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Effects of acetylcholine and carbachol on nuclear large conductance cation channels in rat cerebellar Purkinje neurons

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Acetylcholine and carbachol are known to modulate cell functions by binding to nicotinic and muscarinic acetylcholine receptors, located on the plasma membrane of target cells. However, several acetylcholine-receptor agonists and antagonists have been reported to modulate nuclear membrane channels as well, specifically, LCC-channels (Large Conductance Cation channels), which are one of the most common yet least studied ion channels of the nuclear membrane. In this study, we aimed to test whether acetylcholine and carbachol can interact with these channels and alter their electrophysiological properties. Rat brain cerebella were extracted in an ice-cold environment, fragmented, and roughly homogenized. Later, the homogenized samples were centrifuged, the nuclei-containing fraction was resuspended, and LCC-channels of individual nuclei were examined using a nucleus-attached configuration and voltage-clamp mode of the patch-clamp technique. It was found that acetylcholine, when applied to the intranuclear side, decreases the amplitude of currents passing through LCC-channels and the probability of the channels being in an open state (P_o) at negative membrane potentials. In contrast, when applied to the perinuclear side, acetylcholine increases their P_o at negative potentials while decreasing the amplitude of currents across all the potentials. Carbachol, when applied to the intranuclear side of the membrane, reduces P_o of LCC-channels at -40 mV without affecting current amplitude. In contrast, when interacting with the perinuclear side of the channels, it decreases the amplitude of the currents at all applied potentials without affecting P_o. These findings demonstrate that acetylcholine and carbachol have asymmetric and side-specific effects on LCC-channels at both positive and negative membrane potentials.

Keywords: acetylcholine; carbachol; muscarinic and nicotinic acetylcholine receptor agonist; LCC-channels; patch-clamp; electrophysiology; modulation; ion channels; neurons.

INTRODUCTION

Acetylcholine and carbachol are both well-known agonists of nicotinic and muscarinic acetylcholine receptors [1–5]. The main function that acetylcholine is associated with is its role as a neurotransmitter in the central nervous system, at neuromuscular junctions, and within visceral ganglia. In medicine, it is used in cataract surgery, iridectomy, and keratoplasty thanks to its ability to cause rapid iris miosis. The side effects of its use include swelling and clouding of the cornea. It is produced in nerve terminals by choline acetyltransferase from choline and acetyl-CoA, and later, after being released into the synaptic cleft, is hydrolyzed by acetylcholinesterase into choline and acetate [6]. Acetylcholine is also known to decrease blood pressure by activating NO-dependent vasodilatation [7], and it possibly plays a role in the immune response, as it has been reported to be secreted by T lymphocytes [8]. Like acetylcholine, carbachol is used to induce iris miosis and decrease intraocular pressure. Its effects are very similar to those of acetylcholine. However, while acetylcholine is prone to being hydrolyzed by acetylcholinesterase, carbachol is more resistant to hydrolysis, making the effects caused by carbachol last longer [5, 9]. There are reports that carbachol is also capable of inducing intestinal mucus production and acts as a protective agent during intestinal injuries by strengthening apical tight junctions of the cells [10].

Most of the effects of the described substances are typically associated with their interaction with acetylcholine receptors, located on the plasma membrane of target cells. However, it is documented that certain agonists and antagonists of cholinergic receptors are also capable of interacting with some ion channels of the nuclear membrane as well [11, 12]. It is possible that many cellular proteins that were cholinergically modulated in the past, still retain a capability to be affected by this class of molecules, even though acetylcholine is not commonly found inside cells and usually acts via plasma membrane receptors. In this field, a lot remains unknown, as nuclear channels require high-precision methods to be studied due to their localization. LCC-channels, first discovered on the nuclear membrane of Purkinje neurons and described by Marchenko et al [13, 14], can be characterized by high conductivity (from 179 ± 15 pS in granule neurons to 248 ± 6 pS in pyramidal neurons in the hippocampus), slow kinetics, voltage-dependence, and a high probability of being in an open state [15]. Our research group discovered that their activity can be modulated by nicotine, a strong agonist of nicotinic choline receptors, and also by rocuronium bromide, pipecuronium bromide, and neurotoxin II, which are antagonists of nicotinic cholinergic receptors. In contrast, hexamethonium, methyllycaconitine, and α-conotoxin (all three are antagonists of nicotinic receptors) had no influence on the LCC-channels [11, 16].

In this study, we aim to expand the understanding of choline-receptor modulators, focusing on the effects caused on LCC-channels by acetylcholine itself, as well as by its higher-affinity derivative, carbachol.

