Neuropharmacology - Nistri

Elisa Nerli

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Contents

Chapter 1

Introduction

Textbooks: Rang and Dale's

I'll be dealing with more general subjects: the organization of the NS, the basic theories to understand the action of the drugs and maybe a brief discussion concerning new methods of research in neuropharmacology, in other words things that have emerged during the last few years and we focus on certain aspects on neuro-active drugs and their effects on neurons. The course we are going to run, Chiara and I, implies that you know physiology and anatomy of the Nervous System. To understand clearly neuropharmacology, you need a basic background knowledge in the other fields. During the course we are going to deal with some aspects of anatomy, histology and physiology of the NS.

So, let us deal with specific subject, and the first point is that when you're dealing with the Nervous System, it's very important to understand that there are 3 major categories or subdivisions:

- Central nervous system
- Autonomic nervous system (ANS)
- Enteric nervous system (which is a complex network of neurons in the gut, ENS)

These three systems are structurally and operationally autonomous, although obviously they have cross-modualtion and interactions either via nerve connections or via neurohormones and circulating factors and so on. If you don't know the ANS by chapter and verse don't come to the exam, because this is something you cannot do without if you want to know neuroscience.

1.1 Peripheral nervous system

The Autonomic Nervous System, is comprised of 2 separate sub-systems:

- Sympathetic system
- Parasympathetic system

They are really elementary yet fundamental processes that regulate all our body activities and prepare us to face emergency situations. So if you have a disease like dis-autonomy of the peripheral or autonomic NS you are not going to live very long, because your blood pressure will be completely wrong, you won't have any effective control over the heart rhythm, respiration, gastrointestinal functions and so on. So the ANS is very important. And traditionally if you read the old books of physiology you would find this rather picturesque description of the ANS preparing the body for fight and fear, so if for example an animal is facing an adversary animal and is preparing to have a big fight against it, then it will use a lot of the ANS, with usually contrasting effects between the parasympathetic and the sympathetic system. So almost any body function you can think of, there are a few exceptions, but in general if the sympathetic system does something, the parasympathetic will do the opposite. So there is always a good balance, a good equilibrium in our bodies to have the normal physiological functions. This is what is technically called homoeostasis. So we must keep a good equilibrium between these functions.

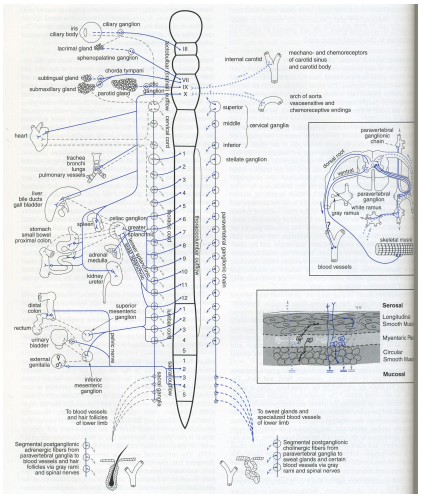

Figure 1.1: Structure of ANS

This is a very schematic cartoon of the lower part of the brain (what is called hindbrain) and the spinal chord. One can see here that there are

various sub-divisions of the hindbrain and particularly the brain stem. Here there is:

- The 3rd nucleus, which is called oculo-motor nucleus (it controls some of the extraocular muscles which move our eyeball)
- Than we have the 7 th cranial motor nucleus which is the facial (it controls the movements of our face, remember that there are 2 major branches of the facial nerve once the nerve goes outside the skull to innervate the various muscles of the face. If somebody has a lesion in the brain, for example a stroke, you may see a droopy lip or a ptosis, which is drop of the eyelid, not so much because there is a lesion of the nerve but because there is a lesion of the motor area or the motor nucleus innervating that particular set of muscles)
- The 9th which is the glossopharyngeal motor-nucleus (innervating the muscles of the throat)
- The 10 th , which is the vagus motor-nucleus or vagus nucleus in general.

T1 vertebra does not correspond to T1 spinal cord: it is a bit undershift. If we look at the red dots, at the level of the thoracic region of the spinal chhord, we'll find clusters of neurons, the *pre-ganglionic neurons* and the first three lombar segment of the lombar region. Doing a coronal section, we'll see a characteristic shape, the butterfly of grey matter with white matter outside. if we look at the grey matter, we identify the dorsal horn and the ventral horn. Looking carefully at this structure, it is arranged in a series of layers, which are 10 and are called *laminae*. Around the central canal, we find the lamina X: that's where we actually find the origin of the sympathetit pre-ganglionic neurons (if we look at thoracic level and in the first 3 lombar segments).

The axons are sent out of the spinal chord and they are called *pre-ganglionic fibers*. What do they do? They travel ad example from T2, leave the spinal chord and go to round bodies, that are *sympathetit ganglia*. The sympathetic ganglia are anatomically arranged in a chain, the *rosary chain*. Whereas some of these pre-ganglionic fibers terminate at the tìlevel of the ganglia, some others actually do not finish there, but they cross the ganglia and continue, going to other ganglia which are located further away from the spinal chord, in the abdomen, like the celiac ganglion.

Pre-ganglionic fiber $\longrightarrow$synapse with post-ganglionic neuron. THe pre-ganglionic neuron decides to fire one or more AP, that will travel until the

end of the fiber (presynaptic terminal), that can do a branching generating multiple synapses. At the branching point, which are critical failure points, if I want to record the electrophysiological response of post-synaptic neurons which is innervated bu a fiber which generates multiple synapse, if we stimulate such fiber, we'll have multiple responses: it may will be failue of the presynaptic spikes at the branching point. If we generate a paradigm to reinforce presynaptic transmission, via *short term plasticity*, for example decreasing the failure probability of the spike at the branch points. How? For example, if we increase extracellular K, depolarize the branches and the signal passes with higher probability.

1.1.1 Synapse

Let assume that there is no failure of spike propagation[1]: the pre-synaptic terminal is filled up with synaptic vesicles containing neurotrasminnter, that in our case is ACh. ACh is synthesized by ChAT (Choline-acetyle transferase): choline is a phosphilipid and acetate comes from the metabolism of cells. This enzyme is a soluble cytoplasmic enzyme: it will be outside the vesicles! We have to push it inside the vesicles: this is achieved by a dedicated transporter which is expressed in the membrane of the vesicles in a very high concentration [5-10 mM]. THis creates a severe biophysical problem, because in solution ACh is weakly positively charged, but if the concentration is high we'll have a high positivity! So, 2 things can happens: ACh will nerver leave the vesicle because of electrical attraction or there is a mechanism to neutralize this positive charge: this is ATP. ATP has 3 Pi molecules, which are negatively charged, that neutralize ACh. When the vesicle releases its content, it will release ACh and ATP.

ACh will be released by the opening of several vesicle, but what is the signal? Calcium, via de opening of voltage gated Ca channels, in particular the *N-type Ca channels* that are responsible for this increase of presynaptic Ca through the activation of SNARE-complex proteins. SNARE proteins are responsible for chaperining and take the vesicles to the terminal, where they fuse and release the content by the process of exocytosis. Then the vesicle is recycled. Ach diffuses through the synaptic cleft and binds to receptors located on the post-ganglionic neuron.

ACh receptors are divided into 2 classes:

- Nicotinic - ionotropic. They are divided into:
  - Muscle type receptors (mAChR)

[1]NOT TRANSMISSION! Spike is all-or-none event!

  - Neuronal type receptors (nAChR), subdivided into:
    - Homomeric
    - heteromeric

- Muscarinic - metabotropic

Nicotinic receptors comprise a huge sub-classification, Muscle type and Neuronal type of receptors. The nAChR are again subdivided into homomeric and heteromeric receptors: the differentiation between these 2 subtypes is due to the composition of the subunits ($\alpha 7$ for the homomeric receptors and $\alpha - \beta$ in the heteromeric). The heterotype is a heteropentameric structure composed by $\alpha 3$ and $\beta 4$ subunits.

Nicotinic AChR

What happens when nicotinic receptors bind Ach? The ions that flows are Na, K and Ca (Ca not very much). The post-synaptic terminal produces an EPSP, a graded response. The amplitude depends on the number of receptors ecc. If EPSP is high enough it reaches the threshold and the neuron generates an AP.

