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Review Article

History of Neuromuscular Blockers

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Received: December 12, 2019; **Published:** January 28, 2020

Abstract

Anesthesiologists administer anesthesia for almost 100 years without the use of the so-called muscle relaxants (from Morton in 1846, to Griffith in 1942). So, we were able to live a century without these drugs so related to our specialty and we have probably under utilized it in some opportunities and we have used it in anothers. I say wrongly called muscle relaxants, because the name is quite universal, because the most appropriate name is that of neuromuscular blockers (BNM), to differentiate them from thats drugs that act at the level of the central nervous system. If we were more purists, this name is not entirely adequate either, because succinylcholine, one of its most conspicuous representatives, does not block receptors and is therefore not a blocker, although it certainly produces relaxation of the striated muscles.

Keywords: *Neuromuscular Blockers; Muscle Relaxation; Narcosis; Analgesia*

Introduction

The introduction of NMBA into clinical practice changed the way of defining anesthesia. Before its existence, endotracheal intubation was an exception and, if muscle relaxation was needed, it was obtained by increasing the inspiratory fraction of inhalation anesthetics, much less safe than the current ones and with obvious risk of respiratory and cardiovascular depression. After the use of NMBA became habitual, a conceptual change took place and anesthesia was defined as a triad formed by three inseparable elements: narcosis, analgesia and muscle relaxation.

The history of neuromuscular blockers can be divided into two periods: the ancient history, referring fundamentally to the discovery and research related to curare and, modern history, referring to the development of molecules that were increasingly specific in their action and with an increasingly better therapeutic safety margin.

The fascinating history of curare

The history of the curare covers about 300 years, from the arrival of the adventurers who followed Columbus after his trip to the New World, the descriptions of death after receiving an arrow or a dart shot by the natives made by Sir Walter Raleigh and, his transfer to Europe by the explorers to investigate what produced the death [2]. It is a romantic story of exploration, personal curiosity, visionary conjectures and a certain degree of serendipity [3].

Columbus's contact with aborigines who will use curare has lent itself to many speculations, but no concrete evidence [4]. After the arrival of Columbus in the New World, colonies were established on the island of Hispaniola (currently Haiti and the Dominican Republic) and Darién (south of the Isthmus of Panama). From there, expeditions arose to the north (Mexico) and to the interior of the South

American continent. The discovery of gold, especially by the explorations of Pizarro, came to Europe as the news of the gold of California a few centuries later, so many sailors without great preparation tried to cross the then called Ocean Sea, some successfully, while others, unaware of the problems of long journeys and the winds of the south, they never reached their destination.

Those who arrived, were subjected to the attack of hostile natives emerging from an impenetrable jungle, armed with spears and darts embedded in a certain substance of mystical and obviously mortal properties (Figure 1). This system was not new; since biblical times, hunters and warriors spread their arrows with poisoned substances to make them more deadly. He had been described by Virgil in the Aeneid and then, in the Middle Ages, used by Celts and Gauls, but replaced by other more efficient weapons, such as gunpowder, in Europe and Asia.

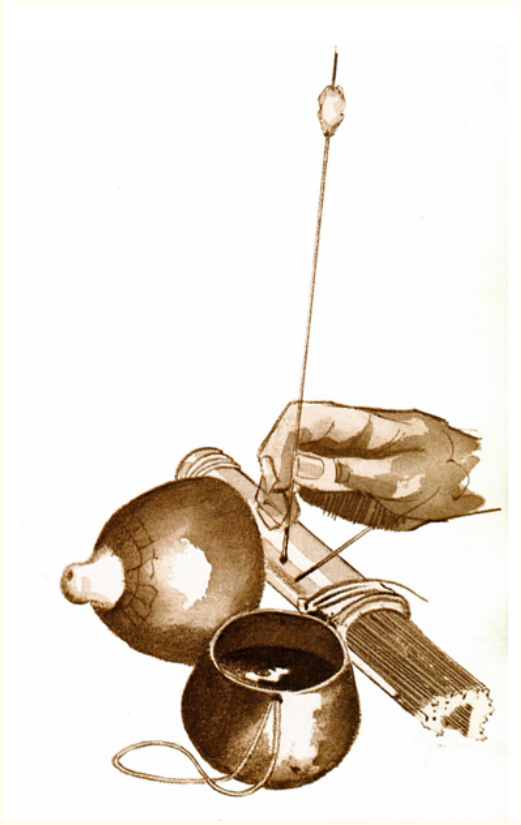

Figure 1: *The arrows, spears and darts of some Latin American tribes were impregnated with a poison that the Spaniards called curare, the product of a mixture of various plant substances (including Chondrodendron tomentosum) and animals.*

Descriptions of the deaths caused by the poisoned arrows in South America seemed in Europe, especially told by eager speakers looking for success, an act of witchcraft. Of course, many of these stories were adorned to impress an anxious audience, unable to discern between the real and the fantastic. These deaths were especially frequent among explorers who approached the Amazon River and the Orinoco Basin, where bloodthirsty warriors native to these regions used a particularly potent poison to exterminate their hunting prey and their enemies.

The conquerors observed that the natives embedded their darts and arrows in a sticky concoction, probably composed of herbs, whose lethal effect was obvious. The death it caused was really horrendous. One of the most frightening aspects of the nature of this type of death was that there seemed to be nothing to prevent it. It was logical that an antidote could exist and, apparently, the natives knew it, but first it was necessary to know what the poison was and how it killed the victims. This poison was obtained from certain herbs and roots of a vine that was soon called "grass" by the Spaniards.

The first details were described by the Italian monk Martín de Angleira, who reported in the Spanish court, around 1514 (without ever being in America), that the poisonous potion was made by a small sect of old women, who kept it sealed by a couple of days and they often died by inhaling their gases [5]. Its composition and manufacturing was kept secret. A later report by Nicolás Bautista Monardes, who also does not appear in the lists of travellers to America, but who was an outstanding Sevillian doctor, author of several works of great value and by some considered as the father of pharmacology, also reports on the danger of the inhalation of the preparation and the horrible death it caused, describing a whole ritual that involved its preparation, surrounding the story of magic and witchcraft. The specific work is called "Two books, one which deals with all the things brought from our West Indies, that serve the ends of medicine, etc." published in Seville in 1569 by Hernando Díaz.