METHODS

All experiments were performed in compliance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. The procedures used in this study were examined and approved by the Bioethics Committee of the Bogomolets Institute of Physiology (Protocol No. 5/25 from 25.06.2025) 3 to 4-week-old Wistar rats were used for the experiments. Nuclei of Purkinje neurons containing LCC-channels were extracted from the rats' cerebella using the technique described in our previous articles [17]. After extracting a rat's brain and isolating the cerebellum, it was treated with the following solution (mmol/l): NaCl – 150, HEPES – 10, EDTA – 1; pH 7.4; t = 4°C in order to cool it and hinder autolytic processes. After that, the cerebellum was cut into slices approximately 0.3 – 0.5 mm thick and placed into Eppendorf microtubes, which contained a K-gluconate-based homogenization solution (mmol/l): K-gluconate – 150, HEPES – 10, HEPES-K – 10; pH 7.2 with protease inhibitors ("cOmplete Protease Inhibitor Cocktail tablets, Roche", Germany). Then the microtubes with cerebellum slices were stored at -20°C in a freezer. When needed, slices in the microtubes were thawed and homogenized with a syringe needle (0.84 mm). The homogenate was then centrifuged at 2000 g, after which the supernatant was removed and the nuclear pellet resuspended in a KCl-based solution (mmol/l): KCl – 150, HEPES – 8, HEPES-K – 12, EGTA – 1; pH 7.2. The same solution was used to fill the micropipettes. The isolated nuclei were transferred to the bath of an inverted microscope ("Leica DM IRB", Germany) and allowed to adhere to the glass bottom for 5 min. After this period, the nuclei were washed with the same KCl-based solution to remove any unattached cellular material.

During the experiment, the test substances were applied at a concentration of 1 mmol/l in two different modes. In the first series of experiments, the solution was applied directly to the bath containing the nuclei, allowing it to interact with the intranuclear side of the membrane after diffusing into the nucleus, while the perinuclear side remained isolated by the patch pipette. In the second series, the test substance was added to the patch pipette attached to the nucleus, thereby interacting only with the perinuclear side of the membrane and

LCC-channels.

Electrophysiological recordings were performed using the patch-clamp technique in the nucleus-attached configuration under voltage-clamp mode. The membrane potential was consecutively fixed at -40 mV, +40 mV, -60 mV, and +60 mV, and the currents flowing through the LCC-channels were recorded. The recordings were subsequently analyzed with Clampfit 10.7 ("Axon Instruments", USA) for each membrane potential and for each mode of substance application.

Two main parameters of the electrophysiological activity of the channels were examined under control conditions and after exposure to the test substances: the open state probability (P_o) and the current amplitude difference between the open and closed states of the channel. P_o was automatically calculated using Clampfit 10.7 as the ratio of the time the channel remained open to the total recording time.

Taking into account that a single patch often contained multiple LCC-channels, the current amplitude difference (hereafter referred to as amplitude) was calculated by subtracting the average amplitude recorded when a channel was closed from the values measured when it was open, thus obtaining the amplitude difference for a single channel-opening event. After performing a series of such calculations at each applied potential, these values were used to construct graphs of the currents' average amplitude.

Statistical analysis was performed using Origin 2018 ("OriginLab Corporation", USA). Results were presented as M ± m. Intergroup comparisons were conducted using a two-tailed paired-samples Student's t-test when measurements were made on the same nuclei under different conditions, and a two-sample heteroscedastic t-test when data were obtained from different sets of nuclei. The difference between the groups was considered significant with $P < 0.05$.

RESULTS AND DISCUSSION

During the first stage, the influence of acetylcholine was examined. It was discovered that acetylcholine applied to the bath and thus interacting with the intranuclear side of the membrane reduces the amplitude of the LCC-mediated currents at potentials of -60 mV and -40 mV ($P < 0.001$, $n = 15$ for both), but does not affect the currents through these channels at positive values of the applied potential (+60 mV and +40 mV). Examples of the recording fragments are presented in Fig. 1A, and amplitude diagrams based on the representative fragments are depicted in Fig. 1B. The decrease in amplitude at -60 mV was 7% (from -14.35 ± 0.26 pA to -13.37 ± 0.23 pA), and 6% at -40 mV (from -9.64 ± 0.19 pA to -9.06 ± 0.23 pA), as illustrated in Fig. 1C.

The probability of LCC-channels being in an open state also decreased when the substance at a concentration of 1 mmol/l was applied to the bath. At the potential of -60 mV, P_o values dropped by 41%, from 0.17 ± 0.03 to 0.10 ± 0.02 ($P < 0.05$, $n = 13$), as depicted in Fig. 1D, with a prolonged effect present even after washing acetylcholine away with 10 ml of KCl-based solution of the previously described composition (not shown, $n = 9$). At the potential of -40 mV, a similar tendency was observed. When applied to the bath, acetylcholine decreased the P_o of LCC-channels from 0.33 ± 0.01 to 0.20 ± 0.03 (39% change, $P < 0.001$, $n = 13$). The described effect also persisted after washing the test substance away with the KCl-based solution (not shown, $n = 9$). The impact of acetylcholine on P_o values of LCC-channels may indicate that acetylcholine has a potent inhibiting effect on their activity at negative membrane potentials. Similar to the results obtained when the amplitude was evaluated, no changes in P_o were registered at the positive membrane potentials.

