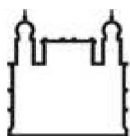




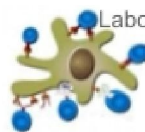
PHARMAVIRTUA



Ministério da Saúde

FIOCRUZ

Fundação Oswaldo Cruz

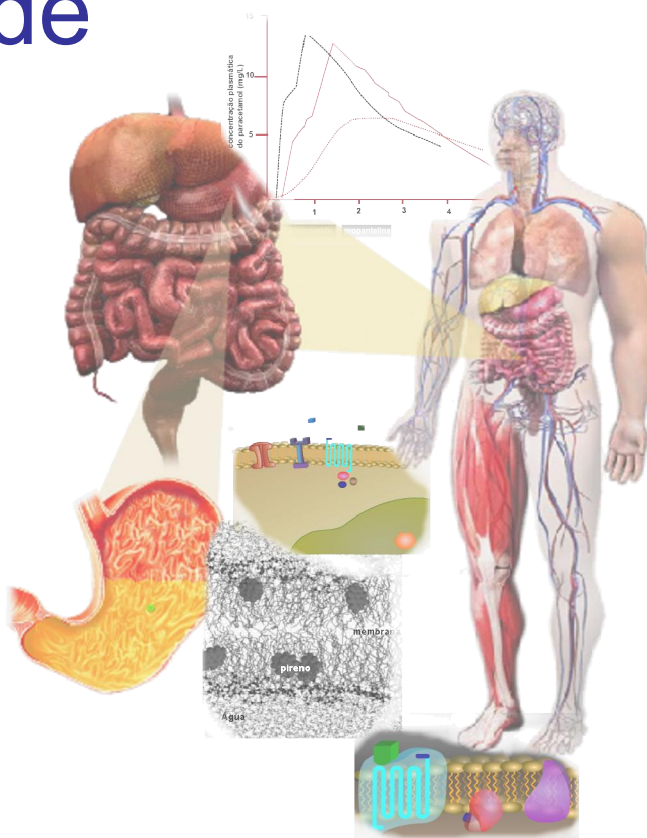


Laboratório de Comunicação Celular

Instituto Oswaldo Cruz - IOC
FIOCRUZ

PHARMAVIRTUA

Contents and Software Guide



Todos os direitos reservados

Pharmavirtua - Ensino virtual de Farmacologia de Fidalgo-Neto; Berçot, FF; Alves, LAA
é licenciado sob uma licença Creative Commons Atribuição-Uso não-comercial-Vedada
a criação de obras derivadas 3.0 Unported



Presentation

For a long time our civilization has made use of herbs, natural products and their extracts both for medicinal and poisonous means. The compounds used in ancient times had therapeutic effects based only on traditional knowledge, however no trace of scientific study was produced to substantiate their use.

Pharmacology was firstly recognized as a formal discipline in the University of Dorpat in 1847 by Rudolph Buchheim. The great progress on the development of organic chemistry synthesis, biochemistry and physiology, significantly contributed to consolidate the practice and the theory of pharmacology.

Pharmacology comprehends the origin, physicochemical, pharmacokinetic and pharmacodynamics properties of compounds as well as its therapeutic utilizations.

No drug is free from undesirable effects, for that reason, its use must be based on solid science. Within the academic environment, pharmacology is supported by different disciplines which make it much complex. In addition, many problems caused by the low quality teaching of the discipline are frequently reported in Brazil and are observed in the day-by-day life like prescriptions errors, side effects among others.

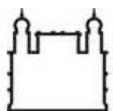
PHARMAVIRTUA attempts to contribute to pharmacology teaching using a hypermedia software format. The software contains the basic concepts of pharmacology, making use of resources such as animations, simulations and exercises.

This is a TEST version developed for practical use during the VACATION COURSE OF THE INSTITUTE OSWALDO CRUZ, FIOCRUZ – 2011 realized by the “Laboratório de Comunicação Celular” – IOC during a week in the month of January.

We hope that PHARMAVIRTUA can provide teachers with an auxiliary tool for the teaching of this crucial discipline on biological sciences as its counterparts.

THIS IS A BETA (TEST) VERSION CURRENTLY BEING UPDATED

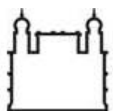
In case of interest, contact us in order to update your current version.





Index

Pharmavirtua	4
Administration Pathways	5
Oral	7
Intravenous	8
Intramuscular	9
Intradermic e subcutaneous	Erro! Indicador não definido.
Inhalative	Erro! Indicador não definido.
Rectal	10
Sublingual	10
Pharmacokinetic	<i>Erro! Indicador não definido.</i>
Absorption	11
Bioavailability	12
Transport	15
Fick's Law	Erro! Indicador não definido.
Liposolubility	Erro! Indicador não definido.
Ionization of acids and bases	Erro! Indicador não definido.
Distribution	22
Biotransformation	23
Elimination	23
Pharmacodynamic	<i>Erro! Indicador não definido.</i>
Receptors	25
Signalization	29
Dose x Response	34
Calculations in Pharmacology	36
Quiz	37
Bibliography	<i>Erro! Indicador não definido.</i>
Help	<i>Erro! Indicador não definido.</i>
Contact	39
Credits	40





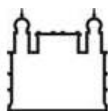
Pharmavirtua

There are several historical records about the benign and poisonous effects of plants and animals by ancient people in China and Egypt before the Christian era (Scheindlin 2001; Sousa 1981). In times where ancient knowledge was the predominant philosophy, pharmacology appeared but only emerged as a science later in recent times, beginning when men attempted to explain biological phenomenon from experimentation and observation. At the end of the 18th century and at the beginning of the 19th century, François Magendie and later his student Claude Bernard developed a series of experimental physiology and pharmacology methods that promote advances in this science. From this period on, following advances in chemistry, biochemistry and molecular biology, pharmacology had developed itself in a way that allowed the study of molecular interaction between drugs and its receptors, its possible effects, and the ability to predict other effects not so desirable (Katzung 2006; Brunton, Lazo, and Parker 2006; Scheindlin 2001).

In its totality, pharmacology comprehends the history, origin, physical-chemical properties of compounds, the biochemical and physiological effects, action and kinetic mechanisms, therapeutics and the drug's use. (Brunton, Lazo, and Parker 2006). A drug can be defined as any chemical agent affecting live organisms physiology (Brunton, Laz and Parker 2006).

Pharmacology is a crucial discipline offered during the professional formation of doctors, nurses, pharmaceutics, nutritionists, physiotherapists and other areas o health sciences, for that reason presents a direct impact on society. International literature presents various signs that appoint to the necessity of revision in the practices of pharmacology teaching (British Pharmacological Society and Physiological Society 2006; Kwan 2002; Faingold and Dunaway 2002; Kwan 2004; Hughes 2003), given the quantities of articles reporting fallacies in the administration of anesthetics (Wheeler and Wheeler 2005), error in the use of medications (Wheeler et al. 2004), errors of prescription (Castilho, Paixão, and Perini 1999; Battellino and Bennun 1993) among many others. In 2005 a study in the School of Medicine in São Paulo revealed that the majority of the adverse effects on patients were due to wrong drugs prescriptions. (Passarelli, Jacob-Filho, and Figueiras 2005).

In this guide, we present a new approach on the contents of pharmacology organized in a hypermedia software format, Pharmavirtua.





Administration routes

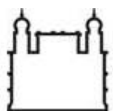
The way in which medication is administered is called [formulation or presentation](#). The available formulations when administered present different drug release times, differences in absorption and consequently differences in its effects. Liquid formulations can generally be absorbed without prior preparations, which then results into much faster effects, compared with the forms where disintegration is the limiting factor. For that reason, the incorrect utilization of particular forms can result in potential damage to the patient.

Forms are prepared with one or more active ingredients and its [excipients](#), which may have several functions such as thickening agents, lubricants, binding agents, preservatives and antioxidants.

Generally drugs are used for topical or systemic applications. The routes are classified as parental or enteral. In the enteral routes drugs are absorbed through the digestive tract. Some examples are the oral, sublingual and rectal pathways. On the other hand, the parental pathways, like intravenous, subcutaneous, intramuscular, inhalative, to name a few, do not present absorption throughout the digestive tract.

Each of the different administration's routes possesses particular advantages and disadvantages that must be considered in different situations. This will guarantee that the therapeutical objectives and the patient's safety are achieved. The rectal route is preferentially used in children not yet capable of swallowing. On the other hand, the oral route is widely used due to its safety, ease and low costs (drug production as well as the techniques involved in the administration).

From the instant a drug is administered to the real observation of an effect, the active product may overcome many steps that can be grouped in three phases: [biopharmaceutical](#), pharmacokinetic and pharmacodynamic. In the biopharmaceutical, the active product is made available to the organism before its absorption.





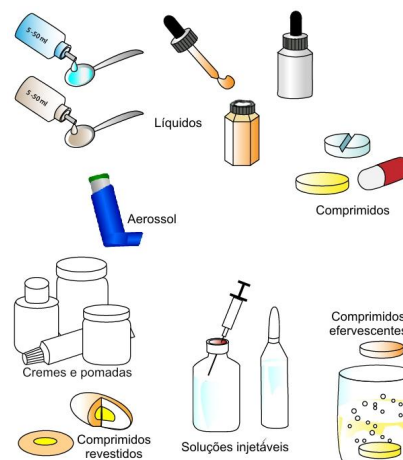
Formulations

The formulations can be subdivided according to their physical nature in liquid formulations, semi-solid and solid. Liquid formulations include suspensions solutions and emulsions. Creams, ointments and gels are considered as semi-solid formulations. In contrast, tablets, capsules, tablets, among many others, are considered solid

The figure shown in the right side presents some formulations:

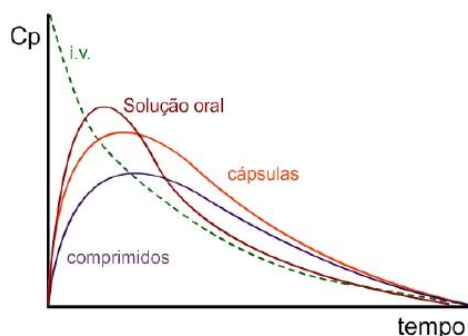
Below are some examples of excipients used in various ways.

