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Review / Revue

Nicotinic receptors and nicotine addiction

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Every year, more than five million people worldwide die from the consequences of smoking, and these deaths, principally from lung cancer, are avoidable. However, there is a formidable obstacle to the prevention of these deaths. Tobacco contains a drug, nicotine, that creates a dependence almost as severe as that generated by cocaine or heroin. How does such a simple chemical substance have such a major effect on human behavior? This question is of even more general relevance. What relationships are established between the chemistry of our brain and its higher functions, such as our decision-making capacity or conscious will? The answer to this question appeared unattainable only a few years ago and, some believe that it may ever remain beyond the reach of scientific analysis. A radical paradigm shift has occurred in recent years, with the gradual convergence of several disciplines that were previously considered entirely separate, such as physiology, pharmacology, molecular biology and cognitive sciences. This article provides an illustration of this.

France has the honor of being the birthplace of two of the founding fathers of this approach, both of whom were members of our Academy of Sciences. Claude Bernard, with his "*Leçons sur les substances toxiques et médicamenteuses*" taught at the Collège de France in 1857, was responsible for establishing an experimental methodology, still used today, based upon the "localization of drugs action". Louis Pasteur, through his early work, demonstrated the existence of stereospecific recognition based on elementary chemistry.

So, what is nicotine and where does it come from? Amerindian populations have used tobacco for thousands of years, in the treatment of disease, to free the body of the "demons" possessing it. This use demonstrated the presence in the plant of an active substance. The Ancient Greeks used the term *pharmacon* to describe material of this type. This led to the development of the concept of "pharmacological agent".

Tobacco was brought to Europe by Christopher Columbus in 1492 and was initially grown as an ornamental plant, until Jean Nicot, in 1560 sent some tobacco powder to Catherine de Medicis, to treat her son, François II, for migraine. This treatment proved successful. The plant was subsequently named after him, *Nicotiana tabacum*. In 1809, Louis Vauquelin isolated the active substance, nicotine. The nicotine molecule is an alkaloid, including a tertiary amine with a pyrimidine ring and a pyrrolidine ring. It has been shown to be effective against migraine and toothache and to act as a stimulant. However, an understanding of its mechanism of action had to wait until the toxic effects of another substance of plant origin, curare, had been elucidated. As early as 1857, Claude Bernard showed that the paralyzing action of curare was neither due to effects on nerves nor to effects on muscles. Instead, curare blocks the transmission *between* nerve and muscle fibers. John Newport Langley, inspired by the work of Claude Bernard, took another step forward of particular importance in 1905. He applied nicotine locally onto the surface of a muscle, causing its contraction,

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and showed that this effect was blocked by curare. He then showed that the muscle surface responding to nicotine was found exclusively beneath the nerve ending and observed that this sensitivity persisted even after denervation. He coined the term "receptive substance" or *receptor*, to describe the "substance in the muscle that binds to nicotine and curare" and distinguished it from the "substance that contracts". At about the same time, T.R. Elliott suggested that a "stimulatory secretion" released by the nerve might elicit muscle contraction. The molecule involved was described as a *neurotransmitter* and later identified as acetylcholine. Nicotine simulates the effect of acetylcholine, and the receptor common to nicotine and curare present on the surface of the muscle is the *nicotinic* acetylcholine receptor. Acetylcholine and nicotine act as agonists of the acetylcholine receptor, whereas curare is an antagonist of this receptor. Acetylcholine is degraded by acetylcholinesterase, which is blocked by nerve gases. By contrast, nicotine is not degraded by acetylcholinesterase, rendering its action much more stable. More generally, our nervous system contains a chemical array of several tens of neurotransmitters. As shown by Tomas Hökfelt, who was awarded the *Grande Médaille* by our Academy, several of these neurotransmitters may be simultaneously present in the same neuron.