When acetylcholine at a concentration of 1 mmol/l was applied through the patch-pipette and acted on the perinuclear side of the nuclear membrane, we registered a statistically significant decrease in the amplitude of the current across LCC-channels at all applied potentials. Fragments of the recordings obtained at this stage are presented in Fig. 2A, and the cor-

responding amplitude diagrams are illustrated in Fig. 2B. At the potential of -60 mV, the amplitude of the currents decreased from -14.35 ± 0.26 pA in control to -11.49 ± 0.38 pA with acetylcholine applied from the perinuclear side via a pipette (20% change, n = 6, P < 0.001). At -40 mV, the amplitude decreased from -9.64 ± 0.18 pA to -7.19 ± 0.10 pA (25% change, n = 10, P < 0.001). Comparing these results with those obtained when acetylcholine was added to the bath, we can infer that the effect of the substance is more pronounced when applied via the pipette and interacting with the perinuclear side of LCC-channels. Also, it is worth mentioning that while acetylcholine did not affect the current's amplitude at positive membrane potentials when applied to the bath, when added to the pipette, such an effect did take place, as shown in Fig. 2C. Specifically, at the membrane potential of +40 mV, the amplitude values dropped from 8.87 ± 0.11 pA in control to 7.25 ± 0.23 pA with the substance in the pipette (18% change, n = 6, P < 0.001), and at +60 mV from 13.27 ± 0.16 pA to 11.17 ± 0.54 pA (16% change, n = 5, P < 0.05). However, at the next step, when the tested substance was subsequently added to the bath, and thus interacted with both intra- and perinuclear sides of the nuclear membrane (mentioned in Fig. 2 as ACh/ACh configuration), no changes compared to the state with acetylcholine present only in the pipette were observed.

The results of P_o estimation with and without

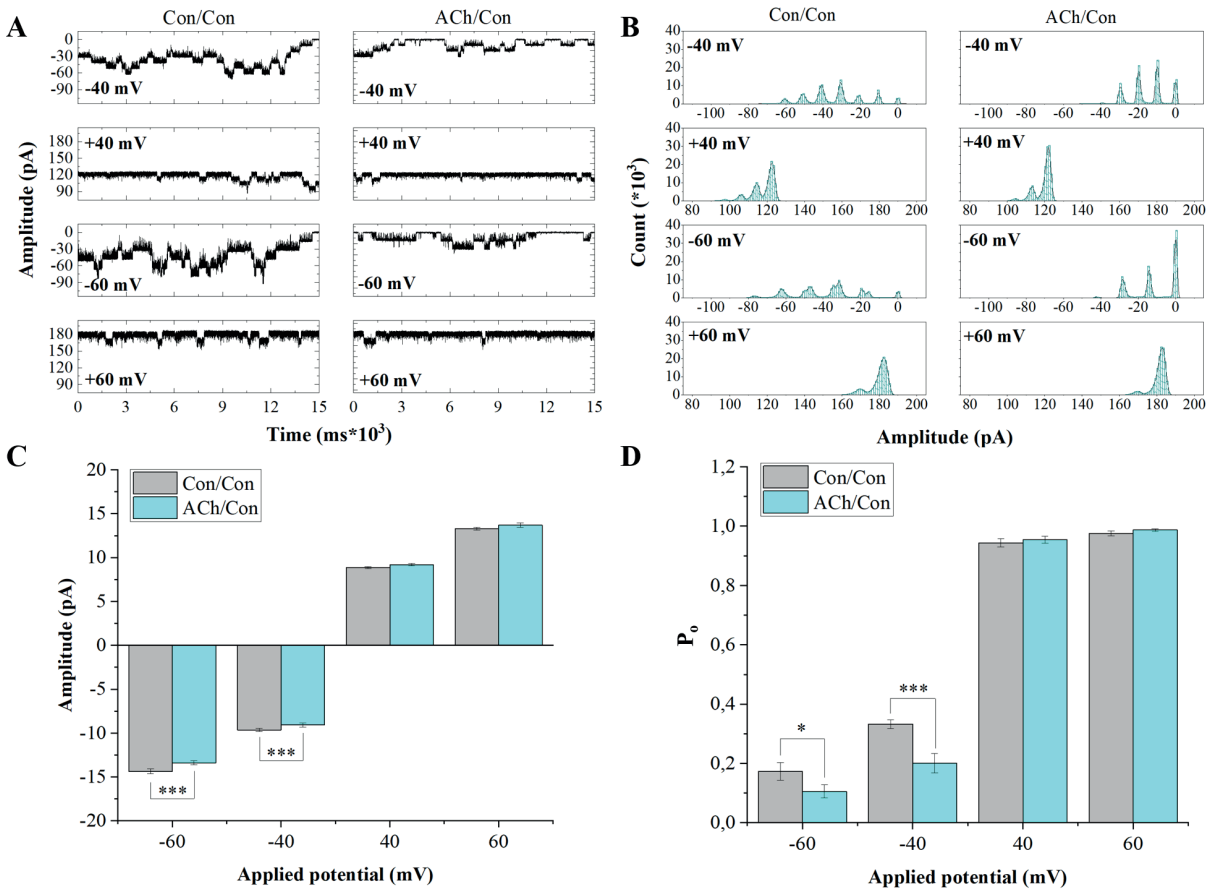

Fig. 1. Properties of neuronal LCC-channels under the effect of acetylcholine (1 mmol/l), applied to the bath. Con/Con means that both the bath and the patch pipette contained control KCl-based solution; ACh/Con corresponds to the condition when acetylcholine was added to the bath, while the pipette was filled with a control KCl-based solution. A – examples of recording fragments. B – amplitude diagrams of LCC-channel currents. C – average amplitude of the currents through LCC-channels. D – impact of the acetylcholine on the mean P_o of LCC-channels. * P < 0.05, ** P < 0.01, *** P < 0.001 compared to control