Dose:
Adjuvants
Aglutinants
Buffers
Dyes
Diluents
Flavourings
Saporificantes
Disintegrants
Lubricants
Conservants
Surfactants
Emulsifiers
Suspendentes



Biopharmaceutic phase

In this context we can mention the drug release which occurs in extravascular administration of a solid form. The release can occur quickly as the case of quick-release forms, or slowly in the slow-release forms. In practical terms is the disintegration of the solid form, then the breakdown into small particles and the subsequent dissolution. To cross biological membranes the active principle must be dispersed in its molecular state in aqueous media at the site of absorption. The dissolution rate depends on their physicochemical characteristics and medium pH at the site of absorption. The pharmacokinetics and pharmacodynamics steps will be discussed elsewhere in this work.



In this graph we can observe the relationship of plasma concentration versus administration time of any drug through an IV route and compare it with plasma concentrations of the liquid forms, capsules and tablets. Note that the liquid form, not to depend on release nor dissolution, it is readily absorbed, resulting in higher absorption rate and higher peak plasma concentration. It is worth to stress that in this example, the capsule has a release time smaller than the tablet, however, given the wide variety of materials for the preparation of capsules, large variations can be observed in this way. **(Click on the graph to see the curves of concentration versus time).**



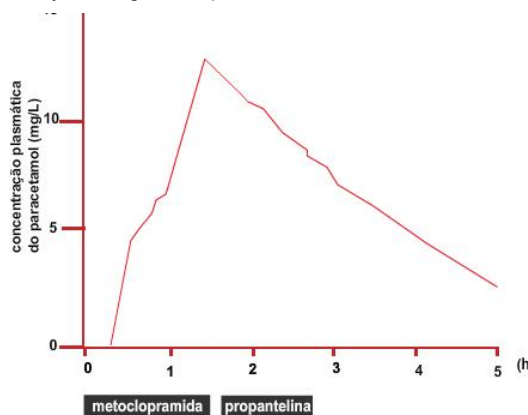
Oral

The oral route is frequently used as the method of delivering medications. It's also considered one of the most convenient pathways due to its both safety and low cost. However, there are disadvantages, such as the administration in non cooperative patients (patients with psychiatric disorders, children and elderly). Additionally, polar drugs are not absorbed through this pathway. Other disadvantages include vomiting resulting from gastric irritation - [irregularities in the absorption due to the presence of food or other pharmaceuticals](#) - or even due to a [pre-systemic metabolism](#), which then results in low plasmatic concentrations of the drug.

Absorption through the oral route is dependent on many factors (physical-chemical and anatomical-physiological). Considering that most of absorption events occur through passive mechanisms, drugs ionization dependent on environmental pH is also an interfering factor. So, it is expected that acidic drugs are best absorbed through the gastric tissue and contrarily, the alkaline ones, throughout the small intestine. Drugs that are degraded by the stomach extremely acidic pH or those that are potentially irritable can be coated with resistant components to this environment.

Factors involved in oral administration that can interfere with absorption.

As already mentioned, the oral administration route is subject to many variables which can result in significant differences in both the speed and the amount of drugs to be absorbed. The concomitant administration of drugs with food can cause changes in the stomach pH for example. The graph illustrates the results of a classic experiment in humans showing the influence of the gastrointestinal tract motility on drugs absorption.



The graph shows the C_p vs. time curve after oral administration of 750 mg of paracetamol in a 22 years old volunteer. The experiment's proposal was to observe the influence of the administration of 30 mg propantheline iv on paracetamol plasma levels as well as the administration of metoclopramide 10 mg iv on the same conditions (Br Med J 1:587-588, 1973).

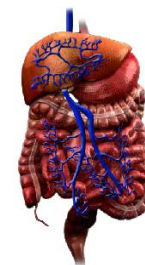


Metoclopramide is a drug used in the treatment of gastrointestinal motility disorders. It is a dopamine blocker, antiemetic and peristalsis stimulator.

Propantheline is an antimuscarinic agent used to treat excessive sweating (hyperhidrosis), stomach cramps or spasms and involuntary urination (enuresis). It can also be used to control the symptoms of irritable bowel syndrome and similar conditions.

Presystemic metabolism

A systemic action is expected to most orally administered drugs. Considering that drugs administered by this route are absorbed throughout the gastrointestinal tract, and invariably pass through the liver via the portal hepatic vein, there is often a significant reduction in the fraction that actually gets to reach the circulation. This phenomenon is termed as pre-systemic elimination or as first-pass effect. This event can substantially reduce the bioavailability of orally administered drugs and thus reduce the clinical response. Because of its big metabolic capacity, the liver is commonly related to the first-pass effect; however, others such as the intestines are also involved in this type of elimination.



Intravenous

In this route of administration drugs are directly administered into the bloodstream and absorption does not occur because of the diverted organism's natural barriers. This guarantees potentially immediate effects characteristic of this route. Only drugs in aqueous solutions can be administered i.v. The oily preparations, those that precipitate, or even the ones that cause hemolysis should not be administered through this route.

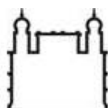
Moreover, irritating drugs can be slowly administered thanks to the relative insensibility of the vessel's walls.

When a single dose of a drug is administered through an intravenous injection it is called as an injection in [bolus](#). Another form of intravenous injection is known as [infusion](#).

Via bolus and intravenous infusion

The main method of intravenous administrations are intravenous injection (iv) and infusion. When a single dose of a drug is intravenously administered to a patient, the dose usually is called bolus iv. The intravascular administration delivers the drug directly into the circulation. When a drug enters the circulation it is rapidly distributed to most tissues because it quickly reaches a dynamic equilibrium between the drug, blood and tissues. This means that a rapid bolus iv injection almost immediately provides an initial high plasma concentration but will begin to fall rapidly due to the processes of elimination.

In contrast, the plasma concentration of a drug administered by intravenous infusion will increase with time until the infusion rate equals the rate of elimination. At this point, the plasma concentration remains constant until the infusion is finished. Infusion has the great advantage because precise plasma concentrations can be maintained over time.





Intramuscular

The muscular route uses skeletal muscle tissue as the absorption site. Several places can be used to apply intramuscular injections, for example, the deltoid muscle in the superior part of the arm, the lateral vast, and the anterolateral vast on the surface of the thighs and on the buttocks. The lateral vast on the anterolateral surface of the thighs is the preferred location for infants and small children, since the buttock's muscles are not yet fully developed. In any situation, the precautionary step of aspirating the needle before the injection is indispensable; to make sure no vessel is hit so that the injection is actually executed.

Generally, in intramuscular injections are used 1-3 inches long needles. The caliber is usually based on the viscosity of the administered material.

Liquid preparations offer rapid absorption rates, contrarily to its oily counterpart. Absorption can vary according to the blood's flow in each patient. Obese or underweight patients can present altered patterns of absorption through the intramuscular route.

Intradermic and Subcutaneous Routes

The intradermic route is restricted mainly to cutaneous tests. The needle is inserted in an almost parallel angle to the skin for drug direct deliver into the dermis. When administered correctly, a small bubble can be observed on the skin surface as a result of the stored intradermic material. The internal arm or back surface are the areas generally used for cutaneous tests and therefore for the intradermic route.

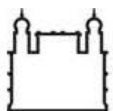
In the subcutaneous administration route the drug is injected below the skin, inside the fat tissue in the connective tissue bellow the dermis. A 45 degrees angle is used; however, it can vary depending on the needle's length. Not more than 2mL can be administered in this route.

After a careful cleaning of the spot in which the needle will be inserted, the skin is lightly pressured, elevated, and pulled away from the muscle. The needle is inserted and the skin is released. Precautionary action must be taken to ensure that no vessel will be hit so that the injection is actually executed.

Inhalative

All volatile gases and drugs can be administrated through pulmonary or inhalative route. Additionally, drugs can be atomized and its tiny drop suspended (aerosol) and inhaled.

Alveoli present a vast vascularized area and due to this characteristic, absorption through this route is fast and virtually free of pre-systemic elimination (first liver passage effect). However, it may present the disadvantage of low dosage precision and vulnerability to irritating drugs.





Patients with lungs deficiency can receive the drug directly into their lungs, like asthmatic or patients baring any other respiratory disorder.

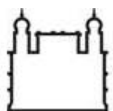
Even though the inhalative route is considered systemic where drugs can rapidly be absorbed, some present essentially a topic activity. This is the case of the bronchodilators administrated through this route whose activity is located on this area with little or no absorption. Drugs produced with this objective present some special aspects like the size of the particles, ensuring a topic effect with little absorption.

Rectal

Approximately half of the administrated rectal route dose is absorbed directly into the systemic circulation. Usually, rectal absorption is not regular and complete, and many drugs can irritate the rectal mucosa, on the other hand, in some cases, it may be rapidly absorbed resulting in a fast onset of effect. Compared to the oral route the rectal route has the advantage of not being influenced by the vomit factor also it can be a solution for those cases in which patient has loss of conscience or a smaller first passage hepatic metabolism.

Sublingual

Despite the small available surface area for absorption, the use of the oral mucosa is particularly important for certain drugs. For example, nitroglycerin is effective when administred sublingually because it does not presents itself in the ionized form, being highly soluble in lipids. Thus, it can be absorbed promptly. Nitroglycerin is also very powerful; relatively few molecules need to be absorbed to obtain a therapeutic effect. As the venous drainage of the mouth is made through the superior cava vena, the drug is also protected from the rapid first pass metabolism in the liver. The first-pass hepatic metabolism is sufficient to prevent the appearance of any active nitroglycerin in the systemic circulation when the conventional tablet is swallowed.





Pharmacokinetics

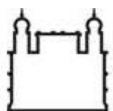
In resumed way pharmacology studies the therapeutic and toxic effects of drugs on organisms, especially man. For this reason it is necessary to determine with maximal precision and detail as possible the way drugs will interact with the body so that the effects are produced. However the effect does not depend only on the drugs and receptors interactions, but also on a set of factors intrinsic to the organism itself.

This is the basis of pharmacokinetic phase also defined as the way the organism works over the drug. Pharmacokinetic comprehends the absorption, distribution and elimination of drugs and study how drugs can reach systemic circulation, its distribution through body's different compartments when metabolized and how they are finally eliminated. The central feature in pharmacokinetic is the establishment of the association of a drug's plasma concentrations over time. The integration of the knowledge obtained in the interactions of drugs and their receptors (pharmacodynamics) and their kinetics in the body determines the drug's final effect which can be desired (therapeutic) or adverse (toxic).

Absorption

For drugs to reach their action sites, it is important for the most part to get into the bloodstream. Once in circulation, drugs can virtually reach all organic compartments. For this, several tissues and / or structures must be crossed such as the gastrointestinal mucosa in the case of orally administered drugs, the alveolar tissues in inhaled drugs, skeletal muscle tissue in intramuscular route among others. Even from a wide variety of tissues and / or structures, the arrival of a drug into the bloodstream depends on its ability to cross biological membranes, common in all absorption sites. The ease with which a substance crosses the membrane is critical to determine the speed and extent of absorption and distribution on multiple organic compartments.