However, the most dramatic descriptions of the "ourari" effect, as one of his lieutenants called it, were made in 1595 after Sir Walter Raleigh's return to Europe from Guinea (Guyana), in which some members of his expedition penetrated the Orinoco basin, including his son who lost his life. The people who received the poisoned arrows were victims of unbearable torment: they remained conscious, with their eyes fixed, but were unable to scream or cry during their agony. The English also believed that there was an antidote, but that even the Spaniards were not using tortures, which they were accustomed to using with the natives (and with their European counterparts who thought differently), they managed to obtain.

The descriptions of the English privateer also lend themselves to doubts because, although educated at Oxford, his vivid descriptions of El Dorado, put a blanket of insecurity about his real intentions or his veracity. Raleigh, probably informed by his men and the Spaniards, describes in this detail the wound with a poisoned arrow: "… because the mortal wound causes the pierced place to support the most insufferable torment in the world and await the ugliest and most regrettable death; sometimes they die wildly crazy, sometimes the intestines leave the belly, and so stinking that no man can bear to heal or care for them… " [6]. Obviously, nothing that the corsair refers to is sympathetic to what we can know today about a "cure" or what those who after Raleigh tried the curare experimentally.

The fear of these stories was such that the need to learn about the nature of this poison was created, so it had to be taken to Europe and studied scientifically. This is how the first experiments carried out on this so-called ourara, urali, urare, woorari, wourali and curare (European attempts to reproduce the native word), were performed at the University of Leiden, Holland, around 1740. There, a English last name Brockelsby, observed that even when the poison was injected into a cat's paw, breathing seemed to be compromised, but the heart was still beating for more than two hours, which meant that the poison did not produce cardiac arrest. However, around 1780, Florentine Abbe Felix Fontana, at the same university, observed his safety orally in humans (as opposed to what happened with guinea pigs and pigeons), his inability to kill the vipers and, definitely, that its vapors did not cause any harm [4]. Thus, the stories spread after the first navigators were wrong. Only studies conducted in Israel at the Weizman Institute more than two centuries later, managed to demonstrate that one of the subunits that make up the acetylcholine receptor in vipers, had a different sequence in the chain of amino acids that make it up (the sequence between the amino acid 128 and 142) [7]. This means that the curare and the venom of the vipers themselves do not affect them and that the response in animals is diverse.

The researcher and explorer Alexander von Humboldt was also involved in the history of the curare. After a series of explorations into the interior of Venezuela made between 1799 and 1804, in 1832 he gave the first version in the west of the way in which the poison in Esmeralda was prepared, "the most isolated and remote Christian settlement of the Upper Orinoco" [8]. Humboldt describes that curare

would be a poison when used on arrowheads and darts, but also a medication when used orally. Probably the oldest description of the use of curare in medicine was made by a friend and fellow Humboldt's explorer, Robert Schomburgk, who tested the "placebo" effect of curare in a malaria attack. He had seen the natives use it for stomach aches, but it was obviously ineffective for one and the other.

The next collaboration in discovering the cause of death of the curare, was made by Charles Waterton, an eccentric naturalist and taxidermist traveller who, on his first trip to what would later be British Guyana, in 1812 at the age of 30, observed the lethal effects of darts and poisoned arrows and got samples of the poison to take them to Europe [9]. If Waterton had not taken samples of the poison to England, the curare's story would have stopped for some time. With these samples he made an experiment by injecting a dose in the leg of an ass after making a firm ligation over the puncture site, observing that the animal walked and fed normally; After an hour, he released the ligature and in ten minutes the animal collapsed and died.

Benjamin Brodie was a distinguished surgeon who studied the effects of a series of plant-based poisons and published his observations in "The Effects of Certain Vegetable Poisons". His version is that he would have obtained the "wourali" samples from Dr. Edward Nathaniel Bancroft, son of a doctor who had practiced in Guyana. From a very different personality to Waterton and an unquestionable scientific rigor, he became president of the Royal Society. After rehearsing and publishing experiments on guinea pigs, cats and rabbits, he made a demonstration similar to that of Waterton in 1812 at the Royal Society in London, this time in a female ass, but without linking the limb. This time the poison, which he now called "woorari", quickly paralyzed the animal and stopped his breathing. Brodie immediately performed a tracheotomy and resuscitated the poisoned animal by maintaining rhythmic ventilation for two hours. The donkey survived several years after the experiment. This is how Brodie finally demonstrated how the curare killed his victims: causing respiratory muscle paralysis, resulting in death by suffocation, without any effect on the brain or heart.

One of the problems in trying to find out what curare really was is that the samples taken to Europe had differences in variety and quantity of ingredients, some of which could deteriorate over time. Logically, the poison varied in its composition according to the region in which it was produced. Those obtained in the Orinoco basin were mainly composed of barks and roots of the *Strychnos nux* vine and called it *Strychnos toxifera*. Since they contained strychnine, convulsive effects predominated, masking the paralyzing effect of curare. On the contrary, those prepared in the westernmost jungle of what is now Ecuador and Peru, in the Marañón river basin, were composed of an extract of vicuñas skin and especially of the *Chondrodendron tomentosum* vine. The latter contained high concentrations of curare and was similar to that used by Waterton and Brodie in their experiments.

Thus, the enigma of discovering how and why curare produced paralysis came, which was carried out by the elegant experiments carried out by Claude Bernard in France, the intuition of Otto Loewi in Vienna and the studies of Sir Henry Dale in London.

