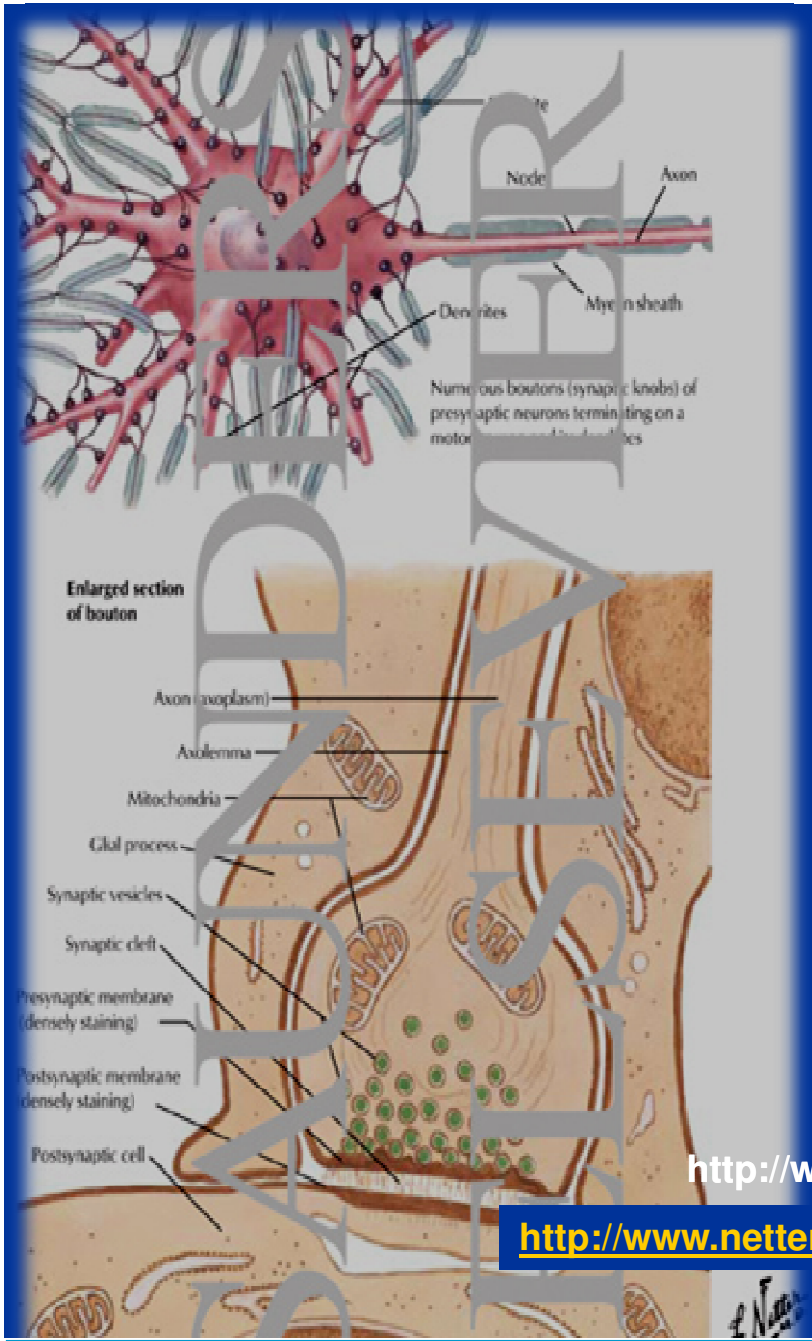


C.5

Drug-receptor binding

Image sources

http://whyfiles.org/225drug_receptors/images/opiate_receptors.jpg

http://www.netterimages.com/images/vpv/000/000/012/12946-0550x0475.jpg

Prof. Dr. Romeo T. Cristina

The term `receiver` was introduced by Paul Ehrlich since 1906 , but the concept was defined later as:

Receptor:

Any biological molecule to which a drug binds and produces a measurable response. (Goodman, 1968).

Proteins that are responsible for transducing extracellular signals into intracellular response. (Lindupp, 1990).

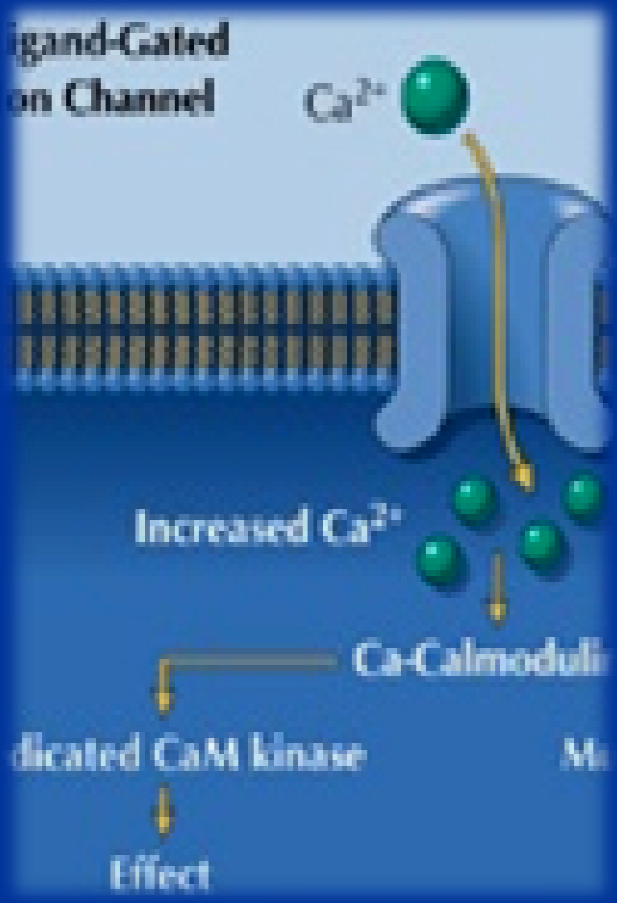
In the current concept,
pharmacological receptors (or pharmaco-receptors)
are *"cells infrastructural configurations that are able to bind more or less specific to the molecules of: drugs, endogenous and toxic substances"*

Pharmaco-receptors
are usually found at cellular level, being placed on the cellular membrane or inside the cells, drugs being able to act on the cells' surface or inside.

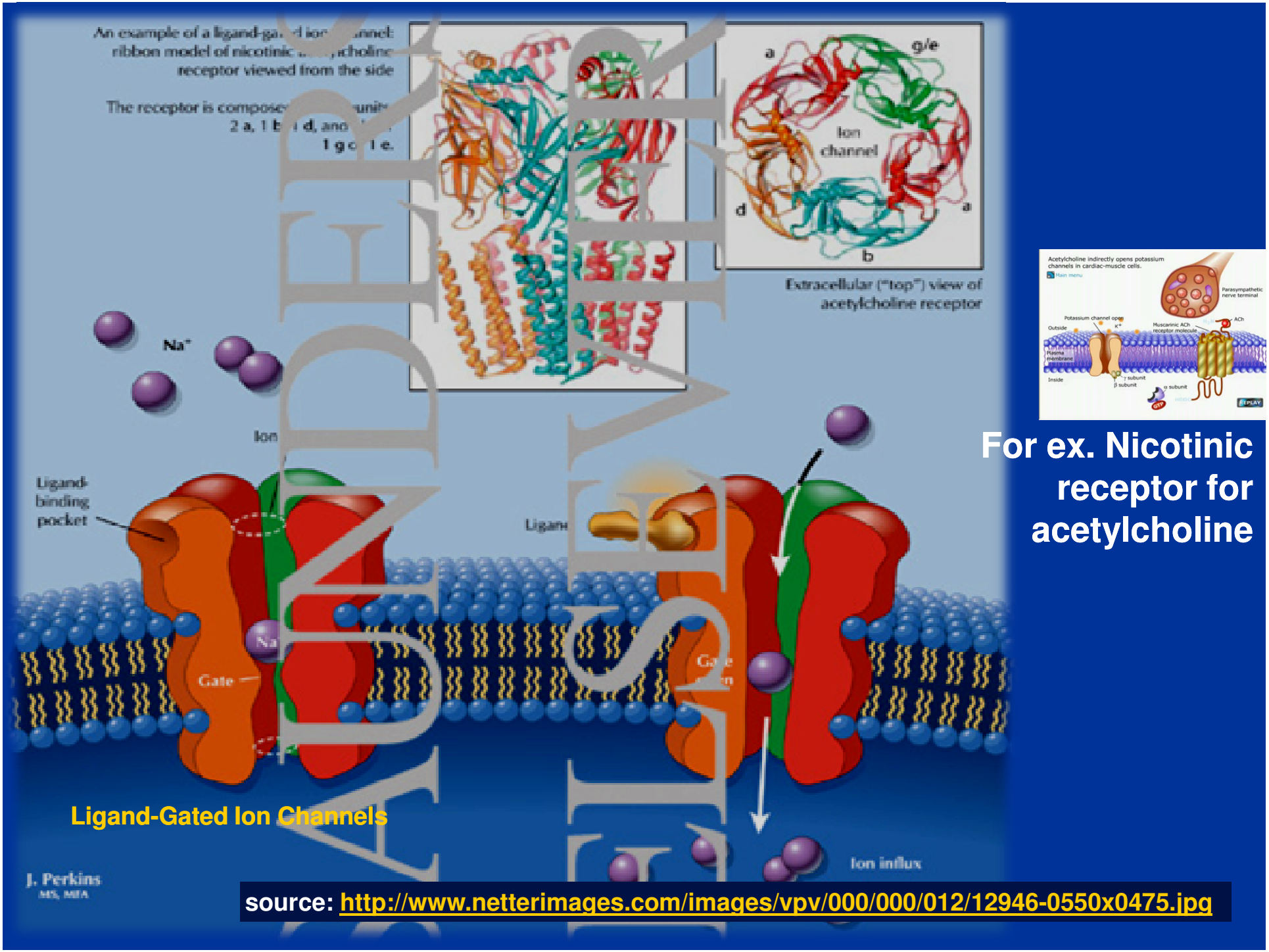
Recent researches have revealed other premises linked to the receptors:

- ✓ Ligand Gated Ion Channels,
- ✓ G-Protein Coupled Receptors,
- ✓ Enzyme-Linked Receptors,
- ✓ Intracellular Receptors.

1. Ionic channels



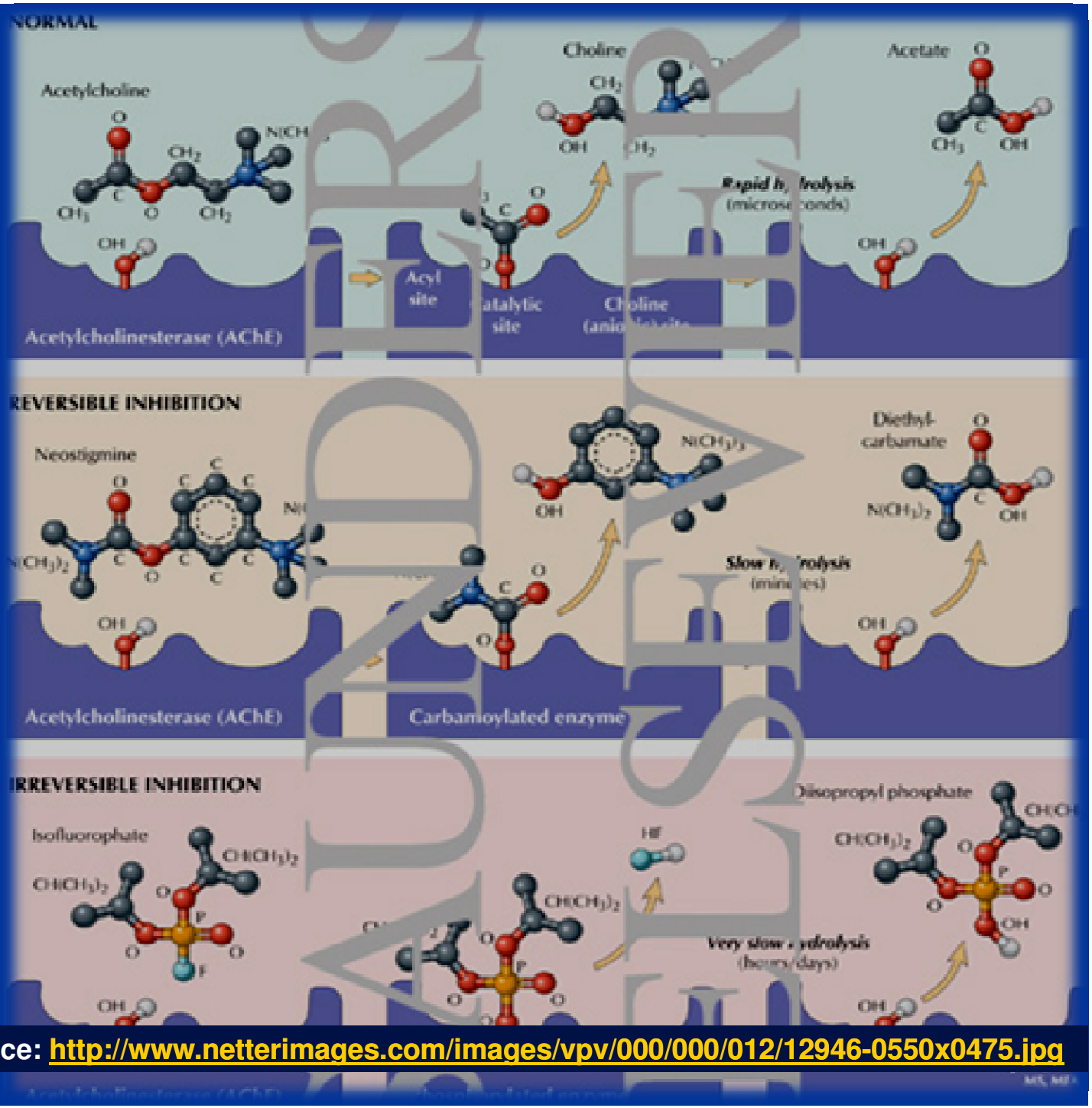
Regulates the flow of ions across the cell membrane.
Ex: Nicotinic cholinergic receptors, GABA-ergics = quick response



