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BLOCKING EXCITATION IN THE NERVE USING AN AJMALINUM-NOVOCAINE MIXTURE

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Abstract

The search for quick-action anesthetics with a long-term effect is one of the most urgent issues of modern neurology. This research aims to study the effect of a mixture of anesthetics with different pharmaceutical dynamics (Ajmalinum and Novocain) on the rate of suppression and restoration of the nerve impulse. The purpose of this research was to study the dynamics of nerve impulse blocking in the sciatic nerve of Rana ridibunda Pallas under the effect of an Ajmalinum-Novocaine mixture.

In this research, nervous excitation was blocked in an intact and denudated (with removed epineural and perineural membranes) sciatic nerves of Rana ridibunda Pallas using an Ajmalinum-Novocaine mixture. The obtained results show that under the effect of the Ajmalinum-Novocaine mixture, the amplitude of the intact nerve's action potential reduced to 50% and was 13.0±0.96 min on average, while the complete blocking of excitation took place in 34.0±2.9 min on average. In the nerve with removed epineural and perineural membranes, complete blocking of excitation under the effect of the Ajmalinum-Novocaine mixture occurred in 12.5±3.1 min on average, i.e. 2.7 times quicker than in an intact nerve. The restoration of the action potential amplitude of intact and denudated nerves with subsequent holding in an extracellular solution takes place on the 25.5±0.61 and 23.38±0.71 hours on average, respectively. Thus, the research demonstrated that the Ajmalinum-Novocaine mixture has a significantly quicker anesthetic effect that Ajmalinum, while said effect lasts significantly longer when compared to the application of a monodrug. In addition, this research opens new horizons for the application of the Ajmalinum-Novocaine mixture as a mixture of substances with different pharmaceutical dynamics, with a view to achieving quick and long-term anesthesia. Keywords: anesthesia, local anesthetics, antiarrhythmic agents, Ajmalinum, Novocaine, Ajmalinum-

Novocaine mixture, Rana ridibunda Pallas, sciatic nerve, denudated nerve, excitation blocking, sodium channels, membrane lipid matrix.

Introduction

The problem of anesthesia is traditionally relevant in both neurophysiology and medicine. Local anesthesia is an important component of general anesthesia. Through contact with the nerve trunk, local anesthetics cause motor and sensory paralysis in innerved areas. The assumption is that the action mechanism of local anesthetics manifests in the blocking of the nerve impulse through the inhibition of the potential of activated sodium channels, which prevents membrane depolarization [1, 2, 3]. However, local anesthetics do not always have sufficient anesthetic activity and can induce various side effects, such as tissue irritation, allergic reactions, and local (direct lesion of nervous tissues, especially with spinal anesthesia) and systemic toxicity (lesion of the cardiovascular and central nervous system) [4, 5, 6]. Therefore, the search for and study of new substances with local-anesthetic activity is a relevant problem of modern physiology and medicine. Some antiarrhythmic agents are known for their local-anesthetic properties [7]. For instance, well-known drugs, such as Lidocaine and Trimecainum are used in clinical practice in both respects – as antiarrhythmic agents and local anesthetics [8, 9]. The indications for using Lidocaine as an antiarrhythmic agent are recurrent ventricular tachycardia and paroxysmal tachycardia with Wolff-Parkinson-White syndrome. Some authors also recommend using Lidocaine for all patients with acute myocardial infarction, with a view to preventing ventricular fibrillation. This is especially important during the first hours following the onset of the disease when attending patients outside intensive follow-up units [10, 11]. Lidocaine is widely used as a local anesthetic in surgery, ophthalmology, dentistry, and otolaryngology [12, 13]. Ajmalinum is an antiarrhythmic agent and a Rauvolfia serpentine alkaloid [14, 15]. This drug blocks sodium channels, which, in turn, increases the threshold of action potential emergence. Sodium channel inhibition decelerates the transit of the excitation wave along the myocardium. Ajmalinum is used in prevention and treatment of ectopic arrhythmias caused by impulse formation disruption (premature ventricular contraction, paroxysmal tachycardia) [11]. Previous studies [16] found that Ajmalinum (an antiarrhythmic agent) had a long-term (20 hours) blocking effect on excitation in the nervous tissues of the sciatic nerve in frog. The flaw of Ajmalinum is the slow development of blocking [16].

In this research, an Ajmalinum-Novocaine mixture was used as a drug that accelerates the blocking of excitation in nervous tissues. The assumption is that Novocaine accelerates the development of the blockade, while Ajmalinum induces its long-term maintenance. This assumption is based on previous studies, which found a quick development

of excitation blocking in the nerve trunk under the effect of Novocaine [17].

