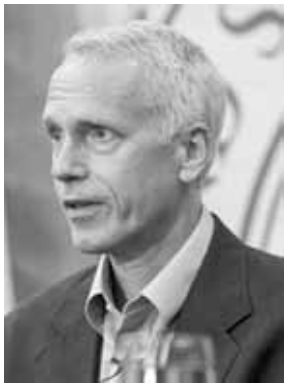
The 2012 Nobel Prize in Chemistry

Brian Halton

School of Chemical & Physical Sciences, Victoria University, PO Box 600, Wellington
(e-mail: *brian.halton@vuw.ac.nz*)

The Royal Swedish Academy of Sciences awarded the 2012 Nobel Prize in Chemistry to ***Robert J. Lefkowitz*** of the Howard Hughes Medical Institute and Duke University Medical Centre, and ***Brian K. Kobilka*** of Stanford University School of Medicine, USA, for studies of G-protein–coupled receptors.



Left: Robert J. Lefkowitz (courtesy of Quivetta Lennon, Duke University, NC); right: Brian K. Kobilka (from Linda A. Cicero/ Stanford News Service)

Each of the billions of cells in the body has tiny receptors that enable it to sense its environment, so it can adapt to new situations. This year's recipients gained the award for ground-breaking discoveries that reveal the inner workings of the *G-protein–coupled receptors* (GPCRs). These receptors form a remarkable modular system that allows the transmission of a wide variety of signals over the cell membrane, between cells and over long distances in the body. Today, we understand the molecular mechanism of how these receptors work in intricate detail, in large part because of the studies by Kobilka and Lefkowitz. Scientists knew that hormones such as adrenalin had powerful effects in 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 obscure for most of the 20th century.

In 1968, Lefkowitz began to use the radioactivity of ^{125}I to trace cellular receptors by attaching the radioactive iodine isotope to various hormones. This unveiled several receptors, among them a receptor for adrenalin, β-adrenergic receptor. His team extracted the receptor from the cell wall and gained an initial understanding of how it works. However, in the 1980s, Brian Kobilka, a newly recruited postdoctoral fellow accepted the challenge to attempt to isolate the gene that codes for the β-adrenergic receptor from the human genome and was, ultimately, successful. When the researchers analysed the gene, they discovered that the receptor was similar to one in the eye that captures light and then realised that there is a whole family of receptors that look alike and function in a similar manner. Today this family is referred to as G-protein–coupled receptors. About a thousand genes code for such receptors, for light, flavour, odour, adrenalin, histamine, dopamine and serotonin, etc., and about 50% of all medications achieve their effect through G-protein–coupled receptors.

The studies by Lefkowitz and Kobilka have proved crucial to understanding how G-protein–coupled receptors function.

Introduction

As human beings we have sensors in our eyes, nose and mouth for light, odours and flavours. Within our body, cells have similar sensors for hormones and signalling substances, such as adrenalin, serotonin, histamine and dopamine. Cells repeatedly use the same basic mechanism for reading their environment through G-protein–coupled receptors, but their nature and mode of action only recently have been determined. We all know how the body reacts to a frightening situation – senses are heightened, e.g., heart rate increases and we are on guard!

In a human being, tens of thousands of billions of cells interact, most of them with distinct roles. Some store fat; others register visual impressions, produce hormones or build up muscle tissue. In order to function, it is vital that our cells work in unison, sense their environment and know what is going on around them. For this, they need sensors. Every human cell is surrounded by a plasma

membrane, a phospholipid bilayer. The membrane makes it possible for the cell to maintain a specific mix of biochemically active species, while preventing unwanted entry of other substances from the outside environment. For proper function, the biochemical machinery inside the cell needs to be able to receive instructions from the outside. Robert J. Lefkowitz and his former postdoctoral student Brian K. Kobilka were awarded the 2012 Nobel Prize in Chemistry for having mapped how the family of G-protein–coupled receptors (GPCRs) work. This family includes the receptors for adrenalin, dopamine, serotonin, light, flavour and odour. In fact, most physiological processes depend on GPCRs and about one half of all medications act through these receptors, among them β-blockers, antihistamines and various kinds of psychiatric medications. Changes in hormone levels on the outside of the cell elicit adaptive changes in enzyme activity on the inside. Odour molecules affect cells in the olfactory epithelium and substances in food influence chemical activities in taste bud cells, which in turn induce electrical signals that transfer information to the brain. Indeed, human cells are constantly communicating with each other and the surrounding environment, which requires a molecular framework and a mechanism for transmission of information across the plasma membrane. Additionally, in the body, signal transmission may take place over long distances. To be able to respond promptly, the brain needs rapid information from our senses, for vision, smell, taste and hearing. Again, this requires a molecular mechanism for transmission of information over the plasma membrane.

