action of glutatione peroxidase is very efficient at converting it into water but you can see that the enzyme requires a constant production of glutathione and if this part of the pathway is compromised then thats benign generated and then hence we get hydrogen peroxide hanging around longer than we what. This can produce free radicals and in turn produce oxidative damage lipids proteins and DNA and this is the orginin of our hemolytic anaemia really. Its the productionof the free radicals that is then damaging our membranes, the red blood cells and damaging other places and hence you get the symptoms of fauvism.





So although thats where the fault lies we can see that then end result is a few stages further removed form that and its to do with the detoxification of these free radicals and hydrogen peroxide. Now there is actually a consiquence of this oxidative damage to varies systems. one being that you can ge damage to the ehmoglobin molecules then they tend to cross link and form these aggregates which we call hines bodies. This is quite typical of people that suffer from fauvism. If you look at there red blood cells under

the microscope then you see these blobs and these represent a aggregate form of haemoglobin which is actually associated withth cell memebrian. The membranes become damaged by the aggregation of the haemoglobin along with the effects of the other reactive oxygen species an fd thats what causes the blood cells to then lyse and you then get the anemia. Now G6PD deficiency is unseals in that its much more common thann any of the other in born errors of metabolism that we have been considering. Its estimated that roughly 400,000,000 people world wide have the defective gene for G6PD and the severity deprends on what mutation is carried in the gene but a lot of people world wide carry it. That really begs the question is there something or other in the enviroment that conferes and advance to someones health by having this condition. It has been shown that some of th G6PD defences have occurred more in certain populations. there eis one example where it causes about a 90% reduction in the G6PD activity and occurs in about 11% of black americans. That variant also occurs in throat the meditatriarina and middle eastern regions and occurs in about 65% of kurdsh jews now you might say what do all these people hav in common. The basic bottom line is that they come form a part of the world where maleria is endemic and it has been shown that having the G6PD defencey actually injures some resistance to maleria. This indicates that the type of deficiencyy in the black americans although they have this reduced amount of G6PD they actually have half the risk of developing malaria. Although it is not wonderful it dose confer and advertge to an individual if you live in an area endemic with malaria. Its not hsatifactuly explained why a G6PD defect causes a resistant to malaria but its recognised that some of the red blood cells that have this which its obviousy the target by the malarial parasite. The rd blood cells lack this G6PD and they make them lass good as hosts for the parasite. The parasite likes to infect the red blood cells but the metabolism in these cells is altered and it may not provide the best environment for the parasites. The other factor may be that the erythrocytes don't last very long. They tend to be more fragile, to brake down and lyse then it may interfere with the activity of the malarial parasite to go through its life cycle and cause the disease.



Chapter 5 (Neurophysiology and Neurochemistry)

This chapter includes

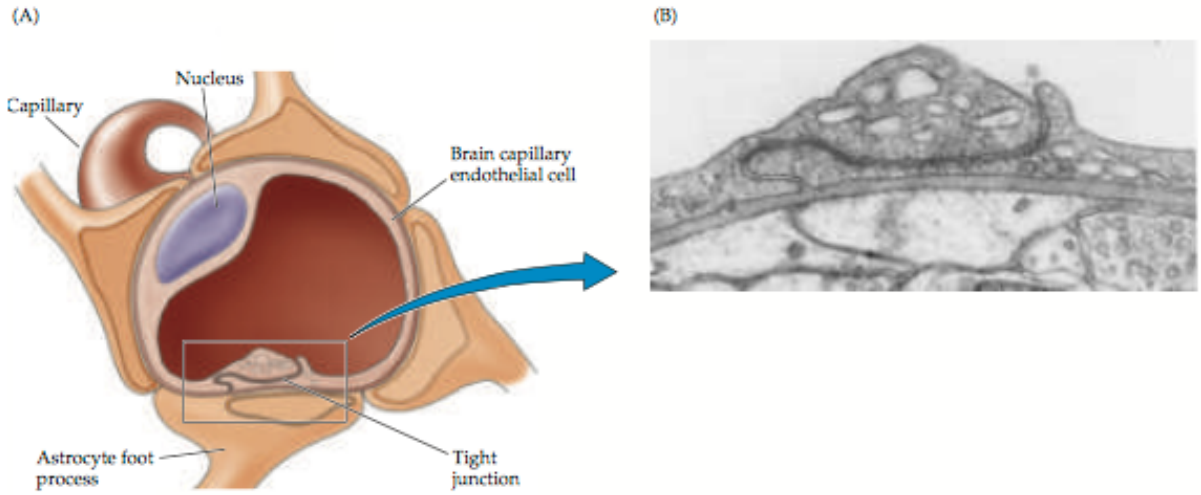
- The blood brain barrier
- Brain isolation
- Glucose
- Teminology

The Blood Brain Barrier

We are going to be moving on from in born errors for now and were going to look into neurophysiology and neurochemistry. The neurochemistry side of our brains is quite well understood but there is still a huge amount we dont understand particulrlay about the relationship between the chemistry thats going on and what we actually observe and behaviour, that sort of thing. We have to contend obviously with the complexity of the CNS and this si where people begin to get a bit lost with the sure complexity. The human brian contains 100 billion neutrons and there connected by some 100 trillion synapses. As there are multiple connections between those neurones. Some people have actually worked out there eis about 100,000 miles of biological wiring in the brain so if you think about that being paked into a space between your ears its incredibly complicated. On top of that there is various problems associated with studying the brain that we will deal with a bit later on. Its not that easy to access the brain in the same way you can access the liver and work out what is actually happening in terms of the chemistry and whats going on. So we have to develop special techniques to get around those problems. Lets start with the provision of energy.

so where dose the brain get its energy from. Well first of all we know that most organs of the body can survive without a blood supply for several hours under appropriate conditions which is why we can do things like heart and liver transplants. Those sorts of organs can be removed from a body under appropriate conditions and can remain in a viable state for several hours before being reconnected to a blood supply you cant do this with the brian. You cant interrupt the blood supply to the brain because fairly quickly within seconds it will lead to unconsciousness and fairly quickly within a few minutes there is cell death. So the brian is completely vulnerable to interruptions of blood supply. Now we might say why is that, why is it so vulnerable. Well part of it is because the brian dosent actually store high level of things like glycogen and energy stores. If we look at the levels of glycogen in different tissue i.e. if we compare the liver to muscle and to the brain. The actual levels of glycogen that is present is in a ration of about 100:10:1 so theres a much higher concentration of things like glycogen in the liver and even muscle compared to the brain so theres whats called a spare storage capacity there. Th brain levels of glycogen are only about 2-4 um per gram of tissue so really quite small quantities of reservers there. The other thing about the brin as well is that its quite restricted in metabolites that its able to work with. We know that with other organs like the liver it can metabolism things like keto acids, amino acids, fatty acids and things like glucose and so on there are a lot of other things that can be used in other organs as energy sources basically. So the brian however or in contrast cannot rely on a lot of thesoe things. A part of the reason for that really is that everything that reaches the brain filters through the blood brain barrier. Any think thats going to get into the central nervous system or into the brain has to get trhrough the BB barrier. The BB barrier was first recognised back in Paul airliche. He was a bacteioogist around in the 19th century. He noticed that if you injected dye intravenously into an animal it would stain the tissue throughout the body of the animal accept the brain. He wrongly concluded that the reason for that the brain had a low affinity of the dye. The dye was going around the body but it didn't actually stain the brian. It was in 1913 that a student called Edwin Goldner who actually shoed that if you inject those dyes into the cerebralal spinal fluid then the brian did become stained and the rest of the body didn’t. This

was then used as the evidence for a barrier stopping the moment from the blood into the brain itself and this formed the basis of what we called the BB barrier.
Its not a physical entity, its not a physical barrier of cells that stops things moving from the blood into the brian, its more of a specialised filtering system and its whats shown in the slide below.

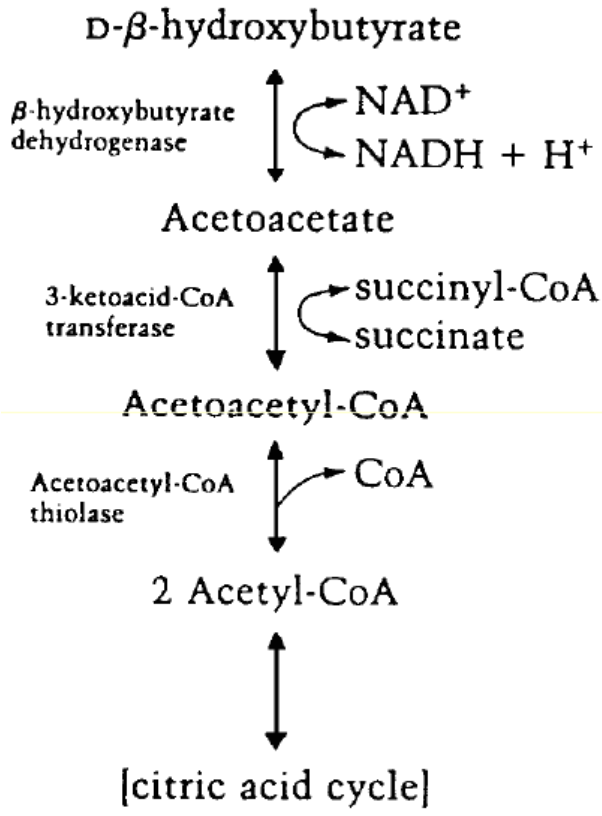


To get baring on it its showing a blood vessel in the brain her eso the blood is flowing out of the screen towards you now any thing that wants to get out through the the vessel not the brain cells themselvs has to go through the barriere system that exists the astrocytes form a tight seal around the blood vessels but there eis a pathway out of the blood vessel through a narrow channel as seen at the bottom of the blood vessel. These are called tight junctions that are tight junctions between cells. This is whats shown to the right in the electron micrograph on the right. Basically anything that wants to get to the brain from the blood ahs to go through the system here and this system has a lot of specialist transporters in it which takes things out from the blood. So things are taken out of the blood as it passes through the tight junction and they are put back into the blood vessel. This means they never get through meaning this si a very selective and sophisticated filtering system. Obviously the existence of the blood brain barrier is important conciseness for studying the brian. The brain biochemistry. If we want to study the metabolism of the liver we could just tak the liver out homogenise it and study it. However if you take a brain out then you disrupt the system of the blood brain abrader and we know that the metabolism of isolated brain tissue is different from the metabolism of the brian in vivo. So the problem is you cant take it out to study it because its never the same organism. There are also consequences for treating the disorders of the brian because whatever drugs you ingest wether taken orally or intravenously have to get past that blood brain barrier and many drugs don't do this and don't get through the BB barrier. particularly things like chemotherapeutics drugs which are completely in affective against brain tumors because they dont pass through to the brain themselves. so there are consequences caused by that. However to say we do know that if you take the brain out of an organism and slice it then the metabolism of those isolated bits of brain tissue is actually not the same as the intact brain so theres another problem there. In vivo we find that glucose is virtually the main fuel for the bria. Isolated brian tissue can use other substrates but in vivo it tends to be glucose. Its recommend that the brain consumes about 120 grams of glucose a day. Thats about 60% of the bodies total utilisation of glucose. So more than half of the glucose is being utilised by the brain. We can also work out that the brian uses 20% of the oxygen in a resting human. If your exercising though your muscles tend to use a lot more but in a resting human then 20% of the oxygen your taking in is actually being used by the brain. You can correlate those two facts and if you do so then you can work out that glucose is completely being turned into carbon dioxide and water. It docent end up in things like lactate. We know about things like muscles for example there are certain curcumstances where your muscles produce things like lactate but in the brain it all ends up a s carbon dioxide and

water. So the brian works very aerobically. They said that because of the blood brain barrier it makes studying the brian a bit comlicated as you cant take the brain out to see whats going on. So people have developed techniques for over coming that problem and one of those methods in wolves what are called artier venous different measurements or A B measurements what we do with these kind of techniques is to measure the concentration of smootheng entering the brain to measure the concentration f say glucose in the systemic arterial circulation. Then you compare that with the concentration of glucose exiting the brain. Typically by sampling from the jugular vein. Basically to find out whats gone on in the middle. So you can work out things like the cerebral metabolic rate (CMR) and that will be equal to the cerebral blood flow ((CBF) multiplied by the artier venouse difference (AVD).

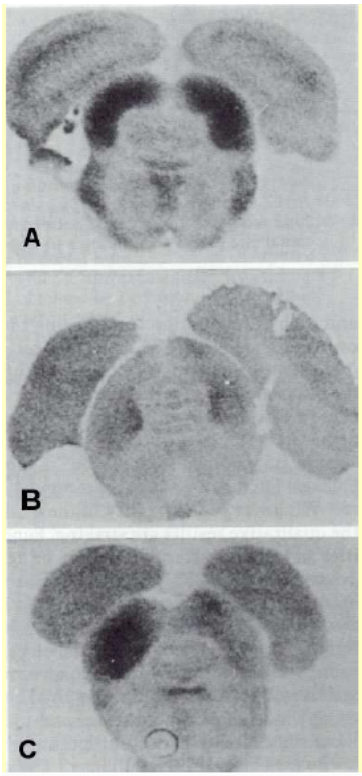
$$CMR = CBF \times AVD$$

All your really doing is measuring the concentration of some substrate going in to the brain measuring the concentration coming out of the brain multiplied by the rate of blood flow through the brain and then you can work out the cerebral metabolic rate or that substrate. Now if you do those sort of measurments you can show theres sort of an inverse relationship between the brains oxygen consumption and body weight. We dont mean the weight on a scales of someone but if you measure the weights and contrasting sizes of someones organs then what you find is that the values for the cerebral oxygen and glucose consumption are in fact about twice those of a dog and about three times for a human being. So the metabolic rate for smaller animals is often quite a bit bigger. So a rat is twice a dogs metabolic rate and about three times as much as a humans. The reason for that is probably that the neurons are more tightly packed in the brains of smaller organisms we have both neurones which are the active cells of our brains and what we call glial cells that become the packing cells or the supporting cels of the brain. Larger organisms like ourselves we tend to have more of these glial cells. Glucose as we know is the major feel source for the brian but isolated brain tissue can make use of other compounds as well. It cant make use of things like fatty acids because fatty acids normally cant pass the blood brain barrier and cannot be utilised by the brain. Howerve under starvation the brain can start to make use of other compounds in particular ketone bodies. So the conditions of fasting or starvation when the glucose is reduced then the brain can start to make use of other things like ketone bodies and in particular acetyl acetate and beta-hydroxybutyrate. under the conditions of fasting people did these cerebral metabolism measurements and what they were able to show is that the amount of ketone used by the brian rose quite significantly in fact the ketones could end up accounting for some thing like half of the oxygen consumption of the brain. So you went from a situation where relatively small concentration of the ketones are being used up to a situation where half of the oxygen being utilised by the brain was being used to oxidise these sort of substrates. As we saw over time the AV difference for things like glucosee were being halved and the brain was actually switching its fuel source under certain conditions. How do they get from the blood into the brain. Well they are passed in by a facilitated diffusion system. Nothing gets into the brin by accident. So these are passed into he brian by a precipitation perfusion system. The pathway seen in the diagram on the right tends to take place in the mitochondria. Its interesting to note though that ketone can act as a fuel source for our brains but they can not be used



to revive people from a hypoglycaemic coma. We know that as individuals as the blood glucose leves fall too low particularly in diabetics then they can go into whats called a hypoglycaemic coma. You might think administering ketone they might revive but no they have to be given glucose or things that can be converted into glucose. The reason being that you need glucose to prime the TCA cycle and it dose other things as well besides being ana energy source. So far we have though about the brain functioning as a discreet organ in the body that utilises glucose as a single energy entity. Of course though we know different regions of the brian are responsible for different aspects of our consciousness and things like the visual process and bits of the brian that carry out different functions and information of those regions can be obtained by seeing which areas of the brain are metabolically active. This is really how people have located things like the cortex or the auditory enters in the brain. They can be found because when carrying out a particular function in the brain then those ares become more metabolically active and take up more glucose and take up more oxygen. We can detect that and we can see which areas are more metabolically active. Classically those thechniques are based on something called carbon 14 labels to the glucose. So classically we would use carbon 14 labeled 2-deoxyglucose. If 2-deoxyglucose is taken up by an organism and metabolised like glucose it will be converted to 2-deoxyglucose-6-phosphate by the action of hexakinase but it dosent go any further it stops. So we take the 2-deoxyglucose feed it to an organism its taken up metabolised to 2-deoxyglucose-6-phosphate by the hexakinase but then it stops so it gets deposited. If its got a C14 attached to it then it is taken up and metabolised to a carbon14 to the 2-deoxyglucose-6-phosphate and then it become deposited in the cells. Because theres carbon 14 there you can detect that by autoradiography. A lot has been done this particular set of slides here. This is a contuse rat and if its exposed to sound then the auditory ares of its brian become active as seen in diagram A. These take up the 2-deoxyglucose and it becomes deposited in those areas that are seen.

Brain isolation



If you obstruct both auditory centers and expose it back to sound then those areas dont become as darkly stained because there not metabolically active because the rat cant here anything. Just to convince people here we have one more auditory channel that is obstructed contralateral side of the brain induces an audible density only on one side. Basically what there saying is that side of the rat that has had its hearing blocked off and so as your aware everything crosses hemispheres but sound docent so the opposite side is paler than the side where the ear is not obstructed. So it shows quite nicely how we can isolate different regions of the brain that have different functionalities. By making use of the fact that those regions of the brain become metabolically active when they are stimulated but of course these measurements require a sacrifice of the organisms or animal where it is exposed to tis stimulus. It is sacrificed. Its brain is removed its sectioned and autoradiographies are formed. This can be done in human volunteers though so we needed other ways or alternatives to this method that docent require sacrificing the organism.

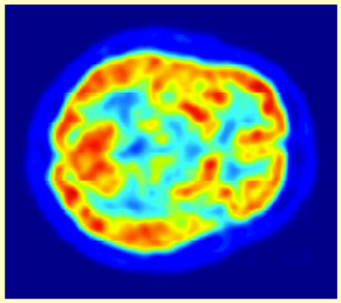
THese techniques are the ones we will discuss now where we have **positron emission tomography (PET)** and **Functional Magnetic Resonance Imagine (fMRI)**

With PET scanning its basically the same experiment as with the C14 2 -doxyglucose but instead of using a C14 label wha they use is an 18F-Flurodeoxyglucose. This si also taken up into the brain and converted into the 6-deoxyglucose derivative

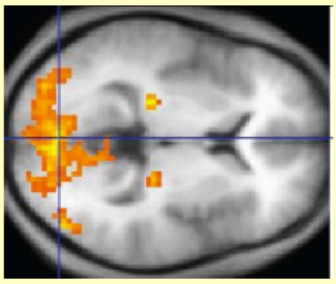
its deposited in those regions of the briain that are metabolically active but in this case instead of sacrificing and sectioning the brain you can detect the emission of positrons. outside of the skull. This can then be used to build up an image of the brain that are metabolically active under a certain stimuls. This is a nondestructive way of doing brain isolation imaging.

Positron Emission Tomography (PET) And Functional Magnetic Resonance Imaging (fMRI) Scan Of A Human Brain

The individual has been given a dose of ^{18}F-Fluoro-deoxyglucose. Red areas show where more of the tracer has been deposited, blue areas where less activity is seen.



fMRI works by measuring the change in blood oxygen levels to regions of the brain that are metabolically active and relies on the fact that cerebral blood flow and neuronal activity are coupled. In this image, increased activity is associated with the yellow regions.



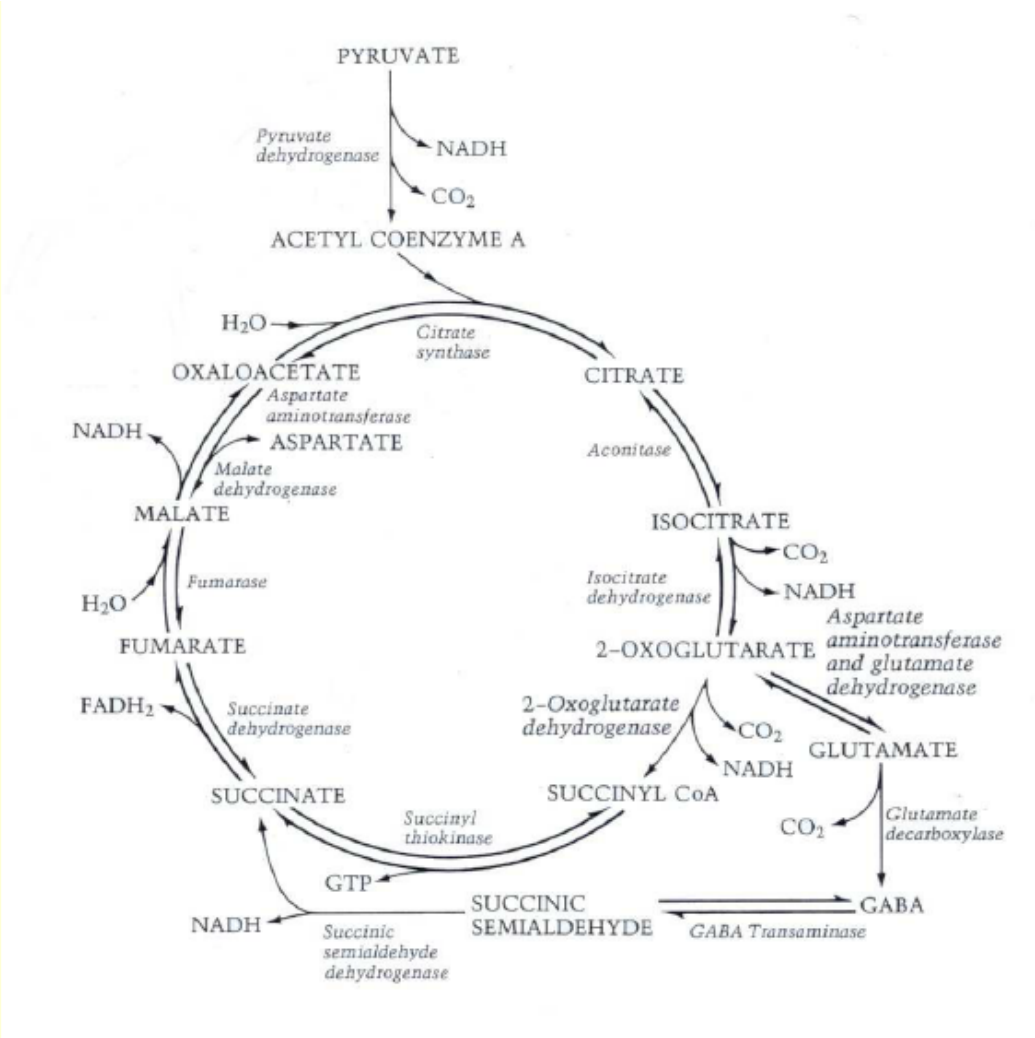
There is another method thats come out in the last few years called functional magnetic resonance imaging or fMRI. This one takes it one stage furthur where it docent actually need anything like deoxyglucose to be ingested by the individual it simply looks at activity of the brian under certain curcumstances. How it actually works is bit beyondd the scope of these lectures but its to do with measuring the changing of the blood oxygen levels of the brian that are metabolically active. So as we say when a part of the briain becomes metabolically active it uses more oxygen and hence the oxygen levels in the blood tend to fluctuate as a result of that. This is what the fMRI is picking up and it shows up as these coloured regions in the brian. So we can see which regions of the brian light up under circumstances and things like that. As with all of these techniquies though we identify different areas or regions of the brain that become more active use more oxygen and more glucose and so on so your basically picking put the metabolic rate of those regions of the brain to determine whats actually going on. Why should those regions of the brain actually become active like that then? well theres one enzyme that is particularly active in those regions when that region becomes metabolically active and that is sodium potassium ATPase. Thats the enzyme that is setting up and maintaining the electrical potentials across the me brains. When nervous activity takes place you have action potential going on and such. this only involves sodium potassium membranes. and these sodium potassium gradients have to be reset when an action potensial has taken place. and that involves hydrolysis of ATP and the enzyme that dose this hydrolysis to pump those ions and then as a consequence those ions have to be generated and thats why they become more metabolically active. So that single enzyme is whats really driving this effect that

we see. so basically when the enzyme operates to change the ATP to ADP its the change in that ATP:ADP ratio which is then being used to trigger changes in the rate of things like oxidative phosphorialtion and so on. In effect what we think of for that particular enzyme is actually called a pase maker enzyme because it sets the pase of the metabolic activity. So the bits of our brain become active in different circumstances. But changes in our metal state such like anxietyy and sensory stimulation, there not really accompanied by changes in the levels of things like the energy phosphates. So things like glucose6-phosphate, you cant examine the changes in the eves of those sorts of substances from normal activity. When sitting in an examination where your working your brian tot he limit your brain is quite capable of ammoniating those levels of sugar phosphates under those conditions and the only way yu start to see the big changes in the levels of the metabolites is in extremes of brain activity. for example if you go to conditions like barbiturate anastasia. If you anaesthetise somebody with barbiturates then you can induce a very suppress state of brain activity. or the other ones actually is hypothermia where the brain energy starts to slow down and you can less less use of metabolightes. So what can you see, well. You tend to get under these conditions, the level of ATP tends to go up as the brian is not as active as you would think it too be. the levels of things like phosphocreatine levels that start to rise. you also get increases in things like glucose-6-phosphate. It reflects a general slowing down of metabolic activity in the brain. The other extreme where brain activity is depressed or generalised seisiure states where you get abnormal activity in the brain. Perhaps the best example is an epileptic fit where someone has had a fit of seizure. Then you see as you would expelct levels of blood flow to increase to the brain. you get a drop in things like ATP which drops by 15% and phosphocreatine can drop by about 50% in the brain under these extreme conditions. So there are variouse levels of extremes that can happen to brian activity. provided that oxygen and blood flow is maintained under those circumstance then the falls in ATP are not generally significant os the brain copes without even though those levels have fallen.