Claude Bernard, Otto Loewi and Henry Dale

Claude Bernard's experiments (Figure 2), fundamental in the history of curare, although much more significant in other fields (the role of the liver and pancreas in the regulation of glucose levels), related Brodie and Waterton's observations and the analysis of the way it acts on the muscle itself. They demonstrated the way in which the curare causes the paralysis of the respiratory musculature and the musculoskeletal system, and thus the death of the animal. That is, it showed where curare acts to paralyze its victims, through a meticulous scientific method.

A colleague of Bernard, Jules Pelouze, took samples of curare to the laboratory, brought from Brazil. The results of the experiments carried out by Claude Bernard with these curare samples carried out between 1844 and 1856 were those that revealed the way in which the curare paralyzed and killed his victims.

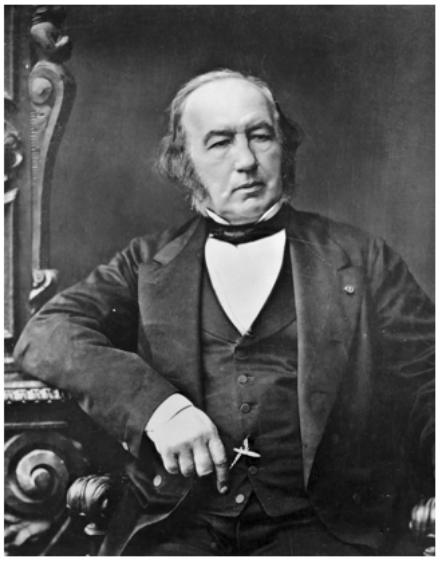

Figure 2: *Claude Bernard (1813-1858), a French physiologist whose experiments in muscle and nerve preparations in toad diaphragms were central to curare history.*

As a result of a series of tests on isolated frog muscles and nerves, Bernard managed to prove that curare poisoned nerve conduction but not muscle contractility. In this way, he was convinced that the curare somehow prevented the messages carried by the nerves to the muscles to produce muscle contraction. Thus, after another series of elegant experiments to discriminate whether the curare acted on motor and sensory nerves or selectively, he managed to demonstrate that the curare acted only by blocking the messages to the motor nerves that reached the muscles, and concluded that selectively and reversibly It poisoned the nerves. His observations were published in the famous book Leçons sur les effects des substances toxiques et medicamenteuses, in 1856 [10]. He was almost right.

At the same time, the development of high-resolution microscopes in Germany allowed Virchow to demonstrate that the organs were formed by independent cells, achieving histology development. Thus, in 1862, Wilhelm Kühne managed to demonstrate a slight swelling at the end of the motor nerve, which ended is a kind of "bulb", and that there was a space that separated him from the muscle. He also observed that the opposite surface was different from the rest of the muscle, thus discovering the motor plate or the neuromuscular junction site.

In 1866, a pupil of Claude Bernard, Alfred Vulpian, concluded in his thesis that this was probably the site of action of the curare. He reported that when it was applied near the motor plate it produced a rapid and deep paralysis, while, if it was applied near the motor nerve, it produced a smaller and slower effect. He concluded that "curare interrupts communication between nerve and muscle fibers". In this case, he was completely right, although he was refuted by Bernard. Teachers do not always recognize the abilities of their disciples, or worse, some are envious of their achievements.

With the invention of the galvanometer and the use of long nerves, such as that of the giant squid, it was possible to demonstrate that a wave of electrical current passed along the nerve when it was activated. Upon reaching the nerve end, the current caused a response, such as the contraction of a muscle or the secretion of a gland. The electrical theory, which postulated that the conduction of a nerve stimulus

to the muscle was the result of an electrical charge that jumped the space between the nerve end and the muscle fiber was not questioned until the 20s of the last century. Famed professor of physiology Charles Scott Sherrington was the biggest advocate of this wrong theory. Those who postulated the possibility that a chemical transmitter was involved were considered dissidents.

It was only between 1934 and 1950, that finally the opinion of the few dissenters to the electrical theory was taken into account and demonstrated, with much patience and perseverance. As a result of this scientific earthquake, with an epicenter in Cambridge, the landscape of human physiology was definitely altered. During that period the experiments were done and the evidence was published that definitively established that a chemical agent, acetylcholine, was involved in the transfer of information from the brain, through the nerves, to the organs under its control, including the striated musculature.

There is a general agreement that the great advance that produced the paradigm shift between electrical theory and the possibility of a chemical transmission was due to Otto Loewi and Henry Dale. Their contribution was recognized in 1936, when together they received the Nobel Prize for their work on the chemical transmission of nerve impulses.

Otto Loewi (Figure 3) was in Cambridge, and under the influence of Dale, committed to the study of the chemical transmission of neuromuscular transmission, though he devoted himself to the study of the autonomic nervous system upon his return to Austria. Through a series of neat experiments carried out in isolated hearts of toads (with their preserved innervation), he was able to demonstrate for the first time that a chemical agent (acetylcholine) was involved in passing the information that was carried by a nerve. His valuable work was interrupted when he was arrested by the Nazis, who occupied Austria in 1938. He was so worried that his discoveries would be lost that, from prison, bribing an assistant, he managed to have his studies sent to a scientific journal [11]. Subsequently, thanks to the efforts of the international scientific community and especially Henry Dale, he was forced to hand over the proceeds of his Nobel Prize to the Nazis and released. Later he was able to continue his teaching activity in the United States.

