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The Nobel Prizes of 2012

Smart cell-surface receptors: On the 2012 Nobel Prize in Chemistry, awarded to Robert J. Lefkowitz and Brian K. Kobilka

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Summary. The 2012 Nobel Prize in Chemistry recognized Professors Robert J. Lefkowtiz and Brian K. Kobilka for their work on β-adrenergic receptors, which have been the paradigm for understanding the mechanism of action of receptors coupled to heterotrimeric G proteins (GPCRs). In fact, the discovery of hundreds of members of this family of cell-surface receptors has provided a detailed understanding of how cells sense their environment. This brief article draws from the summary used by the Royal Swedish Academy of Sciences to support its choice of Lefkowitz and Kobilka for the 2012 award. It also considers some of the diverse applications of GPCRs.

Keywords: G-protein-coupled receptors · adenosine receptors · adrenergic receptors · receptor heteromers

Resum. El Premi Nobel de Química 2012 va reconèixer el treball dels professors Robert J. Lefkowitz i Brian K. Kobilka sobre els receptors β-adrenèrgics, que han estat el paradigma per a la comprensió del mecanisme d'acció dels receptors acoblats a proteïnes G heterotrimèriques (GPCRs). De fet, el descobriment de centenars de membres d'aquesta família de receptors de la superfície cel·lular ha permès entendre detalladament com detecten les cèl·lules el seu entorn. Aquest breu article està basat en el resum utilitzat per la Reial Acadèmia Sueca de Ciències per donar suport a la concessió del premi de 2012, i considera també les diverses aplicacions dels GPCRs.

Paraules clau: receptor acoblat a proteïnes G · receptors d'adenosina · receptors adrenèrgics · heteròmers de receptors

The beginnings and previous breakthroughs

The 2012 Nobel Prize in Chemistry cannot be appreciated without reference to previous work by Ahlquist and Black. In 1948, Raymond Ahlquist (Missoula, Montana, USA, 1914–1983), Professor of Pharmacology at the Medical College of Georgia, USA, published a paper on adrenergic nervous transmission, entitled "A study of the adrenotropic receptors," in the American Journal of Physiology [1]. But the molecular nature of adrenotropic receptors, i.e., receptors for adrenaline (known today as adrenergic receptors), would remain unknown until the work of Robert J. Lefkowitz, one of the 2012 Nobel laureates, in the late 1960s. In fact, Ahlquist's penultimate scientific publi-

cation [2] was a review on "Adrenergic β-blocking agents," which had been developed before β-adrenergic receptors were precisely understood. Indeed, β-blockers can be considered among the most successful drugs in the history of medicine and they remain therapeutically important in a variety of diseases, especially cardiac arrhythmias and hypertension. Due to the reduction in the heart beat rate and other calming effects, they may be used offlabel in stressful situations (for instance before a PhD defense).

In the early 1960s, Sir James W. Black (Uddingston, Scotland, UK, 1924–2010) developed the first β-blockers: propranolol and pronethalol. Although pronethalol never reached the market because of carcinogenicity in mice, propranolol revolutionized the medical management of cardiovascular diseases. According to contrasted information available in the Swedish Academy and on Wikipedia, Sir James Whyte Black developed his research in both the academia and the pharmaceutical industry. One of this main achievements was the developing of the first marketed beta-blocker, propranolol. He also participated in the development of an effective anti-ulcer treatment that consisted of another drug (cimetidine) targeting a GPCR (H2 histamine receptors). Black's contribution to developing effective therapies for two common diseases was noteworthy, and recognized by the 1988 Nobel Prize in Physiology or Medicine. Furthermore, it should be taken into account that the development was possible despite little being known about their targets: β-adrenergic receptors for propranolol, and histamine receptors for cimetidine. Both receptors belong to a superfamily known today as "G-protein-coupled receptors" (GPCRs). It was the β-adrenergic receptor that received the attention of the Nobel Prize Committee in 2012.