Glucose

Now glucose as we say is well known to be th main provider of energy or the energy substrate but ti dose more than that. Its more than just a fuel for the brain. This is evident if you isolate look a the differences in what happens to an isolated brain tissue compared to the in vivo situation. In vitro in isolated slices of cortex you can reduce the amount of glucose concentration down to about 0.5mm before you start to see lower elves of thing like glucose-6-phosphate. You can go down even further to about 0.2mm before you star to see changes in the levels of things like ATP or phosphocreatine. So the brain seems fairly resistant when separated in to brian slices to things like glucose less but the situation in vivo is very differnet. In vivo if the blood glucose concentraten drops below about 2mm then you start to get electrical failure in the brain. this disturbance leads to a loss of consciousness. the brian in vivo is a lot more sensitive to glucose concentration than isolated brain slices would suggest. Those findings indicate to us really that glucose dose something more than acting as a fuel. Basically glucose participates in the synthesis of a number of neurotransmitters in the brain. So its not just a fuel its a starting point for a number of neurotransmitters that carry a number of neural signals in the brain. There is evidencee for this in that if you drop the levels of blood glucose then you start to see the raising of acetyl-coline synthesis being produced as well. This pint to is worth noting that oxygen also is needed for neurotransmitter synthesis. molecular oxygen is involved in the synthesis of some neurotransmitters. Again its the oxygen tension in blood tend stop drop a bit then you can get electrical disturbances in the brain. So what dose glucose actually do apart from provide us as a fuel. Glucose metabolisms in the brian is much like glucose metabolism anywhere else in the body. The pathways of glycolysis, The TCA cycle and oxidative phosphorylation are all identical in the brian. Some pathways like the hexosmonophosphate shunt thats used in the PPP to generate 5 carbon sugars. Thats not particularly active int he brain and the brain as the

brian docent have a huge requirement for five carbon sugars. So thats a fairly low activity. one of the peculiarities of the brain activity though in the brain glucose metabolism is the formation of quite large pools of amino acids particularly aspartate and glutamate and also a compound called GABA. Gaga stands for gama-amino butyric acid. the brain generates quite large quantities of these things and they come from glucose ultimately.



Some figures will help us understand so the concentration of these things are expressed as micromoles per gram of tissue

	Brain	Liver
Glutamate	8.7mm	4.48mm
Aspartate	2.23mm	0.87mm
GABA	2.27mm	0.1mm

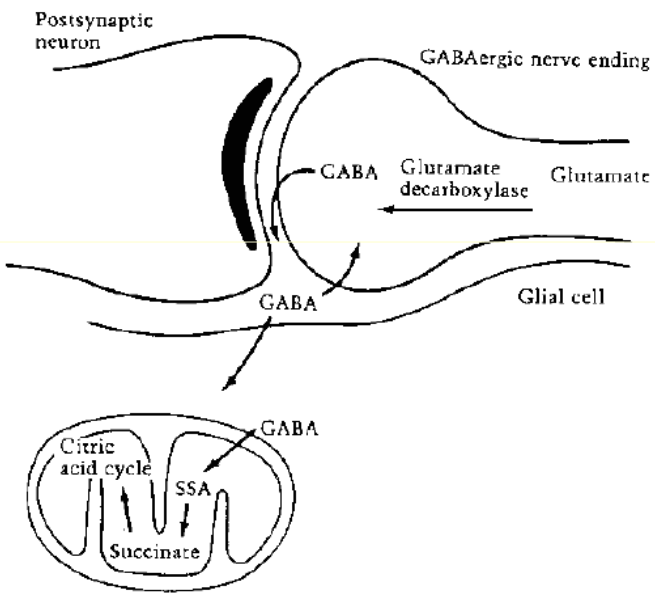
so the brain is producing significantly high concentrations of those amino acids. Glutamate and aspartate have roles as neurotransmitters in the brain. GABA is technically an amino acid although its not a portion amino acid. The brain generates quite large quantities of that because its a neurotransmitter. Homemade

GABA is made in the particular cycle called the GABA shunt and its a short annex really to the TCA cycle. The bulk of the diagram above is just the TCA cycle but the shunt on the side is the way we produce GABA. Its formed from alph-keto-gluterate there which is converted enzyme aspartate aminotransferase and glutamate dehydrogenase to give us glutamate and then glutamate reacts with the enzyme glutamate decarboxylase to form GABA. To complete the cycle then GABA can be metabolised by GABA transaminase in this compound succinct semialdehyde. This si acted on by Succinct semialdehyde dehydrogenase with the elimination of NADH and succinate is formed that goes back in ot ht e cycle. So its sa short annex to the TCA cycle.

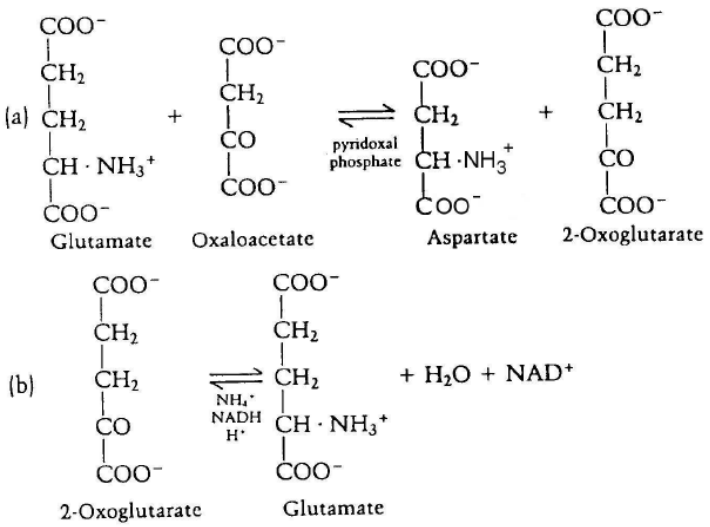
This pathway dose occur in other tissues as we have seen it in the kidneys, liver and the heart but its by far the most active in the brain. now in terms of energy generation involved its not quite as equivalent to the TCA cycle itself. The TCA cycle and GABA shunt produce an molecule of NADHand that sort of thing is equivalent to three ATP's. But whats idffenet is that the TCA cycle generates a GTP at a stage that is equivalent in the GABA shunt. The molecule of GTP is like an ATP equivalnet and that isn't produced in the GABA shunt. So the GABA shunt isn't as efficient i suppose as the TCA cycle. The efficiency isn't what the GABA shunt is about though its about producing GABA. its recommend that about 8-10% of the flow through the TCA cycle actually comes thorough the shunt. so its really just a means of creating GABA.

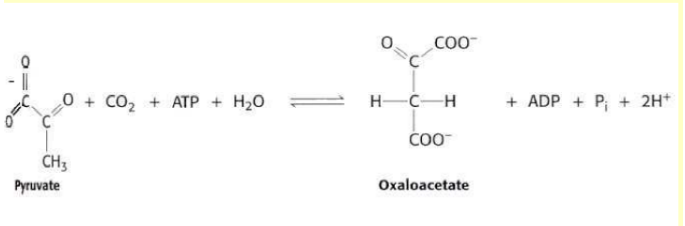
Temrinaology

Now before we go any further we need to go over some terms. Neurotransmitters as we have begun to talk about are chemical subsatances that communicate signals from one nerve cell to another. We can think of them as being divided in to two broad categories. We talk about inhibitory neurotransmitters and there are excitatory ones. So we think of our neurotransmitters as being broadly classified as inhibitory and excitatory ones. The inhibitory ones tend to calm down a nerve cell which dampens the nervous system and reduces the CNA activity. The excitatory ones get things up and going and firing away. Obviously in a normal functioning brain what you get is a combination of the two between the inhibitory and excitatory ones. If something happens to affect one of the two categories then you get an imbalance and then you can get a disturbance in normal brain activity. Now GABA as we have talked about here is an example of an inhibitory neurotransmitter. Its a neurotransmitter that normally calms things down in the brain and its present in quite high concentrations as we have already seen. This concentration here equates to about 1millimolar. Its found in most regions of the brain that happen to be the most abundant of the inhibitory neurotransmitters that found in the found in the brain. The GABA synapse or the GABAergic synapse to use the terminology looks a little bit like the diagram below. Here we have our nerve ending here. We have the glutamate that has been converted into glutamate thats been converted with the enzyme glutamate decarboxylase into GABA The post synaptic neutron is on the left. The GABA itself is placed onto ta nerve terminal it dose its job and then it can either be taken up again in the cycle with the presynaptic nerve or it can be taken up not the surrounding glial cells, taken off to the mitochondria where eit will feed back into the TCA



cycle. This system is then relatively simple and its quite widely used throughout the brain. \we mentioned that its an inhibitor neurotransmitter but there is something that underlies something that was noteced a few years ago. Glutamate decarboxylate requires vitamin B6 as a co-factor or pyridoxal phosphate as a co-factor and vitamin B6 is the precursor of the pyridoxal phosphate. now the significance canme clear a few years ago when it was noted that a number of babies died. These babies suffered from fitting or seizures which in some cases provide to be fatal. Now these babies were being fed on a typee of infant formual. Like artificial milk that didn't contain any vitamin B6 and as a lack of that the babies were suffering from a lack of pyridoxal phosphates. the enzyme glutamate decarboxylase coundnt function properly and they couldn't maintain a level of the neurotransmmitter GABA and as we say the GABA normally inhibits and calms the brain down. So if you lack the inhibitory one the excitatory one tends to become more dominant and leads tothings like seizures in these children. Once it was recognised what the root cause was the infants formula was suplimneted with vitamin b6 but it shows how an understanding of neurochemistry is affective. We also noted as well the concentration of glutamate and aspartate are quite high in the brain, exactly why that is isn't something anyone has fully explained. Its probably only a very small preportion of the toal amount of glutamate or aspartate that act as neurotransmitters. You dont need new the concentrations that are available to act as a neurotransmitter so quite why theres s much, people dont tend to know. Probably only about 1:100th is used for neurotransmission. These ammoniac's as well are used in things like protein synthesis so it has some function. The pathways to the synthesis of these amino acids are quite strait forward. You basically involve them in aminotransferase relations so glutamate, with oxaloacetate makes apsartate and 2-oxoglutarate and 2-Oxoglutarate can be converted to Glutamate. So its a fairly simple reaction in terms of conventions of these molecules with dicarboxycylic acids. One of the concequeces of the extensive formation of things like glutamte and aspartate is that they consume TCA cycle intemrediates. 2-oxogluterate and Oxaloacetate these things are componentss of the TCA cycle. If the components of the TCA cycle actually act like carriers you get acetyl-CoA coming in one side of the acetate. Its the C2 from acetate that are metabolised and excreted as carbon dioxide. Its not the other carbons that make up these other compounds. These act like carrier molecules in the TCa cycle. So if your taking some of these carriers out you start to depleat the TCA cycle of its components. If your generating quite learn quantities of these amino acids then you can depleat the TCA cycle of its componnents. So what we tend to find in the brian is theres quite high activityies of the enzyme Pyruvate carboxylase. Pyruvate carboxylase can take pyruvate it carboxylates it using ATP to form Oxaloacteate and ADP and of course then the Oxoloactate can then go back into the TCA cycle to regenerate this missing compunds ones they get consumed in the formation of these amino acids. That type of reaction we call an anapleurotic reaction (Its a replenishing reaction). there area number of them in the body but this one specifically has quite high activities in the brain and the reason for that is because we nee to regenerate the Oxaloacetate in order to replinish the TCA cycle intermediates and then consume the formation of the amino acids.





Chapter 6 (Neurophysiology and Neurochemistry)

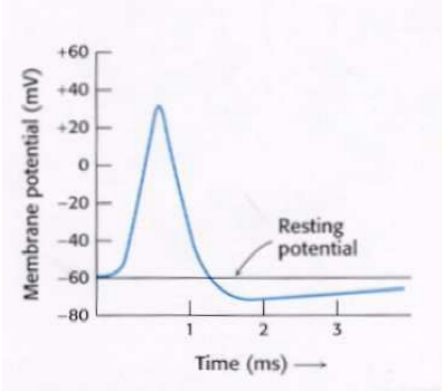
This chapter includes

- Action potensials
- Electrical synapse
- Chemical synapse

Action potensials

Last time we touched on some amino acids like glutamine and aspartate, not to mention the amino acid GABA of which all are used as neurotransmitters. As we can see there are quite high quantities of these compared to other systems. We pointed out that there synthesis are done in quite high quantities and using some of the TCA cycle intermediates and therefor they become depleted and there are reactions like the pyruvate carboxylase that help to replenish the intermediate in the TCA cycle.

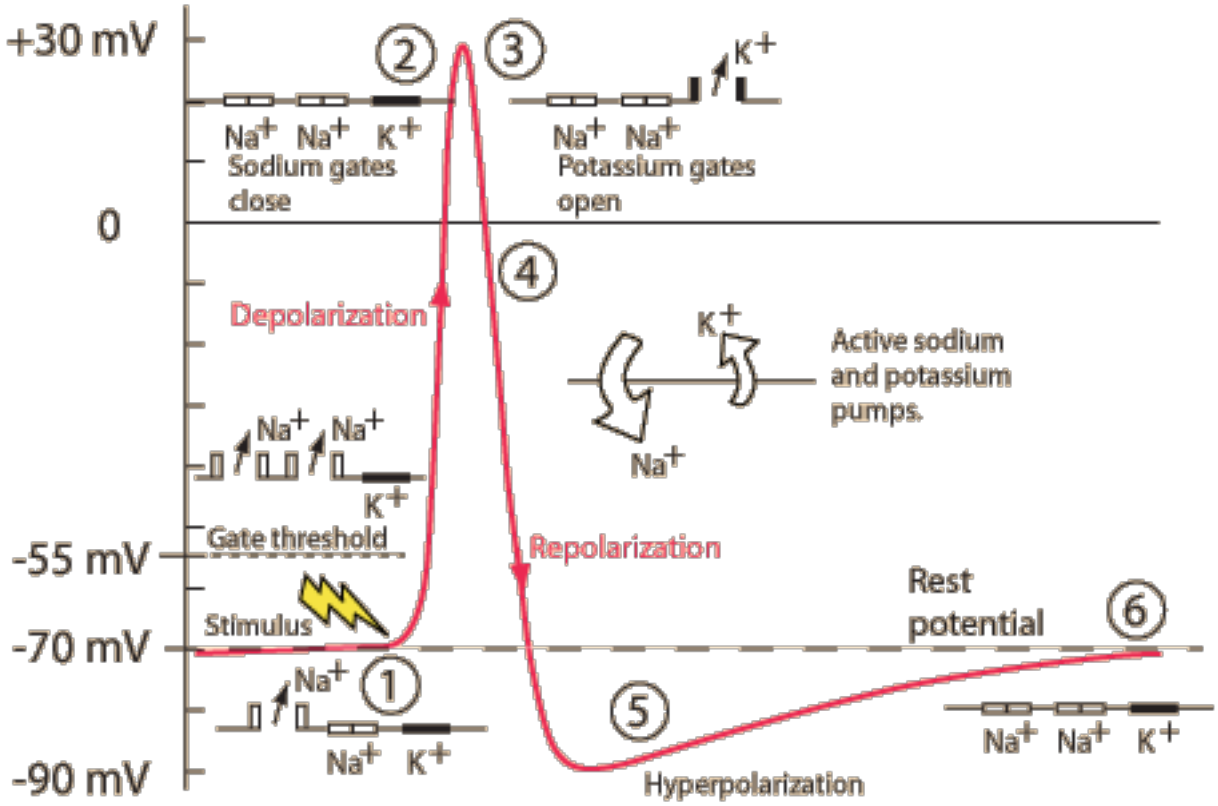
What were going to do today is go on to look at some of the more detailed relationships of the biochemistry of neurotransmission and to understand what a nerve impulse is. Most think of an electrical impuls as like a charge flowing down a wire but a nerve impuls isn't like that it is an electrical impulse traveling down a memebrain but not like a wire.



The internor of a neutron likemost cells of the body contains an ionic difference really so we have a neuron where inside the cell we have a high concnetraion of potassium ions (K+) and a low concentration of sodium ions (Na+) Out side the cell its the other way round where there eis a small amount of potassium and a large amount of sodium. There is then an imbalance set up. Across the membrane that is set up and maintained by our old friend sodium/potasium ATPase. This remember used the energy of ATP topump sodium ions out of the cell and to bring potassium ions in. There is then an imbalance in low inside the cell with high potassium and the opposite outside. This differencain ionic concentration across the membrana means there is a potential difference across the memebrain where there is a voltage across there. In the rest state its wrought -60mV. Now a nerve impuse arises when the meebrain potensial because depolarised. Something starts to trigger the movement of ions across the me brain and the memebrain potential changes from about -60 to about -40 to -50 area. Once that start starts to happen a cascade of the reaction occurs which causes a massive depolarisation of the me brain and the me brain potential swings up as high as the +30mV in the space of about 1mS before it comes back down again to its resting potential of -60mV. In fact you often get an overcorrection to about -70mV before it stabilises back to the -60mV region. And as we said this all happens over a time scale of about a few milli seconds. Now those action potensial arise because a sudden large change in the permeability in the nerve cell membrane to the sodium and potassium ions causes by the ionic flows across the membrane.

If we look at the process in a little bit more detail then we can see what happens to the various ions currents. People associate the potassium ion matched against the changes in the electrical properties of the membrane, now what happens initially is the sodium conductance is what changes first of all. So when the action potential is stimulated to occur the first thing that happens is something becomes more permeable to sodium ions and remember there eis high sodium outside and low sodium inside. So when the meebrain permeability changes to sodium the sodium floods into the cell. Initially it occurs at a rate of about 6000 ions come in per ms per channel that start to come in. this starts the process of, remember there is a slight increase in positive change in side the cell. Then when things start to happen that initially

starts an wash of an explosve oppening of the sodium channels in that me brain that causes massive influx of sodium in that channel so the permeability suddenly increases and once its pushed over the ege it just goes. That officers then causes this big change in the me brain potential as the sodium ions flood into the cell. Almost as quickly as its been switched on though its switched off again and the permiablity drops away very rapidly. Round about the same time the permeability of the me brain to potassium ions starts to increase and it overlaps with the sodium and ofcoruse we have high potasium inside th cell so as the permeability of the membrane to potassium ions increaees there is a large rush of potassium out of the cell. Its then the out flow of potassium ions which is why we see the voltage difference bac down to a resting state once more. And then just as quickly the permeability is deceased and the potassium flow stops suddenly after about 2mS. After this stage now we are back doen to the resting potensial where we might get a slight hyperpolarisation here and then the whole thing is returned to its resting state once more. So that wave of depolarisation then moves rapidly along the cell membrane.

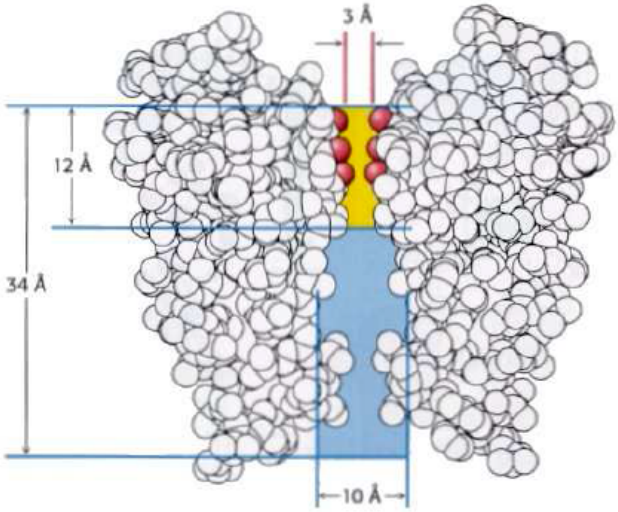


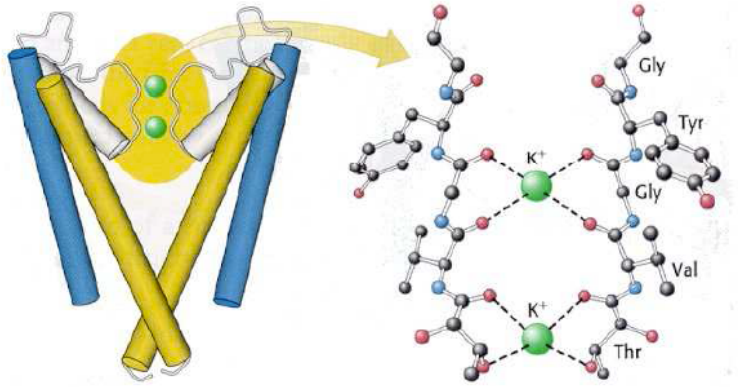
We are looking her eat th cell as a sort of end on but of cours in a cell you have a neve axon and the nerve bodyy to one end. The action potensial propergates along the nerve axon away form the nerve body to the nerve terminal. So the wave of depolarisation is actually going along the nerve until it reaches the snaps of the nerve at the other end. Its not like an electrical voltage or having wie with the current running along it. ITs more like a wave of deolarization spreading along the me brain. As each bit becomes depolarised then it depolarises the next bit andso on. But then the permeability to the ions behind the action potential is shut off again once more so it only travels in the one direction. Obviously for that process to occur though you have to have some sort of potasium channels that are capable of opening and closing rapidly. The whole process hinges on these channels opening and closing within milliseconds.

Sodium potassium channels

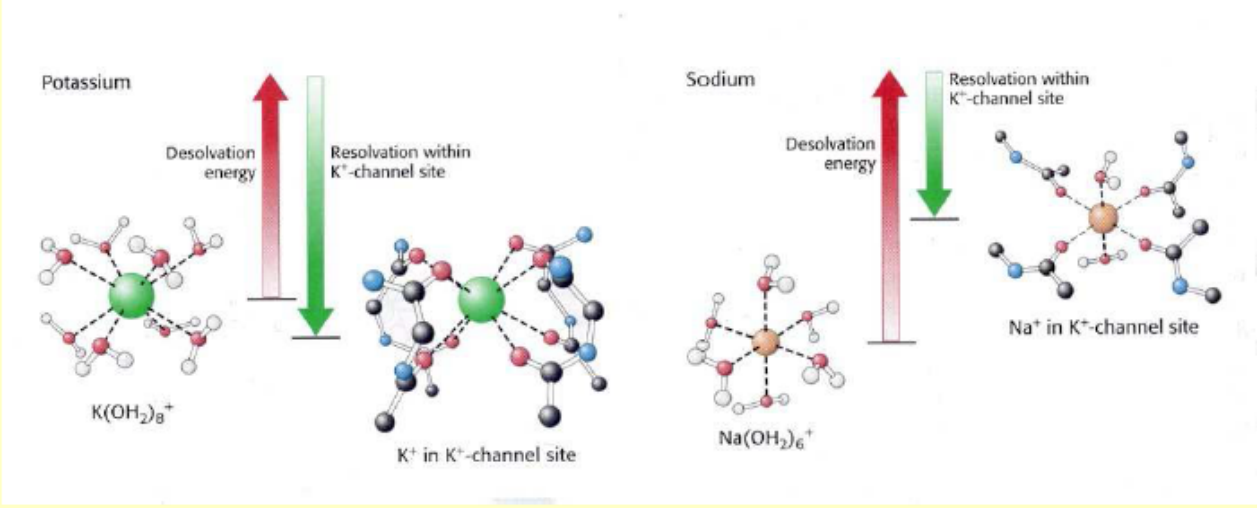
So what are those channels and how do they work? They obviously have to be sensitive the changing membrane potensial there. Therefor they must be voltage sensitive where they sense the change in the membrane potensial here there opening and then there closing again so there sensitive to the voltage difference across the membrane. Both of those ion channels the sodium and potassium ones can actualy be isolated and studied and so people know what they look like in quite a bit of detail. the first one to be isolated was the sodium channel and it consists of a 260KDa polypeptide chain. The potassium one has also been isolated and that is a 70KDa portion but there are four identical subunits. You can think of it as 7x4 = 28 so the sodium one is 4 times as large as the potassium one. Theres quite a bit of sequence homology between the potassium and sodium channels. They do appear to be similar in the way in which they work. This one seems to be a four subunit 70KDa subunit where as this one is a 260KDa unit thats like the four subunits role up togather. Now considering those challenges the first thing we have to answer really is why dose the potassium channel which is about 100 times more permiable to potassium ions than it is to sodium ions. ITs weird because sodium ions are smaller than potassium ions. SO why is it that the sodium ions cant go through the potassium channel when it opens up? The answer to that lies in the free energy costs of dehydrating the ions.

Before a Na+ or K+ ion can get through the channel and thus through the membrane it has too loose its shell of hydration. Because there ionic ions they are hydrated normally. The loss of the hydration to that ion has an energetic cost to the ion. Now the potassium channel is not simply a hole through the membrane, there is a channel but its not just a huole. Basically the potassium ions have to give up there water molecules of hydration before they can pass through the channel and the channel looks a little like the space filling model below.





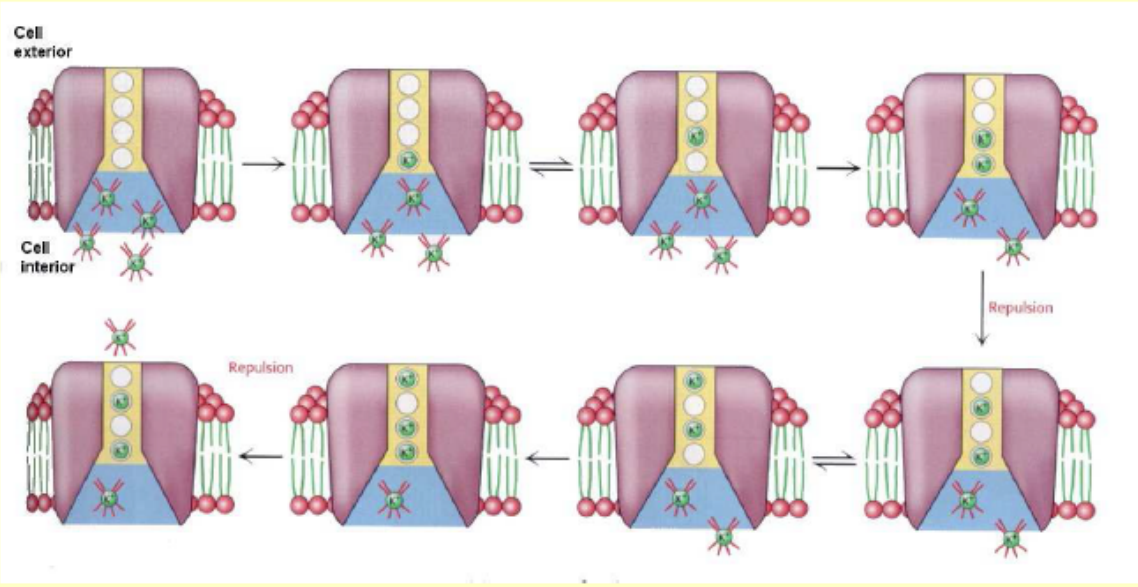
This ions are moving from the blue side to the yellow side so from inside the cell to outside the cell. The channel actually has a pour on the inside of the cell about 10A in width and this allows water or a fully



hydrate ion to enter this part of the channel. Howerve as the ions pass further into the membrane the channel begins to narrow. In order for the ions to enter this part of the channel the hydrated potassium ions have to loose there water molceuls and become dehydrated. Theres an energetic cost associated with that. That energetic cost is ofset by the potassium ions interacting with specific residues in theis part of the channel itself. They can loose the hydration but as they do so they immediately begin interacting with specific groupings in the channel itself so the energetic remain favourable to do that. Sodium ions on the other hand all though there smaller they can get into the channel but they lack the dimensions when they need to interact with those groupings so energetically the sodium ions cant shed its water of hydration and interact with those ions up there because its too small. This shows a selectivity in the channel. There are four subunits involved and there are just two shown here and these grouping of the amino acid that interact with the potassium ions this is a ball and stick model shown below. Where two of the subunits are shown for clarity but the potassium ions are able to interact with the threonine, glycine, tyrosine and valine residues. There is a specific sequence of amino acids orientate there with groupings son them that allow the potassium ions to interact with those groups as they pass through. The sodium ions are too small to gap the bridge between the groupings in the selectivity filter. So because of those differences the energetics of the desolation,resolvation of the two ions is much more favourable towards potassium ions than it is sodium
ions. We are looking really at the potassium channel but explain why sodium ions which are smaller cant get through that channel.