For ex. Nicotinic receptor for acetylcholine

source: http://www.netterimages.com/images/vpv/000/000/012/12946-0550x0475.jpg

The Acetylcholine's activity



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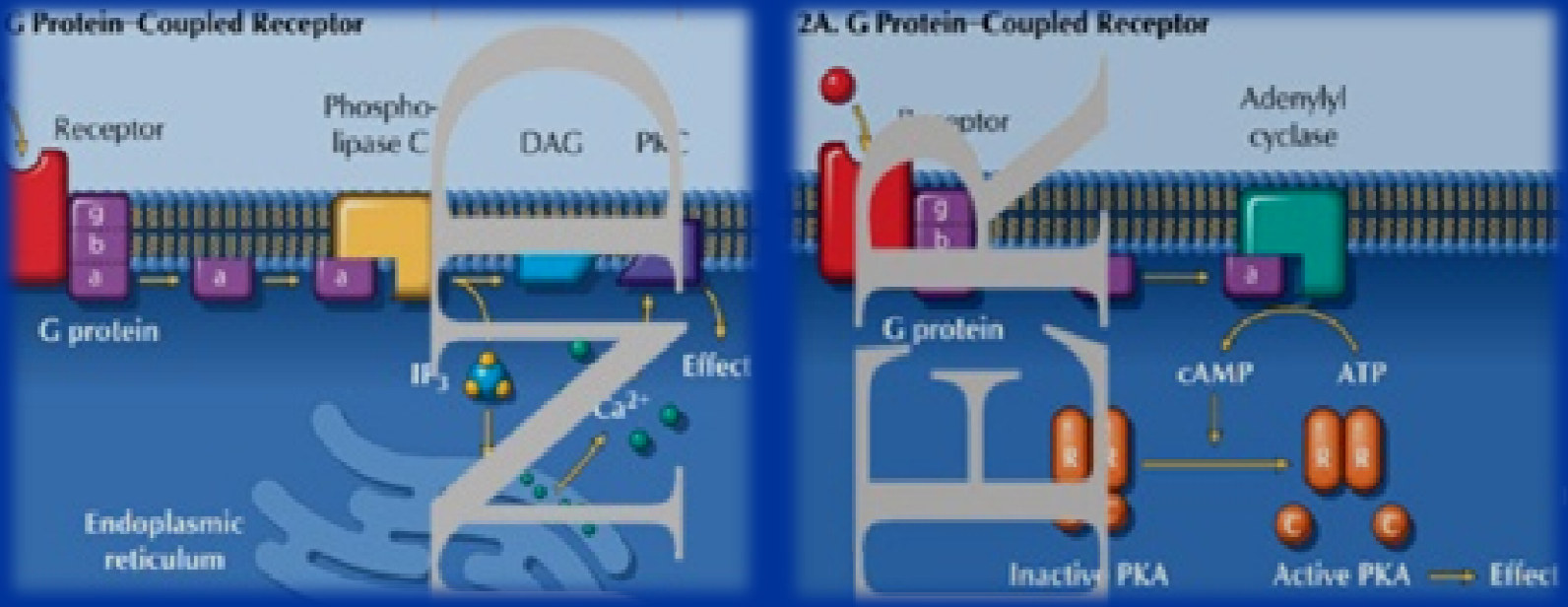
2. G-Protein Coupled Receptors



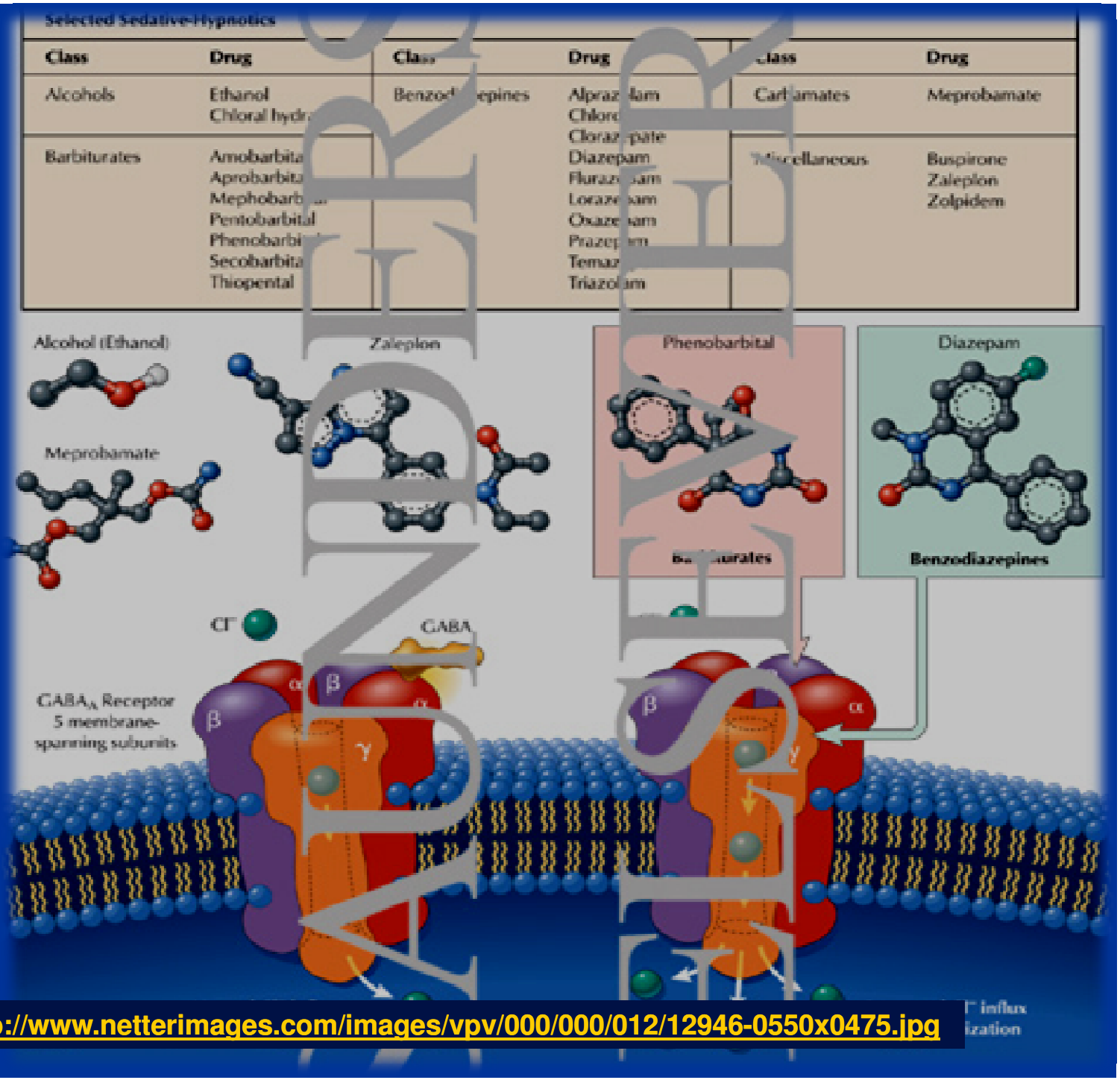
The Nicotinic receptors

- **Stimulated by acetylcholine**
  - **Results :**
    - **sodium influx,**
    - **activation in skeletal muscle contractions.**

The GABA receptors

- **Stimulated by: benzodiazepine, GABA.**
  - **Results**
    - **increased chlorine influx –>**
    - **cell hyperpolarisation**

GABA-ergic receptors



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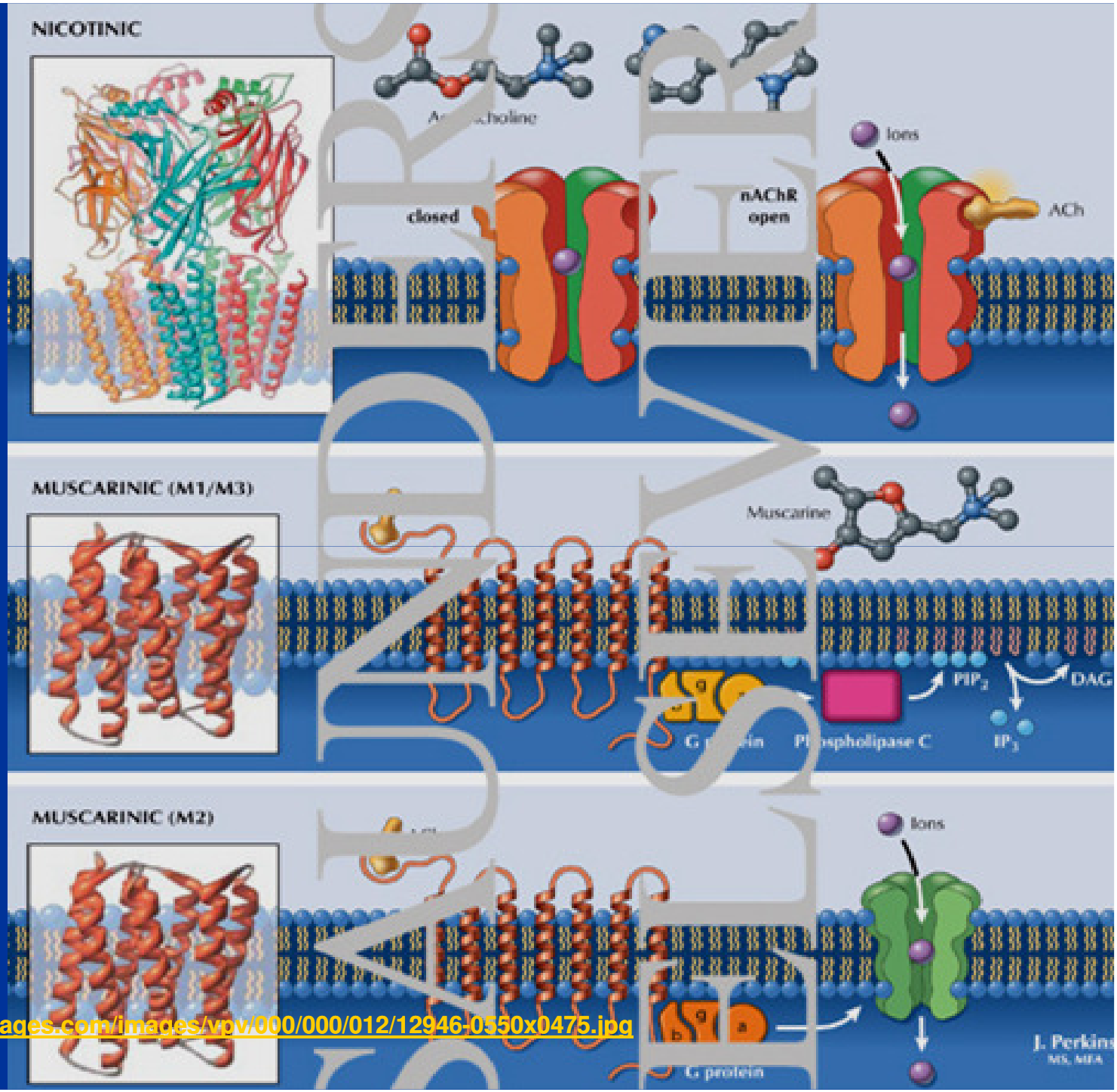
Cholinergic receptors



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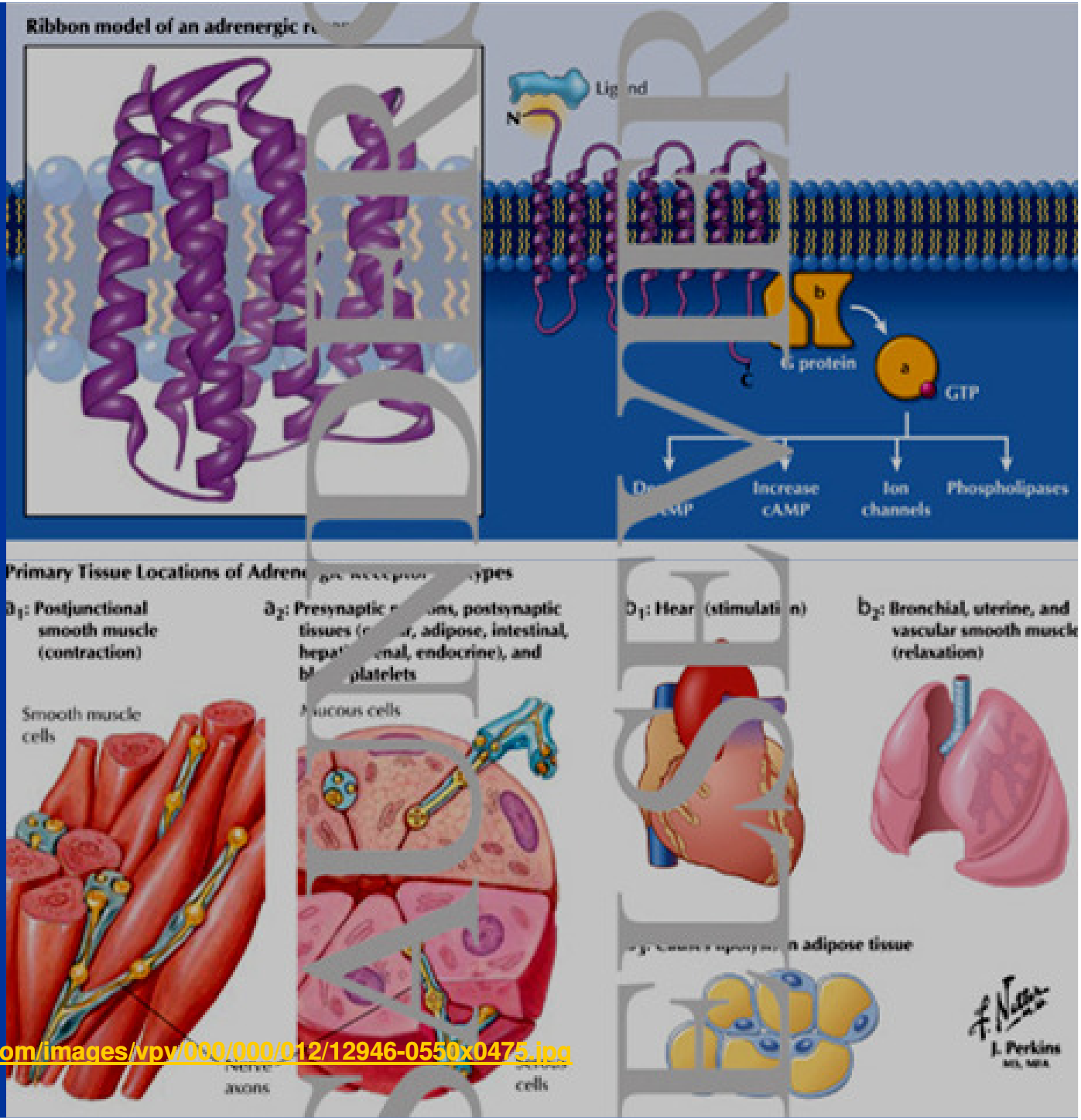
Adrenergic receptors