Molecular nature of β-adrenergic receptors

Robert J. Lefkowitz (New York City, USA, 1943) heads the Howard Hughes Medical Institute at Duke University Medical Center (Durham, North Carolina, USA) and Brian K. Kobilka (Little Falls, Minnesota, USA, 1955) is a professor at the Department of Molecular and Cellular Physiology, Stanford University School of Medicine (California, USA). The two researchers received the 2012 Nobel Prize in Chemistry for: "…groundbreaking discoveries that reveal the inner workings of an important family of receptors that enable cells to sense its environment: G-protein–coupled receptors." The Swedish Academy also noted that: "For a long time, it remained a mystery how cells could sense their environment. Scientists knew that hormones such as adrenaline had powerful effects: increasing blood pressure and making the heart beat faster. They suspected that cell surfaces contained some kind of recipient for hormones. But what these receptors actually consisted of and how they worked remained obscured for most of the 20th Century."

We live through our senses, whose receptors are located on our anatomical surface. Certainly, one of Lefkowitz's key insights was to consider that cells "sense their environment" via sensors/receptors and these are, necessarily, located on the cell surface. In his approach to unraveling the molecular nature of sensors/receptors he selected the adrenaline receptor, which can be easily detected. (Reasons at the origins of other scientific discoveries have been similar. Myoglobin and hemoglobin structures were first determined because these proteins could be easily followed throughout their purification due to their color.) Moreover, adrenaline receptors are abundantly expressed by cardiac muscle cells and can be studied pharmacologically in isolated beating hearts. Lefkowitz's method to detect adrenaline receptors took advantage of the one used to detect receptors in the thyroid, by radiolabeling the ligand, which in the latter case includes the hormone thyroxine. Accordingly, soon in his career Lefkowitz used radioactivity to trace cardiac receptors for adrenaline. As noted by the Swedish Academy: "He attached an iodine isotope to various hormones, and thanks to the radiation, he managed to unveil several receptors, among those a receptor for adrenaline: β-adrenergic receptor. His team of researchers extracted the receptor from its hiding place in the cell wall and gained an initial understanding of how it works." Later on it was demonstrated that adrenaline had quite a number of different targets depending on the cell; thus far, two alpha and three β subtypes of adrenergic receptors have been identified.

While the radioactive detection of β-adrenergic receptors was an important contribution to the field of pharmacology, the obvious next step was to determine their structure. The usual techniques to resolve proteins structure are not readily applicable to proteins such as adrenergic receptors that are embedded in the plasma membrane, i.e., that are surrounded by lipids. Thus, instead, Lefkowitz and his group obtained the mRNA sequence encoding the receptor protein, from which they could easily decipher the amino acid sequence. It was at this moment, as noted by the Academy, that Brian Kobilka entered in scene: "The team achieved its next big step during the 1980s. The newly recruited Kobilka accepted the challenge to isolate the gene that codes for the β-adrenergic receptor from the gigantic human genome. His creative approach allowed him to attain his goal. When the researchers analyzed the gene, they discovered that the receptor was similar to one in the eye that captures light. They realized that there is a whole family of receptors that look alike and function in the same manner."

Interestingly, the βadrenergic receptor was similar to rhodopsin, which is the receptor for light. Solid work from many laboratories in the field showed that all GPCRs had

the same basic structure, i.e. seven transmembrane domains. This observation suggested that these receptors originated from an ancestral gene encoding a cell surface sensor. GPCRs act as sensors for an enormous number of hormones and neurotransmitters active in our cells at every instant. Their importance is reflected in the fact that some 10 % of the genes in the human genome encode GPCRs (Fig. 1)—a percentage higher than that of any other protein family in the human genome; and there are probably even more than the currently estimated 802 GPCR genes in the human genome [3b]. As the Academy put it: "Today this family is referred to as G-protein–coupled receptors. About a thousand genes code for such receptors, for example, for light, flavor, odor, adrenaline, histamine, dopamine and serotonin."

