Conference Dr. Ignacio Chávez: Moving towards molecular medicine

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Introduction

The sole name of Dr. Ignacio Chávez and the academic quality of those who preceded me make me feel excited, aware that I am on the shoulders of great contributors to our medicine.

Medicine takes care of man in a comprehensive form, as a biopsychosocial entity, and it is therefore, like man himself, quite broad and complex. Molecular aspects are but a small facet of the big diamond medicine is, and I will speak about a small fraction of it. I will try to convince you that, in spite of reductionism, it maintains contact with the big questions of medicine. Paraphrasing the poet León Felipe, I will say that "every rhythm of life passes through that window… and death passes too". I will use as a unifying thread my work on receptors, particularly adrenergic, pointing out some general changes that have occurred over the 46 years that have elapsed since I entered the faculty of medicine.

I had the privilege to be admitted to the faculty of medicine of the National Autonomous University of Mexico (UNAM – *Universidad Nacional Autónoma de Mexico*) in 1971, with its director being Dr. José Laguna. Shortly afterwards, our dear professor, Dr. Guillermo Soberón, took office as rector of the university, and the UNAM recovered lost health. I was lucky to have excellent teachers both in basic and clinical stages and in internship. From the second undergraduate year on, I was instructor at the biochemistry department, teaching laboratory practice once weekly, and the rest of the days working, in the afternoons, with Dr. Victoria Chagoya research group, initially at the faculty, and then where what later was to become the Center/Institute of Cellular Physiology. I completed my Master and Doctor Degree studies and went to Brown University, where I performed my postdoctoral training under the direction of John Fain, and then came back to Mexico in 1980, where I have worked at the UNAM Institute of Cellular Physiology ever since, with two short stays abroad, one with Robert Lefkowitz at Duke University and another with Paul Insel at the University of California in San Diego. I express my most sincere gratitude to the institutions that have supported me, to my exemplary teachers and to my students, without the participation of whom a large part of what we have accomplished would be significantly lessened.

When I was a student, receptors were conceptual entities whose chemical nature was unknown. Early in the 20th century, and thanks to the work of Paul Ehrlich with dyes and John Langley with toxins, it could be determined, by competence, that no actions occur at distance, but that agents (hormones, neurotransmitters, autacoids and drugs in general) exert their action by binding to "receptive substances" that now we call receptors[1,2]. In 100 years, we have moved from the concept to the concrete molecular entity; now, we already know that receptors are proteins, the genetic information chromosomal location and, in many cases, expression abundance by tissues. Today it is possible to atomically study the receptors and express them almost at will in model systems. Sutherland had already recognized cyclic adenosine monophosphate function as intracellular mediator, i.e., as a second messenger; the study of protein phosphorylation was startying and, consequently, the study of

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protein kinases and phosphatases; the fundamental concepts of signal transduction or molecular signaling were starting to be generated[3,4]. There was also knowledge that different receptors could be characterized by order of potency as activators or agonists, and blockers or anatagonists[5].

Mi favorite hormone has been adrenaline (and her sister noradrenalin, which acts as a neurotransmitter). This hormone was identified in adrenal gland extracts by Oliver and Shaffer[6], and later it was crystallized by Abel and Crawford[7] in an impure form (probably by catecholic ring oxidation), and by Takamine[8], already in a pure form. This is one of the numerous reasons for the name adrenaline to be preferred. This was an authentic epic accomplishment, which I will not delve in for reasons of time and space[9,10]. The study of its receptors, another epic accomplishment, had its real initiation with the discovery made by Ahlquist, who pointed out that adrenergic actions could be divided in two groups, based on the potency order of different agonists. With a molecular criterion, Ahlquist[5] deducted that there were two types of receptive substances or receptors, which he named alpha and beta.

The development of beta-adrenergic blockers, initially propanolol, by Sir James Black (1988 Nobel Prize), and its therapeutic usefulness in cardiovascular conditions, such as high blood pressure, angina pectoris and some forms of heart failure, underscored the practical importance of basic discoveries[11]. The use of these compounds was found to be contraindicated in asthmatic patients, which placed doctors in big problems. Fortunately, soon it was defined that cardiovascular (predominantly beta-1) and bronchial smooth muscle receptors (predominantly beta-2) belong to different subtypes, and selective agonists and antagonists were developed, which constituted a substantial therapeutic advance[11,12].