In the case of the potassium ions in the diagram on the next page showing the potassium channel. The potassium ion is generally hydrated with about 8 water molecules there and there is an energetic cost in desolating the ion but the energetics of the resolution with the potassium channel there is done so that its pretty much balanced and the ion can shed its water to interact with the selectivity filter. In the case of sodium ions which have about 6 water molecules associated with them there is the energetic cost of desolvating the sodium but because it cant interact properly with the groupings in the potassium channel the resolution energy is smaller so the energy balance is unbalanced and the filter is able to discriminate between the two ions in this way. The second thing about our channels her is that you might expect the desolation resolution malady thats going on is going to slow down that passage of the ions through the channel but in act we know they can flow through at about 600 times per mS. So somehow other the ions can get through that channel quickly and even allowing for the salvation desolation time process thats going on. This si now shown more clearly in the slide below where we see a K+ ion transport channel and how it actually works.

A Model For K^+ Channel Ion Transport

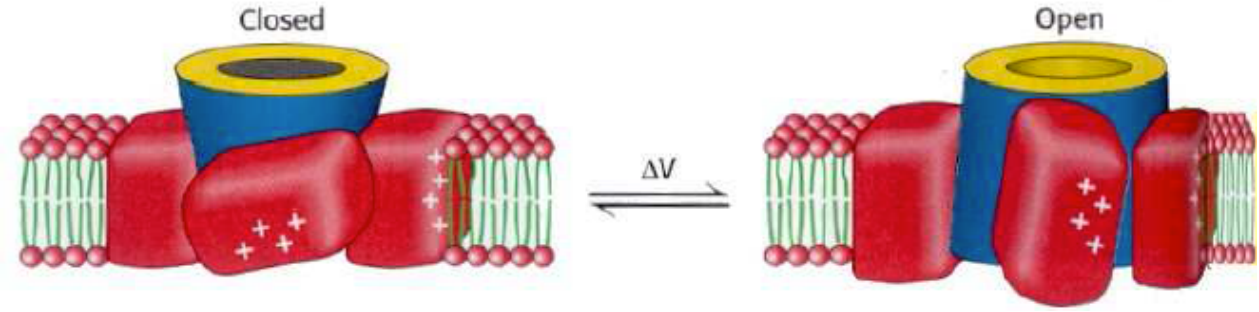


The selectivity filter has four binding sites. Hydrated potassium ions can enter these sites, one at a time, losing their hydration shells. When two ions occupy adjacent sites, electrostatic repulsion forces them apart. Thus, as ions enter the channel from one side, other ions are pushed out the other side.

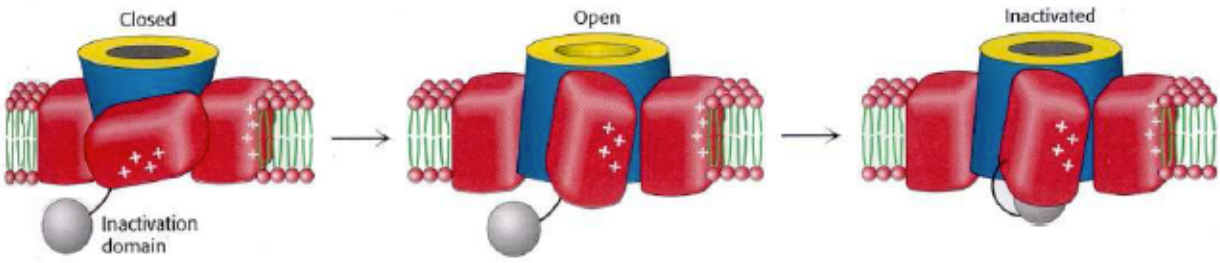
Biochemistry 6th Edn. Berg, Tymoczko & Stryer, Chap 13, Fig 13.21 Freeman

Initially starting with the top left diagram we see the cell interior and exterior. There are some potassium ions with there water of salvation molecules attached to them. They enter the channel and there are four binding sites within the channel. Initially of course is the first potassium ion enters the channel looses its hydration and interacts with the first site. As more ions enter the channel towards the bottom there the ions that havee already entered can move up to higher sites. So we have a situation now where we have a second potassium ion entering and being desolvated and entering at the first binding site. The first ion is then pushed away because the incoming ion has the same chanrge and pushes on the first ion. As more ions enter in to the channel they keep pushing the nextt ion up so as more enter it effectively uses an electrostatic repulsion. Eventually they pop out and become rehydrated once more. The driving force for this is that there is a much higher concentration of K+ on one side of the membrane but there is also some choice in direction with the selectivity filter. Its not a pump it is a channel that is selective for one ion. Basically its selective for potassium ions but its also quite quick in that the electrostatic forces push them through once it starts.

So this explains two things really. It explains how the potassium ions are selected for the potassium channels because of the size of the pour and also that its fairly rapid which can be explained by the quickness of the process. But we still have answer how that opening an closing of the channel is linked to the action potential. so how is it switch on and off, and in particular how dose it sense that change in the voltage across the membrane. Its not on all the time its the action potential that switches it on and switches it off. So we have to explain that. The way in which the model has been proposed for that is the gating of the channel is seen below.



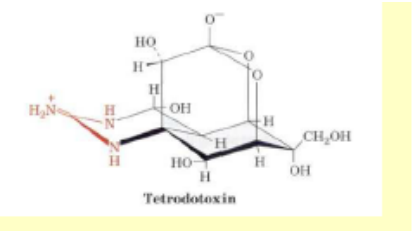
You can believe that the channel pour here you can see on the left is in its closed position where there a re voltage sneaking panels which lie in the down position. We have outpour and the ions that are going to go through it. Then there is a paddle attached to the ion channel there which in the normal position lies in the down position and effectively the channel is then closed. This thing is not open all the time, normally its shut off so that potassium cant come through and the panel provides the shutting off mechanisms. This stops the potassium ions going through that channel under normal curcumstances When membrane depolerisation occurs in that region of the membrane where that ion channel is located. The membrane current courses through that pat of the membrane and that motion pulls the base of the channel apart and opens the channel up. So as see that action potential reach that part of the nerve fiber. where that channel is locate, the voltage sending pals sense that chaning in membrane potential, swing to another position and its like opening the tap. The bottom of the channel becomes wider then allowing the ions to start entering the channel and passing through the membrane.
So now the potassium ions can start to flow out of the cell but when we looked at those ion flows before both the sodium one and the potassium open but then they close. So somethings got to shut it off. Closing that panel again isn't quick enough. The shutting off of theat channel happens more quickly than can be accounted for by that paddel. So something has to happen to shut the swing off. So we know both potassium and the sodium channels can become inactivated within milliseconds. So what mechanism is there for stopping those channels without resetting the pedals. Well the firs clue to how that might arise come from studies in which the plasmic side of the membrane was exposed to trypsin. Tyripsin as we all know is a proteolytic enzyme that cleaves of protein and when people did this and exposed those channels to trypsin you basically cleaved off a bit of the protein. Once you have trimed the protein back with trypsin the channel stays open after depolarisation. Exposing those sides of the channel on the inside of the membrane cleaves a bit of the channel off such that when a nerve impulse arises the channel would open but the panels would still operate but they didn't shut off after woulds. Therefore the trypsin action had cleaved something away that was responsible for cleaving that channel quickly after the action potensial. People were also creating mutants that lacked about 42 residues from the end terminal. So using genetic manipulation they would create mutant potassium channels that lacked about 42 residues from the end terminal and they again open in response to membrane depolarisation but didn't close. So it seems like there is a bit of the protein that is responsible for shutting off that channel very quickly. They took this one stage further and showed that if you put that bit back then you would restore the ability of the channel to close once more. It was quite nice evidence then that there is a bit of the protein responsible for closing this channel off after its been opened. The explanation of that data is the ball and chain model seen below.

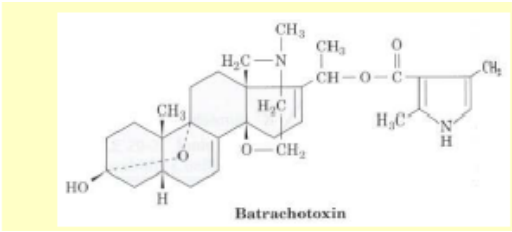


So we see there agin there is our voltage sensing paddle and but it also has this inactivation domain which is often shown as a ball and chain type arrangement. Now normal activation is tethered to the paddel by a chain. Now in the closed state it is located in the cytoplasm and is basically hanging free. Along comes our wave of depolarisation and the voltage sensing paddle swing to the open position and the potassium ions then start to flood out. Once the channel is opened up the ball quickly flies round and plugs the hole. Then when its blue the panel resets itself and the ball drops out. Then the channel goes back to its closed position. This then all happens in the space of a few milliseconds. In a sense you can think of that tethered ball as being like a large tethered cation or large potassium ion that can get through the channel. People have actually done experiments where the alter the length of that tether region and they have shown that if you increase the length of that tethering region then it increases the time taken to inactivate because the ball is able to fly around a bit more before it plugs the whole. If you shorten the tethering domain then it finds the hole more quickly so its quite a nice mechanism.

We have been talking mainly about potassium channels but its believed that the sodium channel and also the calcium channels work in very similar ways. There are voltage sensitive sodium channels and voltage sensitive calcium channels as well. There all believed to act in the same sort of way as that.

Those voltage gated channels though are actually the targets for a number of very toxic compounds and we have learnt a lot about how the channels work by using these compounds to actually study the function of these things but the voltage gated sodium channels which are known to be the site for a number of very toxic substances. There actually very few toxins that affect potassium channels. its the sodium channels that nature seems to be bothered with. We will go through a few examples of those.



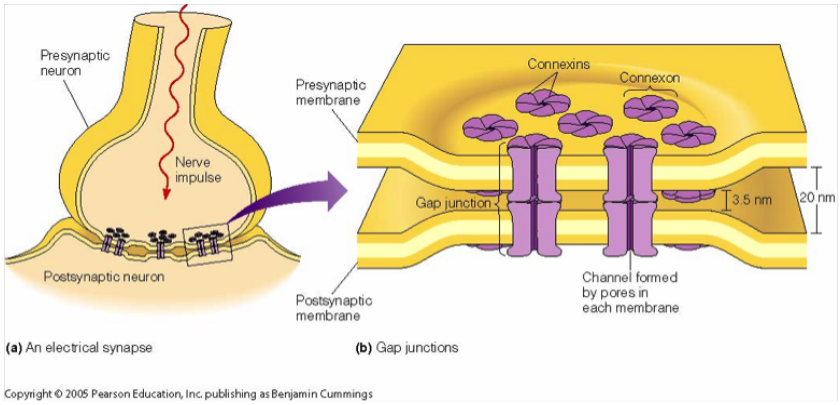


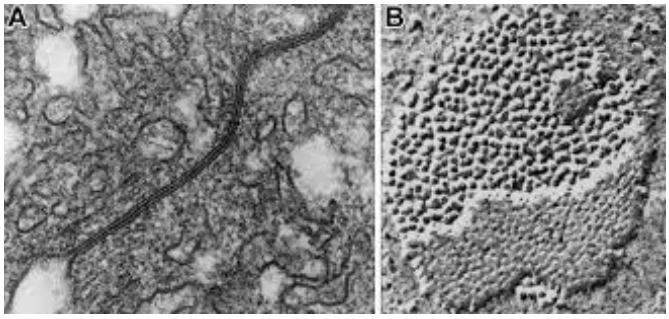
AS we see they have a subtle interest as they have been used in a few studies and things. Tetrodotoxin, saxitroxin and batrachotoxin are three very potent toxins all which act on the voltage gated sodium channels. Tetrodotoxinthe first one we see here is a paralytic poinson that occurs in the liver and intestines of the puffer fish known as FUGU in japan. The puffer fish is a delicacy in Japanese but the the chefs that prepare it have to be very well trained in order to remove the toxic parts of the fish. There have been incidences where people have been poisoned and even died by ingesting the wrong bits of the puffer fish. This one works by specifically blocking the sodium channel. you can understand the paralysis because your blocking neuromuscular transition by blocking the nerve impulses so it causes paralysis. Where you cant breath and things like that. Simerlely the the sodium channel can be blocked by this

compound called saxitoxin which is produced by marine dinoflagellates. which is a toye of plankton responsible for the red tide you sometimes get in the sea. ITs recomended that you don't swim in the sea when you see these red tides but the biggest problem as far as mammals and other animals are concerned is that this saxitoxin is concentrated by feeding shellfish. So a small muscle can contain enough saxitoxin to kill about 50 people. The muscles ingest the dinoflagellates and in doing so concentrate the toxins of these dnioflagelates so that the shel fish becomes incredibly toxic.
The final one is batrachotoxin with is an alkaloid recreated by the colombian arrow poison frog. It is actually in the guinuess book of records as the most potent known venom. It is extremely toxic substance. The reason they are so toxic is because the fatal dose of the TD50 as they call it just 2 micrograms per Kg of body weight in mice. again it specifically binds to voltage gated sodium channels that we find in organisms. But this one instead of blocking the passage of the ions this one renders the me brain high permeable to sodium ions so its works slighltly differently. Bizarrely the Batrachotoxin can be counteracted by tetrodotoxin. Batrachotoxin blocs the channel open allowing sodium ions through the channel but tetrodotoxin actually blocks the channel so they can compensate the other. Might be kl to lick a frog and then eat FUGU.
So we say there interesting to us because they have been used in the study of how these channels work. They are used as experimental tools.

Electrical Synapse

So we have looked now at the process of the action potential. We said before to start a nerve impulse we need to start an action potential, it then runs along the nerve axon to the nerve terminal. The nerve body has is nuclei and the action potential propagated along the axon to the nerve terminal where it reacheds the snaps which is the junction with the next cell. So we are looking to set the propergations of that potential. Now at some stage it reaches that snaps and the signal has had to pass to the next cell and be propagated on down there. At some stage the action potential is going to reach the end of the cell and then transfer to another cell and the signal is passed on to the next one. We have to think a little bit about how that actually works. Now there are a couple of different snaps that exist. Ther is one type known as an electrical snaps. Now the electrical synapse are customized for very rapid conductions of nerve impulses. So the signal can pass form one cell to the next one extremely rapidly. Now what we find when those two cells meat one another there is a small gap between them, and in an electrical snaps the gap is only about 20A in width which is very narrow. Its spanned by things that are known as Gap junctions. This is what the slide here shows where we have and there is a very tight association between those two dnendrties. the gap between the cell apeart to dicier but its actually traveresed by a number of cross bridges. The gap between them has almost disappeared. If we freeze fracture then like the diagram in the micrograph then we can see that they are clustered of pours. The dots you can see are known as connexions that are groups of proteins that form these channels

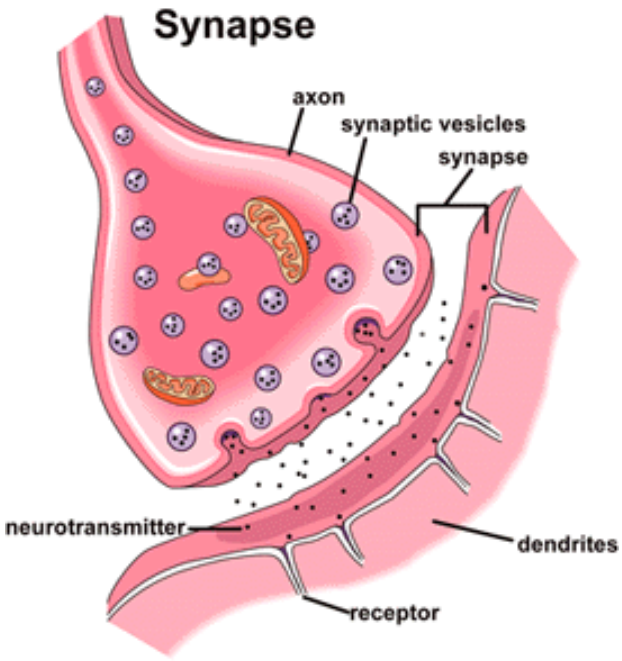




between the two cells. SO this si whats found at an electrical synaps. Those connexions are made up of six portion subunits arrange in a sort of a circle and the circle is about 10nm in diameter with about a 2nm central pour.
That central pour then forms a direct connection between the two cells so when the signal passes from one cell to the next it is effectively passing from one into the other. There is no gap between the two. Its not known how those things form up and how there able to construct a system that allows this channel from oence cell to another. Its small enough though to prevent the cell contencts from flowing between the cells but it dose form a very close connection for very rapid means of transport. So thats an electrical snaps between the two cells.

Chemical synapse

This is perhaps the one that is more familiar where a chemical synapse has a action potential reaching the pre synaptic nerve terminal that causes the relaxes of neurotransmitters. So we get a release of some kind of chemical signal, we have seen a few of these like GABA and things. The release of a chemical neurotransmitter which diffuses across the synaptic cleft to reach the receiving neurone which interacts with a receptor that triggered a signal in the post synaptic neurone. When the electrical one the signal passes almost instantaneously from one cell to the next one but in a chemical synapse the action potential causes a release of a chemical substance that diffuses across the synaptic cleft binds to a receptor on the post synaptic cleft and produces a action potential in the next cell. Typically the gap in that cleft of a chemical synapse is somewhat large to about 200A across. which is about ten times the size of the gap. Typically what you find though is a chemical synapse is a lot slower but because it requires the release of a chemical signal that has to travel and bind they tend to be a little bit slower.



Some final terminology before we go today then is that these synapses can work in different ways we have what are called excitatory synapse and which as its name suggests brings about an excitation in the post synaptic neurone. This causes me bran depolarisation and propagation of the action potential and we also have inhibitory synapses where they have the opposite effect where they alter the post synaptic membrane to inhibit the potentiation of the signal. So we have excitatory ones which which gives the next cell a kick and says right go on send the signal on and inhibitory ones that say shhhh.

Chapter 7 (Neurophysiology and Neurochemistry)

This chapter includes

- Neurotransmitters
- Acetyl choline
- Therapeutics

Neurotransmitters

The last time we started the whole business of action potentials in neurones and how a neurone works with the whole business of neural transimsion. We saw how an action potensial works at the nerve terminal and we saw the synapse and the two major types of them. There are the electrical synapses which are fused with these gap junctions where there are very close connections between the cells and a very fast transmutation between one neurone and the next. Then the other type of synapse we spoke of was the chemical synapse which we are perhaps a bit more familiar with with where the arrival of an action potensail at a nerve junction causes the release of a neurotransmitter which fuses across the gap between two cells which are the pre and post synaptic neurone and then triggers an electrical impulse down the second nerve.

So its the chemical synapses and the neurotransmitteres which we are going to go on to now. With a bit more detail today but the neurotransmitters are vast and there is a large number of them. We cant look at all of them so we are going to look at the main ones and start looking at how they function.

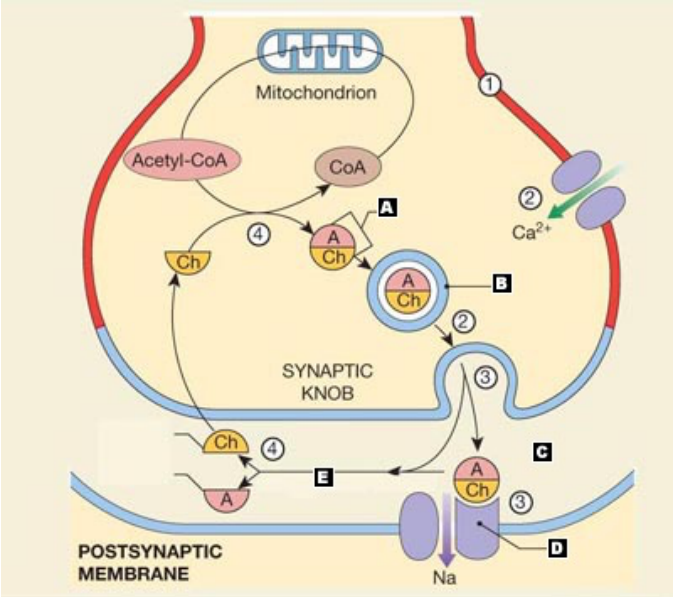
Now the classical sort of euro transmitteres we suppose is acetyl choline. Acetyl choline was the first neurotransmitter to be discovered way back in about 1921 so that one has been known for a long time. It was positively identified as a neurotransmitter for mediating the influence for whats called the vagus nerve which is on a frog heart. The chap who did this was called Otto Loewi and what he did was to refuse the heart of a frog while stimulating the vagus nerve. So basically he has a heart that is stimulated by the vagus nerve. The vagus nerve actually has a lot of control in the body that controls things like gastric recreation but another thing it influences is heart rate. If you stimulate the vagus nerve the heart rate slows down. Basically what loewi did was to take a diffused frog heart like that, stimulate the vagus nerve and he collected the solution form round the outside when its was stimulated. Then he took that profuse and gave it to another heart and low and behold the heart slowed down. So what he was able to show really was the vagus nerve effects on the heart was being mediated by some substance. He could isolate that substance from the solution bathing the heart and when he gave it too another heart it he was able to slow it down. HE was thus then able to prove that there was a substance being released from the nerve that was actually influencing the frog heart. That substance was then later identified as acetyl choline. around about 1936a bit further on people also demonstrated that acetyl choline was also the major neurotransmitter mediating neuromuscular stimulation so this si the calssic thing that acetyl choline is known for. When the nerve impulse comes down the nerve to fibre the muscle contracts and the neuromuscular junction is acetyl choline that stimulates the receptors. SO this was one of the first observations of acetyl choline and what it did. People then found in about 1936 that acetyl choline is a neurotransmitter responsible for transmitting nerve impulses to the muscle and is the major neurotransmitter there. In fact actually if you inject acetyl choline in to the artery supplying muscles it causes the muscles to contract powerfully. As time moved on and reaching around the time of the 1940s there were some ligands starting to be discovered as neurotransmitters. In particular something called

neuroepinphrine or the american term is neuroadrenaline. Normal epinephrine has been show to be a neurotransmitter and then as time goes on again in the 1950's we started finding other things like dopamine. Dopamine is actually biochemically the precursor of neuroepinephrine that was also shown to be a neurotransmitter. Around about the same time a substance called serotonin was discovered and again shown to be a neurotransmitter. Serotonine masquerades under a number of names, its also known as 5-hydroxtryptamine or also 5HT. again this was established in the 1950s as a neurotransmitter. So in the 1950's these were the major neurotransmitteres that were known about.

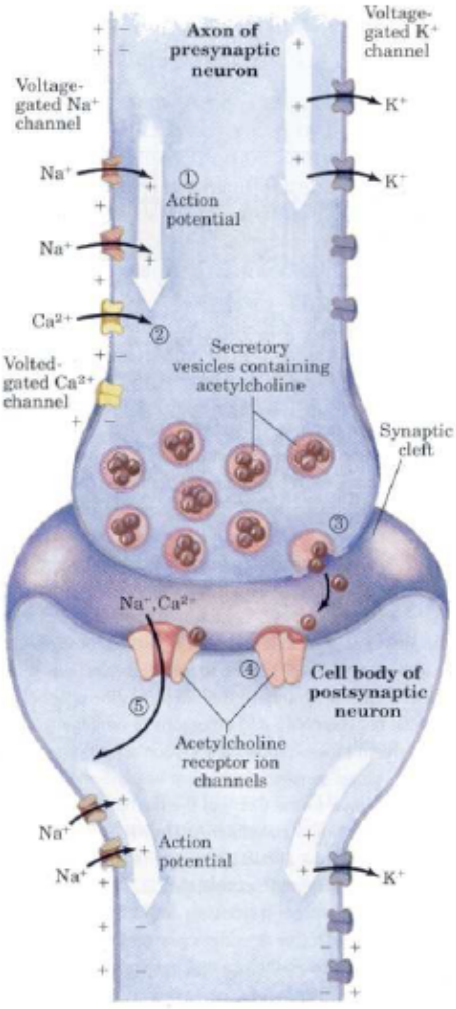
- Acetyl Choline
- Neuroepinephrine
- Dopamine
- serotonine

There was one other substance as well, something known as substance B. Substance B was a portion that was also discovered around that time and was also shown to be a neurotransmitter. So there are four compounds together with a simple peptide called substance B. Substance B gets its rather unusual name from the fact that its a protein but also form the fact that it was originally isolated as an powerful extract. All of this things were localised to the neurones that were using neurotransmitters and as quite potent substances there isn't a lot of them present and there concentration wasn't very dense. They seemed to have a very limited biochemical activity because this is all they do by acting as neurotransmitters. The picture got a lot more complicated as time progressed as the 1950's progressed people started to find more substances that acted as neurotransmitters and some of these things had other roles in the body as well. Four amino acids were discovered, some of them we have already touched upon, things like glutamate, aspartate, glycine and GABA are the classics. These of course are known as proteins amino acids and have other functions in the body. GABA is also technically an amino acid but functions mainly as a neurotransmitter. It was also the case that there were found in other sites in the bodying not just in the neurones, by the time we got to the 1970s about a dozen or so other compounds had been discovered and these were called the neuropeptides. As the name suggests these are small portions. Like acetyl choline and things like that they were specialised molecules particuarly found in the central nervous system and they are compounds like the endorphines. As we say things did get even more complicated than that because people were yet to discover there were other compounds which were known to act as hormones elsewhere in the body but which were also shown to act as neurotransmitters. Things like oxytocin. Well known as a hormone in the systemic part of the body is now shown to act as a neurotransmitter in the CNS. Things like thyroid hormones, releasing hormone (TRH) and its these sort of substances that hada a well known about function that were also being found to be neurotransmitters. So we can show that the system is getting rather more complicated. This all summarises as the different types of neurotransmitters that we actually known. Starting then with the amino acids and culminating in the neuropeptides.