acetylcholine present in the pipette were also intriguing. Contrary to the P_o decrease observed when the substance was added to the bath, its application via the pipette led to an increase in the probability of LCC-channels being in an open state at all of the applied negative potentials. The change of P_o caused by acetylcholine added via pipette is visualised in Fig. 2D. At the potential of -60 mV, P_o values soared from 0.15 ± 0.02 in control to 0.31 ± 0.05 when acetylcholine was in the pipette (107% change, $P < 0.05$, $n = 5$), and at the potential of -40 mV, we registered an increase of P_o from 0.32 ± 0.01 to 0.45 ± 0.03 (41% change, $P < 0.05$, $n = 5$). When acetylcholine was added both to the pipette and to the bath solution, P_o tended to decrease toward the control values at negative membrane potentials, as expected, considering the results obtained when acetylcholine was added to the bath. Nevertheless, this tendency was not found to be statistically significant.

In the second stage of the experiment, we continued the research of nicotinic cholinergic receptor modulators, shifting our attention to carbachol. The results obtained from this stage indicate that carbachol at a concentration of 1 mmol/l is not an effective LCC-channel blocker when applied to a bath solution and interacting with the intranuclear side of the channels. Recording fragments for this configuration, as well as their corresponding amplitude diagrams, are presented in Fig 3A and B, respectively. The