It was also found out that alpha-adrenergic receptors could be subdivided in two subgroups, alpha-1 and alpha-2[13]. However, they were assumed to be isotypes that acted in a similar, yet unknown form. During my posdoctoral training with John Fain, we discovered that these were not receptors that acted in a similar form, as in the case of beta-1 and beta-2, but these were different receptors with couplings to different transduction systems: alpha-2 to adenylyl cyclase inhibition and alpha-1 to phosphoinositide turnover[14]. This indicated that there were three types of receptors for adrenaline: beta (with beta-1 and beta-2 subtypes) coupled in an activating form to adenylyl cyclase, alpha-2, coupled to the same cyclase but in an inhibitory form, and alpha-1 receptors, whose main coupling is to the phosphoinositide/calcium system[14]. This was broadly substantiated by us[15-18] and subsequently by many other groups.

Already back in Mexico, in 1981, and on my first publication as an independent investigator, using vaccine concentrates donated to me by Dr. Mario González Pacheco, from the National Institute of Hygiene, I demonstrated that the pertussis toxin, which is produced by *Bordetella pertussis* bacteria and is the causative agent of whooping cough, is able to block the transmission of inhibitory information from the receptors to adenylyl cyclase[19]; simultaneously and independently, Hazek and Ui[20] reported similar results. Dr. González Pacheco and personnel of the National Institute of Hygiene donated to me large samples of vaccine concentrate that failed to pass the tests due to its high toxicity, out of which I purified the pertussis toxin for my laboratory. Soon, we demonstrated that the pertussis toxin alters affinity for agonists in alpha-2 adrenergic receptors[16,18], but not in alpha-1 adrenergic receptors[18]. This reinforced the idea of different couplings in these receptors and, in addition, Pertussis toxin became an essential tool in the field[21].

Findings made in one field are often of importance for another. This is what happened with the pertussis toxin. There was already some information from Japanese investigators in the sense that pertussis toxin might participate in the protection conferred by the pertussis vaccine. I should mention that the vaccine that was used, standard DPT, contained diphtheria and tetanus toxoids in a suspension of heat-killed *B. pertussis* bacteria. The pertussis component was, then, in a biotechnologically primitive phase. Nevertheless, it was an extraordinary tool, since it dramatically brought morbidity and mortality down in the entire world. Mexico produced its own DPT at the National Institute of Hygiene. In 1984, the results of the use of acellular DPT were published, where the pertussis component consisted of preparations of pertussis toxin detoxified with cross-linkers (formaldehyde/glutaraldehyde). I then proposed the National Institute of Hygiene to test if one of the initial stages of the toxin purification could serve as an acellular vaccine. The result was clear, since it induced an excellent protection using the traditional vaccine testing system itself, and we published the result in 1985[22]. Sometime later we published improvements in the purification and detoxifying process of the toxin for use as an experimental vaccine,

as well as the role of pertussis protomer A as an important antigen in protection induction[23]. We never really managed to draw the health sector's attention and I decided that biotechnology was not my thing. My experiences and some opinions on the subject have already been published[24]. Sadly, economic pressures forced the health sector to abandon the production of DPT and other vaccines, since it became more economical purchasing them already with the new technologies. This broke the national effort to provide Mexico with its own vaccines, which had started with one of our founders, Dr. Eduardo Liceaga[25]. According to information published at its website, Birmex (https://www.birmex.gob.mx/vacbac.html) is in the process of resuming the production of the pertussis vaccine, including the acellular type.

But let's go back to the central topic. With the advances in biochemistry and molecular biology, cloning of adrenergic receptors was started. This enabled to define that the three types of receptor we had found were actually three receptor families, with three members each. Lefkowitz group was the first one to clone eight of them, which, together with his many contributions to the knowledge about their function and regulation, drove him to be awarded with the Nobel Prize in 2012[26-28] (watch the Nobel Conference at: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/lefkowitz-lecture.html). Adrenergic receptors and rhodopsin cloning enabled to determine that these were proteins with seven hydrophobic zones of 20 to 30 amino acids that cross the plasma membrane in an equal number of occasions. All this was confirmed with crystallization. Receptors with this structure form a large family, known as G protein-coupled receptors, seven-transmembrane domain receptors or serpentine receptors.