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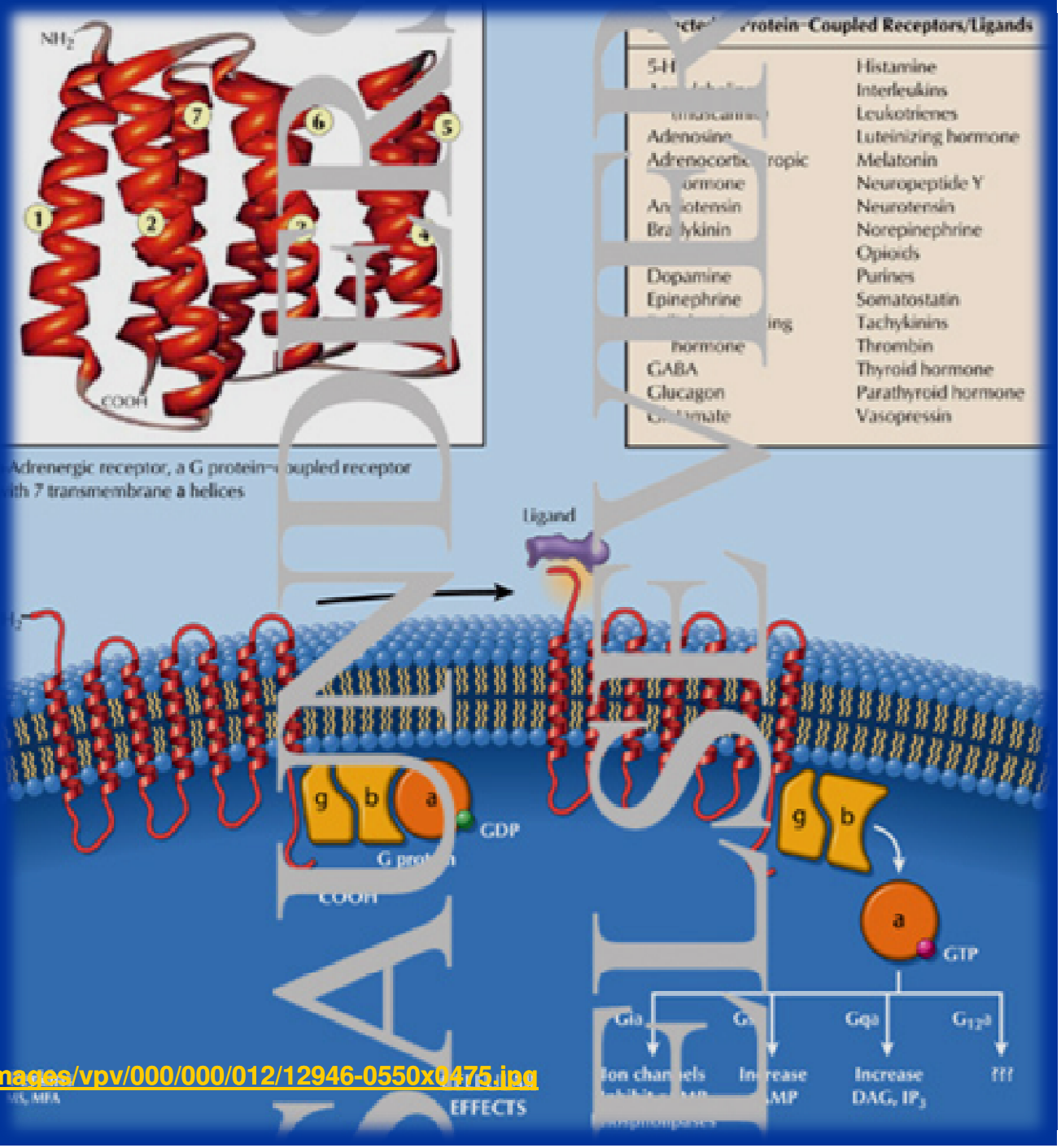
G protein binding receptors



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G -protein

The peptides are linked to the G Protein by three subunits:

- alfa (linked with GTP),
- beta and
- gamma

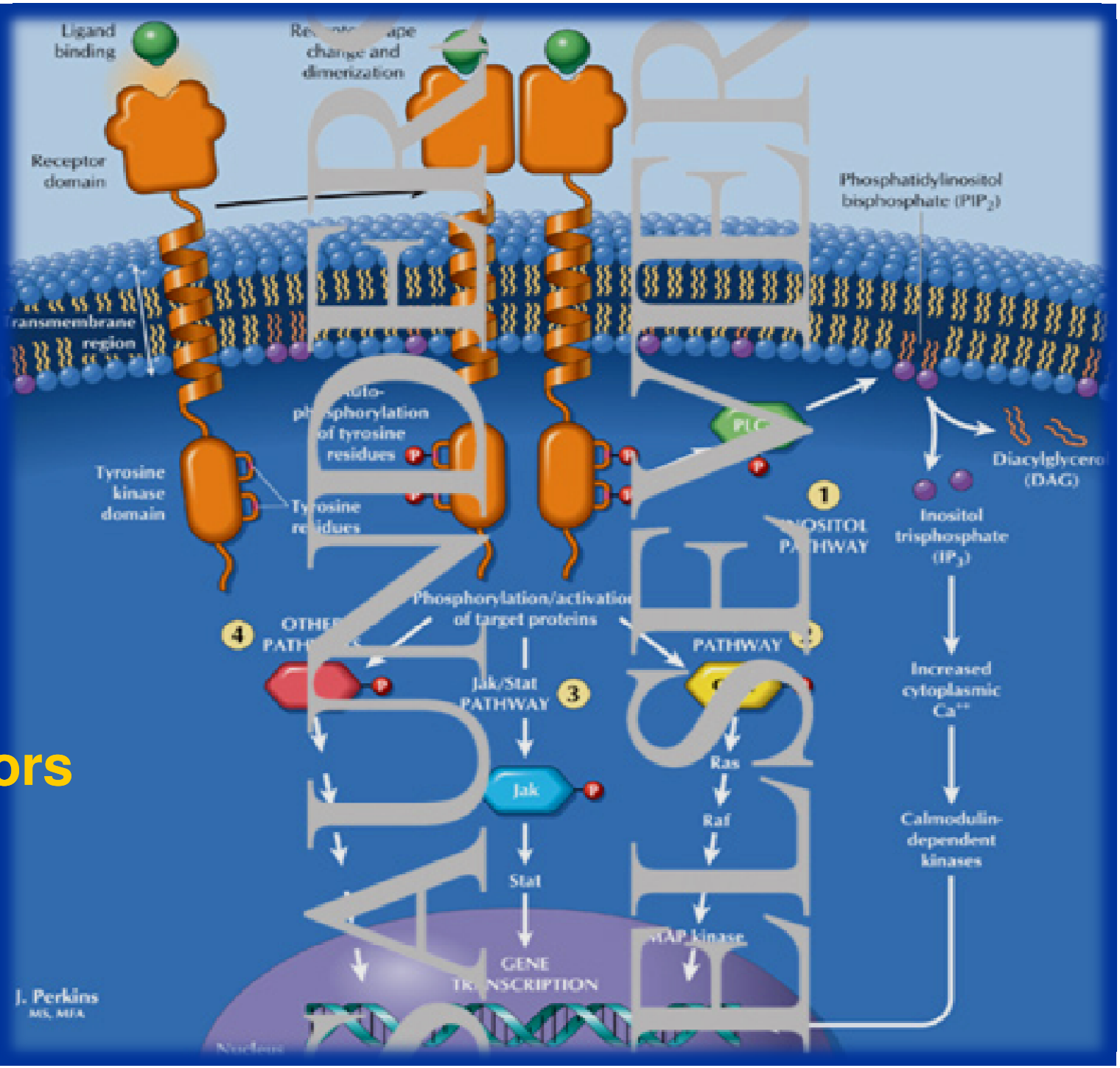
The linkage of the appropriate extracellular ligand = G protein activation

- GTP replaces GDP on the alpha subunit,

During the dissociation of the G Protein the subunits interact with secondary messengers, the answer: from seconds to minutes

For ex. alpha and beta adrenoreceptors

3. Receptors linked to enzymes

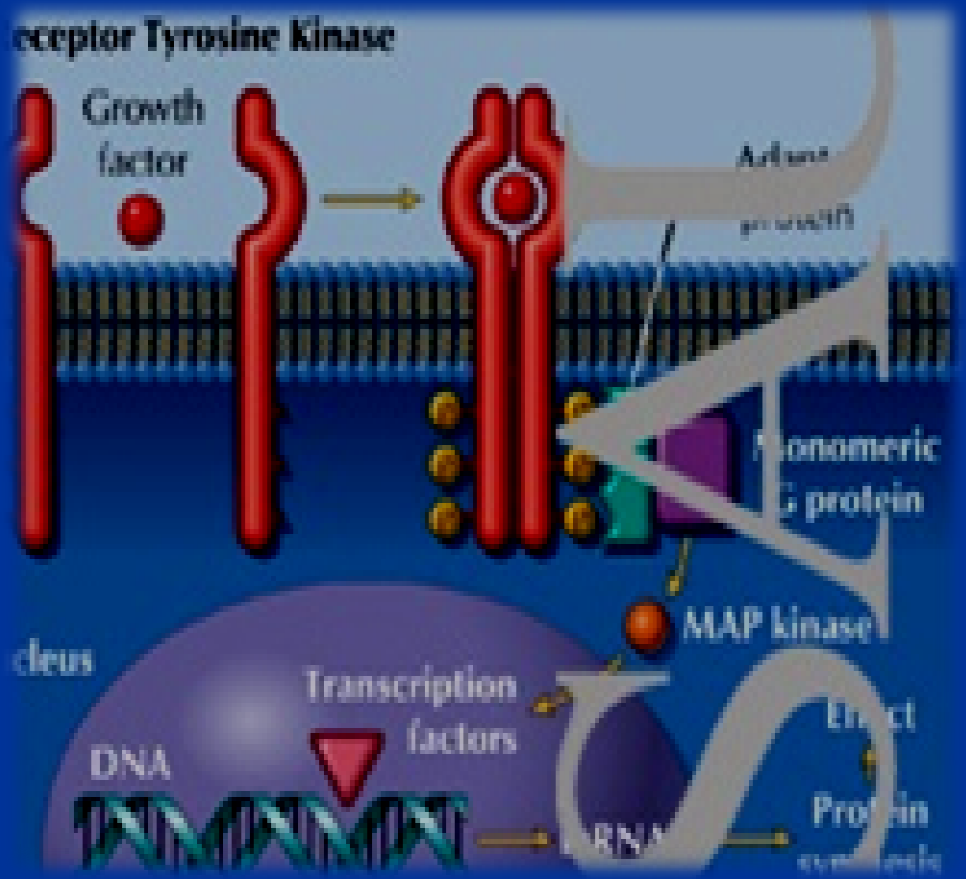


Specific cytosolic enzymatic activity as an integral component of the structure / function.

Binding an extracellular ligand, activates or inhibits the activity of cytosolic enzymes.

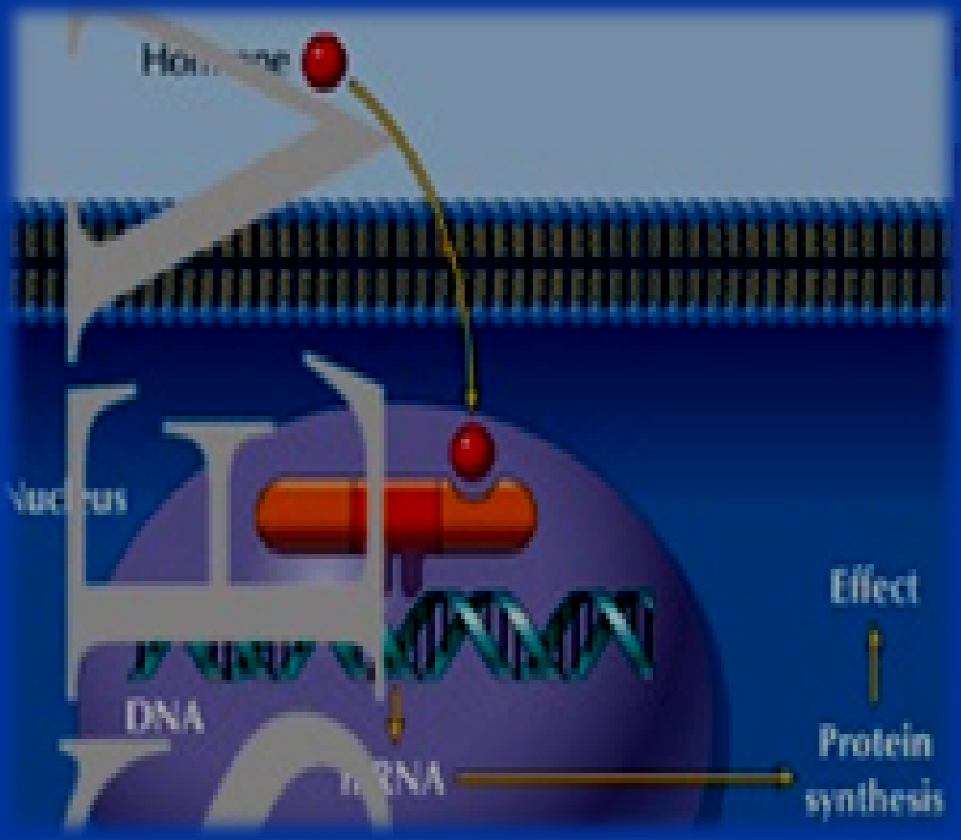
For ex: insulin receptors.

Duration of response: minutes to hours.



For ex. Tyrosine Kinase Receptors

4. Intracellular receptors



Completely intracellular, specific ligands must diffuse into the cell in order to interact with it.

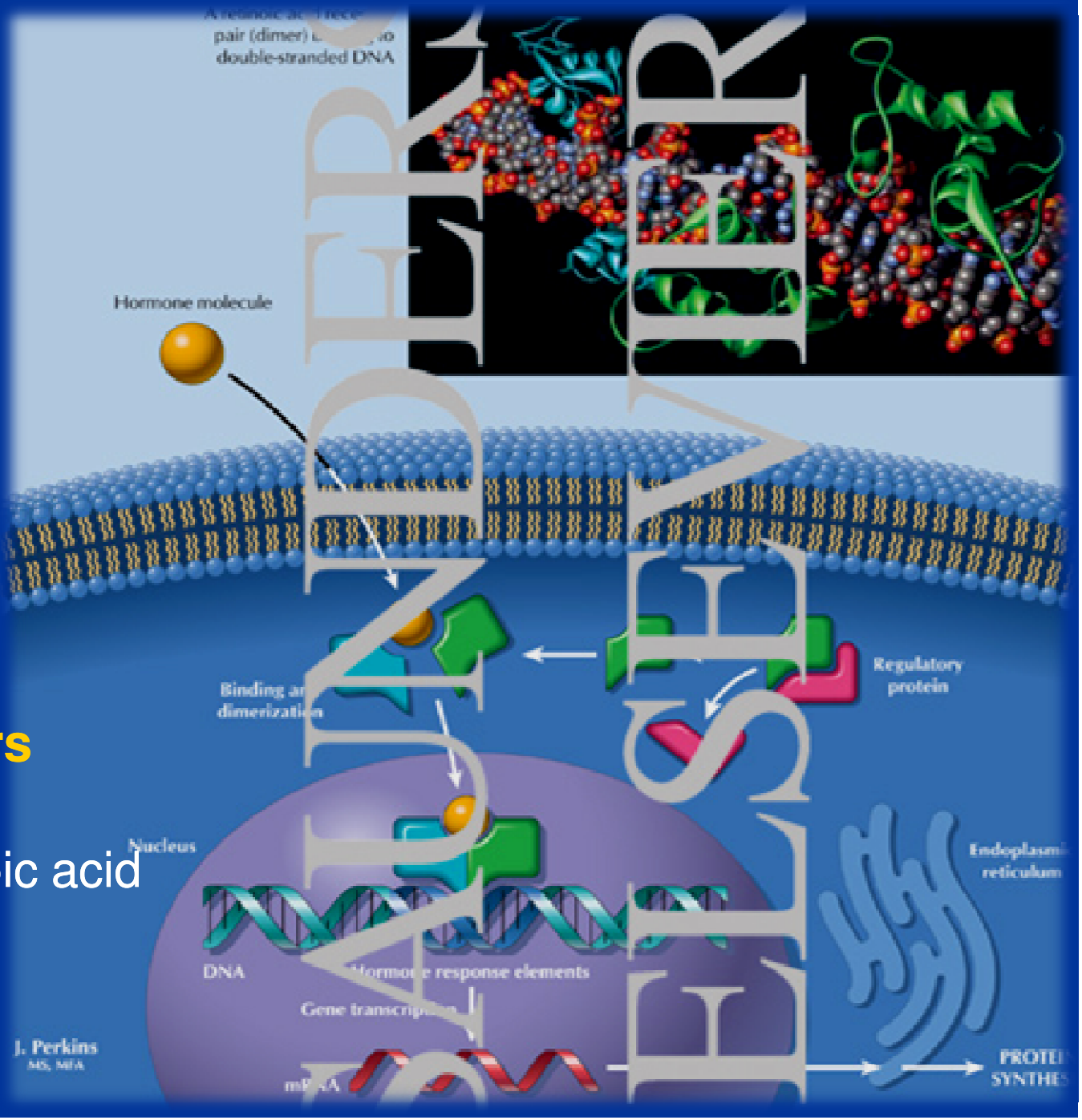
For ex: steroid receptors

Steroid receptors

- The ligand must have a good liposolubility in order to be able to cross the cell membrane.
- In the case of steroid receptor, the activated receptor-ligand complex will migrate to the nucleus, where it binds to a specific DNA sequence, resulting the regulation of gene expression.
- The duration of the response is long: hours or days.