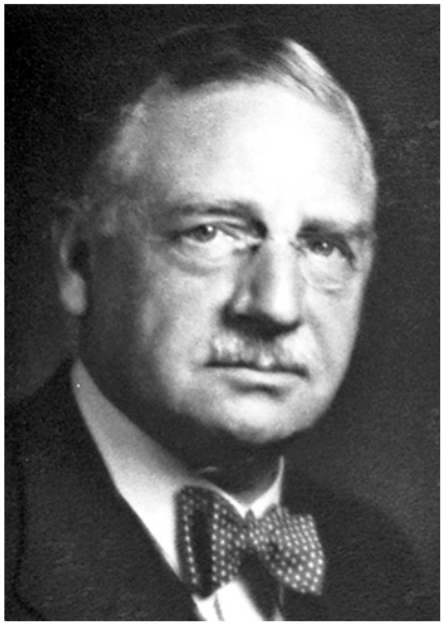

Figure 3: *Otto Loewi (1873-1961), German physiologist of Jewish origin who managed to demonstrate that the nerve impulse was transmitted in a chemical and non-electrical way, and that in the sympathetic nervous system was acetylcholine.*

When Henry Dale (Figure 4) was able to demonstrate to the scientific community that acetylcholine was produced after stimulating a nerve, in the absence of any muscular activity, it was when the curare took a leading place. By stimulating the nerve that innervates an animal's thigh, acetylcholine was produced whether or not the curare was present; This evidence, together with the fact that the acetylcholine injected into the vessel that irrigates the muscle produced muscle contraction, even if the nerve was not stimulated, not only proved the theory of chemical transmission but also demonstrated the way in which the curare acted. The curare had prevented acetylcholine released during nerve stimulation, causing muscle contraction: it had "blocked" the action of acetylcholine in a particularly sensitive area of muscle [12].

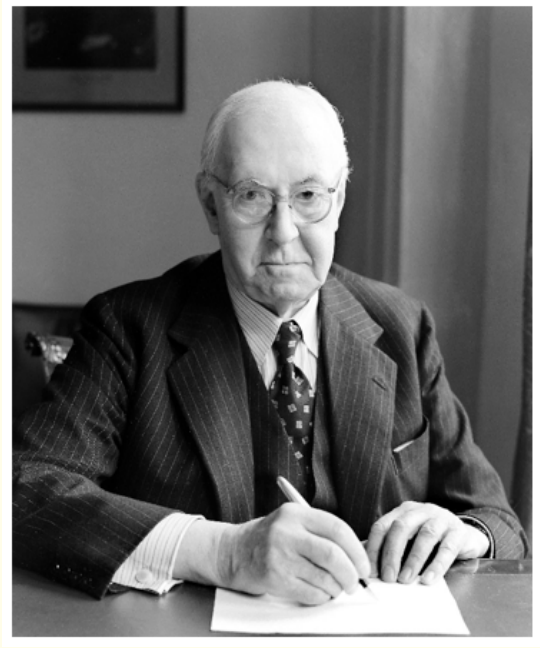

Figure 4: *Henry Dale (1975-1978), an English physiologist who isolated succinylcholine, which allowed, together with Otto Loewi's research, to demonstrate the chemical transmission of the nerve impulse. Both shared the Nobel Prize in Physiology-Medicine in 1936.*

Subsequently two physiologists of the Max Planck Institute in Germany, Neher and Sackman, finally demonstrated how chemical transmitters work at the molecular level and how their action was blocked by the curare. They were also rewarded with the Nobel Prize in 1991. By isolating an acetylcholine receptor from the muscle with electron microscopy, they managed to demonstrate that acetylcholine opened the central pore in a special area of the motor plate, now called the receptor, which allowed sodium ions (electrically charged), will pass through the cell membrane, which resulted in the generation of an electric micro pulse. The production of a small electric current was then the effect and not the cause, of neuromuscular transmission. In addition, this effect was blocked by the curare [13].

Clinical use of curare

Very early, after Bernard's experiments, curare was used rather empirically in diseases that produced muscle contracture, such as tetanus and rabies, and in seizure diseases such as epilepsy. Many descriptions of the use of curare for seizure treatment were published around 1950 and many claimed credit for having achieved therapy. Obviously, the results were not flattering because, although the patients

diminished their contracture, the symptoms manifested again when the effect passed, or even some fell into respiratory depression and death. The alternative at that time was increasing doses of laudanum (opium), which also improved symptoms, but caused patients to fall into respiratory depression and anoxia. The times of artificial ventilation had not arrived.

In the introduction of the curare in clinic the serendipity could not be absent. Richard Gill, former director of a rubber company, decided to settle east of the Andes, in the Amazon basin of Ecuador. He had an accident after falling from his horse that left him with a spastic paresis, probably a spinal cord injury, which made him return to the United States to recover slowly with rehabilitation. During his recovery he suffered severe painful spasms in the right limbs. It was then that he thought about the possible utility of the poison used by the native neighbor to his farm in South America to hunt his prey. This made him return to Ecuador in 1938 to collect enough curare so that the drug could be purified and clinically tested in the United States. So, he gets about 39 pounds of raw curare (around 13 kilograms) [14]. Thus, if Gill had not fallen off the horse, the history of medicine would not have told this essential product so quickly, because until then, the curare was nothing more than a pharmacological curiosity. Of course Gill never fully recovered, remaining with spasticity and tremor episodes. Its use to relieve spasticity was unsuccessful, but it was serendipity that produced the convergence of events that led to the arrival of this large amount of drug to the United States, and that allowed clinical trials in the field of anesthesiology [3].

It was in the laboratories of E. R. Squibb that the curare was purified and standardized for the first time in a commercial preparation. However, it was Harold King in 1935 who isolated and identified for the first time the chemical structure of the curare at Burroughs Wellcome Laboratories in London from the *Chondrodendron* and since the sample had been stored and labeled "Ucayalli river 1871" in a container of bamboo, the purified alkaloid was called tube-curarine [15].

King showed that the chemical structure of the curare was very similar to two acetylcholine molecules, separated by a bulky chemical bond. It was shaped like two hooks, separated by a stem; the distance between both ends had to have a specific dimension. This allowed the ends of the molecule to simultaneously engage as a key in a lock on the receiver, blocking neuromuscular transmission. If the distance between the two molecules varies, only one hook adheres at a time and no blockage occurs. Although we now know that King's formula was not completely accurate, he had the merit of initiating the synthesis of new curare molecules, more selective in their neuromuscular blocking action and with less collateral effects. It is what was later called structure-activity relationship.