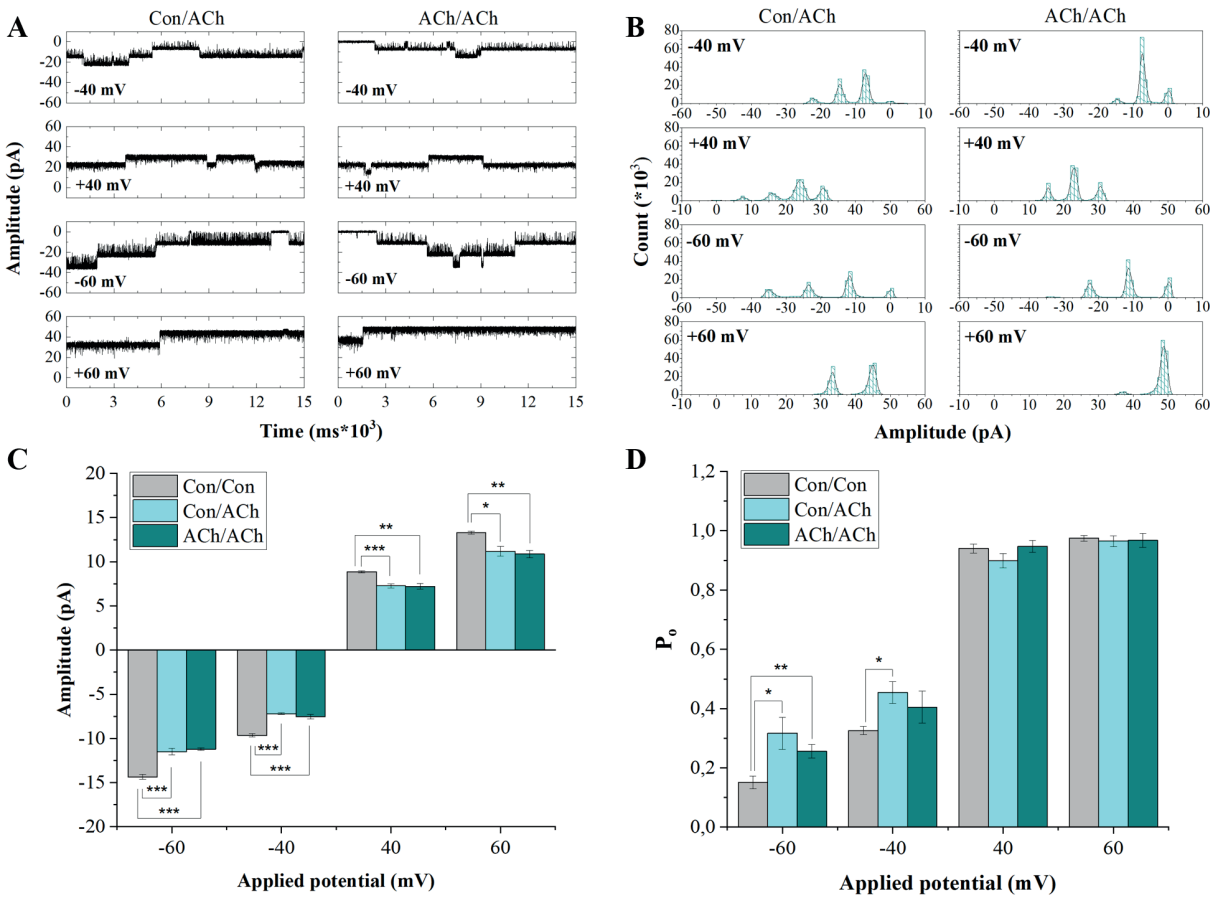

Fig. 2. Impact of acetylcholine (1 mmol/l), introduced to the pipette on LCC-channels of Purkinje neurons. Con/Con means that no active substance was applied on either side of the membrane; Con/ACh means that the bath was filled with a control solution of KCl, while the patch-pipette contained acetylcholine; ACh/ACh corresponds to both the bath and the pipette being filled with the mentioned test solution. A – fragments of the recordings. B – amplitude diagrams of LCC-channel currents. C – current-voltage graphs of LCC-channels. D – average P_o values of LCC-channels. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to control

registered amplitudes were within the range typical for the currents passing through the LCC-channels (-14.79 ± 0.25 pA at the membrane potential of -60 mV; -9.78 ± 0.16 pA at -40 mV; 8.60 ± 0.15 pA at +40 mV; and 12.48 ± 0.45 pA at the potential of +60 mV) and did not differ between control recordings and those with the carbachol at a concentration of 1 mmol/l being added. The amplitude of the currents through LCC-channels at different membrane potentials is shown in Fig. 3C.

Despite no change in amplitude under the effect of carbachol in the bath solution, we did register a slight decrease in P_o values at the potential of -60 mV, with the probability of LCC-channels being in an open state decreasing from 0.23 ± 0.01 in control to 0.16 ± 0.02 with carbachol (1 mmol/l) in bath (30% change, n = 9, P < 0.05). This effect persisted even after the substance was washed out of the sample (not shown, n = 5). The impact of carbachol on P_o of LCC-channels is presented in Fig. 3D.

These results, obtained on Purkinje neurons, correspond with our previous findings [12], as carbachol was unable to influence the amplitude of the currents through the LCC-channels of cardiomyocytes as well. The fact that, despite being unable to influence the amplitude of the currents, carbachol was still able to decrease P_o of LCC-channels draws some attention. It is possible that modulation of the amplitude of the channels and their probability of being in an open state is mediated by the different regions of the interacting molecules.