GPCRs are subdivided into different classes: class A (Rhodopsin-like), class B (Secretin-like), class C (Glutamate Receptor-like), and other (Adhesion, Frizzled, Taste type-2, etc.) [3b]. Some 70 % of all GPCRs are of the class A type. The receptors in this class include those for odorants/pheromones, underlining the importance of smell in evolution. There are also "orphan" receptors, whose endogenous hormones/neurotransmitters are still unknown. In the last 10 years, however, some of these orphan receptors, such as GPR55, now known to be the lysophosphatidylinositol receptor [29], have found their origins.

In recognizing the biomedical importance of the role played by GPCRs in a variety of diseases, the Swedish Academy pointed out that "about half of all medications achieve their effect through G-protein–coupled receptors." Although this percentage was overestimated—the real number lies around 35–40 %—it is clear that the number of "GPCR drugs" far exceeds that targeting any other family of proteins. Some of these drugs are life-saving while others improve the quality of life, for instance in cases of depression or anxiety.

GPCR-mediated signaling

In the last 30 years of the 20th century, hundreds of scientists participated in deciphering the mechanism by which GPCRs communicate the presence of extracellular signals to the cell interior. Because of their work, GPCR-mediated signaling is better understood than that of any other receptor family. Among the key proteins in this process are the G proteins, whose discovery and characterization merited a Nobel Prize in Physiology or Medicine in 1994, awarded to