Over several decades, the concept of "receptor" remained theoretical. Doubts were often raised about the existence of such receptors and many thought that present in tissues in such small quantities it would never be possible to characterize them chemically. This was the case until the 1970s, when the acetylcholine receptor was characterized in my laboratory at the Institut Pasteur. Perhaps I owe this success to the training in zoology I received from my first teacher, Claude Delamare Debouteville. Thanks to him and his naturalist colleagues, I learned that the electric ray (of the genus *Torpedo*) and the electric eel (*Electrophorus electricus*) possess an electric organ extremely rich in synapses all of the same type. The tremendous richness of this organ in cholinergic material was discovered in Paris in the 1930s, by David Nachmansohn [1], who was then working with the late René Couteaux. However, a marker for the selective labeling of the receptor was required. Nicotine and curare were not specific enough. Instead a small protein purified from the venom of a poisonous snake *Bungarus multicinctus* by the Taiwanese pharmacologist Chen Yuan Lee in the 1960s [2] was found to be an ideal tag for this receptor. This protein specifically displaced a radioactive analog of acetylcholine (decamethonium) on synaptic membranes purified from the electric organ *in vitro*. This binding to the membrane was thus postulated to take place at the level of the acetylcholine receptor site [3]. As first suggested by Louis Pasteur in 1886, there was indeed "*un corps actif dissymétrique*" ("an asymmetric active compound") that was involved in the "*impression nerveuse*" ("nervous exposure"). It subsequently became possible to characterize the membrane receptor protein, by dissolving it in "mild" detergents [4], purifying it from our preparations [5] and observing it under the electron microscope [6]. The image of these "centered" rosettes, about 90 Å in diameter, distributed all over the subsynaptic membrane, was particularly striking [6]. We were able to see, for the first time, what a neurotransmitter receptor looks like. This receptor was also the first ion channel to be chemically identified. With a molecular weight of about 300 000, the receptor protein forms a compact bundle of five subunits traversing the subsynaptic membrane. It carries binding sites for acetylcholine and nicotine on its face exposed to the synaptic cleft and contains an ion channel selective for Na^+ and K^+ in its membrane portion [7].

Moving on to the 1980s, several groups around the world, including our own and that of Shosaku Numa in Kyoto, determined first the partial [8] then the complete [9] sequence of *Torpedo* receptor. The structure of the molecule was progressively unveiled.

The structure of the neurotransmitter site first explored by biochemical labeling and site directed mutagenesis (see [7]) is now established at the atomic level, thanks to the discovery by Gus Smit and Titia Sixma of a small protein from a freshwater snail, known as the "acetylcholine binding protein", that can easily be crystallized [10]. This site forms a pocket, flanked by aromatic amino acids that adapts in a tight and complementary manner to the molecules of acetylcholine and nicotine. These observations pave the way to the rational design of nicotinic drugs that adequately fit the structure of the receptor site.

Ion channels had long been problematic for chemists. How can one identify a hole? The first solution, developed in my laboratory in the 1980s, was to use as a photolabel a compound that sterically blocks the ion pore [11]. Another solution, developed by Bert Sakmann and his colleagues, involved the patch-clamp recording of single channel openings following gene mutations [12]. As a result of these approaches, we now know that the ion channel runs along the axis of symmetry of the receptor molecule, is lined by the second trans-membrane domain and contains negatively charged amino acids that control the translocation of Na^+ and K^+ ions.

The receptor molecule mediates the transduction of a chemical signal into an electrical response. But, how

can two topographically distinct sites, the acetylcholine binding site and the ion channel, be bound together at the level of the receptor protein? Quite a while ago, in 1964, in the conclusion of my PhD thesis carried out under the supervision of Jacques Monod and François Jacob, I took the risk to speculate that, in the absence of experimental evidence, an analogy might exist between the bacterial regulatory enzymes on which I was working and the processes underlying synaptic transmission [13]. My thesis focused on the mechanism by which two structurally different molecules, an enzyme substrate and a metabolic signal, could inhibit each other at the level of an enzyme molecule. The data were not consistent with the classical idea of a mutual exclusion by steric hindrance from a common binding site. Instead, they suggested a model in which the two sites concerned were structurally and topographically distinct, thus the term "*allosteric*" [14]. Such *indirect* interaction was postulated to involve a global and concerted conformational change of the protein [15].

In the case of the acetylcholine receptor, the binding sites and ion channel are more than 30 Å apart (see [7]). Could an allosteric transition of this type take place with this receptor? This question has very recently been resolved at the atomic level. Once again, the tremendous diversity of the living world has been of considerable assistance. The most ancient photosynthetic bacteria have receptor channels very similar to the acetylcholine receptor [16]. These receptors are compact, stable and simpler than the animal receptors. They were isolated and crystallized by Pierre-Jean Corringer, a former collaborator of mine who has recently become a colleague. Their structure was then resolved by X-ray crystallography, by Marc Delarue and his collaborators at the Institut Pasteur [17]. The allosteric transition takes the form of a sort of discrete torsion of the molecule, a quaternary twist linking the active site and ion channel and resulting in the opening and closing of the ion channel [17].

Thus, the communication in our brains is based on molecular mechanisms that first appeared on our planet some three billion years ago, not through "intelligent design", but following a long period of Darwinian evolution beginning right at the start of life.