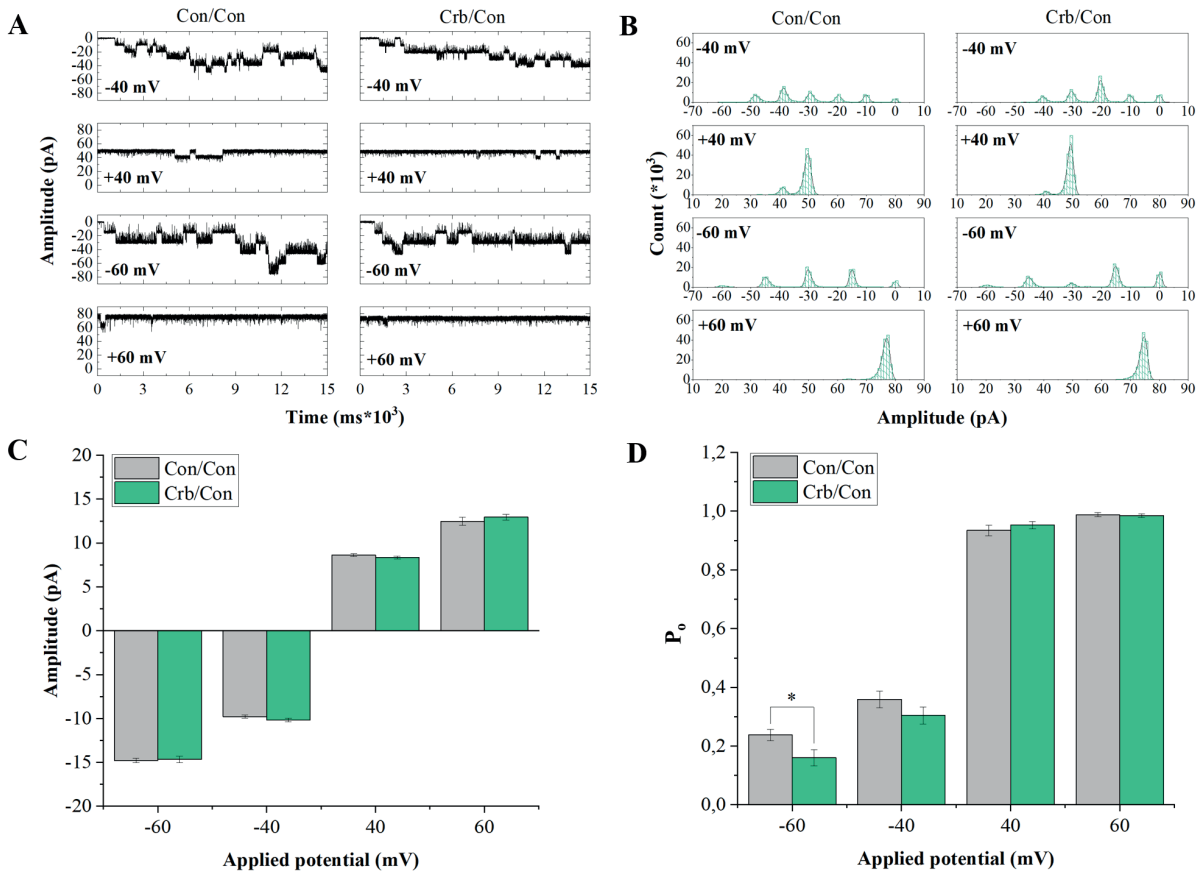

Fig. 3. Influence of carbachol (1 mmol/l) applied to the bath on neuronal LCC-channels. Con/Con means that the active substance was added neither in a bath nor in a pipette; Crb/Con corresponds to carbachol being added only to the bath. A – representative fragments of recordings. B – corresponding to the recording fragments' amplitude diagrams. C – mean amplitude of the currents through LCC-channels. D – influence of the carbachol on the average P_o of LCC-channels. * P < 0.05 compared to control

In the meantime, when carbachol was added to a pipette and acted directly on the perinuclear side of LCC-channels, we saw a statistically significant decrease in the current amplitude at all of the applied potentials. As previously, representative fragments of the obtained recordings are presented in Fig. 4A, and the amplitude diagrams, based on the fragments, are shown in Fig. 4B. At the potential of -60 mV, the amplitude decreased from -14.57 ± 0.18 pA to -11.61 ± 0.47 pA (n = 7; P < 0.001; 20% difference); at -40 mV, from -9.67 ± 0.10 pA to -8.00 ± 0.51 pA (n = 8; P < 0.001; 17% difference); at +40 mV, from 8.76 ± 0.14 pA to 6.84 ± 0.34 pA (n = 7; P < 0.001; 22% difference); at +60 mV, from 12.45 ± 0.48 pA to 11.11 ± 0.23 pA (n = 6; P < 0.05; 11% difference). The graph of the average amplitude of LCC-channels in control and test conditions are shown in Fig. 4C. The amplitude decrease observed when carbachol was applied to the pipette remained unaffected when the acting substance was further added through the duct to the bath solution, confirming the results described previously. Such a difference in the effect of carbachol, depending on which side of the membrane it was applied to, might indicate that the structure of LCC-channels is heterogeneous between the membrane sides as well, and a carbachol molecule possesses motifs that interact with the perinuclear side of these