Because the EPSP is graded, it is also called *electrotonic potential*, meaning it is not self-regenerative, it's a local response which will dissipate in terms of electrical signal and depending on the resistance of the membrane. EPSP cannot go all the way to the target organ because it is not self regenerative, but will propagate electrotonically near by the membrane to activate Na channels to reach the threshold.

In more sofisticated experiments, we try to find with the maximal concentration of ACh how large the maximal EPSP is going to be. What are the factors limiting the synaptic transmission? The amplitude of EPSP, if maximal, has a peak of 0 - +10 mV. Why? The reason is the *reversal potential of EPSP* (eReV). EPSP is caused by a flux of ions, expecially Na and K. Each ion will flow according to its Nerst equation, so the reversal potential of Na is about +30 mV, for K is -90 mV, so the amplitude of EPSP can grow until a maximum which is the algebric summation of these 2 potentials (not precisely, but this is the concept).

ACh must be inactivated because if it will persist it will produces a prolongued excitation. In the synaptic cleft there is Achetilcoline esterase which idrolyzes ACh in Acetyl and Choline: choline will be taken back by an uptake system in the pre-synaptic termina. AChE is present in different forms and there are important drugs acting as an inhibior, like the nerve gases (gas nervini) that bind the enzime and they cannot be taken off. Insect

AChEsterasse is different from the human one, so the pesticids act on that enzyme.

AChE is also a target for a lot of diseases, for example muscle diseases characterized by chronic weakness.

The post-ganglionic neuron becomes activated and it will send its signaling to various organs. The transmitter released by the post-ganglionic fiber is not ACh, but is *noradrenaline* or *norepinephrine* (they are the same thing).

1.1.2 Adrenal medulla

Anatomically, the adrenal medulla are 2 small glands on the upper pole of each kidney. They are composed of a very interest structure: a cortical structure with various layers, that is important because that's where fundamental hormones are synthesized and released, like cortisol, aldosterone. Cortisol controls also the glucose metabolism, and is calles also glucocorticosteroid, while aldosteron is called *mineral corticosteroid* because it rules Na/K exchange.

The cortex is non important for the sympathetic system, for which is important the inner component of the adrenal gland. Open it and have the cortical structure with steroid hormones and the inner structure, the adrea medulla which hosts a very special arrange of neurons $\longrightarrow$this was a model for studying synaptic trasmission. Adrenal medulla is innervated by pre-ganglionic fibers of T6 that do not stops at the level of sympathetic ganglia but forms the splanchic nerve. The fibers of the pre-ganglionic sympathetic neurons goes all the way down to reach the adrenal medulla. There there is a particular structure, because histologically the cells of the adrenal medulla derive from the CNS, while embriologically they derive from neural crest, but they are not neurons! They are called *neuro-endocrine cells* and/or *chromaffine cells* because they can be stained with Chromium. shey retain the same functional properties of neurons, because they produce spikes!They have also a lot of synaptic vesicles, do not have processes and they are rounded cells.

When these cells are activated, depolarized by the EPSP produced by the nicotinic receptors, they behave like a post-ganglionic neuron: they have nicotinic receptors, they are a mixture of homomeric and heteromeric subtype, so they reproduce a ganglion. If they are depolarized to threshold, they generate spikes and the spike activate some peculiar Ca channels. These cells are models for studying voltage gated Ca channels, which allow influx of Ca, activation of exocytosis and release of vesicles. But where these vesicles secrete their content? Into the *blood stream*, they secrete their hormone which

is *adrenaline*.

So, the sympathetic system can use noradrenaline for local synaptic trasmission at the target organ or adrealin for long range transmission: adrenaline triggers the reactions to prepare the body to emergencies. Even if noradrenaline and adrenaline are similar in structure, their affinity for adrenoreceptors is not the same.

1.1.3 Heart

At the level of the heart what is the effect of sympathetic nervous system? (including adrenaline and noradrenaline). Let's thonk about the basic organization of the heart: how can the heart originate a regular rhythm. There are pacemaker cells located in particular in the sinus node: they make sure that a rhythmic electrical signal is regularly generated for the rest of our life. What are pacemaker cells? I can distinguish between conditional pacemakers and stable pacemakers. *The pacemaker is a neuronal structure which generates a rhythm without afferent inputs.* If i take the heart off of the chest and provide nutrients and oxigen, the isolated heart keeps beating. The pacemaker possesses intrinsic rhytmicity independent of inputs. Conditional pacemaker can do that but needs a trigger to go and to shut down. This means a lot of energy supply, in particular from Na/K pump.

If we look at the effects of the sympathetic system of the heart, we have to consider 4 effects:

- Inotropic - it concerns the strenght of the muscle contraction in the heart, so how powerful is the heart muscle contraction and the injection flows of the blood
- Chronotropic - it concerns the frequency of the heartbeat
- Dromotropic - more complex, it is important that the atria and ventricles contrac in a coordinated fashion. If this not happens, the heart looses its rhythmic contraction, for examples if the His bundles are damaged. The speed through the bundles of his is the domotropic effect
- Batmotropic - it concernes the ventricular muscle fibers, which have an intrinsic excitability independent from nerve afferences. This intrinsic excitability, expecially in the ventricle, is called batmotropic effect.

When we activate the sympathetic NS, we release noradrenaline from the neurons and adrenaline (from the adrenal gland). The effets can be summarize like this: sympathetic nervous system increases the frequency of

contraction, the force of this contraction, the velocity of conduction in the heart and also the direct excitability of the muscle fibers.

In the ventricles themselves, the fibers of the ventricles are arranged like a *palizzade*, they are parallel: many heart diseases are characterized by disrhythmia because these cells are disarranged. These cells need a lot of glucose, oxigen, nutrients, otherwise we have a heart attack: this metabolic support is done by branches of coronary arteries that branches from aorta. The principle vessels which supply the metabolism of heart fibers run perperdicular to these parallel fibers (not in the atria). If they run perperdicular, whenever the muscle contracts they are squeezed and the blood circulation stops, so the blood supply to the ventricles is *pulsatory*, not continuous but depends on the actual phase of the muscle contraction. That's why I need time to refill the heart muscle with oxigen, nutrients ecc. If the frequency is too high, I don't have such time and I get necrosis of the tissue that leads to an infart. So, the batmotropic effect is very important because increasing the frequency of beating I deprive the heart of oxigen, so supply and demand are not in an equilibrium.

This 4 effects are all positive: the sympathetic NS produces a positive regulation via *G-coupled receptors* which are divided into α and β. Most of the effects of the sympathetic NS on the heart are due to a sub-group of the β-receptors that are the $\beta 1$. We have also $\beta 2$ and $\beta 3$ and also $\alpha 1$ and $\alpha 2$. $\beta 2$ receptors are extra-cardiac receptors and $\beta 3$ are typically involved in sugar metabolism. There is gluconeogenesis, meaning that liver makes glucose from aa, and glycolisis because glicogen deposits are open, glicogen is split and release glucose: this depends on $\beta 3$ receptors.

Adrenoceptors, in particular $\beta 1$, once activated they in turn activate the synthesis of cAMP, that change conductance of Ca channels because the G-protein coupled receptos activates the AC, the memrbane enzyme that catalyzes the activity of cAMP.

A cardioselective β blocker inhibits $\beta 1$ receptor without affactin also $\beta 2$ and $\beta 3$.

(8.03.2016)

Receptors mediating cymphathetic NS

We have α and β receptors. All these receptors are metabotropic and most of the effects are due to stimulation/activation of AC to produce cAMP and modulation f various effects, like Ca and K conductances.

α receptors If we think at the presynaptic nerve terminal concerning the post-ganglionic neuron and its effector system: this is a gross semplification

because the histology of the sympathetic neuron system shows that there are *varicosity* along the nerve fiber, that can detect the vesicles citaining the neurotransmitter (noradrenaline). Anyway, the receptors $\alpha 1$ are believed to be located on the membrane of the post-junctional cell and they bind noradrenaline. We can also envisage a situation where by in addition to noradrenaline we have circulating adrenaline binding to these receptors.