Acetyl choline receptors



So lets start off in a bit more detail at the archytypical neurotransmitter known as acetyl choline now all pathways that use acetyl choline as a neurotransmitter are known as **cholinergic systems.** This is a diagram of the cholinergic synapse to the right. Acetyl choline as we say was the first neurotransmitter that was identified as a neurotransmitter and is such the best studied and best understood. It is synthesised in a fairly simple reaction between its two components. Acetate which is derived from acetyl-CoA and its second part choline. Choline is a universal substitute and is found in other things like phosphotidyl choline. The enzyme responsible is called **choline acetyl transferase** and it serves to help the coming together of the two parts of an acetyl choline molecule. Choline itself is quite a wide spread substance found throughout the body. It is synthesised primerly in the liver and is transportd to other areas in the body via the blood stream. It is believed there are two major sites for the uptake of choline into the nerve system. There is a high affinity system that seems to linked to sodium drainage and also invovles ATP. So we say this is a high affinity system as its got a high uptake rate into the terminal. The low affinity site seems to act more by diffusion and that seems to be more concerned with phospholipids. As far as the acetyl choline is concerned its high affinity transport is the most important one. That acetate comes form acetyl-CoA and it then is also derived from glucose by the process in the mitochondria. This enzyme choline acetyl transferase has been isolated in pure form from various tissues including the brain. It is a globular protein with a molecular weight of about 67KDa. The acetyl choline must be synthesised which is then stored in little vesicles there on the synapse terminal. Each of those vesicles contains roughly 10x^3 - 10x^4 molecules of acetyl choline and thats how its sits in the nerve terminal waiting for a nerve impulsee to come along. where some of the release of the acetyl choline synaptic cleft and then it diffuses across the cleft to its receptor on the a joining cell. This next diagram shows basically the same thing in bit more detail. Basically what happens is a nerve impulse comes down the nerve fiber. We have present the sodium and potssium channels operating as we saw before to propergate the nerve impulse. When the action potensial arises close to the pre synaptic nerve terminal it triggers the openg of voltage gated calcium channels. This is something we will see as a fairly common theme. Then the concentrationn of calcium outside of the cell is higher than inside so calcium flows into he cell. The influx of calcium is a signal that causes these secretary vesicles to migrate to the nerve terminal.

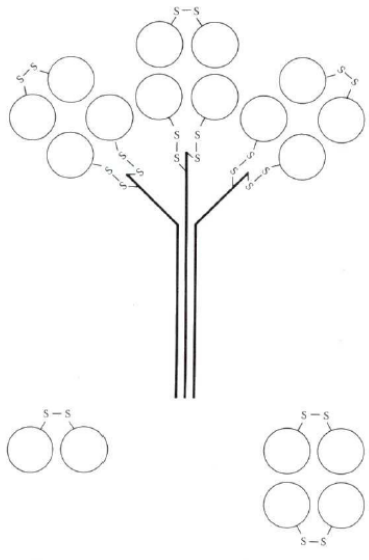


Typically when an action potential arrises it triggers the fusion of about three hundred vesicles to the cell membrane. This causes concentrationn of acetyl choline in the synaptic cleft to rise. IT rises from about 10nM to about 500mM in about 1mS. So the reelse of acetyl choline into he snaptic cleft causes the concentration of the acetyl choline to rise significantly. Its worth noting as this point that there are a number of natural toxins that interfere with that process. We will see some more later on but we willl discuss a few now.

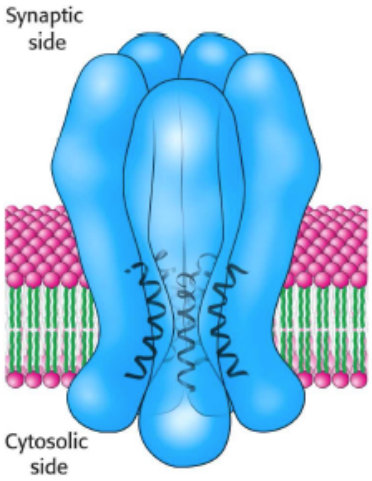
Obviously if you block the acetyl choline receptors than you are putting a block on neuromuscular transmition which causes paralysis. So we find quite a lot of natural toxins work at a level the acetyl choline is set. So a snake for intake would want to deliver enough to paralyse you but not completely overwhelm you. If you can inject an animal with something that paralysis it and stops it running away then its eaier for the snake to eat. A lot of natural toxins are disocovered there. We will go through a few examples again when we talk about the release of acetyl choline from the pre-synaptic membrane. There is a compound called alpha lactrotoxin. Which is a toxin released by the black widow. Basically what that compound dose is trigger a massivee release of acetyl choline. This is instead of the controlled release there is a massive release. It seems to work by opening up the calcium channels which causes a massive influxx of calcium and then a massive release of acetyl choline. This causes paralysis.

Anouther toxin that works at that cite there is the botulinus toxin which is a mixture of about 8 proteins which have molecular weights between 135-170KDa. This toxin is formed by an aerobic bacterium called clostridium botulinum. Its actually as you may have guessed from its name the ingrediant of botox. In the natural world. Clostridium botulinum is often found in aerobic conditions and classical its found in things like tinned meat. So if you had a material and particuarly meat and the meat has gone off then this is one of the bacterium that can grow in it. In this case it is botulinus toxin which is an extreamly toxic substance. Basically what it dose is interfere with the process where the visicles of acetyl choline are released to perfuse with the cell membrane. There are other proteins involved in making these visicles move to the pre synaptic embrace to release there contents and botulinus toxin interferes with that process. The net result of this then is the cause of paralysis. So two toxins that opperate at the release of acetyl choline release.

Assuming this process hasn't been interfered with what happens then. Well the acetyl choline diffuses across the synaptic cleft and interacts with acetyl choline receptors in the post synaptic membrane. Once its interacted with the receptors it has to be gotten rid of because we dont want this continual interaction with the post synaptic cell. The enzyme responsible is called acetyl choline esterase. Basically it carries out the reveres of what choline acetyl transferase dose and splits the acetyl and choline into its separate components which can hen be recycled to make more acetyl choline. Once the acetyl choline has been released it is very quickly replenished. There is quite a tight coupling between the release of the acetyl choline and its synthesis. The acetyl choline esterase enzyme has a very high activity. This is really because you dont want the acetyl choline hanging around in the synaptic cleft and creating more action potensials. You want it to be cut off once its done its job. The esterase is thus very active and quickly homogenises the protein. The enzyme itself its actually like a globular protein and it exists in multiple forms as shown in the diagram here. The reason why this is hasn't been satisfactorily explained why. The simplest form exists as a monomer, but the monomer are often linked togather by a disulphide bridge. You can also get tetrameric forms where two of the dimers link together. And as if that wasn't enough those tetrameric structures can assemble with another protien which is like a collagen type



protein which can bond groups of the together to from the complex seen in the diagram. So again this diagram is the acetyl choline esterase which is responsible for braking the acetyl choline down. So the monomeric units of the acetyl choline esterase are roughly 80KDa.



So lets start looking at the acetyl choline receptors themselves. We have seen how the acetyl choline is produced and we know they bind to the receptors so lets see how that works. We will also see how the receptor leads to an action potensial and how it can be propagated along the post synaptic terminal. What about the acetyl choline receptors themselves? Well basically they are chatogorized into two major types on the basis of ligands that bind to them. We see this quite commonly where the acetyl choline receptor obviously bind acetyl choline and is the actual ligand that binds to and also causes the receptor to respond. We also find that there are lots of different types of receptors, they all respond to acetyl choline but they respond to other drug molecules and different compounds differently. On the basis of that differential responce we can identify the groupings of them. In the case of acetyl choline there are two types that are commonly seen. One of the types repsons to nicotine (the stuff in tobacco) so some of the types of receptors respond to nicotine as well and they are known as **nicotinic acetyl choline receptors**. The other type of acetyl choline receptor responds to a substance called **musteine**. Musteine is a compound known as a tetiary amine which is found in a type of mushroom called the fly agaric mushroom. Which has the latin name ***Amanita muscaria.*** So muscarine is a tertiary amine that is isolated from this fly agaric mushroom and it also will stimulate sub populations of acetyl choline receptors.

Amanita muscaria, commonly known as the **fly agaric** or **fly amanita**, is a mushroom and psychoactive basidiomycete fungus, one of many in the genus *Amanita*. Native throughout the temperate and boreal regions of the Northern Hemisphere, *Amanita muscaria* has been unintentionally introduced to many countries in the Southern Hemisphere, generally as a symbiont with pine and birch plantations, and is now a true cosmopolitan species. It associates with various deciduous and coniferous trees.

Although classified as poisonous, reports of human deaths resulting from its ingestion are extremely rare. After parboiling—which weakens its toxicity and breaks down the mushroom's psychoactive substances—it is eaten in parts of Europe, Asia, and North America. *Amanita muscaria* is noted for its hallucinogenic properties, with its main psychoactive constituent being the compound muscimol. The mushroom was used as an intoxicant and entheogen by the peoples of Siberia, and has a religious significance in these cultures. There has been much speculation on possible traditional use of this mushroom as an intoxicant in other places such as the Middle East, Eurasia, North America, and Scandinavia. The American banker and amateur ethnomycologist R. Gordon Wasson proposed that the fly agaric was the soma of the ancient Rig Veda texts of India; since its introduction in 1968, this theory has gained followers and detractors in anthropological and religious literature. Dead Sea Scrolls scholar John Marco Allegro proposed that early Christianity sprang from cultic use of the fly agaric in Second Temple Judaism, and that the mushroom

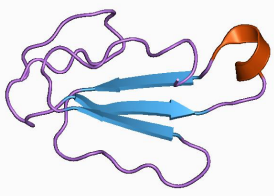
itself was used by the Essenes as an allegory for Jesus Christ. This hypothesis has been met with criticism from academics.

The nicotinic for of the receptor (nicotine choline receptor) is charecterise by a very fast responce time typically 1-2 mS. So once the acetyl choline binds to the receptor within a couple of milliseconds there is a responce. It produces it effects by directly deploring the post synaptic membrane. The nicotinic receptor works by directly allowing an ion current to flow through it and that in tern stimulates an action potential in the post synaptic cell. Basically stimulating an ion channel that comes into the cell which triggered an action potential within 1-2mS. In that type o receptor the receptor molecule itself is actually the ion channel so the receptor itself once it binds the acetyl choline is actually the ion channel itself. These are the types of receptors associated with neuromuscular junctions, when we talk about of receptors in neuromuscular junctions we normally talk about these ones here. So we look at these ones first as these are the better studied of the two types of acetyl choline receptor. We say its an ion channel but it is permeable to sodium and potassium ions so it has sodium and potassium ions flowing through that channel once it has been opened but the influx of sodium tends to be much grater. Largely because the concentration of the sodium outside the cell is much higher. So when this channel opens it tends to be the sodium that flows through it although both can. This is probably the best studied of the receptors because it can be isolated relatively easily. The source of the acetyl choline receptor that we use is not nerve fibres conserved but it actually comes form the electric organs of electric fish which is called torpedo marmorata.

The **marbled electric ray** (*Torpedo marmorata*) is a species of electric ray in the family Torpedinidae found in the coastal waters of the eastern Atlantic Ocean from the North Sea to South Africa. This benthic fish inhabits rocky reefs, seagrass beds, and sandy and muddy flats in shallow to moderately deep waters. It can survive in environments with very little dissolved oxygen, such as tidal pools. The marbled electric ray has a nearly circular pectoral fin disc and a muscular tail that bears two dorsal fins of nearly equal size and a large caudal fin. It can be identified by the long, finger-like projections on the rims of its spiracles, as well as by its dark brown mottled color pattern, though some individuals are plain-colored. Males and females typically reach 36–38 cm (14–15 in) and 55–61 cm (22–24 in) long respectively.
Nocturnal and solitary, the marbled electric ray can often be found lying the sea floor buried except for its eyes and spiracles. This slow-moving predator feeds almost exclusively on small bony fishes, which it ambushes from the bottom and subdues with strong electric bursts. It defends itself by turning towards the threat, swimming in a loop, or curling up with its underside facing outward, while emitting electric shocks to drive off the prospective predator. Its paired electric organs are capable of producing 70–80 volts of electricity. This species is aplacental viviparous, with the developing embryos sustained by yolk and histotroph ("uterine milk") produced by the mother. Mating takes place from November to January, and females bear litters of 3–32 pups every other year after a gestation period of 9–12 months. The newborn ray is immediately capable of using electricity to hunt.

The electric fish is this thing that uses a powerful voltage to stun its pray. The electric organ of these fish is very rich in the nicotine acetyl choline receptors and this is the source of the elect receptor. You can soluble this preparation form the electric organ quite simply and then stage enough cobra toxin has a very powerful affinity for the nicotine acetyl choline receptors.

α-Cobratoxin is a substance of the venom of certain *Naja* cobras. It is a nicotinic acetylcholine receptor (nAChR) antagonist which causes paralysis by preventing the binding of acetylcholine to the nAChR.α-Cobratoxin is a neurotoxin from the venom of certain *Naja genus*, including the Thailand cobra, the Indo-Chinese spitting cobra (Naja siamensis) and the Chinese cobra (Naja atra). The cobras that produce the toxin live in tropical and subtropical regions of The Americas, Africa,



Asia, and Australia. The venom, produced by these snakes, is a mixture of proteins, carbohydrates, and other substances. The venom is only used when the snake needs it for survival, because it costs a lot of effort to produce. If poisoning a subject is not necessary, it can bite without excreting the venom. When the snake does use it, it mostly tries to immobilize or kill its prey.[1]α-Cobratoxin forms three hairpin type loops with its polypeptide chain. The two minor loops are loop I (amino acids 1-17) and loop III (amino acids 43-57). Loop II (amino acids 18-42) is the major one. Following these loops, α-cobratoxin has a tail (amino acids 58-71). The loops are knotted together by four disulfide bonds (Cys3-Cys20, Cys14-Cys41, Cys45-Cys56, and Cys57-Cys62). Loop II contains another disulfide bridge at the lower tip (Cys26-Cys30). Stabilization of the major loop occurs through β-sheet formation. The β-sheet structure extends to amino acids 53-57 of loop III. Here it forms a triple-stranded, antiparallel β-sheet. This ß-sheet has an overall right-handed twist6. This β-sheet consists of eight hydrogen bonds. The folded tip is held stable by two α-helical and two β-turn hydrogen bonds. The first loop is stabilized because of one β-turn and two β-sheet hydrogen bonds. Loop III stays intact because of a β-turn and hydrophobic interactions. The tail of the α-cobratoxin structure is attached to the rest of the structure by disulfide bridge Cys57-Cys62. It is also stabilized by the tightly hydrogen bound side chain of Asn63. In conclusion, the whole is hold together by disulfide bonds and the loops are kept stable by ß-turns and ß-sheets.

So you can prepare a chromatography column with cobratoxin bound to it and the cobratoxin will fish out the nicotine acetyl choline receptors from the soluabalised electric organ.

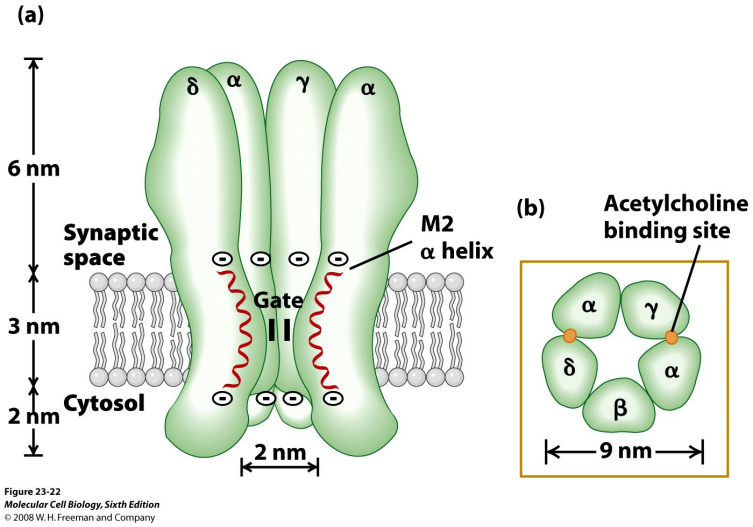


Figure 23-22
Molecular Cell Biology, Sixth Edition
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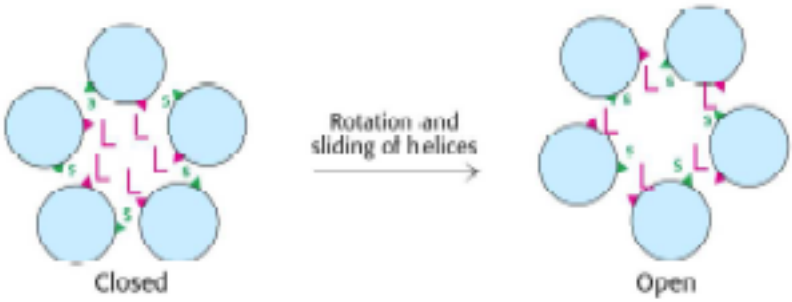
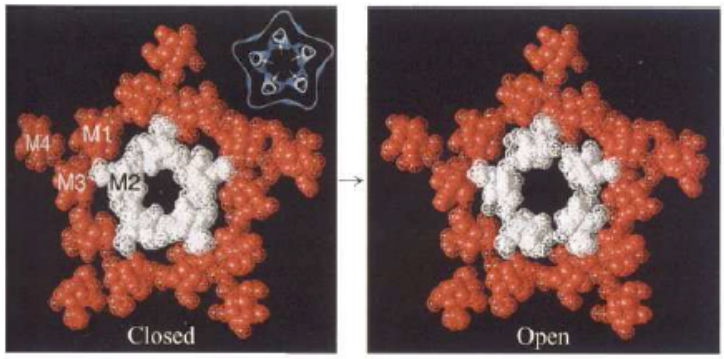
Now people have done this and studied this receptor and it looks a little like this diagram to the right. It actually exists as a pentamer with five subunits and requests as an alpha-2, beta, gamma, delta configuration. There are five subunits and we get two copies of the alpha so its an alpha-2, beta, gamma, delta system. Each of the two alpha chains contains a binding site for acetyl choline. And the whole complex has a molecular weight of around 268KDa. This is the nicotinic acetyl choline receptor. People have cloned the genes coding for those various subunits and its been show to have very similar sequences and it seems likely that basically what happens is there eis a gene duplication that has been mutated over many years to produce the different subunits. Now we say the structure of this has been very well studied and the dimensions of it are very well known.

The structure exists as a kind of cylinder with the a cylindrical pour running through the middle. The mean diameter is about 65A. The receptor sticks out of the memebrain on the synaptic side by about 60A. And about 20A on the other side. The mouth of the channel on the synaptic side is 22A across and it narrows down as it goes through the membrane until it reaches a central region which is about 10A wide. It then

widens out again on the opposite side to about 12A. So people know its dimensions quite well and who it sits in the membrane.
Two molecules of acetyl choline have to bind to th receptor in order to make it function and once two have bound to the outer subunits then the transition form its closed state to its open state occurs extreamly rapidly and happens in about





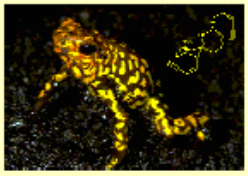
30 microseconds

Under physiological conditions once the channel is open it only stays open for a very short period of time. Probably about 1millisecond or so. This is largely because the acetyl choline which is needed to bind to keep it open is rapidly hydrolysed by the acetyl choline esterase. So we said there was a very reactive enzyme that hydrolyses the acetyl choline quickly and once it has been released the channel closes again. So this gives us an idea of this very rapid repose time that opens very rapidly. That makes it quite difficult to study, particularly if you wan to study the open and closed states to see if there is a difference between them. You might say then ok in an in vitro situation you can add acetyl choline to it with no acetyl choline esterase so the channel will stay open. Even in artificial conditions though when the acetyl choline concentrations can be keep high the receptor spontaneously closes anyway. Its a process called desensitisation and it closes anyway. People no the less have figured out the way in which it works. The pour through the center of the channel there is actually lined with five alpha helices one coming form each of the five subunits. The amino acid sequence of those subunits suggests that they have alternating ridges of small polar residues and large non-polarised groups. So there are alternating riges of large ridges things like isoleucine and things like that and then small polar ones like serine, threonine and glycine. So you have these large and small ridges, it is believed that whennthe channel is in the closed state the helices are rotated such that the large ridges occlude the channel and basically block it off. When acetyl choline binds to the alpha subunits it causes a rotation of the helices and turning the large residues out of the way and the smaller residues take there place. And this is where the molecular spacing for the channel come from. In the closed state there a various helices that we look down upon and the pour in the middle is small and closed off. When the acetyl choline binds the helices rotate and open up the channel. The nature of those alia helices as well also explains why the pour is selective for cations like potassium and sodium and why art the chlorides going through as well? Basically you get a ring of negatively charged residues present aroundd that pour as well and the negatively charged residues tend to reply the chloride ions which are negatively charged as well. So we tend to find that anions cant enter the channal and it tends to be sodium and potassium ions that move through.

We have mentioned befor Lethat the cholinergic synapse is one of the targets for natural venoms and things and we have mentioned two that affect the actual release of acetyl choline but there are many more that affect that acetyl choline receptor. Basically what happens is there are these neurotoxins that tend to cause a blockage of the neuromuscular transmittion that causes paralysis. They praythen tends to die from respiratory arrest. We have given a few examples of the neurotoxins that act at this system and

Neurotoxins Acting At The Cholinergic Receptor

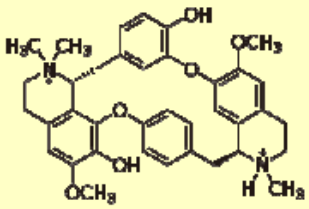
Histrionicatoxin, an alkaloid obtained from the skin of the Columbian arrow-poison frog (*Dendrobates*)



α-bungarotoxin, venom protein from snakes of the genus *Bungarus* , such as the banded krait.

α-cobratoxin from cobras of the genus *Naja*

d-Tubocurarine, an alkaloid obtained from the bark of the South American plant, *Chondrodendron tomentosum*



the top one is Histionicatoxin which is an alkaloid that comes form the columbian arrow poisoning frog. It produces a very notchiouse toxin in its skin.
Alpha bungratoxin is produced from the venom protein produced by snakes from the genus bungarus which is another example of a snake venom that goes on the acetyl choline receptor. Cobras also go for that particular receptor system. They bind quite specifically to the alpha subunit of the acetyl choline receptor to stop it from functioning. Other example here is the delta-Tubocurarine which is an alkaloid taken from the bark of the south american plant chondrodendron tomentosum. This also blocks the acetyl choline at the acetyl choline receptor system. So we say this tends to be the case that a lot of these toxic compounds very commonly go for the neuromuscular junction. There are many others like a sea snake one and so on.

So that was the Nicotinic acetyl choline receptor but we said there was another type of acetyl choline receptor. We are not going to talk as much about that one because its perhaps not quite as well studied. It operats in a totally different way. The nicotinic ion channel opens and closes very fast, the muscurinic one has a much longer lasting response time. It typically operates by a secondary messenger system. Involving cyclic AMP. So the muscarinic type of receptor operate via the G proteins affecting the levels of cyclic AMP and as a consiques as we say the actions of it are much slower and longer lasting. The effects that it has affects smooth muscle, cardiac muscle, secretary cells and has quite a wide range of systems it can affect. IT is therefor not as easy to pin down as the nicotinic ones. There are again a number of toxins that act at the muscerinic receptor and again this gives us clues to what the muscerinic receptor is actually controlling because if we block it we can see whats gonna happen. Couple of alkoloids like atropine which is a tropane alkaloid which comes from the deadly nightshade plant belladonna. Atropine is generally a substance they put into your eyes to cause the pupls to dilate. It gets t same actually

“belladonna” form an italian lady who practised putting drops in your eyes to cause you pulpys to dilate to make them more attractive. Im not sure its successful because ones your dilated everything really blurry and so the poor ladies filrting with the guys with no eye sight could look like anything. Classically though that is what it dose and thats known as atropine. If they are taken centrally these things at low concentrations can have mind altering effects as well. Now a lot of controlled substances because they do have some mind bending effects as well. If you take these substances you can start t hallucinate. These sorts of things were taken by men like edison and such so that they could experience a higher plane and travel the consciousness and stuff. You have to be carful though because taking to much can poison and kill you. So you have to find a plane of finding a higher power and suicide. So thats what they are and they work via the muscerinic acetyl choline receptors.

Therapeutics

We mentioned a couple of substances that are posions of the cholinergic synapse but there are also a number of drugs that can be used at that junction therapeutically. So it docent just relate to toxins and poisons and stuff. There are a number of drugs that block acetyl choline esterase. This is from the enzyme that brakes down acetyl choline the number of drugs that block the action of acetyl choline esterase. If you block the brakedown of acetyl choline it hangs around in the synapse for longer to it intensifies its effect. A couple of thing that are used clinically are Neostigmine and the other one is Physostigmine.

Neostigmine
By interfering with the breakdown of acetylcholine, neostigmine indirectly stimulates both nicotinic and muscarinic receptors. Unlike physostigmine, neostigmine has a quaternary nitrogen; hence, it is more polar and does not enter the CNS, but it does cross the placenta. Its effect on skeletal muscle is greater than that of physostigmine. Neostigmine has moderate duration of action, usually two to four hours.[3]
Neostigmine binds to the anionic and esteric site of cholinesterase. The drug blocks the active site of acetylcholinesterase so the enzyme can no longer break down the acetylcholine molecules before they reach the postsynaptic membrane receptors. This allows for the threshold to be reached so a new impulse can be triggered in the next neuron. In myasthenia gravis there are too few acetylcholine receptors so with the acetylcholinesterase blocked, acetylcholine can bind to the few receptors and trigger a muscular contraction.

Physostigmine
is a parasympathomimetic alkaloid, specifically, a reversible cholinesterase inhibitor. It occurs naturally in the Calabar bean.
The chemical was synthesized for the first time in 1935 by Percy Lavon Julian and Josef Pikl. It is available in the U.S. under the trade names Antilirium and Isopto Eserine, and as eserine salicylate and eserine sulfate. Today, physostigmine is most commonly used for its medicinal value but before it's discovery by western medicine in 1846 it was much more prevalent as a poison. Physostigmine has an LD50 of 3 mg/kg in mice.
Physostigmine has two chiral carbon atoms. Therefore, attention needs to be paid to the synthesis of the correct diastereomers. The 71 syntheses of physostigmine yield 33 racemic mixtures and 38 products of a single enantiomer. The first total synthesis of physostigmine was achieved by Julian and Piki in 1935.[2]
The main goal of Julian’s physostigmine synthesis was to get the intermediate key compound, l-eseroline (compound 10 in the diagram to the left). Then, this compound would be easily converted to physostigmine. In one of his earlier works[3] Julian synthesized the ring of physostigmine from starting

material 1-methyl-3-formyl oxindole, which was discovered by Friedlander. However, he faced the problems that the starting material was expensive, and the reduction of a nitrile to an amine (similar to the reaction of compound 6 to given compound 7 in the diagram) with sodium and alcohol did not result in good yield. In his second work "Studies in the Indole Series III," he had improved the yield of amine from nitrile significantly by using palladium and hydrogen. Although he succeeded in the synthesis of the target chemical, the route had several drawbacks. First, the chemical resolution of compound 8 is unreliable, and the chemical resolution of d,l-eserethole gives optically pure enantiomers after eight recrystallizations of its tartrate salt. Second, the reaction of compound 8 to give compound 9 requires a large amount of Na. In the years since this initial work, many other groups have used a variety of approaches to overcome these problems.

