



G protein coupled receptors and their desensitization

Receptores acoplados a proteínas G y su desensibilización

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In the course of the last forty years hormone receptors, neurotransmitters, autacoids (local hormones) and growth factors have evolved, from being abstract concepts to being perfectly defined chemical entities. Essential elements of their structures, cellular location, functioning and regulation have become more defined all through the last twenty years. Nowadays we know that proteins are the receptors for these elements of intercellular communication. As is the case in all proteins, information for their synthesis is coded within our DNA and is subject to evolutionary process. Due to advances in biochemistry and structural biology, we are nowadays very close to knowing in detail various receptors –primary, secondary, tertiary and even quaternary [in cases when there are subunits] structures– Moreover, molecular genetics allows us to get acquainted with the receptors natural variants (polymorphisms) and to analyze their functional relevance (susceptibility and even resistance to several conditions). Molecular biology allows us to handle DNA and express in cellular models and even in complete (whole) organisms native and mutant receptors to advance in knowledge. Moreover we can modify the abundance or presence of receptors (transgenesis , expression blocking [«knockout»], or expression decrease [«knockdown»]).

According to their location, hormone receptors have been divided into two main groups: those integrated into the plasma membrane from where they exert their action, and those found soluble in cytoplasm and nucleus. The latter are fundamentally transcription modulating factors regulated by their association with ligands (hormones, such as male and female hormones, glucocorticoids, mineralocorticoids, Vitamin D active form, thyroid hormone, or retinoic acid, among many others). Plasma membrane receptors have been divided into three fundamental groups: channel receptors (or ligand-gated ion channels) receptors with enzymatic activity or associated to itinerant enzymes, and G protein-coupled receptors, which are the subject of the present essay.

G protein coupled receptors possess a very peculiar structure. They are constituted by an amino acid chain whose amino terminus is located in the extracellular portion of the cell and the carboxyl terminus is in the cytoplasm, the peptide chain crosses the plasma membrane in seven instances (*Figure 1*). As can be ascertained, transmembrane zones are joined by three intracellular loops as well as three extracellular loops. Due to their structural characteristics, these receptors are also called seven-transmembrane domain receptors and due to their snake –like appearance they are also called serpentine receptors. *Figure 1* shows the receptor extended on the membrane (left drawing), but, in real-life situation, in the lipid bilayer, receptors adopt a very different shape, grouped or agglutinated, as is shown in this same *figure*, (right drawing).

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G protein coupled receptors receive this name since they act basically associating to a family of heterotrimeric proteins, the so called G proteins which are composed of alpha, beta and gamma subunits and which have the ability to join and hydrolyze GTP (3). When these receptors are activated by different hormones and neurotransmitters, they experiment conformational changes which are transmitted to G proteins, which in turn, initiate an activation-inactivation cycle associated to GTP union and hydrolysis (3). G proteins active forms can positively or negatively modulate to different ion channels (mainly for potassium or calcium) and second messenger generating enzymes (*Figure 2*). Typical examples of these enzymes are adenylyl cyclase, which catalyzed cyclic AMP formation and phospholipase C (phosphoinositidase) which catalyzes the formation of two second messengers, inositol 1,4,5, triphosphate and diacylglycerol. These G protein-coupled receptors systems are composed by transmembrane receptors, G proteins and effectors (enzymes or ion channels). The receptor receives the message (hormone or neurotransmitter, that is, the first messenger) in the extracellular face of the plasmatic membrane and induces production, degradation or change in concentration of metabolites or ions (second messengers or coupling factors such as calcium ion, which allow the signal to propagate (spread) inside the cell (*Figure 2*).

It is important to point out that G protein-coupled receptors constitute a huge family. There are receptors of this type for light (rhodopsin), odor and taste receptors, calcium sensors, receptors for many of the main neurotransmitters (adrenaline, dopamine, serotonin, histamine, opiates and cannabinoids among others) as well as many general and local hormones (angiotensin, vasopressin, glucagon, ACTH (adrenocorticotrophic hormone), gonadotropins, prostaglandin, and many others.

Based on information yielded by mammals of which we know the genome (man included) we are aware that these receptors can correspond to between 3 and 5% of coded proteins. That is to say, we possess an enormous amount of this type of receptors. It is, therefore, not surprising to observe that there are many receptor subtypes for a single hormone; for example, adrenalin and noradrenalin share 9 receptors (three alpha-1, three alpha-2 and three beta). The existence of receptor subtypes with differential expression in different tissues, is probably the result of evolutionary selection pressure, which could have granted adaptation advantages through differential regulation. Moreover, a huge window of possibilities is opened for pharmacological treatment of several diseases. It is not uncommon then to find that G protein-coupled receptors are the therapeutic target of at least 40% of drugs used in medical practice. It is as well very probable that a high percentage of drugs of veterinary and dental use act upon these receptors. It is important to point out that there is a great amount of receptors whose natural agonists are unknown (they have been named orphan receptors). This constitutes an extremely wide research area.