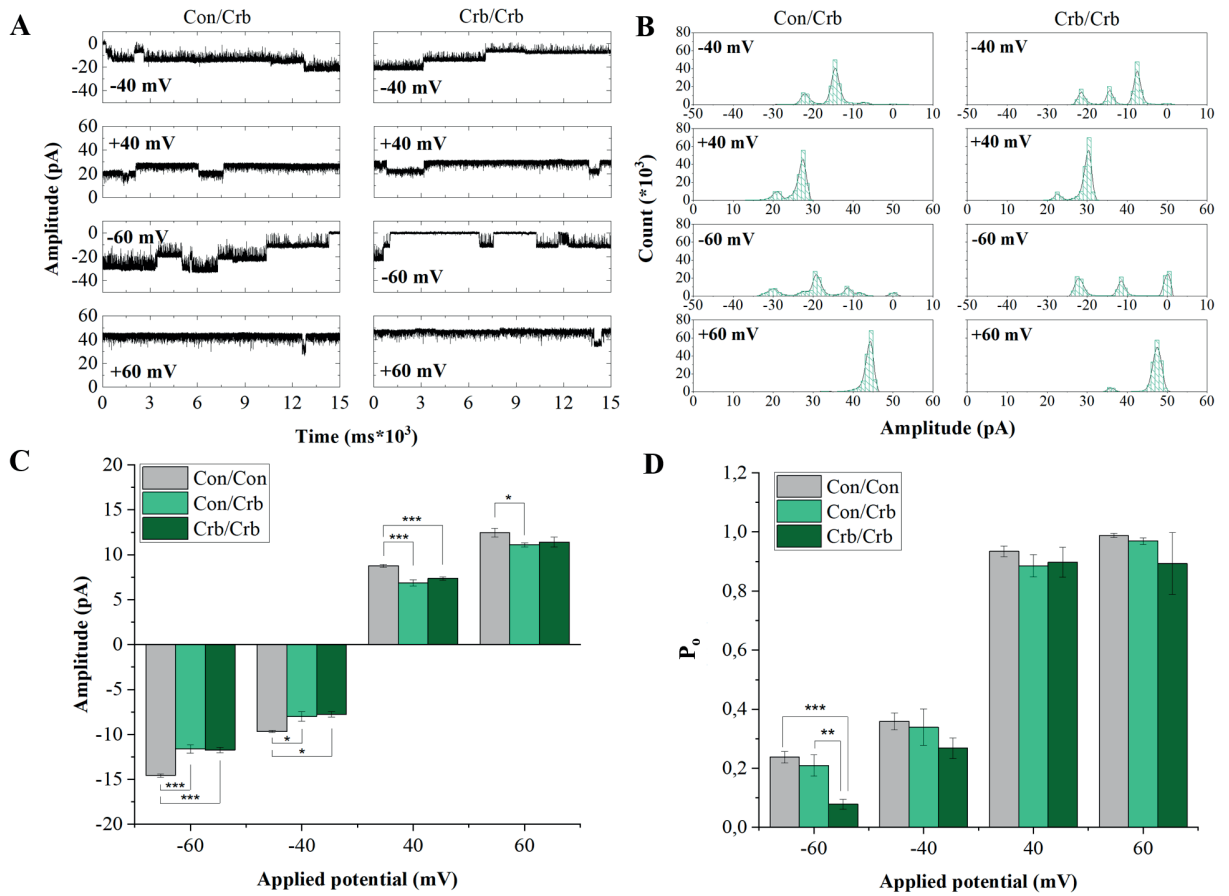

Fig. 4. Influence of carbachol (1 mmol/l) applied to a pipette on LCC-channels of Purkinje neurons. Con/Con means that the control KCl-based solution filled both the bath as well as the patch-pipette; Con/Crb designates the configuration when the bath contained control solution, while the patch-pipette contained carbachol; Crb/Crb corresponds to carbachol being applied to both the bath and the pipette. A – fragments of the obtained recordings. B – amplitude diagrams, which correspond to the fragments from (A). C – current-voltage graphs of LCC-channels. D – influence of the carbachol on the average P_o of LCC-channels. * P < 0.05, ** P < 0.01, *** P < 0.001 compared to groups with different carbachol application method.

channels, but not the nuclear one.

Contrary to the case when carbachol was added to the bath solution, it did not impact P_o of LCC-channels when applied via the patch-pipette. However, as soon as carbachol was added to the bath, P_o dropped from 0.20 ± 0.03 in the configuration with carbachol present only in the pipette to 0.07 ± 0.01 in the configuration with carbachol added to the bath as well (65% change, $n = 4$, $P < 0.01$, membrane potential of -60 mV), as depicted in Fig. 4D. These results further strengthen the hypothesis that the ability of certain substances to modulate the amplitude of the currents through LCC-channels and the P_o of these channels can be independent from each other and be mediated by different molecular mechanisms.

Our findings regarding the effects of acetylcholine and carbachol on the electrophysiological properties of LCC-channels indicate that when added to the bath and interacting with the intranuclear side of LCC-channels, the inhibitory effect of acetylcholine on the amplitude of the currents through the LCC-channels is much less pronounced compared to another agonists of nicotinic cholinergic receptors, nicotine, described by us earlier [11], which caused a decrease in amplitude of the LCC-mediated currents by half already at a concentration of 0.2 mmol/l. A different nicotinic cholinergic receptor modulator, mecamylamine, also had a more potent effect on the amplitude of the currents passing through the LCC-channels, and was capable of decreasing it more than twice as much compared to acetylcholine [17]. Nevertheless, some degree of inhibition allows us to assume the existence of at least some interaction between the LCC-channels and the molecules of acetylcholine, considering the effects of both acetylcholine and carbachol were still more prominent compared to some of the other nicotinic receptor modulators, including hexamethonium, methyllycaconitine (MLA), and alpha-conotoxin PeIA [11].

Despite a relatively low impact on the current amplitude, acetylcholine, when added to the bath, showed a strong capability to decrease P_o of LCC-channels at negative membrane potentials. In the meantime, when acetylcholine was added via the patch-pipette and interacted with the perinuclear side of LCC-channels, this substance increased P_o of LCC-channels, contrary to the effect registered when it was added to the bath solution. This increase in LCC-channels' P_o has not been described before for any of the tested substances. What makes it even more intriguing is that P_o increase was observed simultaneously with a significant decrease in LCC-channels' current amplitude registered at all of the applied potentials and surpassing that of mecamylamine. The difference in the influence of acetylcholine depending on the side of the nuclear membrane to which it was applied suggests potential differences between the structure of intranuclear and perinuclear domains of LCC-channels, but this assumption requires additional verification in future studies.

This hypothesis was further reinforced at the next stage of our experiment, when the effects of carbachol were examined. When carbachol (1 mmol/l) was added to the bath and interacted with the intranuclear side of LCC-channels, we observed no significant changes in the LCC-channels' current amplitude, but a decrease in P_o was detected at the membrane potential of -60 mV. The results remain consistent with previous studies conducted on cardiomyocytes, where no significant changes in amplitude of the currents through the LCC-channels were detected under the effect of carbachol [12]. However, when the same solution was applied via the patch pipette and interacted with the perinuclear side of these channels, we registered a significant decrease in the amplitude of the current through LCC-channels at all applied potentials, while P_o values remained unchanged. Considering these results for both acetylcholine and carbachol, we can assume that the modulation of LCC-channels' probability of being in an open state is independent from the modulation of the amplitude of the currents passing through

them. It is also the first time that an asymmetric action of nicotinic receptor agonists on LCC-channels has been demonstrated. Additionally, it is worth mentioning that no channel flickering was observed upon application of the studied substances; meanwhile, α-cobratoxin, which was also shown to be capable of decreasing P_o of LCC channels, caused flickering alongside its inhibiting effect [16], and so did mecamylamine [17].