Noradrenaline and Adrenaline are chemically called *cathecolamine* because it contains chemical rings of cathecol: interestingly, the synthetic pathway for making NA and A, (noradrenaline is transformed into adrenaline by the enzyme COMT, cathecol o-methyl transferase), NA is one element of synthetic pathway which produces important neurotransmitter, NA, A and also dopamine. Dopamine is important for the mesolimbic system, for release of ipothelemic hormones etc. The synthesis of DA $\longrightarrow$NA $\longrightarrow$A starts with an aa, *tyrosine*, an essantial aa which in the presence of an enzyme which is normally aggregated, TH (tyrosine hydroxilase), tyr will start the pathway to synthesize these transmitters. Depending on the metabolic and synthetic enzyme assay in a particular neuron, the cell will make only DA or prevalently NA etc. TH is the *rate limiting step* in the synthesis of cathecolamines: in neuroscience, it is also useful because if we want to demonstrate the locaton and distribution of nerve cells and fibers making cathecolamines, we look for this enzyme and we can isualize it by fluorescence etc. If we don't find it, we cannot have DA, NA or A. In some experimental models we want to deplete the concentration of DA, NA etc $\longrightarrow$block TH.

NA is stored into vesicles in relatively high concentration (not as much as Glu or ACh) and by exocytosis Ca dependent will be released into synaptic cleft. THe transmitter will bind to $\alpha 1$ receptors: NA can also bind β receptors, but with a slightly lower affinity. NA after the binding activates the intracellular second messanger and the pathways goes on, but how the effect is terminated? For ACh we had AChEsterase, while in this case the system is different.

It is operating by a system of *uptake*: the concentration of NA is taken up by a transport system and stored inside the presynaptic terminal/varicosity. This transport system is very important: there are 2 subtypes, one with high affinity and one woth low affinity, because we don't want to loose noradrenaline. THis transport system is important and is dependent on the concentration f extracellular Na: it is a *Na-dependent transport system* and also *energy dependent*, so it can also go against gradient. NA is transported back into the pre-ganglionic fiber from where it is released.

Why is the transport system so important? If there is a deficit or a problem with this transport system there will be a persistent high concentration of NA $\longrightarrow$eccessive activation of sympathetic NS. What concerns us most

is that the transport system is a target for mechanism of action of most of drugs, like anfetamines that inhibit the re-uptake of NA. This phenomenon is important als for cocaine, an inhibitor of re-uptake on NA: there is a strong hyperactivity of sympathetic NS and also of NA neurons in the brain. There are also *more useful* application of upake system inhibitors: some *antidepressant* drugs will produce an improve of the behaviour by inhibiting the re-uptake.

revise anatomy

The dopaminergic neurons, the serotoninergic neurons and the noradrenergic neurons in the brain originate from distinct nuclei: 2 nuclei are the major source of projection of NA fibers and SHT fiber to all the brain, the *L.C.* or *locus coeruleus* for the cathecolaminergic neurons, in the midbrain, and *rafe nucleus* for the serotoninergic neurons, also in the midbrain. SHT belongs to the *biogenic amines*. THe rafe nucleus will send its projections to many areas of the brain.

Now we have NA free, not bound because the transporter will discharge NA ino the presynaptic cytoplasm as a freely diffusible substance, and we have the vesicles. 2 things can happen:

- Some NA will go directly into the vesicles, contributing to the refilling, but not very much
- The rest will be inactivated and transformend into an inactive metabolite by MAO, monoamine oxydase. THis metabolite will be escreted by the urines

MAO enzyme is important: on one hand because there was a class of pharmacological inhibitors of MAO and these drugs were also antidepressant, potentially dangerous drugs that leads to major side effects (blood pressure and heart function) and are not used nowadays. This category (MAO enzymes) comprehends subgroups and subtypes: some subtypes are distributed in only some type of neurons in the brain $\longrightarrow$different affinity and kinetics. For example the idea is to block the transformation of DA into NA or, in particular, the disruption of metabolic activation of DA for example in Parkinson's disease. One of the prototypes of these chemical substances is called *deprenyl*.

We have oxidation of NA inside the cytoplasm of the post-ganglionic neuron $\longrightarrow$generation of inactive metabolite + recycling of some NA. The control of the activity of the sympathetic NS is vitally important and we don't want hyperactivity that can lead to hypertension, fibrillation etc. We have to define a series of systems to control and prevent overactivity of SNS: uptake of NA, oxydation of NA and a third system, the $\alpha 2$ *receptors*. Whereas $\alpha 1$ mediates the typical effect of NA and A on the post-junctional membrane,

$\alpha 2$ are foun in the pre-junctional membrane: they are metabotropic receptor and their role is to inhibit too much release of NA.

How do $\alpha 2$ receptor operate? They are a *negative feedback*: when there is too much Nt in the synaptic clefs, it will bind the $\alpha 2$ receptors and have a negative effect. How this negative effect is achieved? By decreasing Ca entrance by *blocking voltage activated Ca channels* via intracellular second messenger activated by the binding of NA to $\alpha 2$ receptors.

What happens with MAO inhibitors? These are potentially dangerous drugs: we potentiathe the SNS effects and we produce a lot of NA released which is non-vescicular. If we block MAO there will be free NA in the presynaptic terminal cytoplasm: some will go into vesicles, the rest is going to leak out the membranes, that will go into the synaptic cleft. This is not the usual release of NA, but a *leakage of NA* through a non-CA dependent mechansim. This NA non vesicular release will not be important for the $\alpha 2$ receptors, since this is a Ca-independent release $\longrightarrow$raising levels of extracellular NA. How is this possible? How can we skip the vesicular secretion? One possibility is *reverse operation of the transport system.* pushing the neurotransmitter out if there is a high concentration in the cytoplasm, since all the transporters are reversible. Another possibility is to be released by the *leak channels*, membrane pores through which certain substances can permeate and pass: connexins, pannexins etc, but there are others. For certain transmitters like Glu and GABA, the nonvescicular release appears to be very important: for GABA there are specialized types of GABA receptors.

β receptors $\beta 1$ receptor is responsible for positive effects on the heart, activating cAMP etc. Outsie the heart, $\beta 3$ receptors metabolic-like are involved in the regulation of sugar metabolism. In the lungs, there are minimal α receptors, only on the blood vessels of the lungs, but there are $\beta 2$ receptors: for many years histologists have searched for the presence of sympathetic fibers along bronchi. We have receptors of smooth muscle cells of trachea and bronchi, but *there is no innervation*! They are very important for physiological and pathological consideration: (physiological) if I'm running and I want to dilate the bronchi so that the air flow higher $\longrightarrow$I need broncodilation and this can be achieved by *circulating adrenaline.* Under stress, the adrenal medulla chromatine cells release adrenaline in the blood stream, adrenaline is a very powerful ligand that will bind to $\beta 2$ receptors on smooth muscle cells of respiratory system, producing broncodilatation. If I have asthma, I'll take some Ventolin inalers in which there is a calibrated dose of a selective $\beta 2$ agonist: it is undesirable to activate $\beta 1$ receptors, so Ventolin administration will deliver a fixed dose of $\beta 2$ agonist. In the leaflet with antiasthme drug,

there is written to not overexcess with the dose, that could lead to side effect of the heart, because the $\beta 2$ receptors will be no more selectively and wil lactivate also $\beta 1$ $\longrightarrow$*loss of selectivity of the subtypes.*

If major allergy attack or acute anaphylapsis, the best treatment is to inject adrenaline, because it will produce the maximum broncodilatation.

We have seen excitation of the heart and inhibition of the lungs, without innervation but via circulating adrenaline: on the gastrointestinal tract, the effect of the sympathetic system is by large also inhibitor $\longrightarrow$relaxation of the smooth circular muscles of the gut, of the small and large instestine. Adrenaline (EPInephrine) and Noradrenaline (NE): both have a very strong

Table 10–2
Comparison of the Effects of Intravenous Infusion of Epinephrine and Norepinephrine in Human Beings[a]*

EFFECT	EPI	NE
Cardiac		
Heart rate	+	–[†]
Stroke volume	++	++
Cardiac output	+++	0,–
Arrhythmias	++++	++++
Coronary blood flow	++	++
Blood pressure		
Systolic arterial	+++	+++
Mean arterial	+	++
Diastolic arterial	+,0,–	++
Mean pulmonary	++	++
Peripheral circulation		
Total peripheral resistance	–	++
Cerebral blood flow	+	0,–
Muscle blood flow	+++	0,–
Cutaneous blood flow	—	—
Renal blood flow	–	–
Splanchnic blood flow	+++	0,+
Metabolic effects		
Oxygen consumption	++	0,+
Blood glucose	+++	0,+
Blood lactic acid	+++	0,+
Eosinopenic response	+	0
Central nervous system		
Respiration	+	+
Subjective sensations	+	+

[a]0.1 to 0.4 μg/kg per minute. [b]*Abbreviations:* Epi, epinephrine; NE, norepinephrine; +, increase; 0, no change; –, decrease; †, after atropine; ‡. After Goldenberg *et al.*, 1950.