The first curare purified by Squibb laboratories was called Incostrin and was used curiously in Nebraska, to reduce seizures associated with seizure therapy with Metrazol, a drug used in psychiatry as an alternative to electroshock. The psychiatrist, surnamed Bennett, reported that patients stopped having seizure fractures, using "half the necessary dose of Incostrin that produced respiratory paralysis". However, the introduction in anesthesia itself belongs to the enthusiastic and persistent Lewis Wright.

Wright, trained as an anesthesiologist at Bellevue Hospital in New York, was able to observe the difficulty associated with producing good surgical conditions when opening the abdomen and relating it to the use of curare, which he had observed using Bennett in seizure prevention. His anesthesiological baggage made him think about administering more relaxation and less anesthesia in the sickest patients, since the way to do it was then deepening the anesthesia. Funded by the Squibb laboratory, he tried to convince opinion leaders in anesthesiology in the United States at the New York Congress in 1940. He convinced his former head of the Bellevue, Dr. Emery Andrew Rovenstein, who used the same dose suggested by Bennett, ended the patient in respiratory arrest, requiring artificial respiration (something not very common in 1949), until he slowly regained ventilation. The experiment was repeated with fewer doses with similar results, so Rovenstein decided to quit, considering that the drug exceeded the safety margin. He had a similar experience with Dr. Stuart Cullen, one of the few anesthesiologists at the time in the United States with research experience. Cullen tested the drug in dogs with fatal results. The failure with Rovenstein was caused by the potentiating effect of curare with ether. At that time, respiratory depression was

associated with excess anesthesia, and patients were not intubated. Cullen's failure, due to a great interspecies difference from curare, which in dogs produces a histamine release incompatible with its clinical use.

But Wright was not willing to give up. In another meeting of anesthetists, without giving up, he contacted Dr. Harold Randall Griffith, head of the Department of Anesthesiology at Homeopatic Hospital in Montreal (future Queen Elizabeth Hospital), trained in England and responsible for having recently incorporated cyclopropane in Canada. Unlike American anesthesiologists, Griffith get used endotracheal intubation, to avoid respiratory depression, by manually assisting them. Finally, Griffith agreed to try the Incostrin, although with some suspicion, and with much preparation in case of an incident. The first trial took place on January 22, 1942, this time using cyclopropane, which does not produce the potentiating effect of ether blocking. Thus, together with his colleague Enid Johnson, Griffith used Incostrin in 25 patients without any complications, publishing his results in the American Journal of Anesthesiology in 1942 [16] thus opening a new era in anesthesia. Soon the fact was recognized in Europe as "a milestone in anesthesia". A paradigm had been changed and a new chapter in the practice of anesthesia had been opened.

Other publications followed in the United States [17], where Incostrin was always used with caution because of the lack of preparation of anesthesiologists and the practice of the specialty by nurses not suitable for airway management. After the war, in 1946, Drs. Cecil Gray and Jack Halton used d-tubocurarine - the synthetic curare that the Burroughs Wellcome laboratory had prepared - in 1,000 patients in Liverpool, presenting it at the Royal Society of Medicine, in London, with the title: "A Milestone in Anaesthesia?" [18]. Thus, began its widespread use in Europe, where anesthetists had a medical preparation and proper management of the airway, allowing a total neuromuscular block, managed with artificial ventilation.

Now, if you want to be attached to the facts and not to the most widespread history, the first to study curare in animal experimentation and administer it in general anesthesia was Arthur Läwen, a surgeon from Leipzig in 1912. Although the result of a study conducted in 7 patients undergoing general anesthesia to facilitate closure of the abdominal wall was published, Läwen could not continue using it due to lack of supply and his contribution to the history of anesthesia has almost always been forgotten [19].

In relation to the clinical use of curare, two schools were developed. In the United States, probably induced by early experiences, they considered it inherently dangerous. In addition, the practice of anesthesia was mostly performed by non-specialists. Thus, only small doses of curare were recommended, as a general anesthetic supplement. In England and the rest of Europe, the approach to the use of curare was different, using totally paralyzing doses, with ventilation control and a more superficial anesthesia. England was the only country where anesthesia was administered almost exclusively by qualified doctors and later anesthesiologists, with a scientific reputation since the time of John Snow. The result was that the standards of anesthetic practice and research were the best in the world.

At present, especially with the development of drugs with a large safety margin, the English technique prevailed, so that the majority of patients receive a paralyzing dose of NMBA, ventilation is controlled and an antagonist is administered at the end of the procedure. With this technique, special care should be taken that muscle relaxation does not hide superficial anesthesia.

After 25 years of curare use in England, a study presented at the Royal College of Surgeons revealed a 30% decrease in anesthetic cause mortality, whose cause could largely be attributed to the use of NMBA and a better understanding of Physiology related to its use [3]. However, a study conducted in 1954 by Beecher and Todd [20] which received a lot of publicity, showed that, on the contrary, the use of curare sextupled the number of postoperative complications and deaths. The study covered a period of 4 years between 1948 and 1952 in a total of 600,000 anesthesia's. A series of contradictory articles that refuted one another were published, but the conclusion of the famous Harvard article was soon modified. The increase in mortality was due to the acceptance of patients increasingly committed to surgery and

poor safety standards of anesthetic management in the United States, in which partial paralysis, with poor airway management, was used by paramedical personnel or poorly trained doctor and no residual block reversal was used.

Modern history

Until 1967, curare was the most affordable NMBA available in our specialty, and its cardiovascular safety margin was insufficient. With the development of coronary surgery, the hypotension that caused the release of histamine began to be a problem and tried to find drugs with a safer profile, both hemodynamically and metabolically.

In 1946, Daniel Bovet would discover gallamine triethiodide, a compound with three quaternary nitrogens, which would be introduced by Huguenard and Boué in 1948 and that had some advantages over d-tubocurarine [21]. It was the first synthetic NMBA used in the clinic, but its extremely vagolytic effect and exclusive renal elimination made it soon unpopular, especially after the appearance of pancuronium.