Nuclear receptors

For ex.
Receptor for retinoic acid



Structure:

- the receptors have one or more active centers
- the active groups of the pharmacon will be fixed on the active centers.

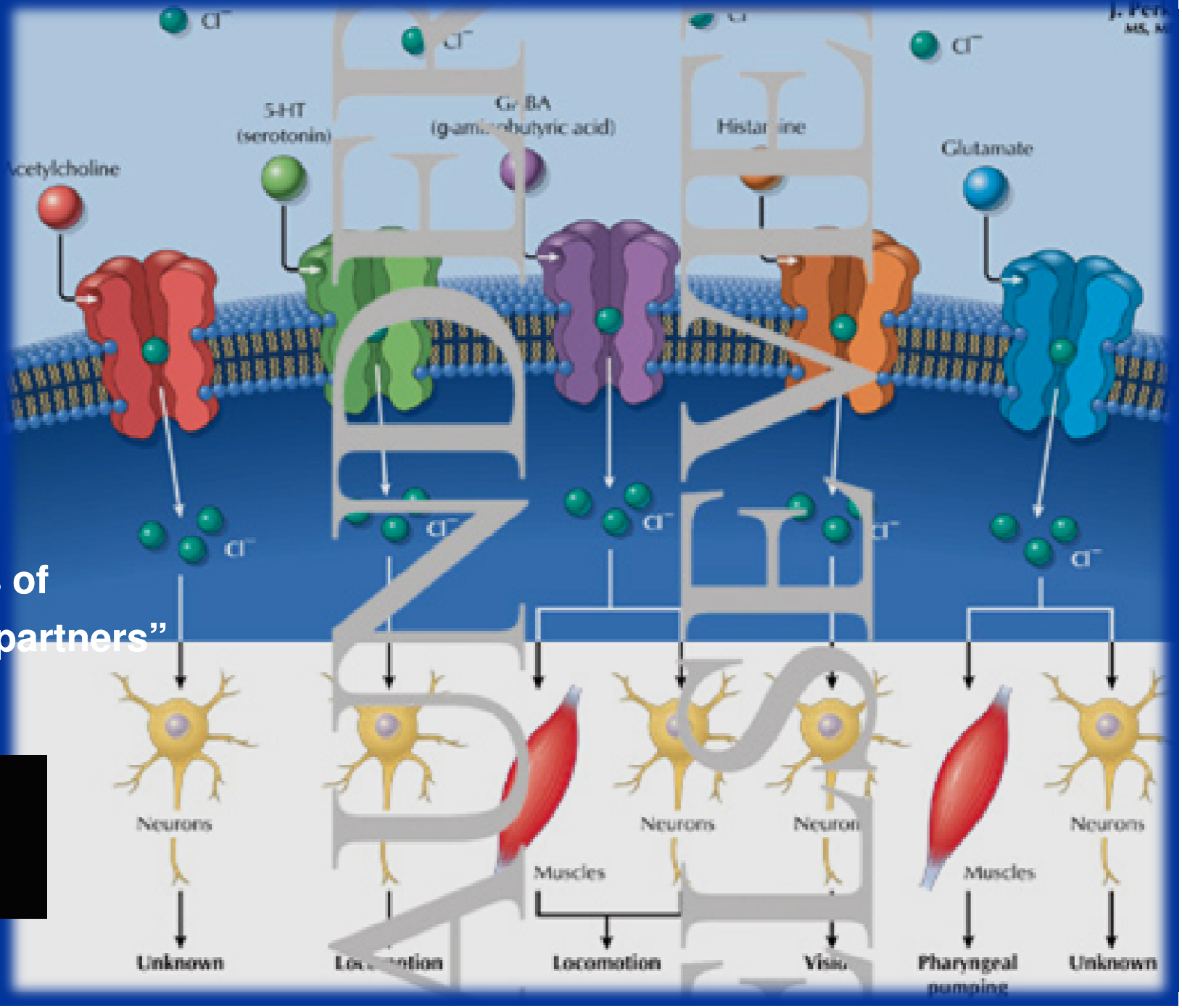
Preliminary aspects

of drug-receptor interaction

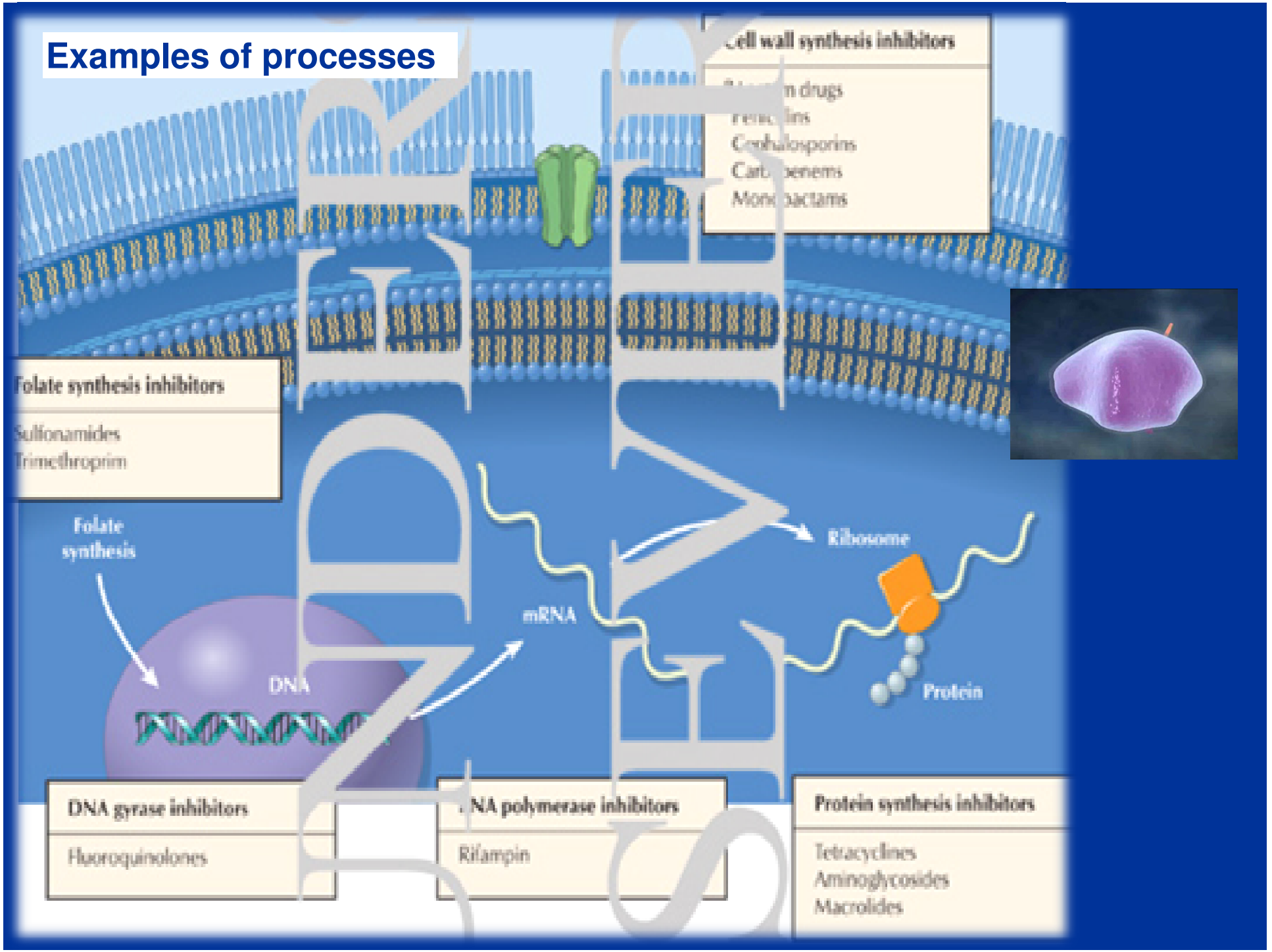
The Receptor theory starts from the following principle:

A substance will become active at a cellular level when a specific "molecular reaction partner“ will be present.

Examples of
“Reaction partners”



Examples of processes



This reaction partner (Receiver-R) must have specific qualities, so that a substance (or group of substances) could form a chemical bond with it (whose type does not play, usually, any pharmacodynamic role).

As a result, the changes in physicochemical properties of biological response from the action place, will constitute an "excitation" that will trigger "the effect".

In general , in case of fixation, are formed the following types of connections:

- hydrogen bonds,
- non-polar (Van der Waals),
- ionic and
- covalent.

The binding process has two stages:

- the first one, pharmacokinetic:

  the accumulated drug binds to the receptor in order to form drug-receptor complexes.

- the reaction is reversible and depends on the affinity between the substance and the pharmaco-receptors.

The second stage is: pharmacodynamic

The drugs' effect is the result of substance-receptor interaction, due to biophysical, biochemical and physiologic modifications of the substrate, on which receptors are fixed.