Literary sources describe cases of using mixtures of various anesthetics, with a view to modifying the duration of analgesia and the latency of the commencement of blocking [18]. Our previous studies also described the acceleration of nervous tissue blocking using a mixture of Trimecainum and CO2 [19]. However, the mixture of Trimecainum and CO2 did not prolong the anesthetic effect [19]. Nerve blocks are frequently used in medical practice. In this case, the anesthetic is administered along the nerve, which disrupts the nerve impulse and causes a loss of sensitivity in the innerved area [20, 21]. Therefore, studying the dynamics of nerve impulse blocking under the effect of a local anesthetic on the nerve trunk is of practical interest. Materials and Methods Experiments were conducted on intact and denudated sciatic nerves (n=20) of Rana ridibunda Pallas at 18-22°C [17, 22]. After preparation, the nerve was placed into the following solution (mM per 1 l): NaC1 – 114; KC1 – 2, 5; CaC12 – 2, 0; the pH of the solution was maintained at 7.35 using a HEPES-10 buffering agent. The exterior membranes of the nerve were removed under an MBS-2 binocular microscope. After being held in the solution for 30-40 min, the nerve was placed in a humid chamber to two pairs of electrodes: proximal – stimulating and distal – pickup. Silver plates were used as stimulating electrodes; calomel electrodes were used as pickup electrodes. The nerve contacted the pickup electrodes via glass tubes filled with agar-agar. At the start of the experiment, the action potential (AP) amplitude of the nerve in response to singular maximum stimuli with 0.1 ms duration was registered. After the registration of initial data, the nerve was placed in an extracellular solution containing 10 mM of Ajmalinum and 10 mM of Novocaine. The nerve was removed from the solution with the mixture periodically (after 5-10 min), with a view to registering the response of the nerve to singular stimulation.

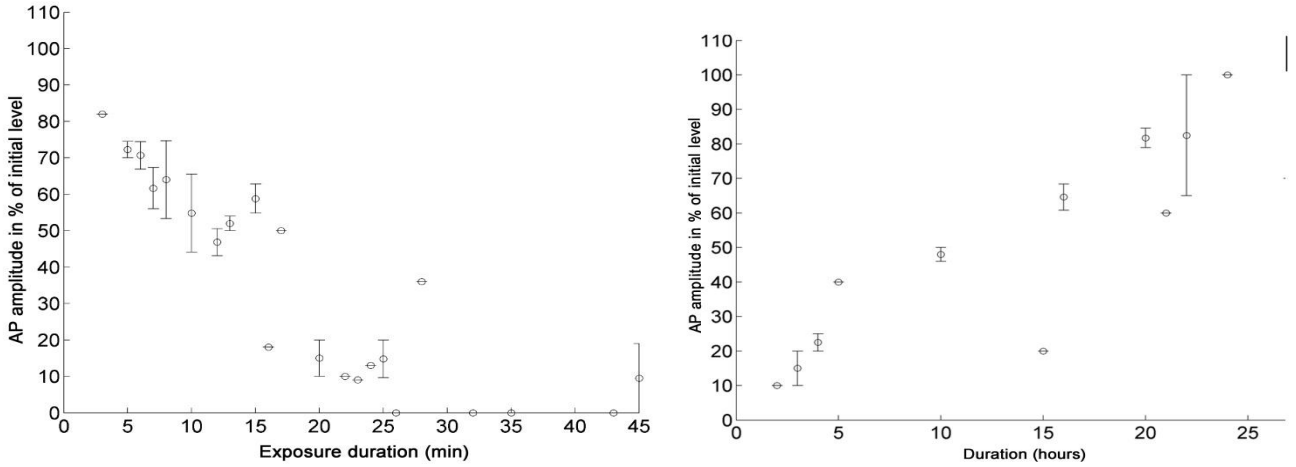
After the AP amplitude dropped to zero from the initial level, the nerve was placed in an extracellular solution, with a view to determining the time during which the drug mixture washed off, which was determined based on the restoration of the AP amplitude to a singular stimulus to the initial value. The stimulation of the nervous tissues in the sciatic nerve was conducted using a custom-made computer-controlled square-wave pulse generator. The registered nerve APs were supplied to the cathode amplifier with input-capacitance compensation and then recorded on a personal computer via a DISCO USB-oscilloscope. The neurograms were processed using Mathcad 14 software.