The receptor – an elusive enigma

At the end of the 19th century scientists began to experiment with the effects that adrenalin has on the body. They discovered that it makes the heart rate and blood pressure increase and that it also relaxes the pupils. They suspected that adrenalin worked via nerves in the body, and so they paralyzed the nervous system of laboratory animals only to find that the effects of adrenalin were still manifest. They concluded that cells must have some kind of receptor that enables them to sense chemical substances in their environment, e.g., hormones, poisons, and drugs. However, for decades, all attempts to find these receptors failed. Scientists wanted to understand the size, shape and nature of the receptors, and how they conveyed signals to the cell. They knew that adrenalin was administered to the outside of the cell, and that it led to changes in its metabolism that they could measure inside the cell. Each cell has a wall, a membrane of fat molecules that separates it from its environment. Thus, the essential questions are: "How did the signal get through the wall?" and "How could the inside of the cell know what was happening on the outside?" The receptors remained unidentified for decades even though drugs were developed that specifically have their effect through recognition by one of these receptors. In the 1940s, the noted American pharmacologist Raymond Ahlquist examined the response of different organs to various adrenalin-like substances. His work led him to conclude that there were two different types of receptors for adrenalin: the α-receptor that primarily makes smooth muscle cells in blood vessels contract, and the β–receptor that primarily stimulates the heart. It was shortly after this discovery that the first β–blockers were developed. Now β–blockers are among the most frequently used heart medicines. Undoubtedly, such drugs produced effects in the cells, but how they did so remained a mystery. We now know why the receptors were so difficult to find: they are relatively few in number and are mostly encapsulated within the wall of the cell. After some twenty years Ahlquist began to feel lost in his theory about the two distinct receptors, writing at about the end of the 1960s, "To me they are an abstract concept conceived to explain observed responses of tissues produced by chemicals of various structures." At about that time, Robert Lefkowitz, started the studies that have stamped his mark on the history of these receptors.

Luring receptors out of their hiding places

Lefkowitz was a high-achieving young student with his mind set on becoming a cardiologist. However, he graduated at the height of the Vietnam War and was required to do his military service. For him this was prescribed in the US Public Health Service laboratory at the National Institutes of Health in Bethesda, Maryland. There, he was presented with the challenge of finding the receptors! Lefkowitz's supervisor suggested attaching ^{125}I to a hormone so that as the hormone binds to the surface of a cell, the radioactivity would make it possible to track the receptor. However, it was essential also to confirm that the coupling of the hormone to the cell took place on the outside of the cell and that it was this that triggered a process already known to take place in the interior of the cell. Were Lefkowitz to succeed, there could be no doubt that he had, in fact, discovered a biologically functioning receptor. He began by working with adrenocorticotropic hormone (also known as corticotropin or corticoliberin) that stimulates the production and release of corticosteroids in the adrenal gland. However, there was no real success until the second year of study when, in 1970, he published two articles, one in *Proceedings of the National Academy of Sciences,*[1] the other in *Science*,[2] outlining the discovery of an active receptor. This achievement led him into full-time research and, subsequently, he was recruited to Duke University in North Carolina. In new laboratories, Lefkowitz formed his own research team and, while he was never to become a cardiologist, he worked on heart disease and began to focus on receptors for adrenalin and noradrenalin, the so-called adrenergic receptors or adrenoceptors. Using radioactively tagged substances, including β-blockers, his research group examined how these receptors work and eventually, and with great skill, they managed to extract a series of them from biological tissue. Meanwhile, knowledge about what happens inside cells grew, and researchers found that what they call G-proteins were activated by a signal from the receptor. The G-protein, in turn, triggered a chain of reactions that alter the metabolism of the cell. Thus, by the early 1980s, scientists were beginning to understand the process by which signals are transmitted from the outside of the cell to its inside.