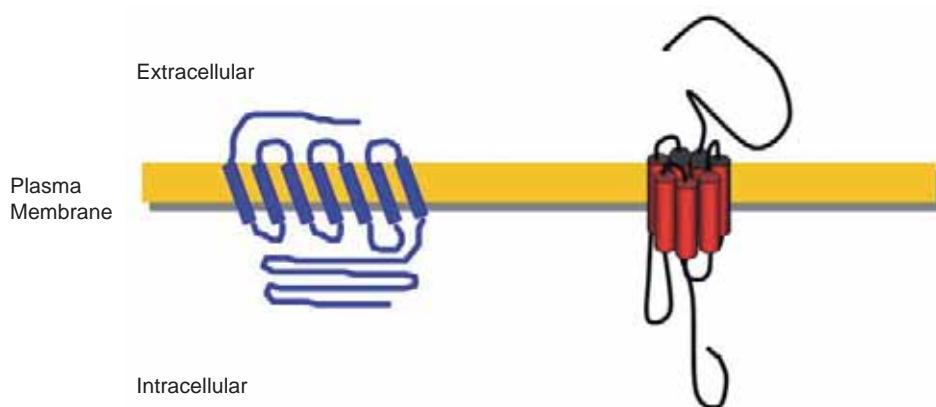


Figure 1.

The actions of hormones and neurotransmitters mentioned in this paper, generally possess an almost instantaneous start and an equally rapid shutdown. The fact is well known that, when an agonist is applied, as, for example pressor amines like adrenaline or dopamine, an intense and immediate action is triggered which abates with time; if a second dose must be administered, frequently the response is attenuated, and later doses tend to have even less effect. This process is called desensitization or tachyphylaxis and is observed daily, especially in hospitals' intensive care units. This has led to the general belief that it constitutes an exclusively pharmacological effect. We do not agree. This is a physiological process of sensitivity adjustment which continuously takes place in our cells. Studies performed on culture cells, have enabled us to establish several desensitization stages. In the initial phase, which takes a few minutes, there are changes in the receptor's state of phosphorylation which «freeze» it to an inactive state or one with little activity. Later, the receptor is internalized into vesicles and this decreases the number of receptors in the membrane, this latter process is called downregulation. The whole desensitization process starts in minutes, but can take many hours. Internalized receptors can be degraded or recycled to the plasma membrane (Figure 3). If the stimulation is very prolonged or takes place intermittently with high frequency, changes also take place at the level of the receptors' synthesis.

Experimental work carried out during the last ten years has shown that receptors phosphorylation and dephosphorylation take place on a continuous basis, especially in the carboxyl tail and the third intracellular loop. These phosphorylations constitute points to which several proteins associate. Among these are the beta-arrestins, which allow the formation of macromolecular complexes for receptor internalization. As an example of desensitization, let me mention this: we all perceive, upon entering a dimly lit place, such as a theater or cinema, when the session is already started, that we have to wait some seconds or minutes until we are able to gain a certain amount of vision, in a similar manner, if we enter a highly illuminated establishment, we do not gain acute vision until a few minutes are elapsed. These adjustments to light sensitivity take place in our retina, in the retinal rods and cones, where the receptor to light, rodopsin is phosphorylated and dephosphorylated.

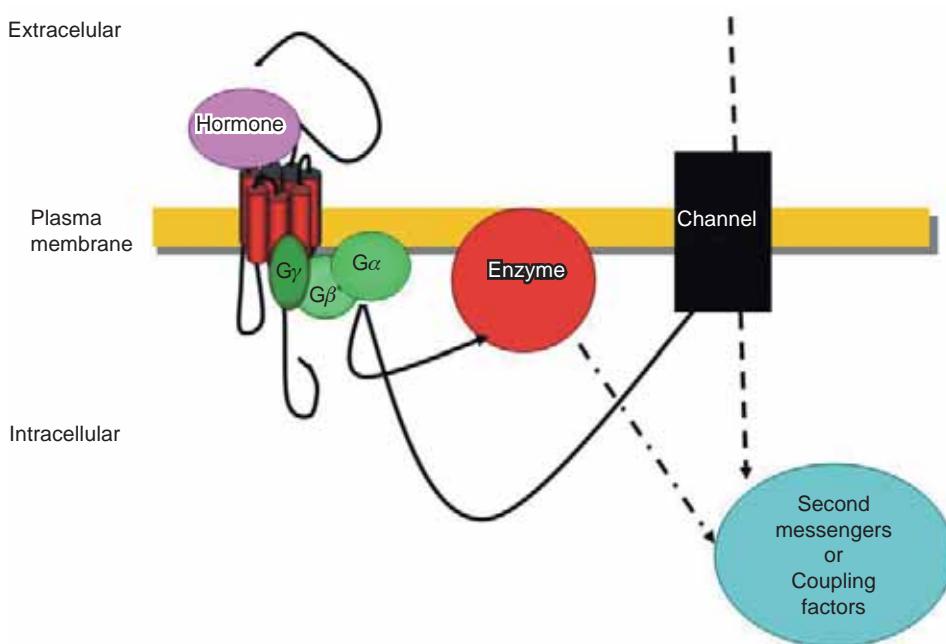


Figure 2.