By chance, in the same year 1945 two independent groups, Barlow and Ing, and Paton and Zaminis, independently synthesized the decamethonium, which was introduced in clinic in 1949 by G. S. W. Organe [22]. This is how the methonium group appears for the first time in the history of NMBA, demonstrating a large difference in neuromuscular blocker or ganglion blocker activity according to the length of the chain between 2 ammoniums; among them, suxamethonium (or succinylcholine in the United States), whose quality of blockage and rapid onset of action became part of the list of ideal characteristics of a NMBA.

In 1906 Hunt and Taveau had described the effects of succinylcholine and a series of choline analogues on blood pressure in cats, but they never noticed its muscle relaxant effect, as the trials were done on cured animals [23]. Thus, its neuromuscular blocking effect was discovered only after 43 years by Bovet and Phillips [24]. Finally, it was introduced in clinical practice in Europe by Brücke in Vienna by 1951 [25], Thesleff in Stockholm by 1951 [26] and Foldes in the United States by 1952 [27]. It was precisely the description of Foldes in his article that answered the reason for its validity: "Compared with other relaxants, succinylcholine has several advantages, the most relevant being its easy control, which allows almost instantaneous changes in the degree of muscle relaxation. With succinylcholine, both the increase and decrease of muscle relaxation, take less than a minute". The rapid start of the relaxing effect, its rapid reversal and its economic price are factors that have probably favored the permanence of its use despite its side effects.

In 1957 Professor Daniel Bovet received the Nobel Prize in medicine for his studies related to NMBA gallamine and succinylcholine, which were (and succinylcholine remains), very useful in anesthesia. Bovet's credit is unquestionable, but the synthesis of both drugs was made by Ernest Foumeau and Reid Hunt respectively; Unfortunately, these two scientists have been largely ignored [28].

Two false concepts would guide the search for new NMBA for several years: that the presence of two quaternary nitrogens with a distance of 1.2 to 1.4 nm between them was essential and that the incorporation of them into a rigid structure of heterocyclic rings facilitated the blockade.

The review of the definitive structure of d-tubocurarine made by Everett changed the line of research [29]. Thereafter it was accepted that neuromuscular blockade could occur with molecules with a single quaternary nitrogen, and that the distance between them was less important. Factors such as stereochemistry, the presence of functional groups that favored the metabolism or degradation of the molecule, the degree of hydro or lipophilicity that could modify the pharmacokinetics (distribution and elimination) and the molecular design to reduce or suppress side effects acquired importance. The effort was focused on looking for an agent considered as "ideal NMBA", developing two lines of research, which have delivered to the anesthesiological arsenal the most used agents today. Thus, from ~~45~~ natural or totally synthetic products, about 45 drugs were developed, all of them derived only from two chemical groups: steroids and benzylisoquinolines [30,31].

They were only left out of these groups, which are worth mentioning if we are talking about history, c-toxiferine I and diallyl-nor-toxiferine (alcuronium), which hybrid molecule suggests a steroidal structure by name, but which is more well a curare. It had an ephemeral pass, with a rapid replacement when pancuronium was marketed, due to its great ganglioplegic effect [32].

Amino steroids

Pancuronium was the first synthesis NMBA with amino-steroidal structure and neuromuscular blocking properties used in clinical practice [33]. The story begins in the 60s from the synthesis of maluetine, a steroidal alkaloid isolated from the bark of the *Malouetia bequaertiana* shrub, which produced neuromuscular blockade. After 4 years of research Hewett and Savage synthesized an amino steroid with two groups of quaternary ammoniums, and in a remarkable chemical engineering work, they joined two acetylcholine-like fragments to a rigid androstane steroid ring of 17 carbon atoms, to get the specific action of neuromuscular blockade (Figure 5) [34,35]. It was introduced in clinic in 1967 by Baird and Reid [36]. Although similar to d-tubocurarine in terms of its onset and duration of action, its cardiovascular safety margin was much better, without presenting histamine release and ganglion block, although it blocked cardiac muscarinic receptors, producing a moderate vagolytic effect.

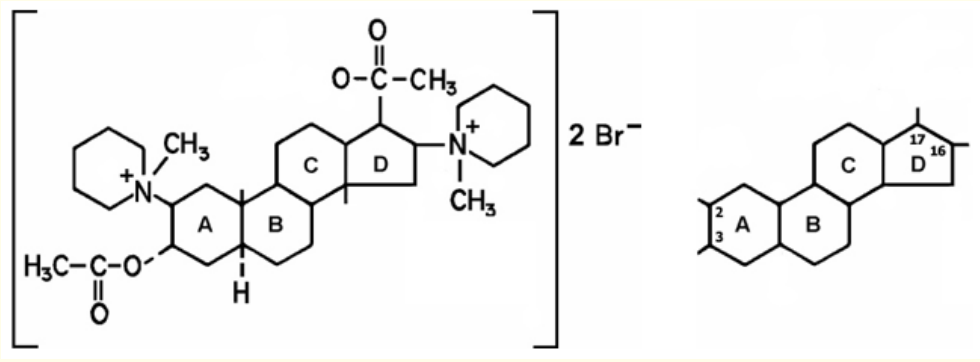

Figure 5: *Pancuronium (left): fragments similar to acetylcholine, attached to a rigid androstane steroid ring (right), from which compounds of similar structure were synthesized, by substitutions at positions 2, 3, 16 and 17.*

The first step to improve pancuronium occurred in Hungary, where the synthesis of pipecuronium, which differs from pancuronium in substitutions 2 and 16 of the androstane nucleus, resulted in a long-lasting muscle relaxant, but without vagolytic effect. This agent, much better than its precedent, was not used in the West until many years later, as a result of the lack of commercial exchange during the Cold War [37].