In some cases, to observe an effect it is not necessary for the drug to reach the bloodstream, for example, orally administered drugs for the treatment of infections in the tract itself, reducing gastric or other drugs topically administered. However, as already mentioned, in most cases drugs need to be absorbed in order to be effective. In addition to absorption, other pharmacokinetic phases also depend on the drug's passage through biomembranes, like distribution and elimination.





To study the absorption or, more generally, the passage of drugs to several compartments it is useful, therefore to discern some biological and physico-chemical properties of biomembranes as well as drugs related ones. The biological membrane is composed of a bilayer of amphipathic lipids with their hydrocarbon chains forming a continuous hydrophobic phase, and a polar region externally to the bilayer. The phospholipids in the bilayer can move laterally, which results in great fluidity and flexibility. In addition, membranes have large electrical resistance and relative impermeability to highly polar compounds with the exception of water, which passively crosses the bilayer. Similarly, those lipophilic drugs will cross more easily cellular barriers resulting in more efficient absorption processes. On the other hand, most drugs have some affinity with water; otherwise it would not be transported by blood or other fluids to their sites of action. The size and space shape, the solubility, the degree of ionization are important features of a drug to define its potential permeability through membranes.

Therefore, absorption describes the rate at which a drug leaves its administration site and the degree by which it should occur. Many biological and physical-chemical are involved in this process; however, it is possible to simplify all these processes using an extremely important pharmacokinetic parameter called bioavailability, which describes the amount of a particular drug that reaches its action site or biological fluid from the point where it was initially administered. Bioavailability will be discussed later.

Alveoli

Alveoli are structures found in the last portions of the respiratory tract formed by a single layer of epithelium supported by a delicate connective tissue with a large capillary network. Such characteristics give almost immediate absorption. Gaseous and volatile drugs can be administered by this route, as well as solutions atomized or aerosol. Another significant advantage is the reduced first passage effect from inhalation. On the other hand, it is a difficult route of dose adjustment due to individual anatomical variations.

Skeletal Muscle

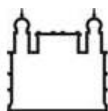
Skeletal muscle tissue is made up of long bundles of cells. Each group is surrounded by bundles of connective tissue. The blood vessels reach the muscle through the conjunctive septa forming an extensive capillary network. The intramuscular route of administration is subject to many variations, such as blood flow to the muscle group and the amount of body fat. In general, the drugs in aqueous solutions are absorbed rapidly while those present in oily solutions absorption slower.

Gastrointestinal Tract

The gastrointestinal (GI) tract, especially the small intestine is the site of major absorptive capacity. Apart from presenting simple epithelial tissue and a highly vascularized connective tissue, the epithelium has microvilli that increase the absorption area. In pharmacology, the oral route is chosen for GI tract for absorption because of its safety and economic advantage. Drugs taken orally, in general, suffer first pass metabolism which occurs immediately after absorption. This fact can be easily understood because the entire vascular network of the GI tract reaches the liver through the hepatic portal system.

Bioavailability

The desired pharmacological effects occur as a result of a specific drug plasmatic concentration that we call target concentration (TC). Concentrations greater than the TC may result in an increased





potentials of toxic effects occurrence, on the other hand, plasma concentrations below TC results in sub-therapeutic doses. Thus, it is extremely important that TC is reached in the time time required for positive therapeutic effect. A number of factors are involved from the time the drug is administered until the time it actually reaches the bloodstream. The animation below illustrates some of the phenomena involved in this kinetic, taking as example an administreted oral drug. Thus, the bioavailability or physiological availability (F) is defined as the amount of active ingredient (drug) absorbed from a pharmaceutical form that reaches the systemic circulation. The study of the bioavailability (F) also includes the speed at which the phenomenon occurs.

CALCULATION OF F

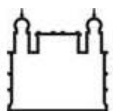
As mentioned earlier, the pharmacokinetics seeks to study and understand the effects of the plasma concentration of a particular drug as a function of time. The best way to represent these relationships is through the use of a plasma concentration graphics (Pc) versus time (t). From this relation we can also obtain information on bioavailability. In pharmacokinetic and availability studies, it is common to evaluate the area under the curve (AUC) of plasma concentrations versus time from time zero to infinity. Thus AUC is determined mathematically as:

$$AUC_{0 \rightarrow \infty} = \int_0^{\infty} C \cdot dt$$

Meaning that we should calculate the entire area under the curve of a graph $C_p \times t$. This information represents the amount of drug, from a predetermined form, dose and route, which effectively arrived to the systemic circulation. The intravenous route of administration presents an AUC equal to the administrated dose, or as we shall see ahead, an $F = 100\%$.

Generally, the bioavailability of a pharmaceutical form drug and administration route is related to the administration of this drug intravenously, which is considered as $F = 1$ ($F = 100\%$), since the amount of drug administered is equal to what effectively reaches the systemic circulation. When the bioavailability is obtained from the comparison with the intravenous route, this is called absolut bioavailability. In contrast, when the bioavailability is obtained from the comparison with diferent routes it is called relative bioavailability. In this case F can be obtained from the equation below.

$$F = \frac{AUC_{oral}}{AUC_{i.v.}}$$



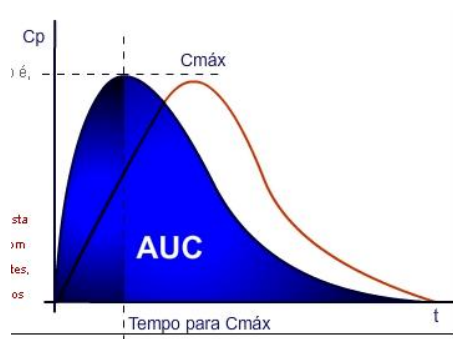


In cases of impossibility of comparison with the same doses:

$$F = \frac{AUC_{oral} \times dose_{i.v.}}{AUC_{i.v.} \times dose_{oral}}$$

Bioavailability is a useful parameter for comparing different forms, routes, including the different marketed drugs. This concept is the basis for approval of generic medicines, that is, a generic drug must have the same bioavailability of the equivalent brand name. When this feature is achieved among others, drugs are considered bioequivalent. Thus, generic drugs are bioequivalentes to their respective brand-name drugs, or also called references drugs.

Note that the F value alone is not present the precision required for this comparison. Other important data to evaluate is Cmax and time to Cmax. Curves presenting similar AUC and Cmax may have significantly different time to Cmax, resulting in different kinetic behavior, as well as different therapeutic results. (Refer to Figure opposite)





Transportation

The knowledge on how molecules cross cell membranes is very important for the understanding of the absorption and its resulting effects. Many drugs currently used alter specific transport mechanisms across membranes to generate its effects. Some drugs are used to treat ulcers and gastritis by inhibiting the pumping of protons into the stomach, other medications that block ionic transport are used in patients with heart failure among many other examples. Furthermore, after absorption drugs should reach various organic compartments which are referred to as distribution step also dependent on many of the transportation mechanisms presented here.

Passive and Active Mechanisms

Molecules can be transported according to its calorie necessity. Passive transport requires no energy so it may occur directly through the membranes or with the aid of proteins, always in favor of the concentration gradient. On the other hand, active transportation uses ATP high energy bonds to move molecules against its concentration gradients.

Diffusion

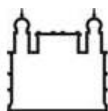
All molecules are in constant movement. When they are concentrated in one area, they spread homogeneously all over the place in which they are located. The diffusion of drugs across membranes can occur in two ways, directly through the membrane (facilitated diffusion), or with the help of membrane proteins (transporters) which will decrease the transport activation energy (facilitated diffusion). In passive transportation, the transported species always move towards a decreasing electrochemical gradient and no accumulation occur over the equilibrium concentration.

Simple Diffusion

Hydrophobic and low molecular weight molecules can easily cross membranes passively by diffusion; however larger polypeptides depend on other mechanisms to reach circulation, for example, facilitated diffusion or active mechanisms. Ions do not cross membranes by simple diffusion because they are electrically charged.

Facilitated Diffusion

Passive transportation can be facilitated by membrane proteins. To cross membranes, charged or polar drugs, must break their interactions with water and only then diffuse. This requires large amounts of energy. Membrane proteins use an alternative route for transportation in order to decrease





this energy requirement for that reason, transportation proteins constitute a significant fraction of all proteins encoded in the genome. Such molecules are primarily divided into channels and carriers. Channels are fast transporters proteins when compared to carriers. Proteins belonging to the carrier's family are also able to perform an energetically unfavorable type of transportation that will be discussed later (active transport).

Primary active transportation

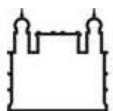
Active transportation is an essential mechanism for living organisms. Through the use of active transportation, cells are able to accumulate solutes above the equilibrium achieved with passive transport. The active transport is thermodynamically unfavorable, occurring only when coupled to energy-generating processes like the process of ATP breakdown and the concomitant flow of different chemical compound down to its electrochemical gradient. Active transportation which uses ATP molecules breakdown is termed as primary and that connected to a flow of compounds in favor of a gradient is referred to as secondary active transport.

Secondary active transportation

Unlike the active mechanisms that use ATP on ions and molecules transportation against its concentration gradient, secondary active transport uses ionic gradients formed by the primary transport of Na^+ or H^+ as the "driving force" for other solutes transportation. Whenever a flux with a favorable gradient is produced, energy is then released and directed to create work.

Endocytosis / exocytosis

Molecules and ions can cross the plasma membrane by using different mechanisms. Cells are also able to transfer in blocks to its interior, great amount of macromolecules (proteins, polysaccharides, polynucleotides) and even particles that are visible under an optical microscope, such as bacteria. This block transportation is always accompanied by plasma membrane morphological changes. This block transportation can be termed endocytosis, when compounds are transported to the intracellular environment, and exocytosis when transported to the extracellular medium.





Fick's Law

Drugs absorption occurs predominantly via passive diffusion process. This phenomenon, which does not depend on energy, is considered to be as order one, ie, it reaches equilibrium when the concentrations are equal on both sides of the membrane. Drugs presenting this kinetics are weak acids and bases, as well as all non-electrolyte organic compounds. This behavior is described by Fick's first law:

$$\frac{dQ}{dt} = \frac{D.S.\Delta C}{e}$$

Where

dQ/dt is rate of passage through membrane

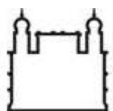
D, is the diffusion coefficient

S, is the contact area between the compound and membrane

ΔC, is the concentration difference between means is the membrane thickness.