The number of publications on receptors has increased exponentially over the last few years, with 652 000 articles published on receptors in general and 45 800 articles published on the diverse receptors of acetylcholine. The field has become considerably diversified. Other receptors and ligand-gated ion channels have been discovered, including the receptor of the inhibitory neurotransmitter GABA, the target of benzodiazepines, a group of tranquilizers that French people too frequently abuse. Many other receptors are linked not to a channel, but to a G-protein, whereas another group, such as the *Toll* receptor studied by our Academy President, has another mode of action. However, all these molecules are allosteric membrane proteins. From general anesthetics to tranquilizers, and from antipsychotic agents to cardiovascular effectors, about 46% of the drugs in current use target membrane receptors [18].

In this immense repertoire, the acetylcholine receptor remains a target molecule of choice, known at the atomic level and involved in key brain functions affected by major diseases in humans. There are 17 genes encoding subunits of the nicotinic receptor in our genome and these subunits assemble together to create a broad diversity of receptors distributed in various territories throughout the brain. How can we elucidate the role of acetylcholine in its multiple functions in the sleeping and waking cycle, attention and learning, reward systems and, of course, tobacco addiction? The strategy that we have developed makes use of the many possibilities provided by genetically modified mouse models [19]. It involves several steps: invalidation of the gene encoding the subunit defined, followed by quantitative analysis of the physiology and behavior of the animal; then re-expression of the gene at a critical site in the brain using a lentiviral vector developed three years ago in my laboratory by Uwe Maskos [20].

The experiment was carried out with the gene encoding the beta 2 subunit. In the absence of this subunit, mice lose the ability to self-administer nicotine. Another simultaneous change in behavior is also observed in these mice: rather than meticulously exploring and searching their environment, they run continually from one area to another, they navigate. The site chosen for re-expression of the gene — the ventral tegmental area — was not selected by chance. This region houses the body of neurons containing dopamine, a neurotransmitter specialized in the reward process. Mice treated with the lentiviral vector re-acquire the behavioral traits they had lost: they begin to self-administer nicotine and to explore their environment again [20]. The nicotinic receptor containing the beta 2-subunit thus serves as a critical link between the internal release of acetylcholine and the dopaminergic reward. Thus, we are beginning to understand, step by step, the molecular mechanisms involved in the acute effects of nicotine and those involved in the consequences of chronic nicotine usage. Long-term adaptation occurs in the expression and function of the subunits of the nicotinic receptor, and a functional reorganization of the brain circuits ensures the homeostasis of cognitive functions in the presence of

nicotine. However, the chronic presence of the drug becomes indispensable.

Several subunits of the nicotinic receptor are involved in the establishment of this dependence. Others are involved in the appearance of withdrawal symptoms when people quit smoking. Remarkably, three independent epidemiological studies this year have highlighted the importance of three genes encoding subunits of the nicotinic receptor as predisposing to lung cancer: alpha3, alpha5 and beta4 [21]. Despite being aware of the risks involved, smokers continue to smoke, they are unable to enforce their will to stop smoking. In this respect, there is a loss of conscious control. Brain imaging in adolescent heavy smokers subjected to withdrawal has revealed a decrease in frontal cortex activity. Perhaps this brain network, which includes the frontal cortex and which Stanislas Dehaene and myself propose to be involved in the access to consciousness [22] is particularly susceptible to nicotine?

These results have stimulated considerable pharmaceutical research activity focusing on nicotinic receptors, particularly in the domain of tobacco dependence, but also for diseases linked to smoking in pregnant women, such as Sudden Infant Death Disease and Attention Deficit Hyperactivity Disorders in children. Paradoxically, they have also led to pharmaceutical research in the field of degenerative diseases, such as Alzheimer's disease, Parkinson's disease and even depression, for which nicotinic agents might serve as neuroprotectors and cognitive enhancers.

An understanding of the allosteric mechanisms involved in the mode of action of acetylcholine and nicotine has led to major changes in drug design. Whether one target the receptor site or the ion channel, one needs to consider the dynamics of the conformational changes occurring: one kind of "molecular key" opens the site or channel and a different one closes it, depending on the allosteric state it stabilizes. Unexpectedly, a unique category of molecules, known as "allosteric modulators" has been discovered to modify these dynamics by binding to a new type of site located in the transmembrane domain. There are serious hopes to develop efficient therapies originating from this new pharmacology.

Progress in brain chemistry and major advances in neuroscience in general raise important ethical questions, as one might expect. We know it is possible to use this knowledge for ethically unacceptable purposes, as the development of nerve gases or the various attempts to control human behavior beyond the will of the human subject. It is our responsibility and that of our Academy to oppose these advances. It is also our responsibility to use this knowledge to build, together, a future for humanity that would lead to a more harmonious and peaceful life for everyone in our planet.

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