The gene – a key to new insights

In the 1980s, Lefkowitz decided that his research group should try to find the gene that codes for the β-receptor.

This decision was crucial to the award of the Nobel Prize. The gene contains the code that is read by the cell when it joins amino acids together to create a protein, such as a receptor. If the research group could isolate the gene and read the blueprint for the β-receptor, insight as to how the receptor works would be gained. At about that time, Lefkowitz hired a young doctor, Brian Kobilka, whose fascination with adrenergic receptors was born of experience in hospital intensive care where a dose of adrenalin could make the difference between life and death. The hormone opens up a swollen respiratory system and speeds up the heart rate. Kobilka wanted to study the power of this drug in its smallest molecular detail, and he approached Lefkowitz and his team of researchers and joined them.

During the 1980s, trying to find a particular gene in the body's genome was akin to finding a needle in a haystack and so this technically very challenging project was slow to advance. However, Kobilka had an ingenious idea that made it possible to isolate the gene and then, with great anticipation, the researchers began to analyze its code. This revealed that the receptor consisted of seven long and fatty (hydrophobic) spiral strings of amino acids – so-called helices. This told them that the receptor probably winds its way back and forth through the cell wall seven times. It had the same number of strings and same spiral shape as previously found for rhodopsin, the light receptor in the retina of the eye. This led them to ask whether these two receptors were related, even though they had completely different functions. Robert Lefkowitz later described this as "a real eureka moment". He knew that both adrenergic receptors and rhodopsin interact with G-proteins on the inside of the cell. He also knew of about thirty other receptors that work via G-proteins. The conclusion was, then, that there had to be a complete family of receptors that look alike and function in the same manner. Since this ground-breaking discovery, the puzzle has been assembled piece-by-piece, and now detailed knowledge about GPCRs – how they work and how they are regulated at the molecular level – is known. Lefkowitz and Kobilka have been at the forefront of this entire scientific journey, and in 2011, Kobilka and his team of researchers reported a finding that crowned the work.

Imaging adrenalin effects – a crystal structure

After having isolated the gene, Brian Kobilka moved to the School of Medicine at Stanford University in 1989. There he set out to obtain an image of the receptor, something regarded by most scientists as unattainable. For Kobilka, this programme took many years, as imaging a protein involves many complex steps. Eventually, he and his group were able to obtain a suitable crystal for X-ray analysis. What needs to be noted here is that the bulk of protein crystal structures have been gained from water-soluble entities whose solubility facilitates crystallization. Far fewer researchers have managed to obtain the structure of a protein located in the fatty membrane of the cell. GPCRs are by nature very mobile (they transmit signals by moving), but inside a crystal they have to remain almost completely stationary. Getting one of them to crystallize was, therefore, a major challenge that took Kobilka over two decades to solve, finally being achieved in 2011.[3] They obtained an image of the receptor at the precise moment that it transfers the signal from the hormone on the outside of the cell to the G-protein on the inside of the cell. The image, published in *Nature*,[3] shows new details about GPCRs, including what the activated receptor looks like when it opens up a void where the G-protein likes to bind. Such knowledge surely will be useful in the future development of new pharmaceuticals.

Life needs flexibility

The mapping of the human genome has revealed close to a thousand genes that code for GPCRs. About half of those receptors receive odours and are part of the olfactory system. A third of them are receptors for hormones and signalling substances, such as dopamine, serotonin, prostaglandin, glucagon and histamine. Some receptors capture the light that hits the eye, while others are located on the tongue and give us our sense of taste. Over one hundred receptors still present challenges as their purposes have yet to be established. Besides discovering many variations in the receptors, researchers, with Lefkowitz and Kobilka in the lead, have found that receptors are multifunctional, because a single receptor can recognize several different hormones on the outside of the cell. Moreover, on the inside, they not only interact with G-proteins, but also with proteins called arrestins, a small family of proteins important for regulating signal transduction. The realization that these receptors are not always coupled to G-proteins has seen them increasingly referred to as seven-transmembrane receptors (7TM), after the seven spiral-shaped strings that wind their way through the cell wall. The receptors' number and flexibility enable the fine-tuned regulation of cells that life requires.

References

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Additional information is available from: *www.nobelprize.org*