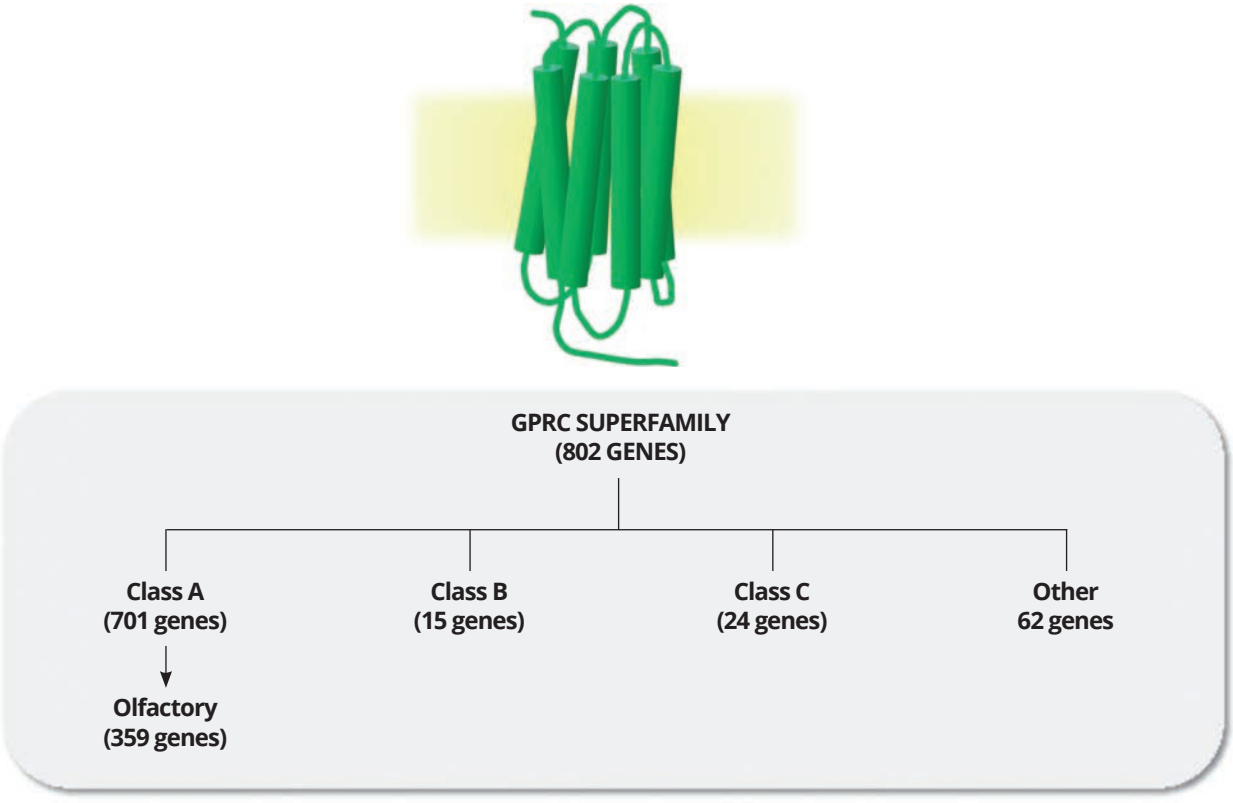

Fig. 1. Classification of the human GPCR family. The main subfamilies are: class A (rhodopsin-like), class B (secretin-like), and class C (glutamate-like); other includes Frizzled, taste type-2, and unclassified receptors. Modified from Bjarnadóttir et al., 2006 [3b]. The number of GPCR receptors in the human genome is not yet known; therefore, the numbers in the figure are estimates from data in Pubmed and Wikipedia.

Alfred G. Gilman and Martin Rodbell for "their discovery of G-proteins and the role of these proteins in signal transduction in cells." Further information on these G proteins and their action as mediators between GPCRs and intracellular components can also be found at the Nobel Prize web site. The first G proteins to be identified and characterized were those that regulate, either positively (Gs) or negatively (Gi), the activity of adenylate cyclase, the enzyme that produces cAMP, an important intracellular messenger. Indeed, the title of Gilman's Nobel lecture was: "G Proteins and Regulation of Adenylyl Cyclase." In the meantime, a number of other G proteins and cAMP-independent signaling pathways have been discovered.

The history of GPCRs took an unusual turn with the discovery that they can bind to a variety of proteins other than G proteins. Consequently, GPCRs are now more accurately referred to as heptaspanning membrane receptors. It was Lefkowitz who, in 1987, reported that adrenergic receptors interact with a protein he named β-arrestin, because of its apparent involvement in receptor deactivation [3]. Lefkowitz and colleagues later observed that β-arrestin also participated in G-protein-independent signaling. They reported that β_2-adrenergic receptors signal the relevant mitogen-activated protein kinase signal transduction pathway via an arrestin-dependent pathway that is independent of G protein coupling [41]. Adenosine receptors are likewise able to bind to a number of proteins, some of them extracellular, such as adenosine deaminase, and others intracellular, such as caveolin, the heat shock cognate protein hsc73, and alpha-actinin [4,5,12,15,20,22,35–38]. The full diversity of GPCR-related signaling options stems from the ability of these receptors to form homodimers and even heteromers. Recognition of these features provides many new opportunities to exploit the structural and functional diversity of these receptors, e.g., in drug discovery (see below).

GPCR structure

The final paragraph of the statement of the Royal Swedish Academy of Sciences in support of its choice for the 2012 Nobel Prize in Chemistry is: "The studies by Lefkowitz and Kobilka are crucial for understanding how G-protein–coupled receptors function. Furthermore, in 2011, Kobilka achieved another breakthrough; he and his research team captured an image of the β-adrenergic receptor at the exact moment that it is activated by a hormone and sends a signal into the cell. This image is a molecular masterpiece—the result of decades of research."

There has been a general consensus in the GPCR field, which year after year seemed ripe for a Nobel prize, that the award would have not been possible without the resolution of the 3D structure of these proteins. This piece of the puzzle was greatly aided by Kobilka's contribution, in which he helped to solve the structure of G-protein-coupled β_2-adrenergic receptors (Fig. 2) [33]. In contrast to soluble proteins such as myoglobin or hemoglobin, which are easily isolated and crystallized and their 3D structure readily resolved using X-ray diffraction, membrane proteins pose a challenge, as their purification and crystallization are difficult.