Both of them are reversable inhibitors of acetyl choline esterase, the difference really is in there Lipophilicity (how lipid soluble they are) physostigmine can penetrate the blood brain barrier but the neostigmine cant the clinical use of these things is in the treatment of things like glaucoma where ether is quite a high pressure in the eye. there is also a condition known as myasthenia Gravis. Myasthenia Gravis is an autoimmune disease in which the body produces antibodies again the acetyl choline receptros. This is normally characterised by muscle weakness and wasting and stuff like that. Treatment with these drugs basically makes the acetyl choline hang around in the neuromuscular junction fo longer. So if these patients have got to reduced level of the acetyl choline receptors then of course the few receptors that remain have more chance of being stimulated if the acetyl choline is around for longer.

More powerful inhibitors of acetyl choline esterase tend to be very poisonous. Particularly things like nerve gases and things that have been developed are irreversible inhibitors and again they block the neuromuscular junction. They have very little use normally, some times used as insecticides.

Just to finish off the substance tubocurarine is a substance that basically competes with acetyl choline for the binding site on the receptor and it inhibits depolarisation of the nerve and causes paralysis. It used to be used therapeutically in surgical applications. Basically if you a surgeon the last thing you want is muscle contraction so by administering the nerve agents can stop the nerve impulses and induce paralysis. Today it is largely achieved by the compounds and things like succinyl choline which works in exactly the opposite way which causes a persistent depolarisation. Where as tubocurarine acts to inhibit depolarisation succinyl choline causes a persistent polarisation and the muscle relaxation is the same. AS we say this is quite commonly used in surgical. There is a down side of course where if you paralyse someone with these sorts of drugs then you have to ventilate them or they stop breathing and die anyway.

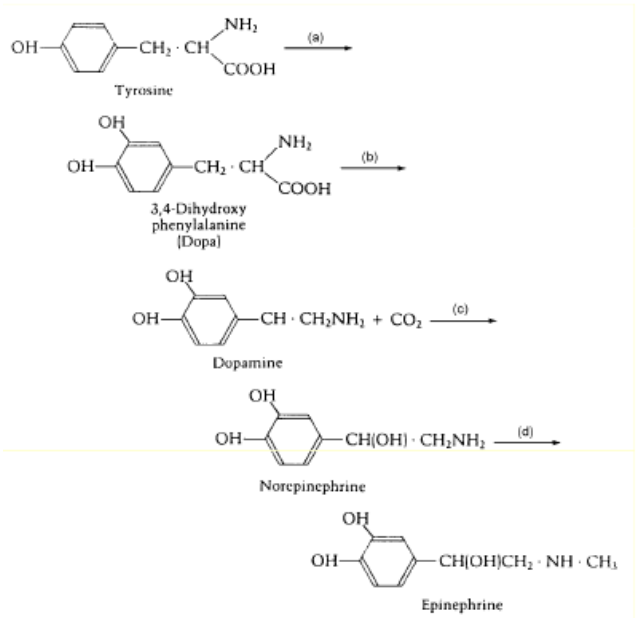
Chapter 8 (Neurophysiology and Neurochemistry)

This chapter includes

- Catecholamines
- Catabolism of the Biogenic Amines
- Dopamine and serotonin

Catecholamines

Last time we looked at the collinergic system and we saw how things interfere with the cholinergic system tend to cause things like paralysis and so on. What were going to do today is go to look at a few other neurotransmitter systems. These are what are called the Catecholamine neurotransmitter sytems and the indolamine which is the next one that concerns things like seratonins and things like that. They get there names form the fact that these catecholamines possess this catecholic group consisting here (the benzene diol grouping that you get is technically called a catachol). They do mastered under a number of other names as well. there known as things like the monoamines you also here them referred to as the biogenic amines but there al the same thing. The synthesis of them is quite a simple process. You start off with tyrosine at the top and this is converted initiallinto the compound 3-4 dihydroxyphenylalanine or more commonly referred to as DOPA. The enzyme responsible form this is tyrosine hydroxylse. Second stage of the reaction invovles the conversion of DOPA to Dopamine and thats catalysed by the enzyme aromatic acid decarboxylase. And then finally of from Dopamine to norepinephrine by the enzyme dopamine beta hydroxylase. That enemyme dopamine beta hydroxylase is the thierd one in the sequence here and it is only precent in neurones that are considered noraganeric neurones thats to say those that secret nuroepinephprine or noradrenaline and there for the pathway stops at that point. So how dose that actually work? tyrosine starting compound is available from tissue pools and its very commonly distributed. Its transported into the brain form the blood stream across the blood brain barrier and there are specific transporters that take it across. It enter by whats called the neutral amino acids transporter system and thats also responsible for the things like tryptophan and various toher amino acids its not particularly specify for tyrosine. So the first step of the process is changing tyrosine to DOPA and that enzyme (3-4 dihydroxyphenylalanine) appears to be the slowest one. So therefore it is probablyy the rate limiting enzyme in that pathway. The molcular weight of tyrosine hydroxylase varies a bit depending on

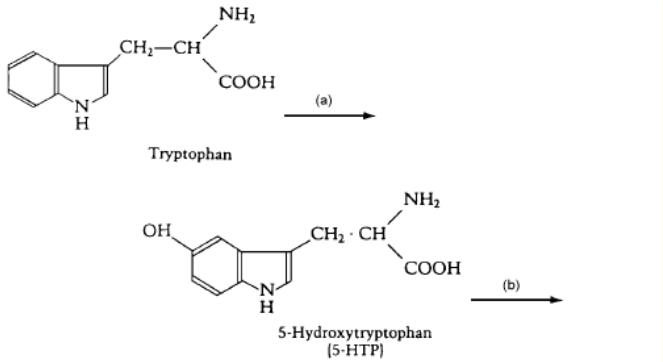


Enzymatic reaction	*Enzyme*	*Cofactors*
(a)	Tyrosine hydroxylase (EC 1.14.6.2)	Tetrahydrobiopterine Molecular O_2, Fe^{2+}, NADPH
(b)	L-Aromatic acid decarboxylase (EC 4.1.1.28)	Pyridoxal phosphate
(c)	Dopamine β-hydroxylase (EC 1.14.17.1)	Ascorbate; Molecular O_2 Cu^{2+}
(d)	Norepinephrine *N*-methyltransferase (EC 2.1.1.28)	*S*-Adenosylmethionine

the source but the one that comes form the human brane tend stop have a molecular weight somewhere around 200-250 thousand molecular weight range. It seems to consist of a tetramere where each of the monomeric units have a molecular weight of about 60 thousand. So it tends ot come as a tetrameric thing where ehe individual components are around 60, 000 molecular weight units. Its controlled by things like phosphorylation, you can phosphorilate tyrosine hydroxylase and there are a number of protein that are phosphorialted. Things like protein kinase A and C will do it as well as some of the calcium calomelin type of proteins. When its phosphorylated it tends ot become more active.

So any stimulus that ranges the level of cAMP calcium or the daily glycerol will all increase the tyrosine hydroxylase activity. Because its activated by phosphorylation anything that stimulates the things like cAMP, calcium ion concentration or the levels of diacyle glycerol that will activate those things and phophorialte the first enzyme and help the pathway.

So the next stage is the conversion of DOPA to Dopamine and its carried out by the enzyme aromatic acid decarboxylase. Its sometimes called DOPA decarboxylase because of its invovlemnt in the pathway but it is not action specific to DOAP. Quite often it has the more general name Alomatic decarboxylase. That enzyme is quite widely distributed in the body. Its not linked to the brain its also found in the kidney and the liver as well. The enzyme typically has a weight of around 85-90 thousand molecular weight units. Its considerably more active than tyrosine hyroxylase and is even considered 100 times more active. So as a consequence of that enzyme being so active mean you dont get much DOPA sitting around in the cell. As fast as its formed its converted to Dopamine. The next enzyme in the sequence is enzyme number C known as dopamine beta hydroxylase which serves to convert the dopamine into norepinephrine. Basically it hydroxylates the side chain. Again that enzyme has been isolated and as a tetrameric glycoprotein has molecular weight of 75,000 molecular weight units. Theatre one of the compounds that we have talked about, the catecholamines is called serotonin. Its grouped with the dopamines and things like that but chemically speaking its an indole amine because it has the indole grouping seen in the digram to the right.



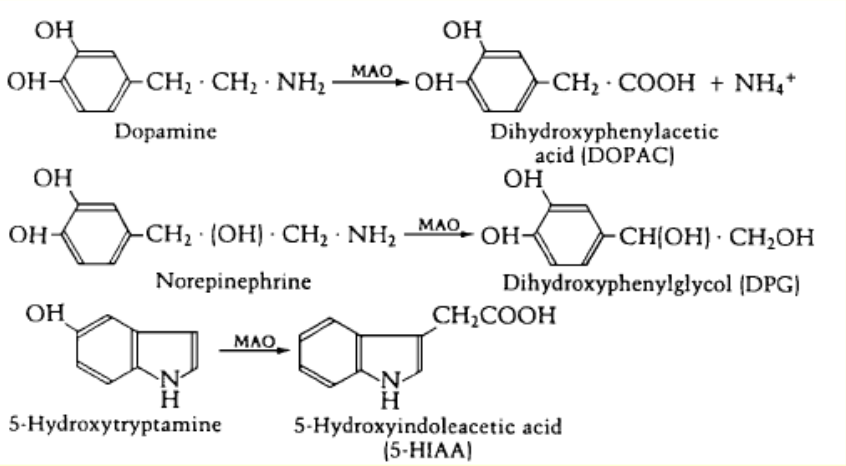
Enzymatic reaction	*Enzyme*	*Cofactor*
(a)	Tryptophan-5-hydroxylase (EC 1.16.4)	Tetrahydrobiopterine, molecular O_2, Fe^{2+}, NADPH
(b)	L-Aromatic acid decarboxylase (EC 4.1.1.28)	Pyridoxal phosphate

Its sythnthesised in again in a very short pathway but this time its starting material is tryptophan rather then tyrosine. The first enzyme is tryptophan-5-hydroxylase which hydroxylates the tryptophan to form the compound 5-Hyrdoxytryptophan or also known as 5-HTP. That enzyme is found chiefly in the brane and has a MW of around 230,000 MWU. It also exists as a tetramereand makes use of quite a lot of co-factors as well. The second stage of the synthesis, the conversion of 5-hydroxytryptophan to 5-hydroxytryptamine, also known as serotonin, is

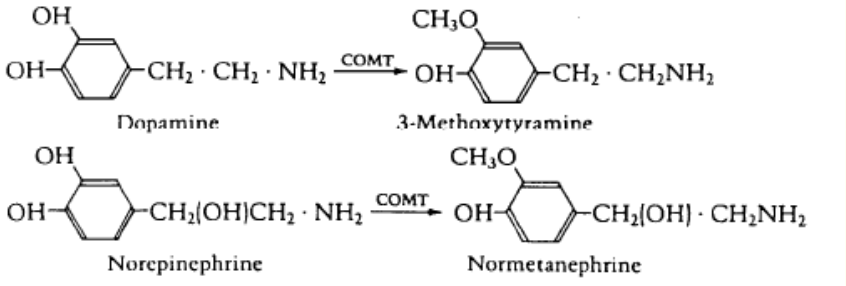
catalysed by our old friend aromatic acid decarboxylase. Almost certainly the same enzyme that we saw in the other pathway, so as we said not specific to one pathway. Now According to the synthesis of these compounds they tend to synthesis in the cytoplasm of the nerve cells and then the neurotransmitter is loaded into the vesicles by whats celled a acicular monoamine transporter. So it sits there ein the visicles waiting to be released and the release of the neurotransmitter is again a calcium driven process, very similar to the cholinergic system. Remember the cholinergic system we saw how an action potential comes down an axon but to get to the nerve terminal it has to induce the opening of the voltage gated channels. Calcium comes into the cell and its the rise of intracellular calcium which then causes the visicles to migrate to the membrane to release the neurotransmitter. Exactly the same thing happens with this. Its a calcium dependent process. So the neurotransmitter has been released in to the cleft, it diffuses across the gap, binds to its receptors, triggers a signal but then you have to get rid of it. So the way in which its gotten rid off is its taken up again into the surrounding cells by a sodium dependent high affinity transport system. So there are actually transporters that will pick up the serotonin and the dopamine and transport them back into the cells where they can be broken down and metabolised further. So those high affinity transport system that pick the things back up are driven by the sodium gradient. Once they have been taken back up into the cells they are broken down and there are a couple of enzymes involved in the catabolic metabolism of the biogenic amines.

Catabolism of the Biogenic Amines

There are two enzymes involved first is the monoamine oxidase (MOA). This can operate on dopamine, norepinephrine and serotonin. The other enzyme is catochol-O-methyl transferase. That docent work on serotonin. It can work on dopamine and nuroepinephprine but not on serotonin. The firs reaction seen here which is the MAO one occurs in the mitochondria. This is because eMAO is a mitochondrial enzyme. This one her the methylation reaction happens in the cytoplasm. the methylation pathway (the lower of the two) appears to be the less important. Most of the monoamines are broken down by MAO but we think about 10% of the Dopamine



Enzymatic reaction of monoamine oxidase (MAO)



Enzymatic reaction of catechol-O-methyl transferase (COMT)

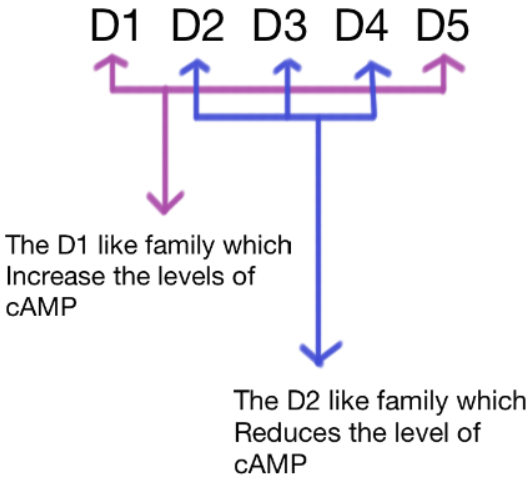
and norepinephrine and the broken down by the methyltyion pathway. Looking fir of all at the MAO pathway, it is a pathway used throughout the body and not just in the CNS. We can also find it in the liver he kidney and some other places. The one thats isolated from the pig brain has a MW of around 102,000 MWUs. That enzyme exists in a number of different forms, they tend to be known as monoamine oxidase A and B. So there is more than one type of it and the differences really are based on there different affinity for various substrates and inhibitors. The way in which the catochol-O-methyl transferase works is basically a transfer of a methyl group onto the hydroxyl group and thereby inactivating the neurotransmitter. That methyl groups actually comes from a compound S-adinosarmethionine. Once these neurotransmitters have been inactivated the enzymes are recreated into he blood stream and out of the body. So we have seen the biogenic amines but what do they actually do? Well when we looked at the cholinergic system it was fairly easy to see that its concerned with neuromuscular transition and if you mess with it you cause paralysis. These systems involving the catecholamine and the indole systems are much more complexx in terms of how there used and regulated. The biogenic amines actually act on a number of nerves both in the CNS and PNS. they tend to work on the sympathetic nerves of the peripheral organs (the sympathyic nervese system is the one we don't have any control over). They also act on the lymbic system, the lambic system is some pathways in the CNS in the brain which is concerned with things like emotional states, mood, behaviour and that sort of thing. So these nerurotransmitteres work in the lytic system hence there connection with things like the sympathetic nervous system. The consequencee then you find with the drugs that affect the biogenic amines tend to have some sort of neurological effect or sytiactric effect. Most of the drugs perscribed for psychiatric conditions often work on these systems here. Just as we saw there are subcategories for cholinergic receptors like the nicotinic and muscogenic type of receptors so there are also subcategories for the biogenic amines.

If we look first at the nuroagenergic ways so we are looking at the receptors for norepinephrine they tend to be called noragenergic receptors. There classified as alpha and beta adreno receptors. Probably the best known and most important examples of the adreno receptors are the ones that control things like heart rate and contraction of the heart. Some of the pathway in the peripheral nervous system operate to control the heart rate and the contraction of the heart are governed by this system. Drugs that block the alpha receptor for example, things like the drug phentolamine are used for the preventing the contraction of smooth muscle. Smooth muscle like that which surrounds the blood vessels. We known that arteries are surrounded by smooth muscle and that artery can be told to constrict the artery and in doing so the blood pressure is raised. Things like phentolamine work on the alpha-adreno receptors relax that smooth muscle around the blood vessels and tend to lower the blood pressure. So they are used in treatment of things like hypertension. To complicate matters further we now know the alpha adreno receptors are themseleves sub-divided into a number of diffenret types, they are known as alpha1 and alpha2 adrenoreceptors.

The alpha1 types tend to be excitatory in there action while the alpha2 tend to be inhibitory. So we can start to see the complexitity of the nervous system and how it works. Now those excitatory and inhibitory receptions tend to be a second messenger one and we tend to find that the alpha2 tend to inhibit the action of adenylate cyclase, where as the alpha1 report tend to increase cellular calcium concentrations via the phosphinosityl system. The beta adreno receptor operate in the same sort of way actually and tend to be some of the better known compounds. Compounds that bloc the beta receptors are known as

beta blockers. Classic examples include drugs like propanilol and another called oxypilinol and these drugs are whitely used in the control of high blood pressure. Quite a high proportion of older people to control high blood pressure and hypertension. They work by decreasing both the force and the rate of contraction of the heart so they tend to lower the blood pressure as a result. Again ther is more than one type of beta adreno receptor, there are actually three of them. There is beta 1, 2 and 3. Its the beta 1 form that is found in the heart the beta 2 form is found in smooth muscle and the beta 3 type is found in the smooth muscle which is made affective by those drugs like propapaniliol. All three of these types are linked to adenylate cyclase and the cAMP as the secondary messenger system. So thats the adreno receptors and those are the ones working with just with norepinephrine.

Dopamin and serotonin

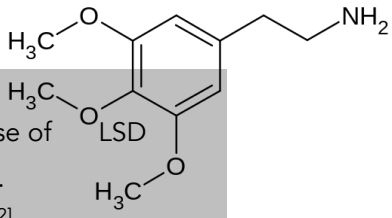


What about dopamine receptors then. Well surprise surprise they get complicated too. The dopamine receptors or also known as the dopaminergic receptors. They exist int he CNS and they are classified into a number of types often denoted as D1 and D2 and that sort of thing. There are believed to about 5 different types of dopamine receptor. Its generally considered that those five types of dopamine receptors can be loosly classified into a D1 like form. So we have the D1 like family of receptors and the D2 like family like receptors. So although there are five types they are either D1 like or D2 like. The D1 family includes D1 itself and D5 as these are the D1 and D5 receptors and they tend to operate via activation of adenylate cyclase again which causes a rise in cAMP levels. The D2 like family tend to inhibit adenylate cyclase so they reduce the level of cAMP. So they all seem to work via cAMP as a second messenger. Some of them increase the levels and some decrease the levels of cAMP.

The classification of serotonin receptors again i complicated. studies have suggested there may be as many as seven different types of receptor for serotonin and they tend to be called things like 5HT1 unto 5HT7. 5HT comes from the 5-hyrdoxytritomine. With the acceptation of the 5HT3 form which tends to affect the sodium and potassium flux through the membarne all of the other ones tend to operate through G protein coupling systems. So of the seven types there the 5HT3 form operates via a ligand gated ion channel that basically controls the flux of sodium and potassium ions through the channel and the others tend to operate through the G protein coupling systems. Each one of these seven forms can be further sub divided into A, B and C types so we can start to see that the picture for serotonin can start to get complicated. They also all work via diffenret methods for raising and lowering the levels of different things.

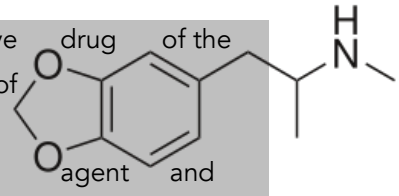
We said that the dopamine and seratoneric systems tend to to opperate on the lambic system and the CNS and tend to control things like mood, behavousre and things like that. So its not surprising that a

number of the common drug abuse tend to work on this system here. There are a number of drugs that afec this system but also because this system is so vast that and drug may play an effect on the system somewhere. There is a slide missing but it shows some compounds that are very similar to dopamine. they include things like mescaline Mescaline is like a structural analog of dopamine.



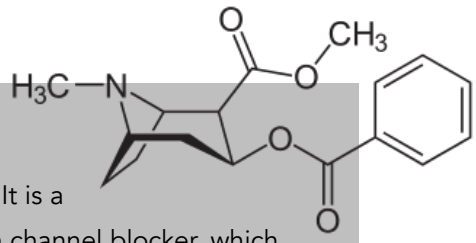
Mescaline or **3,4,5-trimethoxyphenethylamine** is a naturally occurring psychedelic alkaloid of the phenethylamine class, known for its hallucinogenic effects similar to those of and psilocybin. It shares strong structural similarities with the catecholamine dopamine.
It occurs naturally in the peyote cactus (*Lophophora williamsii*),[1] the San Pedro cactus[2] (*Echinopsis pachanoi*) and in the Peruvian torch (*Echinopsis peruviana*), and as well in a number of other members of the Cactaceae plant family. It is also found in small amounts in certain members of the Fabaceae (bean) family, including *Acacia berlandieri*.

It is a product of the peyote cactus and its well known to induce a schysophrinic type state so. Mescaline is an analog of dopamine that causes a mind bending experience. Another compound is amphetamine or speed as its street name. It is also a dopamine analog in the way it works. Along wit its derivatives where the best known one is 3,4, methylenedioxy-methamphetamine or MDMA.



MDMA (contracted from **3,4-methylenedioxy-methamphetamine**) is a psychoactive drug of the substituted methylenedioxyphenethylamine and substituted amphetamine classes of drugs that is consumed primarily for its euphoric and empathogenic effects.
Pharmacologically, MDMA acts as a serotonin-norepinephrine-dopamine releasing agent and reuptake inhibitor.
MDMA has become widely known as "**ecstasy**" (shortened to "**E**", "**X**", or "**XTC**"), usually referring to its tablet street form, although this term may also include the presence of possible adulterants. The UK term "**Mandy**" and the US term "**Molly**" colloquially refer to MDMA in a crystalline powder form that is relatively free of adulterants."Molly" can sometimes also refer to the related drugs methylone, MDPV, mephedrone or any other of the pharmacological group of compounds commonly known as bath salts.

This is a derivative of amphetamine and also cocaine



Cocaine (INN) (**benzoylmethylecgonine**, an ecgonine derivative) is a tropane alkaloid that is obtained from the leaves of the coca plant.[5] The name comes from "coca" and the alkaloid suffix "-ine", forming "cocaine". It is a stimulant, an appetite suppressant, and a nonspecific voltage gated sodium channel blocker, which in turn causes it to produce anaesthesia at low doses. Biologically, cocaine acts as a serotonin–norepinephrine–dopamine reuptake inhibitor, also known as a triple reuptake inhibitor (TRI). It is addictive due to its effect on the mesolimbic reward pathway. At high doses, it is markedly more dangerous than other CNS stimulants, including the entire amphetamine drug class,[7] due to its effect on sodium channels, since blockade of Nav1.5 can cause sudden cardiac death.

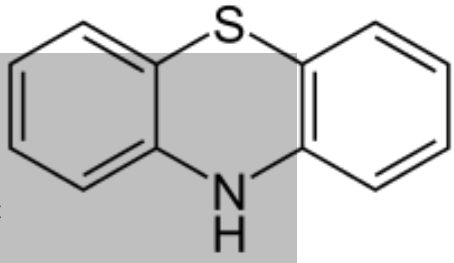
Unlike most molecules, cocaine has pockets with both high hydrophilic and lipophilic efficiency, violating the rule of hydrophilic-lipophilic balance. This causes it to cross the blood–brain barrier far better than other psychoactive chemicals and may even induce blood-brain barrier breakdown. Furthermore, many MAT inhibitors are not reinforcing like cocaine, and has led some to postulate another mechanism, such as DAT "inverse agonism" to play a role in cocaine's pharmacological mode of action.

These things like amphetamine, cocaine and such. The way in which they work is they tend to inhibit the repute of dopamine. Dopamine is released form the dopaminergic neurone but instead of it being taken back up again and gotten rid off these compounds tend to inhibit the reuptake of dopamine so its tends to hang around longe in the synaptic cleft. The effects are quite well known and amphetamine is quite a powerful appetite suppressant. Now there is a story behind that as some years ago in the 1960s people didn't fully appreciate the dangers off it and it was quite commonly perciacbed as a diet pill. It was common for people to go to the doctors whop wanted to loos a bit of weight to be prescribed slimming tablets and they were based on amphetamien. They were quite effective but they did of course have the side effects where people became addicted to them more than anything else and they of course became band for that purpose. Cocaine has a similar sort of effect where it inhibits the appetite. They are both stimulants and cocain is also whats called a euphoriant. However you don't get something for nothing and these things as well as making you feel good can have some severe effects on your cardiovascular system. Basically they can induce abnormal rhythms in the heart. We saw that some of these compounds are used for controlling things like heart rate and things like that and so obviously things like cocaine and amphetamine and so on will affect the heart rate. They can induce things like arthymias that are abnormal rhythms of the heart. That in turn can lead to all kinds of complications. You can get a coronary infarction or a heart attack of susuptual individuals. You can also get some cerebral vascular effects so thats to say some effects on the blood flow in the brain and that of course can cause some major problems. They can all be explained by the effects of these catocholimines on the cardiovascualr system. So the catacholimines affect the cardiovascualr system so these drugs can interfere with that tend to interfere with the heart and vascular system. Young responsible healthy people tend to get away with it but older people don't sometimes and there are many cases of waging rockstars who have taken stuff like cocain or something and then keeled over. There was a member of the Who that was a good example.

There is also another side effect to it as well and amphetamines for example. Amphetamines when they are degraded they tend to form a neurotoxin something called 6-hydroxydopamine now this is a neurotoxin which is toxic to the nerve cells. Normally that would be de toxified by our old friend MAO but amphetamine is an inhibitor of MAO so we get a sort of a double wammy where you are getting the substance reduced as your body tries to get rid of these substances. They intern inhibit the enzyme and otherwise brake down the 6 hydorxydopamine. So intoxication of theses substance manifest themselves in a variety of ways where they get a tremour of shaking when you overdose. you can also get dilutions, hallucinations and paranoid behaviour.