Figure 1.2: Table to summarize and compare differences between Adrenaline (EPI) and Noradrenaline (NE)

ability to produce arrhythmias and the *heart rate* is enhanced by A and NA.

The *systolic blood pressure* goes up strongly in both cases: hyperactivity of sympathetic nervous system will kill those who have cardiac disorders. Both NA and A have intense positive effect on the systolic blood pressure. The *diastolic pressure*, the minimum pressure, we found a less dramatic effect: NA ++, A +0- : the reason is that if A vastly increases the blood flow to the large leg muscles, by binding $\beta 2$ receptors and producing dilation of the small arteries, and therefore more flow goes to the muscles, then the general diastolic pressure depends on the general resistance of the circulaitng system, so we may have a high maximum pressure but an unchanged minimum pressure because we are simply diverting blood to the muscles.

Then, we also observe that A, via $\beta 2$ binding inactivation, will visodilate and direct blood flow to the major organs in the abdomen, in particular *liver*, to support metabolism: the combined vasodilation of large muscle and abdominal organs will make no change in minimal pressure. NA has less effect: it will not change a lot large muscle flow because it prefers α receptors.

1.1.4 Blood pressure

What happens to blood pressure when we administer some drugs?

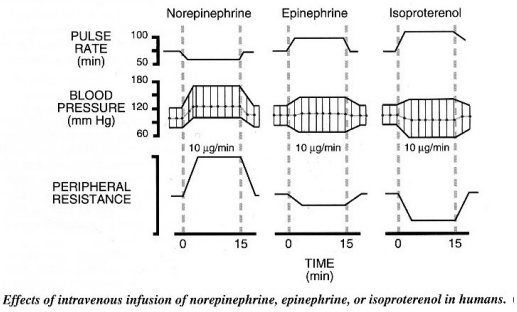

Figure 1.3: Effects of intravenous infusion of NA, A or isoproterenol in humans

The parameters measured in Y axis are *pulse rate*, *blood pressure* and *peripheral resistance*.

Noradrenaline

In the first experiment, NA is administer at the time indicated by the vertical dashed line and we can follow the changes: there is a *gradual* fall in the pulse rate which goes from 75/min down to 60/min. At the same time we have a gradual rising of systolic blood pressure that remains to a steady

plateau throughout the period of administration. The change is diastolic or minimal pressure are indicated by the lower line: the increase is less dramatic than the sistolic pressure one. The man between the values of systolic and diastolic pressure is the average pressure. The bottom record shows changes in peripheral resistance acocmpanied by the observed changes in heart rate and blood pressure: the resistance is largely increased by administration of NA and at the end of the infusion it gradually goes back to the control. Peripheral resistance is measure in arbitrary units, so we don't have real numbers.

We know how $\alpha 1$ receptors mediate constriction of arteries and the sistolic pressure measure in the big arteries: activation of these receptors increases the sistolic blood pressure. There is a mixed effect in the diastolic because there is also some $\beta 2$ vasodilation, but it is important that the peripheral resistance goes up, due to the constriction of the small arteries, the ones just before the capillaries, which are strongly vasoconstricted by $\alpha 1$ receptor activation.

The large rise in blood pressure, expecially the sistoli one, is associated with a drop in pulse rate: this might seem a paradox. This is a *reflexed response*: when blood pressure goes up ($\alpha 1$ receptor mediated) inside large arteries, in particular aortic arch an charotid biforcation, at that level we find 2 categories of *sensory receptors*, not chemically activated receptors, which pick up a signal for the sensory system. These are:

- Chemoreceptors: responsible to sense and pick up how much oxygen and CO_2 there is in the blood, so they will be the terminal.

- Baroreceptors: what we are interested in, they are *pressure transducers* so respond to changes in blood pressure and are a negative control over the risk of high blood pressure. They pick up the large rising sistolic pressure produced by NA in the figure below, signal this to the brain stem, in particular to the NTS region, *nucleus of the tractus solitarius*, a structure important in controlling our blood pressure. NTS has projections to other brain stem nuclei, including the X nucleus which contains the vagus: activation of the vagal fibers will produce bradichardia. This is a self-protecting mechanism via the baroreceptors, which stimulates vagal fibers to produce bradicardia: this is the reason why we see the pulse rate falling from 75 down to 60.

Despite the fact that NA activates $\beta 1$ receptors of the heart, the overall effect of injection of NA is *bradichardia*. If we take an isolated heart and inject NA, we have an increase heart rate. The effect of NA in the obody is *reflex-mediated* by the brain.

Adrenaline

A significantly increases the heart rate and this is primarely due to the high affinity of A for the $\beta 1$ receptors of the heart: from 75 to 90. If we look at blood pressure changes, the effects are different from those of NA: increase of the sistolic blood pressure which is only half of the effect seeing with NA and the diastolic pressure goes down to 60 mmHg. These changes in blood pressure induced by A are readly accounted for by what happens to peripheral resistance: it goes clearly down because the activation of $\beta 2$ receptors at the level of large volontary muscle and intrabdominal organs will divert the blood flow to those tissue, will produce therefore an overall decrease in peripheral resistance $\longrightarrow$diastolic blood pressure will go down because reflex the basal level blood pressure and also the sistolic is not so high because of the vasodilation in this important district which represents the major pool of circulating blood.

Isoproterenol

It is predominantly a synthetic β agonist, also called *isoprenaline*: it is not selective for β subtypes of receptors, so cannot distinguish between $\beta 1$ and $\beta 2$. The effects on the 3 parameters can be summarized by the overall effects of β receptors on the human body. Isoproterenol will produce a large increase of pulse rate, much more the one of NA and A, because there are other effects mixing this, so this is a clear $beta1$ response. The response is not accompained by a large increase on blood pressure because isoproterenol is not acting on α receptor, so the increment in sistolic pressure is probably completely to to the rise of heart rate, but overall it is a relatively small change if compared to the basal level. Because isoproterenol is beta selective agonist, it will generate a fall in diastolic pressure which is even more intense than the one observed with A and the pressure goes to values closer or lower than 60 mmHg $\longrightarrow$due to strong activation of $\beta 2$ receptors because the peripheral resistance is a lot lower than the one seen for A and NA. With adrenaline we had a mixed response: α produces vasocontrsiction, β produces vasodilatation, so the overall vasdilatation wins, but the effect is not so intense. Here we have a pure β mediated response, so a large reduction of peripheral resistance and a strong fall in diastolic pressure which cannot be compensated by the enhancement in the heart rate.

If we look at the time course of the response to isoproterenol, it is clear that the decline of the response is very slow: the return of pulse rate to the basal level will take more minutes than for Na and A, where the return to control level is very fast. Isproterenol is synthetic, so not a substrate for

uptake and MAo oxydation: to eliminate its effects we need time, because the end of the effect is due to diffusion and excretion which are slow processes.

Chapter 2

Parasympathetic system

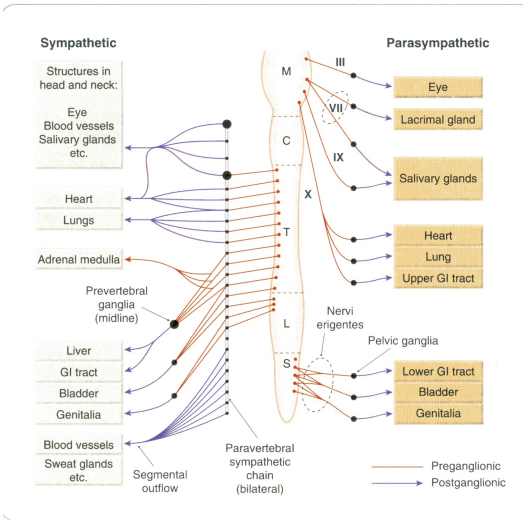

Figure 2.1

It tends to do the pposite than sympathetic one. It originates from 2 distinct area of CNS: *midbrain* and *sacral region of the spinal chord*, the 2 extremes of the CNS. This arrangement means that, as a general rule, these axons drive through a long way to the target organs. In the midbrain, the pre-ganglionic neurons of the parasympathetic system are found in nuclei closely associated with motor nuclei: some of them will control movement of the eye, others will control the movement of various head muscle. For example an important motorn neuron for the movememnt of the joue, other for controlling muscles of the face (VII facial nucleus). These ganglionic neurons are not part of the motor nuclei, becuause they are voluntary!