Drug

▼

Receptor

▼

▼

▼

▼

Signal

▼

▼

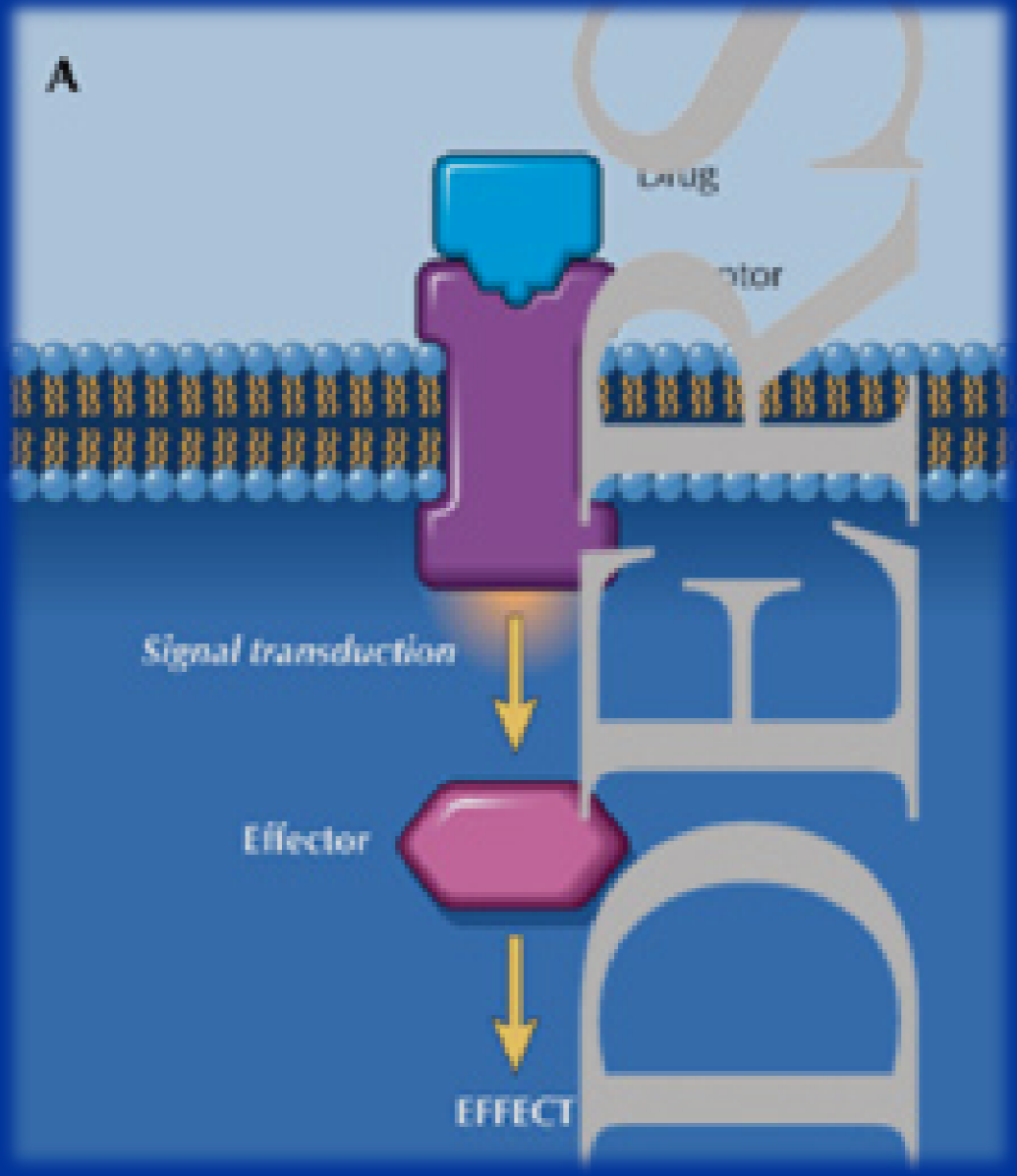
Effector

▼

▼

▼

Final effect



The possible situations

Drug

▼

Receptor

▼

▼

▼

Signal

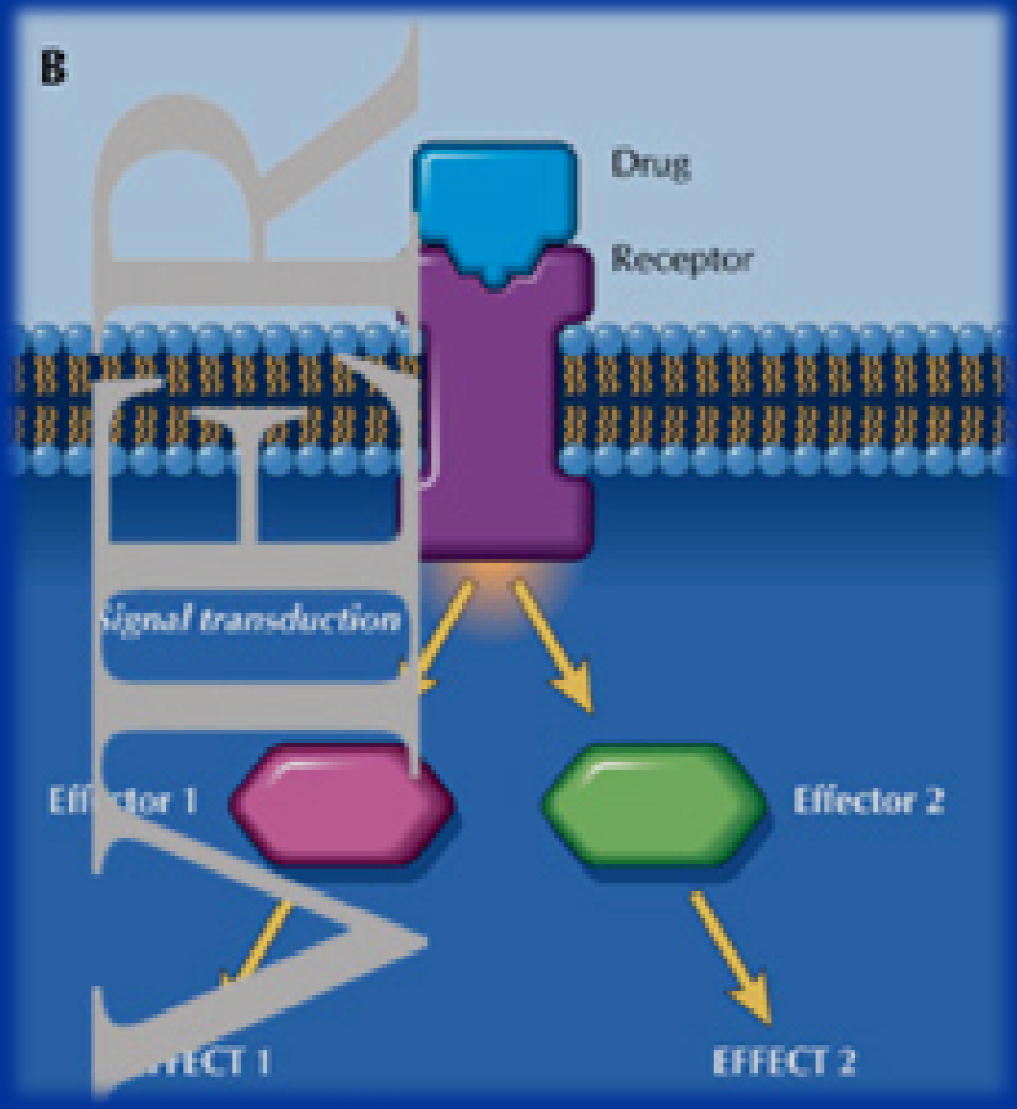
▼

▼ ▼

E1 E2

▼ ▼

Ef.1 Ef.2



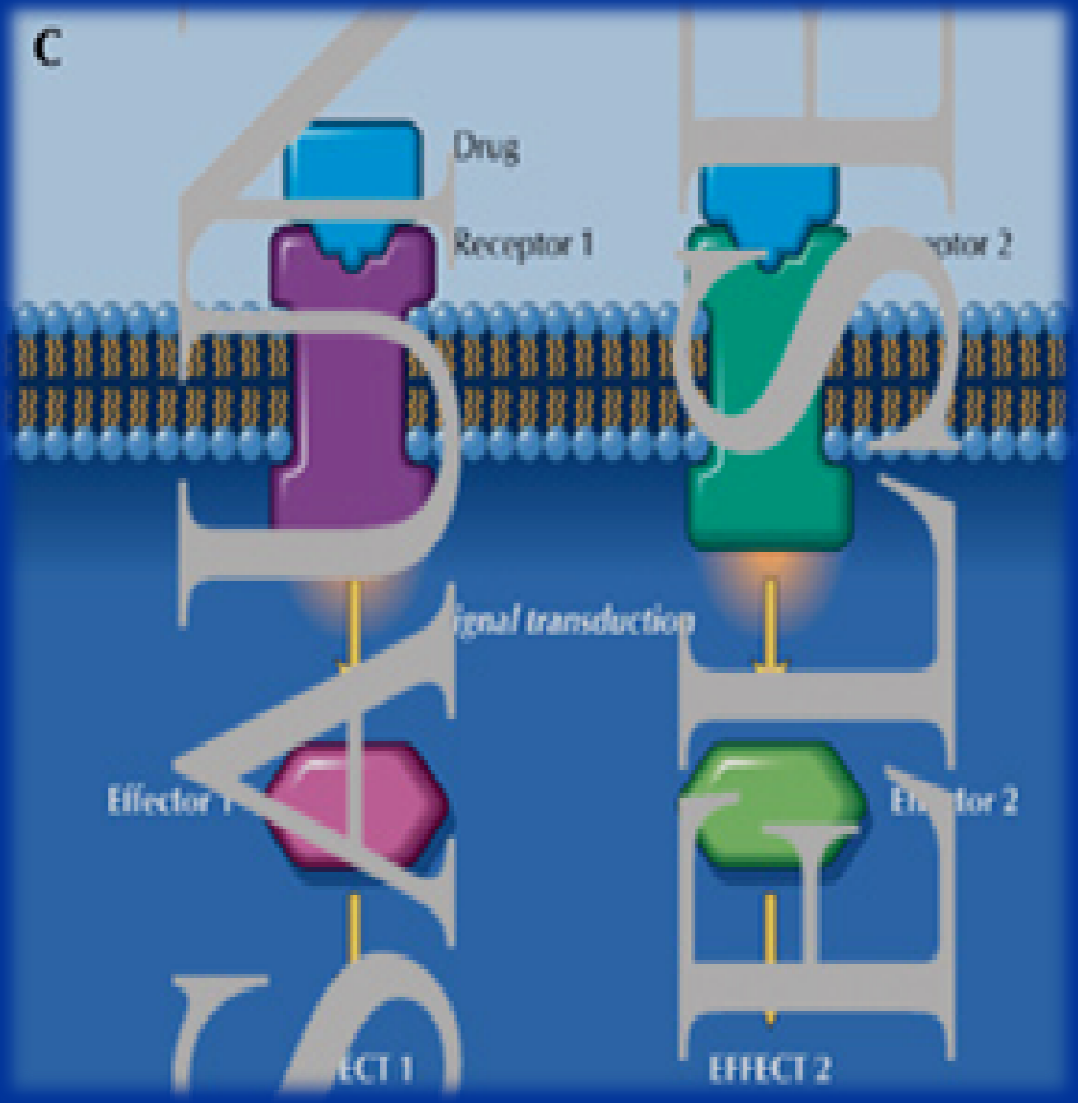
Drug

Rec1 Rec2

S1 S2

E1 E2

Ef.1 Ef.2



Of greater importance for determining the number of receptors and their properties is the rate of binding of agonists and antagonists.

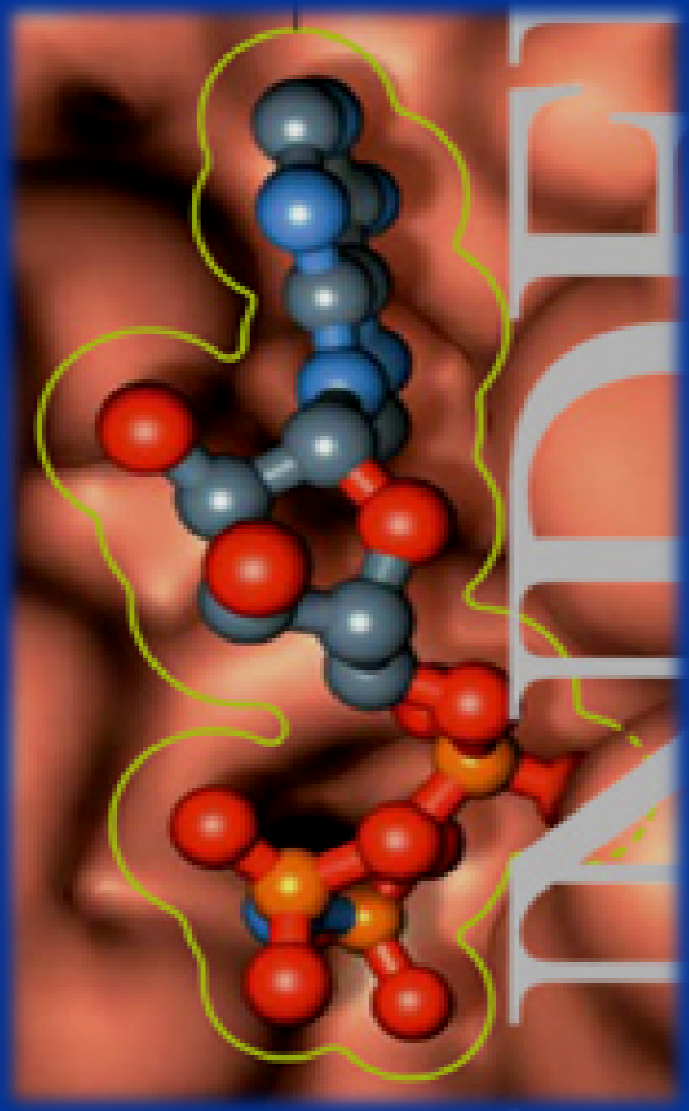
This consists in: *measurement of the binding specific capacity* **using radiolabelled material (of radioactive isotopes H^3, C^{14}).**

Activity

and receptor’s characterization

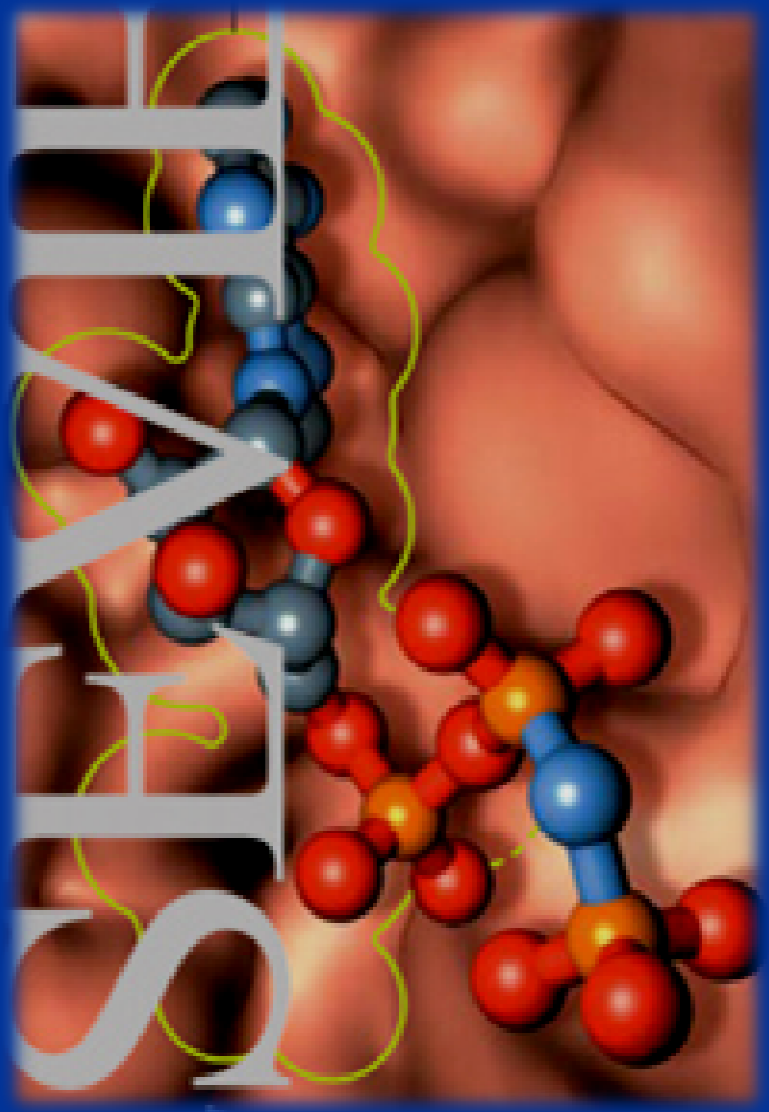
The *structure-function* relationship of receptors is based on the receptor representation (because when the receptor is assigned to specific chemical, physicochemical and physical properties, it is self explanatory that the agonist has an additional structure).

Possible situations:



1. an enantiomer can occupy an entire binding location.

2. an enantiomer can occupy only a part of a binding location.



About the biological effect of a chemical coupling certain assumptions can be made, but with reservations.

However, it can be applied one type of principle:

- modified structure - an effect that is applied to obtain analogous preparations in pharmacology:
if a substance is found to be effective, then the active parts of the molecule should not be modified.

For example:

- **phenothiazine (neuroleptic substance)** insignificant changes in the cycle and the carbon chain in **position 10** of the phenothiazine molecule =
- **benzodiazepine**
- **saline diuretics** etc.

Receptors

mode of action

The receptor concept was sustained in analogy with enzymes, comparing receptors with the active centers of enzymes.

In this way, the classic model **"key-lock"** was applied in drug action explanation:

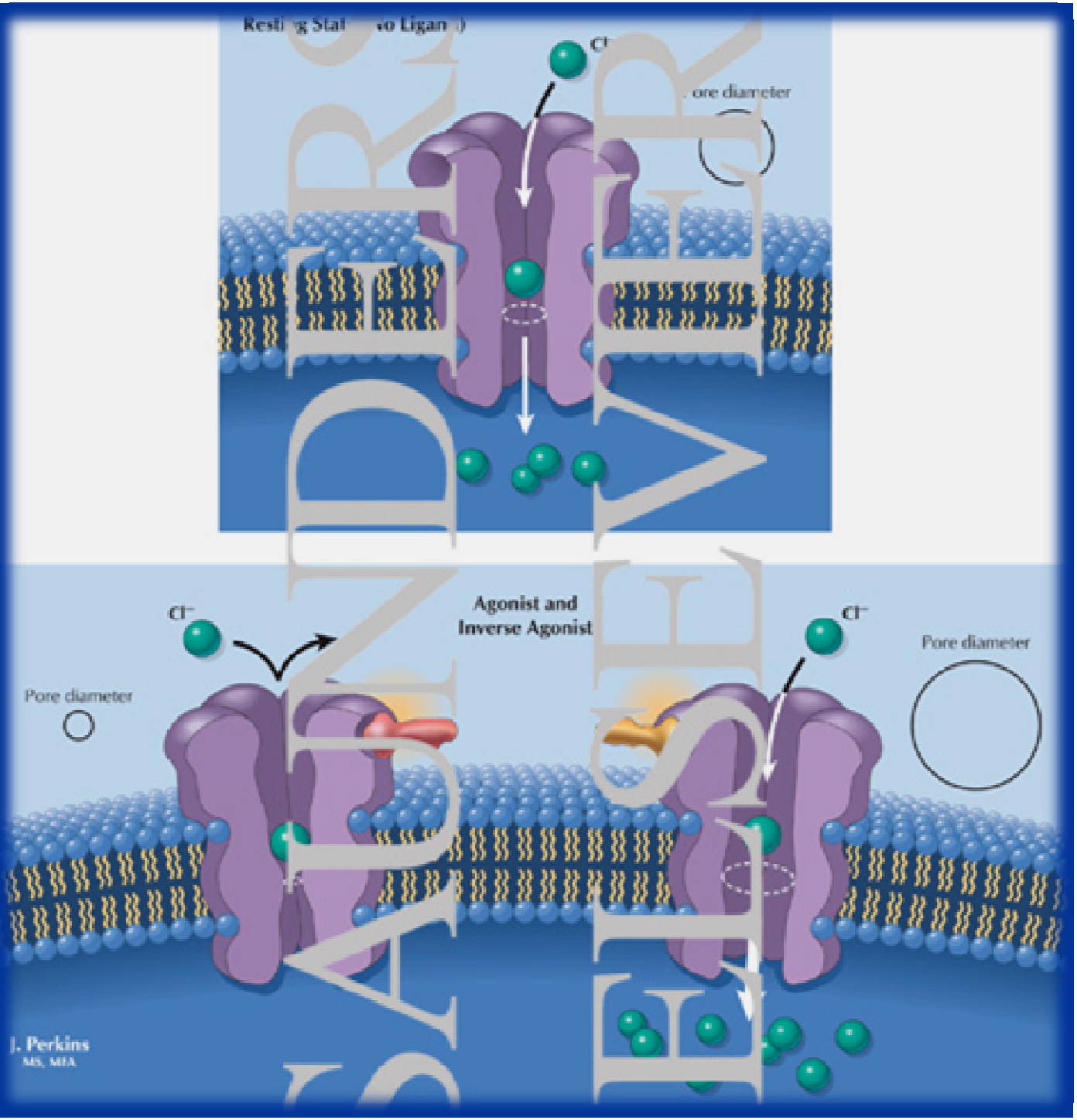
- the **“key" is the drug,** and
- **the “lock" is the receiver.**

Changing the form of the “key”, will clearly affect its ability to "come and open the lock."