This law, largely used in pharmacology, presents some peculiarities. The diffusion coefficient (D), for example, can be considered as a constant because it depends on the substance and drug's characteristics. D is stable at constant temperature, but influenced by the drug's partition coefficient, its solubility and ionization state in different pH media. Thus, the absorption is dependent on the concentration difference (Δ C) and the contact area. Given a practical situation, the blood concentration will always be below the membrane concentration which makes Δ C difficult to defeat. This is mainly due to local blood flow that ensures drug's rapid distribution the reduction of concentration at the site of absorption. In this context, rapidly absorbed drugs are considered as an exception.

Considering that blood concentration is always inferior to membrane location concentration, a large absorption area will be more efficient than large membrane concentrations. The intake of oral tablets with water is justified, by the fact that water would serve as a medium that would "spread" drug over a large area at the site of absorption.





Lipid solubility

Lipid-soluble molecules permeability.

Plasma membranes serve as diffusion barriers allowing intracellular and extracellular concentrations to be kept quite different. The relative impermeability of water-soluble compounds was assigned based on the membranes' lipid "nature". Experiments in the past have shown that soluble compounds in nonpolar solvents (ether or olive oil) enter the cell more quickly when compared to similar molecular weight water-soluble compounds. The permeability of the plasma membrane to a substance increases with its "lipid solubility".

The lipophilicity has an important role in the processes of transport of bioactive molecules across biomembranes and their interactions with its respective receptors. The interactions between drugs and membrane are extremely important in the development and evaluation of new drugs, the understanding of its therapeutic and adverse effects as well as their availability in the organism.

Thus, the lipophilicity is a molecular property that expresses the relative affinity of a solute (drug) by aqueous or organic phase. The lipophilicity is, among others, the result of intramolecular forces between solvent and solute.

Partition coefficient

As already mentioned, the molecular lipophilicity is the property that expresses the relative affinity of a solute (drug) by the aqueous or organic phase. The lipophilicity is, among others, the result of intramolecular forces between solvent and solute.

The most common way of expressing lipophilicity of compounds is the partition coefficient, which is defined by the ratio between a substance concentration in the organic phase and its concentration in the aqueous phase. A higher partition coefficient results most of the time, in the increased bioavailability of the drug. From the year 1960, the logarithm of the n-octanol/water partition coefficient ($\log P_{o/w}$) has been used as a quantitative measure of the hydrophobicity / lipophilicity and still represents one of the most useful physical and chemical properties used in medicinal chemistry.

Importantly, there are numerous variables to consider when studying the transport of drugs across membranes. For example, for compounds with the same partition coefficient, the permeability





decreases as molecular weight increases. Others, such as charge, shape, molecules ionization are also relevant to the process, and will be further discussed.

Legend: Note that this is a didactic representation of the partition coefficient obtained. Take into account that several immiscible liquids can be used, unlike those depicted (water / oil). We use to find the n-octanol/water partition coefficient, but also others such as butanol, chloroform and olive oil are used to represent the organic phase. It is also important to note that the partition coefficient is expressed by the ratio of quantities between the two phases. Thus, values greater than 1 denote greater affinity for the numerator medium and, on the other hand, partition coefficients smaller than 1 shows greater affinity for the denominator medium.

Example: Partition Coefficient oil /water of $X = 2$;

where [X] organic phase = 10 ai

[X] aqueous phase = 5 ai

(ai) arbitrary units

Physical-chemical differences and drug absorption

Two [cardiac digitalis or cardiac glycosides](#) can be used to study pharmacokinetic differences due to the partition coefficient. They are mainly from vegetable origin and are used for centuries. Some of their products are digoxin and digitoxin, which present half-lives of 40 hours and 6 days respectively in humans. Both drugs are drugs of [low therapeutic index](#). Digitoxin, unlike digoxin, is extensively metabolized by the liver. In addition to the significant differences in metabolism, we also observed differences in solubility between the molecules. Analyze the structures bellow which differ only in their hydroxyl radical (see arrow). This difference directly influences the partition coefficients. The different solubilities and metabolic differences already mentioned make these two drugs to present a significantly different pharmacokinetic behavior.

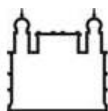
Cardiac digitalics

Digitalics compounds are used for the treatment of congestive heart failure for centuries. Generally they have a steroid nucleus containing lactone at position C17 and one or more glycosides in C3. They are found in plants of the family of apocinaceas like the Digitalis lanata and Digitalis purpurea. Besides plants, some types of frogs present these glycosides which function with toxin against predators.

The digitalics action mechanism seems to be highly selective and potent inhibitors of the sodium and potassium transport across cell membranes. As a result, it is observed positive inotropic effects explained by the increased rate of shortening of the cardiac muscle. In fact it is observed a shift upward to the left in the ventricular function curve, so that the increase in stroke work is generated by a given pressure or increase in filling volume.

Some other examples are digitalic compounds are digitoxin, digoxin, and methyl digoxin lanatoside. In Brazil the digitalic generic drug can be found along with the brand names. Digoxin is administered orally and during emergencies, can also be administered intravenously. Due to its unique pharmacokinetic characteristics (high lipid solubility and high half-life) digitoxin is rarely used in clinical practice. Digitalis is used in severe left ventricular systolic dysfunction - congestive heart failure - atrial fibrillation.

There may be some toxicity due to digitalics use (which may be related to hypokalemia due to diuretics), causing symptoms such as nausea, vomiting, diarrhea, confusion, ventricular extrasystole, visual disturbances, AV block to complete heart block. In pregnant patients the risks and benefits should be evaluated.





Therapeutic Index

The therapeutic index (also known as therapeutic ratio), is the comparison of the amount of a therapeutic agent that causes a desired effect in relation to the amount that causes death. Quantitatively, the ratio between the 50% lethal dose (LD 50%) therapeutic dose and 50%. Drugs with high therapeutic index are relatively safer than those with a low rate. The mathematical expression of the therapeutic index is shown in Eq 1.

Eq 1.

$$IT = \frac{DL_{50\%}}{DE_{50\%}}$$

Drugs such as diazepam have a therapeutic index of 100. Others, such as digoxin have an index of 2 or 3. Dimercaprol, theophylline, warfarin and lithium carbonate are also drugs with low therapeutic index. The therapeutic index is presented as an imprecise measure of drug safety. Others such as the margin of safety or security reasons are more precise by considering other different outcomes of death and efficacy of 50%.

Ionization of acids and bases

Most drugs are weak acids or bases present in solution in both ionized and non ionized forms. The non-ionized molecules are generally more soluble in lipids and therefore can spread easily throughout the cell membrane down the concentration gradient. On the other hand, the ionized molecules are usually unable to penetrate the lipid membrane due to its low lipid solubility.

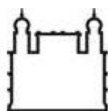
Additionally, the ionization constant (pK) is a physicochemical property of extreme importance because it is able to express, depending on the chemical nature of the compound and the pH of the medium, the relative percentage of ionized and non-ionized forms. Beyond the importance of pharmacokinetics already discussed, pharmacodynamics phase can also be influenced by the pK as ionized compounds may interact with amino acid residues present in the active sites of receptors.

Why are ionized molecules not well absorbed?

Polar and charged species are normally found solvated by water molecules hampering the process of passive absorption. As uncharged molecules do not react the same way, they can easily cross membranes.

Acidic drugs in alkaline medium

Here, we consider the absorption of drugs in the intestinal environment slightly basic. The compound in question is a pKa 4.0. acid. The drug appears predominantly ionized, which reduces the





rate of absorption in this situation. From the same reasoning, basic drugs would be mostly non-ionized, resulting in high rates of absorption in this context.

Acidic drugs in acidic medium

In this animation we can observe the absorption of an acidic drug with $pK_a = 2.0$ in an acidic environment with $pH = 4.0$. This situation may be comparable to absorption in the human stomach. It is observable the greater prevalence of non-ionized and therefore higher rate of absorption of the alleged compound in the gastric environment. It is worth noting however, that the histological organization of the stomach does not have the ideal characteristics for absorption when compared to the simple cylindrical epithelium and their microvilli that increase significantly the absorptive capacity of the small intestine. The opposite is also true where basic compounds have a higher rate of absorption in the alkaline medium.

Revisão do conceito de Pk

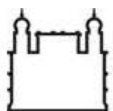
The pK of an acidic or basic group is the pH value in which protonated species (ionized) and not protonated (nonionized) are in equal concentrations. The relationship between pK_a and pH can be mathematically represented by the Henderson-Hasselbach equation:

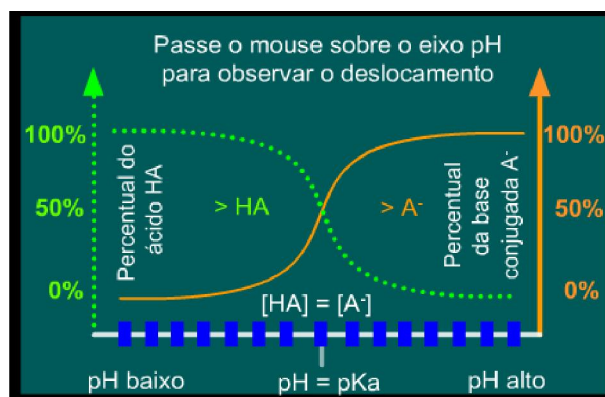
$$pH = pK_a + \log [A^-] / [HA]$$

where $[A^-]$ represents the deprotonated form and $[HA]$ represents the protonated one. The same relationship holds true for bases, with $[B]$ replacing $[A^-]$ as the deprotonated and $[HB^+]$ replacing $[HA]$ as the protonated form. It should be emphasized that the Henderson-Hasselbach equation is valid for a given acid or base even when various acids or bases are present.

Simulation

Changes in pH values alters the proportions of the acid form and its conjugate base. The graph shows the free acid (green) and its conjugated base A^- ionized (orange) concentrations. At acidic pH , in a non-ionized (HA) and in media with alkaline pH , the compound is predominantly ionized (A^-). In medium with a pH close to pK_a , the compound is presented as a mixture of the two forms.

