Neuropharmacology - Florio

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Contents

Todo list

Chapter 1

Neuropharmacology - introduction

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Books: Molecular Neuropharmacology (Nestler, Hyman) and Basic Neurochemistry (Siegel) and Rang & Dale's pharmacology (Rang, Ritter).

Oral exam with Nistri.

Pharmacokinetics and pharmacodynamics.

What is Pharmacology? Pharmacology is the science that deals with the origin, nature, chemistry, effects and uses of drugs, a lot of things. It includes:

- Pharmacognosy, just natural products
- Pharmacokinetics, means the movement of drug
- Pharmacodynamics, is what professor Nistri is going to explain you, which means what a drug does in your body to change pathological situation
- Pharmacotherapeutics
- Toxicology, toxic effect usually depends on the dose.

We can say that:

- Pharmacokinetic is what our body does on the drug
- Pharmacodynamic is what the drug does on our body

Chapter 2

Pharmacokinetic

The study of the movement of drugs in the body, starting from the absorption, then distribution into the body, reach the tissue with the molecular target and then excretion from the body. It can also be metabolized in the process called *biotransformaton*. After absorption, at the beginning of the distribution, the drug can be biotransformed. *The drug goes in the body, it is distributed along the body, it is metabolized and then is eliminated.*

We'll study the processes that enable the drug to achieve the right concentration at the target tissue in order to give the pharmacological action: not too high, not too low. If too low $\longrightarrow$no pharmacological activity; if too high $\longrightarrow$side effects.

To reach the target the drug has to move through barriers: translocated from the administration site to the target. Meanwhile, there are metabolism and elimination that reduce the concentration of the drug at the target tissue.

ADME: Adsorption, Distribution, Metabolism and Excretion.

Absorption Unless you give a drug by injection, a drug has to translocate into different compartment. The mechanisms for absorption are the same that enable a drug to *exit* the body.

Distribution Starts when the drug has reached the blood stream and from the blood stream goes to the target tissue: to do this, the drug has to go through different barriers, made up by cell membranes of capillaries, cells etc.

Drugs can be widely distributed or distributed in certain compartments: the distribution as an effect on the concentration of the drug at the site of action, on excretion and biotransformation.

Metabolism A lot of enzymes are responsible for the metabolism of drugs. Each drug can be metabolized into different compounds: the same compound can undergo metabolism from different enzymes. These enzymes are responsible for the transformation of endogenous compound, but take advance of the presence of the drug. Biotransformation can activate or inactivate drugs, but also change the pharmacological activity (increase or decrease).

Excretion The drug is removed from our body, mainly through the kidneys.

2.1 Overview

There are different point from which the drug can be absorbed: gastrointestinal tract, lungs or via injeciton. The distribution is achieved by the blood stream: the drug has to reach the blood stream to be distrbuted into the tissues. From the blood stream, the drug can be eliminated. From the liver, all the drug that are take by oral intake have to go into the liver before distribution. Into the liver we have enzymes that transform the drug, reducing the active form of the drug in a significan manner (*first pass metabolism*).

After oral intake, the drug has to pass through the liver and then goes into the blood stream (in the cava) and through the heart and from the heart to the tissues. From the liver, the drug can be secreted into the cistifellea and eliminated by the feces; there is also the possibility that the drug secreted into intestino can be reabsorbed to pass again through the liver and pass again though the blood stream.

The drug can reach the molecular target, but there is also *free drug*, because in the blood stream the drug can bind to plasma proteins and such drug cannot leave the circulation. A drug can also in eritrocites ecc.

Drugs can also be stored in some tissues, in particular *fat tissue*, but also bones or liver. When a drug is stored, these tissues cannot be used for pharmacological activity: the drug has to leave those tissues, reach the blood stream and then give the pharmacology product.

2.2 Enteral vs Parenteral administration rules

Enteral: the drug has to go into the enteral tract, parenteral are all the other groups

2.2.1 Parenteral

Intraveneous administration: no absorption and the distribution starts immediately ⟶rapid onset of the pharmacological action. We can also administrate the dose that cleaves the concentration that we need. So, we have a precise delivery of the dosage. Disadvantages are, apart from the risk of infection (does not exist anymore), that if you administer quickly the drug, we can obtain a very high concentration of the drug at the beginning that could lead to toxic reactions.

The entire volume of blood is 6 L in an adult; the cardiac output is 6 L/min ⟶in one minute a drug administered by the intraveneous rule can make a tour of our circulatory sistem. So, 30 s is a time enough to dilute the drug.

Intramuscolar administration: there is a quick absorption, because it is made into the skeletal muscle in gluteus or deltoid, which are highly perfused of blood and the drug doesn't reach the gastro-intestinal tract, so there is no first pass metabosilm. Disadvantage is that we cannot use a huge volume, it can damage the tissue, it may be painful ant the absorption can be variable.

Pulmonary administration: convenient because there is a large surface area for absorption and it's also easy to tritate the dose through different dispensers. It is a nice way for drugs that cannot be taken by oral rule, like peptides; however, the administration require the coordination of movements (see spruzzino per asma) and the delivery is not so precise.

Topical administration: into skin, eye, sinovia. Usually by this rule the drug is not absorbed, so side effects are minimal, and there is no first pass metabolism.

2.2.2 Enteral

Oral administration: most frequent administration rule because it is safe, natural, non-invasive, economical. After oral administration, not all the drugs can reach circulation due to first pass metabolism and to other factors that can change the amount of drug that is distributed. It depends on the activity of the intestinal tract: if there is high motility, the time of contact will decrease. Some antibiotics can be not absorbed if complexed with Calcium and so on.

Sublingual administration: particular, put the drug under the tongue, where the mucosa is thin. This mucosa is highly perfused, so the drug can be absorbed quickly ⟶rapid onset. Another advantage from the capillaries under the tongue is that the *ranina*, a really small vein under the tongue, goes right through the cava, skipping the first pass metabolism. Disadvantages:

the drug, in order to be active, should be potent, because we can administer a little amount. If in liquid form, it should not be lesive for the mucosa.

2.3 Absorption

Is the process by which a drug moves from the site of application and reach the blood stream or the limphatic system. To do this, the drug has to go through *cell membranes*, which are selective barriers.

Who can go through the membranes? Small uncharged compounds (H_2O, oxygen), lypophilic compounds. Hydrophylic compound cannot, very small compound like ions cannot either. We have compounds that can cross all the barries but other has to take advantage of different entities. For glucose, we have a transporter, for ion we have channels: VOC and ROC. Passive diffusion, active or facilited transport and passive diffusion through channels.

Ripartition coefficient values are low.

2.3.1 Passive diffusion

Most of drugs move through the body for absorption and excretion by passive diffusion. It depends on different characteristics of the drug and of the environment, like thickness of the cell membranes, surface area, degree on ionization. There are drug dependant and drug independent characteristics.

Drugs dependant characteristics that enable it to go through the membrane by means of passive diffusion are:

- Concentration gradient
- Lipid solubility
- Degree of ionization

Passive diffusion: 2 compartments with a *concentration gradient* that with time reach the equilibrium. In the body this is difficult to reach because of the removal of the drug. This gradient is the meas force that make the drug moving.

Lipid solubility is a property of the drug and depends on its physiochemical properties. It's important to have a way to define the degree of lipid solubility of a drug: this is the first characteristic that we can measeure for a drug, is a hallmarck of the drug when the *lipid/water partition coefficient* is measured. This index of lipid solubility is measured putting the drug in a tube with oil and water, then mix very well, wait until the 2 phases will

appear (water and oil) and then make a ratio and higher this ratio, higher will be the lipid/water partition coefficient.

We can plot partition coefficient with permeability: there is a good linear correlation.

Degree of ionization: most drugs are weak acids or basis, so they can be present in ionized or non-ionized form depending on the pH of the environment. A weak acid tends to dissociate in $H^+ + A^-$. Basis do exactly the opposite, they accept H^+. The ionized form cannot go though the lipid bilayer: only the non-ionized form can go through $\longrightarrow$the consequence is that if we have an acidic drug in an environment with low pH like stomach, the acidic drug will bind the H^+ and be undissociated; on the contrary, if pH is high, the acidic drug will be more prominent in the dissociated form. Exactly the contrary for weak basis: if pH low, ionized form will predominate; if pH high, the non-ionized form will predominate. This is important for the ability of the drug to cross the membrane.

Acidic drugs are best absorbed in the stomach (es Aspirine) and basic drugs are best absorbed in basic environments. We can play with pH to change the form of the weak acid/basis in order to increase absorpion or excretion. To increase absorption of an acidic drug $\longrightarrow$acidify the environment. To increase elimination $\longrightarrow$alcalinize the environment. Ex: barbiturices can be eliminated more quickly if we alcalinize the urines, in case of overdose.

Another consequence is the *ion trap*: a drug is absorbed easy and can be put in an environment where its dissociated form and it's difficult to remove it.

We want to measure the tendecy of acidic and basic drug to change its behavior by changing pH: we can measure a number which is characteristic for these drugs, the *pKa*, that is the pH value at which the drug is 50% in the ionized form and 50% in non-ionized form. If pH = pKa $\longrightarrow$equilibrium. Knowing pKa we can predict the degree of absorption of the drug depending on the pH of the environment. For example, an acidic drug with pKa = 4: this drug at pH 4 will be 50% ionized and 50% non ionized. If we change the pH of only 1 unit (pH = 3), the non-ionized form reaches 90%, so small changes in the enviroment deeply affect the balance between the two forms.

Also thickness of the membrane and surface area affect passive diffusion. Bigger the area, bigger chance to be absorbed and easier to be distributed. All these characteristics (drug-dependent and drug-independent) can be reassumed by the Fick law:

$$\frac{dQ}{dt} = \frac{PA}{h}(C_p - C_t) \tag{2.1}$$

where dQ/dt is the diffusion rate, A is surface area, C_t is the drug concen-

tration in the tissue, C_p is the drug concentration in the plasma, $C_p - C_t$ is the concentration gradient, h is the thickness of the membrane and P is the oil/water partition coefficient. The concentration gradient is the real important factor: when the concetration gradient decreases, the velocity also decreases. The smaller is the concentration gradient, the smaller quantity will be eliminated: that's why we never eliminate a drug.

Passive or simple diffusion depends on the *concentration gradient*, doesn't need energy and is not saturable.

2.4 Facilitatied diffusion

These 2 least characteristics are important for the other important mechanism to drugs to go into the body, the active trasport and the facilitated diffusion, which are both saturable and the active transport also need energy.

Apart from the saturability, these 2 kinds of transports have another thing in common, the *presence of transporters*. These transporters have almost the same phase: transporters are proteins and can be recognized because they are proteins with 12 transmembrane domains linked by extracellular and intracellular loops. All the transporters can bind molecules, change their conformation after the binding and changing the confromation they can move the molecule from one side of the cell to the other side. Sometimes the molecules that binds through these transportes or carriers needs also an ion in order to change conformation and move the compound in or out of the cell.

Where are located these transporters? They are expressed in polarized cells or cells facing the lumen of an organ or in the baso-lateral part. We have trasporters expressed in the apical or in the basolateral prt of the cell and the *uptake* occurs when the substrate goes form outside to inside the cells and *efflux* when the compound is moved from inside to outside of the cell. Endothelial cells are an exception because the blood is facing the apical part of the cell.

Almost all the cells express transporters, but they are expressed more in gut, lungs, kidney, that are the interphae between circulation and outside of the body (for removing the substances).

So, there are 2 kinds of transporters, those that mediated translocaiton of molecules using energi, *active transport*, and those who mediate facilitated diffusion. The most recent classificatio is primary active transport for active transport and electrochemical-potential transporters for those which mediate facilitated diffusion. They have in common the structure and the fact that they belong to a superfamily: for active transport, ABC, *ATP-binding cas-*

sette: there are 7 families of active transporters. They use hydrolisis of ATP to move compounds against their concentration gradient. The superfamily of facilitated diffusion transporters is SLC, *solute carrier*.

Facilitated diffusion: transporters are divided on the bases of requiremen of ions in symport and antiport, that are 2 kinds of co-trasport. Apart from glucose, we have a lot of endogeneous substances like aminoacids, metals, neurotransmitters. Antidepressant drugs bind to serotonin receptors, like Prozac. The characteristics of facilitated diffusion:

- No need of energy
- Direction of transport according to electrochemical concentration gradient
- Saturable : since they are proteins and are expresse din limited number on the surface of the cell, they can be occupied totally and after that there is no way for other molecules to be transported
- Selective : with a certain degree of overlapping.

The neurotransmitter transported families are DAT, NET, 5HTT. They can be organic cationic tranporters OCT or organic anion tranrporters OAT. They have endogeneous ligands or exogeneous ligands.

Transporter localization in intestinal epithelia, hepatocytes (also in the part facing the canalicola, where bile is secreted), kidney proximal tubules and BBB. In BBB several transporters are responsible for the efflux of the drugs out of the brain into circulation, giving significant contribute to the real BBB.

(2.03.2016)

2.5 Active transporters

Coupled wth intrinsic ATPase activity, using hydrolizys of ATP it pushes agains concentration gradient the ligand. These carriers are *saturable* and *selective*. These transporters are called Primary Active transporters in which there are 2 superfamilies:

- P-ATPase - ex. SERCA
- ABC - subgroups compreending the multidrug resistant transporter MDR. This group has been identify trying to explain why patient treated with anti-cancer drug with time the cancer resists to different drugs. They identifyed these proteins: in resistan patients there

was an increased expression of these transporters with time, so drugs that can permeate the cancer cells with passive diffusion were extruded by the cells via these transporters ⟶resistance.

2.5.1 ABC proteins

They are a large family of gene with homology sequences, critical for moving a wide range of substrates, like aa, sugars, ions and metabolites. 1 000 of these proteins have been identifyed and one is the *P-glicoprotein*. The molecular structure is 12 transmembrane domanis, intra and extracellular loops and 2 well-conserved ATP-binding site and the sequence near these binding site is responsible for the ATPase activity.

The glicoprotein are cellular-efflux pumps, responsible for resistance of tumor cells for chemotherapy, expressed on *apical* membrane of intestine, liver, kidney, BBB, testes and adrenals. Their expression can modify the pharmacokinetic of a drug which is substrate of this lipoproteins.

At the level of the intestine in the *enterocytes*, they are responsible for reducing the absorption of the drug. In the kidney is responsible for efflux of the drug from blood into the urine, so responsible for active elimination of the drug. In the liver, these carriers are responsible of efflux of the drug in the bile and for elimination through intestine. In the BBB they can extrude drug, so they reduce the entrance of drug into CNS and limit drug distribution, so they are determinant in the *pharmacokinetic* of drugs.

Why P-glycoprotein is the Gorilla of the BBB? Because its expression in the endothelial cells limit the entrance of the drug. For Methadone for example, if we KO the protein, the dose absorbed is increased.

Fura-2 is a dye for studying the movement and increase of intracellular Ca: it can permeate the cells and then it is transformed by an esterase in a polar compound that cannot exit. This form of Fura-2 is a substrate for P-glycoprotein, so you can measure the rate of extrusion of the drug by measuring the rate of extrusion of the dye.

These carriers are expressed in luminal or up-luminal position. In BBB, the expression of different ABC occurs.

2.5.2 Vesicle-mediated transport

Endocytosis or pinocytosis or receptor-mediated endocytosis. Insulin for example bind particular receptors at the level of the coated pit, so the vesicle can transport that from one side of he cell to the other side.

2.6 Distribution

In order to distribute the drug, it has to go in blood stream and then reach target outside the circulation. Distribution depends on drug-related conditions and drug-unrelated. The first ones are the chemico-physical properties of the drug; the seconf ones are:

- Entity of perfusion of the tissue
- Ability to bind to plasma proteins like albumin
- Tendency of the drug to accumulate in the tissue
- Presence of barriers

Circulation reaches every part of our body.Capillaries have only endothelial cells and basal cells and at this level the drug can exit from the blood stream. Before the capillaries there are small veins with smooth muscle cells, responsible for the pressure: capillaries enhance the drug to reach in a capillary fashion the tissues.

Revise circulation

2.6.1 Entity of perfusion of the tissue

The entity of perfusion differs from organ to organ. Liver and kidneys receive 1350 mL/min, the 27 % of the cardiac output. Skeletal muscle are good perfused, also skin. Heart is poorely perfused by the coronary, fat is less perfused, so it is easier to distribuet the drug in liver than in fat.

Also the type of capillary is important. Capillaries are different in differet organs:

- Sinusoids: in the liver, they have interaction of the basal membrane and gaps between the endothelial cells. Hydrophilic drugs, polar drugs can leave easly these capillaries to go into the tissues. For lipophilic druf, they go through the membrane by passive diffusion
- Continous: no gaps in the basal membrane, the endothelial cells are tightly connected by tight junctions. They are present in smooth muscle cells and BBB, so it will be difficult for a polar drug to distribute into tissues
- Fenestrated: a continuous basal membrane and gaps between endothelial cells. The presence of these discontinuities in the endothelium is not enough to enable the drug to leave the capillary and reach the tissues: there is also a role of *pressure*. Starting from the arteriolar

part through the venous part, the hydrostatic pressure decreases from 32 mmHg to 15 mmHg: it pushes the water of the blood out in the extravascular interstitium. There is also another pressure, the *osmotic pressure* that is due to plasma proteins: 25 mmHg and is stable, doesn't change from arteriolat to venus part. This allows to take water inside the capillaries. there is also a *colloid pressure* in the interstitium $\longrightarrow$in the arteriolar part there is a positive pressure that enables the exit of water with hydrophilic drug inside; on the other part, the effect of the colloido-osmoti pressure preveals, so there is absorption of water and solutes into the venus part.

So, water exit from the arteriolar part and is re-uptaken in the venus part: there is filtration and absorption. The net flow is 3 L/day.

Plasma proteins are produced by the liver; if we have cirrosis, the plasma proteins are reduced and in patients we see a big round belly $\longrightarrow$because the lack of plasma proteins reduces the absorption of liquids, the water is prevalent outside the circulaiton in the interstitium.

2.6.2 Plasma proteins

Albumin is the most abundant, then $\alpha 1$ and 2 and β and γ. They are lipophylic so cannot stay in the blood, which is acqueous. Albumin binds bilirubin, folic acid, Vitamin C, Barbiturates. $\alpha 1$ can bind steroid hormones (via transcortine).

Albumin can bind different kind of drugs and form complexes that are reversible. Only the free drug can reach the site of action going through the capillaries and permeating the membranes. The binding to plasma proteins influences the pharmacokinetic and the pharmacodynamic. Only free drug can be eliminated because it's the only that can be filtrated at the glomerular level (70 kDa is the limit for proteins to be filtrated at the glomerular level). Only free drug is metabolized and secreted.

For drugs that have a high degree of binding the plasma proteins, it's difficult to deal with because the binding to plasma protein makes a reservoire of the drug into the body from which the drug can be removed to give the pharmacological action in a not precise way. The complex protein-drug depends on:

- Physiochemical properties of drug
- Drug concentration
- Affinity of the drug for the protein

- Total quantity of plasma proteins

For a drug that has a high tendency to bind to proteins, a consistent decrease of proteins can affect the quantity of the free drug: the dose to administer to a patient is usually standard until you know that there is a disease etc. If you administer a standard dose of a drug that has a higy tendency to bind, like 95% of the total dose, in the presence of a lower concentration of plasma protein we'll have a higher quantity of free drug, so the levels can be higher than the pharmacological effect, so it can reach a concentration taht could give toxic effects. Plot of concentration of albumin and % of patients with adverse reaction: the increase of adverse effects is related to the decrease in plasma proteins concetration.

Drugs highly bound to plasma proteins generally persist in body longer, because the lower concentration of the drug, the higher the availability of plasma proteins, so the protein binds and unbinds from the protein, there is a continuos equilibrium between bound and unbound drug. When drug concentration decrease, we'll have more sites to bind it $\longrightarrow$slower elimination of the drug and reduction in distribution.

Apart from the possibility to see toxic effect in patients with low plasma protein concentration, a more frequent possibility is the interaction of two drugs simultaneously administred. Drug is present at high degree of % of binding (95% to plasma protein and 5% free). If we administer another drug that binds to plasma protein and compete to drug A: if the other drug can displace just 5% of the drug A from the plasma protein, so afther this displacement the % of A bound is 90% $\longrightarrow$incerease of free drug of 100% (because now it is 10% instead of 5%) $\longrightarrow$we can have *toxic effect*.

In case of drug B that is 50% bound and 50% free, if we administer another drug that displaces of 5% (like before), we will have an increase of the 10% of the free drug (from 50% to 55% = +5% and 50%/5% = 10)

Drugs that are highly bound to plasma proteins are more difficult to deal with because their behavior can be unpredictable: there could be displacement by endogeneous compounds.