The motor nuclei controlling the eye bowl voluntary movements are the

III, IV and VI. For the topic of parasympathetic system we are interested in the III motor nucleus, located in the mesencephalon: we find the *Edinger-Westfal nucleus*, the site where there are pre-ganglionic parasympathetic nurons. There are differences btw motorneurons which innervates the extrinsic muscles of the eye eand the motorneurons innervatig face muscles: the motorneurons for extrinsic eye muscles, innervate special muscle fibers which can twitch at very high speed, like no other human muscles. These motorneurons have special muscle fibers and also some special motorneuron proterties as neurons: they are typically *resistant to motorneuron diseases*. Patients with ALS, even at the very later stages of the disease they can move the eyes. However there are other pathologies that affect these neurons.

The pre-ganglionic fibers run to a ganglio, the *ciliary ganglion* near the eye: there is the synapse btw the pre-ganglionic and post-ganglionic neuron of the parasympathetic system. The pharmacological properties are similar: ACh release by pre-ganglionic neurons and acting on post-ganglionic neuronal nicotinic receltor $\alpha 3$ $\beta 4$ subtypes of alpha7 receptors. At the level of postganglioni membrane, ACh will produce an EPSP that, if large enough to reach threshold, will activate Na channels to produce a spike.

(15.03.2016)

The pre-ganglionic neuron send long axons to the ciliary ganglion (bilateral) and there they establish synaptic contacts with the post-ganglionic sympathetic neuron which goes to the eye. THere are 2 major areas of parasympathetic innervation in the eye:

- Ciliary muscle: it is connected up to the lens of the eye through a series of ligaments. It is important to control the *eye accomodation* to focus on near or remote objects. The lens acts as a mobile, flexible compoennt which direct the focus of the object on the retina. BY flattening or bulging the lens, the parasymp system is important to see correctly the objects.

- To adjust how much light is going inside the eye: changing the diameter of the pupil. If we have a constriction, we call id *myosis*; if we are in a dark environment, the dilatation is called *mydriasis*. The parasymp system constrict the pupil to have myosis; there are no fine nerve terminals of the sympathetic system, but there are receptors for adrenaline: if they get very excited, we have mydriasis (like when we are upset) profuced by adrenoreceptors that capture adrenaline released from adrenal medulla.

The eye drop that the doctor put to dilate the pupil are drops of muscarinic R blockers of parasympathetic system.

2.1 Basic pharmacology

When we get to the target organs, ACh is again the neurotransmitter, responsible for myosis and vision accomodation. The effect of ACh is mediated by *muscarinic receptors*, G-protein coupled receptors. The name comes from the effect of the drug, muscarin, a component of some poison of mushrooms, *Amanita muscaria*. They are activated by muscarin and traditionally the antagonist is *atropine*, a substance coming from a plant, *Atropa Belladonna*, which is also a poison if taken at high concentration. These substances have constrasting effects.

The cranial component of PS has a lot of other effects, for example it will stimulate secretion of tears from lacrimal glands and of saliva from salivary glands. If someone is taking an anti-muscarini drug, used against motion system (for sea ache etc), he/she my have dry mouth, because they block secretion of saliva, dry eye etc. It is not rare to see peripheral effects due to block of muscarinic reeptors.

2.1.1 Parasympathetic effects

The origin of pre-ganglionic neurons, in case of cadiac regulation, is in the pons at the level of the *vagal nucleus*. There is also a large cluster of these reganglionic neurons which, via the vagus, goes through the neck all the way down to the heart.

Sinus node cells as pacemaker or bursters: they generate a tightly packed group of cells that produce a series of AP which go on and on and if I measure the distance btw 2 AP it wil lbe the *heart rate*. If we look at the AP generated by cardiac cells, we see a different shape from the AP of a neuron. THe depolarizing phase si the *up-stroke* of the action potential, due to activation of Na conductances; then a *down stroke* due to K conductances (different K channel types), then we have *AHP* (after hyperpolarization), activated by Ca activated K conductance.

put image on the sheet in quaderno

If we look at the cardiac AP, we see that it is very different: there is a *delayed hyperpolarization*, a plateau due to activation of voltage dependent Ca conductances. This AP is long: it is important if I want to contract the muscle, because I need a strong and long depolarization (in the brain I don't need that). We may have a little bit of an AHP, but not like the neuronal spike, then we have to get back to be ready to produce the next AP. The sinus node cells have an intrinsic rhythmicity, so they don't need an afferent input: the isolated heart keeps beating (if it has oxygen, nutrients etc). Do we know why the next spike is produced? How can the burst produce the next spike?

These cells use a current, I_h, a subthreshold current (H indicates that it is activated by hyperpolarization): I_h is due to Na and K conductances, so it has a reversal potential around -40 mV. How can the parasympathetic system affect this physiological activity?

THe vagal branch of the PS acting on the heart has 2 primarly effect:

- Negative chronotropic effect
- Negative ionotropic effect

So, the vagal acivity is mainly an inhibitory one. Heart was used for the first chemical synapses demonstration. Otto Levi experiment: he got 2 isolated frog hearts, which keep beating in vitro for long time. If the dissection is carefully made, we can stimulate electrically the vagus nerve and see that the heart is slowing down in frequency and strength. The took the liqquid from the first heart and put in the second heart (unstimulated) $\longrightarrow$slow down of the second heart also. This substance was ACh.

How in the eye we have an excitatory effect of the parasympathetic system? ACh released by vagal fibers will modulate I_h inhibiting it and the distance between 2 spikes will be longer. That is old stuff! Let assume a sinus node cell: it is possible to do a patch clamp experiment where by the glass microelectrode we break the memrbane of the cell and record directly the electrical activity. If I apply ACh or muscarin in the bath, I will see a response, because these substances will act on muscarinic receptors expressed on the membrane of the cell. These are G-protein coupled receptors $\longrightarrow$cascade $\longrightarrow$blocking of I_h currents $\longrightarrow$inhibition of sinus node cells, mediated by intracellular second messenger. It is possible to do an outside out configuration of patch clamping: we can deep this membrane into various solutions and record from single channel. If we apply ACh into the pipette, it works: we don't need intracellular second mesenger or cytoplasm, but only close vicinity btw the K channels and the receptor for ACh (not I_h), that are activated by ACh and they vastly increase the conductance of the cell, producing inhibition. The interval btw one spike and the next becomes longer and longer and the frequency of heart rate drops: this is a *protein protein interaction*. This interaction produces either inhibitin or activation.

To summarize, the PS effect on the heart is an inhibitory effect due to ACh on muscarinic receptor that activated K conductances via protein-protein interaction to inhibit thesinus cells. The mechanism via which muscarinir receptor activates K conductances is not clear: if you look at the membrane topographically, the memrbane has islands which are called *lipid rafts*, which moves and substances can pass more readly through the membrane and channels and receptor can change their conformation becominc more active. If a

protein can move there quickly, it can produce conformational changes that can also activate other channels.

Atropine will have the opposite effet: if there is a dangerous bradichardia, it is necessary to inject Atropine to inhibit the PS system to raise the heart beat frequence.

There is also an *M-current* that is a subtype of K channels which is selectively inhibited by muscarin: this is interesting in ganglia and brain neurons also. It is one of the mechansism via which neuropeptides can modulate the activity of brain neuron.

The kuscarinic receptors are not all the same, but there are subtypes: M1, M2 and M3. M1 subtype is only and exclusively found in the *stomach*: it facilitates the secretion of gastric juice. M2 is found in few peripheral tissue but most muscarinic receptors in neurons, spinal chord and ganglia are M3. Regardless structural composition, they work with G-protein and express different sensitivity to muscarin (it depends on structural composition).

2.1.2 Effects on gastrointestinal tract

Promote gastric secretion, contraction of the smooth muscle of the gut.

If we activate a lot of $\beta 2$ receptors, the blood pressure can go down. There is not very much effect by parasympathetic system on the vascular tree: the effects are small. What happens is that If we administer an ACh-like agent, we will activate nicotinic receptors on the adrenal glands and activate the release of adrenaline. There is an effect on the bronchi, in which PS via M3 receptors will produce broncocostriction.

In gastrointestinal tract, SS produce an inhibition of motility whereas M3 receptor of PS always incease motility and M1 in particular stimulates gastric acid secretion.