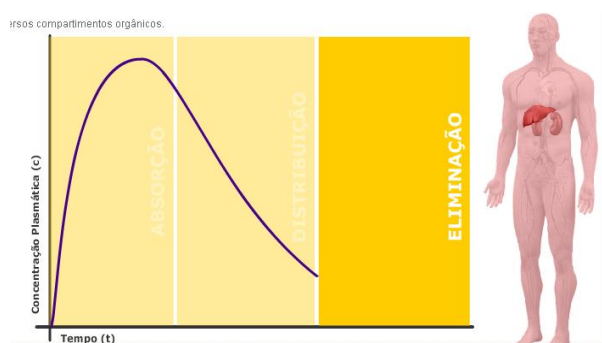




Distribution

After absorption for the case of extravascular administration, the drug enters the circulation. In this compartment, the drug binds to plasma proteins, erythrocytes and other cells and eventually leaves the vascular space, reaching the interstitial fluid and tissues. From a practical aspect, drugs must reach the tissues for their actions to initiate. With the exception of fibrinolytic agents, anticoagulants and some others, the product should be distributed for their actions to be observed. At this point, we observe a decrease in the curve $C_p \times \text{Time}$, attributed to the pharmacokinetic distribution phenomenon. Explore the animation below by clicking on the different steps and associate with the pharmacokinetic model to the human side.

The distribution occurs basically in two phases, one dependent on cardiac output and blood flow and other the phase two, dependent on the diffusion rates between different organic compartments.





Biotransformation

Biotransformation is a critical pharmacokinetics step not only from the pharmacological point of view, but also toxicologic, since virtually any compound that reaches the bloodstream can be converted into metabolites with favorable physical and chemical elimination essentially through the kidney.

Thus, the biotransformation of xenobiotics (foreign and non-nutrients compounds) is the result of a set of chemical reactions, enzyme catalysis, capable of producing metabolites of increasing polarity, compared to the unaltered drug. Thus, we consider that certain drugs metabolites are always more soluble than the original compounds not metabolized.

The result is the production of soluble metabolites more easily eliminated by the kidneys. However, this set of chemical reactions, may also produce active metabolites, or simply have them pharmacologically active. Another possibility is the conversion of a drug, initially without activity, to an active metabolite. This process is called bioactivation, and has great relevance in some diseases' etiology. Some cancers can be explained by the ability of our metabolic systems to produce highly reactive and carcinogenic compounds.

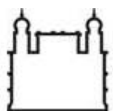
The biotransformation is divided into two phases, each of them from the well-defined enzymatic families' activity. Phase one is performed predominantly by CYP450, a hepatic monooxygenase system, anchored in the endoplasmic reticulum membranes, dependent on electron donors and capable of inserting or removing functional groups, turning the molecule less apolar (lipid soluble), and more reactive so that the second phase will continue.

On the other hand, the biotransformation second phase, also called conjugation, is carried out by enzymes (UGTs, NATs, UGSTs) that are able to combine phase I metabolites with endogenous compounds highly soluble. The conjugates are easily eliminated by the kidneys.

Elimination

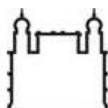
Since our body is capable of producing polar molecules (metabolites), our excretory systems are responsible for their elimination from the body. It is worth noting that even the non-metabolized drugs can be eliminated as long as they present a minimum level of polarity. Thus, from a urine sample after the use of drugs, we can find the parent drug and metabolites in unchanged form.

The elimination of drugs from our body is made through the renal route, lung (still able to eliminate nonpolar compounds), and the biliary-fecal among other processes. It is worth noting that all the pharmacokinetic properties are common to all stages, ie, all events controlling drugs absorption can be applied to other pharmacokinetic phases.





Finally, please note that the pharmacokinetics division in stages is purely didactic. In a real model all steps occur at the same time, the observed difference is basically in relation to prevailing rates for each of the phases. Explore this animation and notice the various pharmacokinetic phases occurring at the same time.





Pharmacodynamics

A Pharmacological effect is the result of chemical interactions between a drug and its specific targets in the organism. This concept was initially proposed by Paul Ehrlich which used to state that a drug will not act unless it is connected. This is a central concept in pharmacology specially when searching for the knowledge of drug action mechanisms. This is the definition of PHARMACODYNAMICS, ie, the study of a drug biochemical, physiological and molecular processes and its action mechanisms. The knowledge of drug x receptor interactions as well as their quantitative (dose) relation with the generated response is a crucial factor for the use of drugs in therapy.

Drugs act by interacting with its specific organic sites, however structurally nonspecific interactions and specific ones can also be observed in most pharmacologically useful drugs. Structurally nonspecific drugs present their actions according to their physical and chemical properties like antacids that once ingested neutralize gastric pH by reacting with stomach producing HCl parietal cells or in the case of general anesthetics which react nonspecifically with cell membranes turning them less excitable. On the other hand, structurally specific drugs - most of the useful therapeutic drugs – bind to specific entities called receptors. This way, pharmacodynamics investigates drugs interactions and its receptors including all effects produced by these interactions.

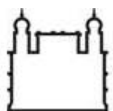
Receptors

Chemical signaling is the primary control mechanism of biological functions from a single cell level to a whole organism. Receptors have as primary function the recognition of endogenous chemical signals but also can act as important experimental target for drug use. For that reason receptors play a central role in basics of pharmacology.

Several of the active properties isolated from animal and plants are known from ancient time, however, only after the discovery of the benzene ring in the 1860s, the notions of “key-lock” emerged.

Nevertheless, the concept of receptor began to be consolidated only in the following century through Paul Ehrlich’s observations on antiparasitic effects of organic synthetic compounds followed by the J. Langley’s experiments with curare and nicotine. Many of the current knowledge about receptors were possible only after the development of biochemical and molecular biology techniques.

Receptors determine in large part quantitative relations between a drug dose and concentration and its pharmacologic effects (see dose x response). Pharmacokinetic factors may also influence pharmacological effects such as metabolic changes as a result of genetic variations that are often involved in dose-response relationship.





Receptors characterization

Any molecular and cellular structure participating in a homeostatic process in the organism in general can act as a pharmacological target and be considered a receptor. Thus, the term receptor has been used to denote any macromolecule in which a drug can bind to trigger a biologic response. From a pharmacological perspective, enzymes, nucleic acids, structural and transporter proteins can be considered receptors as they often participate in the physiologic processes maintenance.

In receptors at least two domains can be identified, the drug (ligand) - binding domain and an effector domain (spread of the message). Several techniques used to elucidate these molecules structures and functions are available today.

Most of receptors are proteins or glycoproteins inserted on cells lipid membranes or present in cell nuclei. Possible this is due to the polypeptide structures capable of providing necessary forms diversity and specificity. Such receptors are classified according to its function in four receptors superfamilies in which all members of the same superfamily will present the same general structure and the same action mechanism. However individual members of a superfamily tend to exhibit variations in the amino acids residue sequence in some regions and also in extra and intracellular domains dimensions. Each of the superfamily is divided according to their endogenous ligand characteristics.

Superfamilies

Type 1 superfamily

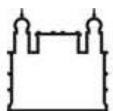
In this superfamily receptors are inserted into cell membranes and present in general four to five subunits that cross the membrane to form a central pore.

Ionic channels are examples of this group of receptors. Generated response of channels operation is virtually immediate occurring in microseconds. The opening of a channel allows the ions passage into and out of the membrane. Some factors such concentration and electric gradient determine ion passage and direction through channels. The relative passage selectivity occurs through sites known as ionic filters which interact with certain ions. This way we can find sodium, potassium, chloride selective ions channels among others (see figure).

Once activated, ion channels undergo a change in their conformation resulting in the passage of ions, for that reason they also are known as gated channel. Generally they can be presented in different conformational states, the rest state – closed - in which ion cannot cross the channel, the activated state – open - in this state it is possible for ion to cross the channel and a state known as desensitized in which even in the presence of the activation signal there is no response of the channel.

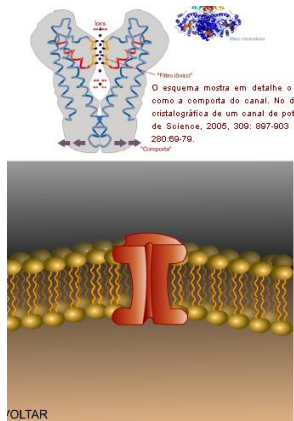
Some drugs are able to quickly produce this third level of structure which results in blockage of the channel activity.

As an example, the nicotinic receptor which upon activation by acetylcholine (ligand-dependent channel) allows the influx of sodium ions resulting in the depolarization of the cell. On the other hand,





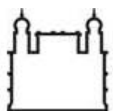
other channels can allow the entry of negative ions, like the GABA receptors which once activated by gamma-aminobutyric acid produce an influx of chloride ions hyperpolarizing the cell. Activation of GABA and the resulting hyperpolarization seems to be the benzodiazepines main action mechanism, drugs traditionally used as sedative-hypnotics.



Legend: The diagram shows in detail the ionic filter as well as the channel gate. In detail the crystal structure of the potassium channel. (modified from Science, 2005, 309: 897-903 and Science, 1998, 280:69-79).

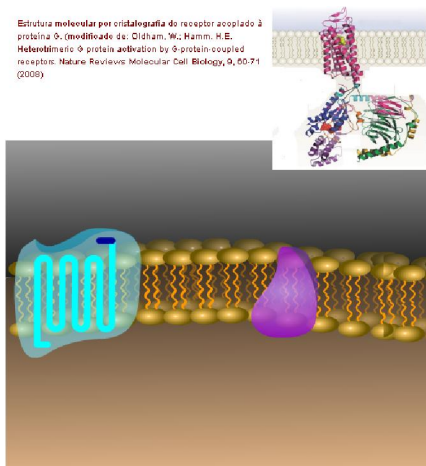
Type 2 superfamily

These receptors present a single polypeptide chain with seven transmembrane domains resembling a serpentine which cuts right through the cell membrane exposing its N-terminus to the extracellular medium and the C-terminus to the intracellular medium. Receptors from this family are also known as G-protein coupled receptors because they activate G protein upon its own activation. G protein have three subunit (alpha, beta and gamma), which can activate an ionic channel or an enzyme active site. The resulting cascade activation produces a molecule known as second messenger, which is the responsible for the generated biological responses. G protein activate two main enzyme classes, adenylate cyclase and phospholipases, each producing its own second messenger. Several endogenous mechanisms are regulated by this type of receptor. As an example, a muscarinic receptors, the beta adrenergic adrenalin receptor.