2.6.3 Tendency to accumulate in tissues

We see how the concentration of the drug into the blood changes with respect to time. This drug is Tiopental a barbiturate: the drug concentration decrease quickly (gradient of passive diffusion). For this drug administered intravenously, the reduction of blood concentration is accompained by a distribution into different organs, starting with highly perfused organs like brain and viscera: the drug enter and the exit to be distributed into poorly per-

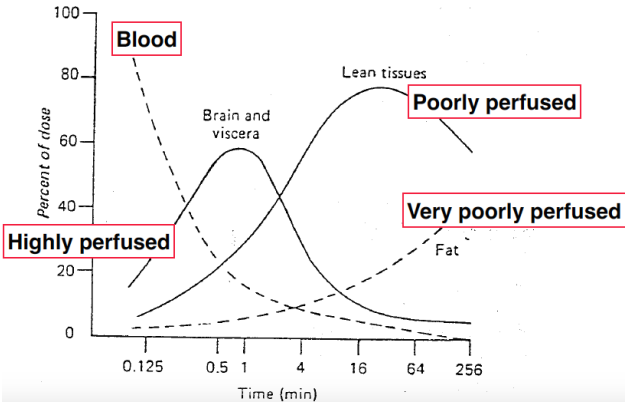

Figure 2.1: Distribution of Tiopental

fused organs. In very poorly perfused organs like fat, the entrance is slower. This is for highly lipophylic drug.

Other drugs have different behaviors:

- Some drugs like Eparine remain largely in the vascular system (they are anticoagulant). They also have high degree of binding to plasma protein
- Drugs that have low molecular weight and are polar, water soluble, for example Ethanol. They distribute throughout the body water in a uniform manner
- Drugs that have a tropism for certain tissues, for example iodine in thyroid, tetracycline in Ca rich tissues (bone, teeth), highy lipid soluble compounds (in fat tissue)
- There are drugs that have a non-uniform distribution which depends on the physical proterties of the drug, rade of diffusion etc.

Is there a parameter that give us immediately an idea of the entity of distribution of a drug? Yes, it is called *apparent volume of distribution* V_d, a useful indicator of the type of the distribution pattern that characterizes a drug. V_d tell us the relationship between the concentration of the drug in the *blood* with respect to the amount of the drug in the *body*.

1. The blood is the only organ from which we can easly misure the concentarion of a drug
2. The blood is just in the middle between absorption and distribution, secretion, elimination.

Of course we must know the dose that we administer. The amount is related to the plasma concentration by the V_d: Amount $= V_d \cdot conc$. The blood is made up by 50% of water and 50% of blood cells. 6 L of blood (3 L of water). We have a total quantity of water in body that is 40 L, 60% of body weight. Of these 40 L, 28 L are inside the cells (40% of body weight), while 10 L (15% of body weight) in the interstitial fluid and 3.5 L (5%) in the blood and lymph. These 3 compartments are linked.

Bath tube model

Bath tube with some water inside, put a dye inside the water, wait until it is well distributed, we know the amount of the dye: when the color is uniform, we take a sample of water and measure the concentration of the dye into the water. Then we relate these 2 values: the known amount od the drug administered with respect to the plasma concentratio (mg/mL). For example, amount administered is 500 mg and the concentration 10 mg/ml $\longrightarrow$50 L.

What if we put a sponge in the bath tube? Some of the dye will enter the sponge. If we measure the concentration of the dye, it will be lower because some of the dye is sequestered by the sponge (this is an example of how the fat tissue works). This time we have 500g/1mg/mL = 500 L.

The red herring model

If we put fish that bind the drug, the drug now is not unifromly distributed: take a sample and find a higher concentration of the drug, 100 mg/mL and the distribution volume will be 5 L, this is why we call V_d the apparent volume of distribution. So, V_d is teh volum in which the drug appear to be distributed in order to have the same concentration of the drug that we have in the blood.

Some examples: Wafarin has a low V_d. while clorochina has an incredible high V_d. SOmetimes we find the V_d corrected for Kg.

2.6.4 Ability to cross barriers

In particular BBB and blood cerebro-spinal barrier.There are no pores in endothelial membrane, there are transporters in endothelial cells and less protein concentration in interstitial drug, so a lower colloidal pressure. How can drug go through the BBB?

revise BBB

With the mechanism of *passive diffusion* for lypophilic drugs and *carriers for influx* for aa, nucleosides, glutathione, or *receptor mediated transcytosis*

for insuline, transferrin for iron and finally *carrier for efflux* (active transport).

For example, the expression of P-glycoprotein can affect teh brain concentration of certain drugs like protein inhibitors Indinavir, Nelfinavir and Saquinavir. In yellow the concentration obtained in KO animals for P-glycoproteins and in red the wt animals $\longrightarrow$important effects! This effects depend also on the content of the transporters: if the number of transporters increases, the efficiency of removing the drug or internalize it increases $\longrightarrow$the expression of P-glycoprotein can be regulated! Drugs in the case of cancer at the level of BBB acts as *transcription factors*: this is a mechanism that we will find several times, the mechanism by which a drug acts as a transctiption factor. Both in human and rodents, intracellular receptor has been identifyed, it's *SXR, steroid and xenobiotic receptor*. This receptor can bind drugs and the binding enable the complex drug-receptor to translocate into the nucleus, where the receptor can bind DNA through a specific sequence, the *responsive element*. In tis way it can regulate the expression of different proteins, in this case of P-glicoprotein, increasing its expression.

By this mechanism also anticonvulsants can produce their pharmacological activity. It is necessary to increase the dose of the drug in order to achieve he effect that was initially found: this is called *tolerance*, increasing the dose to obtain the same effect. This is just one mechanism of tolerance, the induction of P-glycoprotein.

We also have evidence that this increase of expression of P-glycoprotein is a cosequence of the anti-epileptic activity itself: it seems to pe prostaglandin-E2 that with intracellular receptor can interact with DNA to increase the expression of P-glycoprotein.

Blood cerebrospinal fluid barrier: the barrier is made up by the cells of the choroid plexus that presents tight junctions whereas capillaries have gap in the endothelial cells, so hydrophilic and lypophilic drugs can enter in the intesritium, while cannot cross the basal cell (?).

There are some points in which we don't have BBB: in the *chemoreceptor trigger zone*, where it can be detected the presence of exogeneous or toxic compound. This zone is connected with the *vomiting center*: the idea is to protect the organism from possible toxic substances inducing vomitin. The vomiting center receives afferences from larinx, pharinx and stomach. Disease of liver, pritoneum and urinary tract can stimulate the vomiting center. The dopamine through its receptor D2 acts as the neurotrasmitter that links the chemoreceptor trigger zone with the vomitin center. D2 R antagonist can be used to inhibit this reflex.

2.7 Metabolism

(3.03.2016)

Highly lypophilic drugs tends to remain longer in our body, so biotransformation can be seen as a way to reduce lipophilicity of a drug in order to enhance elimination. We can distinguish 2 different phases for biotransformation:

- Phase I, Non-synthetic: the enzymes operate a change in the substrate to add/unmask a group that can make the drug more soluble in water (oxidation, reduction, hydrolysis)
- Phase II, Synthetic: an endogeneous group is added to the drug, like a methilic group, a glucoronic acid etc. The compounds that undergo this phase becomes more soluble in water.

Drug metabolism phase I does not results always in increase in hydrophilicity or inactivation of the drug. We can also have:

- Conversion of an inactive drug into an active metabolite $\longrightarrow$*pro-drugs*.
- conversion of active drug into a metabolite that has per se a pharmacological activity (phenacetin becomes paracetamole)
- The drug is metabolized into a toxic compound (methanol converted into formaldehyde) that can affect the integrity of the cell, causing necrosis, or act on DNA as pro-tumoral compound

For Phase II, the result is almost always *inactivation* of the drug, with few exception: morphine conjugated with glucuronic acid is still active.

Phase I does not always occur before phase II, it can also be the contrary. One compound can be metabolized by more than one enzyme and the metabolite can be metabolized itself in another compound, still retaining pharmacological activity. Flunitrazepam (antidepressant) undergo methylation, reduction, hydroxylation.

First pass metabolism affects oral administrated drugs. The drug must pass through the liver before reaching circulation. Liver is rich in enzymes able to mtabolize drugs, so this passage can reduce the amount of drug that reach the circulation. It reduces the *bio-availability* of the drug.

Another way to classify enzymes responsible for methabolism is:

- Microsomal: can be isolated by homogenization and centrifucation of the cells and they are found in SER. These enzymes can catalyze hydroxilation, reduction, hydrolysis and their expression can be changed

by drugs and age, so they are unstable. The most important is *cytochrome P450*. They are more abundant in the liver

- Non-microsomal enzymes: expressed all over the body. They catalyze the same reactions, the big difference is that their activity is stable throughout the life.

2.7.1 Cytochrome P450

It's a superfamily of enzymes and it can catalyze mainly *oxidation* but also other reaction. These enzymes are present in almost all the cell but the highest levels are in the liver. Endogeneous substrates are steroid hormones, fatty acids etc, even if selectivity of these enzymes is very low. The expression of these enzymes can change due to genetical or environmental factors. These enzymes can be induced or inhibited by drugs: this puts the bases for *drug interaction*. Furthermore, there is a great variability between individuals, due to *polymorphism*: the human cytochrome superfamily comprehends 18 families, 43 subfamilies and more than 60 genes which differs in specificity for inhibitors etc. There is a specific nomenclature: CYP is abbreviation of cytochrome, thes a number for the family, a letter for the subfamily and another number for the gene/enzyme.

The members of the same family should have at least 40% of identity, while members of a subfamily should have more than 55% of identity. Why such a high variability? Maybe because evolution due to selection pressure, due to the variability of dietary compounds. The content of tyr changes adapting to the environment, synthesizing new compounds in orde to defend themselves from animals. Also expression of cytochrome has changed. It has been identtifyed a small clan of people in Etiopia with a limited number of one family of cytochrome: this maybe because this population has been isolated from other clans with access to very limited differences in food. We have to remind that not all the families of CYP are important for metaboolism: a 50% of drugs are substrates of CYP3A4 and CYP3A5, then 25% are metabolized by CYP2D6 etc.

The variability is due to *genetic* and *environmental* bases.

Genetical

Very high polymorphism. We can have deletion of the gene $\longrightarrow$no enzyme $\longrightarrow$no metabolism of the drug by CYP.

On the other side we can have a higher that normal enzyme activity, due to the presence of 2 more genes codifying for the same enzyme $\longrightarrow$increased

metabolidm by CYP.

Whew there is one signle gene codifying for the CYP, we can have a normal enzyme and normal metabolism or the enzyme can be unstable (metabolism reduced) or have an altered substrate specificity (different metabolites are produced). Therapeutically, no metabolism or increased metabolism are 2 important conditions, because in the population statistically there is a lot of people with no CYP gene, the so called *poor responders* but also people with more than one gene, the *ultrarapid metabolizers*.

For the poor responders, the metabolism of the drug is lower that the mean of the population: this leads to a high increase of the drug level using the normal dosage $\longrightarrow$high risk for *toxic reaction*.

For products like codein there is no response because the drug is not metabolized into an active form. For the ultrarapid, the response to the drug is lower or there is no response at all after the administration of the normal dose.

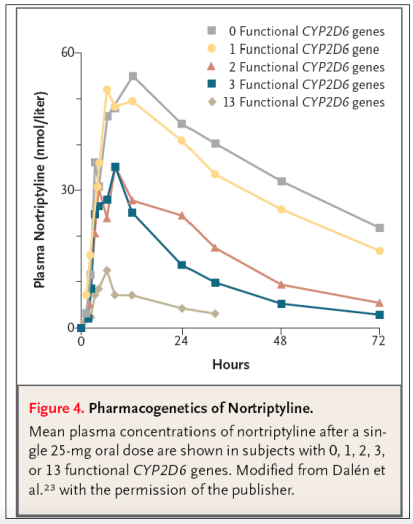

Figure 2.2: Pharmacogenetics of Nortriptyline

The concentration of a drug in the plasma changes with respect to time. The yellow one (the second higher one) is the pattern of a person with 1 functional gene: it is not so different from the pattern of the person with 0 genes (grey, the higher one) because the specificity of this enzyme is not oso high and if the enzyme is not present, other enzymes will take its place.

The *polymorphism* is not an hallmark for CYP: other enzymes like the acetyl-transpherase has at leat 2 phenotypes (slow and fast) $\longrightarrow$this condition may be the reason why slow acetylator delevops peripheral neuritis and fast

acetylators are prone to hepatic toxicity (there is the formation of high levels of toxic metabolites).

Environmental (acquired)

Drugs can induce *ex-novo* synthesis of enzymes or inhibit enzymes, in particular the microsomal ones.

For *inhibition*, the drug can bind to the enzyme and occupy the site for other drugs.

Induction can really affect the response to drugs. There are several drugs that can induce the *ex-novo* synthesis of enzymes, like phenytoin, tobacco smoking, phenobarbitone. This means that one drug can also impact on the metabolism of other drugs that are substrate for the same enzyme. Induction means an increased expression of the enzyme, so bottom line is that induction can *reduce* the amount of drug for the therapeutic activity. Induction may be a way to adapt to environmenal pollutants, a way for the body to reduce the toxicity of exogeneus compound. It *decreases the effect of drugs*: this can lead to *tolerance*.

The mechanism by which a drug can induce new synthesis of enzyme is the same for P-glycoprotein, the difference is in the *receptor* to which drug binds, it is *Aryl hydrocarbon* receptor (AhR). The drug or the exogeneous compound binds to this intracellular receptor, the receptor binds additional proteins that mantain inactive the receptor itself, then the complex drug-receptor translocates into the nucleus, HSP and other small proteins unbind, the receptor finds on the DNA its responsive element sequence $\longrightarrow$increase syntehsis of CYP.

This mechansim is peculiar also of steroid hormones.

Inhibition The drug causes inhibition of the enzyme, there is no metabolism of the drug and of other drugs that bind the same enzyme. Therapeutically, this increases the plasma level of other drugs that are substrate of the same enzyme, because they are not metabolized (interaction).

2.8 Elimination

Different organs are involved in drug elimination, but *kidney* is the main one. Every renal disease reduces drug elimination $\longrightarrow$increase plasma level of the drug. Other sites are the gastro-intestinal tract, the salivary gland:

rifampicin is an antibiotic escreted by salivary glands, used for dental extraction; stomach for morphine; large intestine for drugs that are not absorbed, like drugs used for intestinal infection; the liver through the bile for ampicilln and fidampicine; sweat or lacrimary liquid for rifampicine, which give a red color; lungs have a big area for absorption and elimination of drugs, in particular volatile anesthetics; milk during lactation for morphine or amphetamine.

We will focus on the kidney. The kidney is very high vascularized, we have the afferent arteriole and efferent that forms the glomerulae, but then also a vascularization all over the nephrons (vasa recta). Of all the blood that reaches the kidney per unit of time, only a small part (1/5) reaches the glomerulae, all the other goes into the tubuli.

revise kidney system

The drug elimination by the kidney is due to the presence of three processes:

1. Glomerular filtration: hydrophylic drugs can be filtered, so from the blood into the tubular fluid
2. Reabsorption: from the tubular fluid again into the blood
3. Secretion: a drug from the blood is secreted into the tubular fluid

Secretion and reabsorption are based on the presence of *transporters*, for reabsorption also passive diffusion for lipophilic drugs.

2.8.1 Filtration

For glomerular filtration we have different pressures that works:

- Hydrostatic pressure of blood (45 mmHg)
- Colloid-osmotic pressure that tends to retain drugs into the blood stream (-28 mmHg)
- Hydrostatic pressure into the Bowman capsule (-10 mmHg)

The resulting filtration pressure is 7 mmHg.

Only drugs with less that 70 kDa can be ultrafiltrated and only free drugs can be ultrafiltrated. So, for tubular filtration, we must consider:

- Size of the molecule : < 70 KDa
- Filtration pressure : 7 mmHg
- binding to plasma proteins.

2.8.2 Reabsorption

For tubular reabsorption, in the proximal tubuli, we can have both *passive diffusion* and the presence of *specific transporters* for facilitated diffusion: reabsorption goes along concentration gradient. The degree of reabsorption is related also to the pKa of the drug compared to the pH of the urin, if the drug is acidic or basic. Increasing the pH of urin you can faciliate the excretion of acidic drugs; if you reduce the pH of the urin you can increase the excretion of basic drugs.

2.8.3 Secretion

We have active transporters at the *distal* level. These transporters are saturable: this is important for the competition of drugs for the same transporter, or between endogeneous compounds and drug (for example uric acid and salicilates $\longrightarrow$increase of uric acid is responsible for the gotta. If you take aspirin, you worsten the pain because aspirin will compete for the transporter and it will be eliminated whereas acid uric will accumulate, worstening the clinical condition).

Usually transporters shows a high affinity for drugs with respect to plasma proteins. The binding to plasma proteins will reduce the amount of drug available for distribution and for therapeutic effect: the binding of the drug to plasma protein in this case *increase the velocity of elimination* by secretion. Why? This is a dinamic thing: the drug continuosly bind and unbind from its binding site, so it will bind preferentially to the binding site for which it has higher affinity.

For active transport, the drug that is not bound at that moment will bind the transporter and will be eliminated. Usually the affinity of drug for plasma protein is lower than for transporter.

The amount of drug that is escreted by urin is equal to amount filtered through glomerula - amound reabsorbed into renal vein + amount secreted into tubular luliman fluid. We need a parameter for the rate of elimination of the drug: so, pKa, partition coefficient, distribution volume and *clearance* (Cl).

Clearance We measure clearance in this way:

$$C_x = \frac{U_x \cdot V}{P_x} \tag{2.2}$$

If the concentration of the drug in urin U_X is high, the drug goes into the urin. The bigger is U_x, the lower is the concentration in the plasma P_x. This

value is corrected by the volume of the urin formed in a given time V. So, clearance is measured into mL/min.

So, the clearance is the volume of blood from which the drug is removed in a given time. The rate at which kidneys excrete solute into urine is equal to the rate at which the drug disappear from the plasma.

Kidneys are highly perfused by the 25% of cardiac output (6 L/min): it is 1.25 L/min. Considering that the 50% of blood is water, the 50% of 1.25 is 0.650 L/min of plasma water that reaches the kidney every minute. Only 20% of 0.650 L/min is filtered by glomerula (0.130 L/min) $\longrightarrow$glomerular filtration rate (GFR). the remaining 80% of blood reaches the tubula via the vasal recta, the peritubular capillaries etc: this is 0.650 - 0.130 L/min. If you find a value of clearance for a drug that is equal to GFR, you can usuppose that the drug has been ultrafiltrated but no secreted and not absorbed. If C_x is lower than GFR, the drug underwent to reabsorption; if C_x is between 0.13 and 0.650 L/min, the drug is also secreted. Glucose $C_x = 0$ $\longrightarrow$glucose is totally reabsorbed: it is present in urin only in diabetic conditions. The maximum value of C_x is 0.650 L/min, the minumum is 0 L/min.

GFR and the higher value for drug secreted can be determinate by administation of acido para ammino epurico: this helps evaluating renal function, whereas creatinin is used to evaluate the glomerula function. Creatinin is an endogeneous compound produced by skeletal muscle activity.

Bioavailability (F) it is another pharmacokinetic parameter, like C_X and V_d. It is the fraction of a drug that after oral administration reaches the systemic circulation in an un-altered form, so it is available for the pharmacological activity (active form). If you administer the drug by endoveneous route, the bioavailability will be 100%, because you skip the first pass metabolism. F has no unit because it is a ration between the availability after oral or other route with respect with endoveneous route.

Oral availability can be affected by several reasons, all base of the amount of drug absorbed, the amount of drug metabolized and so no. Reduced availability of the drug after oral administration is due not only on the first pass metabolism in the liver, but there are several reasons like failure of disintegration or dissolution of the pharmaceutical preparation, the low pH of the stomach could contribute to hydrolysis of the compound, bacteria into the intestinal tract and then the presence of the P-glycoprotein, responsible for reducing absroption of drugs and the presence of enzymes, that are present also into the intestinal epithelium in high concentration (CYP in particular). Duodenum has almost 50% of CYP with respect to the liver, 10% in the ileum.

Al these factors contribute to reduce the availability of the drug. Valporate has a F of 100%, Gentamicin is less that 5%.

How can we measure F?

We plot the variation of the concentration of the drug with respect to time: we can see how plasma concentratio increases quickly until a peak is reached (absorption is the most important process in this phase). At the peak there is equilibrium between absorption and elimination, then after the peak a decrease in plasma concentration to 0. From this graph we can individuate the maximal concentration C_{max} obtained at the peak level after administration. The time needed to reach the peak is t_{max}. The lowest concentration starting by which we can observe the pharmacological effect, the *minimun effective concentration*. We can see the duration of action, which is the therapeutic window. We can calculate the AUC, *area under the curve*, which integrates all the quantity of drug that is present between the lag time until the total elimination of the drug.

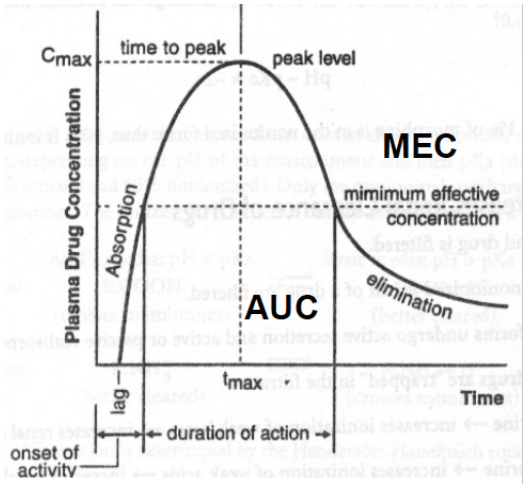

Figure 2.3: Plasma level curve after oral administration

So, administering a drug intravenously and by mouth (at the same dose) we can compare the AUC of the 2 different administrations: we make the ration between the 2 oral and intravenous and obtain the F value (oral availability).