Chapter 3

Molecular pharmacology

Dealing with theories and methods to measure the effect of drugs.

We have to consider the definition of *receptors*: in most cases, we are dealing with proteins on the membrane or in intracellular organelles or in the nucleus and the receptors are proteins which accepts a ligand and produce a response. In general, most drugs will bind to a membrane protein: some of them are receptors. Some drugs can bind to ion channels, for example Cl or K: these are typically not receptors. We also have enzymes, like ACh acting on nicotinic receptors and being rapidly hydrolyzed by AChE, an esterase on which ACh binds but it is not a receptor. We also have *carriers*, as for the transport of NA to limit the duration of the effect of sympathetic transmitter. There are some drugs that can act without receptors, like bicarbonate when we have an indigestion, used to stimulate gastric secretion.

In biology, whether we deal with human or not, there are 2 major cathegoies of responses: graded or all-or-none, and for them we need a different approach. The larger the concentration of the drug, the higher is the response for graded response (reaching a plateau). If I measure AP or mortality etc, I have an all-or-none response. We will focus all the time on *graded responses*.

Another important definition is the *dose* or concentration of a drug, which are not strictly the same thing. Dose is the amount of substance which I administer to a person/isolated tissue/culture and I know exactl how much it is, but I don't know how much will bind to the receptors. The dose is what I star with, the *concentration* is a value that I measure.

If we are using doses and concentration, we have 2 variables: an independent variable (the dose), because I decide how much I want to test, and a dependent variable (the concentration). I will plot the dose on the x axis and the concentration (response) on the y axis. There is another set of definition that we should remember: the binding of a drug to the receptors implies that there is a chemical binding btw these 2entities, but it will not determine if

the binding is successful to start a response or if the drug is an agonist or an antagonist. If we use radio-active compounds, we can label the receptors and measure the interaction, but we don't know the result of the interaction.

Agonist is a *ligand*[1] that binds to a receptor an alterates the propportion of them that are in an active form, resulting in a biological response. Conventional agonists increase the proportion of receptors in the active form: it's a matter of probability.

Antagonist is a drug or a ligand that reduces the action of another drug, typically an agonist. Many antagonists act at the same receptor as the agonist: if the antagonist and the agonist go to compete for the same receptor, is it the type of interaction surmontable or not? Can it be reversed? If I take atropin to block muscarinic receptor, is this block surmontable? Is it atropin blocking that receptor forever?

Modulator is a ligand that increases or decreases the action of an agonist by combining with a distinct (allosteric) site on the receptor macromolecule. So, givevn alone, a modulat has usually no effect, but in the presence of the agonist it can increase or decrease the action of the agonist. An example are modulators of $GABA_A$ receptors like benzodiazepine, increasing response to GABA by an allosteric modulation.

Receptor Cellular macromolecule directly and specifically concerned in chemical signalling between and within cells. Combination od a hormone, neurotransmitter, durg or intracellular messenger with its receptor initiates a change in cell function. The regions of the receptor macromolecule to which endogeneous agonist bind are referred to...

3.1 Radioactive receptor binding technique

Prepare a sample of cells or isolaed membranes which contain the receptor population and the study, like $\beta 1$ receptors and I'm inerested if a new drug can bind these receptors. I'm investigating the interaction btw receptor and drug. THe idea is to take the sample of membranes, apply a small amount of the radio-active label compound, which can be H3 or C14, allow to equilibrate and bind the available receptors, then start apply the unlabelled compound in *serial dilutions* $\longrightarrow$equilibration with the tissue and, after a

[1] Neutral like drug, we don't know the final effect

long time, separate the memrbanes from the rest of the solution (filtration or centrifugation), then use a liquid spectrometer to measure the radioactivity. I want to know whether my "cold" compound has displaced the radioactive compound at what concentration. The receptor binding is *saturable*, the non-

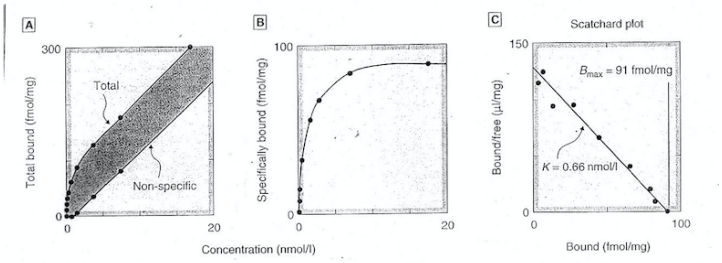

Fig. 2.2 Measurement of receptor binding (β-adrenoceptors in cardiac cell membranes). The ligand was [^{3}H]-cyanopindolol, a derivative of pindolol (see Ch. 11). A Measurements of total and non-specific binding at equilibrium. Non-specific binding is measured in the presence of a saturating concentration of a non-radioactive β-adrenoceptor agonist, which prevents the radioactive ligand from binding to β-adrenoceptors. The difference between the two lines represents specific binding. B Specific binding plotted against concentration. The curve is a rectangular hyperbola (equation 2.5). C Scatchard plot (equation 2.7, p. 16). This gives a straight line from which the binding parameters K and B_{max} can be calculated.

Figure 3.1

specific binding is unsaturabl because there are not receptors involved. If I plot the ligand concentration (the adding of cold compound) agains the total compound, so how much radioactivity I found in the tissue at the end of thee xperiment, I obtain the first plot which comprehends specific and non-specific binding. I havev to subtract by the total response the non-specific binding, to obtain the specific binding. To obtain the value of non-specific binding, I put a high concentration of cold compound (mmol) and I look at hte proportional biding, present into the *excess cold compound*. I obtain the second graph, an hyperbolic funciton, in which concentration of drug is plotted against the specifically bound (subtracted point by point from the first graph). Non specific binding is measured in the presence of a very large concentration of non-radioactive compound which prevents the radioactive ligand from binding to β-adrenoreceptors.

The third graph is the *Scatchard plot* which plots how much radioactive compound I find in my sample (x) vs the ratio of bound/free concentration. So, this is the transformation of the hyperbolic graph. This plot can provide useful information about drugs: it can tell me if I made mistakes if I don't have a straight line, from which i can derive the value of K, which expresses numerically the affinity of the ligand for the receptor. In tis way I can rank the receptor affinity of several drugs. i can also take the intersect of the straight line with the x axis and extrapolate the B_{max} value, that indicates the amount of receptor present in that sample per milligram of proteins.

(22.03.2016)

The second graph (conveerting hyperbolic curve into a straight line) is o extrapolate data in a simply way. It is possible to rearrange data so that we can work with the linear equation:

$$y = ax + b \tag{3.1}$$

where a is the slope and b is the intercept.

The main goal is to linearize the analysis plot and every thing starts with the *law of mass action*, according to which we can consider drug-receptor interaction as a bimolecular interaction in which there is A, the test molecule, and R, the receptor. The combination of A with R will generate a reversible complex RA governed by the law of mass action where by:

$$\frac{[R][A]}{[RA]} = K \tag{3.2}$$

$$y = \frac{[RA]}{[A]} \tag{3.3}$$

$$x = [RA] \tag{3.4}$$

$$y = \frac{1}{K} + \frac{R_t}{K} \tag{3.5}$$

K is an equilibrium constant. the law of mass action implies equilibrium conditions. Substituting 3.3 and 3.4 in eq.3.2, I obtain eq. 3.5.

From this particular plot I can reach the 2 values I'm looking for, K (equilibrium constant) and R_t (the number of available receptors.

3.2 Agonist's action at equilibrium

Remember that with the law of mass action we are dealing with equilibrium conditions: if we are interested in the agonist, I can plor te drug concentration vs the effect (in % of the maximum effect, I_{max}). This must be a saturable response, it goes with receptor's availability.

I can then calculate the IC50. The IC50 wil ltell us the *potency* of the drug in a class of compound, so how much of the drug we need to activate the receptor. For example, IC50 of ACh for nicotinic receptors is smaller than nicotin value. It is not convenient to obtain the IC50 from a curve (see first graph): let's transform the hyperbolic plot in a *sigmoid curve* with $log[drug]$ agains the % of the maximum effect which is still plotted in a linear scale, so this is a *semilog graph*.