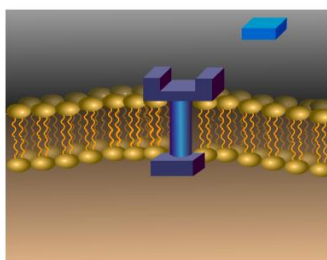
Estrutura molecular por cristalografia do receptor acoplado à proteína G, (modificado de: Oldham, W.; Hamm, H.E. Heterotrimeric G protein activation by G-protein coupled receptors. *Nature Reviews Molecular Cell Biology*, 9, 60-71 (2009))



Legend: Molecular structure of G protein coupled receptor by cristalography. (modified from: Oldham, W.; Hamm, H.E. Heterotrimeric G protein activation by G-protein-coupled receptors. **Nature Reviews Molecular Cell Biology**, 9, 60-71 (2008))

Type 3 superfamily

Receptors from this family are also known as tyrosine kinase receptors. They present a single helix transmembrane subunit connected to large extra and intracellular domains. The extracellular domains of each tyrosine kinase present variations capable of binding to different types of ligand while the intracellular domain present a tyrosine kinase residue as integral part of its structure. The binding to an extracellular domains region results in protein conformational changes which lead to tyrosine kinase autophosphorylation. Such phosphorylated residues appear to be high affinity site which can react with specific intracellular proteins. This event triggers an complex biochemical cascade that results in a biological response.

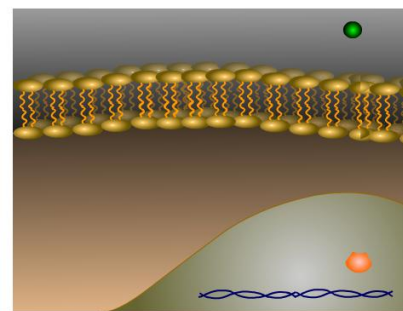


Legend: Deregulation of tyrosine kinases (TK) is associated with numerous diseases including cancer, diabetes and inflammatory diseases. Inhibition of these receptors then becomes relevant as pharmacological targets. Inhibition of TK is well established for treatment of chronic myeloid leukemia, and several other types of solid tumors. As an example, imatinib binds to the TK extracellular active resulting in useful pharmacological responses. The Figure shows Tk's structure and conformation and in details the binding of imatinib in its active site. (Modified from: *Nat Struct Mol Biol*. 2008 Oct; 15 (10):1109-18)



Type 4 superfamily

This class of receptors is endogenously activated by thyroid steroid hormones among others. They are not found inserted into the lipid membrane instead they are located inside the cells as intracellular proteins. Nowadays it is accepted the existence of two large intracellular receptor families, one group is found in the cytoplasm which reach the nucleus after binding to their ligands and the other group such as LXR and FXR receptors that are entirely located into the cell nucleus. LXR is a liver receptor whose ligands are 22-OH cholesterol and FXR also known as farnesoid receptor which binds to chenodesoxicoloico acid. From a biochemical perspective these are large proteins containing from 400 to 1000 amino acids. Once activated by their ligands these receptors are able to regulate gene transcription which leads to the production of an specific messenger RNA (transcription) and subsequent protein synthesis (translation) resulting in biological response (the animation does not distinguish cytoplasmic receptors).

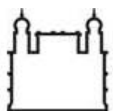


Signaling

Communication was fundamental for the establishment of our society as we know it today. The cave drawings and tribal rituals were the first records of human communication. Throughout evolution communication has expanded from writing through inventions like the telegraph and telephone to an unlimited borderless via the internet.

By analogy, we can say that multicellular organisms, as we know them today, followed different evolutionary path through several signaling mechanisms that allow cells to communicate with each other coordinating each cell activity for the benefit of the whole organism.

However, communication is not exclusive or a privilege of multicellular organisms, it is intrinsic to life and manifested itself as a necessity. When the first organisms – the prokaryotes – appeared billion of years ago, in parallel the need to understand, recognize and interact with the world around was developed. With evolution, organisms began to develop internal signaling mechanisms, between the same specie and different ones.





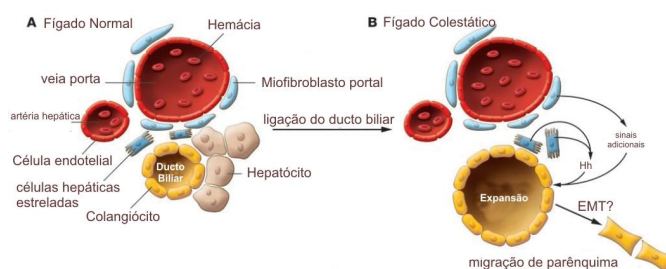
A higher complexity level of communication was reached by metazoan. Their cells developed a complex communication network ranging from small “rumors” spreading throughout the neighborhood, [paracrine signaling](#); “intimate secrets whispers”, [cell-to-cell direct communication](#)- via surface receptors and gap junctions; the “monologue”, cell talking to itself – [autocrine signaling](#) - communication by mail with a well established receiver and deliverer, [endocrine signaling](#); even an intricate net of “cables and connectors”, [neuronal communication](#).

Over the course of evolution on human physiology, biochemistry and molecular biology knowledge, man became able to interfere with these intricate signaling mechanisms. This is the situation in which pharmacology plays a role, where drugs would interact and interfere with the organism communication mechanisms to produce beneficial physiological alterations that we call pharmacological effect.

Paracrine signaling

Among the different ways of communication present in organisms, paracrine signaling, perhaps is one of the first “sketches” of communication, it has proven so effective that has been conserved throughout evolution and can be found from unicellular to the multicellular organisms are more developed.

The paracrine signaling is the release of signaling molecules - local mediators - to target specific cells that are in the neighborhood. Nitric oxide (NO), to have short half-life - 50 to 10 seconds in the extracellular space - is a good example of local mediator resulting in potent vasodilation. Many studies in pharmacology from the involvement of this mediator have been performed, resulting in the development of important drugs such as sildenafil (Viagra®).



Congenital and acquired diseases of the biliary tree or cholangiopathy represent significant morbidity in children and adults. In the later stages of the disease, cholangiocytes no longer proliferate resulting in loss of bile ducts, increased fibrosis and eventually cirrhosis and liver failure. Several researchers have proposed that the epithelial-mesenchymal transition is an important mechanism for both the proliferation of cholangiocytes as for fibrogenesis in biliary tract diseases. This transition seems to happen through a kind of paracrine signaling between hepatic stellate cells and epithelial cells of the bile ducts. In response to liver injury a paracrine signaling is initiated resulting in histological changes common to these conditions. J. Clin. Invest. 118:3263-3265 (2008).

Autocrine signaling

Another type of communication found both in bacteria and in metazoans is the autocrine signaling, which we call a true “monologue” in this type of cell secretes signaling molecules that will act on its own receptors

Direct cell-cell communication – Endocrine Signaling and neuronal communication.

In higher organisms there are two additional ways of communication for soluble substances: endocrine and nervous system. In both systems chemical messengers are used by cells to talk to each other (Snyder, HS, 1985).

The nervous system is responsible for accurately perceive the environment to respond and change extremely fast for any necessity.

Neurons, are nervous system highly specialized cells that upon excitation trigger an action potential or electrical impulse that travels along the axon and passes into another cell by direct transmission through electrical synapses, or through



chemical synapses through release of a chemical signal- the neurotransmitter- which operates in specific target cells, muscle cells, glandular or other neurons. The neurotransmitter binds to specific receptor located in the membrane of the target cell, promoting chemical changes in the cell membrane and inside the cell itself (Snyder, HS, 1985 and Changeux, JP, 1993).

Electrophysiological studies have shown that different neurotransmitters may produce different effects on synapses. However, a single neurotransmitter can produce different responses in different receptors. When acetylcholine acts on muscarinic receptors, found in intestine smooth muscle cells it promotes the closing of the channels allowing potassium efflux from the cell, creating a gradual and prolonged excitation in the muscle, but when it binds to nicotinic receptors, cationic channels found in the skeletal muscle endplate, leads to a depolarization of this plate by the activation of voltage-dependent sodium channel, promoting a fast-twitch muscle.

In 1980, a group from the Pasteur Institute and other groups have shown that alone, the acetylcholine receptor protein has all the elements required for chemical transmission of an electrical signal - a binding site for acetylcholine, an ion channel and a mechanism for engaging their activities. This way, the receptors that constitute what we call neurotransmitter receptors superfamily and ion channels are known as "neurotransmitter-gated ion channels" plays a key role in mediating the conversion of chemical signals into electrical signals (Changeux, JP, 1993).

Signals transmission by cell's nerve endings usually occurs via specialized cell junctions called synapses, which ensure that a neurotransmitter is released to the target postsynaptic cell rapidly and specifically.

Unlike neuronal communication that is more specific, extremely fast and can reach up to 100 meters / second, hormones act in an indirect and much slower way. While there are also autocrine and paracrine mechanisms, the classical form of communication is the hormone found in the endocrine system, where a gland releases circulation hormones that can act on cells or organs anywhere in the body in a process that can take several hours (Snyder, HS, 1985).

The endocrine system and nervous system together coordinate the activity of billions of cells in "superior" animal to integrate the body's response to changes in its external and internal environment.

Although morphologically different, in the speed of signaling and in the concentration required for signaling - the hormones are highly diluted in the bloodstream and neurotransmitters can reach high local concentrations, the receptor's affinity - hormone's receptors have high affinity by its ligands while the neurotransmitter receptors have, in general, low affinity enabling rapid dissociation of neurotransmitter and completion of the response; specificity - the specificity of the endocrine system is obtained by the presence of specific receptors for different hormones in the nervous system in most cases, the specificity is determined by the contact between the nerve extensions and the target cells although, some neurons secrete into the bloodstream, these two systems are not so different in molecular terms, both operate through special messenger molecules that interact with specific receptors on target cell, and several studies have shown that certain messenger molecules are used by both systems.

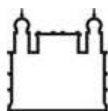
Norepinephrine, a hormone, is released by the adrenal gland and acts by stimulating contractions of the heart by dilating the bronchial tract and lungs and increasing the contractile force of muscles of the arms and legs. On the other hand, norepinephrine also acts as a neurotransmitter as the main system mediator.

General properties of receptors and signaling

Some function patterns can be observed in most of the already known signaling mechanisms and even with a profuse offer of distinct biological signals, organisms may take advantage of only few evolutionary well conserved signals recognition mechanisms.

Signal transduction is [specific](#) and sensitive. Specificity is the result of a precise molecular complementarity mediated by weak non covalent forces. In multicellular organisms specificity is explained by the fact that only some cells present receptors for a given signal. Sensitivity on the other hand is regulated by three basic factors, the high affinity of receptors and their respective signals, cooperativity of ligand-receptor interactions and finally, by the [signal amplification](#) through enzymatic cascades. In pharmacology this is an aspect of fundamental importance because it guarantees a good match of drugs actions in specific places. On the other hand, the binding of a drug to a not wanted receptor (binding on secondary sites), may explain in part the occurrence of side effects.