Advances occurred not only with adrenergic receptors, but with many other of the same family. In addition, knowledge on the human genome and of that of many organisms has enabled to know at which moment of evolution these receptors appeared and how they gradually turned into a huge evolutionary success. Seven-transmembrane domain receptors appeared approximately 1,200 million years ago[29]. They are already detected in fungi, yeasts and plants, and gradually become more abundant in vertebrates, particularly in mammals. In humans, some 600 different receptors are estimated to exist, out of which only in half is the physiological ligand known. On the other hand approximately 300 receptors the ligand is unknown, and they are therefore called "orphans"; for the same reason, we have little information about their function. They are a field of enormous interest for the pharmaceutical industry.

Molecular knowledge of G protein-coupled receptors is also allowing for them to be grouped in families, based on similarities in their sequence. Currently, there are several classifications, and one of the more widely used divides them into 5 groups: those of the rhodopsin family (the most abundant and with various subgroups), those of the secretin family, those of the glutamate family, those of the family of flavors and frizzled, and a complex group of adhesion receptors[30].

As previously mentioned, there are already surprising advances on the structure of receptors based on atomic-resolution X-ray crystallography. Brian Kobilka, a former student of Lefkowitz and also a trained doctor, has made an extraordinary work on this subject, which rendered him sharing with him the Chemistry Nobel Prize[27,28,31]. Studying the beta-2 adrenergic receptors, Kobilka achieved their crystallization in the presence of agonists (active) and antagonists/inverse agonists (inactive). A comparison of their structure shows that antagonists induce for the upper structure (extracellular) to become narrower, to tighten, which elicits then opposite change at the lower part, i.e., to open and in a very amplified form. In addition, by performing a co-crystallization with G protein, he managed to demonstrate that the opening of the receptor at the intracellular portion enables a much wider interaction with G protein loops, in particular with its subunit alpha. All this drives to G protein wide rotation, which is part of signaling initial activation (watch his interesting and educative Nobel Conference at: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/kobilka-lecture.html)https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2012/kobilka-lecture.html).

Receptor expression in system models allowed demonstrating that they are not in a completely inactive form, but that some of them have basal activity and that this has physiological importance (see, for example, references 32 and 33), and is even relevant in the pathogenesis of some conditions[34-36]. This is changing pharmacology, since it has made us aware that the idea of agonist/antagonist is not everything. Today, we talk about full agonists, partial agonists, classic antagonists, allosteric modulators, internalization inducers and biased agonists[37-39]. This has already had clinical application consequences. One example is the case of fingolimod (Gilenya ®, Novartis),

which has already found a therapeutic niche in the treatment of multiple sclerosis. This agent is an agonist with very brief action on sphingosine 1-phosphate receptors, which has a bias towards receptor internalization and degradation, actually acting as a long-lasting functional antagonist[40,41].

Molecular biology has also enabled adding labels to proteins in order to follow them from synthesis to degradation. One of them, the green fluorescent protein, has been widely used[42]. This protein has allowed for us to follow the receptors, under the action of different agents, during their internalization to intracellular compartments and even on their recycling to the membrane[43]. Related technologies, such as Bioluminescence Resonance Energy Transfer (BRET) and Fluorescence Resonance Energy Transfer (FRET), by means of energy transfer, enable to determine whether there is association between receptors and other cellular proteins[44-48].

The knowledge that has been acquired over the past 25 years is way over that of the previous 25, and the advance will surely keep on accelerating. This has occurred in all areas of medicine, from molecular medicine to the world of images (tomography, magnetic resonance imaging, positron-emission tomography, echocardiogram, etc.) or to interventional medicine (the world of stents, pacemakers, valves, filters, etc.), just to mention a few. However, these technical-scientific advances are only elements to improve the quality of services provided to the patient. There is no substitute for a good doctor-patient relationship, for a detailed clinical history or for a good examination. Medicine is a profoundly human profession and it should stay this way: our objective is comprehensive health of the human being.

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