The hyperbole becomes sigmoid and there is a plot with a linear part, so we can extrapolate the values from that part. This simple scheme also

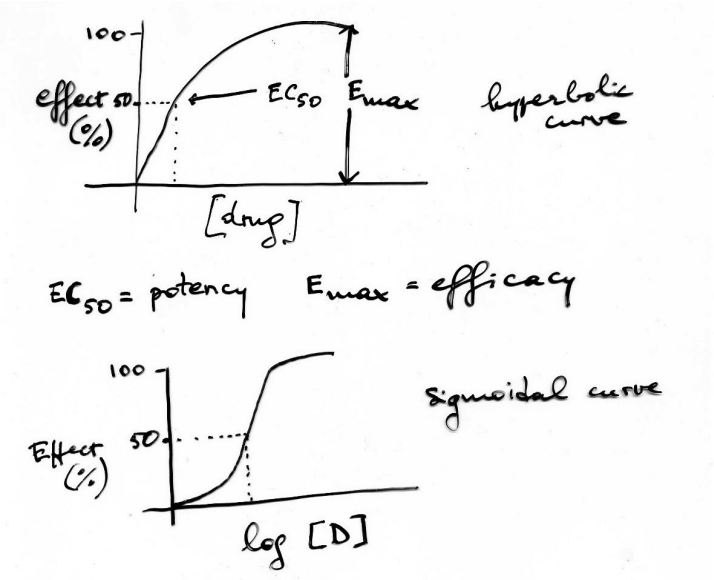

Figure 3.2

introduces the concept of *efficacy*, not synonimous with potency. Potency indicates what concentration of that drug I need to activate the receptor, while the efficacy is a different concept: it means wheter a particular drug can produce or not the maximum response. There are some drugs which, even using very large concentration, will not be able to generate maximum response. These drugs are called *partial agonists*. We can have 2 separate values describing the proterties of a drg: potency and efficacy. If we cannot reach 100% of the effect, we say that the efficacy is less that one and that we are dealing with a partial agonist.

In figure 3.3 we see 2 sigmoidal curves of 2 different drugs: These are real examples: in figure 3.3 we can see some nice sigmoidal curves from 2 different preparations, frog muscle preparation with nicotinic AChR in smooth muscle with 2 different agonists, histamin acting on histamin H1 receptors and ACh acting on AChR. We can detect nice sigmoidal curves with the linear part whih allow us to calculate EC50 values.

3.2.1 Law of mass action

The law of mass action applied to a Bimolecular reaction of constituent A and B which will give C and D at the equilibrium. There are 2 constants, a rate of forward reaction, how fast the reaction proceed from left-right (k_1), and a rate of reverse reaciton, how fast the reaction goes from right-left (k_2), because these are reversible reactions at equilibrium conditions. At

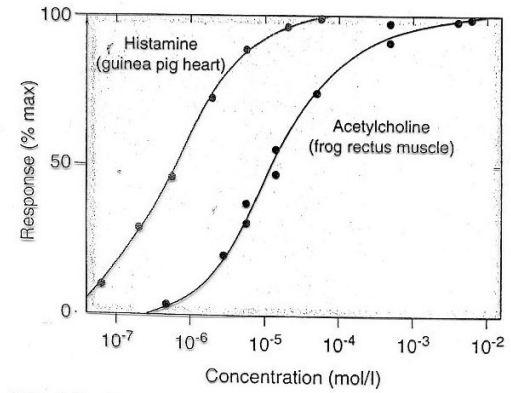

Fig. 2.3 Experimentally observed concentration–effect curves. Though the lines, drawn according to the binding equation 2.5, fit the points well, such curves do not give correct estimates of the affinity of drugs for receptors. This is because the relationship between receptor occupancy and response is usually non-linear.

Figure 3.3

equilibrium conditins, the forward and reverse reactions must be similar, otherwise the raction will go onw way or the other. We can calculate the dissociation constant K, the ratio btw reverse over the forward reaction, $\frac{k_2}{k_1}$.

Let's move to receptors by apply these basic concepts and we can arrive to what is the important point, an equation relating to occupancy. We have potency, efficacy and occupancy. *Occupancy* is the fraction of receptors bound by a given drug. If maximum occupancy, we have bouond all the receptors. The occupancy is somewhat relate to the response of the tissue or the cell, because occupancy is a graded response (larger [drug], larger the effect):

$$\frac{Y}{Y_{\max}} = \frac{x}{x + K_x} \tag{3.6}$$

where K is the ration of the reverse relation over the forward one (k_2/k_1). We use the *Langmuir equation*, or the isoterm of Langmuir, because it is applied to gases when the T is constant. He calculated the binding of a gas to the surface, but we can transport that equation to pharmacology and drug-receptor interaction. His binding of the gas becomes our occupancy which became equal to the fraction of the maximal response.If I use a semilog plot, that's how I get the sigmoidal curve.

impara bene!

3.3 Classical theory of receptors

For our own purpose, we can look at the classical theory: using Langmuir equation etc, this theory implies some importnat points:

- The magnitude of the effect is directly proportional to the number of RA complexes
- The maximum effect occurs when all the receptors are occupied (this might not be true)
- By taking 50% of the maximum response and measuring the IC50, I can say that thas concentration is equivalent to the drug dissociation constant (the ration btw reverse and forward k). If I take 1/K, i get the *affinity constant* for the drug.

Affinity and dissociation constants are distinct and different values. In operational terms, the K_A or dissociation constant is usually referred to the *apparent dissociation constant*, because we cannot measure the drug concentration at the level of the plasma membrane exactly. Then I can write the Langmuir equation:

$$\frac{Y}{Y_{\max}} = \frac{[x]^n}{K_D + [x]^n} \tag{3.7}$$

when n indicates the number of sites of each R molecule to which the drugs bind. Sometimes we need 2 or 3 molecules to activate the receptors, like for AChR or $GABA_A$ receptors, so the equation has been modified for the value n. Doing the log, we obtain the *Hill equation*, which is expressed graphically as the Hill plot and n becomes the *hill coefficient*:

$$log\left(\frac{Y}{Y_{\max}}\right) = nlog[x] - logK_D \tag{3.8}$$

This is a log-log plot, necessary to linearize the equation and to get to something like equation 3.6. Regardless of the log units, we can immediately see that this is a resentation of a straight line equation, where n is the slope and K_D the intercept. We can find out when we need more than 1 molecule to activate the receptor. If I take the effect of the response at 50% of the masimum, the left term becomes 0, so K_D (dissociation constant) become equal to $[x]$.

3.3.1 Hill equation

Hil lequation is a three parameter equation of a non linear relationship btw 2 variables, x and y:

$$y = \frac{y_{max} x^{\alpha}}{C^{\alpha} + x^{\alpha}} \,. \tag{3.9}$$

vedi slides

By substitutin the 2 variables by C and E,

I plot the log[c] on x and % of max effect on y: I can get sigmoids which have the linear part of different slope, even if I get always the maximum effect. Hill coefficient of sigmoidicity (α) will indicate the slope of the curve. In this graph, using the Hill equation, the blue curve corresponds to a $\alpha = 2$ and $EC50 = 1$. If I take the dashed line, I have $\alpha = 1$ and $EC50 = 1$, but the stipness of the reaction is very different comparing the red and blue lines. In the black dotted lines, the $\alpha = 2$ but $EC50 = 5$: changes in the Hill coefficient, even if with drugs of equivalent potency, can produce very different doseresonse curve, so they indicate the sensitivity of the system to changes in drug concentration.

If we get to the Hill plot (log-log), we can extract the K_D and the α.

The kinetics of an enzymatic reaction can be calculated with the M&M equation: the veocity of the control reaction was dependent on the concentration of the substrate. This sigmoidal curve is sigmoidal on linear coordinates! That is what happens when we have a reaction which needs more than 1 molecule of substrate/agonist to activate the receptor/enzyme.

Let's see electrophysological recordings of changes in Vm from a fish electroplaque, interesting for the nicotinic receptors kinetics. Even this is plotted on linear axis: if we do the log, we obtain a stip line, because we have 2 molecules of agonist (if we have only one, the lower kine would be the curve and α would be lower). changes in Vm are expressed as $E - E_0$ mV: this is the difference btw the observe Vm (E) and the reversal potential (E_0), so this is the *driving force*. We can assume that the E_0 for ACh is near 0, that's why the response has been plotted in mV.

In experimental research, we are never sure that this is truely an equilibrium phenomenon, so we can say *steady-state*. These are graded responses and the response in proportional to the concentration of RA complex. Number 9 is considered unvalid nowadays, because we have *receptor cooperativity*.