From the basic ring of the androstane, and by a series of substitutions, especially at the level of carbon atoms 2, 3 and 16, 17, all other compounds of steroidal structure (vecuronium, pipecuronium, rocuronium and rapacuronium) were synthesized. All have two quaternary nitrogen atoms, located at a distance of 11.1 Å [38]. Figure 6 shows the androstane nucleus, with the different substitutions that have given rise to the NMBA of the amino steroid group. Two series of closely related monoquaternary compound can be identified, of which vecuronium, rocuronium and rapacuronium were marketed (the last was soon taken off the market, as we will say later). A series of 16-N-methyl compounds, varying only the ester substituent at position 17: vecuronium (17-acetyl), Org 9489 (17 propionyl) and Org 9453 (17-butyryl). The other series consists of 16-N-allyl monoquaternary compound, varying mainly, but not exclusively, the ester substituent at position 17: rocuronium (17-acetyl), rapacuronium (17-propionyl) and Org 7617 (17-butyryl). Rocuronium also has other substituents and a deacetylation at position 3, which make it stable in aqueous solution.

Compound	R2	R3	R16	R17
Vecuronium	Piperidine	Acetyl	N-methyl-piperidine	Acetyl
Org 9489	Acetyl	Piperidine	N-methyl-piperidine	Propionyl
Org 9453	Piperidine	Acetyl	N-methyl-piperidine	Butyryl
Rocuronium	Morpholino	Hydroxyl	N-allyl-pyrrolidine	Acetyl
Rapacuronium	Piperidine	Acetyl	N-allyl-piperidine	Propionyl
Therg 7617	Piperidine	Acetyl	N-allyl-piperidine	Butyryl

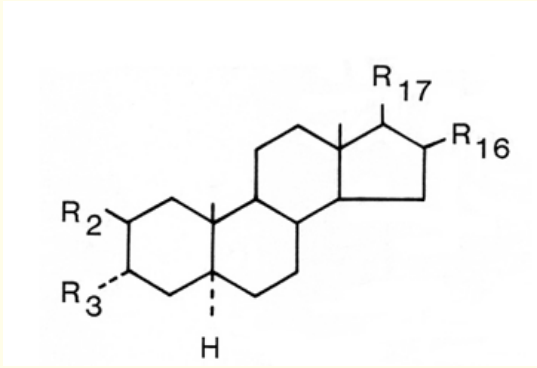

Figure 6: *Molecular structure of a series of muscle relaxants of the amino steroid group synthesized by Organon Laboratories, three of which have been marketed.*

The focus was now on the search for a NMBA that was called "ideal", of shorter duration and shorter onset of action [39]. Thus, begins the race to simultaneously investigate the search for a compound of intermediate duration, with the lowest possible cardiovascular effect; The result being the incorporation of two drugs that lived together for almost twenty years: vecuronium, in the group of amino steroids, and atracurium in the benzylisoquinoline group. Then, trying to obtain a non-depolarizing agent with a start time similar to succinylcholine, the synthesis of rocuronium and rapacuronium was achieved.

Savage was able to recognize that the stimulating activity of cardiac muscarinic receptors that produced tachycardia with pancuronium resided in one of the quaternary ammoniums of the end of the androstane nucleus (in ring D) and the neuromuscular blocking effect in quaternary nitrogen of the other end (in ring A). Thus, by demethylating the quaternary nitrogen of ring A, he managed to synthesize vecuronium, an agent of intermediate duration of action (neither as long as pancuronium, nor as short as succinylcholine), but devoid of any cardiovascular effect. Vecuronium was introduced in clinic in 1980 [40,41].

The next major advance in the development of the NMBA of the amino steroid group came from the work of Bowman, who in 1988 established that in the NMBA of this chemical group the speed at which the blockage occurred (onset of action) was proportional to the potency of the agent. That is, the drugs with less potency (those that required more doses to produce the same effect), had a faster onset of action [42]. Thus, arose rocuronium and rapacuronium. Several chemical modifications were made to the vecuronium molecule, reaching rocuronium bromide, which structurally differs from vecuronium in 4 positions of the steroidal nucleus: it has a 2β-morpholino group, a 3α-hydroxy group and a linked 16-pyrrolidine function to a 16-N-allyl group [43]. Rocuronium has a profile very similar to vecuronium, but in adequate doses it achieves a time of onset of action very similar to that of succinylcholine. It was introduced in a clinic in the United States in 1995 [44].

The last contribution to the pharmacological arsenal of the BNM of the amino steroid group, worthy of being mentioned historically was rapacuronium [45]. It is the 16N-allyl-17β-propionate analog of vecuronium. It was the first non-depolarizing NMBA that combined the attractive characteristics of rapid onset of action and short to intermediate duration of action [46]. Attention was drawn to the rapid approval made by the FDA in 1999, when the drug was just beginning the clinical phase of research, when there were few studies in animals and very few studies in humans published in the medical literature. Soon, two years after its introduction into the clinic, severe bronchospasms were reported, which caused the manufacturer's laboratory to quickly remove it from the market [47].

Benzylisoquinolines

Shortly after the publication of the correct structure of the d-tubocurarine made by Everett in 1970, coincidentally, John B. Stelanke, investigating an alkaloid of the tubers of a Mediterranean plant, *Leontice leontopetalum*, suggested a completely novel and unknown approach in the mammalian metabolism. One of the main constituents of said plant, petalin, which was a simple quaternary benzylisoquinoline salt, had some structural similarities with d-tubocurarine and in addition, suffered the opening of a ring and conversion to an open chain tertiary amine in the presence of a mild alkaline medium.

It was an old process that had been called by the chemists "Hofmann Elimination", in honor of A. W. Hofmann who, in 1851, in the same year that Claude Bernard concluded his experiments with the curare, had described that at 100° C, quaternary ammonium salts can decompose in a strongly alkaline medium to form a tertiary base. More than 120 years later, Stenlake, observing that a strong alkaline medium and a high temperature was not essential for this process to occur, envisioned the possibility of synthesizing a NMBA that could be self-destruct in the body, activated by the mild alkaline medium of the physiological pH and without the intervention of liver or kidney mechanisms.