Similarly, an antagonist is a drug able to enter into the lock, but unable to trigger the response.

He will remain there to prevent the entry of the "appropriate" key.

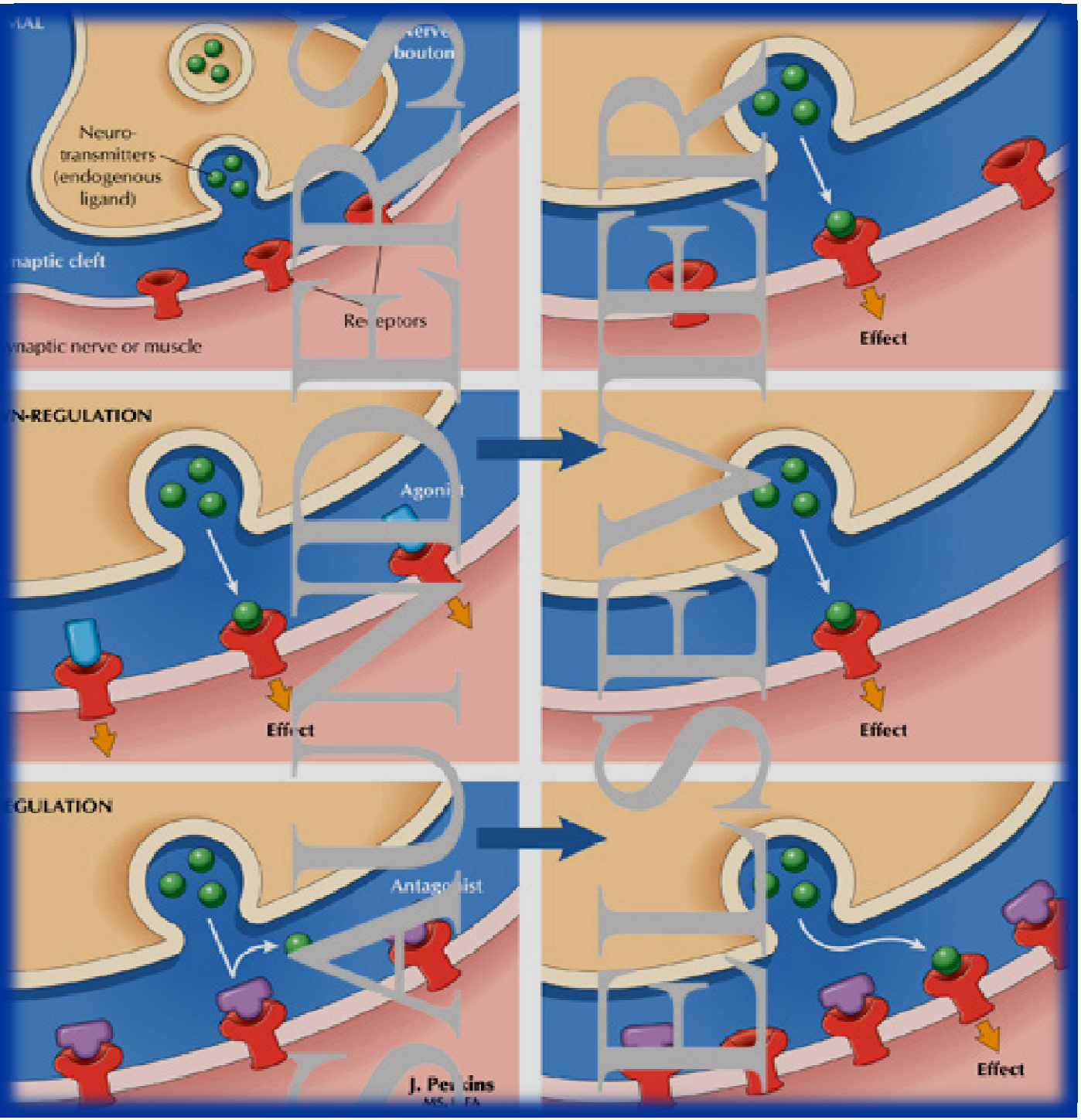
Possible situations



Drugs specificity for the receptor

It happens often that a drug's name is used to identify the population of receptors of cells in which they react.

Possible situations



A problem of particular interest was explaining the drug action immediate reversibility (e.g. by successive washes in isolated tissue preparations), when the drug action is dependent on a chemical bond between the drug and target.

The current hypothesis is that: drug molecules that are disorderly moving in biophase will couple with their receptors only when they are close enough to them.

Covalent and **coordinative** links are formed by certain drugs, as **a feature** of their own specific action (ex: organophosphate anticholinesterases).

Energetic links of this type are stable and many times **characterize the drugs** who have a **high action duration.**

Whereas the drug's approaching to the receptor is favorized by the electrostatic forces, that function on long distances (e.g. ionic coupling potential), short distances forces of weaker bonds are numerous, and thereby, more important for the association recovering when there is a high degree of complementarity.

Coupling of a drug to receptor is classified as:
with **high affinity** / **low capacity.**

Demonstration of the coupling is not sufficient to highlight the place where the drug acts.

The sites where drugs are binded, but is not produced any type of associated effect, most of the time, are with:
high affinity / **low capacity** (e.g. serum albumins).

They are also known as:
drug **"acceptors"** or **mute"** receptors.

Drug molecule can be switched from the receptor when its kinetic energy is increased by thermic collisions, at a level that exceeds the coupling power.

Neuromuscular junctions are among the few detectable histological entities that contain numerous receptors placed on the cell surface.

Changeux et al. showed that nicotinic receptor for acetylcholine (250 kDa glycoprotein) contains 5 functional subunits, where two are identical.

Transport mechanism (pore, channel or ionophore) through whose opening and closing pulse allows passage of sodium ions through the membrane is the one who initiates the response.

The authors have issued the idea that the affinity of coupling sites **is not constant.**

In the presence of the agonist, the conformation of the binding sites is changing, meaning that the affinity for the agonist will grow.

During the active phase, the ionic gate will open transitory, and the physiological / pharmacological response will be able to install.

If the agonist is still present, the coupling affinity will reach a high level, while the ionic gates will be closed.

The reduction or absence of a response, that follows it, is called **desensitization.**

Both activation and **desensitization** are prevented in the presence of a competitive antagonist, for which the site found in state of repose has a higher affinity than the activated or desensitized site.

A big part of the available information regarding the transmembrane ion channel activity and the ways in which drugs or other ligands can modulate their activity, were recently discovered:

- an ultrafine micropipette is applied on the plasma membrane area that contains nicotinic receptors (for ex: terminal motor neurons).

Using this technique it was possible to define the concentration of acetylcholine required :

- to produce the opening of ion channels,
- to establish the duration of opening,
- the size of the flow of sodium ions that enters the cell

and

- the effect of drugs.

In the attempt to propose some models that are able to explain the action of drugs, was introduced the **mobility** term, with reference to individual receptors from the surface of the membrane.

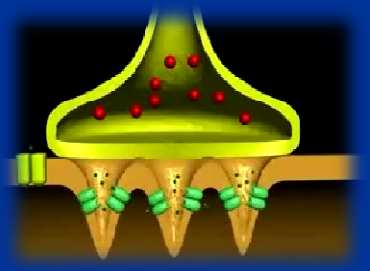
It has been suggested that the phenomenon of coupling may induce a change in the membranar receptor conformation (or of a group of associated receptors).

The result will be **opening a pore** in the membrane, allowing **ionic flow realization.**

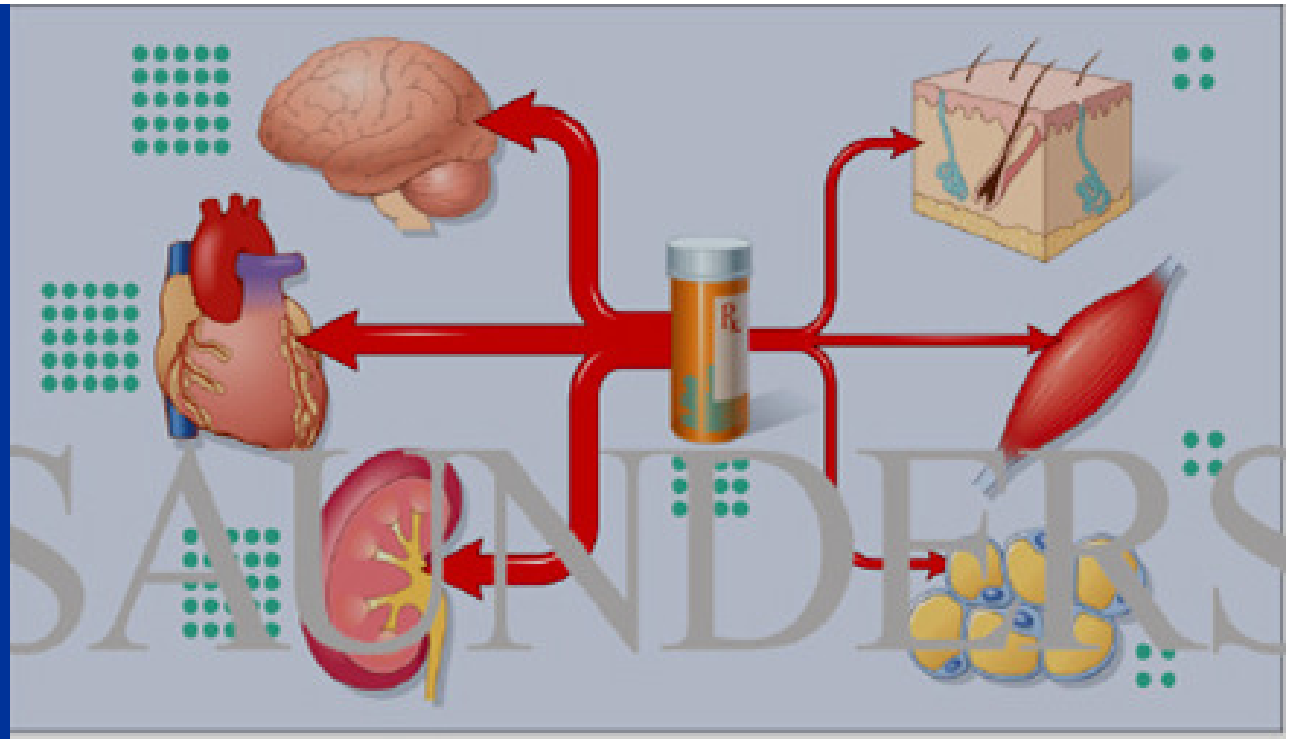
Further, **membrane depolarization** will be produced.

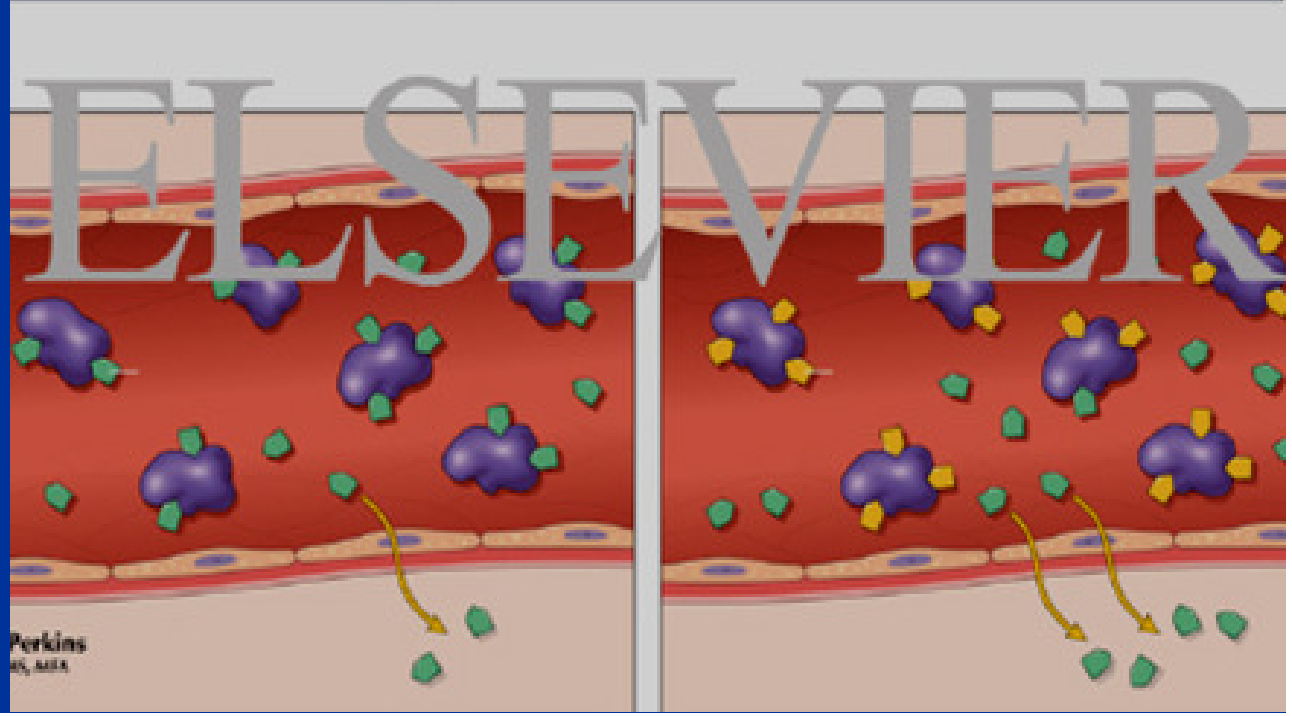
The ability to move laterally is totally compatible with the fluid mosaic model of the structure of plasma membrane.

Drugs that can cause opening or blocking the membranar channels for potassium are, also, of interest for regulating muscle tone of blood vessels.



Nicotinic acetylcholine receptor





Some drugs can affect muscle tonus, although each class works on other group of receptors.