Rhodopsin, which is very abundant in the retina, was extracted by Palczewski and collaborators [30,31], facilitated by their use of mixed micelles of nonyl β-D-glucoside and heptanetriol. Thus, a highly purified protein preparation could be crystallized from solutions containing varying amounts of detergent and amphiphile; these crystals provided the first structure of a seven transmembrane protein [31]. The laboratory of Palczewski also carried out

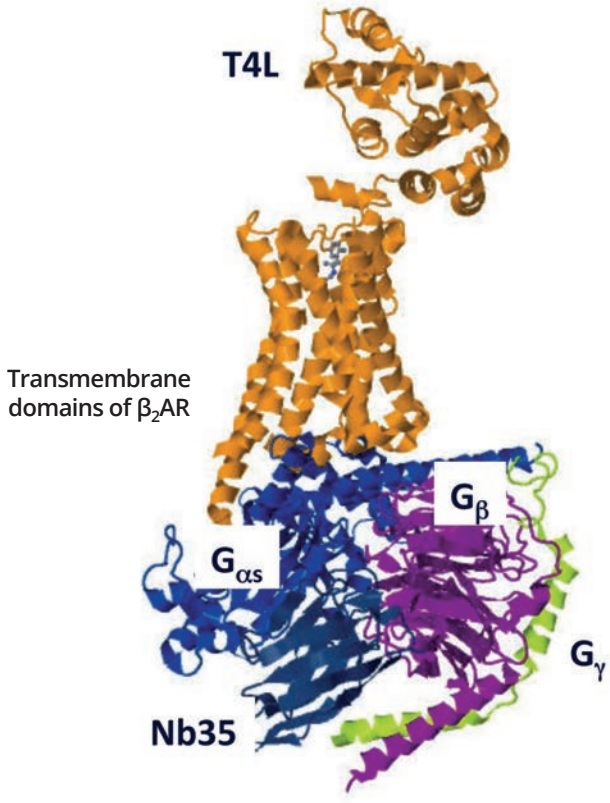

Fig. 2. Structure of the β2-adrenergic receptor-Gs complex. The overall structure shows the β2-adrenergic receptor (β2AR) bound to an agonist and engaged in extensive interactions with a heterotrimeric Gs, which is composed of Gsa, Gb, and Gg subunits. A specific nanobody (Nb35) binds to the Gs protein between the a and b subunits. Crystallization, for purposes of structure resolution, is facilitated by NB35 and by the lysozyme of T4 bacteriophage (T4L) fused to the N-terminus of the β2-adrenergic receptor. Data taken from the protein data bank cited in the report by Rasmussen (2011) [33]. (Reprinted by permission from Macmillan Publishers Ltd.).