F can also be used to evaluate the *bioequivalent*. Generic drugs are drugs for which the patent has expired: it is necessary to demostrate the bioequivalence between generic and patent drug. This is done measuring F, so the t_{max}, C_{max} and AUC and these vaule must be similar with no statistical variation. These compounds must have the same bioavailability and the same rate of absorption.

(7.03.2016)

2.8.4 Elimination half-time

The time needed for a drug to reduce its concentration by 50% with respect to the initial concentration. It depends on the characterizstics of the drug and it is linked to Vd and Cl of the drug, where Cl indicates the velocity by which a drug is removed from the blood into the urine and Vd indicates how far is the drug from the blood stream. For example, for a drug with a high Vd and 3 different drugs with different Cl values: look how different are the half-time of these different drugs. The highest is for the drug that is also reabsorbed and the lower for the drug that is secreted. We can see that a drug that is also secreted actively and have a very short permanence time, has also a small Vd. On the contrary, the highest time of permanence is fod a drug that has a high Vd and is also reabsorbed: there is a link between these parameters.

Half-life $t_{1/2}$ is the time that takes the drug concentration in the blood to decrease to one half of its initial value. To mesure this parametes, the drug must be administered endovenously, so we are sure the bioavailability is 100%. The unit is time (min, h, days). The link between Cl and Vd is:

$$Cl = kV_D\,. \tag{2.3}$$

In the graph we have the rapresentation of variation of plasma concentration of a drug with respect to time: We administer endovenously the drug

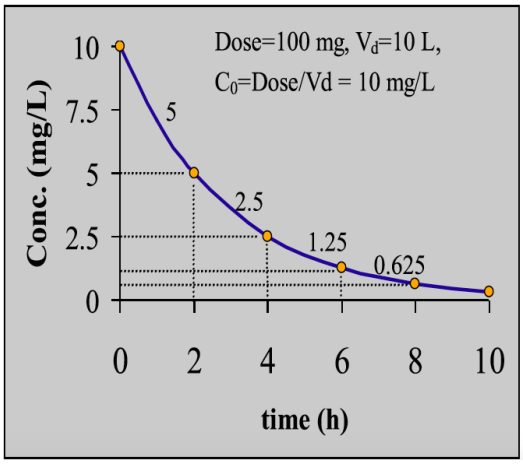

Figure 2.4: Rate of elimination curve

and look at the amount of drug in the blood taking samples of blood and

measuring the plasma concentration. From the relationship btw the dose administred and the Vd value, we can also extrapolate the *initial concentration* of the drug in the plasma (at time 0) C_0. This is done dividing the dose for the Vd of the drug.

We can see that if C_0 is 10, after 2 minutes the concentration is reduced by 50%, so we can say that the half time elimination of this drug is 2 min. After other 2 minutes (4 minutes), the concentration in the plasma is reduced again by 50% and after other 2 minutes (6 minutes), we have again 50%. At time interval equal to the half-time of elimination, the amount of drug eliminated is always 50% with respect to the initial concentration. Why? We have 3 main mechansims by which drugs go through the membranes: passive diffusion, facilitated diffusion and active transport. The last 2 are due to the presence of transporters, the first one is based on the Fick law and the concentration gradient is the force: here, the reduction of concentration gradient reduces the amount of drug that is eliminated and also the velocity of elimination.

The rate of elimination: by passive diffusion we have a *linear kinetic* or first order. We have to give the drug endovenously. As the drug is eliminated, the concentation in plasma decreses, so if a drug follows a first order kinetic, the rate of elimination is proportional to the plasma concentration at any time in any moment and decrease with time as the plasma concentration decreses.

This prameter $t_{1/2}$ is useful when we have to administer the drug cronically for the right interval of time: the most simple thing is to *predict how long it will take to a drug to be eliminated.* This is 6 times the half-time $(6 \cdot t_{1/2})$. Why this is a linear kinetic? The data in the graph are experimen-

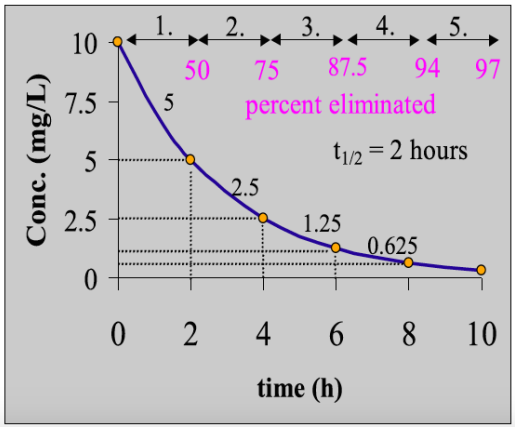

Figure 2.5: Use of $t_{1/2}$

tal, so these points are the results of an interpolation of the datas. Usually, we change something in order to obtain, instead of a curve, a line: we can do this by changing the y axis, putting *log of concentration* instead of concentration ⟶the curve become linear and we obtain a semi-log graph: From this

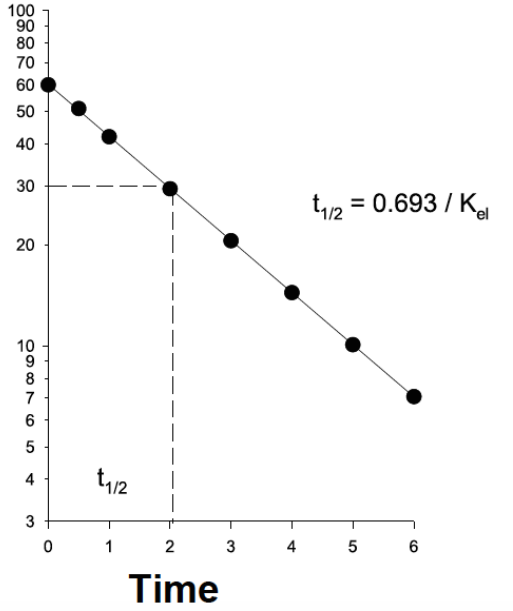

Figure 2.6: Rate of elimination curve in semilog scale

graph we can immediately and more precisely see the time corresponding to the half time and see that we need more than 6 times to reach the 0 concentration and calculate the half time just dividing the elimination constant K_{el} (slope of the curve) by the natural logaritm 0.693 ⟶we obtain $t_{1/2}$:

$$t_{1/2} = \frac{0.693}{K_{el}}\,. \tag{2.4}$$

Principle of linear pharmacokinetic

Linear (first order) pharmacokintic: elimination is not saturable (non-capacity-limited) and the rate of drug elimination is directly proportionate to the plasma concentration of the drug. Half time for first order kinetic is *independent from the dose* and it will not change until something else will change, like Cl value.

Nonlinear pharmacokinetics

There are drugs that follows a non-linear kinetics, like drugs carried through membranes by transporters, enzymes, so the drugs which elimination depends on proteins that are number-limited and saturable. The amount of drug eliminated by nonlinear pharmacokinetic is constant with time if all the transporters are saturated.

The rate of elimination is:

$$RE = \frac{V_{max} \cdot C}{K_m + C} \tag{2.5}$$

which is the Michaelis-Menten equation. We cannot calculate the half time because it depends on the quantity of the drug that we administer. The rate of elimination is constant, irrespective to plasma concentration. These drugs are more difficult to deal with: they can accumulate, reach toxic concentration if we administer the 2 doses too close etc.

This is how the pattern of nonlinear drug appear: in a semilog graph.

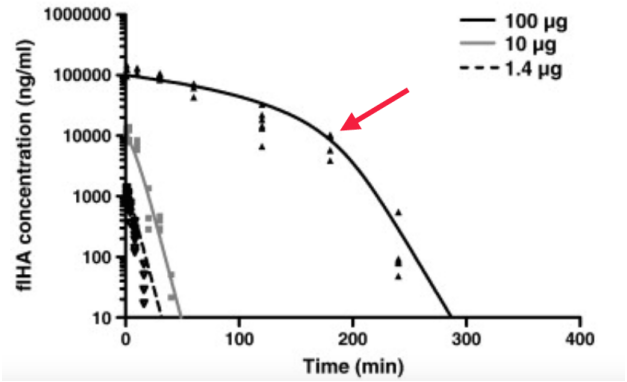

Figure 2.7: Rate of elimination in a nonlinear pharmacokinetc

It is not linear, with time only little amount of drug is eliminated until we reach a point (red arrow) under which the transporters are no more saturated, so we see an elimination pattern like the linear one.

For alcohol the nonlinear pharmacokinetics depends of saturation of the metabolizing enzymes that have a very very low affinity, s it is difficult to go under the limit of saturation.

If the drug is administred at the lower dose (than the saturation concentration, 100 μg) we have the black patterns: below this value, we have a pattern that resembles to the linear one. We cannot measure a real $t_{1/2}$ and we cannot predict how long it will take for the drug to be eliminated because it *depends on the dose*.

2.9 Dose-effect relationship

In the middle between pharmacokinetic and pharmacodynamic. We can study this relationship building the *dose-response curves*: both the intensity and duration of the effect of drug are strictly dependent on the dose that we administer and on the concentration of the drug at the target site. We have to administer a dose in order to obtain a concentration high enough to give the pharmacological response. A very low dose will give a low concentration that is enough to reach the target but not to give the pharmacological effect; in order to have tha pharmacological effect, the concentration of the drug must reach and go above a *threshold concentration*.

Those response curves can be build up into 2 different manners, different for the end-point that we want to reach:

- Graded: the endpoint is to observe the *effect in relationship with the dose*. It means that we have a continuous scale: we observe how gradually the effect changes with respect to the dose. This kind of curves are measured in vitro and you can study for example how cAMP levels increases in a cell population, how aorta rings contract in the presence of a drug and so on. This relates the dose to the effect;

- Quantal: the endpoint is to observe *if we have an effect or not*. We observe if the effect is present or not: an all-or-none. We use only data form observation. This curve is done on populations and they observe if a certain dose causes the effect or not.

Why we are interested into these curves? With the graded curve we have an idea about the *potency* of a drug, in terms of concentration. The quantal curve give us an idea of the *mean dose* that is efficacious in most of the person.

2.9.1 Graded dose-response curve

Different concentration of the drugs on x axis and record the response on the y axis, reported as the % with respect to the maximal effect: we have a rapid increase of the effect, then a plateau and after that if we increase further the drug concentration we cannot increase the effect. The effect is the consequence of the bind of the drug to a specific target: enzyme, receptor etc. M/L is molarity. From the linear graph we can obtain the so called *EC50*, which is the concentation of the drug that gives the half maximum effect. We can build up another graph, the semilog, to easly determine the EC50:

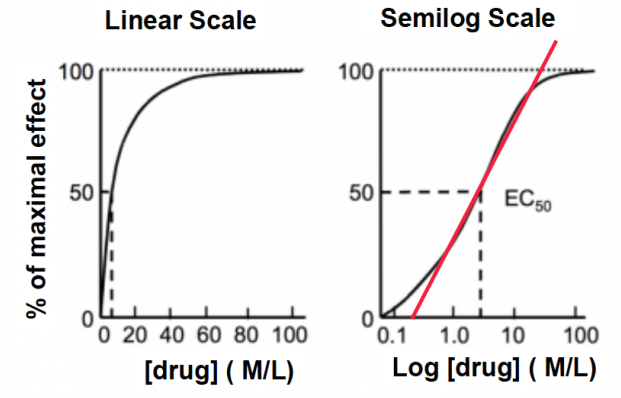

Figure 2.8: Graded-dose response curve

we obtain a sigmoidal pattern. The central part of the sigmoid is *linear* and comprehends the datas from 16 to 84% of the effect:

EC50 is the index of affinity of a drug for its target, but it is not the *true* affinity: the true affinity can be determined only with the *radio-lingand binding study*, in which we have the receptor obtained by an homogenated brain and the ligand labelled and we put them together $\longrightarrow$obtain the K_d value (dissociation constant), which is the true value for affinity. EC50 is somehow related to K_d, but they are never the same, because here we are looking at an effect, so we have intracellular effects before seeing the effects, while with radio-ligand binidng we have only the receptor and the ligand and we see the pure activity.

2.9.2 Quantal dose-response curve

This curve is based on *normal distribution* of biological variables. Most of the biological variables are normally distributed:

Glycemia, levels of hormones etc can be represented by this curve. The response to drugs also follow a normal distribution: the high proportion of a population will respond to the same dose of drug: we can determine the amount of a drug that is supposed to be effective in a population. We also have some peple which are resistants and need a higher dose in order to obtain the pharmacological affect, but also the contrary.

We saw something like this talking about enzyme induction or inhibition: some people can be much sensitive of resistant to drugs. The dose can be determined by this curve plus or minus one standard deviation of the mean and we find 16% and 84% that correspond to the limits outside +1 or -1 standard deviation.

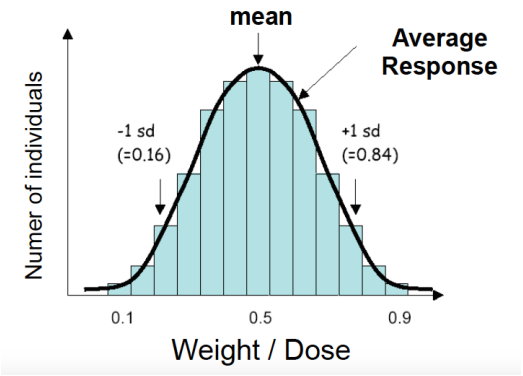

Figure 2.9: Quantal-dose response curve

To build this curve in vivo, we have a population, start administering increasing doses and we'll observe if we have the response or not, for example if the blood pressure decreases by 1 or 3 mmHg: then record the number of subjects that responds to that drug. If you put these numbers into a graph ⟶normal distriution. We can record the *ED50*, the dose required to produce the pharmacological effect in 50% of the population.

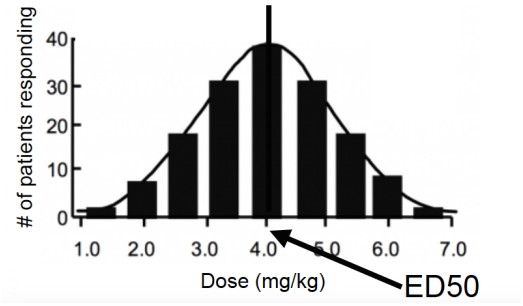

Figure 2.10: Quantal-dose effect curve: frequency distribution

From the same datas obtained from the quantal study we can build another graph: the *cumulative frequency dose-response curve*, recording the subjects that responds to the first, second, third etc dose and adding to the second dose the number of individuals responding to the first dose, to the third the number of individuals responding to the second dose and so on. Notice that the scale is logarithmic and we have a sigmoidal pattern from which we can measure again the ED50.

When we face a dose-response curve, first ask what is on the Y axis:

- If number of individuals ⟶quantal curve
- If an effect ⟶graded curve

We can do other things: we can compare two different curves, for example a curve that studies the hypnosis as effect of a drug and one (with the same drug but at higher dose) that studies the lethal dose. We can compare the ED50 with the LD50, the dose that causes death of the 50% of the population. It is important that these 2 values are quite far away, quite different! Of course, this distance in doses btw ED50 and LD50 gives us an idea of how safe is a drug, but as always we need numbers to have an immediate idea of that: this is called *therapeutic index*. It is the ratio between LD50 and ED50:

$$TE = \frac{LD_{50}}{ED_{50}}\,. \tag{2.6}$$

If therapeutic index is 1, the same dose can give both therapeutic effect and death $\longrightarrow$the higher is the therapeutic index, the most safe is the drug. In the linear portion of the sigmoid we have also a linear proportion btw dose and response, whereas in the marginal part (nonlinear) btw 16 and 84% of the curve we don't have a linear relationship. The therapeutic index does not take in account this nonlinear part of the curve: there could be an overlapping of the 2 doses! We have a dose that can cause death in 1% of the animals that is also a dose needed for a pharmacological effect in the most resistan patients. How can we calculate a dose that is effective in 1% of the population? There is a manner, but it's very diffucult; however, juts know that we have some tables, the *probit tables* that can extrapulate from our datas also the dose that give a so small response. This is a statistical method. Having LD1 and ED99 we can calculate the *margin of safety* instead of TE: we have the ratio between these 2 values:

$$MS = \frac{LD1}{ED99} \tag{2.7}$$

The margin of safety give us a better idea of how much safe the drug is.

In another graph we have straight lines instead of sigmoids: on Y axis we have the probit value. We can see that drug A, therapeutic and toxic effect, is more safe than drug B, therapeutic and toxic effect, because for drug B there is the *overlapping* of both therapeutic and toxic effect. This overlapping is not seen for drug A. These are 2 different drugs: however, the slope of both drug A and B are parallel (therapeutic and toxic effect). This has a meaning in pharmacology: by a parallelism beween curves we can say that toxic effect is an extension of pharmacological effect. For example, an anticoagulant drug can cause emorragia, which is a toxic effect: if the 2 curves (therapeutic and toxic) are parallel, we can presume that the mechansisms of action of these 2 effects are the same. If there is no parallelism, we can presume that the molecular mechanisms underlying the pharmacological effect and the toxic

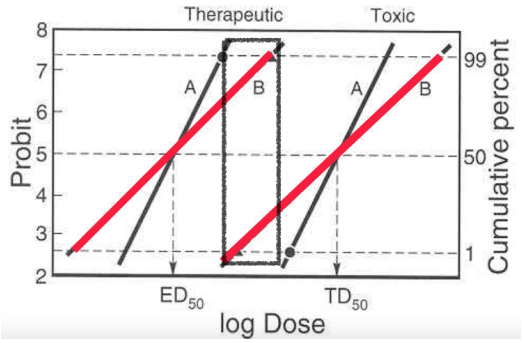

Figure 2.11

effect are different ⟶the drug is acting on 2 different targets. For example, antipsicotic drugs that blocks dopamine receptors can also block muscarinic receptors: the side effects are due to the ability of the drug to recognize different receptors from their primary target of action. This is due to lack of selectivity, which is a frequent condition for different drugs.

(9.03.2016)

Chapter 3

Pharmacodynamics

Let's define 2 important properties:

- Affinity
- Efficacy

We can measure them and this can help us to predict a lot of things about potency of the drug, ability to give pharmacological response etc. From dose-response curves we can measure EC50, a inch for the potency of the drug: it is a mirror of the potentcy and we can define it using the K_d.

Affinity is the predisposition of a drug to interact with its binding site. Highest the affinity, stronger the binding.

Efficacy is the ability of the drug to give cellular response, and this appens because the drug can induce confromational changes and the molecule for which the drug binds. It depends on the changes that the ligand can impone on the receptor. For pharmacology, we'll see that receptors are a limited numbers of the possible targets of drugs: they are the molecules that can interact with a ligand and give cellular response, for example enzymes modify a substrate, so ideally they are receptors, because receptors are molecules that bind the ligand and give response.

Considering metabotropic receptors, the drug binds and unbinds continuosly: higher the affinity, higher the propensity to stay bound. For efficacy, the ability to give response, it is due to changes into the receptors after the binding of the drug.

3.1 Affinity

We can depict the interaction between a drug and its receptor as a dynamic interaction: D + R can form a complex DR with k_1 and k_2 as association

and dissociaton constant. The intensity of the effect will be proportional to the quantity of the complex drug-receptor.

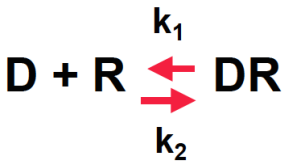

Figure 3.1

In the graph we see different dose-response curve, different drugs. On X axis in semilog scale the concentration and the response on the Y axis: there is a difference btw the drugs, that is the EC50 value which decreases and the highest this value, the smaller is the potency of the drug. EC50 is related to the affinity. The true affinity of a drug is given by radio-ligand binding assay

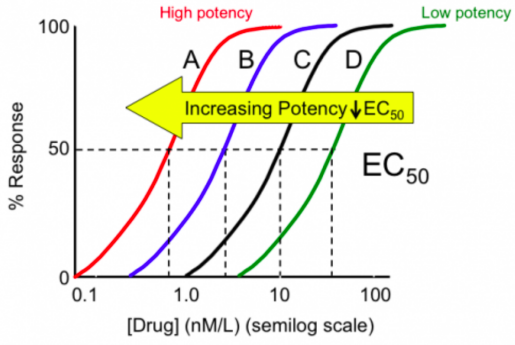

Figure 3.2: Concentration-response curve

in which we need molarity, K_d (the drug concentration that occupies the 50% of receptors at the *equilibrium*). In this experiment we have to define the time at which the complex DR is stable, so an equal quantity of drug binds and unbinds.