The observation that the inhibition potency of the substance may drastically change depending on the application method deserves attention as well. As we showed, acetylcholine, while being a weak inhibitor of LCC-channels, still had some confirmed effect on the amplitude of the currents passing through these channels, contrary to carbachol, to which the channels remained inert when it was applied to the bath solution. However, when applied via the patch-pipette, carbachol turned out to be as potent as acetylcholine. Taking into account that many studies of LCC-channels modulators were performed by the means of adding the test substance directly to the bath with the nuclei-containing solution [11, 12, 16], our findings can potentially open new usage perspectives for the previously studied substances, as though some of them don't modulate LCC-channels activity when interacting with their intranuclear side, they still may be capable of doing so when applied to the perinuclear side instead.

CONCLUSIONS

From the obtained results, a series of conclusions can be made:

Acetylcholine at a concentration of 1 mmol/l, when applied to the bath solution, decreases both P_o (up to 41%, depending on the membrane potential) and the amplitude (up to 7%) of the currents through the LCC-channels at negative membrane potentials, but has no impact on positive ones.

When applied via the patch-pipette, acetylcholine at the same concentration decreases the amplitude of the currents through the LCC-channels at both negative (by up to 25%) and positive (up to 18%) membrane potentials, and increases the probability of the channels being in an open state at negative potentials (by up to 107%).

Carbachol at a concentration of 1 mmol/l doesn't decrease the LCC-channels' current amplitude when applied to the bath solution, but decreases their P_o values at negative membrane potentials (up to 30%).

When applied to the pipette, carbachol (1 mmol/l) exhibits an ability to decrease the amplitude of the LCC-mediated ion currents at all of the applied potentials (by up to 20% at negative and up to 22% at positive ones), but has no effect on P_o.

Compared to previously studied nicotinic cholinergic receptor modulators, acetylcholine and carbachol are characterised by a relatively weak ability to inhibit the amplitude of the currents through LCC-channels when applied to the bath, and are more effective when added via the patch pipette. The ability of acetylcholine to decrease P_o when applied to the bath is similar to that of mecamylamine and higher compared to carbachol. However, the increase in P_o of LCC-channels caused by acetylcholine applied to the patch-pipette has never been shown before for any other substance, and was not observed for carbachol either.

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relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ВПЛИВ АЦЕТИЛХОЛІНУ ТА КАРБАХОЛІНУ НА ВИСОКОПРОВІДНІ КАТІОННІ КАНАЛИ ЯДЕР НЕЙРОНІВ ПУРКІНЬЄ МОЗОЧКА ЩУРІВ

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Відомо, що ацетилхолін і карбахолін модулюють функції клітин, взаємодіючи з нікотиновими та мускариновими ацетилхоліновими рецепторами, розташованими на плазматичній мембрані клітин-мішеней. Однак деякі агоністи та антагоністи ацетилхолінових рецепторів також здатні модулювати канали ядерної мембрани, зокрема LCC-канали (Large Conductance Cation channels), які є одними з найпоширеніших іонних каналів ядерної мембрани, і, водночас, найменш дослідженими. Метою роботи було оцінити ефективність впливу ацетилхоліну і карбахоліну на електрофізіологічні властивості LCC-каналів. .Мозочки щурів виділяли при охолодженні на льоду, подрібнювали та грубо гомогенізували. Пізніше гомогенізовані зразки центрифугували, ресуспендували фракцію, що містила ядра, і досліджували LCC-канали окремих ядер методом patch-clamp у конфігурації nucleus-attached та режимі фіксації потенціалу._Встановлено, що ацетилхолін при аплікації з внутрішньоядерного боку зменшує амплітуду струмів, що проходять через LCC-канали, та ймовірність їх перебування у відкритому стані (P_o) при негативних мембранних потенціалах. Проте, діючи на перинуклеарну частину каналів, ацетилхолін збільшує їх P_o при негативних потенціалах та зменшує амплітуду струму при всіх потенціалах. Карбахолін при аплікації з внутрішньоядерного боку мембрани зменшує P_o LCC-каналів при -40 мВ і не впливає на амплітуду струму крізь них. Однак при взаємодії з перинуклеарною частиною каналів він зменшує амплітуду струму при всіх прикладених потенціалах і не впливає на P_o. Ці результати свідчать, що ацетилхолін та карбахолін мають асиметричну дію на LCC-канали по різні боки ядерної мембрани як при позитивних, так і при негативних мембранних потенціалах.

Ключові слова: ацетилхолін; карбахолін; агоніст мускаринових та нікотинових ацетилхолінових рецепторів; LCC-канали; patch-clamp; модуляція; іонні канали; нейрони.

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