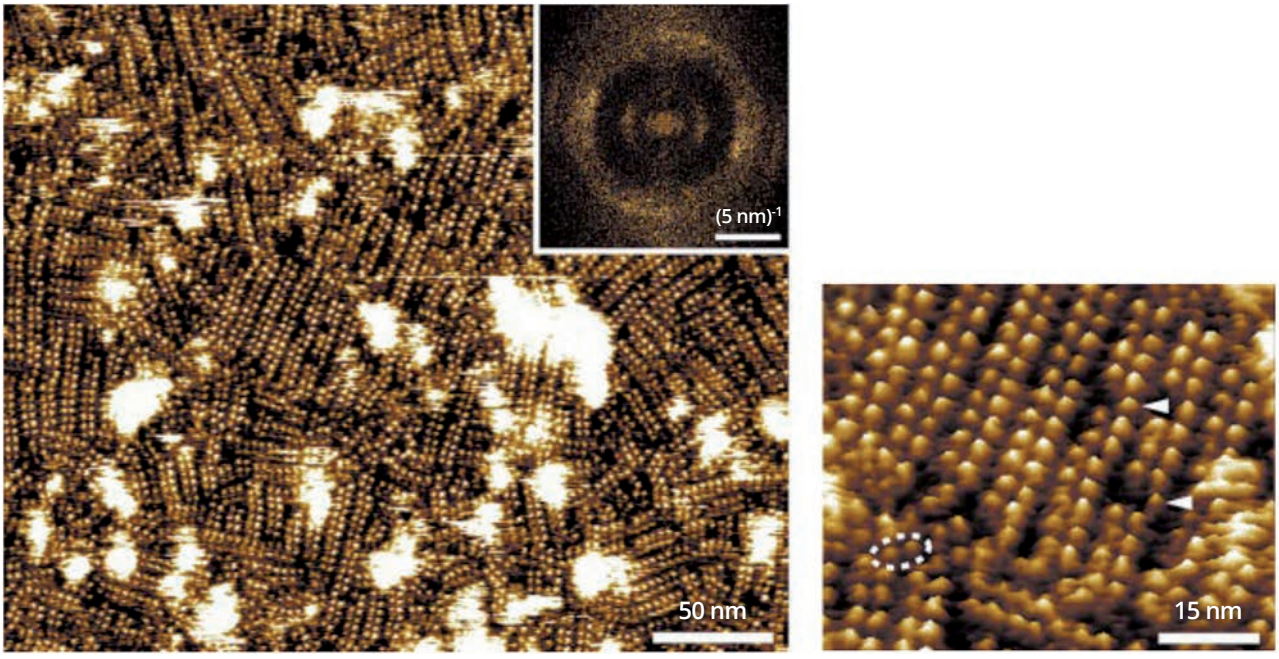

Fig. 3. Atomic force microscopy images of dimer arrays of rhodopsin in native retinal disc membranes. (From [17]; reprinted by permission from Macmillan Publishers).

pioneering work using infrared-laser atomic-force microscopy to reveal the native arrangement of rhodopsin, which forms paracrystalline arrays of dimers in mouse retinal disc membranes [17] (Fig. 3).

The methodology for other GPCRs that are less abundant in natural sources is far more complex and involves the design of fusion proteins containing part of the receptor and motifs that facilitate crystallization. For example, a common strategy to promote crystallization is to fuse the protein with the lysozyme from T4 bacteriophage and to delete the carboxy-terminal tail, which has high conformational flexibility [42] (Fig. 2). These pseudo-receptors are over-expressed in heterologous systems and then further purified and crystallized using specific approaches. The first receptor structures resolved accordingly were those of the β_2-adrenergic [10,34] and the A_{2A} adenosine [23] receptors. The contribution of the laboratory of Raymond Stevens, at the Scripps Research Institute in California, to obtaining these structures was fundamental.

GPCRs in pharmacology

An appreciation of the terms agonist (a compound that activates the receptor), antagonist (one that triggers the inactive conformation of the receptor), and allosteric modulator (a compound that modulates the effect of the agonist) is essential to understanding receptors for hormones or neurotransmitters and the activities of the drugs that bind to them. GCPRs are not the only cell-surface receptors, as these also include ionotropic and enzyme-linked receptors, which are not coupled to G proteins (Fig. 4). However, as noted above, GPCRs are the most abundant membrane receptors in mammals and they have led to the recognition of "partial agonists," "full agonists," "inverse agonists," "neutral antagonists," "biased agonism," and "selectivity."

The signal given by a partial agonist is lesser than that achieved by the full agonist, which is usually the natural hormone/neurotransmitter. Classical antagonists are known today as neutral antagonists although many compounds initially considered as neutral are indeed inverse agonists, that is, they bind to the receptor but counteract the response of agonists and reduce the constitutive activity of GPCRs. This definition makes sense only if the GPCR is active even in the absence of agonists. Such constitutive activity was indeed shown by Lefkowitz and his group, using mutant or over-expressed receptors in heterologous cells [13,25]. There are also several diseases, such as congenital night blindness and male precocious puberty, that are caused by mutations in GPCR genes such that the encoded receptors have high constitutive activity [40]. Furthermore, many (non-mutated) GPCRs display natural constitutive activity, which has raised interest in inverse agonist development in drug discovery programs.