3.2 Efficacy

The ability of a drug to give a mesurable response. We have a concentration-response curve with semilog scale: We'll have drugs that can give 100% of the response, but other cannot, like drug B and C. A drug that gives the maximal response that can be obtained *in a certain tissue* is called *full agonist*. Drugs

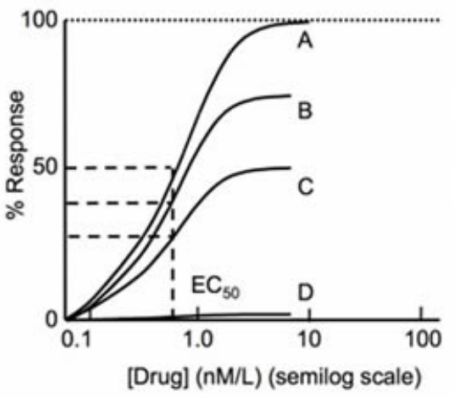

Figure 3.3

that in the same tissue cannot give the maximal response are called *partial agonists*. Drug B is a response that is equal to 80% of the effect od drug A and drug C gives a response that is 50% of drug A.

We also have *antagonists*: they give very low response or no response at all. Antagonist are useful and common drugs because they works antagonizing the effect of endogeneous compound. A β blocker is a compound that inhibits the binding of noradrenaline to $\beta 1$ and $\beta 2$ receptors. Antagonists don't have efficacy but they have affinity.

3.3 Models

3.3.1 Drug receptor theory

The effect of a drug is due to the amount of DR, but also depends on a constant, α, which is named *intrinsic affinity or efficacy*. If we can measure affinity of a drug by measuring K_d, we can also measure the intrinsic activity of a drug, the value of α, just making a ratio between the maximal effect obtained by an agonist and the maximal effect of the full agonist. Drug B, which was 80% of the full agonist, will have an α value of 0.8.

α value is btw 1 and 0: 1 is 100% of the response (full agonist), 0 is for a drug which is an antagnist.

$$Effect = \alpha \cdot [DR] \tag{3.1}$$

So, α is specific for each tissue: the same ligand can act as full agonist in one tissue and as a partial agonist in another tissue. Why? Because tissues are different, so all the machinery transducing the signal can be different.

The model is that to give an effect is not enough the formation of the complex receptor-drug DR: it has to happen the change of the receptor to another conformation which is active. The binding of the drug to the receptor is bound to give a *perturbation*. In order to explain partial agonist and partial antagonist, the receptor has to switch to an active conformation. Possibly, the full agonist is pushing on the receptor population in the active, whereas the partial agonist produces a submaximal response in that tissue and cannot shift all the receptor population inot active form. For an antagonist, the drug binds to the receptor $\longrightarrow$no response, no measurable effect, so it cannot at all push the conformational change.

We also have ligands like the green one in the graph that give an effect, but the effect is the opposite of that of an agonist. They are called *inverse*

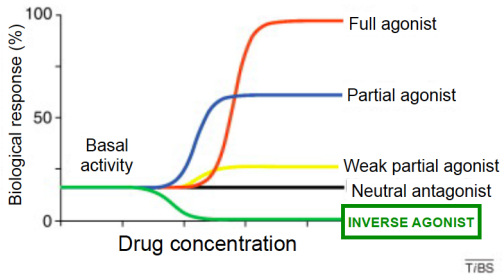

Figure 3.4

agonists and they have been described not for G-coupled receptors but for ionotropic $GABA_A$ receptors, target of benzodiazepine, used as ansiolitic drug. A group of molecules, beta-carbolines, bind to $GABA_A$ receptors and give an ansiogenic effect!

Inverse agonism has been discovered recently: in order to see the effect of an inverse agonist, you must have a sensitive experimental setup able to detect the basal activity of what we are looking at, to appreciate the reduction. Several classical antagonists have been re-studied and found out that they are inverse agonists, like β-blockers. Inverse agonist has affinity and efficacy. The complex DR* can be in equilibrium with D + R* (* is the active form) and R* can spontaneously change its conformation to become inactive, but also the inactive R can change its conformation to become active. Not all the receptors have this ability to be in the active conformation in the absence of ligand: R* in absence of the ligan is called *receptor with constitutive activity*. Not all the receptors can be contitutively active, for example the subtype 4 of dopamine receptor has a high tendency to be active: the probability for a receptor subtype to be present in constitutive

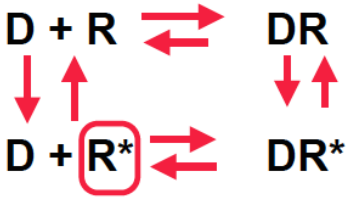

Figure 3.5: Inverse agonism

form depends first of all on the aa sequence, that can change spontaneously from the inactive to the active form.

The *inverse agonist* is a ligand that binds to the inactive form of the constitutively active receptor (R) and shift the population of the active receptors into the active form (R*). The drug by itself is able to change the conformation of the receptor. Inverse agonism takes advance of the receptors that are in active conformation without the presence of ligand (endogeneous ligand!).

The simple drug-receptor theory has been complicated by experimental evidences: these models are created in order to explain some effects that are observed during experiments and that cannot be explained by the current model of D-R interaction.

3.3.2 Biased agonism

Or ligand-selective functional agonism. A ligand binding to a receptor can give 2 different responses: response A (stimulation of PCL) and response B (stimulation of PLA2). The ligand interacting with this metabotropic

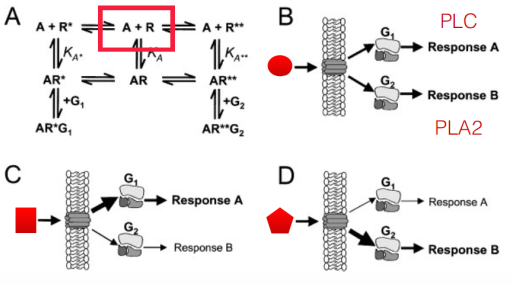

Figure 3.6: Biased agonism

receptor can change the conformation of the receptor in at least 2 different

conformations, one able to interact with G_1 and one with G_2. This complex AR can interact with 2 different G proteins. Every ligand that is able to bind to this receptor will give the same response, activation of response A and response B. This is not true! Other ligands, like C and D, that binds to the same receptor of ligand B, changes the conformation of the receptor with high preference for one conformation: for example, activation of PLC more than activation of PLA2.

The ability of a drug to select one pathway of response is called *ligand-selective functional agonism* or biased agonism.

This is true not only for this cascade of events, but also with regard to all those mechanisms that are responsible for the end of the receptor activity: for example sensitization of G-coupled receptors, it is following phosphorylation of the receptor, binding of β-arrestin etc. Different ligands are more propense to give the off-response to change the receptors in order to start quickly all the machineries that are responsible for the shut off of the receptor response. We will se an example talking about oppioids, one that can give dependence and one not, even if they bind to the same receptor.

Receptors coupled with G-proteins are not stati entities: they can spontaneously change their conformation. Not only the receptor can change confromation, but also different agonists acting on this receptors can give different conformational changes in the receptor itself. The interaction with G-proteins is due to the binding to the third intracellular loop (ribbon representation).

3.3.3 Antagonists

Naloxone is an antagonist for the opioid receptor. Why antagonist are important? Because they can block the effect of endogeneous ligands. We can have smooth muscle relaxation in the arteriole due to the blockade of $\alpha 1$ receptor. There are 2 kind of antagonism:

- Competitive antagonism: due to the ability of a drug A to bind to the same bindig site of the antagonist I. We can see the pharmacological effect of an antagonist in the presence of an agonist. The first dose-response curve is due to an agonist: if we add a certain concentration of a competitive antagonist, antagonist binds instead of the agonist to the binding site and can reduce the ability of the agonist to give the response. Since the antagonist is competitive, increasing the concentration of the agonist we'll reach the maximum effect, because the binding of agonist and antagonist is reversible and is constantly changing, so antagonist occupy the receptor when agonist is not bound and vice

versa

- Irreversible antagonism: the antagonist binds with a covalent binding to the binding site that is shared with agonist. In this way, increasing the concentration of the irreversible antagonist, it will occupy all the binding sites and, since the bind is not reversible the agonist will not bind to the receptor. In the presence of the irreversible antagonist, the dose-response curve is shifted and the efficacy is reduced, so we'll neve obtain the maximum effect

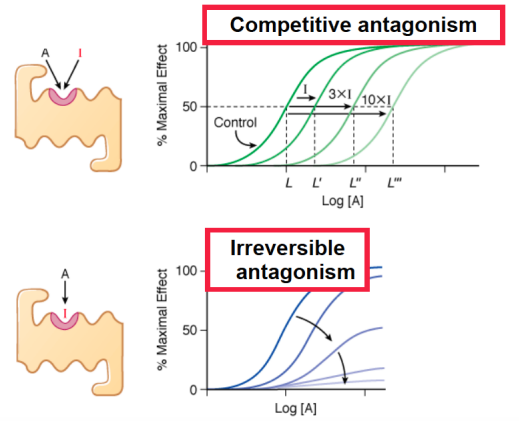

Figure 3.7

Irreversible antagonist is for example the aspirin, monoamino oxidase (MAO) inhibitors for treatment of pressure.

In order to compete for the binding site of the agonist, an antagonist must have *affinity*. Can we measure this affinity using dose-response curve? Efficacy is 0. Yes, we can do it with *schild plot*:

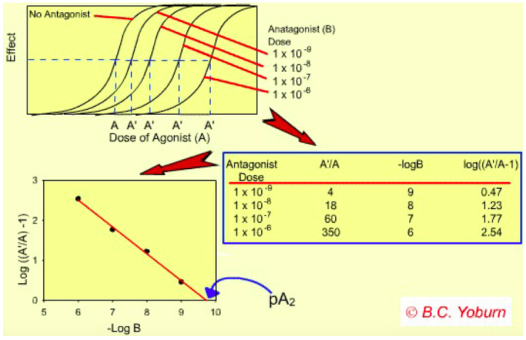

Figure 3.8: Schild plot

We see the shift of the dose-response curve of an agonist in the presence of different known concentration of the antagonist. We can measure the different EC50 values for the agonist and then build a graph using the antagonist dose, the ratio btw EC50 of the agonist in the presence of the antagonist, make the negative log etc.

Schild plot is a question for the examination

We have other kinds of ligands apart from agonist and antagonis: *allosteric ligands*.

3.3.4 Allosteric ligands

Allosteric drugs bind on their own binding site on the receptor, that is different from the binding site for the agonist, which is the *orthosteric binding site* (for the agonist). How can an allosteric drug change the response of the receptor? Allosteric drugs can change the receptor conformation in order to change the shape of the orthosteric binding site, so having an effect on the agonist, changing the affinity of the agonist. They also can change the conformation of the receptor to affect the *efficacy* of the agonist, in positive or negative manner. Negative or positive with regard to what?

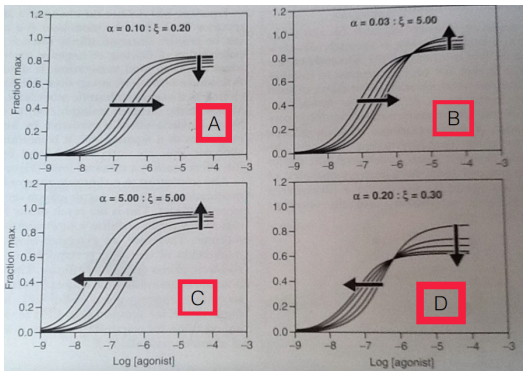

Figure 3.9

The first curve is the agonist curve (look at the direction of the arrows), the others are the curves of the agonist in the presence of the allosteric modulator: its presence decrease the affinity (shift to the left of the dose-response curve) and also the efficacy. The other possibility is that the presence of the allosteric modulator can reduce the affinity but increase the efficacy. We can have a positive modulation for both affinity and efficacy: in this case the agonist alone is the last curve (look at arrows). Finally, we can have increase in affinity and decrease in efficacy.

An example of positive allosteric modulators are *benzodiazepines*: bind to $GABA_A$ receptor, modify the conformation of the reeptor increasing the

affinity of GABA for its own binding site; barbiturates make the same thing. A positive modulator can increase both affinity and efficacy or only affinity or only efficacy. The idea is to find drugs that can modulate the response of the receptor selecting favorable effects: benzodiazepines are used to treat epilepsy, anxiety, eye's muscle relaxation but also have negative effects on cognition, memory. We would like to find compounds that retain the ability of ansiolitic effect without making effects on the cognitive part. Also Glut receptors NMDA: we want to retain memory effects without the cytotoxic effects.

(10.03.2016)

3.4 How drugs act: targets

Suggested by Paul Ehrkuch (1854-1915): pharmacology is a young science. He believed that in order to give a pharmacological response, drugs must bind to a target. We'll discuss the proteins that bind drugs, in 4 groups:

- Receptors
- Voltage dependent ion channels
- Enzymes
- Transporters

Of course there are other targets that are not proteins. We call *receptors* those proteins whose function is to recognize and respond to endogeneous chemical substances. All the other proteins with which drugs interact are called *targets*, not receptors.

To act therapeutically, a drug must bind selectively to a target with high affinity (possibly) and selectivity means *specificity* to recognize one receptor and one target and to not have affinity for another target. It is impossible to find a drug that is totally specific: increasing drug concentration makes possible the interaction with other targets. If I increase the affinity of the drug, I need a lower concentration so the drug will be specific. At the present time we use low affinity drugs, because it is possible to obtain a therapeutic effect using more than one drug, with different kind of action. This will be more useful that a highly selective drug.

Among the different targets, we have the receptors in sensus strictu that comprehends more than 45% of the total drug target, even more if we include nuclear receptors; ion channels, nucleic acids, hormones, enzymes (30%) and other target that are still unknown.

3.4.1 Transporters

All the transporters that mediate active or facilitated transport share the same molecular structure, 12 TSM domains with intra and extracellular loops. This classification of drug targets is based on *molecular structure*. Some examples of drugs that interact with transporters: the drug that bind to the transporter is not called agonist or antagonist, but that terminology is reserved to endogeneous substances or to drugs that bind to receptors. In the case of transporters, we talk about *inhibitors* and *false substrate*.

Antidepressant are often noradrenaline transporter inhibitors. Non selective inhibitor of the MDR transporter is *probenecid*, used with pennicilline in order to increase the permanence of pennicelline into the blood, because it is a substrate of MDR transporters. Duretics act on Na/K/Cl cotransporter, while digitoxin is an inhibitor of the Na/K ATPase to treat cardiac insufficiencies.

3.4.2 Enzymes

We have again inhibitors, those drugs that inhibit in competitive or irreversible way, or false substrates. For example, inhibitors of AChesterase are used to inhibit the symptomes of Alzheimer; organophosphates binds to nervine gases. Aspirin is an irreversible inhibitor of oxigenase; L-NMMA and L-NAME are inhibitors of nitric oxide syntase; meclobemide is an inhibitor of MAO, is an antidepressant. L-DOPA is a false substrate of DOPA-decarboxylase for treatment of Parkinson's disease.

3.4.3 Voltage dependent ion channels

VOC to regulate cell permeability to Na, K and Ca. We have *blockers* and *modulators*. Blockers usually are drugs that find their binding site inside the pore of the channel, so they occlude the channel, whereas modulators are *allosteric modulators*, so their binding site can affect the time of opening or the frequency. For local anestetic, lidocaine or tetrodotoxin (from pesce palla), are blockers, while veratridine is a modulator of Na channels. For Ca channels we have cadmium as blocker and the dihydropiridines as Ca modulators. ATP-sensitive K channels expressed in the pancreas, they are physiologically blocked by ATP, so when the energy inside the cell is high and ATP levels are high, these channels are blocked; modulators of these channels are drugs used to treat diabetes, like sulfonylurea derivatives, that can remove the block of ATP, so the cell depolaryze and release insulin.

For VOC we can talk about a common structure: they are usually made

up by a single chain of aa that forms 4 domains each formed by 6 TSM domains. These 4 domains will form the channel with inside the pore and all these domains have in the 4th TSN, the S4 or *voltage sensor*, so when Vm changes, this changes also the conformation of the channel so that S4 moves up.

There are also other conserved part: btw 5th and 6th S domain there are aa responsible for the conductance of the channel, so they can choose which ion will go through the channel; then a loop responsible for *inactivation* of the channel, like a gate that can change conformation and moove to close the pore from the inside. VOC, when Vm is at rest, they are present in the resting state and the intracellular gate is open, then during depolarization the channel opens and during repolarizing phase the intracellular gate closes.

The role of VOC is that of enable the AP initiation and propagation in excitable cells. VOC, in particular Na VOC are often regulated by ancillary subunits: the main subunit is the α subunit and there is also these ancillary subunits, like $\beta 1$ subunit: the expression of this ancillary subunit is *tissue dependent*.

Na channels

A large family. The nomenclature is given by the IUPHAR committee: Na_v stays for the channel in general, the first number is the isoform and the second number is the splice varials. We have at leat 9 different isoforms ($rNa_v 1.1$, 1.2 etc). The isoforms are given by aa that changes btw the different classes: this difference is shown in %. The consequence of having different aa is that each isoform will assume a small different conformation, so the characteristics can be different from some aspects, apart from the fat that usually there is also a different expression of different isoform in different tissues. Muscarinic receptors are all G-protein coupled receptors, but we know at least 5 subtypes (M1, M2, M3, M4 and M5) that changes for a small aa sequence and from different expression in the body. M1 in CNS and M3 is glandular.

So, differences in the conformation means also a different regulation: some aa can be Pi, and Pi is the most powerful way to change the activity of a protein. There are differences in the sequence $\longrightarrow$different conformation of the channel $\longrightarrow$possibility to find molecules that can interact specifically with the different isoforms. All the drug that acts on Na channels interact with α subunits are local anestetic, antiepileptic, antiaritmic drugs etc.

We have a lot of binding sites that have been identified for natural toxines, that have been very useful to understand the physiology and pathology of these channels, as well as K and Ca channels. We see the hypothetical binding sites for some of these toxins on the α subunit: these binding sites have

been identifyed in Na channels. TTX, derived from blowfish, Saxitoxin from dinoflagellates that are filtered by muscles and are responsible for the paralitic shell-fish poisoning, μ-conotoxin from see snail called conus geografus. The bind to receptor site 1.

Then receptor site 2: we have veratridin, batracotoxin, aconitin from ranucnolacee and grayanotoxin form ericacee leaves. Few years ago it was forbidden to make honey from these flowers.

We have other neurotoxin receptor sites, all of natural origin, like site 4 for β scorpion toxin etc. Most of them acts as allosteric positive modulators, apart those that bind to the site 1 that acts as blockers, and can modulate time opening and frequency of opening of these channels. We can presume that all these toxins evolved in animals or plant in order to defen theirself.

Ca channels

Ca is important in regulating a lot of functions: contraction, secretion of ntr, gene expression etc. Mde up by a big α subunit, ancillary subunits $\alpha 1$, $\alpha 2$, β and γ. They can be intracellular and extracellular subunits: their expression changes the characteristics of the channel and their expression variates btw different kind of cells. Another dendrogram and we see that there are at least 10 genes that encode for different Ca channels: Ca_v denotes the VOC, 3 families with diffrenent nubmer 1, 2 and 3 and each family has different isoforms, so with respect to Na voltage channels we can distinguish 3 groups:

- L-type: L stays for long lasting, these channels once open remain opened for relatively long time. Localized in skeletal muscle, cardiac muscle: their funciton is to initiate contraction and secretion. Drugs are calcium channel blockers uset to treat hypertension, arythmias.

- T-type: T means transient, so they open and close very fast. They show specific localization in cardiac muscle, skeletal muscle and neurons, they are calcium channels blockers-sensitive.

- P/Q, N and R type, where N stays for neuronal. Mostly expressed in the CNS, on nerve terminals and are responsible for the release of neurotransmitters. THese channels are not sensitive to the calcium channels blockers, whereas toxins have been useful to distinguish btw the 3 different subtypes: agatoxin from the spider or marine snails for P/Q, conotoxin for N-type and SNX-482 for R-type.

K channels

Comprehends the largest group of ion channels, almost 40 genes, that are different with respect to Na and Ca channels. They are involved in regulation of cellular volume, Vm, neuronal excitability, secretion etc. Whereas Na and Ca channels are made up by a single main α subunit with 4 domains, K channels can be formed all by 4 single domains made by 6 STM: 4 subunits made 1 channels, or 2 subunits made by 4 STM or 4 subunits made of 2 STM. The latter group comprehends the channels activated by G-proteins.

It is believed that K channels were the forst to develop, they are the most ancient, and the others channels derive fro mthe evolution of K channels. The most recent seems to be the Na channel.

6 STM comprehend the channels activated by cyclic nucleotides and the transient resting potential channels: K_V, K_{Ca}, CNG and TRP. They are frther complicated by the presence of ancillary proteins, not only β subunits but also calmodulin, the presence of different splicing form, the presence of subunits that can change the functions and since they are formed by 4 different subunits, they can be formed by the same or different subunits (homotetramers of heterotetrames).

4 STM (2 subunits) group comprehends 18 different channels, 15 genes: they are involved in the leakage of K at the rest, in stabilization of Vm and they can be regulated apart from the Vm also by chemical and physical stimuli, like changes in pH, oxygen tension, mechanical stetch, G-protein coupled receptors etc.