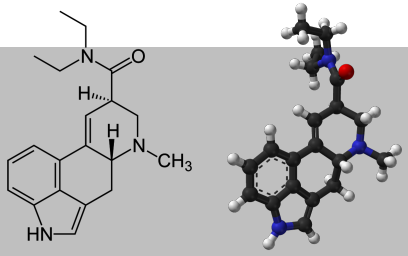
Its not all bad news though there are some reasons to use these drugs quite legitamtly that operate at the level of the dompinergic system. Typically they are used to control the disorders of movement or so

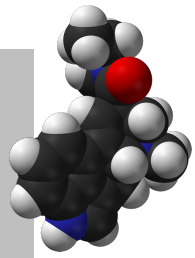
called choreas. The word has its origin in the sam was choreography or dance like. Perhaps the best known of the dopamine receptor blockers are a family of drugs known as the phenothiazines.



Phenothiazine is an organic compound that occurs in various antipsychotic and antihistaminic drugs. It has the formula $S(C_6H_4)_2NH$. This yellow tricyclic compound is soluble in acetic acid, benzene, and ether. The compound is related to the thiazine-class of heterocyclic compounds. Derivatives of the parent compound find wide use as drugs.
The compound was originally prepared by Bernthsen in 1883 via the reaction of diphenylamine with sulfur, but more recent syntheses rely on the cyclization of 2-substituted diphenyl sulfides. Some of the pharmaceutically significant derivatives of phenothiazine are not prepared directly from phenothiazine, although some of them are.

They are very powerful tranquillising type compounds. So in movies where someone is going bazaar and the guys int he white coats come dashing in and administer something to a patient that immediately calms them down is these sorts of drugs and classically it was phenothiazine. These things are also used in the treatment of neurological disorders like schizophrenia perhaps the side effect of these is that because there linked to these choreas here the side effects of these drugs is that there very good at suppressing the more horrid symptoms of schizophrenia. On of the classic symptoms of this disease are the hearing of voices and commands and people telling them to do things and the phenothiazines are very good at suppressing this paranoid behaviour that people are listening in to your thoughts. But because there not an absolutely specific drug they tend to affect the moment functions of the body as well. People of these types of drugs sometimes develop abnormal sorts of moments. Particularly of the facial muscles so they tend to pull faces without any control over it. One other drug that we didn't get to see was is the compound LSD. This stands for lysergic acid diethyl amide

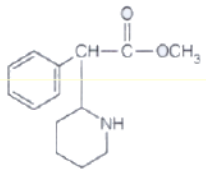




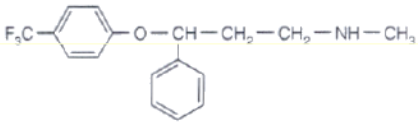
Lysergic acid diethylamide abbreviated **LSD** or **LSD-25**, also known as **lysergide** (INN) and colloquially as **acid**, is a psychedelic drug of the ergoline family, well known for its psychological effects, which can include altered thinking processes, closed- and open-eye visuals, synesthesia, an altered sense of time and spiritual experiences, as well as for its key role in 1960s counterculture. It is used mainly as an entheogen and recreational drug. LSD is non-addictive. However, acute adverse psychiatric reactions such as anxiety, paranoia, and delusions are possible.
LSD was first synthesized by Albert Hofmann in 1938 from ergotamine, a chemical derived by Arthur Stoll from ergot, a grain fungus that typically grows on rye. The short form "LSD" comes from its early code name *LSD-25*, which is an abbreviation for the German "Lysergsäure-diethylamid" followed by a sequential number. LSD is sensitive to oxygen, ultraviolet light, and chlorine, especially in solution, though its potency may last for years if it is stored away from light and moisture at low temperature. In pure form it is a colorless, odorless, tasteless solid. LSD is typically either swallowed (oral) or held under

the tongue (sublingual), usually on a substrate such as absorbent blotter paper, a sugar cube, or gelatin. In its liquid form, it can also be administered by intramuscular or intravenous injection. Interestingly, unlike most other classes of illicit drugs and other groups of psychedelic drugs such as tryptamines and phenethylamines, when LSD is administered via intravenous injection the onset is not immediate, instead taking approximately 30 minutes before the effects are realized. LSD is very potent, with 20–30 µg (micrograms) being the threshold dose.

LSD is again well known as a hallucinogen that makes people see things and so on. ITs supposedly not addictive and very potent. Most of the deaths that have arisen form LSD come form the fact that people who have taken it though they could fly and jump out of windows. IT is said not to be harmful as well but that probably a little bit of an over simplification as there is some evidence that people that are susceptible to a metal illness can be pushed over the edge by taking substances like that. The way this seems to work is by the 5HT receptors. It is well known that it seems to cause its effect by activating the 5HT2 receptors (a hlucinogen working via these serotenergic receptors). There are other compounds that work on the serotenergic system and this drug pholoxitien of prozac is one of the most widly perscribed substance in a america its an antidepressant and the way in which it works is by blocking the re-uptake of serotonin. It makes the serotonin hang aroundd in the synaptic cleft for longer.



Ritalin
(methyl phenidylacetate)



Prozac
(fluoxetine)

Also related to serotonin is the compound methyl phenidylacetate or ritalin. Again you may have heard of that one as it has been in the news. IT is often used to treat children with suffer with this hyperactivity attention deficite disorders. They are given this stuff to calm them down. There is often some debate as to wether medicating children is an effective way to treat a disorder. IT again works by elevating the levels of serotonin in the brain and seems to clam kids down.

Just to finish of with a coupe of other examples to give you an idea of the various different types of activity that is controlled by the dopaminergic and serotinergic systems. The serotonin receptors are also targets of drugs like Ondansetron which is a drug used to treat post operative nausea and chemotherapy induced nausea some of you may know again certain types of operations or drugs can cause quite violent nausea and sever sickness that can be debilitating. These sorts of drugs like Ondansetron are good at controlling that nausea. They tend to work on the 5HT3 receptors so it just shows you the wide range of different behaviours and different effects that can be controlled by this system. Simply other drugs that act as antidepresants work on the serotinergic system there.

Ondansetron (INN), originally marketed under the brand name Zofran, is a serotonin 5-HT_3 receptor antagonist used to prevent nausea and vomiting caused by cancer chemotherapy, radiation therapy, and surgery. It has little effect on vomiting caused by motion sickness, and does not have any effect on

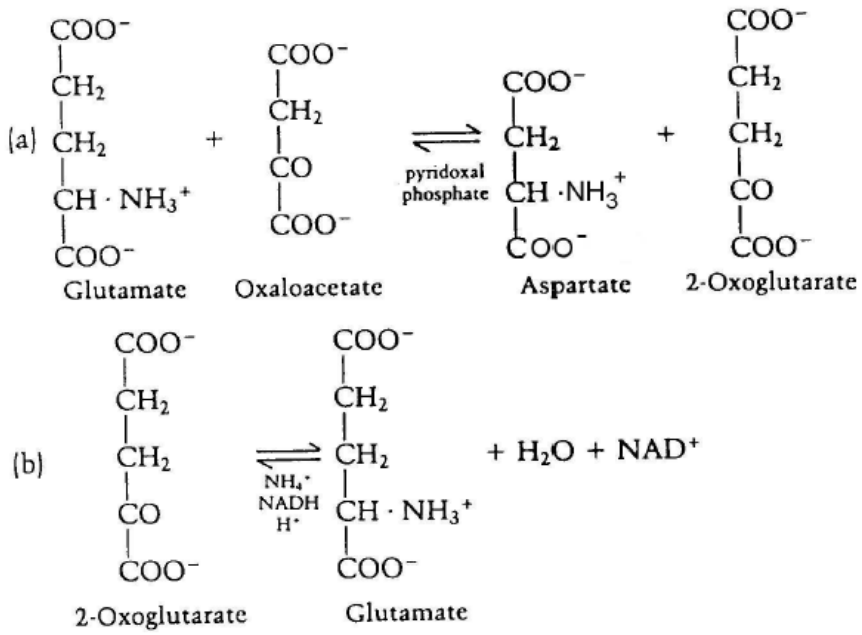
dopamine receptors or muscarinic receptors It is on the World Health Organization's List of Essential Medicines, a list of medications needed in a basic health system.

Chapter 9 (Neurophysiology and Neurochemistry)

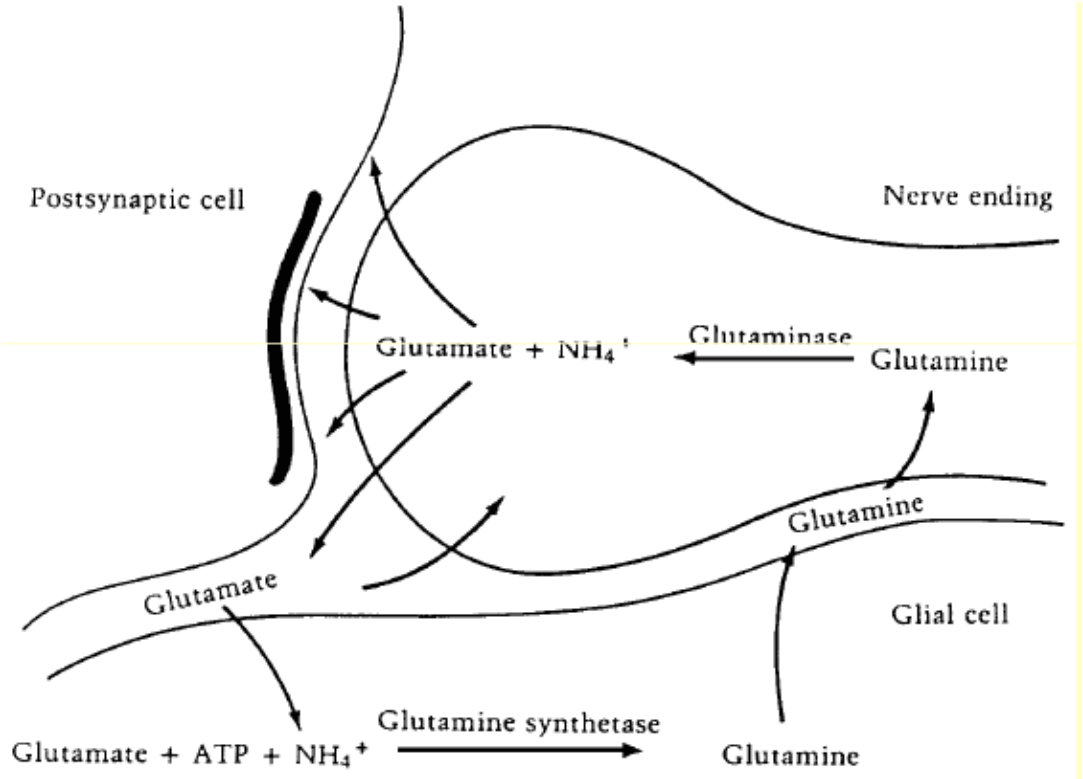
This chapter includes

- Amino acid biosynthesis
- GABA and Glycine

Amino acid biosynthesis

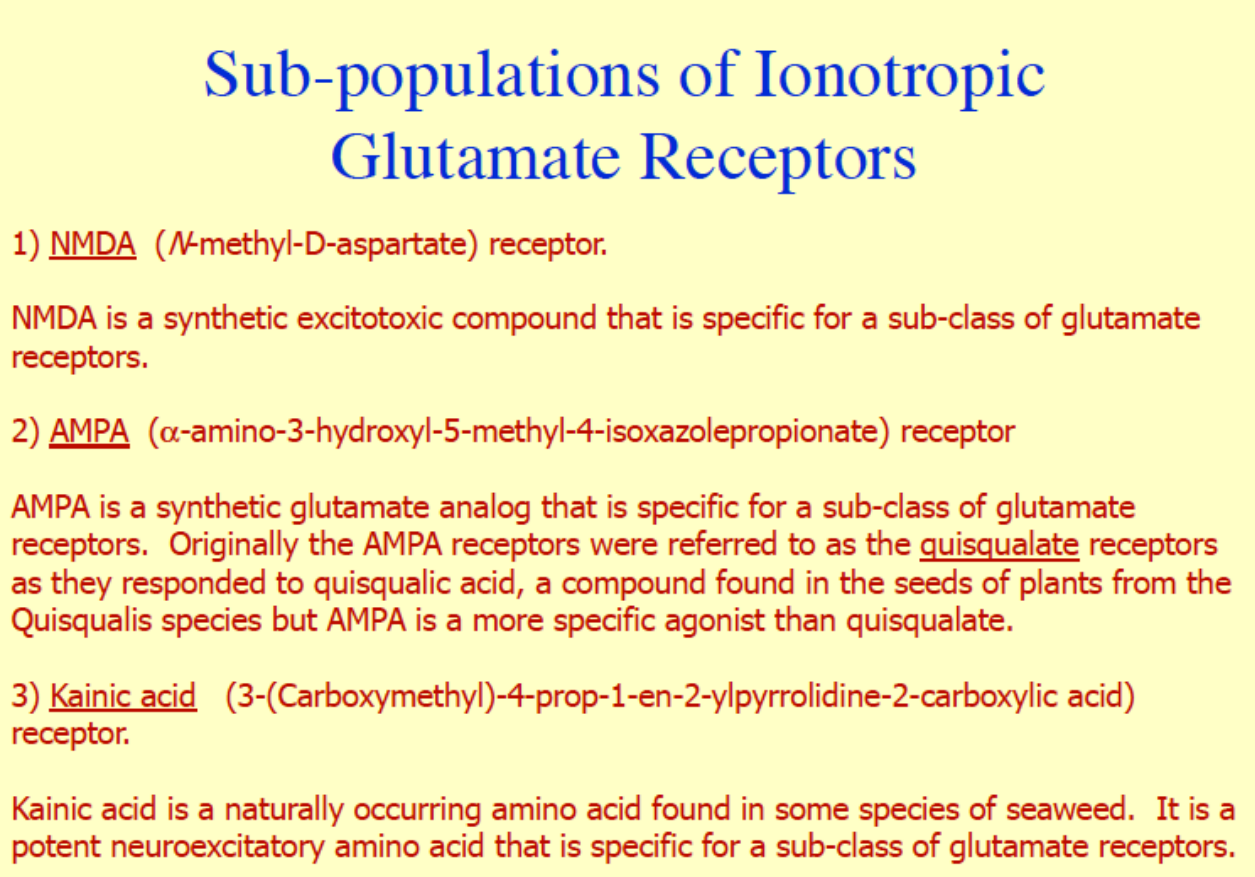


The way its gotten rid of is with these high affinity transport systems that are taken up by the sodium gradient and they are processed in perhaps a glial cell the enzyme glutamine synthetase here takes the glutamate converts it too glutamine and then everything can be recycled back to a neurotransmitter so its a fairly simple cycling process. Glutamate is generally acknowledged to be probably the most important nerotransmitter in brain functions. Nearly all of the excitatory neutrons that you find in the CNS are glutaminergic. Thats to say they use glutamate as a neurotramitter. So is a very ubiquitous neurotransmitter in the CNS. Its actually recorded that over half of all the synapses and the excitatory synapses use glutamate as a neurotransmitter in the CNS. there is actually a consequence of that actually because you have a lot of synapses using glutamate as a neurotransmitter it plays an important role in clinical neurology. When the brain is injured glutamate can be released form a lot of cells in the brain, this can be an excessive and because a lot of neurons in the brain use this as a neurotransmitter that causes an over excitation of those neutrons and that process is called excytoxicity. And as we said before it is caused by a massive release of glutamat ein the brain and can cause an over stimulation of those neurones and synapses that use glutamate as a neurotransmitter so you can imagine that lots of neutrons start firing of inappropriately and that causes an exocytoxicity and it can actually cause cell death. Because they are firing continuously because of the excessive amount of glutamate they can literally excite themselves to death.

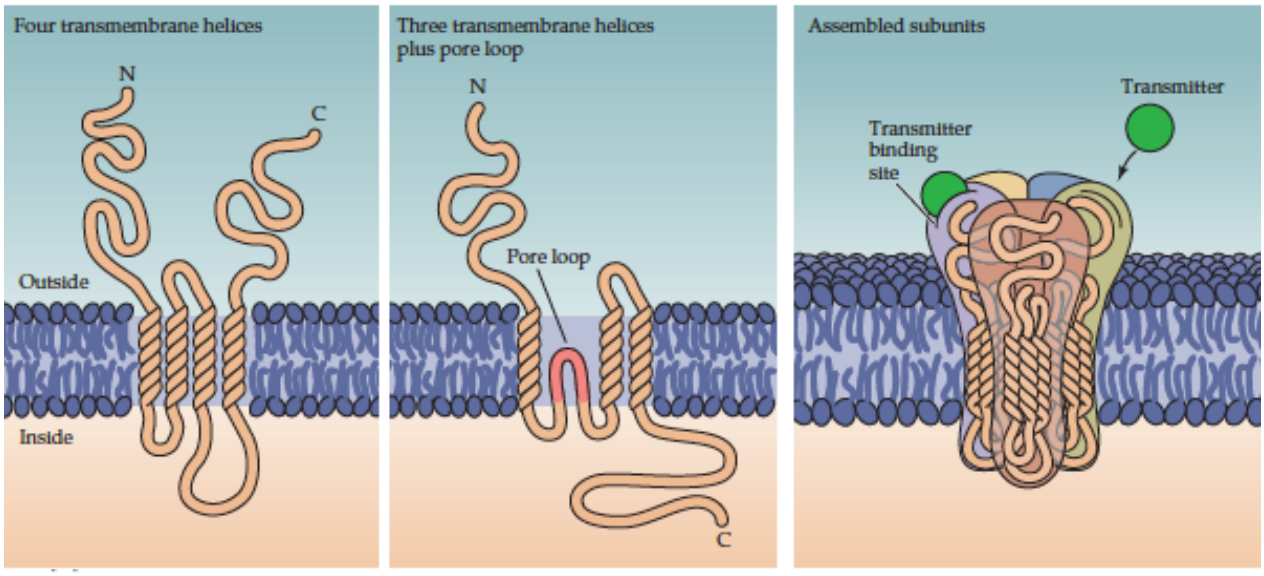


This has been extensively studied because as we say it can arise after brain injury and its believed that a lot of the damage thats cause to the CNS after an injury actually occurs after the initial trauma. So you can imagine that when someone is involved in a road traffic accident or suffer a blow to the head they suffer some damage to the brain. Thats the initial sort of effect but then this process goes on after the initial impact has taken place and as we say it continues to cause further brain damage after the initial process. People have speculated that if you could somehow blockade the glutamate receptors at least on a temporary basis you might be able to stop some of that brain injury that occurs after the initial injury occurs. So there is quite a lot of interest in helping brain injuries after an initial trauma. Its not just RTAs that can suse this mage either it can also follow other types of injury. Say people that suffer intense electric seizures is basically when someone suffered an epileptic fit that keeps going on and on and on and its doesn't stop and that kind of prolonged seizure state can cause a cytotoxic effect that it caused by repeated firing of the neurones. This is part of the rational behind when they cool the brain. One of the ways in which people can be prevented form experiencing this sort of brain injury is if the brain scooped and the patient is heavily sedated it kind of calms down the CNS until its had chance to recover. Possibly the most recent case is micheal schemata who suffered from a skiing accident and was in an induced coma for a long time. Part of that again is to do with this long term damage form occurring and giving the brain chance to recover. So glutamate is a very important receptor in the CNS and it wont surprise us to know that there are a number of different receptors for glutamate. They are distinguished on the basis of there response to other agonists. There are three subpopulations of inotropic glutamate receptors. Ionitropic means they use ions as the species being transmitted and when glutamate binds to the glutamate receptor it allows that passage of sodium and potassium ions. So those are ionotropic glutamate receptors so we say there are three are three categorises of them. In the body of course there all responding to glutamate as there transmitter. We know they can respond to other substances as well.

First class of them are the NMDA glutamate receptors, there names as we said after the substance we know that can stimulate them. NMDA is N-methyldiaspartate and this is basically a synthetic excitotoxic compound specific for one of those cases of glutamate receptors. The second category are the AMPA ones.



AMPA is actually also a synthetic glutamate analog and it si specific for another class of glutamate receptors. In some of the literature you may see it referred to as the quisqualate receptors. This quisqualate is a seed found in the plants of quisqualis species. Again this stimulates the category of the glutamate receptors. AMPA has largely succeeded that now and it is a bit more specific in what it goes for. the quisqualte can trigger other things as well. So there usually known as AMPA receptors now.
In some of the litrature you will see the receptoros referred to as quisqualate receptors but it is not as specific as AMPA so now they are referred to as AMPA receptors. The third class are these Kainic acid ones, and agin this is a naturally occurring amino acid found in some species of sea weed. Basically we have three subclasses of glutamate receptors established on the presence of these other compounds that they will respond too. Nodal three of those receptor types are inotropic, now thats to say they allow the passage of these sodium potassium ions in and out of the cell when they bind glutamate but in some cases these receptors can also allow the passage of a small amount of calcium ions as well. That has consequences as we will see in minute. Now the receptor structure of these things is shown in the slide on the next page. Basically there formed from a combination of a number of subunits. These are either four or five units that have these subunit names here in the table. Different synapses have different



Receptor	AMPA	NMDA	Kainate	GABA	Glycine	nACh	Serotonin	Purines
Subunits (combi-nation of 4 or 5 required for each receptor type)	Glu R1	NR1	Glu R5	α_{1-7}	α1	α_{2-9}	5-HT_3	P_{2X1}
	Glu R2	NR2A	Glu R6	β_{1-4}	α2	β_{1-4}		P_{2X2}
	Glu R3	NR2B	Glu R7	γ_{1-4}	α3	γ		P_{2X3}
	Glu R4	NR2C	KA1	δ	α4	δ		P_{2X4}
		NR2D	KA2	ε	β			P_{2X5}
				ρ_{1-3}				P_{2X6}
								P_{2X7}

combinations of these receptor subclasses so you can begin to see again the complexity of the central nervous system. There is not glutamate receptor or or type of synapses there are many different types and there characterised by these different numbers of these subunits here. So here is a huge diversity and complexity of the central nervous system here. The NMDA receptors in particular have specific properties and this is just a diagram here that shows a diagrammatic structure of the the NMDA receptors (seen on the next page). As we said before they allow the passage of sodium potassium ions so we see at the top there there is glutamate bound to the receptor subunit and the passage of sodium ions not the cell as its coming down the concentration gradient and potassium ions leaving the cell as they pass down there concentration gradient. We also noted that calcium ions can pass through these receptors as well so theres a small flux of calcium as well. Calcium of course is used to coming into the cell because theres a low calcium concentration inside. And as you know calcium itself is a second messenger molecule. So the influxx of calcium can arise the intracellular calcium level and that can in turn influence the functioning of other pathway with in the cell. so its another way in which this cell can work. The other curious thing about these types of receptors tot is that in many cases they have acquired a precentce of whats called a co-agonist in this case its glycine. So before the recpetor can open it docent require just the binding of its normal agonist glutamatee it also requires the binding of a co-aganoist called glycine. The other curiouse thing of course is this magnesium get stuck in the middle of it as well. And as you can see the magnesiumm acts like a kind of a block. The magnesiam finds its way in form the outside and acts like a

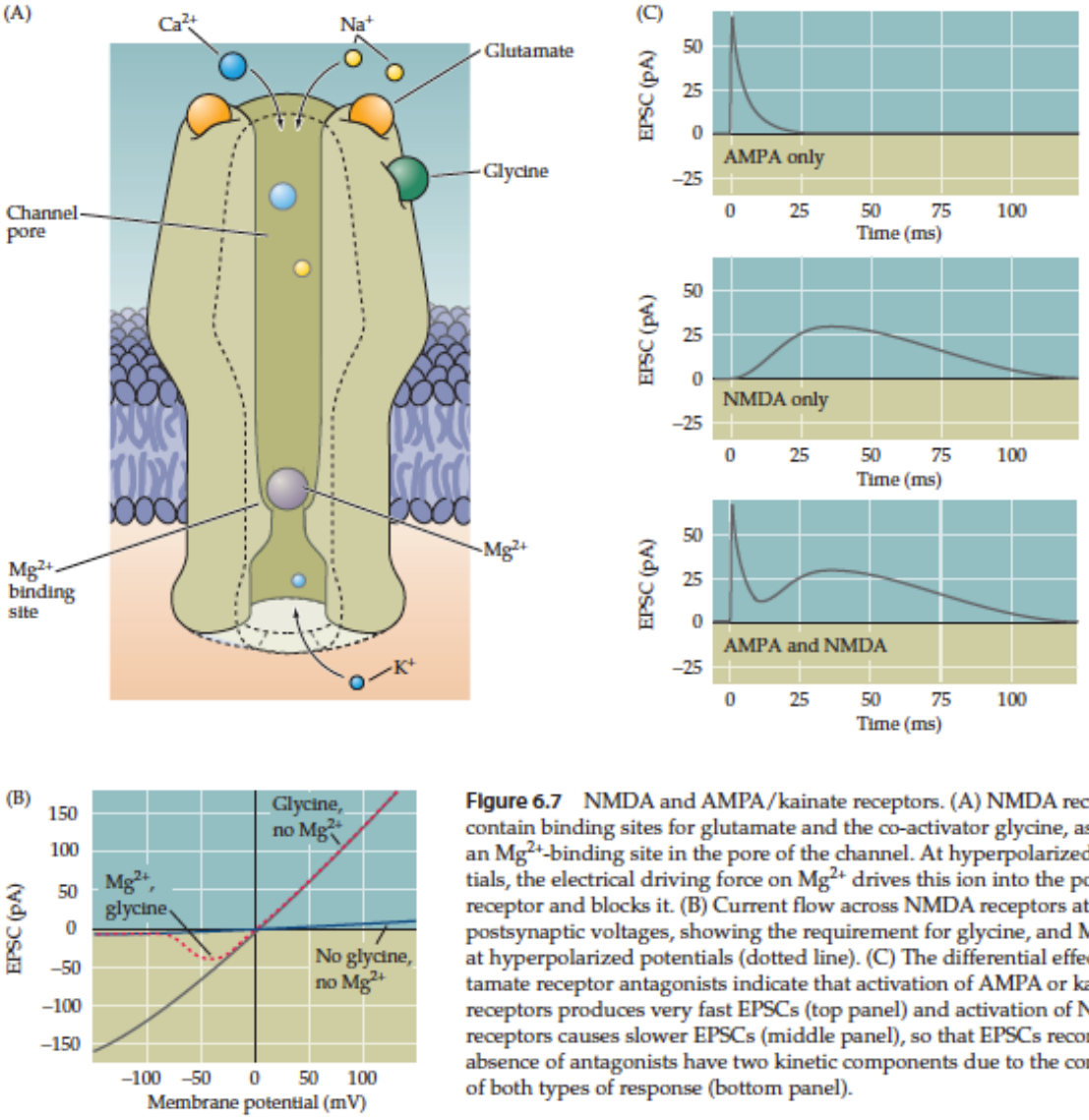
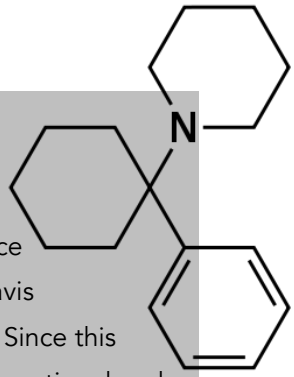


Figure 6.7 NMDA and AMPA/kainate receptors. (A) NMDA receptors contain binding sites for glutamate and the co-activator glycine, as well as an Mg^{2+}-binding site in the pore of the channel. At hyperpolarized potentials, the electrical driving force on Mg^{2+} drives this ion into the pore of the receptor and blocks it. (B) Current flow across NMDA receptors at a range of postsynaptic voltages, showing the requirement for glycine, and Mg^{2+} block at hyperpolarized potentials (dotted line). (C) The differential effects of glutamate receptor antagonists indicate that activation of AMPA or kainate receptors produces very fast EPSCs (top panel) and activation of NMDA receptors causes slower EPSCs (middle panel), so that EPSCs recorded in the absence of antagonists have two kinetic components due to the contribution of both types of response (bottom panel).