An additional event common to most of the signaling mechanisms is called [signal amplification](#). Amplification is the result of the activation of enzymatic cascades. Once activated, receptor is able to activate additional enzymes that will in turn activate another group of enzymes and so on. This mechanism will produce intense cellular responses from a small signal and in only few milliseconds the enzymatic cascade will generate an amplification of many orders of magnitude.



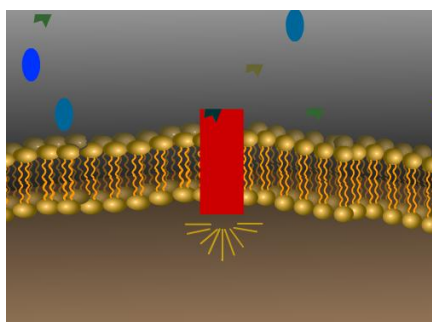


Modulation of receptors sensitivity can occur by a mechanism not entirely understood known as [desensibilization or tachyphylaxy](#). The activation of this mechanism results in the drug's efficacy decrease when it is repeatedly administrated in a short period of time. Another phenomenon is pharmacological tolerance which similarly will decrease drug's efficacy after a long period of use. It has been suggested that [desensibilization](#) would represent a receptor's conformational alteration after some signals leading to a weak or abolished response while tolerance correspond to a physiological response (pharmacokinetics or pharmacodynamics) resulting in decrease on drug's efficacy.

The last characteristic worth a comment on transduction systems is [integration or modulation](#) which consists in the receptor's capacity in receiving multiples signals and produces a modulated response of the received signals. This characteristic would explain the phenomenon known as physiological antagonism, in which a drug is able to produce an antagonist response to an existent endogenous signaling.

Animations legends

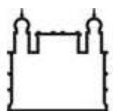
Specificity

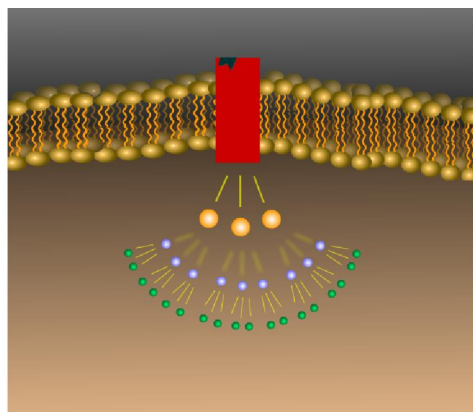


Signals are relatively receptor-specific.

Only the complimentary signal to a binding site will be able to activate the receptor generating a biological response.

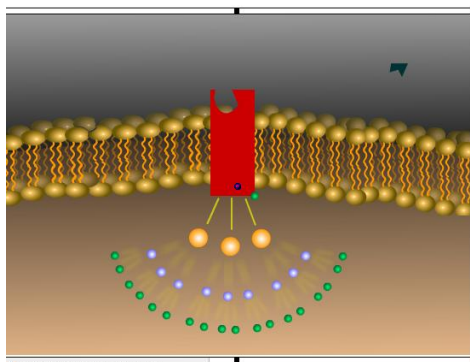
Amplification





The intracellular receptor domain when altered in their structural conformation after ligand activation is able to initiate an enzymatic cascade from a single or few signals.

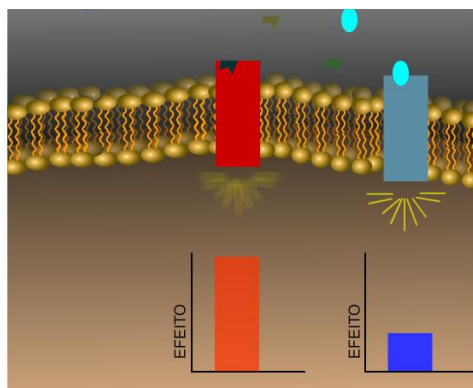
Desensitization



Even though the events that follow receptor activation are not entirely identified, some authors suggest that the activation signal (not shown) would be able to alter the binding site which then would decrease ligand affinity and respectively an inhibition of biological responses. In the example shown above, binding on allosteric sites would be able to promote affinity differences in the primary site.



Integration



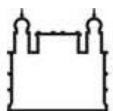
In the example above a given signal generates a characteristic response and a different signal activating its respective receptor is able to generate another antagonistic response. However, when both signals are simultaneously activated there is an integration of the generated effects. We can represent it generically by:

$$\begin{aligned}\text{Ativação do Receptor1} &= \uparrow [X] \\ \text{Ativação do Receptor2} &= \downarrow [X] \\ \text{Receptor1} + \text{Receptor2} &= \Delta [X]\end{aligned}$$

Dose x Response

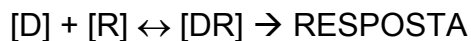
The idea that bigger doses would equally generate bigger effects is a common belief to most of people. However, differently, when dose is increased it is observed the decrease in response, additionally some doses would not even generate a perceived increase in effect. This relationship describes a hyperbolic curve usually known as dose-response curve.

In the nineteenth century, even before any knowledge about receptors, it was believed that the effects of drugs were determined by the Law of Mass Action. A.J. Clark, in the 1920s, developed the theory of occupation. He postulated that the drug binding to several identical and independent receptor sites resulted in activation of these sites, and the corresponding magnitude of the response was proportional to the number of receptors occupied at equilibrium. In his opinion, the maximal response occurs when all receptors are occupied, and that the response would cease on the dissociation of the ligand from its receptor.





Then,



Where D = ligand R = receptor e DR ligand-receptor complex.

And according to the proposition, the effect is proportional to the complex DR concentration.

Effect and $\propto [DR]$, and the maximal effect then: **Maximal effect ($E_{\max}$) $\propto [R_t]$** ,

Where R_t is the total amount of receptors.

For a given dose:

$$\frac{E}{E_{\max}} = \frac{[DR]}{[R_t]}$$

The affinity of a given drug to its receptor can be measured by the dissociation constant described by:

$$K_D = \frac{[D][R]}{[DR]}$$

Through analysis of the above equation we can observe that low K_D values represent greater affinity by its receptors, in the same way, there is an equation that express the affinity coefficient K_a :

$$R_t = [R] + [DR]$$

And by substituting the RT equation on KD equation:

$$K_D = \frac{[D]([R_t] - [DR])}{[DR]}$$

Rearranging

$$K_D = \frac{[D][R_t]}{[DR]} - \frac{[D][DR]}{[DR]}$$

Then:

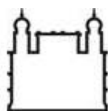
$$k_D = \frac{[D][R_t]}{[DR]} - [D]$$

and

$$K_D + [D] = \frac{[D][R_t]}{[DR]}$$

$$\frac{K_D + [D]}{[D]} = \frac{[R_t]}{[DR]}$$

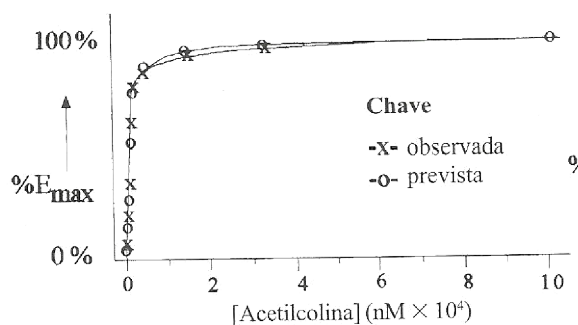
$$\frac{E}{E_{\max}} = \frac{DR}{R_t} = \frac{[D]}{K_D + [D]}$$



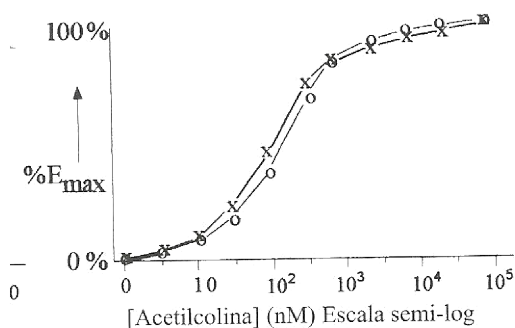


By substituting the anterior equation by the fractions equation:

That can be expressed by:



Or transformed by:



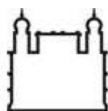
Agonists

Simulation:

1. Choose the antagonism type.
2. Add the agonist.
3. Add the antagonist.
4. Progressively increase the antagonist dose.

Calculations in Pharmacology

Here you will be able to simulate similar laboratory activities through calculation, solutions, dilutions among others.

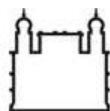




Quiz

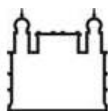
References

- Albuquerque, E. X., Pereira, E. F., Alkondon, M., & Rogers, S. W. (2009). Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol Rev*, 89(1), 73-120.
- Azam, M., Seeliger, M. A., Gray, N., Kuriyan, J., & Daley, G. (2008). Activation of tyrosine kinases by mutation of the gatekeeper threonine. *Nat Struct Mol Biol*, 15(10), 8.
- Baláz, S. (2000). Lipophilicity in trans-bilayer transport and subcellular pharmacokinetics. *Perspectives in Drug Discovery and Design*, 19, 21.
- Barreiro, E. J., & Fraga, C. A. M. (2001). *Química Medicinal: As bases moleculares da Ação dos Fármacos*. Porto Alegre: Artmed.
- Bayburt, T. H., & Sligar, S. G. (2002). Single-molecule height measurements on microsomal cytochrome P450 in nanometer-scale phospholipid bilayer disks. *Proc Natl Acad Sci U S A*, 99(10), 6725-6730.
- Brunton, L. L., Lazo, J. S., & Parker, K. L. (2006). *Goodman & Gilman: As Bases Farmacológicas da Terapêutica* (Vol. 11): McGraw-Hill.
- Buchwald, P. (2000). Modeling liquid properties, solvation, and hydrophobicity: A molecular size-based perspective. *Perspectives in Drug Discovery and Design*, 19, 27.
- Calabrese, E. J., & Baldwin, L. A. (2001). Hormesis: U-shaped dose responses and their centrality in toxicology. *Trends Pharmacol Sci*, 22(6), 285-291.
- Chang, Y., Ghansah, E., Chen, Y., Ye, J., & Weiss, D. S. (2002). Desensitization mechanism of GABA receptors revealed by single oocyte binding and receptor function. *J Neurosci*, 22(18), 7982-7990.
- Christopoulos, A. (1998). Assessing the distribution of parameters in models of ligand-receptor interaction: to log or not to log. *Tips*, 19, 7.
- Clark, B., & Saameli, K. (1970). Pharmacological properties of a new beta-receptor blocking agent. *Triangle*, 9(8), 300-308.
- Colquhoun, D. (1987). Molecular neurobiology. A new type of ion-channel block. *Nature*, 329(6136), 204-205.
- Colquhoun, D. (2006). The quantitative analysis of drug-receptor interactions: a short history. *Trends Pharmacol Sci*, 27(3), 149-157.
- Colquhoun, D., Dreyer, F., & Sheridan, R. E. (1979). The actions of tubocurarine at the frog neuromuscular junction. *J Physiol*, 293, 247-284.
- Colquhoun, D., & Sakmann, B. (1985). Fast events in single-channel currents activated by acetylcholine and its analogues at the frog muscle end-plate. *J Physiol*, 369, 501-557.
- Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., & MacKinnon, R. (1998). The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. *Science*, 280(5360), 69-77.
- Duncan, R. (2006). Polymer conjugates as anticancer nanomedicines. *Nat Rev Cancer*, 6(9), 688-701.
- Gabrielsson, J., & Green, A. R. (2009). Quantitative pharmacology or pharmacokinetic pharmacodynamic integration should be a vital component in integrative pharmacology. *J Pharmacol Exp Ther*, 331(3), 767-774.
- Gadsby, D. C. (2009). Ion channels versus ion pumps: the principal difference, in principle. *Nat Rev Mol Cell Biol*, 10(5), 344-352.
- Gandhi, M., Aweeka, F., Greenblatt, R. M., & Blaschke, T. F. (2004). Sex differences in pharmacokinetics and pharmacodynamics. *Annu Rev Pharmacol Toxicol*, 44, 499-523.
- Gill, E. W., & Rang, H. P. (1966). An alkylating derivative of benzilylcholine with specific and long-lasting parasympatholytic activity. *Mol Pharmacol*, 2(4), 284-297.