Biased agonism describes the activation of different signaling pathways by different molecules acting on a given receptor [39]. It is an important consideration in the design of mole-

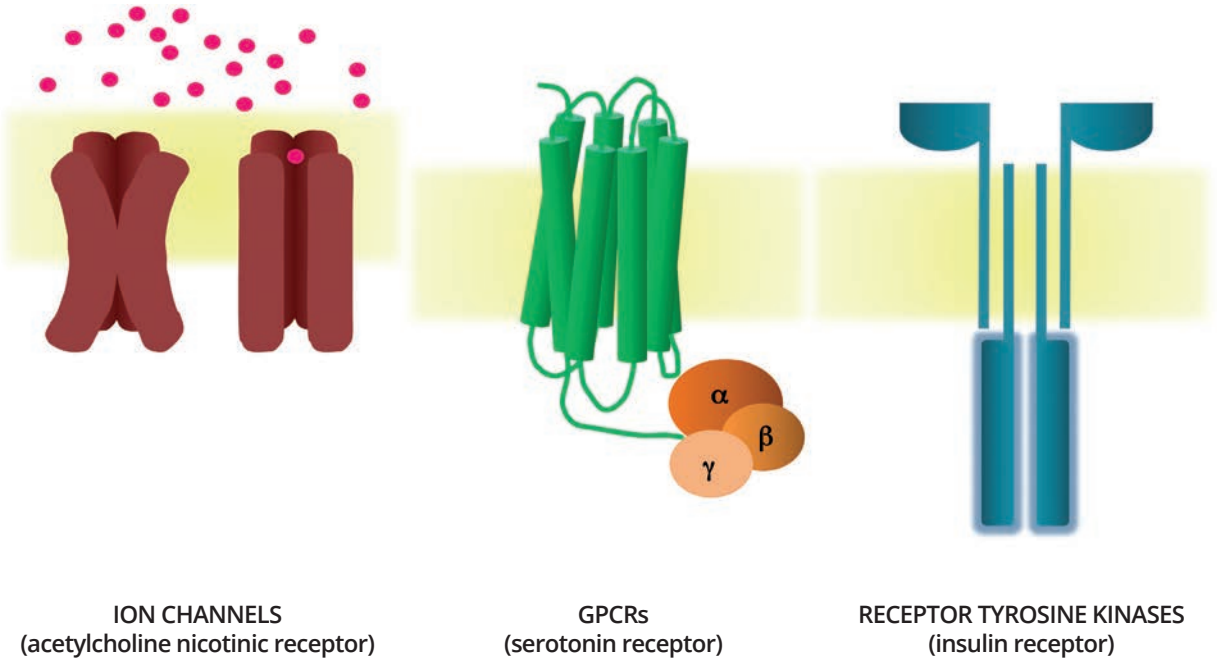

Fig. 4. The three main types of cell-surface receptors. Examples are given in parentheses.

cules with therapeutic potential. Selectivity is another important concept in receptor pharmacology and drug discovery. A selective compound that binds, e.g., to β_2 adrenergic receptors, would have at least 100-fold more potency for this subtype than for any other subtype of adrenergic receptor.

The future: structure and function of GPCR heteromers

The impressive research leading to the 2012 Nobel Prize in Chemistry was centered on "monomeric" receptors, including a seminal work co-authored by Leftowitz, in which the mechanism of action of a single GPCR molecule coupled to one G protein was described [14]. Unlike membrane receptors of other families, e.g., T-cell receptors and insulin receptors, the expression of class A rhodopsin-like GPCRs as monomers on the cell surface was a common assumption. But this view is progressively changing based on findings, such as those from our laboratory, in which we determined that A_{2A} receptors are present on the cell surface as homodimers [6]. Atomic force microscopy images of retinal rhodopsin also show GPCRs as arrays of homodimers (Fig. 3). Recently, Kobilka's laboratory reported that the μ-opioid receptor crystallizes as a two-fold symmetrical dimer through a four-helix bundle motif formed by transmembrane segments 5 and 6 [26]. It is therefore likely that many cell-surface receptors are in the form of homodimers. Exceptions may occur, i.e. there are receptors that may be found as monomers in the cell surface. Homodimers have led to a dimer-based pharmacology that is very robust in defining useful parameters for drug development [9,18,19]. Yet, from a functional point of view, the monomeric or dimeric structure of a given receptor is largely irrelevant. This is not the case for heteromer formation. Indeed, if GPCRs deserve another Nobel Prize it will likely be for novel insights into their heteromeric forms.