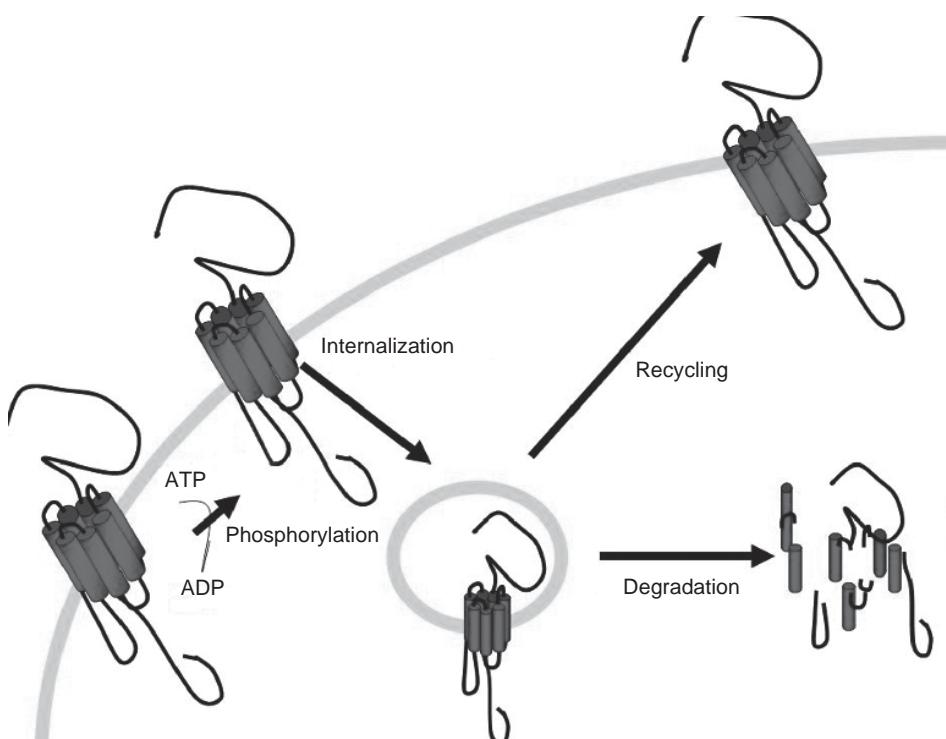


Figure 3.

Present studies in this field are focused in finding the phosphorylation regulation of various receptors, identifying the protein kinases which participate in phosphorylation, as well as phosphatases which participate in dephosphorylation; defining the specific affected sites (mainly serine, threonine and tyrosine residues), as well as the molecular events which participate in the signal shutdown and its recovery. There is no doubt that the coming years will bring advances in functional and structural knowledge of these receptors.

SUPPORTING REFERENCES

1. Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev* 2001; 53: 1-24.
2. García-Sáinz JA. Adrenaline and its receptors: one hundred years of research. *Arch Med Res* 1995; 26: 205-212.
3. García-Sáinz JA, Romero-Ávila MT, Molina-Munoz T, García-Pasquel MJ. G-protein -coupled receptor-receptor tyrosine kinase crosstalk. Regulation of receptor sensitivity and roles of autocrine feedback loops and signal integration. *Current Signal Transduction Therapy* 2008; 3: 174-182.
4. García-Sáinz JA, Vázquez-Prado J, Villalobos-Molina R. Alpha 1-adrenoceptors: subtypes, signaling, and roles in health and disease. *Arch Med Res* 1999; 30: 449-458.
5. Kenakin TP. Cellular assays as portals to seven-transmembrane receptor-based drug discovery. *Nat Rev Drug Discov* 2009; 8: 617-626.
6. Lefkowitz RJ. Historical review: a brief history and personal retrospective of seven-transmembrane receptors. *Trends Pharmacol Sci* 2004; 25: 413-422.
7. Penn RB, Pronin AN, Benovic JL. Regulation of G protein-coupled receptor kinases. *Trends Cardiovasc Med* 2000; 10: 81-89.
8. Tobin AB. G-protein-coupled receptor phosphorylation: where, when and by whom. *Br J Pharmacol* 2008; 153(Suppl 1): S167-176.