Although an antagonist can inhibit the action of a group of drugs by blocking its receptors, the tissue may still respond in a characteristic manner if a drug that will activate another type of receptor is administrated.

in the case of nicotinic cholinoceptors, a demonstration often cited, is alpha-bungarotoxin, a toxin extracted from the snake venom, that will connect to the receptor with high affinity and specificity.

The nature of receptors

The accessibility of studying enzymes, as well as the facility to estimate the concentration of the substrate and product has allowed enzymology as science to advance and provide a wealth of valuable concepts in the study of receptors.

The classic receptor from the surface of the cell is, normally, an **included lipoprotein** or a lipoprotein that penetrates the plasma membrane of the cell, just as the active site of an enzyme is known to be a small portion located within a folded protein.

The substrate **will bind** with the active site that will catalyze a change in the substrate structure.

The key - lock analogy requires a **"rigidity"** of the reactants and, therefore, it is not entirely compatible, since the vast majority of drug molecules are **"flexible"** as structures.

Some enzymes suffer changes of the chemical conformation, and the induction of **a change** in the conformation of the receptor can be necessary for the drug action.

Clearly "rigidity" implies (requires) a complete specificity of the drug for the receptor.

Such a specificity is not absolutely necessary, the enzymes can be inhibited irreversibly, just like some receptors, by molecules that are covalently bound to their active site.

A false substrate can be coupled with an enzyme, which later will be separated at **a much lower rate** than the appropriate substrate.

Likewise, an antagonist who binds to the receptor, does **not cause any response** and remains coupled to the receiver a relatively **long time.**

Various enzymes, that can catalyze the same reaction were called **isoenzymes.**

The differences in the apparent sensitivity of the receptor at a number of activator drugs have led to the concepts of:

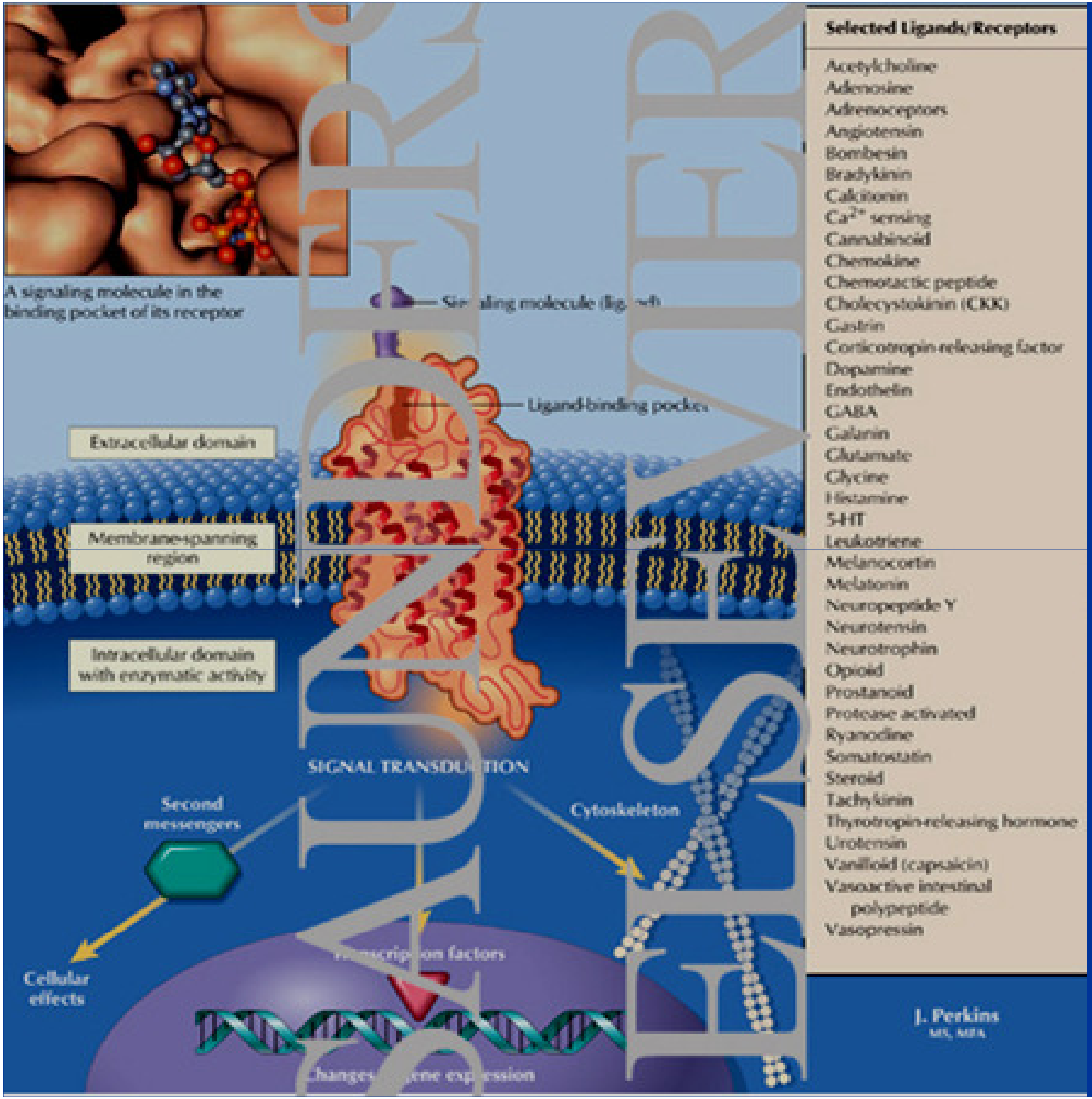
- ***isoreceptors*** (e.g. alpha & beta receptors for adrenaline)

and

- ***allosteric*** **which describes a change in shape induced by an enzyme, after coupling to a site other than the active one, with a different substance than the normal substrate.**

The combination of the so-called allosteric site may result in allosteric activation or inhibition of the enzyme by changing the access to the active site.

This mechanism was (also) used to explain the ability of a drug to alter the action of another drug at the receptor site.



Specific Ligands/ Receptors

Comparison of the mechanisms of action of the most important mediators that engage and activate their specific receptors (after, Brander, 1991)

Mediator	Membrane cell	Within the cell	Action	Effect	Conclusion
Steroid	Diffuses through the membrane	Couples the receptor protein to the DNA	Allows transcription of genes	Increased synthesis of regulatory proteins that are specific steroid	Catabolism of these regulatory proteins
Nicotinic agonist (A.co)	Coupled to ligand-receptor recognition, Open the ion gate ; membrane is depolarizing	[Na +] increases [K +] decreases [Ca^{2+}increases	Releases the Ca^{2+} ions and opens electrosensible channels for Ca^{2+} ions	Myofibrils are shortened by coupling $Ca^{2+}$and muscles are contracting	[$Ca^{2+}$is reduced by exiting the cell
Beta-adrenoceptor agonist or (adrenaline)	Binds to the β-adrenoceptor that will complex the G protein. this will bind GTP and will activate adenylatecyclase.	ATP→cAMP; the cAMP concentration increases	Activates proteinkinase A-dependent on the cAMP	Phosphorylates (activates) the target enzyme and the action is expressed, for ex: lipolysis	Phosphatases deactivate the enzyme, phosphodiesterase hydrolyze cAMP.
Calcium ions	crosses channels for Ca^{2+}	The majority will couple with CAM	CAM –Ca^{2+} activates the dependent kinase. modifies membrane proteins,Ca^{2+} binds to Troponin C	The function in question is positively modulated. Permeability changes. Muscles contract.	Ca^{2+} ions are pumped out of the cell. Enzymes are inactivated by dephosphorylation
Alfa – adrenoceptor agonist or (noradrenaline)	binds to receptors that mobilize Ca^{2+}, Activates phospholipase C, cleaves PI bisphosphate to IP3 and DAG.	IP_3 diffuses into ER DAG comes into contact with Proteinkinase C	Activates the ER receptor, proteinkinase C is activated	Releases bound Ca^{2+}, and the dependent function is stimulated, for ex: vasoconstriction Phosphorylates target proteins, for ex: nicotinic cholinoceptors	Recycled in the membranar .PI

Therefore, there where not unexpected the cases that demonstrate drugs' ability to inhibit "in vitro"

Although, it does not automatically mean that this mechanism is relevant for the same drug "in vivo".

Demonstration of the process depends on establishing a relationship between:

- dose,
- local concentration and
- the degree of inhibition of the response.

The inhibition of enzymes can be realized through several mechanisms:

- the active site can be **irreversibly blocked** by an antagonist who binds covalently (e.g. heavy metals)

Reversible inhibition can be achieved by using agents that are structurally related to the physiologic substrate, but dissociate slowly from the enzyme (e.g. physostigmine and cholinesterase).

Such agents can act (also) as **"false substrates"**, that can be **"processed"** by enzymes in a fake product.

Drugs can inhibit enzymes by interfering their synthesis or by removing essential cofactors.

Another example is the case of benzimidazoles, where they interfere with enzymatic systems (fumarate reductase and succinate decarboxylase), ATP synthesis sites, essential for the energetic metabolism of helminths.

One way to characterize the receptor consists in isolating and studying them.

Initially researchers tried to isolate the nicotinic acetylcholine receptors in a tissue, in which they are in a high density namely the fish's electric organ.

The *"in vitro"* binding of an agonist can not be made in the same manner as for the *"in vivo"* and, therefore an accurate assessment of the functional capacity of receptors is not yet possible.

Isolation

and identification of receptors

Active sites of enzymes,likewise, can be perceived as receptors.

In order to trigger a certain excitations it is necessary to:

- achieve a number of coupled sites
- couple a number of receptors per unit time (the coupling rate of the receptor)

Recognizing the role of cytosolic receptor protein in binding with steroids (by binding to the nucleus and by inducing the synthesis of a specific structural or regulatory protein) has clearly established the mechanism that connects the binding of a chemical messenger at a specific site and the expression of a characteristic cellular response of that messenger.

The definition

of agonists and antagonists

Substances that stimulate receptors are called agonists.

For example, morphine is fixed on the opioid receptors (OR), with analgesic effects and depression of the respiratory center.

Nalorphine, its antagonist, will keep the analgesic action but will produce a stimulating effect of the respiratory center (so it is used as antidote in morphine poisoning).

The agonist, is a drug that can be coupled with a receptor and causes a **positive response** in the tissue where receptors are located.

The maximal response

is considered the response with an intensity that can't be overcomed by subsequent agonist administration (by increasing the agonist concentration).

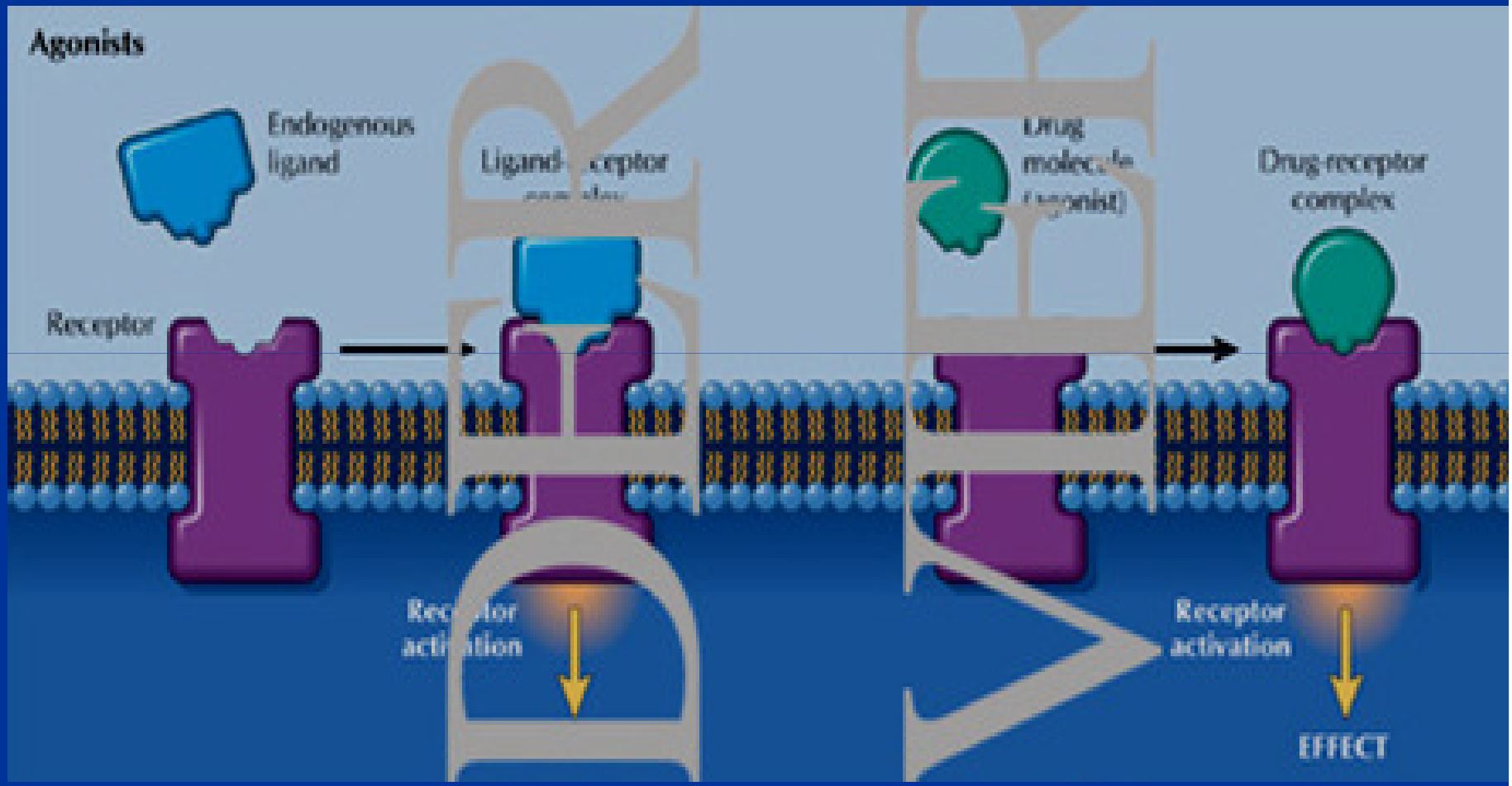
Body potent agonists (such as acetylcholine, norepinephrine and histamine) are identified by a **high coupling / decoupling speed.**

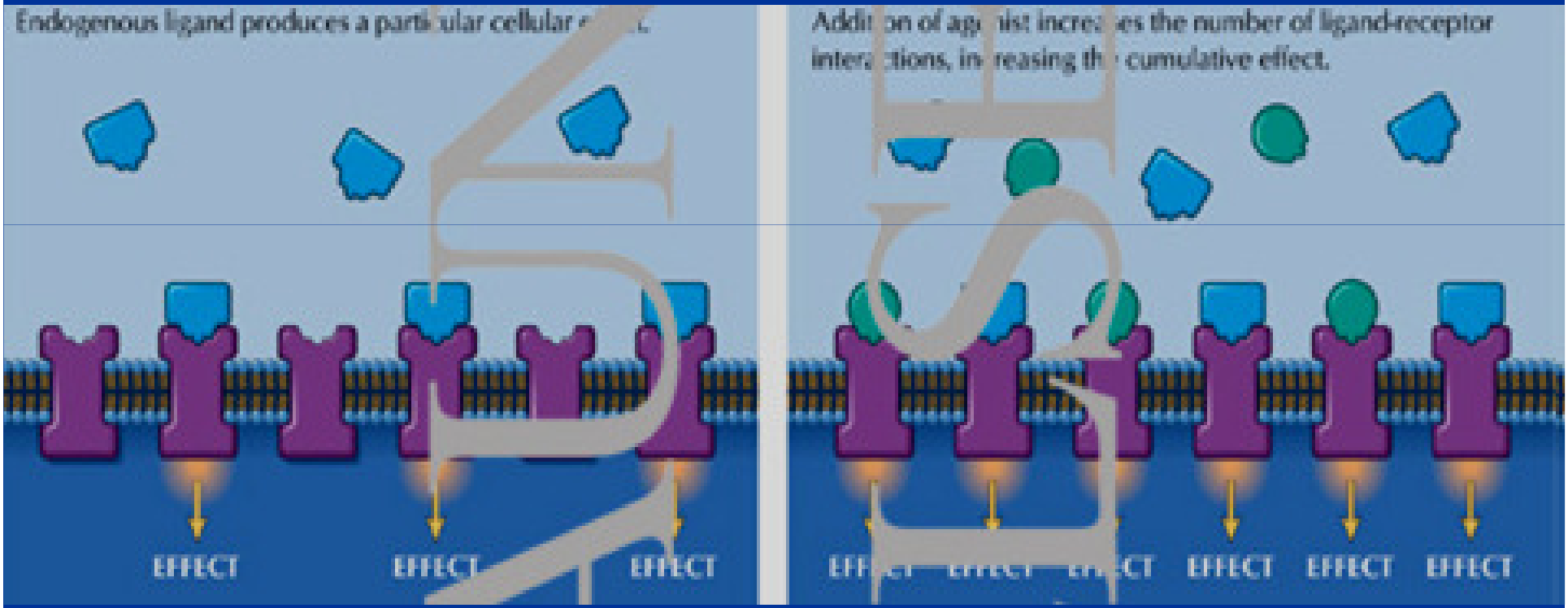
In this context, the image about the receptors' utility, regarding the competitive antagonism of substances is clarified.