- Goutelle, S., Maurin, M., Rougier, F., Barbaut, X., Bourguignon, L., Ducher, M., & Maire, P. (2008). The Hill equation: a review of its capabilities in pharmacological modelling. *Fundam Clin Pharmacol*, 22(6), 633-648.
- Greenbaum, L. E. (2008). Hedgehog signaling in biliary fibrosis. *J Clin Invest*, 118(10), 3263-3265.
- Hohme, S., Hengstler, J. G., Brulport, M., Schafer, M., Bauer, A., Gebhardt, R., & Drasdo, D. (2007). Mathematical modelling of liver regeneration after intoxication with CCl₄. *Chem Biol Interact*, 168(1), 74-93.
- Katzung, B. (2006). *Farmacologia Básica e Clínica* (Vol. 9): Guanabara Koogan.
- Kenakin, T. (2004a). G-protein coupled receptors as allosteric machines. *Receptors Channels*, 10(2), 51-60.
- Kenakin, T. (2004b). Principles: receptor theory in pharmacology. *Trends Pharmacol Sci*, 25(4), 186-192.
- Kumagai, A. K., Kang, Y. S., Boado, R. J., & Pardridge, W. M. (1995). Upregulation of blood-brain barrier GLUT1 glucose transporter protein and mRNA in experimental chronic hypoglycemia. *Diabetes*, 44(12), 1399-1404.
- Langley, J. N. (1905). On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. *J Physiol*, 33(4-5), 374-413.
- Limbird, L. E. (2004). The receptor concept: a continuing evolution. *Mol Interv*, 4(6), 326-336.
- Lipinski, C., Lombardo, F., Dominy, B., & Feeney, P. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46, 23.
- Long, S. B., Campbell, E. B., & Mackinnon, R. (2005). Crystal structure of a mammalian voltage-dependent Shaker family K⁺ channel. *Science*, 309(5736), 897-903.
- Lu, H., Qu, G., Yang, X., Xu, R., & Xiao, W. (2011). Systemic elimination of de novo capsid protein synthesis from replication competent AAV contamination in the liver. *Hum Gene Ther*.
- Navia, M., & Chaturvedi, P. (1996). Design principles for orally bioavailable drugs. *DDT*, 1(5), 11.
- Nelson, D. (1998). Metazoan Cytochrome P450 evolution. *Comparative Biochemistry and Physiology Part C*, 121, 8.
- Nelson, D. R., Zeldin, D. C., Hoffman, S. M. G., Maltais, L., Wain, H., & Nebert, D. (2004). Comparison of Cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics*, 14, 18.
- Nesher, M., Shpolansky, U., Rosen, H., & Lichtstein, D. (2007). The digitalis-like steroid hormones: new mechanisms of action and biological significance. *Life Sci*, 80(23), 2093-2107.
- Rauma, M., & Johanson, G. (2009). Assessment of dermal absorption by thermogravimetric analysis: Development of a diffusion model based on Fick's second law. *J Pharm Sci*, 98(11), 4365-4375.
- Roden, D. M., & George, A. L., Jr. (2002). The genetic basis of variability in drug responses. *Nat Rev Drug Discov*, 1(1), 37-44.
- Schadt, E. E., Molony, C., Chudin, E., Hao, K., Yang, X., Lum, P. Y., Kasarskis, A., Zhang, B., Wang, S., Suver, C., Zhu, J., Millstein, J., Sieberts, S., Lamb, J., GuhaThakurta, D., Derry, J., Storey, J. D., Avila-Campillo, I., Kruger, M. J., Johnson, J. M., Rohl, C. A., van Nas, A., Mehrabian, M., Drake, T. A., Lusi, A. J., Smith, R. C., Guengerich, F. P., Strom, S. C., Schuetz, E., Rushmore, T. H., & Ulrich, R. (2008). Mapping the genetic architecture of gene expression in human liver. *PLoS Biol*, 6(5), e107.
- Shanks, R. G., Wood, T. M., Dornhorst, A. C., & Clark, M. L. (1966). Some pharmacological properties of a new adrenergic beta-receptor antagonist. *Nature*, 212(5057), 88-90.
- Sikka, R., Magauran, B., Ulrich, A., & Shannnon, M. (2005). Bench to Bedside: Pharmacogenomics, Adverse Drug Interactions, and Cytochrome P450 System. *Acad Emerg Med*, 12(12), 9.
- Soskic, M. (2005). Modeling the Octanol-Water Partition Coefficients by an Optimized Molecular Connectivity Index. *J. Chem. Inf. Model*, 45, 9.
- Thomas, G. (2003). *Química Medicinal: Uma introdução* (2 ed.). Rio de Janeiro: Guanabara Koogan.
- Urban, J., Clarke, W., Zastrow, M., Nichols, D., Kobika, B., & Mailman, R. (2007). Functional Selectivity and Classical Concepts of Quantitative Pharmacology. *JPET*, 320, 13.





- Urban, J. D., Clarke, W. P., von Zastrow, M., Nichols, D. E., Kobilka, B., Weinstein, H., Javitch, J. A., Roth, B. L., Christopoulos, A., Sexton, P. M., Miller, K. J., Spedding, M., & Mailman, R. B. (2007). Functional selectivity and classical concepts of quantitative pharmacology. *J Pharmacol Exp Ther*, 320(1), 1-13.
- Viswanadhan, V. N., Ghose, A., & Wendoloski, J. C. (2000). Estimating aqueous and lipophilicity of small organic molecules: A comparative overview of atom/group contribution methods. *Perspectives in Drug Discovery and Design*, 19, 13.
- Wang, Y. D., Chen, W. D., & Huang, W. (2008). FXR, a target for different diseases. *Histol Histopathol*, 23(5), 621-627.
- Wang, Y. D., Chen, W. D., Moore, D. D., & Huang, W. (2008). FXR: a metabolic regulator and cell protector. *Cell Res*, 18(11), 1087-1095.
- Werck-Reichhart, D., & Feyereisen, R. (2000). Cytochromes P450: a success story. *Genome Biol*, 1(6), REVIEWS3003.
- Wyllie, D. J., & Chen, P. E. (2007). Taking the time to study competitive antagonism. *Br J Pharmacol*, 150(5), 541-551.

Help

Pharmavirtua presents an intuitive and friendly interface. Anyone used to surf the internet will not find it difficult to use all the features available. In general, texts are arranged as hyperlinks, ie, clickable words that will draw user away to a different site for more detailed information on the subject.

Another feature widely used in the software is the animation of important concepts in pharmacology, both as figures or graphs. So, explore with your mouse all presented program areas and prove all potentiality of Pharmavirtua.

Any doubts, criticism or suggestions, please write to us.

Contacts

Antonio A Fidalgo-Neto
<http://lattes.cnpq.br/3582131045080938>

fidalgo@ioc.fiocruz.br

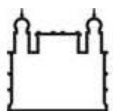
Filipe Faria Berçot
<http://lattes.cnpq.br/7831957955269307>

filipeberçot@gmail.com

Luiz Anastácio Alves
<http://lattes.cnpq.br/8572675732158244>

alveslaa@ioc.fiocruz.br

Laboratório de Comunicação Celular
Instituto Oswaldo Cruz – Fiocruz
Pav. 108 - Sala 36 e 39
Av. Brasil 4365, Manguinhos
CEP-21045-900, Rio de Janeiro, RJ., Brasil
Fone: ++ 55 21 2562 1815
www.lcc.kftox.com





Credits

Antonio A Fidalgo-Neto – Professor de Farmacologia, Doutorando IOC - FIOCRUZ
Filipe Faria Berçot – Designer em educação
Luiz Anastácio Alves, MD, PhD – Chefe do Laboratório de Comunicação Celular
Giuliano Lima – Designer giuliano.lima@papilliodesign.com.br
Eloana Baldo – Estagiária em design e animação educativa
Rafaela Nascimento – Estagiária em design e animação educativa
Frederico Barreto – Estagiário em design e animação educativa
Ricardo Riedel – Estagiário educação em ciências
André Bonavita, PhD – Professor de Farmacologia UFRJ e Pesquisador Visitante do Laboratório de Comunicação Celular
Anael Viana – Professor de Farmacologia e Bolsista do Laboratório de Comunicação Celular
Rômulo Bezerra – Professor de Farmacologia
Paula Fonseca, PhD – Professora de Fisiologia e Imunologia
Anderson Correa – Estudante de Doutorado
Cristina Souza, PhD – Vice-Chefe do Laboratório de Comunicação Celular
Ivone Ventura – Secretária do Laboratório de Comunicação Celular
Pedro Teixeira – Pesquisador Visitante do Laboratório de Comunicação Celular
Renato Matos Lopes, PhD – Pesquisador do Laboratório de Comunicação Celular
Robson Xavier, PhD - Pesquisador do Laboratório de Comunicação Celular