2 STM (4 subunits) comprehend ATP-sensitive K channels and the G-protein coupled K channels. The intracellular loop regulate the link ofthe channel to G-protein. There are several neurotransmitter that can stimulate the opening of this K channels through their G-coupled receptor. For example, opioids, ACh in heart. We hage G-protein of the inhibitory pat tha can inhibit AC activity, but also inhibit voltage gated Ca channel through the $\beta\gamma$ subunit and stimulate the opening of K channels. Then, the overall result of inhibiton of Ca channels and stimulation of K channels determines a reduction of the possibility of the nerve ending to be excited $\rightarrow$hyperpolarization of Vm due to opening of K channel, so decreasing the probability of stimulate htese cells. These channels are iportant in regulating the funciton of the heart, but also other organs, and changes (genetically determined) of this class of channels have been linked to different diseases: epilepsy, alteration or abolishing of Kir 4.1 can cause deafness etc.

3.4.4 Receptors

The existance of receptors has been postulated last century: the real classification is recent. Before the discover of the receptor and cloning of the receptors, they had to wait until the 90's. We have orphan receptor which have been identify by their structure to be G-coupled, but they are orphans because their endogeneous ligand is not known yet.

There are 4 kind of receptors:

- Ionotropic: ligand gated ghannels
- Metabotropic: G-protein coupled
- Enzyme-linked
- Nuclear receptors

They differ from the structure. Since their structure is difference, they also mediate different responses on the bases of time: ionotropic, since responsible for the movement of ions, they give a fast response (ms). G-protein coupled give origin to a cascade of second messenger with activation of protein kinases and changes in [Ca], so they act in minutes. Then enzyme-linked receptors, they also regulate Pi of substrates, but the response is seen later (hours) as well as the response of nuclear receptors.

Receptors withis a family are present in several subtypes which shows similar strucutre but different aa sequence, tissue distribution, pharmalogical structure and regulation $\longrightarrow$this will affect the secondary and tertiary structure. They also differ for their regulation. The hetergeneity of different receptor families derives from the presence of different genes, at least 7 different receptors for serotonin, 5 subtypes of the 3 receptor for serotonin etc. There can be different for the translation phase of the gene to form splicing variants (also post-translational modifications).

For ligand-gated, 4-5 subunits and each subuit is made up by 4 STM. Each STM faces the pore and 5 or 4 of them form the channel. This is the structure of a nicotinic receptor: ACh binds btw $\alpha\gamma$ and ... The subunits can be the same or different. The location is CNS, ganglia and skeletal muscle and depends on the combination of $\alpha\beta\gamma$ subunits. The $\alpha 7$ are permeable to Ca while the other are permeable to Na.

For G-protein coupled: 7 STM.

For enzyme-linked receptors: they are made up by 2 subunits, each one has single STM. In the active form, these 2 subunits form a dimer: for example, NGF receptor dimerize, the conformation changes, it is transmitted from the extracellular portion to the intracellular one and this enables the

intrinsic enzyme activity of the intracellular portion to be active. All growth factors receptors enzyme-linked receptors and this is a tyr-kinase activity. There is a small group of enzyme linked receptors with different intrinsic enzymatic activity: inseat of tyr-kinase is a *guanilate-cyclase activity*, actinf on GTP to produce cGMP. This family comprehends the natriuretic peptides, only 2 subtypes of these receptors that are also expressed in the sea urchin.

For nuclear receptors, see cytochrome B. Only drugs that have a high lipid-water coefficient can use these receptors. For example, vitamine D, steroid hormone, glucocorticoid etc. There is the binding of vitamin D to intracellular receptor $\longrightarrow$translocation in nucleus $\longrightarrow$regulation of transcription. Why this is very complicated? Because the interaction with nuclear receptors results to an increase or a decrease in expression of other proteins: for vitamine D increase of Ca binding protein, alcaline posphatase, but reduction of c-myc $\longrightarrow$regulation of cell proliferation and differentiation. For glucocorticoid we have a decrease and increas eof inflammatory proteins.

(14.03.2016)

Ionotropic receptors

GABA and Gly are both inhibitory, because the ion that permeate these 2 channels is Cl $\longrightarrow$hyperpolarzation. Then we have excitatory transmitters, ACh and $5-HT_3$ serotonin receptors. They are permeable to Na and Ca. The receptors activated by Glu are NMDA, AMPA and Kainate: these receptors are made up of 4 subunits, 2 a 2 uguali. 2 dimers bind together to from the true receptor. AMPA receptor has a big extracellular portion.

As well as the voltage dependent channels, also the receptor operated channels can undergo conformational changes, shifting to resting conformation to an activated one and to desensitization. Same thing for AMPA receptors.

Then we have another endogeneous neurotransmitter that have channels as receptors, ATP: it is a large and complicated family of channels and no other neurotransmitter has ionotropic receptors. We can easly suppose which kind of receptors a neurotransmitter can bind. Receptors with intrinsic kinase activity are activated by neurotrophic factors.

G-protein coupled receptors

Include the more common drugs used. β-adrenergic receptors were the first to be identifyed and they are the most studyed. Very big family, diversified. There are more than 3 different subfamilies based on the structure:

1. Family 1: for cathecolamines, small molecules, odorants, small hormones etc. The binding site for the ligand is almost inside the structure of the receptor, in the *allosteric polar pocket*

2. Family 2: for GLu, it has a simle structure with big extracellular domain, because they bind molecule with high MW

3. Family 3: Glu or GABA metabotropic receptors (GABA B). The extracellular portion is bigger and forms the *vineous trap*, able to bind ligand (Glu $\longrightarrow$changes in conformational structure and goes very near to the surface of the receptors, pushing the ligand inside this structure

The off signal is Pi of the intracellular loop by specific protein, the G-protein receptor kinases GRK, that Pi the activated receptor, and this Pi change the conformation of the receptor, so it can recognise *arrestin*, physically bound to the Pi receptor, conseals the receptor from G-protein. The receptor can remain in the membran but not respond to the ligan (desensitization) or it can be *internalized* in a coated pit vesicle and then can be destroyed at the lysosomal level or recycled back to the membrane.

When the receptor is degrader, the total number of receptor on the surface of the cell is reduced, so we call it *downregulation*. The reduction of the number of receptors after continuous or prolongued exposition to an agonist and the desensitization means that the cell cannot respond less to the endogeneous ligand and also to drugs: it is a pharmacodynamic manner to induce *tollerance*, the need to increase drug concentration to obtain the same effect than at the beginning.

G-protein coupled receptors have a lot of putative recognition sites for different cellular component: arrestin, GRK, Pi, SH2 and SH3 domains, highly conserved aa sequences present in different regulatory proteins. SH2 and SH3, when they bind each other and to regulatory protein and to the receptor, on the G-protein recept act as a brick, they physically tight together different proteins in order to transduce the signal. There are sites for dimerization, for the G-protein etc. Often, G-protein coupled receptors do not act alone, they can form a *dimer*. This interaction through estracellular and intracellular binding sites that changed composition and characteristics of the receptor.

The G-protein is bigger than the receptor itself and has 3 domains. G-protein are called G because they have GTPase activity: 4 main classes on the bases of the structure of the α subunit, with different isoforms (21 α). G-protein coupled receptors are the most abundant, they recognize a lot of endogeneous ligand: NA, DA, 5-HT, GABA, Glu, Ca etc. Depending on the G-protein they bind to, they can activate different pathways. When we talk

about G-protein coupled receptor we think at the acute effect of activation; most of them when activated can give a *long term effect* due to interaction with protein synthesis. This effect can be important: the smooth muscle cells at bronchial levels can be relaxed through activation of $\beta 2$ adrenergic receptor or contracted by histamine: this conctraction or dilation is an acute effect. However, there is also a long term effect: substances that can contract, on the long term also increases the number of smooth muscle cells, in order to potentiate the physiological response. In ashma, there is an hyperproliferation of smooth muscle cells, so it's important to have a dilatation, to have a relaxation of smooth muscle cells because it counteracts the hyperplasia and hypertrophya of smooth muscle cells: it happens at vascular level. This is mediated by activatio of MAPKinase, ERK etc, increasing synthesis of new proteins.

put scheme

Protease activated receptors

We have also *protease activated receptors*: it has the ligand inside the structure. The N extracellular terminus is a substrate for protease, like trypsin, trombin, factor of coagulation cascade etc. Protease cut the extracellular portion so that what is left (white portion) can move and activate the receptor, acting as a ligand! These receptors are widely distributed, like in the stomach where they stimulate secretion of mucus, gastric acid, then modulate smooth muscle activity. They can also be activated by small peptides. They are still G-protein coupled receptors.

Effectors

Adenilyl cyclase AC, 2 domains of 6 transmembrane sequences, linked by intracytoplasmatic groups. It has a structure similar to a transportes (12 STM). When it is activated, cAMP is produces. During cAMP production there is also an efflux of cAMP outside the cell: this is still not clear why there is such waste of energy. Maybe AC is itself a transporter able to extrude cAMP, but extracellular receptors for cAMP have not been identifyed, so possibly it can be a substrate of ectoenzymes, by formation of cAMP $\longrightarrow$Adenosine, which is a member of transmitters of the purinergic sistem. AC enzymes are a big family, which means that we have different form that differ on the basis of aa sequence, tissue expression, regulation and pharmacology. In mammals, there are at least 10 distinct AC isoforms that differ from aa sequence, tissue expression and regulation mechanisms. Regulation mechanisms are always the same: Pi, activation of PKA, PKC, CaMK.

Presence of a binding site for purine and pirimidines nucleotides on the catalytic site and an allosteric site that binds forskolin: to increase cAMP concentration we use forskolin, derived from a plas, Oleus Foskori, used by indians to treat asthmatic attack, burning the plant and inalating, causing dilatation of smooth muscle cells (it is very lypophilic).

PLC It can produce inositol-3P and dyacil glicerol, so we have 2 outputs. IP3 regulates intracellular levels of Ca. PLC has different isoforms, 3 classes that have some regions in commons: aa sequence, catalytic domain. Only in β isoform we have a domain for the interaction with G-proteins, whereas γ isoform contains SH2 and SH3 sequences that can act as a link point for other proteins, so they are different in their sequence, distribution, regulation and for their pharmacology. There are enzymes that can bind together to give different responses: in a receptor with intrinsic tyr-kinase activity we'll have a lot of sites to recognize these proteins etc.

Ras-ERK pathway ...

put scheme

PI3K pathway The most recent and the most complicated.

This is a family of enzymes with different sequence, pharmacology and tissue expression. The intracellular signaling is very well organized. Even if the cell membranes act as a wall to protect the cell from the external environment, the cell has, a part from receptors, other manner to communicate: there are a lot of extracellular enzymes that do the same thing of intracellular enzymes, like nucleoutidase, adhesion molecules, phosphodiesterases. Intracellular enzymes are quite often exposed to extracellular milieu.

Chapter 4

Opioids

Papaver Somniferum. Opioids are extracted by the fruit, cutting the capsle and collecting the dense liquid inside, which contains a lot of active compounds, first of all, *morphin* (10% in the opium juice). There are other compound with almost the same chemical structure of morohin, like codeine, thebaine. Codeine is used to treat the cough, it is an antitussive drug: it is methabolized in vivo into morphin, so it has an analgesic property. Thebaine has not a pharmacological use, because it causes disphoria, contrary of euphoria, an alteration of the mood, a bad feeling.

We also have compounds with different chemical strucutre, like papaverine, used to relax the smooth muscle of gastrointestinal tract, noscapine to treat the cough and that may have antitumoral activity. Of course, we have also drugs that are similar to morphin, derivatives from semisynthesis of total synthesis: *eroin* is an acetate semisynthesis compound. This group of morphine-like drugs are all agonists, even if we have antagonists like aloxon or partial agonists as moprenolphine. The pharmacological properties of morphine are known from centuries, however larger used only at the beginning of the 18th century, when morphine was used to keep calm babies.

4.1 Actions of morphine

In CNS and gastrointestinal tracts.

Starting from CNS, the most important is *analgesia*: it is a very potent pain-killer for acute pain in particular, like post-operative pain or in neoplastic patients. This effect is related to antoher activity of this drug, the *sedation*, detachment from the environment and increase of somnolence, together with *euphoria*, part of the analgesic activity is due to sedation and euphoria: Patients feel ain but they do not care.

In some patients, morphine can also cause dysphoria and allucination with a very low degree. The other effects are not so good: frequent nausea and vomiting, respiratory depression, inhibition of the respiratory center, so the center is not more sensitive to an increase of CO_2. We also have depression of the cough reflex (that's why codeine is used), miosis and pupillary constriction, an effect of hyperdosage of morphine. When a patient loose consciousness, we are able to say that it is a morphine intoxication if the pupille is constricted.

At the peripheral level, the eccets on gastrointestinal tract we have the ability to increaase the tone and reduce motility $\longrightarrow$costipation. We also have contraction of the biliary sphincter, which can cause itterus.

Other actions can be related or not on the presence of morphine receptor: morphine can induce the release of istamine, with urticaria and itching or broncocostriction as consequences. We can have also hypotension and bradicardia and the ability to modulate the immunitary system in a negative manner, due to the presence of morphine receptors on most of the cells of the immunitay system. In this way, all the immunitary system can be modulated. How morphin acts?

Presence of receptors that can recognize the drug: this idea was corroborated by pharmacological evidences. Almost in the 70', at least 3 subtypes of morphine-like drugs exist:

- Mu receptors, from morphine, now we call them MOR
- Kappa receptors, from Ketocyclazocine, now called KOR
- Delta repetors, from deferent vessesl, called DOR

THere are also 3 groups of endogeneous ligands (endorphines)

- Beta-endorphins
- Dynorphins
- Enkephalins

The receptors, cloned in the 90', are all G-protein coupled receptors. Orphanin FQ/nociceptin receptor has bee identifyed, it does not recognize a classical non-selective antagonist for oppioids receptors, naloxone.

These endogeneous ligands are peptides: we have Leu enkephalin and Met enkephalines, that are penta peptides (5 aa) and the first 4 are in common also with β endorphines and Dynorphin. Endorphin is a much longer peptides. Apart these classic ligands, other have been identifyed loke Orphanine, endomorphine 1 and 2 etc.

The synthesis of these peptides is particular like for many peptides that act as neurotrasmitter: they are not synthesized by themselves, but included in a sequence from which they are cut off, so they have precursors and β endorphine sequence for example is expressed n a precursor that is called *pre-proopiomelanocortin*. From this long sequence can be optained β endorphin, lypocortinsm melanocyte stimulant MSH and ACTH, the adenocorticotropy ormon.

Precusor for enkephalin is *preproenkephalin* that hase several sequences to obrain Met-ENK, 5 sites. So, we have the possiblity to obtain more than 1 molecule from these precursors, like those involve din synthesis of 5-HT, DA, A. Dinorphine A and B, leucin-enkephalin etc. The most abundant transmitters are the pentapeptides, they are synthesized in the CNS, in particular in the regions responsible for controlling pain, they are relased during stress, pain or anticipation of pain and are also present at the periphery in gastrointestinal tract, adrenomedulla and immune system. Once released from the vesicle,s these molecules can interact with the receptor and there is an OFF signal, so enzymes like peptidases. At least 2 enzymes seems to be involved in the catabolism, one is neprelisin and the other one is aminopeptidases: these are extracellular enzymes but linked through transmembrane domains, no intramembrane enzymes. They have additional function, apart for acting on endogeneous opioids: neprilisyn can degrade other peptides; aminopepetidase N can also act modulating other cellular activity like proliferation interacting with extracellular molecules $\longrightarrow$multitasking enzymes.

We have a lot of ligands and 3/4 (if consideric noreceptin receptor NOP) receptors. THer eis no a correspondence btw one peptide and one receptor, with exception of orphanine which interacts with its own receptor and does not recognize MOP or COP receptors, whereas for example DOP cereptors recognize hormons and opioids. MOP have a preference for beta-endorphines. There is no degree of selectivity among the endogenenous ligands and the receptors, wherease with other drugs that can be used to dissect very well the receptor system because there are selecitve agonist and selective atagonist, this selectivity is important to discriminate what action is mediated by which receptor.

Among the agonists we have *morphine* and *methadone*: the big difference is that methadone is used to treate dependence by morphine and the half-time, shorter for morphin (2h) than for methadone (14–40h). Antagonists like naloxone, used in case of intoxication after hyperdosage in order to displace morphin from its own receptor. THen we have partial agonists such as *buprenorphin*, used mostly during surgical procedures to reduce the pain.

All these receptors are *G-protein coupled receptor*, in particular inhibitory G-rpotein. They reduce activity of AC, of VOC for Ca (N and P/Q chan-

nels, expressed n CNS) and activate K channels (inhibited by G-rpotein). These 3 actiity are a remark for different neurotrasmitter: muscarinic receptors coupled to inhibiroty G protein can do the same thing ⟶reduciton of excitability of the nerve reducing transmission of the pain stimulus.

We also have the activation of MAPK, so a long term response.

4.2 Classification of opioids receptors

There is a high number of ligands (11) and a small number of receptors (3–4) with poor selectivity for the ligand. This leads to complications about the possibility of a more complicated classification, but the existence of different subtypes is still under debate.

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Paradox: whereas for many neurotranmsitter we have one ligand and different receptors subtypes, in this system we have a limited nuber of receptors and an abundance of ligands. There are pharmacological evidence that suggests that this system could be more represented: MOP antagonist can inhibit some actons of morphin, for example analgesia but not respiratory depression ⟶there are some subtypes of receptors. Experiments with KO animals with regard to more receptors can inhibit all the responses of this receptor.

put scheme

Do opioid receptor subtypes exist? Yes, at least 3 of MOP etc . Another hypothesis is the presence of at least 3 different mechanisms:

- Splicing of a common gene for one receptor
- Functional selectivity
- Possibility of dimerization btw receptors

There are experimental results that can corroborate this hypothesis.

In the splicing, some exon can be present in one protein and other in another protein ⟶different proteins, different conformation, different features. For example, exon 1 gives analgesic activity; exon 2 can confere the analgesic activity to another ligand.

In *functional selectivity*, different ligands binding to different receptors can induce different conformational changes, or can affect differently the desensitization mechanisms of the receptor (Pi by GRK + arrestin). This is due to a prolongued response to an agonist ⟶internalization (downregulation) or resensitization. So, not only the ability to interact with different protein but also to show a preference for desensitization mechanism. Gi coupled receptor with stimulation of AC. The difference btw morphin and DAMGO,

used only experimentally, is that morphin stimulate the PKC pathway and favours the desensitiation of the reeptor, whereas DAMGO can stimulate the activity of GRK $\longrightarrow$internalization of the receptor. The lack of internalization due to morphin is due to the high degree of tolerance as a consequence to the abuse of morphin, while DAMGO is less prote to give tolerance.

In *dimerization*, there are both extracellular and intracellular site that favor the dimerization. When 2 receptors dimerize, they can achieve this into 2 different manners:

- Putting in contact 2 of the transmembrane portions of the receptor, 5 and 7
- within the same transmembrane domain, but opening the structure of the receptors

The difference in interaction can give different properties: in the dimers, both the receptors can interact with G-protein, whereas in this example just one of the 2 recetors can make contact with a G-protein. These 2 possbilities giev also the possibility to affect signal transduction induced by dimerization of these recepors and can explain also the differences in the efficancy. Dimerization can be obtained starting from 2 or more receptor, and we can have omo- and hetero-dimerization: we can have the same 2 receptors (omo), 2 different receptors but of the same class (homo) and heterodimers of G-Protein Coupled Receptrs of 2 different classes (for example μ-R and CB1-R).

These experiments are done forcing a cell to express a receptors that is not usually expressed: the omodimer is normally inactive and the ligand can stimulate the dissociation; an omodimer gives a strong response, than the stimulation of the 2 separately, and it is still associated with internalization; omodimers with MOR and DOR gives different responses, because in absence of DOR, the response to the MOR receptor is reduced. For heterodimers, we have different combinations. The response of the receptor can be enhanced or reduced depending on which dimer it will form.

4.2.1 The opioid system paradox

Different receptors activated by the same ligand; differences in patterns and or efficiency of intracellular signal; duration of action of the endogeneous ligands; differences in intracellular trafficking of the receptors as a function of the receptor and of the ligand.

MOP receptors mediates all the most important activity, like morphin; analgesia, repsiratory depression, tolerance, euphoria. DOP receptors are more involved in respiratory depression and analgesia. Analgesia is divided

into supraspinal, psinal and peripheral: this shows how are distributed the opioid receptors in the pain pathway. KOP receptors mediate disporia and allucinations and NOP have an antagonist activity with regard to supraspinal analgesia and actively mediate spinal analgesia.

4.3 Pain pathway

Pain is the sensorial and emotionall experience due to a real or potential tissue damage. We distinguish acute and chronic pain: acute is the useful one because it triffers responses in order to reduce or avoid pain and the consequent damage. The chronic is called unuseful, it usually comes with adaptive mechanisms that can also increase the pain perception.