A series of experiments was conducted to study the dependence of the AP amplitude on the duration of exposure to the Ajmalinum-Novocaine mixture. The initial AP amplitude was registered. The deviation of the AP amplitude in % of the initial level was measured after 5-10 minutes; the average values of the AP amplitude and SE were calculated.

The statistical treatment of experimental data was carried out using the Mann-Whitney U test. Results In the nerve with intact membranes, the Ajmalinum-Novocaine mixture within 13.0±0.96 min reduced the AP amplitude to 50% of the initial value; the complete blocking of the excitation occurred after 34.0±2.9 min on average (Figure 1, A). Subsequent restoration of the nerve's AP amplitude in the absence of the Ajmalinum-Novocaine mixture occurs within 22.5±0.61 hours (Figure 1, B).

Figure 1. Excitation blocking in the intact sciatic nerve using the Ajmalinum-Novocaine mixture

A – AP amplitude reduction in the intact nerve under the effect of the Ajmalinum-Novocaine mixture (n = 19); B – AP amplitude restoratio in the intact nerve during holding in the extracellular solution (n = 6).



A B

Figure 2. Effect of the Ajmalinum-Novocaine mixture (10 mM of Ajmalinum – 10 mM of Novocaine) on the AP of the intact sciatic nerve. A – action potential that emerges in response to the maximum singular stimulation of the nerve in Ringer's solution. B – same, after 13.0 minutes of exposure to the Ajmalinum-Novocaine mixture. C – same, after 20 hours of nerve washing in the extracellular solution.

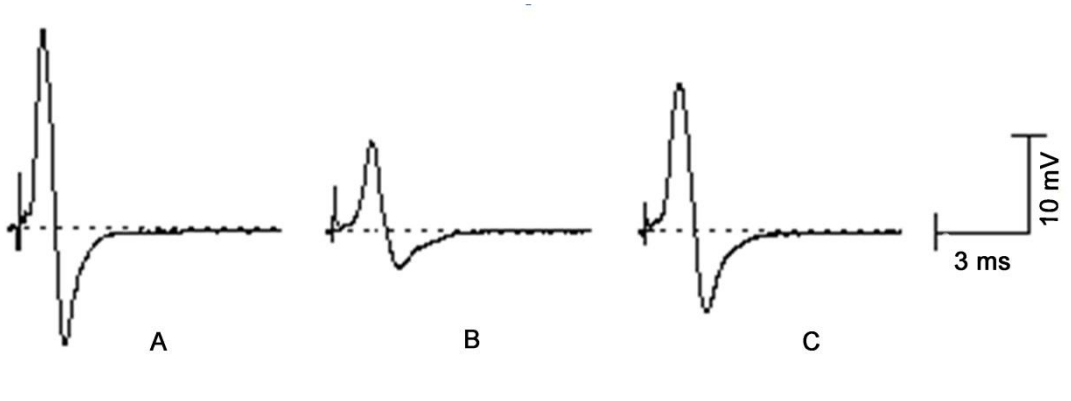
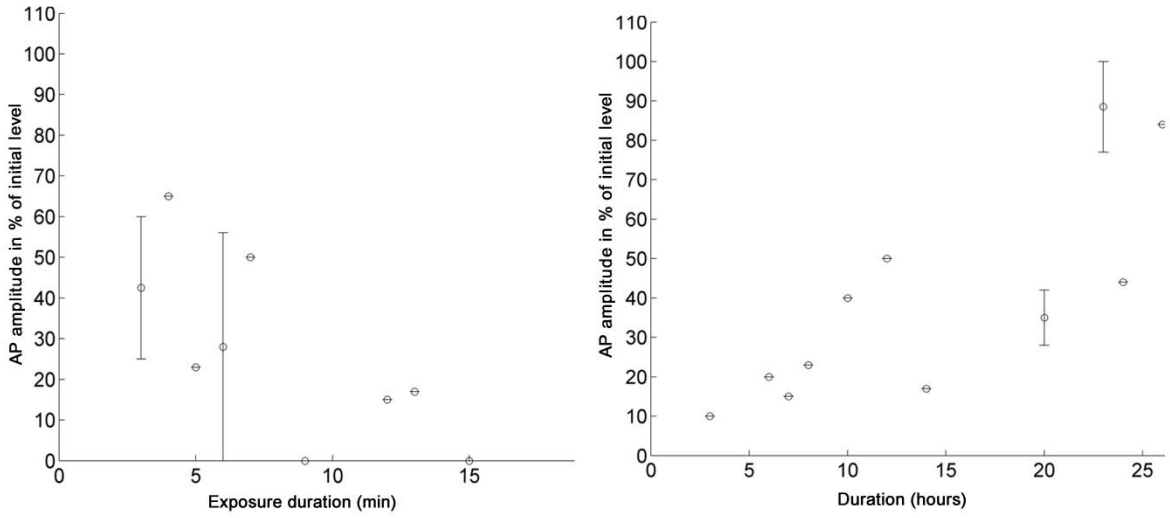