A GPCR heteromer is defined as a macromolecular complex composed of at least two different receptor units, both functional, with biochemical properties that demonstrably differ from those of the individual components [16]. In fact, heteromer-specific signaling relies on a precise quaternary structure of the whole complex [28]. The first reported heteromer for a given neurotransmitter was that formed by the kappa and delta opioid receptors [24]. The first identified heteromer formed by two receptors for two different neurotransmitters/neuromodulators was that containing the adenosine A_1 and dopamine D_1 receptors [21]. Several other heteromers were subsequently reported, proof that the GPCR heteromer receptor field is gaining momentum in the 21st century. There are two open questions concerning the molecular aspects of heteromerization: the size(s) of the heteromer(s) and the receptor: G protein stoichiometry. Finding answers to them will require imaginative approaches and powerful techniques, in-

cluding resolution of the 3D structure of macromolecular complexes formed by heteromers and multiple G proteins. At present, complexes consisting of three different receptors (heterotrimers) and dimers of heterodimers (heterotetramers) have already been reported [7,27].

From an evolutionary point of view, heteromers seem to provide diversity in hormone- or neurotransmitter-mediated responses. The dopamine receptors subtypes 1 (D_1) and 2 (D_2) offer an appropriate example of functional diversity. Whereas D_1 is coupled to a Gs protein and D_2 to a Gi protein, the D_1-D_2 receptor heteromer couples to a Gq protein. G proteins are the mediators that control the concentration of second messengers, with Gs and Gi controlling cAMP and Gq controlling Ca^{2+} levels. Thus, while individual receptors signal via cAMP, the heteromer signals via a totally different cascade, one that is triggered by calcium ions [32]. Another example of the differential role of heteromers is provided by the adenosine A_1-A_{2A} receptor heteromer. Adenosine regulates glutamate release from cortical neurons; the regulation of neurotransmitter release via A_1 receptors is negative but it is positive when A_{2A} receptors become activated. The mystery of why nerve terminals express both A_1 and A_{2A} receptors to regulate glutamate release was solved when heteromers of A_1-A_{2A} receptors were identified and their functions elucidated [8,11]. Specifically, these heteromers constitute a switch mechanism by which low and high concentrations of adenosine inhibit and stimulate, respectively, glutamate release. By intra-heteromer crosstalk, the neuron senses the extracellular concentration of adenosine and responds accordingly [8,11]. In summary, the heteromer may both increase and decrease neurotransmitter release, something that would be impossible to achieve with just one adenosine receptor subtype. Promising applications can be expected as novel heteromers are identified and novel heteromer-specific functions are discovered.

Drug development based on GPCR monomers, such as screening drugs in heterologous systems expressing a single receptor, was successful in the 20th century but, at least thus far, progress in the 21st century has been slow. Perhaps one way to accelerate GPCR drug discovery is to focus on GPCRs and heteromers. A two-state dimer receptor model is now available to understand the mechanism of action of GPCRs and to interpret data obtained from drugs interacting with dimers, and even from mixtures of monomers and dimers [9,19]. By contrast, heteromers are distinct entities such that a given drug will have different affinities and different efficacies depending on the heteromer. Cell models expressing receptor heteromers would allow the identification of novel pharmacological profiles [9] and would broaden the therapeutic potential of drugs targeting GPCRs while lowering the incidence of side effects.

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