We have 3 ascending afferent nerves that convey pain sensation and one efferent, descending. The frst one of the ascending starts in the periphery through the spinal cord, the second one from spinal cord to thalamus and the third one from thalamus to higher centers, like prefrontal cortex, cingulate cortex etc. The signal from the periphery is integrated and we have descending pathways that control the overall response.

4.3.1 1st order neurons

These nerves are mainly of 2 kind of fibers, the *Aδ fibers*, with fast conductance speed, myelinated, sensitive to some kind of noxious stimuli, in particular mechanical stimuli, they convey pain sensation from localized points of the body. We have also *C fibers*, slow conductance speed, amyelinated, respond to thermic insults, chemica, pressure and are more responsible for diffuse and strong pain.

These fibers have thir body into the dorsal rostral ganglion and they make a synapses in the spinal cord (dorsal horn). Apart from pain that can arise from chemical insilts, these nociceptors are also activatedy by a lot of mediators released after tissue injury. These mediators are Bradykinin, 5-HT, H^+, ATP, histamine, NGF. When these fibers are activated, they can also activate in an *antidromic manner*: fibers that can in turn release other mediators, like substance P that can activate the receptors of neutrophils, or mastocytes that release histamine which contribute to the stimulation of firs order fibers, or calcitonin-G related peptide and substance P that can cause dilatation of arteriole and increased permeabilization of the vessels.

4.3.2 2nd order neuron

It starts from the spinal cord, decussate and reaches the thalamus, where the sensation goes to the prefrontal cortex and the cinguate cortex. There are ta lot of connection between the second order and the third order neurons, with the amigdala, nucleo accumbens, hippocampus that are related to the emotional compoent of pain. We have 2 other important nuclei, PAG (periacquetuctal grey) and RVM (rostral ventral medulla) with descending nerves that modulates the response to pain. These 2 nuclei are connecdet through descending pathway from the higher centers. The ascending 1st order, 2nd order, 3rd order and the descending pathway, starting from the cortex (lateral prefrontal cortex). 2 different colors because the efferent control system has both facilitatory and inhibitory effect. In red we have the facilitatory nerves, the *ON cells* and in green the inhibitory efferent fibers *OFF cells*, from PAG and RVM. We have in yellow serotoninergic fibers, that are part of the inhibitory pathway.

Opioids are part of this inhibitory controlling mechanism: how MOR receptors can activate the OFF cells in order to reduce the pain. The nerve release opioids that can bind the receptors expressed on the excitatory cells that mediate pain (ON cells): they inhibit these cells reducing pain. Opioids receptor are also expressed on the GAGAergic neurons that project on the OFF cells: inhibiting this inhibitory pathway, there is a stimulation of the OFF cells $\longrightarrow$no pain. This is at the level of RVM.

However, opioid receptors are expressed in all the three afferent pathways plus the descending one: from the cortex to amigdala and hippocapus, then PAG, RVM (respiratory center) and dorsal root ganglion neurons (1st order).

Sedative effect or detachment from pain sensation: it was believed that analgesic effects of opioids were related to the expression of this system is higer CNS; it has been shown that opioids receptors are present also in periphery, for example in the first order neuron, where they can reduce the AP, so the transmission of pain signal. They are also at the end of the 1st order neuron, and there they can inhibit the release of the transmitters related to pain, so reducing the ability to activate AP in the second order neuron. Opioid receptors are also expressed at the beginning of the 2nd order neuron and contribute to reduce the transmission of the pain signal up to the third neurons.

We have also the terminal of the descending neuron from brainstem, releasing 5-HT and NA: it contributes to modulate the activity of the second order neuron, reducing its activity $\longrightarrow$reduction of pain sensation.

Fibers from locus coeruleus secrete NA $\longrightarrow$important not to feel pain.

There are A beta fibers (mechanoceptors) that project to the dorsal hor

in substantia gelatinosa: the fibers from SG inhibit the second order neuron, reducing pain sensation. These area is also activated by the descending inhibitory pathway.

4.4 Mesolimbic dopamine pathway

Called *reward circuit*: it is activated when there is something that could give pleasure, like when we eat, make sex, stay with people. Dopamine is the neurotransmitter of this circuit, that starts from the ventral tegmental area VTA and goes to the nucleus accumbens Nacc and to the prefrontal cortex PFC. This pathway is present also in rodents. In VTA, Da fibers that roject to Nacc are under control of GABAergic neurons and also of Glutamatergic neurons.

The GABAergic neurons in VTA are tonically active: they release GABA and GABA has receptors on the dopaminergic neuron and mintain under inhibitory control the release of DA. When this system is activated, there is an *increased release of dopamine*. Several drugs, almost all the drugs that can cause dependence and abuse, cause an increased out of control release of dopamine (disinhibition). How? The MOR receptors on GABAergic neuron: their activation causes the inhibition of the GABAergic neurons, so there is a reduced release of GABA, the tonic inhibitory effect is removed and DA is released at higher levels.

Same thing for cannabinoids: they have receptors on the GABAergic neurons, Gi coupled receptors $\longrightarrow$inhibition of GABA's release. Nicotin directly activate DA fibers through nicotinic receptor. Benzodiazepines can induce dependence and abuse: GABA released by the neuron can reduce the activity of the GABAergic neuron itself (there are GABA receptors, activated by benzodiazepines) $\longrightarrow$no more GABAergic effect on DA neuron $\longrightarrow$augmented release of DA. Different subunits that made up these receptors confer diffferent activiy to the receptor and, apart from the different composition, have different expression in CNS and also differ in pharmacology. The GABA and the DA neurons express both benzodiazepine receptors, but with a different kind of subunits: $\alpha 1$ on GABAergic- and $\alpha 3$ on dopaminergic-neuron. The GABA receptors expressing $\alpha 1$ subunit are responsible for the tendency of abuse of benzodiazepine, but not for the ansiolitic effect: if we could find a drug that can just activate $GABA_A$ receptors which express a different subunit from $\alpha 1$, we would have a drug that is not prone to give abuse, retaining the ansiolitic effect.

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Chapter 5

Psychosis

Detachment from reality characterized by different symptomes, such as inability to stay in contact with reality, disturbance of the mood with inappropriate reaction to different situations and impared cognitive functions.

There are different symptomes classifyed in different disorders: schizophresia is a thinking disturbance and it can be a progressive disease, or be characterized by alternance btw remission of the symptoms and relaxing. It s associated with an impairment in social interaction an in occupational funtioning. It is characterized by a reduction of lifespan of 15-25 years. THe symptomes can be distinguished into 3 groups: positive, negative and cognitive impairment.

Positive symptomes: hallucinations, delusion and behaviour disorders. We have inability to give the right weight to things that happens. Positive because there is a reaction to the environment.

Negative symptomes: apaty, inability to make contact with other people, anhedonia (inability to take pleasure), lack of motivation, apathy. These symptomes are usually progressive and are more difficult to be treated. The presence of negative symptomes make the prognosis less favourable and are more resistant to therapy than the positive ones.

Cognitive ipairment: deficit in memory and attention.

5.1 Etiology

The etiology of schizophrenia is still largely unknown: it is based on genetic predisposition and environmental stress, there can be anatomical changes or not and the onset is in the late teens-eaarly twenties. Could afflict 1% of the population. It is equally distributade btw male and female, but in females it is milder, the symptomes are less impressive. There are biological correlates:

- Heritability
- Genetic factors
- Environmental stressors
- Neurodevelopmental hypothesis

For heritability, in monozygote tweens, the risk to develop the disease is 50% if the other is affected, 17% in dizigotic tweens: we can see the dicrease of the risk with the distance by relatives affected, so the genetic component is important.

More than 100 genes have been proposed to be involved in the etiology of schizofrenia, highly polygenic: the idenfityed genes are involved in different functions. Mostly are involved in Glu pathways, involving AMPA receptors and NMDA receprots, genes involved in Ca channels and in the machinery for release of neurotransmitters and Glu in particular. Even the enzymes serine racemase is involved: it is a modulator of NMDA receptors.

On the basis of genetic predisposition, also *environmental stressors* have a role: they can start to work before the onset of the symptomes, like maternal stress, maternal nfections, pregnancy and birth complication but also nutritional deficiencies, autoimmune diseases, head injury, chilhood injury, abuse of substamces.

Considering the environmental stressors acting before the birth, the neurodevelopmental hypothesis make sense: the environmental stressors can impair the normal progression and maturation of CNS; impedin the maturation of progenitors, neuronal migration, dendritic arborization, leading to manifestation of the disease *later* in adolescence or early adulthood.

Before birth, environmental stressors and genetic factors play together, then after birth we have that these 2 factors alterate the glutamatergic cynapse density, decresing it; we also have alteration in myelination, of maturation of interneurons and alteration of the mesocortical dopaminergic projections, so at least 2 neurotransmitters have an important role (DA and Glu) in alterating the development of CNS.

There can be or not *anatomical changes*: the more frequent alterations are enlargment of cerebral bentricles, atrophy, enlargment of the basal ganglia. The fisrt theory about etiology start putting DA: maybe it could be caused by an abnormal exagerated activity of DA. This increased activity of DA pathway should be responsible of the symptomes of schizophrenia: evidences are on pharmacological bases, for example L-dopa, a precursor of DA, is able to produce psychotic symtomes.

5.1.1 Dopamine receptors

G-protein coupled receptors, we have 5 different receptors, the 1 and 5 are positively coupled with AC, whereas 2, 3 and 4 are negatively coupled with AC and can also stimulate the activity of K channels and inhibit Ca channels.

These subtypes of receptors have different tissue distribution: D1 are expressed in striatum, prefrontal cortex, hypothalamus, D5 are expressed in the limbic system, D2 in striatum and limbic system and pituitary gland, D3-D4 in refrontal cortex and limbic system. DA in CNS has a resticted distribution in 4 pathways:

- Nigrostriatal pathway: from substantia nigra to basal ganglia, it contains 80% of total DA in CNS and it is part of the extrapyramidal system, involved in controlling the voluntary motility, the fine tuning. Ipoactivity of this pathway due to the death of this pathway results in Parkinsonian symptomes.

- Mesolimbic pathway: from VTA to Nacc (limbic nuclei). It is part of the reward circuit and it is involved in modulating behaviour responses to activate reward-motivation response. Over activity of this pathway is believed to be responsible for the *positive symptomes*.

- Mesocortical pahtway: starts from VTA and goes to the frontal cortex (cingulate gyrus), it is involved in motivation and emotional responses and it seems to be associated with both positive and negative symptomes, regulating mood in positive and negative manner. In particular, a reduction of the hyperactivity of this pathway is believed to be responsible for the efficacy of some drugs to reduce the negative symtomes.

- Tubero-infundibular pathway: from hypothalaums to pituitary gland (posterior lobe), controls the release of prolactine in a negative manner. If the receptors that take under control the release of prolactine are blocked by an antagonist, there is an over release of prolactine that can give galactorrhea.

- Chemoreceptor trigger zone: detect the presence of toxins in blood and transmitters that inform the vomiting center is DA.

Dopamine theory of schizophrenia : Drugs that increase Da, like cocaine, apomorphine, bromocriptine give a positive behaviour similar to schizophrenia symptomes. Apomorphine, in animals it is used as a model for schizophrenia (stereotyped behaviour): repetition of the same acts like

earing, sniffing etc. On the contrary, drugs reducing DA activity are antagonists of post-synaptic D2 receptors and can control the positive symptomes of schizofrenia. there are evidences agains the simple dopamine hypothesis: if it was true that schizophrenic patients have a higher activity of DA pathways, DA metabolite should be increased in cerebral fluid of these patiens, but this is not the case. Antagonist of D2 receptors are not always efficacious (ineffective in 30% of patients); antagonist should act in a very short lag of time, based on their mechanisms of action, on the contrary the therapeutic effect is seen after a couple of weeks, so antagonist of the receptor by itself is not suffiient to explain the therapeutic effect. There are drugs recently introduced that have low affinity for D2 receptor but works very well.

There are drugs like LSD and phencyclidine, as well as anphetamines and cocaine, that can cause these symptomens: the first is an antagonist for serotonin, hte second an antagonist of NMDA receptors. Phencyclidine, blocker of NMDA receptor, mimics negative and positive symptomes. Ketamine, a non competitive blocker of NMDa receptors reproduces these symptomes, in particular it impair the activity of the prefrontal cortex.

Phencyclidine has been used as a model to study etiology of schizophrenia: after acute administration, it is a model for positive synmptome and when administer chronically it is a model for negative symptomes. Ketamine can cause *hypofrontality*: it can be studied by functional imaging technique and there is a reduction of metabolic activity in the prefrontal cortex.

vedi slide

5-HT has a modulatory role, in particular on dopaminergic neurons.

Now we think that many neurotransmitter are involved in schizophrenia, like Da, 5-HT, ACh, neuropeptides, Glu, GABA.

5.1.2 Drugs

Is schizophrenia for live? Recent researchers think that there are few cases of total rescue from the disease, but it's still a debate. Until now, it has been treated with *antipsycotic drugs*:

- First generation: typical drugs
- Second generation: atypical drugs (for their receptor profile)

The first one introduced was chlorpromazine, a serendipitous discovery: it reduced positive symptomes. Haloperidol (serenase), the drug normally used in case di riposo to keep them calm. More recently we have Molindone, but the real change in 80's with *clozapine*, which opened the way for the synthesis of second generation drugs like Pisperidone, olanzapine, Quetiapine. THe difference btw typical and atypical drugs: the typical have as side effect

the *ability to induce Parkinsonisms*, whereas the atypical have just minimal Parkinsonian side effect. The improvement of the therapy with aypical drugs is also related to their ability to control boh positive and negative symptomes, while typical was only to treat positive one. Atypical are often also effective in patients that did not respond to the therapy with the typical.

Typical drugs have affinity for D2 recpetors and act as atagonist: their efficacy correlates woth affinity towards D2 rceptors. The antagonism is responsible of both efficacy and side effect. These drugs have affinity also for other receptors, like AChR, 5-HT etc. The affinity correlates with the dose that is effective (linear relationship). The antagonist gives different effect depending on the localization of the receptor: if the block of D2 receptors occurs in the mesolimbic pathway $\longrightarrow$reduction of positive symptomes. In mesocortical pathway $\longrightarrow$increase of negative symptomes; in pituitary gland, we have endocrine changes; in basal ganglia $\longrightarrow$extrapyramidal syndrome, Parkinsonism induced by drug, neuroepileptic maignant syndrome (very dangerous). Neuroleptic because antispycotic drugs are also called neuroleptic. The neuroepileptic malignant syndrome is characterized by an increase of body temperature, muscle rigidity, impairment in sweating and it seems to be associted with polymorphism of D2 recpetors. it is potentially fatal (usually): dantrolene, a drug that increases Ca, is effective in counteracting the symptomes (but we don't know why).

put graph

Typical antipsychotic are also effective against muscarinic AChR, causing constipation, urinary retention, alteration of memory; blockade of histamine H1R causes sedation, weight gain; blockade of $\alpha 1$ receptos can cause arteriolar dilation and venodilation. Typical drugs have some *advantages*: efficacy against positive symptomes and low cost.

Atypical antipsychotic drugs have less ability to give parkinsonian side effects, they are often effective also against negative symptomes but they are expensive. The difference in efficacy between these 2 drugs (typpical and atypical) derives from a different affinity with respect to the typical towards receptors like the other subtypes, like serotonin and α-adrenergic receptors, they still bind D2 receptors but there is a balance with D1. They all have a high affinity for 5-HT2 receptors: the ability to recognise this receptor contribute to the producion of less side effect, responsible for the antispychotic effect and improve congition.

Atypical drugs for 5-HT2 receptors can be administer in lower concentration that typical one, because they have a higher affinity for 5-HT2 that typical one.

The blockade of 5-HT receptor is important because serotonin has an inhibitory effect on dopaminergic neurons, so its blockade in nigrostriatal pathway could enhance production of dopamine, reducing parkinsionan ef-

fects.

Also drugs of the second generation have side effects, in particular changes in weight, ability to increase the probability to develop diabetes, living sedation (quietapine, used also for depression). Clozapine has high propensity to induce agranulocytosis, so blood monitoring was imperative, and unduces seizures. As we said, the hypothesis with regard to the fact that these drugs are efficacious after 2 weeks is that these drugs can also modulate the levels of neurotrophic factors: reduced expression of BDNF and its receptors.

5.1.3 Absorption and distribution

Most of these drugs are absorbed easly, but in erratically manner. There is first pass metabolism (significant) so bioavailability from 20–60%, they can bind with plasma protein, so there could be interaction with other drugs and a large V_d, so they are slowly eliminated. They are totally metabolized, expecially by conjugation with glucuronic acid and the metabolites are inactive, Phenothiazines is an inducer of the drug that metabolize itself.

They are excreted by the renal pathway with half-time btw 10–24h. The metabolites can be foun in urine several month after the discontinuation of the administration.

Chapter 6

Depression

(21.03.2015)

Depression is represented by different forms of mood disorders, it is a common disease, heterogenous and often incapacitating psychiatric disorder, so a depressed person has a reduced ability to interact with people, is unable to walk in a proper manner, it is a person whise ability to interact affectively with relatives is reduces. Depression affects 15% of population and difference with psychosis is that it can occur at any age. Depression is twice common in humans with respec to man and, as many psychiatric disorders, the etiology is unknown. Clinically, we have there are three categories of depression:

- Unipolar depression: the mood is constantly depressed, or major depressive disorder
- Dysthymia: less severe but more chronic form
- Bipolar disorder, or maniac-depressive disorders. Bipolar because the person who is affected have an alternance of depressed mood and high mood.

Which are the symptoms? We have *biological* and *emotional* symptomes.

Biological symptomes are mainly: disturbance of sleep (hypersomnia or somnia), changes in appetite and weight, both an increase or decrease; an hallmark is the lack of energy and fatigue to face daily duties, poor concentration and memory.

Emotional symptoms are very different. They have been put in a list that are the criteria for the *diagnosis* of major, depression, the "Diagnostic and statistical manual of mental disorders", that states that at least 5 of these symptoms must be present for at least two weeks:

- Depressed mood

- Loss of interest or pleasure: nothing can make the person happy
- Anydonia
- Feeling of worthlessness: feeling guilty for something
- Tendency to commit suicide.

6.1 Etiology of depression

It is a mixed pattern of genetic, developmental and environmental factors:

- Poor nutrition during childhood
- Lack of maternal care
- Physical stress and depletion of nutrients
- Physical trauma
- Emotional trauma
- Loss of job, divorce or death
- Illness can be also a base
- Drugs can induce depression
- Hormones can play a role (like glucocorticoids, estrogens, like in post-partum depression which can be heavy, with suicide too)
- Endogenous: no external causes.

6.1.1 Genetic factors

There are several genese involved in the tendency to develop depression, in particular polymorphism in:

1. Serotonin transporter (SERT)
2. Serotonin 5-HT 2A receptor subtype
3. FK-506 binding receptor: involved in the regulation f activity of glucocorticoid receptor.

Serotonin transporter (SERT). It is due to the presence of several points in the sequence of the gene that encodes for SERT: SNPs, alternative splicing, VNTR and alternative polyadenylation sites. We also have a connection of the expression of different polymorphism due to a predominance in black part of short-short form in Asians with respect to African or American, where only 21% of people have that tranporter. On the basis of the presence of short, long, ultrashort etc polymorphism, it has been hypothesized the difference in the activity of the transporter: this polymorphism has been hypothesized to be related to the *velocity of the activity* of the SERT.

FK-506 binding receptor. Glucocorticoids are important hormones for adjusting our physical and emotional performance in stress conditions. We can live without of glucocorticoids, but only without any kinf of physical and emotional stress. They can regulate the metabolism of glucose in danger and stress conditions. They are highly lipophilic and their receptor is an *intranuclear receptor* (4th type, responsible for changing the expression of different proteins acting as transcription factors). An example is *cortisol* that can bind to its receptor (GR) that is present in the cytoplasm in a silent form, bound to heat shock proteins and FKBP5 protein. When cortisol bidns to its receptor, this protein is removed, so the receptor will translocate in the nucleus, intecact with its responsive element to bind the DNA. FKBP5 can bind to the complex of receptor and cortisol as a regulatory feedback mechanism, reducing the signal.

In the presence of a polymorphism of these proteins, the interaction with GR starts the synthesis of more copies of this inhibyroty protein FKPB5, increasing the negative loop. So, there is a *reduced response* to corticoids, or resistance to their activity. There will be also a reduced regulation of GR sensitivity and stress hormones system regulation.

6.2 Theories of depression

There are 3 theories:

- Monoamine theory: the first one that appeared
- Neuroendocrine theory
- Trophic and neuroplasticity theory

We can uderstand that these 3 theories overlap one each other. We now believe that there is a mix of the efficiencies of alteration of these 3 pathways, in order to give origin to depression.

6.2.1 Monoamine theory.

The mood is controlled by at least 3 monoamine: DA, NE and 5-HT. These neurotransmitter have pathways starting from different nuceli: NE fibers from locus coeruleus, both in ascending manner toward the cortex and discending through the brainstem, 5-HT fibers from the Raphe nuclei and DA fibers from VTA[1] to project to limbic and cortical area.