This observation gave rise to a series of studies whose objective was the synthesis, in 1981, of a bicuaternary compound with structural characteristics that allowed a competitive neuromuscular blockade and that suffered an easy degradation in physiological conditions of pH [48]. A drug of shorter duration was thus obtained than the muscle relaxants used then (d-tubocurarine, diallyl-nor-toxiferine and pancuronium), more predictable in terms of potency and duration of blockade and without cumulative effects, whose main attraction has been its original elimination route: atracurium, introduced in clinic in 1984, just like vecuronium.

Subsequent research showed that atracurium has at least three important metabolic pathways: Hofmann elimination, ester hydrolysis and elimination through organs, in which the liver probably has the greatest participation. Over the years there has been great speculation about the relative importance of each of these pathways. Older studies suggested that Hofmann Elimination was the primary route, but subsequent studies have shown that, under normal conditions, elimination through organs is the most important, reaching 60%. More than half of the remaining 40% is the responsibility of ester hydrolysis and not the Elimination of Hofmann, contrary to what was initially proposed [49].

Atracurium and vecuronium would compete for more than 20 years in the NMBA market, and it continues to be used for its unique characteristic of liver and kidney elimination independence, something that has never been achieved with the NMBA of the amino steroid group. A whole myth circulates in relation to the great battle waged by competing laboratories, to introduce an agent of intermediate duration, which finally occurred simultaneously in 1984. In fact, the investigation of both NMBA was made under strict secrecy in rival laboratory facilities, located at a few blocks away in Glasgow.

Atracurium is actually a racemic mixture of 10 stereoisomers and produces some degree of histamine release. Of these 10 isomers, 6 have been tested as NMBA, since the other 4 are very difficult to synthesize. The 6 isomers of atracurium studied vary their onset and duration of action inversely proportional to the potency of the block [50]. The 1R cis-1'R cis isomer was finally selected, the only one of

the 6 that does not release histamine and normally constitutes 15% of the racemic mixture of atracurium, obtaining a more predictable drug from the pharmacodynamic point of view, the besylate of cisatracurium, incorporated by the FDA in the United States in 1996 [51].

The Savarese group also developed the mivacurium. Chemically it is a benzylisoquinoline derivative, with an ester bond and two choline-like fragments; it differs from atracurium by the presence of an additional methylated phenolic group and in the relative position of the ester groups. Thus, it is a NMBA metabolized by plasma cholinesterase, with an onset of action similar to the NMBA of intermediate duration, but a shorter clinical duration than all non-depolarizing NMBA. It was introduced in clinic in 1988 [52].

Consequences

When analyzing the natural selection of NMBA developed from the synthesis of d-tubocurarine, two periods can be verified:

Period 1942-1980

During the course of this period, products derived from the chemical modification of natural products (*Chondodendrum spp, Strychnos spp, Erythrina coralloides*) were developed, and only secondarily some purely synthetic compounds. Of the large number of NMBA developed in this period, many of them had an ephemeral life. As a highlight, during all these years 15 products have been marketed, 8 of which come from only three different molecules. In chronological form:

- 1942: Tubocurarine
- 1946: d-Tubocurarine Chloride
- 1948: Gallamine
- 1947: Mephenesin
- 1949: Decamethonium
- 1950: Metocurine
- 1951: Succinylcholine
- 1951: Benzoquinonium
- 1952: Laudexium
- 1955: Hexafluorenium
- 1959: Hexamethylene carbaminocolin bromide
- 1961: C-toxiferine I
- 1962: Dialyl-nor-toxiferine
- 1967: Pancuronium bromide
- 1972: Fazadinium

The practical reality of these first 40 years, is that having stopped using most of them (fazadinium and hexamethylene persisted until 1990), the anesthesiologist was primarily managed with:

Non-depolarizing: d-tubocurarine

- Metocurine
- Gallamine

- Alcuronium
- Pancuronium.

Depolarizing: Succinylcholine.

1980-2020 period

During this period the great development of synthesis NMBA, coming from the substitution of different radicals, took place in only two chemical groups: the benzylisoquinolines, which had had a precursor in the benzoquinonium and the amino steroids, which had debuted with the pancuronium. Since then, two large pharmaceutical conglomerates compete for the NMBA market, and with the exception of pipecuronium, which was developed in Eastern Europe and then absorbed by one of them, all new muscle relaxants have been synthesized in their laboratories. In these 40 years of pharmacological research, a series of muscle relaxants that have a much more selective action, less side effects and great versatility of indications are made available to anesthesiologists. In chronological form:

- 1980: Pipecuronium
- 1984: Atracurium
- 1984: Vecuronium
- 1988: Doxacurium
- 1994: Mivacurium
- 1995: Rocuronio
- 1996: Cisatracurium
- 2000: Rapacuronium

The practical reality of these last years has been a tendency to decrease the use of long-lasting NMBA. Succinylcholine has less and less use in adults every day and except in the difficult airway management, its use is contraindicated in children. Pipecuronium, being the best long-lasting drug, ceased to be marketed in many countries. Pancuronium, due to its diminishing use, is also not currently available. The tendency has been that rocuronium has been replacing vecuronium and cisatracurium has not had the same process with respect to atracurium, especially since its long onset on action is incompatible with the impatience of some anesthetists. Mivacurium is the only non-depolarizing muscle relaxant with a short duration and has several applications for current clinical use. Rapacuronium, as everyone knows, was born dead.

Conclusion

The NMBA were a milestone in the practice of clinical anesthesia. Nevertheless, all those that are currently in use have some limitations and the need for an "ideal" NMBA persists, although research in this area has diminished because agents with large safety margins have been reached. Probably the ideal NMBA of today is asked much more than Savarese in the 70s. Today it is necessary to have a rapid onset of action (such as succinylcholine), which does not accumulate, whose metabolism is independent of liver and renal function (such as atracurium, cisatracurium and mivacurium), which is easily reversible (with an agent that has no side effects) and free of side effects, especially hemodynamics (such as vecuronium and cisatracurium). That hybrid does not exist and is not part of this story.

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