- agonists are substances that are bound to receptors and that will induce modifications of cell properties (high affinity and "intrinsic" activity)

- competitive antagonism binds reversibly to the same receptor and will not induce any change (high affinity and absent "intrinsic" activity), but will block some receptors (e.g. decreases the concentration of active receptors) and in this way the agonist will lose efficiency (e.g. acetylcholine- atropine, acetylcholine-D-tubocurarine, noradrenaline - sympaticolitics, histamine- antihistamines).

1. Agonists


Agonists
Endogenous ligand
Receptor
Drug receptor complex
Receptor activation
Receptor activation
EFFECT


Endogenous ligand produces a particular cellular effect.
EFFECT
EFFECT
EFFECT
Addition of agonist increases the number of ligand-receptor interactions, increasing the cumulative effect.
EFFECT
EFFECT
EFFECT

On the presence or absence of the appropriate receptors will depend if a cell will respond to the administration of a chemical messenger type:

- exogenous or
- endogenous.

The nature of any cell response to its receptor activation depends on the cell.

It produces the response that represents its usual function (e.g. muscle cells are contracting if membrane depolarization at the neuromuscular junction exceeds a critical level).

2. Antagonists

Antagonist

is considered a drug that, when is administered before or concurrently with an agonist will diminish or abolish the agonist' response.

It is said that antagonism is:

- **permanent,**
- **irreversible or**
- **non-competitive,**

if the intensity will remain unaffected in the presence of increasing concentrations of agonist.

The antagonism of a drug toward the generating capacity of response, at another medication, is a negative response that can be played using the dose - effect curves.

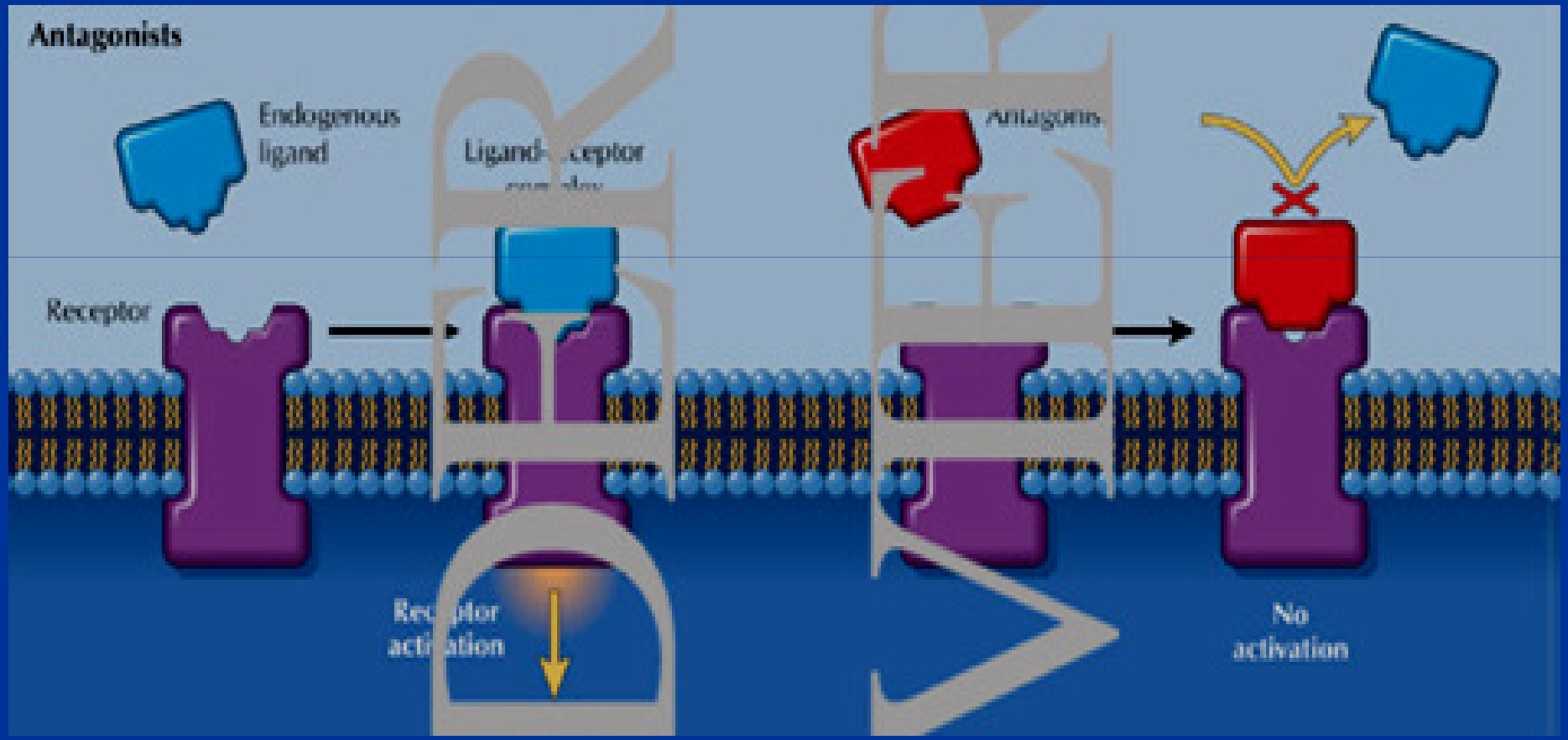
By definition, the antagonism is non-competitive when, in the presence of an antagonist, the agonist is no longer able to produce the maximum effect, regardless of the increase of its concentration.

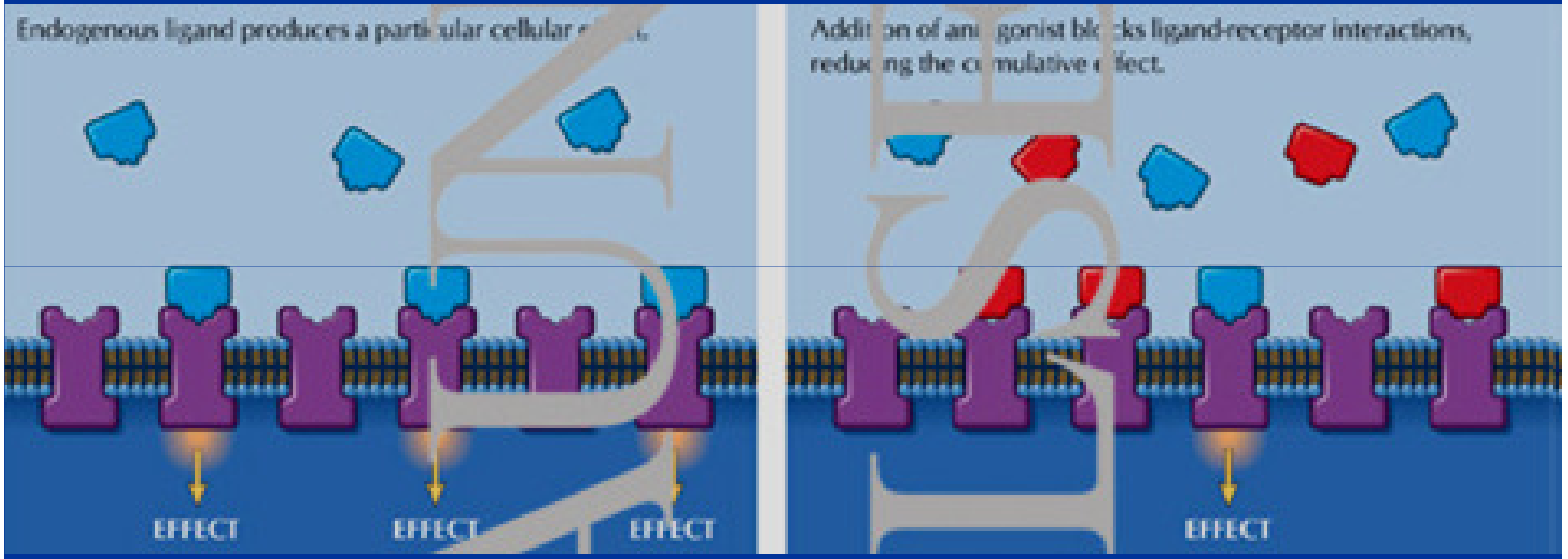
In this situation, dose-response curves of agonist in the presence of antagonist's gradually increasing concentrations will become progressively less inclined, and the maximum possible effect will decrease, as the concentration of the antagonist increases.

If the increasing agonist concentration reduces or exceeds antagonism, the antagonism is called:

- ***temporary*,**
- ***reversible* or**
- ***competitive.***

Antagonists




Endogenous ligand produces a particular cellular effect.
EFFECT
EFFECT
EFFECT
Addition of antagonist blocks ligand-receptor interactions, reducing the cumulative effect.
EFFECT

Competitive or non-competitive reduction** of the response to an agonist are types of **pharmacological antagonism.

When a drug reduces the effect of another drug by inducing a contrary response, by activation of other receptors, we talk about a physiologic antagonism.

For example,

antihistamine drugs pharmacologically block the action of histamine, but effects of histamine can be obtained also with adrenaline.

In the treatment of poisoning, the continuous drug absorption through the gastro-intestinal tract, sometimes, may be prevented by transforming the toxic substance into an insoluble form.

3. The secondary messengers

There where many progresses accomplished during the last years, concerning the identification of the binding path for certain chemical messengers and specific receptor proteins from the membrane, the way this path can intensify or diminish the cellular function.

In most cases this phenomenon consists of a modified rate of entrance or synthesis of the so-called secondary messengers.

- In this case, in contrast with the complex:

  steroid hormon – cytosolic receptor protein, the **drug receptor** complex will no longer be the final intracellular effector.

- In this case, the integration of the drug-receptor couple and **the activation** of the cells functional apparatus will be made by an **intracellular secondary messenger.**

Several chemical mediators, including:

- neurotransmitters,
- endocrine hormones and
- tissue hormones,

After the complexation with the membrane`s receptors, they can determine the activation / inhibition of adenylate cyclase enzyme at the membrane level.

The concentration of cyclic AMP from the cell will increase, and consequently, the mediator will react as an intracellular messenger, and with the help of protein-kinase A, will adjust those enzymes that mediate cells' characteristic response.

This is the process from where the secondary messenger term derives.

Essentially, drug-receptor complex has an increased affinity for adenylatecyclase, forming a large complex.

In fact, a protein realizes the bond between the complex: drug + receptor + adenylate cyclase and binds nucleotide guanosine triphosphate (GTP).

Adenylate cyclase's maximum activity requires the presence of the combination:
drug-receptor + coupled protein + GTP + enzyme.

Cyclic GMP is another intracellular messenger synthesized by the guanylate cyclase enzyme.

A practical consequence of this is that the levels of cyclic nucleotide concentrations can be monitored.

Muscarinic cholinoceptors can be **enzymatically mediated.**

They are able, after coupling a muscarinic agonist, to bind at the **cell's membranes interior** with the protein that binds **guanosine 5'-phosphate (G protein).**

It has been demonstrated that this complex is able to:

- **inhibit** adenylate cyclase,
- **activate** guanylate cyclase,
- **increase** the potassium ions conductance (in the heart)
- **reduce** the conductance of potassium ions (in CNS)
- **ease** triphospho-inositol-phospholipase activity.

So:

the action will be dependent to the **site,** in each of these cases, because the receiver will be coupled to a different G protein.

Calcium ions

Are also considered intracellular secondary messengers with a great importance.

Free concentrations may be increased not only by membrane depolarized agents acting on receptors coupled to the membrane pores (e.g. acetylcholine) but also through several other mechanisms (e.g. medications, hormones or neuro-transmitters) that act on mobilization receptors of the calcium ions.

E.g. calcium ions are involved in the arahidonic acid release from the phospholipidic membrane by activated phospholipase, initiating the synthesis of prostaglandins and leukotrienes (which are extracellular messengers).

While previous researches were focused on the calcium entry through the electro-sensitive channels in the electrically excitable cells, as a mechanism to increase the calcium ion concentration in the cytosol, the current studies, performed on endoplasmic reticulum (ER) from muscle fibers, have revealed that the release of calcium coupled inside the cell, can act as an alternative mechanism that leads to the same result (increased intracellular concentration).

Later, it was revealed the involvement of membrane phospholipids in the cellular turnover of calcium ions.

In this case, **phospholipase C** (enzyme activated by the receptors located in membrane) cleaves the **phosphatidyl inositol (PI)** in:

- **diacylglycerol (DAG)** and
- **inositol-1-phosphate,**

stage in which the intracellular calcium ions will be released.

DAG is a secondary messenger that activates protein kinase C before it is resynthesized in the PI.

Recently, it has been demonstrated that:

- phospholipase C hydrolyzes a second phospholipid, phosphatidyl inositol-bisphosphate (PIP2), releasing:
- DAG and inositol triphosphate (IP3), the latter being a powerful calcium bound "liberator" to the endoplasmic reticulum.

Like DAG, IP3 is recycled to the membranar PI.

Conclusion:

The molecular structure is the first determinant for the medicinal activity of a chemical substance.

The type of response produced depends on the location's normal functioning, at which, the molecular properties of a drug allow it to:

- **persist,**
- **accumulate and**
- **bind.**

Thank you for your attention!