They are all involved in *modulation of mood* and emotionality: the 3 different areas have overlapping parts. DA is mostly involved in reward system, whereas 5-HT controls sleep and drive and also motivation, and NE for the ability to concentrate in something, reaction. They seems to be involed also in cognitive function, aggressivity etc. 5-HT and NE seem to be involved in regulating mood, since it has been observed that all drugs that can increase the level of 5-HT and NE or agonist of these two receptors are all effective as antidepressant.

Depression is due to a reduced activity of 5-HT and NE activity, in connection with an upregulation of 5-HT and NE receptor. In untreated depressed patients there is an up regulation of NE and 5-HT receptors.

Depletion of 5-HT and NA do not induce depression in healthy people. As for antipsychotic drugs, antidepressant drugs are not effective in all patients. The effectiveness of these drugs takes at least 2–3 weeks to give an effect, whereas they immediately increase the level of neurotransmitter or very quickly stimulates the receptor.

6.2.2 Neuroendocrine theory.

Depression shares with chronic stress some basic physiological mechanisms. Cronic because we have some mechanisms of response in acute stress whereas we have different mechanisms during chronic stress. In the acute stress we have both neuronal and hormonal responses. The neuronal responses are due to signaling that starts from prefrontal cortex (possible danger) and through the amygdala and goes to Red nucleus of stria terminalis $\longrightarrow$responsible for stimulation of the adrenergic system with autonomical and motor responses, like tachicardia, sweeting, restness.

On the other hand, from the PAG the behavioural responses, the stereotyped reaction like freezing, facing stressfull situation, mobility, panic. The hormonal response involves *glucocorticoids*: in acute stress due to release of glucocorticoid, our body can adjust to stressfull condition, both methabolically and emotional. This acute hormonal response is based on the so called *hypothalamic pituitary adrenal axis* (HPA). Again, the dangerous situation

[1] Ventral tegmental area

from prefrontal cortex goes to hypothalamus, that releases hormone called *corticotropin releasing hormone* pr factor (CRH). From the portal system, this hormone reaches pituitary gland in the anterior part, where interacts with its own metabotropic receptor and stimulates the release of another hormone, the *adrenocorticotropic hormone* (ACTH). This hormone, through the blood, can reach the surreal gland and here it finds its own receptors (metabotropic) that stimulate the release of GC (glucocorticoids). The adrenal gland emits cortical part, the medulla releases adrenal part.

GC have receptors in almost all tissues and in this way it can regulate the response to stress. They also regulate their own release, acting with a *negative feedback* on the release of adenocorticotropine, but also at the level of hypothalamus CRH and ACTH and has receptors in the hippocampus. In this manner, the release of GC is perfectly regulated, because the release of GC is under control of circadian circle, so the level of GCs in the blood follows a daily pattern.

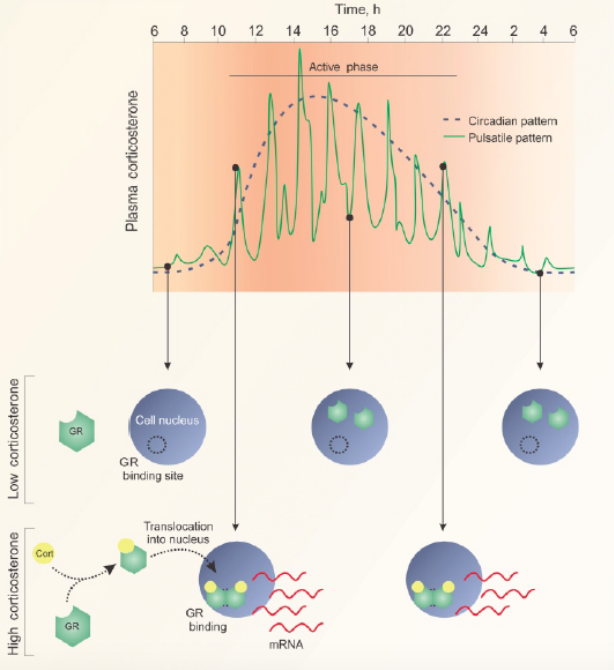

Figure 6.1: Regulation of the hypothalamic-pituitary-adrenal (HPA) axis: circadian pattern.

The peak of GC level in human is about at 8 am and then gradually reduction with minimum during the night. The dotted line indicates the integration of different peaks of secretion of GCs.

Chronic stress

When there is a chronic stress, there is a *disfunction of the release of GC*. The correct feedback mechanism is missingand as a consequence there is an increase of GCs levels without a feedback regulation. These prolongued levels of GCs will affect both metabolic and emotional situation. There is a mis-regulation of levels of glucose, lipids (see slide), there is an exaust of the energy because of consumption of glucose, proteins; a disregulation of immune system, alteration in patterns of sleep and all this can contribute to depression.

Cushing's syndrome is due to increase of GCs: depression in another hallmark together with propensity to infection due to alteration of immune system and loss of skeletal muscles and so on. Whereas the normal levels of cortisol and GCs are in a normal control, low and equal to the levels of GCs in people with psychiatric disorders that are not depression, in depressed patients the levels of cortisol in blood can be very very high. Even if same patient have normal levels of GCs, they respond in different manner to the *Desamethasone suppression test.* In the first pannel of GCs levels in healthy

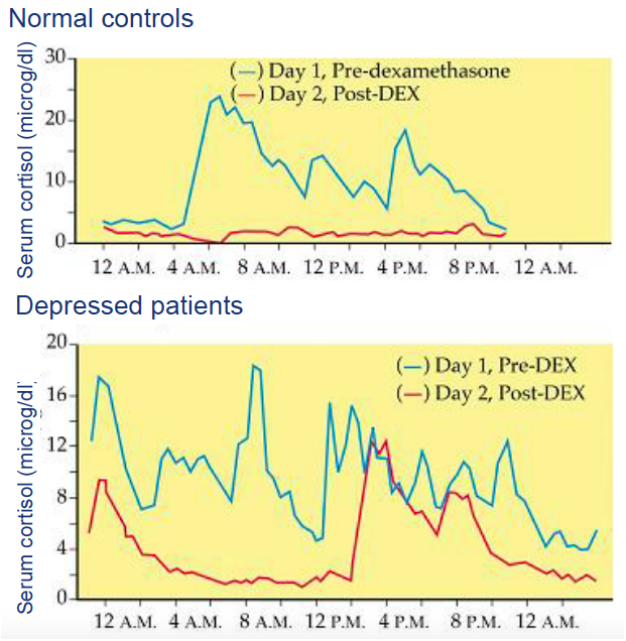

Figure 6.2: Dexamethasone Suppression Test.

person (blue): in healthy persone, somministration of dexamethasone (red line), a synthetic GCs, acts like endogeneous GC in a feedback manner to inhibit further release of GC, so cortisol levels will be lower. It doesn't work in

depressed patients, after DEX there will be the same cortisol pattern during the day.

On the contrary, DEX suppression test doesn't work in depressed patients: irregular levels of GCs and still after DEX (second pannel). DEX has a high affinity for GC receptor.

The central role of hypothalamus

Hypothalamus has a central role in this feedback, in particular PVN nucleus of hypothalamus, which is responsible for the release of CTR (corticotropic-releasing factor). So, added to the normal feedback mechanism due to GCs on PVN and on hippocampus, we can see also fibers from amygdala and hippocampus that regulate positively and negatively the corticotropin-releasing factor. Thos nucelus also receives inputs from the monoamine pathway. Also the release of CTR has a role in the response of stress: apart from regulating the release of ACTH, it has receptors at the level of the brain in different area where it can regulate mood, increased anxiety, increased locomotion, decreased food intake, sexual behaviour and sleep, so by itself it exhert different activities connected to depression.

Most importantly, the level of corticotropin-releasin factor are positively modulated by GC, so GC can increase the level of corticotropin-releaseing factor.

Effects of stressors have also been studied in mouse model, in particular newborn mice: in mice with the normal level of maternal care, indeed, looking at hippocampus, amygdala, pituitary glan, adrenal cortex, we see that serotonin levels in hippocampus are high or normal, as the metabolites of serotonin. The development of hippocampus was normal, so normal neurogenesis. Also the levels of growth factors responsible for development of neural structures, like BDNF or NGF are high. These are coupled with a mood and anxiety: in neglected mice we saw an increase of corticotropin releasing factor.

Hippocampal volume is reduced, development impaired, there is a decrease of level of 5-HT and growth factors and the response of the HPA axis[2] is increased with higher levels of circulating cortisol, and the mood is more towards axious behaviour, with symptomes of depression.

If this kind of experiments stess the role of GCs in the development, as etiological base for depression, it gives hints of inolvement of irregular development of certain circuits, like hippocampus and amigdala: this takes us to the third theory of depression.

[2]Hypothalamic-pituitary-adrenal axis (si pronuncia ipa-axis).

6.2.3 Trophic and neuroplasticity theory

Depression is often associated with reduced levels of neurotrophic factors, mainly BDNF. This can contribute to development of depression and atrophy of hippocampus. This hypothesis is corroborated by fundings showing that therapy with antidepressant drugs can increase the expression of neurotrophic factors in hippocampus. How? With *long-term signal transduction*, that we recalled talking about G-coupled receptors:

- cAMP $\longrightarrow$PKA $\longrightarrow$Pi of CREB $\longrightarrow$transcription factor expression, that can increase the levels of trophic factors
- Inhibitory G-rpotein coupled receptor through the MAPK cascade

In normal condition, there is a balance between trophic factors, monoamines and other neurotrasmitters, like Glu that in depressed patients are at lower levels, and GCs can decrease these levels; a reduction of the synapses: this can be restored by treatment with drugs that are presently in our hands. There are hints also for involvement of other neurotransmitters in the etiology of depression that involves a lot of circuitry.

6.3 Antidepressant drugs

There is a normal alternation of high and low mood in normal people. Mood is also regulated by several genes, so stressful events can decrease the mood. From this condition, we can obtain usually a recovery if the intervention is early: in the case of late intervention it is more difficult to recover.

Treatment phases in unipolar major depression: first of all a treatment for weeks, then months in order to obtain remission and avoid new episodes of depression and go on for 1 or more weeks to obtain the full recovery:

Which drugs do we have to use? There are 3 big categories of drugs:

- Mono amine uptake inhibitors (serotonin, noradrenaline and dopamine), divided into 4 groups:
  - Tryciclics: first generation drugs
  - Selective inhibitors of 5-HT reuptake
  - Atypical
  - Newer
- Mono amine oxidase inhibitors (MAOI)
- Mono amine receptor antagonists

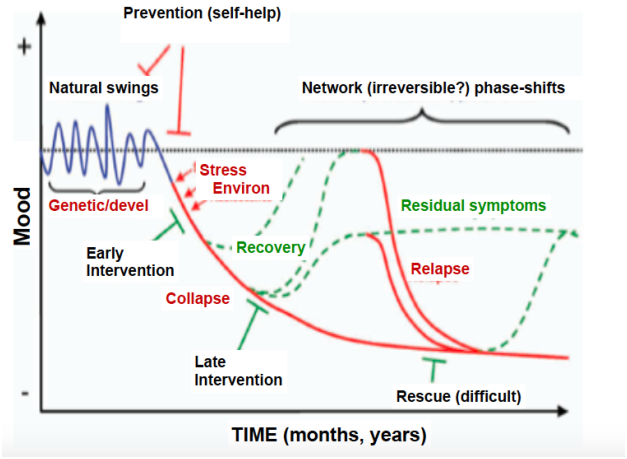

Figure 6.3: The life-cycle of major depression and its treatment.

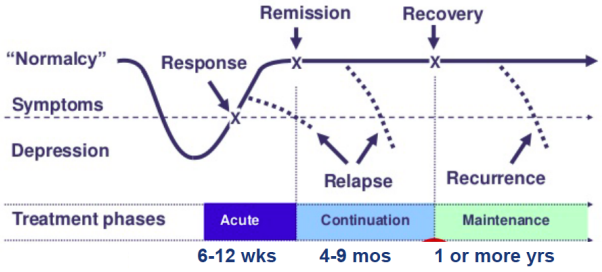

Figure 6.4: Treatment phases in unipolar depression.

6.3.1 Selective 5-HT uptake inhibitors

These are the most used drugs. 5-HT in involved in everything and is responsible of a CNS pathway. The nuclei are in the medulla and pons, in particular in raphe nuclei, and they project all over CNS with descending pathway. They are involved in mood, memory, anger, appetite, circadian control and rhythm, respiratory drive.

5-HT receptors

There are at least 7 subtypes of receptors. They are all GPCRs, with the exception of the subtypes 3, which are ionotropic receptors.

We have 5-HT1 coupled with inhibitory G-protein, gorup 2 coupled with G_q and the other coupled with stimulatoru G-protein G_s. 1, 2 and 3 subtypes are the most studied among these receptos.

Serotoninergic transmision is regulated like noradrenergic or dpaminergic:

precursor, triptophan, can reach the nerve through a specific transporter, then we have hidrylases and 5-HT is packed in vesicles due to the presence of specific transporters and then released by calcium-dependent signal. Once released, it can act on its own postsynaptic or presynaptic receptors $\longrightarrow$off-mechanism, which relies on the presence of transporters that are responsible for the uptake of5-HT that can be again packed in vesicles or metabolized by MAO. In particular, transporters and MAO are the target for 2 types of antidepressant, the selective 5-HT inhibitors and the MAOI.

Signaling of the 5-HT1A receptors Coupled with G inhibition proteins, it can also activated K channels and inhibit voltage-dependent Ca channels. This is also for opioids receptors, muscarinic receptors couple diwth inhibitory G-proteins. These receptors mediate a reduction ofthe excitability. Indeed, the presynaptic 5-HT1A are auto-receptors that are expressed on serotoninergic neurons and reduce the amount of serotonin that is released and that can interact with postsynaptic receptors (1, 2 or 3 subtypes).

There is an increased expression of these presynaptic auto-receptors in depression, with reduction of the postsynaptic $\longrightarrow$if there is an increase of expression of autoceptors, there is a reduced release of serotonin.

This is the premise in order to understand how these inhibitor of 5-HT reuptake works. We should explain also why, in the case of this drug, the therapeutic effect is seen after 2-3 weeks of treatment. The inhibitors of 5-HT reuptake works immediately, so they cause an early increase of 5-HT in the synaptic cleft, and this is probably also connected with increase of synthesis and release of 5-HT. what happens when the levels of 5-HT are increased? 5-HT acts on presynatic receptors reducing the activity of the serotoninergic neurons.

However, with time, there is an adaptative reduction i nthe expression and down-regulation of the serotoninergic autoreceprots, so the inhibitory brake is removes and we have higher levels of 5-HT after 2-3 weeks, that can act on post-synaptic neurons.

Desensitization fist and downregulation after of the presynapti 5-HT autoreceptors are the basis of the efficacy of these drugs, together with stimulation of the post-synaptic HT1 receptors. There is also an increased synthesis and release of neurotrphins which will promote neurogenesis, expecially at hippocampal levels.

Increase of 5-HT is also bound to stimulate the 5-HT2 receptor, and this is responsible for the adverse effect of the inhibitors of serotonine-reuptake. It is still not clear the mechanism by which these inhibitors can enhance the release: there is a blockade of the transport, but also an enhanced release.

Maybe we have an inversion of the direction of transport? It has to be clarifyed which is the mechanism that induces changes in the expression of neurotrophins.

Some examples of these drusg are Prozac and Fluoxetine. Advantages: they are more safe than first generation drugs; however, simultaneous administration of other drugs should be avoid, because it will increase 5-HT at very high levels, causing 5-HT syndrome that can be fatal. We can also treat other conditions like anxiety. The subset eficacy can be explained as anxiety in atients has some biological moment shared, even if physician still tends to separate depression from anxiety. This is kind of clear-cut difference btw these 2 deiseases.

The other possibility is to see that anxiety was treated with BZs, but now we are conscious of the tendendcy to abuse of BZs.

The most important side effect of these drugs are nausea, anorexia, insomnia, sexual disfunciton, aggressive behaviour.

6.3.2 Tricyclics (serotonin/norepinephrine reuptake inhibition)

First generation drug, they can inhibit re-uptake of both 5-HT and NA. Furthermore, they also have affinity for the receptor acting as antagonist. they can block also histamine and muscarinic receptors. We have some examples: Imipramine.

Advantages: they are old drugs, so low cost, they have long clinical history so we know theur behaviour very well, subset efficacy (chronic pain and dyspepsia).

Disadvantages: lethal overdose, sexual dysfunction, weight gain, blockade of AChR like constipation. These antidepressant drugs were used to commit suicide etc. They can interfere with ventricular funciton, in particular with Q period.

6.3.3 Atypical antidepressants

DARIs, dopamine uptake inhibitors (not 5-HT and NA), e.g. bupropion which have efficacy in treat depression and mild side effects, used also for nicotine dependance (more effective).

6.3.4 Newer antidepressants

Bigger group. We have 4 classes:

- Second generation Serotonin Noradrenaline Reuptake Inhibitors
- NaRIs: selective noradrenaline reuptake inhibitors
- NaSSAs: noradreanline re-uptake inhibitor and specific serotoninergic antidepressants
- SARIs: serotonin reuptake inhibitors and receptor antagonists.

There is a precursor aa with its own transporter on the vesicles, then NA is released, can act on its wn receptor post-synaptically or pre-synaptically ($\alpha 2$ receptors, which are autoreceptor), then NA transporter ad monoamino-oxidase that metabolize NA. Transporters have 12 TSM: we have quite similar

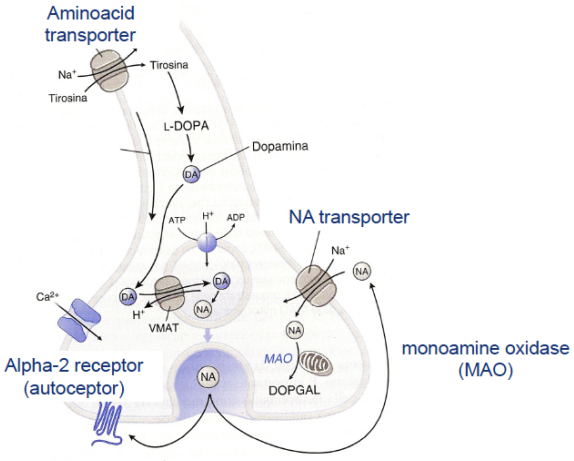

Figure 6.5: Presynaptic regulation of noradrenergic transmission.

transporters for NA, DA and 5-HT. Overlap of ability to transport different substrates, so the specificity is not high. The second generation of NA and 5-HT drugs are inhibiting both transporters.

Second generation SNRIs can reduce anxiety and can be used in bipolar situations. They have different side effects in common with first generation (anticholinergic side effect, increase of serotonin in the periphery, hypertension and tachycardia).

Reboxetine (NaRIs) block NA reuptake, but the efficacy is not very high. This group are more effective than the first generation, but again more safe.

The NaSSAs act by blocking NA A2 auto-receptors, present in the terminal of noradrenergic cells. The antidepressant effect is believed to derive from the ability of these drugs to block the A2 presynaptic NA receptors that are located on those terminals of noradrenergic nerves that make synapses with

serotoninergic nerves. This adrenergic terminal exerts an inhibitory effect on the 5-HT nerves, so by blocking the $\alpha 2$ receptors, this drug reduce the brake so there is more NA released that cannot act anymore on the 5-HT1 receptors, so there is an increased release of 5-HT. These receptors decreases NA release.

The same drug can also act by blocking 5-HT2 or 5-HT3 receptors, that are responsible for the side effects of 5-HT, leaving free the 5-HT1 receptor. The mechanism of these drugs is blocking the brake of 5-HT due to $\alpha 2$ receptors inhibition and antagonist of the other 2 5-HT receptors. Examples: Mirtazapine, Risperidone and Olonzapine.

Advantages: ansyolitic effect due to the blockade of HT2 receptors, low incidence of nausea and vomiting (blockate of HT3 receptors) and fast onset of activation with respect to other antidepressant drugs, because there is a quick stimulation of HT1 receptors.

Disadvantages are sedation for stimulation of presynaptic autoceptors and weight gain.

6.3.5 MAOI (mono amine oxidase inhibitors)

MAO comes into two isoforms: MAO-A and MAO-B. They are intracellular enzymes found notonly in CNS, but also in the intestinal tract.

The selectivity of these 2 groups of MAO: MAO-A has more affinity for 5-HT, whereas MAO-B is more selective for DA, unless affinity of 5-HT and NA. We are more interested in blocking the activity of MAO-A. Now we have drugs that can block with a certain degree of selectivity either MAO-A and MAO-b. Selective inhibitors of MAO-b, like rasagilin, are used for treatment of parkingson's disease, because they increase DA levels. For depression, we have selective inhibitors of MAO-A like Meclobemide and Ladostigil. We also have non-selective inhibitors for both subtypes.

The first MAOI were irreversible and not-selective and had important side effect, the *Cheese reaction*. In the intestine, MAOI are responsible for the metabolism of tyramine. Tyramine is present in red wine and cheese: it has an activity towards adrenergic receptors. Using non-selective and non-reversible MAOI, there is *no metabolism of tyramine*, that can act on adrenergic receptors, giving hypertension crisis. Newer drugs which are reversible inhibitors of MAO-A are less responsible for this Cheese reaction because tyramine can be metabolized by the MAO-B that are present both in intestine and liver, expecially those expressed in liver have 50% of the entire activity. So, inhibit MAO $\longrightarrow$increase of NA. Now we use the reversible MAOI.