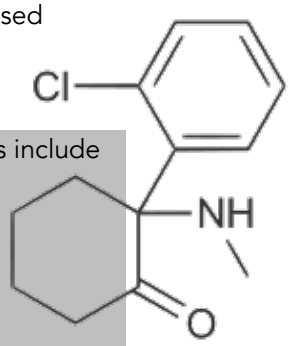
blockage in the channel there. So the receptor only becomes permianleto these ions like sodium, potassium and calcium. When that block is removed. The magnesium block can be removed under certain circumstances particulrly ehen you get depolarisation of the postsynaptic cell. Now what causes that depolorisation well withe the post synaptic cell can receive a lot of inputs form the other synapses which can polorise it. Or it can receive repetitive depolarisation passes from the pre synaptic cell. This looks like it is just complicated but its believed that that kind of response is the basis of information storage and memory. In certain parts of the brain these receptor subclasses particularly the NMDA ones ar believed to be used in the formation of memories. Again people don't understand how memoriess are formed in the brain or how memories are stored. This however is believed to be one potential mechanism by which a memory can be set up and stored. The NMDA recentor again the target of a number of drugs. some of which can be abused, there is a compound called phencyclidine. This also mascarades under another name like PCP or angle dust. It was originally made as an anaesthetic so thats what it was originally used for. but it also had some delirium and hallucinatenic effects so its use in human medicine has largely been stopped now. It dose largely still go on in veteran medicine but not in humans.

Its interesting though that these substances are abused but we can tell important information form them because if people take small doses of PCP it tends to produce signs of intoxication people stagger and there speech becomes slurred. At higher doses it can produce a sort of hallucination effect and such a state is commonly accompanied by aggressive behaviour so people high on these sorts of substances can often become aggressive. The other curiosity and this links up with the memory side of this as well. is that these agents are quite high potent anethesitc agents. Thats to say they tend to make you forget things and this fits in quit uniquely in that some of these receptors may be involved in laying down and the keeping of memories. So basically people who are high of PCP go out and get involved in punch up but then cant remember it after woulds. It comical but you can see the biochemical understanding of why that is. This PCP we know interacts with the NMDA receptor and it is an anethestic reagent so it ind of fits in.



Phencyclidine (a complex clip of the chemical name **1-(1-phenylcyclohexyl)piperidine**), commonly initialized as **PCP** and known colloquially as **Angel Dust** and by many other names,[1] is a dissociative drug. PCP was brought to market in the 1950s as an anesthetic pharmaceutical drug but was soon taken off the market in 1965 due to the high prevalence of dissociative hallucinogenic side effects. Likewise ketamine was discovered by Parke-Davis researchers as a better-tolerated derivative for use as an anesthetic pharmaceutical drug. Since this time a number of synthetic derivatives of PCP have been sold as dissociative drugs for recreational and non-medical use.
In chemical structure, PCP is a member of the arylcyclohexylamine class, and, in pharmacology, it is a member of the family of dissociative anesthetics. PCP works primarily as an NMDA receptor antagonist, where it blocks the activity of the NMDA receptor. Like most hallucinogenic drugs, there is risk of abuse with PCP. In the 1970s, the US media demonized PCP and PCP users giving many outlandish claims including that PCP gave users superhuman strength.
As a recreational drug, PCP may be ingested orally, smoked, insufflated or injected.

There is another drug that is known to interact with the NMDA receptor and that is ketamine. Again it is a drug that has become a bit notorious and become known as K or special K on the street. It is a substance that we mention because again its a substance that can cause hallucinations. Ketamine is also used therapeutically as well. It is used in legitimate medicine that is used as an alginic or painkiller.



Ketamine (INN) is a medication used mainly for starting and maintaining anesthesia. Other uses include sedation in intensive care, as a pain killer, as treatment of bronchospasm, as a treatment for complex regional pain syndrome and as an antidepressant. It induces a trance like state while providing pain relief, sedation, and memory loss. Heart function, breathing and airway reflexes generally remain functional.
Common side effects include a number of psychological reactions as the medication wears. This may include agitation, confusion and psychosis among others. Elevated blood pressure and muscle tremors

are relatively common, while low blood pressure and a decrease in breathing is less so. Spasms of the larynx may rarely occur.
Pharmacologically, ketamine is classified as an NMDA receptor antagonist, but it also acts at numerous other sites (including opioid receptors and monoamine transporters). Like other drugs in its class, such as phencyclidine (PCP), it is classified as a dissociative agent.
Ketamine was first developed in 1962. It is on the WHO Model List of Essential Medicines, a list of essential medicines that should be available in a health system. Its hydrochloride salt is sold as Ketanest, Ketaset, and Ketalar. Its use as a recreational drug has been associated with several high-profile deaths.

So ketamine acts both as an apathetic and a painkiller. ITs quit commonly used to treat severely injured people. If people are involved in traumatic accidents or a severe injury then its is actually avery good substance to deaden the pain and anaesthetise them a little bit. Under controlled conditions it wont cause hallucinations as its given at low doses in combination with other things. In the wider world people can abuse it and take too much.

Ketamine is given in conjunction with things like morphine and thats how they keep the hallucinations out. Ketamine is widely administered to people who suffer injuries and it is one of the basics of emergency medicine. IT can also produce the anethstic affects and its one of these drugs known as a date rape drug because it can be used to cause them to forget things. So there is a kind of nasty side to these things as well. Basically it is used and operates via the NMDA receptor.

This dose then give a tantalising insight into how memory might work as these effects link to the chemistry of the brain and what we observe can help us understand the physiology of these sorts of things. So thats glutamate and we wont say much about aspartate because aspartate is very much like glutamate.

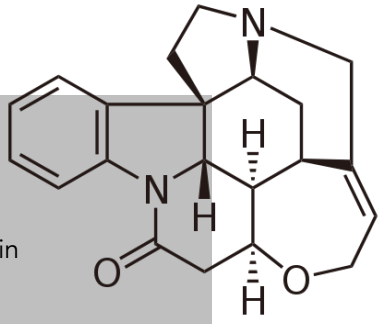
GABA and Glycine

GABA and glycine are two inhibitory amino acids. Glutamate is the excretory version of this and the inhibitory ones tend to calm things down. We have talked about the synthesis of GABA when we spoke about glucose metabolism so were not going to revisit that again. The synthesis of glycine is a fairly simple mechanisms as well. It course primary by the action of a single enzyme, Glycine is formed by the action of the enzyme serine trans-hydroxylmethylase and basically that converts serine not glycine so its a trans amilation reaction it just take serine to convert it to glycine. It wont surprise you to learn that both of those nuerotransmitters are released in a calcium dependent type of transmission. There inactivation again happens via a high affinity uptake system so they are released from the nerve terminal they bind to the receptor and do there job and there taken back up again by a high affinity sodium link transport system. Now both glycine and GABA act as inhibitory neurotransmitters by increasing the permeability of the post synaptic cell to chloride ions. If you do that basically what happens is the post synaptic cell

becomes hyper polarised. So by allowing chloride ions to enter the post synaptic cell and it becomes hyper polarised and that makes it more difficult to trigger an action potential in it. So thats how it can dampen things down. Glycine and GABA increase the chloride conductance into the post synaptic cell they hyper polarise it. It makes it more resistant to becoming excited and hence they acts as inhibitory neurotransmitters.

We should mention now that when eve you have an signal traveling from one cell to another its not always just a single synapse. Its not just one cell going to another one and a particular cell might be a number of synapses coming in from other cells. Some of them inhibitory some excitatory. So if the inhibitory ones are firing it makes that cell less likely to be induced by an excitatory one.

There are actually very few drugs that block the glycine receptor and this is one of the reasons why its so difficult to study. but there is one that is a well known inhibitor of the glycine receptor known as strychnine.



Strychnine is a highly toxic (LD_{50} = 0.16 mg/kg in rats, 1–2 mg/kg orally in humans), colorless, bitter crystalline alkaloid used as a pesticide, particularly for killing small vertebrates such as birds and rodents. Strychnine, when inhaled, swallowed or absorbed through eyes or mouth, causes a poisoning which results in muscular convulsions and eventually death through asphyxia. The most common source is from the seeds of the *Strychnos nux-vomica* tree.

Strychnine is an alkaloid produced by some plants and its well known as a poison. In days gone by it was sold quite freely as a rat poison. It is however also a very potent blocker of the glycine receptor. Because glycine is a nuero =inhibitory transmitter you might image that if you block that inhibition the CNS becomes excitable and that is exaxtly what happens. This manifests its self by causing convulsions or seizures and that sort of thing. This works eventually by causing respiratory failure. There is actually a rare condition that is believed to be linked to the glycine nuerotransmitter system and again there are a few clues as to what it dose. This condition is called Hyperekplexia and its a rare inherited condition believed to be linked to a defect in the glycine neurotransmitter system.

Hyperekplexia ("exaggerated surprise") is a neurologic disorder classically characterised by pronounced startle responses to tactile or acoustic stimuli and hypertonia. The hypertonia may be predominantly truncal, attenuated during sleep and less prominent after a year of age. Classic hyperekplexia is caused by genetic mutations in a number of different genes, all of which play an important role in glycine neurotransmission. Glycine is used by the central nervous system as an inhibitory neurotransmitter. Hyperekplexia is generally classified as a genetic disease, but some disorders can mimic the exaggerated startle of hyperekplexia.

Hyperekplexia is a condition in which people have an exaggerated surprise response. They suffer to an extent to which ther is bad startle response and thats generalised stiffness and they become quite rigid

and they more or less become paralysed. Its believed to from a defect in the glycine receptor system. So people might actually fall over. There is a species of goats called fainting goats and if there startled they freeze and fall over. This is actually a different response although its looks the same.

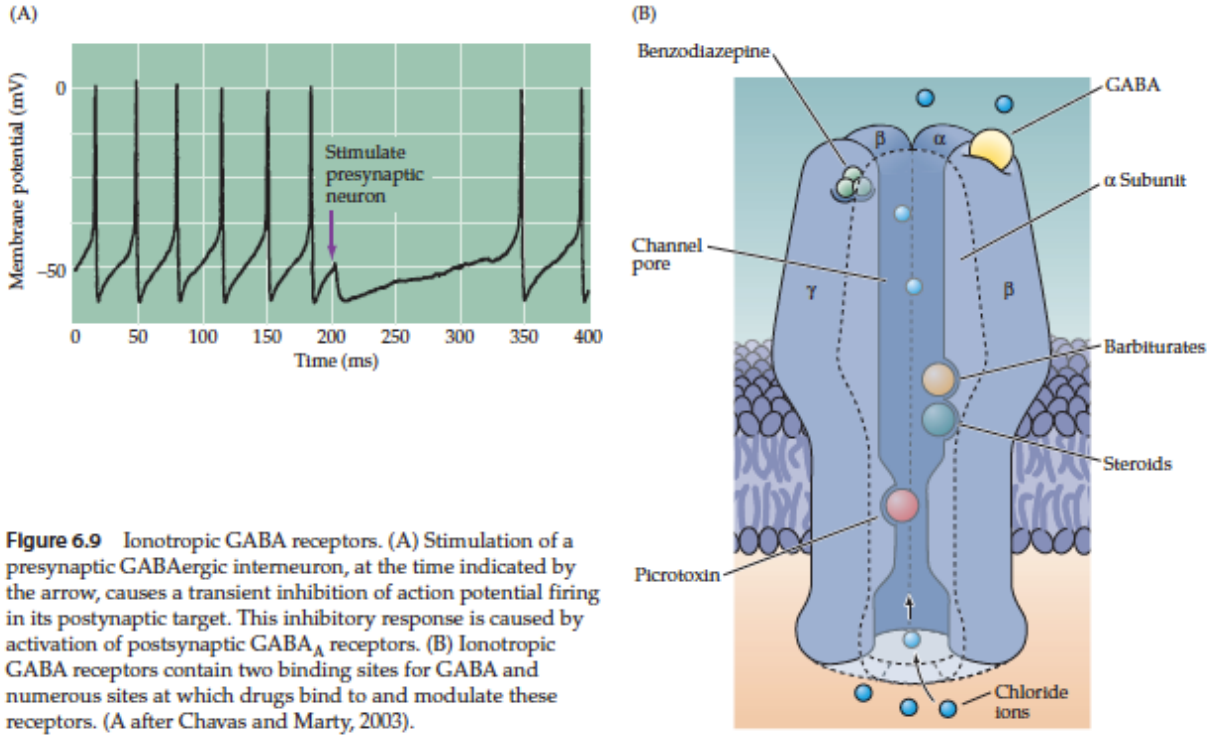


Figure 6.9 Ionotropic GABA receptors. (A) Stimulation of a presynaptic GABAergic interneuron, at the time indicated by the arrow, causes a transient inhibition of action potential firing in its postsynaptic target. This inhibitory response is caused by activation of postsynaptic $GABA_A$ receptors. (B) Ionotropic GABA receptors contain two binding sites for GABA and numerous sites at which drugs bind to and modulate these receptors. (A after Chavas and Marty, 2003).

Moving on to the GABA receptor This again is an inhibitory system and there are three different types of receptors that respond to GABA there known as GABAa GABAb and GABAc. The GABAa and GABAc receptors are again ligand gated ion channels. So thats to say when the ligand GABA binds to them it opens an ion channel and in this case allows chloride ions to flow through. The GABAb receptors are different there linked to what are called metabotrophic systems But the other two are ionotrophic ones. Basically when they are stimulated by a GABA binding to them they open an ion channel and allow chloride ions to flow into the cell. This makes them less responsivee to becoming stimulated. The GABAa one is probably the best understood and it consists of a pentamer where there are five subunits there known as the alpha, beta, gamma, delta and Rho subunits. Named after the first five letters of the greek a;phabet. In addition to those five subunits there are six alpha subunits which are variations on the alpha subunit and three types of the beta and gamma subunits. So the receptor its self is made up of five subunits and in various combinations and we know there are six different alphas and three different beta and gamma subunits. These all as well have subtly diffenrt responses and ways in which they work. There are a great many compounds that are known to work at the GABA receptor, basically because its an inhibitory synapse, if you block it from functioning then you will cause fits, seizures, convulsions and that sort of thing. Compounds like picrotoxin is a plant substance known to bind to the GABA and it basically blcoks the ion channel. Picrotoxin comes from a shrub called anamerta coculus. It is a plant substance known to act at the GABAa receptor and it is known to act as a convulsant. Barbiturates act in the opposite way as they bind to the channel and keep it open. So in affect it you stimulate this system is will

have a sedative effect and as we all know these are very powerful sedatives and cause somebody to fall asleep. It is exactly the effct you would expect from an inhibitory neutotranmitter system. This is actually a classic way in which people were put to sleep during surgery and there is a substance called sodium thiopentone. Basically the infusion of these things takes a few second as you see in surgery when people are put to sleep it is a very powerful sedative. IT is largely superseded these days by a substance called propofol. Propofol also acts at the level of the GABAa receptor. This is 2,6-diisopropylphenol and propofol is just its trade name. This stuff hit the headlines recently with the stuff that Michael jackson was addicted too and his doctor gave to him and its now used to put people to sleep. Its use in preference to the other because its quicker acting and there are fewer side effects and so on.

Chapter 10 (Neurophysiology and Neurochemistry)

This chapter includes

- Histamines
- Neuropeptides

Histamines

Histamines are loosely classified from H1-H4 and we looked at the H1 last time. The H1 and the H2 are the major ones really where the H1 types mediate things like smooth muscle contraction and these are the kind of ones that are involved in things like hay fever. So the classic things like anti-histamins such as benydril tend to go for the H1 type receptors. The H2 receptors though are also quite interesting. The H2 type of receptor is coupled to the histamine mediated recreation of gastric acid (the stomach acid that is produced by everyone). Thee have been the focus of much investigation because a lot of people suffer from excess stomach acid and basically in the past people who suffer from the excess stomach acid would often end up suffering from gastric ulcers which is a brake down of the lining of the stomach. In sevear cases that can cause bleeding or even perforation of the stomach. This is a very serious occurrence and can even be life threatening condition. Classically the way it is treated is for people to have whats called a gastrectamy which is basically a survival removal of the stomach. Its not a major operation but then its not something that can be done lightly. However people reasoned that if you can blockagee the H2 histamine receptors then you could prevent the excessive release of stomach acid and there by produce some sort bypass for a surgical intervention. A whole family of drugs where developed to blockage the H2 histamine system. The classic one is a drug called cimetidine which is a H2 receptor antagonist.

Cimetidine INN is a histamine H_2-receptor antagonist that inhibits stomach acid production. It is largely used in the treatment of heartburn and peptic ulcers. It has been marketed by GlaxoSmithKline (which is selling the brand to Prestige Brands) under the trade name Tagamet (sometimes Tagamet HB or Tagamet HB200). Cimetidine was approved in the UK in 1976, and was approved in the US by the Food and Drug Administration for prescriptions starting January 1, 1979.

The **H_2 receptor antagonists** (**H2RA**) are a class of drugs used to block the action of histamine on parietal cells (specifically the histamine H2 receptors) in the stomach, decreasing the production of acid by these cells. H_2 antagonists are used in the treatment of dyspepsia, although they have been surpassed in popularity by the more effective proton pump inhibitors.
The prototypical H_2 antagonist was cimetidine, developed by Sir James Black and Smith, Kline & French (now GlaxoSmithKline) in the mid-to-late 1960s and first marketed in 1976; sold under the trade name Tagamet, cimetidine would later become the first ever blockbuster drug. The use of quantitative structure-activity relationships (QSAR) led to the development of other agents—starting with Ranitidine, first sold as Zantac—which has fewer adverse effects and drug interactions and is more potent.

This was marketed under the trade name Tagamet and these drugs were very effective at blocking the H2 receptors that were responsible for the recreation of this stomach acid. They reduced the recreation of stomach acid and almost eliminated the need for a major surgery. This Tagamet was the first one but there were others like Ranitidine.

Ranitidine (trade name Zantac) is a histamine H_2-receptor antagonist that inhibits stomach acid production. It is commonly used in treatment of peptic ulcer disease and gastroesophageal reflux disease. Ranitidine is also used alongside fexofenadine and other antihistamines for the treatment of skin conditions such as hives. Ranitidine was discovered and developed by scientists at Glaxo Pharmaceuticals, now a part of GSK.
It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.

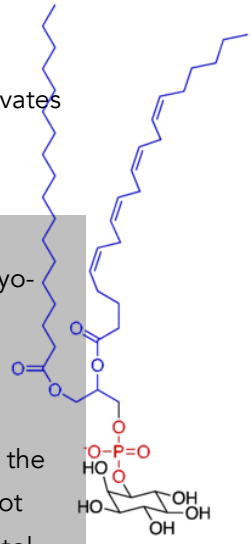
This one was used under the trade name Zantac. Again this is an H2 receptro antagonist that reduced the recreation volume of stomach acid. Some of the drug compaines made a lot of money out of these things because surprise suppurates they were effective but some may also be familiar with the something that goes with this story. That some years after these things were invented and quite widely marketed a couple of australian doctors noticed that a couple of patients who suffer from gastric ulcers almohad an infection with a bacterium called helicobacter pylori. And thes two reasoned that maybe the problem with the gut was die to this bacterium. That was partly responsible for the stomach ulcers. They basically tried treatming some people with antibiotics to get rid of the bacterial infection and low and behold in many cases the ulcers resolved them selves as well. The antibacterial agents at the time were a lot cheaper than the H2 receptor antagonists so antibacterials are normally the first go to treatment for excess stomach acid.

Just to compleat the circle really, nothing eer stands still and in fact actually today these drugs are less frequently used and people have developed specific proton inhibitors of the proton pump called omeprazole

Omeprazole (INN) (also distributed under the brand name **Prilosec**) is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease, laryngopharyngeal reflux, and Zollinger–Ellison syndrome.
Omeprazole is one of the most widely prescribed drugs internationally and is available over the counter in some countries.
It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system.

Omeprazole is not actually an inhibitor of the H2 receptor but an inhibitor of the proton pump that is used to treat stomach acid problems. (as a side note these proton pumps are not holy effective because although they limit the production of stomach acids like sulphuric acid they do not limit the production of bile acids which can actually overcompensate for the loss of gastric acid). All the histamine receptors

opperate by G proteins so all of them exert a reaction through G proteins and the H1 receptor activates phospholipase C and phosphatidylinositol signalling pathways.



Phosphatidylinositol consists of a family of lipids as illustrated on the right, a class of the phosphatidylglycerides. In such molecules the isomer of the inositol group is assumed to be the myo- conformer unless otherwise stated. Typically phosphatidylinositols form a minor component on the cytosolic side of eukaryotic cell membranes. The phosphate group gives the molecules a negative charge at physiological pH.
The form of phosphatidylinositol comprising the isomer *muco*-inositol acts as a sensory receptor in the taste function of the sensory system. In this context it is often referred to as PtdIns, but that does not imply any molecular difference from phosphatidylinositols comprising the myo- conformers of inositol.
The phosphatidylinositol can be phosphorylated to form phosphatidylinositol phosphate (PI-4-P, referred to as PIP in close context or informally), phosphatidylinositol bisphosphate (PIP_2) and phosphatidylinositol trisphosphate (PIP_3). All lipids based on phosphatidylinositol are known as inositides, or sometimes phosphoinositides.

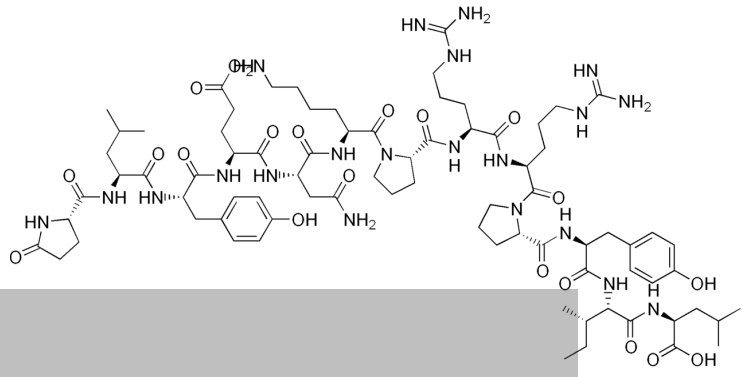
\the H2 forms tend to be via the G proteins and usually activation of adenyl cyclase and raising the level of cAMP.

Just to conclude the discution on those then, this is obviously talking about the effect of thes ehsitmaine receptors in the peripheral nervous system. But histamine dose have a role in the CNS as well and in the CNS the histamine is concerned with arousal and tension. That explains why histamines that cross the blood brain barrier and things like benadryll cause drowsiness. You have seen on the packages of these drugs that they mention not to drive or opperate heavy machinery because they may cause drowsiness and the reason they say that is because they have histmines that cross the blood brain barrier and inhibit the histamine neurotransmitter system thats concerend with alertness and attention and things like that.

Neuropeptides

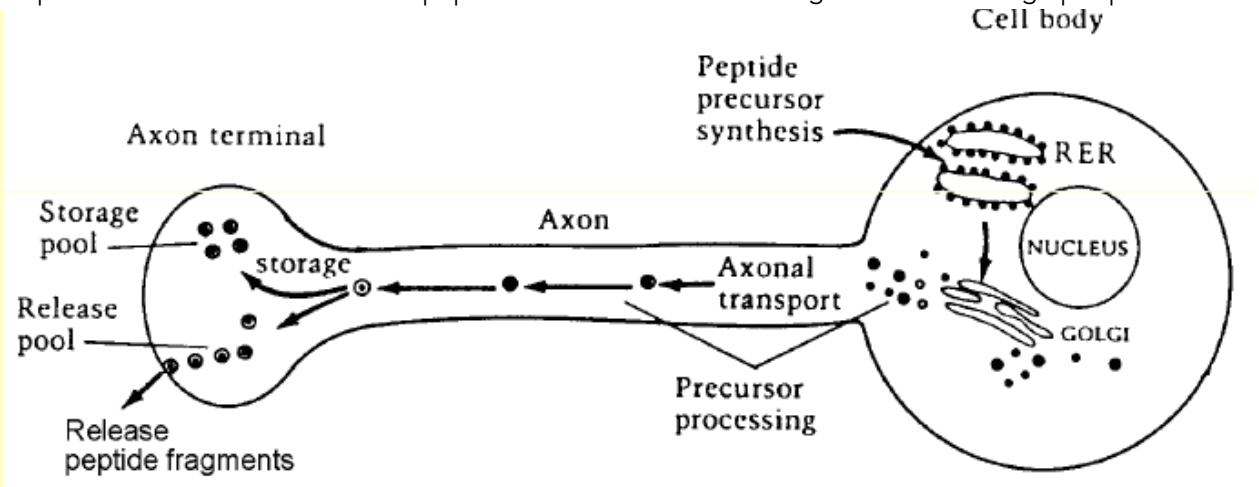
Now so far the neurotransmitters we have been considering have been simple molecules like histamine and dopamine and things like that but there are higher classes of neurotransmitters which are quite a bit more complicated and principle these are the neuroactive peptides. As the name suggest the neuropeptides are small proteins and again they set up a neurotransmitter function in the central nervous system. There different from other neurotransmitters we have been talking about though in that they are extremely potent. We saw that these are not present at very high concnetrations but the neuropeptides are of even lower concentrations than things like dopamine and GABA. To give you some examples lucine enkephalin. The concnetration of that tin the hypothalamus is about 170 pico moles per gram of weight. There is another one called neurotensin that has a presenece of about 55 pico moles per gram of weight. These are extremely low concentrations. To give you something to compare it too, if you have dopamine it is present at about 4.96 nano moles per gram of wet weight, serotonin is present at about

11.36 nano moles per gram of wet weigth. and GABA is high still at about 1.96 micro moles per gram of wet weight.