Figure 2, A shows the action potential of the sciatic nerve that emerges in response to a maximum singular stimulation. Figure 2, B shows the action potential after the nerve was held in the solution with the Ajmalinum-

Novocaine mixture for 13 minutes. Figure 2, C, shows the restoration of the nerve's AP amplitude after 20 hours of holding in the extracellular solution. Experiments were also conducted on denudated nerves – with removed epineural and perineural membranes. In this case, the Ajmalinum-Novocaine mixture reduces the AP amplitude to zero after 12.5±3.1 min on average, i.e. 2.7 times quicker than in the nerve with intact membranes (Figure 3, A). The differences in the mean values were statistically significant, according to the Mann-Whitney U test, $p<0.01$. Apparently, the external epineural and perineural membranes are a considerable barrier for the passage of the Ajmalinum-Novocaine mixture. The restoration of the AP amplitude of the denudated nerve during its washing in an extracellular solution lasted 23.38±0.71 hours (Figure 3, B).

Figure 3. Excitation blocking in the denudated sciatic nerve using the Ajmalinum-Novocaine mixture

A – AP amplitude reduction in the denudated nerve under the effect of the Ajmalinum-Novocaine mixture (n = 4); B – AP amplitude restoration in the denudated nerve during holding in the extracellular solution (n = 4).



A B

Thus, the Ajmalinum-Novocaine mixture is an anesthetic with a long-term effect. Exposure to the Ajmalinum-Novocaine mixture induces quick blocking of excitation in the tissues of intact and denudated nerves, as well as its prolonged maintenance.

Discussion

Previous studies [16] showed that exposure to 10 mM of Ajmalinum reduced the AP amplitude of an intact nerve to 50% of the initial level within 120.0±2.6 min.Under the effect of the Ajmalinum-Novocaine mixture, the AP amplitude of an intact nerve drops to 50% of the initial level 9.2 times quicker than under the effect of Ajmalinum. The differences in the mean values were statistically significant, $p<0.01$. With subsequent washing of Ajmalinum by holding the nerve in a large volume of solution, the AP amplitude restored slowly – within 20.0±1.0 hours [16]. The

relatively slow development of excitation blocking under the effect of Ajmalinum is probably caused by the large molecular mass, hydrophobic properties, and peculiar spatial structure of the Ajmalinum molecule, which consists of several condensed aromatic heterocycles [16]. With washing of the Ajmalinum-Novocaine mixture by holding the nerve in an extracellular solution, the AP amplitude also restores slowly – within 22.5±0.61 hours. It was shown previously [17] that Novocaine at a concentration of 10 mM at pH – 7.35 induces quick blocking of excitation in the tissues of an intact nerve (AP amplitude reduction to 50% of the initial level takes place within 18.4±1.46 min). The AP amplitude of an intact nerve during holding in a solution after exposure to Novocaine restores itself within 78.1±5.7 min. The restoration of the AP amplitude of an intact nerve after exposure to the Ajmalinum-Novocaine mixture is 17.3 times slower than that after exposure to Novocaine. Thus, the Ajmalinum-Novocaine mixture provides for prolonged maintenance of excitation blocking. Despite the significant difference of the Novocaine and Ajmalinum block, the action mechanisms of these substances are apparently similar. Both drugs are tertiary amines and are in two forms in the solution: neutral and positively charged (protonated). Novocaine and Ajmalinum can interplay with open or inactivated channels [1, 2]. According to the modulated receptor theory, neutral molecules of anesthetics pass into the cytoplasm through the lipid matrix of the membrane [16, 23, 24]. Under the effect of the low pH of the cytoplasm, part of the anesthetic's molecules are protonated and enter the sodium channel in charged form once the activation gates open [24, 25, 26]. In neutral form, these anesthetics interplay with inactivated sodium channels and fixate the inactivation gate in closed state. The conclusions of this research are as follows: 1. The Ajmalinum-Novocaine mixture is a quick-action drug with a prolonged anesthetic effect. 2. In nerves with removed epineural and perineural membranes, complete blocking of excitation under the effect of the Ajmalinum-Novocaine mixture occurs 2.7 times quicker than in intact nerves. These results offer a new approach to studying local anesthesia; they can be widely used in clinical practice.

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