They cause also anxiolysis. Disadvantages: we should reduce cheese and red wine and cannot be used with the selective inhibitors of serotonin uptake.

6.3.6 SARIs

SARIs have quick effect on anxiety, they act as antagonist towards the 5-HT2 subtypes, like Nefazodone and Trazodone. Advantaged is that they have quick effect on anxiety. Disadvantage is sedation. Nefaxodone is very effective in reducing anxiety in depressed people with respect to other antidepressant drugs. To stress this, we can see the different symptomes related to diseases of CNS, that are related with different diseases, like depression ,anxiety, schizophrenia, chronic pain or stroke.

The agonist that are selective for 5-HT1 receptors, like esapiron, have also activity as anxiolytic drugs, but their efficacy on depression is still under debate.

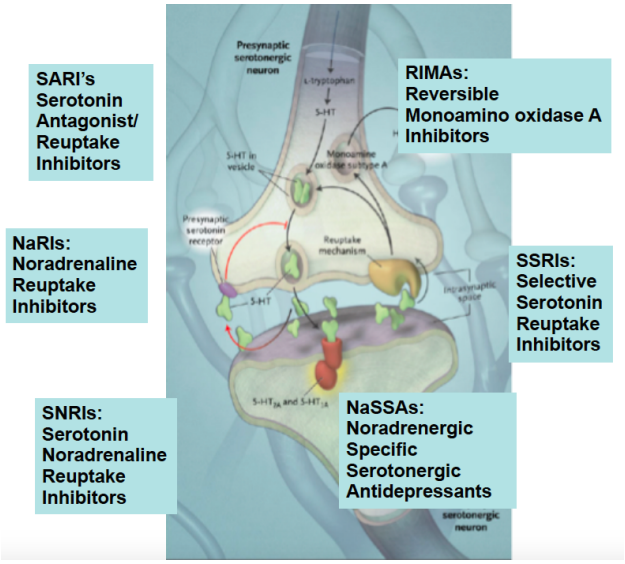

Figure 6.6: Riepilogo

We still don't have a drug or a group of drug that can act on all the patients with depression. The quest goes on and one direction is related to the *MARTA*, multiacting receptor targeted agent. The idea is to have drugs highly selective for one target, we have drugs that can act on more than one substrate. This strategy is now used also for drugs acting on the opioids system and other systems involved in control of pain. It is also an approach for new drugs to treat psychosis, because Multitarget mechanism may be more effective: they are better tolerated, because they are supposed to have

lower degree of side effects, they have complementary components of action and thus a greater chance of controlling both the mood deficits of depression and other symptoms.

Inhibitors of 5-HT reuptake with antagonism towards the A2 receptors are better tollerated that simple 5-HT reuptake inhibitors. These MARTA drugs are build with this principle in mind: putting together together drugs that act only on monoaminergic target and that, at the same time, do not act on target related to the adverse effect, plus some moiety targeted towards different receptors.

Clinical validated drugs, like inhibitors of transporters or antagonist of 5-HT2 receptor plus the antagonist of $\alpha 2$ receptor, or as a complementary target a non-monoaminergic targets. Antagonism of neurokinin can result in reduction of anxiety and pain; antagonist of GABA b receptors are related with depression nd anxiet; melatonin agonist to improve sleep. These strategies can increase efficacy, reduce the onset of action, reduce the adverse effect and are better tolerate.

There are other possible targets that can be included in MARTA agents: GCR, corticotropin-releasing factor CRF, GABA and NMDA or AMPA receptors, nicotinic receptors etc.

6.3.7 Pharmacokinetics

They are absorded, reach therapeutic concentration in few hours, little bit longer for inhibitors of 5-HT uptake, they have significant first pass metabolism, which is inhibited by alcohol. High level of protein binding: this is a problem when more than one pack of drug that binds to plamsa protein are taken together. They cross BBB.

There are variations in the half-time:

Chapter 7

Bipolar disorder

(23.03.2016) We have major depression or unipolar depression. A milded for depression is *bipolar disorder*, in which the mood switch from mild, severe depression to a mild, moderate mania. This shifting in the mood has stil unknown etiology and a totally different pharmacology.

Symptomes of the high phases are mania, emotional and biological stress, very quick way of talking and acting in impulsive manner. Psychiatric symptomes are similar to those of psychosis, then feeling very high, very well.

Mania: feeling very high on life. We see red part with high concentration of glucose.

We use anticonvulsants and lithium carbonate. The atypical antipsicotic are used during an excess in a very high moment to calm down the patient. The therapy goes on with anticonvulsants like Carbamazapine, topiramate etc, that act inhibiting the activity of voltage dependent Na/Ca channels. SOme of them, like topiramate, can act also increasing the activity of $GABA_A$ receptors, reducing the excitability of neurons. lithium carbonate has been for many years the first choice drug for treatment of bipolar disorder: its mechanism of action is inhibition of re-synthesis of inositol-3P. TThere are several molecular target for this ion: it can inhibit also the activity of glycogen synthase kinase (GSK-3α), inducing apopthosis and intracellular pathways that lead to cell damage.

Independently from mechanism of action, Lithium as a neuroprotective effect and can stimulate neurogenesis, so maybe it is protective against neural damages. Block activity of GSK $\longrightarrow$block apoptosis. Lithium carbonate has a very narrow therapeutic window: the patient should be monitored for Li levels in plasma, because of the several adversal effect, in which we have weight gain, nausea, hair loss, hyperparathyroidism confusion, motor impairment. However, Li can be substitued with anticonvulsant, with fewer side effect than Li and can be used also for acute attack.

Chapter 8

Anxyolitic drugs

Anxiety is a normal reaction to stressful situations and it is useful because during anxia we have activation of sympathetic system. Anxiety gives the possibility to recognize the danger and to react, depending on the kind of danger (external or internal). Normal anxiety is an advantage.

Pathological anxiety, like chronic pain, is unuseful: it is an exagerate response to a stimulus (internal or external). Anxiety is divided into 2 groups:

- Conditioned or learnt anxiety: anxiety that starts knowing that we have to face a stressful condition, before an examination for example
- Unconditioned or innate anxiety: reaction to something even for a situation we have never lived before, for example when we are at the edge of a skyscraper or darkness.

This distinction is made on what we believed are the areas involved: for depression and unconditioned anxiety, we have an output from amigdala that receives input from medial/prefrontal cortex $\longrightarrow$activation of autonomic noradrenergic system, whereas for the signal starting from the periacqueductal grey we have a stereotyped behavior, inability to react. Conditioned anxiety seems to involve different nuclei from the amigdala, in particular the inputs from the cortex (thalaus and ipothalamus) reach the amigdala through the basolateral nuclei and from the amigdala, the response is projected to several other regions, like the *frontal cortex*, responsible for choice, knowing what you are going to face, memory of emotional events; *hippocampus*, where memory is stored, responsible for the consolidation of memory of stressful events; *striatum* and other nuclei of amigdala for autonomic and somatic response.

How can we say we are anxious? There a re symptomes due to activation of noradrenergic system: tachicardia, increased sweating, weeping and gastrointestinal disorders. An axious person tells what he feels, so there are

verbal complains, impaired social contact. Anxiety means a lot of things: the disorders are classified in different groups:

- Generalized anxiety disorder GAD
- Phobic anxiety: agoraphobia, fear of animals etc (special phobias) and social phobia
- Panic disorders: acute attack of fear, whereas in GAD panic is almost always present
- Obsessive-compulsive behavior: people cannot refrain to do or think to different things
- Post-traumatic stress disorder

8.1 Causes

Normally, causes of anxiety are stressful situations: at work, for school, personal relationship, financial stress, stress from medical illness etc. These are more or less the same symtpmes that can cause depression. Something in the etiology is in common between anxiety and depression: often these 2 conditions are both present in the patient at the same time. Hippocampus and prefrontal cortex change the expression during life, for example glucocorticoid receptors expression is maximal in adult, while in prefrontal cortex it is maximal in adolescent.

Respiratory illness as asthma, cardiovascular disease, metabolic diseases like hypoglycemia. We have also drugs that can cause anxiety: anphetamines, cocaine, caffeine, sympathomimetics like epinephrine, dopaminergic drugs. Dome drugs like barbiturates, alcohol, oppioids, benzodiazepines can cause anxiety with the withdrawal of the drug.[1]

8.1.1 Anxyolitics

For the relieve of pathological anxiety we can use several drugs: to reduce anxiety without interfere without causing sedation or, when sedation is deepned, with tdrowsiness and decease of reaction time, then increasing the dose we usually have hypnosis, confusion, depression of respiration etc.

[1]Sindrome di astinenza che si manifesta alla sospensione del farmaco: si manifesta con ansia.

- Benzodiazepines: No more the first choice drug because they give dependence, abuse and tolerance.
- Selective serotonin uptake transporter inhibition These are the first drugs now used.
- Agonist of $5 - HT_{1A}$
- 5-HT2A, 2c etc — see slides
- Antagonist of β andreno-receptors Such as propranol, in particular when symtpoms of activation of adrenergic nervous system are prominent.

Other drugs are those with anxyolitic activity like TCAs, antihistaminic agents (because they are present also in medications that can be sold without medical prescription). We are looking for new drugs, like CCK_B receptor antagonist.

Generalized anxiety disorder drugs: these drugs need 2-3 weeks befor starting to be effective. The second line therapy are benzodiazepines, which has a large therapeutic margin.First line therapu for obsessive-compulsive — see slides
disorders are SSRI. — see slides

8.1.2 Benzodiadepines

Acts on $GABA_A$ receptors, they can be used also for other diseases' treatment.

$GABA_A$ receptors are target also of barbiturates, that were used before benzodiazepines to treat anxiety and have a narrow therapeutic window, giving depression of CNS leading to coma and death.

$GABA_A$ receptors are target of drugs used to treat epilepsy, convulsion, anhestetic: these drugs act with non specific mechanism of action, just reducng the excitability of the nerve making the membrane more thick, so that the signal is difficult to be transmitted. $GABA_A$ receptors are expresse din different areas and modulate different behaviours, anxiety, cyrcadia rhythm, congnition, learning, memory etc. The alteration of these receptors is present in different psychiatric disorder. $GABA_A$ receptors belong to the *Cis-loop pentameric superfamily* and they have been identifyed after nicotinic receptors. There are differences in the strucutre of subunits forming the channels: ATP ionotrpic receptor has 2 STM, $GABA_A$ receptors and nicotinic receptor 5, Glutamate receptor has a pin inside and is tetrametic.

Even if each receptor is made of 5 subunit, the number of subunits in higher: we have at least 19 different subunits and the most important one

is the $\alpha(1-6)$, which differ fro aa sequence, then we have β, γ, δ, etc (8 classes). However, there is a restain: not all the subunit can be used to form any kind of receptors. The subunits assemble in a restricted manner. Thesee different receptors made up by different subunits are different for aa sequence, expression, regulation and pharmacology and functions. Most of the $GABA_A$ receptors are composed by alpha, beta and gamma subunits, 2 alpha, 2 beta, 1 gamma or different combinations.

There are different binding sites, not on one subunit but btw 2 subunits as well as the binding site for GABA.

Benzodiazepines act as allosteric positive modulators and increase the affinity of GABA for its own receptor. Since they are modulators, they cannot act in the absence of GABA: this is why they are so safe, because they cannot cause like barbiturates the opening of the channel by themselves. If we have positive allosteric modulator for these receptors, we have foud also compound that act as *negative allosteric modulators*, called *allosteric inverse agonist*, which facilitate or reduce the activity of the endogeneous ligand. β-carbonin were he first compound found that act as inverse agonist, but now we know several benzodiazepines that can anct as agonist, partial agonist and antagonist and inverse agonists.

The binding site for benzodiazepines is recognized also by drugs with a different structure, like zolpidem, an allosteric modulator. $GABA_A$ receptors is made up by different subunits and we know that the subtype of α subunit is responsible for *affinity* and *efficacy* of the drug. Among the total $GABA_A$ receptors, 80% of them can bind diazepam with high affinty.[2] We have a 10% with low affinity and another 10% with no affinity. $GABA_A$ receptors which do not recognize benzodiazepines expresse 4 and 6 subtype of α subunits.

Among igh affinity receptors, 90% can recognize with high affinity zolpidem, 10% do not: the latter express the $\alpha 5$ subunit. The receptors that recongize both have to exress $\alpha 1$, 2 or 3 subunit: in particular, the $\alpha 1$ subunit have a very high affinity for zolpidem. The sbunit is repsonsible for affinity and efficacy, so which is the effect mediate by these different subunits. We can see in different colors the different subunits, from 1 to 3 and 5, because 4 and 6 are insensitive to benzodiazepines. We have differnt functions associated with α subunits: benzodiazepines can be used to treat anxiety because they have anxiolytic effect, but they also mediate sedation and they can induce sleep. They also have anticonvulsant effect, they cause relaxation of skeletal muscles and these are all pharmacological effect that can be used to treat different conditions like insomnia, to reduce anxia, to relax skeletam muscles contracted, to treat epilepsy. Side effects are addition,

[2]Diazepam is the reference ocompound

expression on the GABAergic neurons responsible for break of the release of dopamine. They also cause amnesia, short term amnesia. $\alpha 1$ subunit seems o be responsible for sedation ,anticonvultant activity ans amnesia, whereas the presence of $\alpha 2$ is responsible for the anxiolytic effect leads to muscle relaxation and anxiolysis.

Zolpidem can bind with high affinity to $\alpha 1$ receptor, indeed is more potent as hypnotic, sedative drug with respect to the anxyolitic effect, whereas $\alpha 5$ seems to be related to the amnesic effects, so the receptors with this subunit are highly expressed at the level of the hippocampus. This evidence was the reason why several new compounds have been synthesized with an inverse agonist activity with respect to receptors with $\alpha 5$ subunit in order to act as a memory enhancer.

Antoher interesting thing is to see how different is the expression of the receptors which express δ subunits: these receptors have smaller Cl conductance but the time of opening is longer and they do not desensitize. It is believed that they are also expressed at synaptic level, but are responsible fro modulation of synapses to mediate the *tonic inhibition* of the synapses and not the phasic inhibition, due to the action of GABA on the receptors in the synaptic cleft.

Effects

Some effects are not wanted, like sedation, congitive impairment, ataxia and tolerance/dependence. There is a relationship btw plasma concentration of benzodiazepines and the different effects: with high doses amnesia, sedation at very lower concentation so as anticonvulsion and anxiolytic effect. They can be used or for sedative-hypnotic efect to induce sleep or for anxiolytic effect and this is based on *pharmacokinetic properties*. The drugs that are absorbed very quickly are used to induce the sleep; if the absorption is slower, they can be useful to treat insomnia. Midazolam is udeful to sleep inducer, Diazepam not (20-50 hours), it is good to treat anxiety. With longer half time we have also the effect during the day time, so sedation and cognitive impairment.

Another pharmacokinetic aspect: most of BZs have active metabolites, so the prolongued effect is due not only to the drug itself but also to its metabolites and some of them has an half time greater than the parent compound. Diazepam (valium) has 2 metabolites, *desmethyl-diazepam* and *oxazepam* and this contributes to its long time of action. A lot of BZs have the same metabolites.

These drugs are very safe, but they has to be used for short period, max 2–

4 weeks, otherwise they can give dependence.[3] With respect to barbiturates, they are safer because they are just allosteric modulators and they have additional mechanisms of action altering the turnover of monoamins. Adverse effects: long term use can cause abuse, dependence, cognitive imapiment, motor impairment etc. The risk icreases with age with concomitant alcohol and other drugs that can cause sedation. Drugs that are highly lipid soluble tend to remain longer in our body, so the probability to intefere with other drugs is higher. At high doses, BZDs can give excessive sedation, impaired coordination, confusion and memory loss and tolerance and dependence is often observed.

BZDs dependence mechanism

After a long time they can give withdrawal syndrome, with convulsion (rare). The mechanism by which BZDs can give dependence is due to the inhibition of the inhibitory GABAergic neuron, that normally controls the dopaminergic neuron, so the release of dopamine is reduced. If the inhibition is inhibited, there is an increased release of dopamine. On the GABAergic neuron we have receptors with the $\alpha 1$ subunit, whereas on dopamine neurons we have GABAergic receptor with $\alpha 3$ subunit. If we are able to find BZDs that recpgnise only receptors with $\alpha 3$ subunit but not the $\alpha 1$, we'll have BZDs with no ability to give addiction and tolerance. Flumazenil, a BZD receptor antagonist that can be used to treat overdose of BZDs has a short half life.

BZDs are safe, however they have additive effects with all the other drugs that are depressant of the CNS: when we take other drugs that are sedatives, BZDs should be avoides, as with alcohol. There are drugs that can be sell without ricetta, like antihistaminic drugs, that can act with BZDs to increase the dangerous effects.

[3] With the molecular mechanism saw las time.

Chapter 9

Animal models

For the simylation of psychiatric diseases. They are useful in order to try to reproduce behavioural and physiological features that are indicative of the emotional state, the etiology of the disease and the effect of therapeutic intervention. In a perfect experimental animal model, etiology symptomes and treatment responses should be identical to the human one. This is difficult to obtain!

Most reliable for anxiety are rodents, mostly based on etological observation of the animal, simple to infere emotional changes, whereas is more difficult to find them for depression. Pathological anxiety is mediated by different circuits than normal anxiety: what we can obtain from certain models based on innate anxiety can be not predictive fr the treatment of conditioned anxiety. We have models for the conditioned response, based on the etological observatoin of the animals like exploration, social behavior, stress-induced modifications and miscellaneous observation. Since we need always more models, marble burying or anxiety test battery are useful: the first one is done by putting marbles in the cage of the animal and see it the animal start to cover these marble, see if it makes stressed the animal; the latter one is an oval in which the mice can run and is exposed to a stimulus as the smell of a cat and record how faast the mice run.

Other models are based on conflic test. Conditioned responses implyes the training of the animal: the most common one is the *elevted plus maze*, made by a device which has the frm of a cross, elevated by 16 cm from the floor and 2 of these arms are open and 2 are closed. The animal doesn't need to be trained before and is treated with the substance that we want to study, then put in the middle of the cross. We can observe its behavior based on differencies, for example how long the animal stays in the open space with respect to the closed one: the less it stays in the open part, less anxious they are (usually they are afraid of open spaces, of light, they tend to stay in the

closed part). We can register also the deeppings, how many times the animal puts the nose to look down (curiosity) or an index of anxiety is when the animals try to splat on the wall to hide. We can collect a lot of indications from this test.

We also have the circular plus maze, so we can see the freezing behavor, it does not know where to go. We also have the *open field test*: if the drug is an anti-anxiety one, the animal wil lexplore the open space with less fear and we can measure the distance that it covers. We can also evaluate if the autonomic system is activated or not, counting defecation and urination.

Both these tests have been done in a very controlled environmnent: no noises, no visual clues, everything that can disturb the animal should be avoid.

The *hole board* take advantage of the idea that relaxed mouse will explore more: it can be tricky because if you are not observing by yourself but only looking at the dataa that are connected to the hole in which the animal can put its head and look down, we could also collect the cases in which the tail is in the hole (e il sensore scatta). *Light/dark test*: a box divided into 2 parts, in the middle there is a door: we can measure in 5 minutes sessions how many times the mouse goes in and out by the door, how long it stays in the open space (with light). So mice, so relaxed, tryed to jump out :P

The *social inetraction test* is made wuth 2 animals in the same cage and see how they interact: we can look at freezing (one of the 2 does not move anymore), if the posture is defensive/aggressive, vocalization, jump, aggression etc.

Fear potentiated startle test based on conditioned behavior: to teach an animal that there is a condition that can give anxiety, so we need to train the animal. Associating a feet-shock with a flash of light, the animal pairs the 2 things. We can train the same animal to ear a sudden noise, then put these 2 things together: light the animal without the shock but with the noise, we have an increase of the response (exagerated startle).

More sofisticated models are based on genetically modifyed animal (or obtained by selection).

9.1 Model for depression

A limited number: maybe the most sensitive is the *social dominance model*. There are other extremely simple models, like the *swimming test* in which the animal is in a very high glass maze with water inside (level of water is low so it cannot jump out). We see how long it takes to the animal to stop swimming (stop trying to escape).

One of the most sensitive at all is using instead of a rat the *Tupaia belangeri* (male), that is a rare animal living in the boreal forest, more similar to humans than to rodents, and also its behavior! This is a *psycosocial test model*: we need 2 male animals in 2 different cages near one each other: we see that one will assume a leader role, the other defeat will develop depression. The changes in behavior and organism of the defeated animal are similar to the human ones: increase of glucocorticoids in plasma and urin, reduced sleep etc.

Morris water maze: see how long it takes to the animal to find out a specific place and remember it. The place is a platform in a circular big pool with water, put the animal inside and observe the trajectory until the platform (a place to rest). After 8^{th} the animal remembers where to go.