Neurotensin is a 13 amino acid neuropeptide that is implicated in the regulation of luteinizing hormone and prolactin release and has significant interaction with the dopaminergic system. Neurotensin was first isolated from extracts of bovine hypothalamus based on its ability to cause a visible vasodilation in the exposed cutaneous regions of anesthetized rats. Neurotensin is distributed throughout the central nervous system, with highest levels in the hypothalamus, amygdala and nucleus accumbens. It induces a variety of effects, including: analgesia, hypothermia and increased locomotor activity. It is also involved in regulation of dopamine pathways. In the periphery, neurotensin is found in endocrine cells of the small intestine, where it leads to secretion and smooth muscle contraction.

now because lucine enkephalin and neurotensin are present at such low concentrations you might expect there method of synthesis is very different from the simple metabolic pathways and basically there mode of synthesis follows a pattern that we see for other protein hormones and proteins that are recreated from the cells. These are synthesised on the RER like any other protein and usually in the form of a much large and what are called pre-protein. We known that proteins that are to be recreated from the cell have a pre sequence on them and these neuropeptides are no differnet. There generated as a large pre-protein



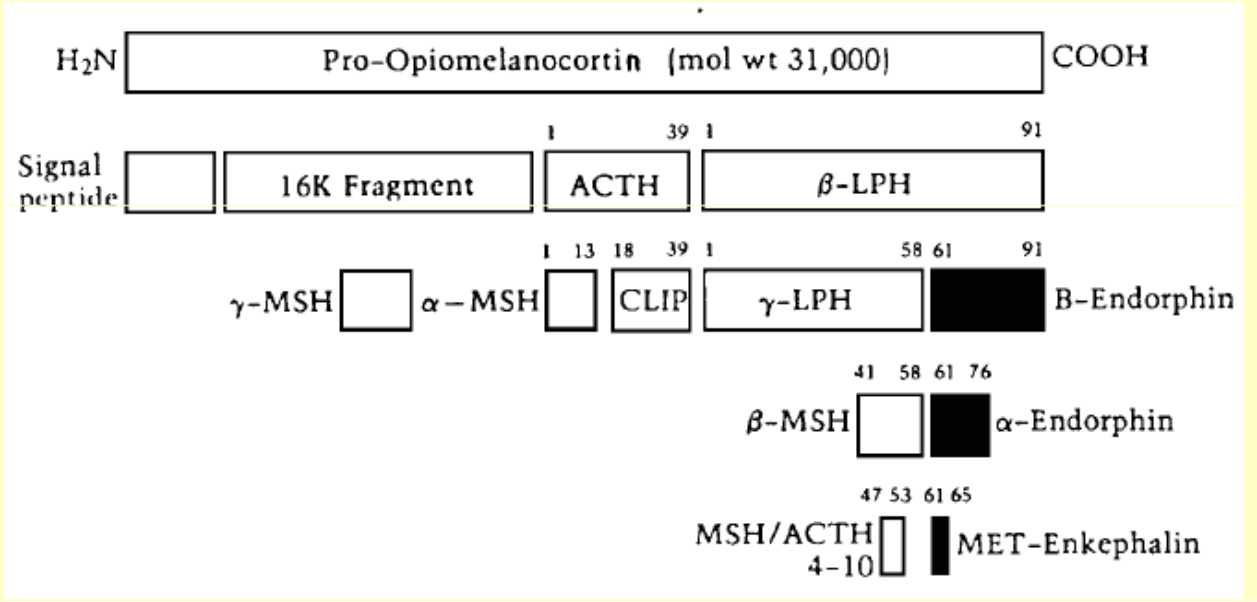
initially and that is then shuttled of too the golgi complex where there is further processing of the protein tends to occur and then there packaged up in the vesicles and shuttled off down the axon or the nerve fiber as part of the axonal transport system.

To the axon terminal where they end up being stored until which time they are released from the nerve terminal and come of and then interact with the post synaptic neurone. Once they have done there job they cant then be taken back up again because there proteins so basically they are just degraded by peptidase enzymes. Now one of the first neuropeptide neurotransmitters ot be identified was something

called substance P. This was identified way back in the 1930s so its actually been around a lot longer than the many of the other neurotransmitters, or was certainly identified before some other the other neuropeptides we looked at. It gets its unusual name from in the early days, it was a standard extract that was used and the name has stuck ever since. Its structure wasn't determined though until the 1970s so the way it was shown to consist of some 11 amino acids. Now substance P mediates quite an array of effects. It stimualtes the contraction of smooth muscle. it also a powerful enhancement of salivation and causes people to salivate. The technical name for this is a sialodog as it creates salivation. It has also got a number of other central nervous system effects as well. So it has quite a range of things that it dose. Now the actions of substance P are quite slow and precipitant reactions. not like acetyl choline and such were the reactions are fairly fast these things are quite slow long lasting types of response. The interaction of substance P with its receptor tend to cause changes in phosphatidyl turnover and also you get the mobilisation of some cellular calcium. So phosphonosilying system that changes the levels of calcium and thing like that. Substance P also occurs int he spinal cord and this has led to the idea that it is involved in pathways concerned with pain mechanisms and pain sensation. So its believed that in the spinal chord substance P has a central role in the pain signalling pathways. Antagonists of substance P (an antagonist is something that blocks the action of the agonist). So the agonist in theis case is substance P and the antagonist blocks the action of substance P. Antagonists tend to have an analgesic action and thats to say they block pain. Substance P itself tend to sensitize people to pain and reduces the reaction time so sending pain or inducing a stimulus. Substance P then dose seem to have some role in the pain sensing mechanisms. We also find that analgesics like morphine for example (morphine is an opioide analgesic) that basically inhibit the release of substance P or block its actions. So substance P has been known but during the 1970s in particular people began to discover a lot more of these nueropeptides

Leucine enkephalin	Try-Gly-Gly-Phe-Leu-OH
Methionine enkephalin	Try-Gly-Gly-Phe-Met-OH
β-Endorphin	Try-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Gly-Gln-OH

and there is a whole family f them that tend to be called the opioid peptides because they interact with the opiate receptor that morphine and such tend to bind to. You can see from there structures that they tend to bind too peptides and you can see how they are related to one another.
First of all we have lucine enkephalin and methionine enkephalin which are simple pentapeptides (5 amino acids) and you can see if you look at the methionine enkephalin it is the first little bit of the beta endorphine so methionine enkephalin is actually a part of the larger molecule of beta-endorphin and these molecules have pretty powerful effects in the algesic mechanisms. The situation gets even more complex though if we look at where things like beta-endorphine come from because these molecules are them selves part of even larger molecules as seen below.



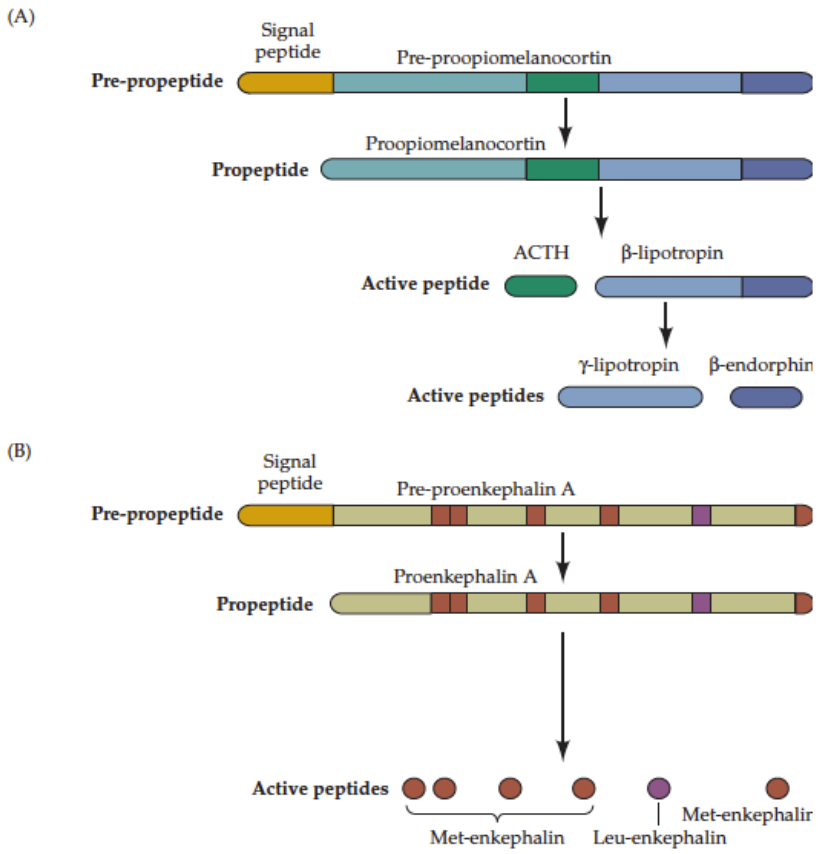


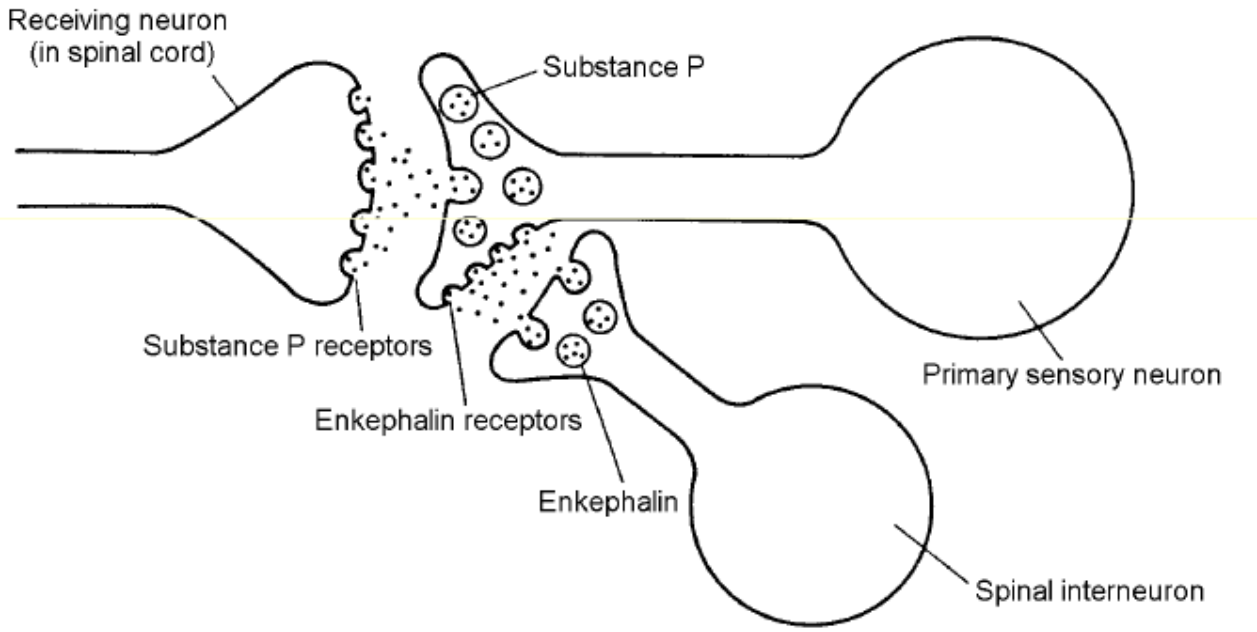
Figure 6.14 Proteolytic processing of the pre-propeptides pre-proopiomelanocortin (A) and pre-proenkephalin A (B). For each pre-propeptide, the signal sequence is indicated in orange at the left; the locations of active peptide products are indicated by different colors. The maturation of the pre-propeptides involves cleaving the signal sequence and other proteolytic processing. Such processing can result in a number of different neuroactive peptides such as ACTH, γ-lipotropin, and β-endorphin (A), or multiple copies of the same peptide, such as met-enkephalin (B).

As we can see in the diagram here the methadone enkephalin is part of the beta endorphins molecule. We can think of this as being the molecule in the diagram and the beta endorphins molecule is actually a small part of the molecule above. There is another molecule called alpha endorphin which is itself also a

part of this. These molecules here is beta melanocyte stimulaiting hormone which is actually another molecule part of this. there is also gamma lipotrophin we wont go into these but we can see that all of these bio active molecueles are part of a larger precursor. So our beta endorphin and the gamma lipotrophin molecule are actually part of a beta lipotrophin molecule. And all of these things start there life as pro-opiomelanocotrin, so remember when we said that these things are synthesised by the RER as large precursor molecuels. This pro-opiomelanocotrin is the thing thats made initially and that thing has at its end the signal peptide which signals the protein to be recreated outside the cell and then having down its job is cleaved away. Ther is a 16KDa fragment there which has melanocyte signalling hormone as part of it. there is also these other parts and the the beta endorphin and such are smaller parts of this precursor molecule that can be made by successe cleavage of the pro-opiomelanocotrin. Thats only one of these large precursor molecules and there are a number of others. We have a few examples here, the other ones are called pre-pro-enkephalin and pre-pro-glynophin which by itself generates further opiod peptides. Those three mother proteins can give rise to about 20 opioid peptides. So by clipping bits off you can generate around 20 different opde peptides. Were not going to say much about the receptors because basically there not very well understood. But there are three main classes that can be referred too and there known as the Mu, delta and kappa receptors. As we say the method of action of the opiod peptides at these reports is not well understood. They all stimulate an inhibitor neurotransmission in the brain and all seem to be related to inhibitory pathways in the brain and they all seem to be coupled to G proteins but that is quite a simplification of whats going on. There called the opioid peptides because they interact with the receptor that was known to be the binding site for things like morphine. In fact we can think of it the other way round because people discovered that there was a receptor for morphine in th brain before these things were discovered. If you think about it why shouldd we have a receptor for something like morphine in our CNS. morphien is not a natural component of our bodies so it suggest that the receptor that morphine binds to is actually a receptorr for something else but the something else wasn't known at the time. Theres a drug called naloxone which is a morphine antagonistt, thats to say it blocks the actions of morphine and naloxone will also block the actions of the opiode peptides. so again its another bit of evidence to show that they are part of the same receptor system.

Naloxone (INN, BAN, USAN) marketed under the trade name **Narcan** among others, is a pure opioid antagonist. Naloxone is a medication used to counter the effects of opioid especially in overdose. It will usually reverse the depression of the central nervous system, respiratory system, and hypotension. Naloxone may be combined with opioids that are taken by mouth to decrease the risk of their misuse. Use may cause symptoms of opioid withdrawal including: agitation, nausea, vomiting, a fast heart rate and sweating among others. In those with previous heart disease further heart problems have occurred. It appears to be safe in pregnancy after having been taken by a limited number of women.
It was developed by Sankyo in the 1960s. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. In most developed countries, naloxone is required to be present whenever opiates or opioids are administered intravenously to combat accidental overdose.

So what do these opiode peptides do and what do we notice about them? well people have done experiments were they have injected these tings into he brain. where they are injected inter cerebrally. The enkapanlins themselvs are not particularly painkillers but beta endorphine is very powerful if injected directly into the brain. Interestingly if its repetidly injected into an experimental animal then the animal will become addicted to. In the same way people become addicted to morphine and things like heroine (which is a relative of morphine). These drugs like morphine are very powerful painkillers and they can also be addictive and the same is true fro the beta endorphine where its seen if it administered repeatedly then you can become addicted to it. This is where you build tollerance to it and physical dependence and addiction to it. If you withdraw beta endorphine then you tend to get withdrawal symptoms in these animals. in the same way you withdraw morphine and heroin and such. Beta endorphienhas some interesting like with acupuncture. There is no explanation for how acupuncture works but it dose seem to work and people have speculated that acupuncture can increase the levels of beta endorphines in the cerebral spinal fluid and the brain. It has therefore been speculated that the way in which acupuncture works is by deadening the pain by the release of beta endorphines in the CNS. So these things are released they do there job in the CNS and such. There released in a calcium dependent manner exactly as we have seen for other types of neurotransmitters so calcium levels rise in the cell and this triggered the release of these compounds. As we say there then not taken up again they are

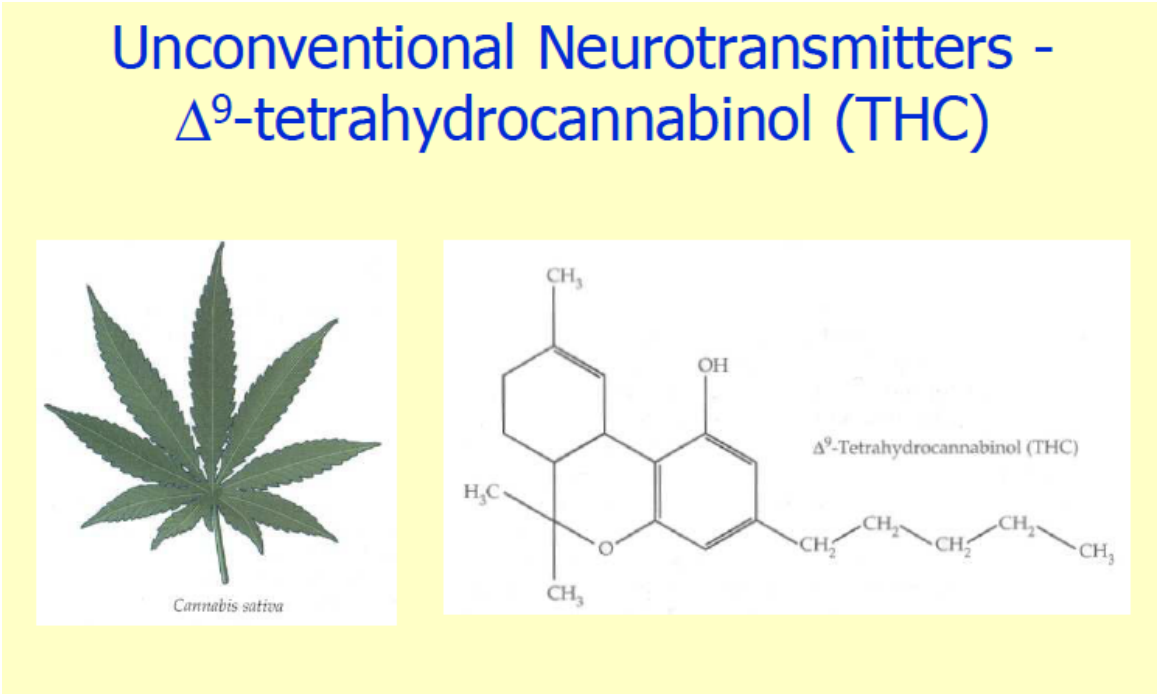


degraded by these peptidase enzymes. In the case substance P we mentioned that these things are mentioned in the pain receptors and pain deadening in the spinal cord. This is one idea of how there believed to work. So its probably a simplified diagram but this is how a primary sensory neurone looks that we would find throughout the pain sensory mechanism. so if somebody pricks there finger or put there hand over a flame or something. Its the primary sensory neurone that says ouch and it starts sending a signal and it is believed to release substance P into the spinal cord. Substance P will bind to its receptors on the receiverg neurone and the signal goes up to the brain to give an ouch response. You also have these spinal interneurones and its believed to be these that release the enkephalin which binds to other receptors on the primary sensory neurone and it inhibits the release of substance P and

deadpans the sensory response. Its a simplisticc idea but its the way in which these are though to have worked as the pain sencing mechanisms. Of course they have to work to get you to move away from a pain sending stimulus but we also don't want a dibilitating pain every time you have a minor injury. so this is a kind of mechanism that modulates that response.

Unconventional neurotransmitters

So thats the opiod peptides but the final group of neurotransmitters that we are going to talk about briefly are the so called unconventional neurotransmitters. These are all fairly conventional things that are released from nerves, bind to receptors and trigger some sort of responce somewhere but there are a group of what are called unconventional neurotransmitters. They are neurotransmitters but they work in a very different way. They have somethings in common with neural transmitters for example they are released in response to the calcium levels but they are unconventional in that there not usually stored in vesicles in the nerve terminal. These things are not stored in that way. They are often associated with whats called retrograde signalling. This is where the nerve signalling begins to get more complicated than we though previously. Retrograde signalling is where a post synaptic neurone signals back to the pre synaptic one. So the nerve signal travels backward. So normally nerve signal comes down due to action potensial the vesicles move to the pre synaptic membrain due to calcium flux, the neurotransmitter is released into the synaptic cleft and the receptors on the post synaptic neurone are triggered and carry out a response. In the retrograde system that signal triggers a release of the retrograde neurotransmitter to be released and come back to and bind to a receptro in the pre synaptic neurone and then modify its release of the neurotransmitters. Now there are a couple of compounds that have been identified to work in this retrograde system. And one is the substance below.



This substance is tetrahydrocannabinol (THC) which is the active ingredient in cannibis and the way in which this works is it binds to a receptor in this retrograde signalling. Tetrahydrocannabinol is not a naturally occurring retrograde molecule but it dose opperate in that way. The actual compound that this stuff binds to or the receptors that it binds (there are a couple of them) anandamide which is an unconventional neurotransmitter and basically anandamide is synthesised from phophodidic precursors like phosphoethanolamine where there is an N-acyltransferase existing on the end of to give N-aracadonile phosphotidylethanoloamine which is then acted on by phospholipase C to generate the anandamide molecule and this is one of these retrograde signalling molecules. The name anadoamide buggs in some curiosity that comes from Sanskrit for bliss and amide on the end. Some of these sort of molecules are quite soothing and they calm down the system. There is another one and this is 2-arachadonile glycerol and this again is a molecule that can be synthesised by two different pathways. Starting at the it can either go through phospholipase C to give a 1,2-diacylglycerol which is then acted on by a lipase which gives an arachadiyl glycerol or you can come at it another way phospholipase A1 to generate a lyosphosphtidoanecotol or phospholipase C to give arachadiyl glycerol. either way.

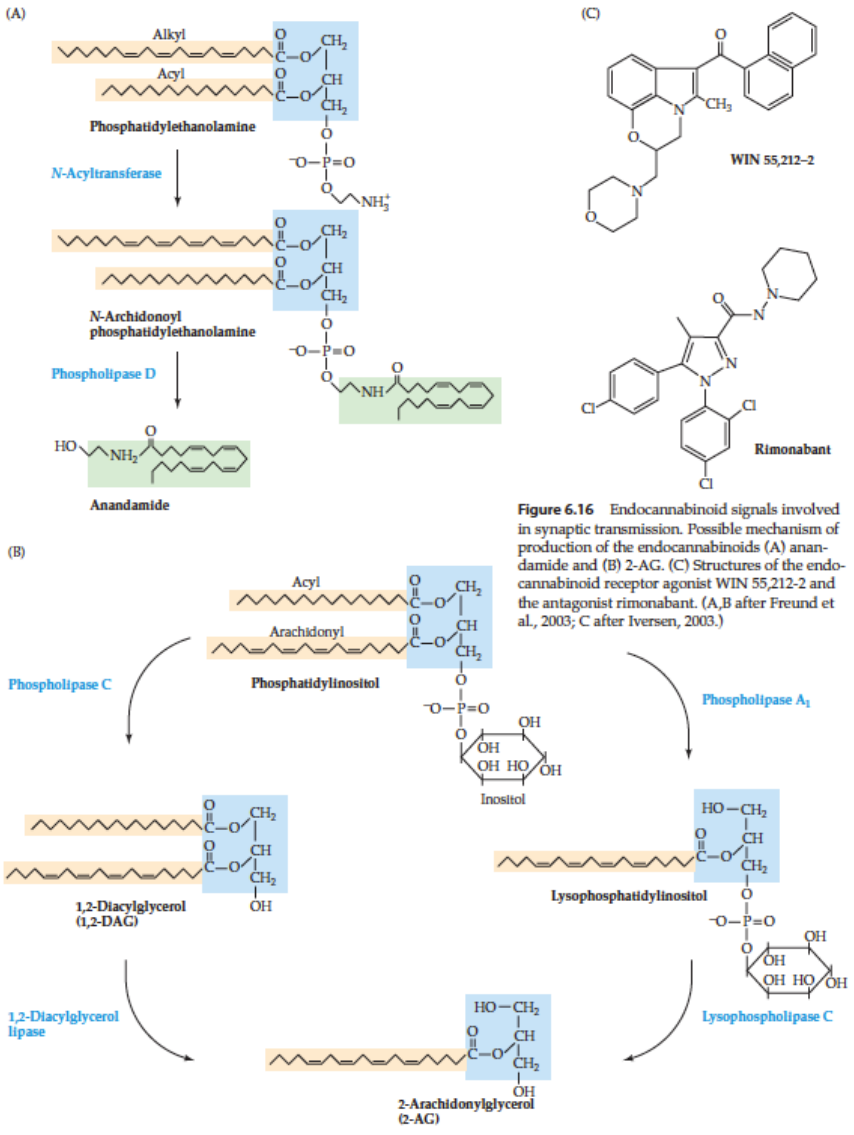


Figure 6.16 Endocannabinoid signals involved in synaptic transmission. Possible mechanism of production of the endocannabinoids (A) anandamide and (B) 2-AG. (C) Structures of the endocannabinoid receptor agonist WIN 55,212-2 and the antagonist rimonabant. (A,B after Freund et al., 2003; C after Iversen, 2003.)

These are the naturally occurring substances that perform this function in vivo so anandamide and 2AG are naturally occurring retrograde signalling molecules so they are known exist in the body. THC is not natural but ti work on the same system now how doe it actually work. Well it seems that the production of those endocannabinoids as they are generally known as. Are stimulated by a second messenger system in the post synaptic neurone. Typically what happens is that once the post synaptic neurone is stimulated the calcium concentration in there tends to go up. Thats the signal for the synthesis for these sort of molecules. Now there are at least two types of receptor on the pre synaptic neurone. And thy tend to be known as CB1 and CB2. These are the two canabannoid receptors which bind these things. CB1 is a G protein couple report system and the way in which it works is like this. In certain regions of the brain and particularly the GABA receptor system, the endcanabannoides serve as retrograde signals to stimulate the release of GABA so there particularly important in the recreation of GABA. Now what happens is you have a signal come alongg and you have your neurotransmitter which could be GABA, which stimulates the post synaptic neurone The calcium levels rise and this causes the synthesis of these retrograde neurotransmitters. there released and go back to the pre synaptic neurone there where they bind to the CB1 receptor system and then once they have bound it inhibits the release of GABA from the pre synaptic nerve, so there is kind of a feedback system.

Its not very known quite how activating the CB1 receptor brings about the release of GABA but its believed to involve the effects on voltage gated calcium channels of the pre synaptic cell so some mechanisms can actually cause lockage in the release of GABA.