3.3.2 Competitive antagonist

Dealing with the pharacology of ANS we have a lot of antagonist, beta blockers, muscarinic blockers etc. Let's assume to have an antagonist: in the

TABLE 1.2. *General assumptions in occupation theory*

1. The interaction between a drug and a receptor is bimolecular and readily reversible.
2. A response results from equilibrium or steady-state (pseudo-equilibrium) occupation of receptors. In the presence of a competitive antagonist, there is equilibrium occupation of the receptors by the antagonist.
3. Either a graded response is obtained from each individual cell, or the tissue behaves as a syncytium.
4. A response results from a stimulus that is in turn proportional to the concentration of agonist-receptor complexes.
5. For any given tissue, a stimulus or response is independent of time; thus, the stimulus-response relationship is characteristic of the particular tissue.
6. The concentration of the unbound drug near the receptors can be measured or assumed to be equal to the concentration of unbound drug in the external solution bathing the tissue. Therefore, a negligible amount of drug is taken up by the tissue and receptors.
7. Specific irreversible drugs can inactivate some of the receptors without modifying the stimulus-response relationship.
8. The stimulus to any individual organ is the sum of the total number of agonist-receptor complexes. A maximal stimulus occurs when all of the receptors are occupied.
9. The occupation of one receptor does not affect the tendency of other receptors to be occupied.

Adapted from ref. 22.

Figure 3.4: General assumptions in occupation theory

presence of an agonist and an antagonist, the Langmuir equation is:

$$\frac{E'}{E_{\max}} = \frac{x'}{x' + K_x(1 + \frac{a}{K_a})}\,, \tag{3.10}$$

where K_x is the dissociation constant of the specie x. The competitive antagonist a will occupy the same site of the agonist (substrate), so there is a competition btw the 2 drugs. Without the agonist, the antagonist alone produces no response. In the presence of both antagonist and agonist, the log dose-responses curve is shifted to the right by the $(1 + \frac{a}{Ka})$ factor, but it retains the same slope and maximum. If we take equal responses in presence or absence of the antagonist, respectively E' in the presence of the antagonist and Ein absence, and their size is the same,[2], then I can write the *dose-ratio equation*:

$$\frac{x'}{x} = (1 + \frac{a}{K_a})\,. \tag{3.11}$$

This equaiton enable us to calculate the dissociation constant K_a of the competitive antagonist. If x' is twice as much as x, the ratio is 2, so I can take the negative log of [antagonist] and call it pA_2.

What is the pA_2? It is the negative log of [antagonist] in the presence of which it is necessary to double the [agonist] to produce the same response.[3] It is an importnat parameter that not only can tell me the activity, the potency of an antagonist. How can I get it?

[2]Indicating an equivalent number of active recpetors.

[3]Something similar to pH

With the *Schild plot*, a log-log plot appliable to antagonist, that linearizes what we have said above:

$$log(\frac{E'}{E} - 1) = loga - logK_a\,. \tag{3.12}$$

This plot must have 1 as slope for the antagonist. Let's tr to measure the antagonist of a beta-blocker: isoproterenol ancting on the heart response. Then I take the antagonist (propranolol): I need more isoproterenol, so the sigmoid is shifted to the right, retaining the same slope and the same maximum. If i put more propranolol, I have another shift.

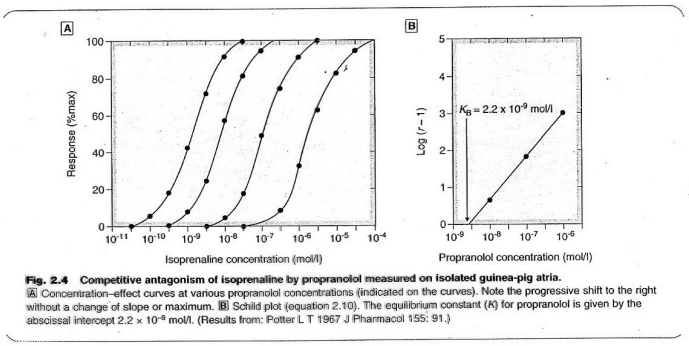

Fig. 2.4 Competitive antagonism of isoprenaline by propranolol measured on isolated guinea-pig atria. A Concentration–effect curves at various propranolol concentrations (indicated on the curves). Note the progressive shift to the right without a change of slope or maximum. B Schild plot (equation 2.10). The equilibrium constant (K) for propranolol is given by the abscissal intercept 2.2 × 10^{-9} mol/l. (Results from: Potter L T 1967 J Pharmacol 155: 91.)

Figure 3.5: General assumptions in occupation theory

I can calculate the pA_2 of propranolol: I take equal responses in control and in presence of antagonist, so I see the dots on the plot and I see which concentration of isoprotrenerol I need to have a certain effect (control and with antagonist) and I can make a plot of $Log(r-1)$[4], against propranolol concentration: the intercept with x axis is K_a.

3.4 Desensitization

Desensitization is a rapid lost of receptor-mediated response during continuous or repeated administration of an agonist. This is a general phenomenons: many receptors, in particularly ionotropic receptors, are very prone to desensitizarion (AMPA, $GABA_A$, Glycine receptors). We can speculate whether it is limiting the duration of synaptic response. The idea behind desensitization is that the receptor, once bound by agonist, it become occupied (RA)and then active (RA*).

[4]Where r is the ratio of the response in presence of antagonist over the response in absence of antagonist

The first record (with microelectrode from single frog NMJ fiber) shows end-plate potential at slow rate. Very quick application of ACh (iontophoresis), then in the second recording, via a second electrode in extracellular space, a little bit of ACh is applied so that the change in Vm is minimal (2-3 mV). During the application of ACh, the aplitude of a single responses to pulses of ACh becomes smaller and smaller. When ACh application is stopped, the small responses return to the intial amplitude, so continuous application of ACh has *desensitized* the receptor so that subsequent pulses of ACh give a much smaller response. If I apply a large, prolongued dose of ACh, the desensitization is long, and responses are very minimal. The agonist is completely inhibiting the agonist affinity.

After desensitization, recovery is very good, even if desensitization was very intense, and this intensity depends on how strongly the receptors are activated, so how large the initial depolarization is. How do we measure desensitization, that is a rate limiting property for many receptor responses?

Let's apply an agonist: increase of the response, reach a peak and falls down. I stop agonist aèplication, the response goes back to base line, then applying the same agonist at the same concentration I see a smaller response that declines to a plateau. I wait some time and I get again the same response that before (baseline). We have a scheme to calculate desensitization to give numerical values: i want to know how fast is desensitization, how strong or intense it is and how long it lasts. I measure the speed at which desensitization develop from the *decline* of the response from the peak to the plateau. I take the time constant of the decline, which can be ms or s: that will tell me the speed at which desensitization is inhibiting the receptor. How intense? I take ratio btw plateau and peak response, because I assume to be under *pseudo-equilibrium*. Finally, how long desensitization lasts? I take the *time to recovery* as index of desensitization, so the time necessary before I can produce the full recovery of the response.

So, 3 parameters: *speed* as time constant of response decline, extent or *intesity*, ratio btw desensitized responses at plateu and peak and finally *duration*, so the time necessary to recovery. This is well exemplifyed by the response of nicotinic receptors to nicotin: fast initial peak (inward current in pA) and during continuous application of nicotin, which is not very long, the response desensitize very quickly to a plateau. I take the time constant of desensitization as the speed of onset of the process, the ratio btw plateau and peak and the time to have a full recovery.

The simplest possible scheme implies that the receptor can bind the agonist and is converted into an active complex, RA* from which it is converted into an inactive complex in which the agonist is bound but the receptor is desensitized, AD. It slowly dissociates into desensitiez receptor and agonist,

and finally we go back to the inital step.

This is a circular scheme of receptor activation and desensitization which has a number of implications: there is a population of receptors which are desensitized but the are not bound to an agonist, and also desensitized receptors bound by 1 or 2 agonist molecules etc. If we use the appropriate rate constant we can reproduce exactly the responses we have seen experimentally.

The transition from active receptor state to desensitize and recoery back to the nrmal state is highly regulated by *intracellular mediators*: the Pi state (by PKC or PKA) of intracellular domains of nicotinic AChR will facilitate the returning to restng state, while de-Pi by calcineurin wil lhelp to remain in a desensitized state. because these are Ca-dependent, desensitization is a *modular process.*