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Effects of pilocarpine and platyphylline on nuclear membrane large-conductance cation channels in rat cerebellar Purkinje neurons

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Large conductance cation channels (LCC-channels) are abundantly expressed in the outer and inner nuclear membranes, but a specific antagonist of these channels remains to be identified. Given that many previously discovered modulators of these channels include N-cholinergic receptor agonists and antagonists, this study aimed to assess the blocking effects of M-cholinergic modulators, such as pilocarpine and platyphylline, on LCC-channels. The experiments were performed on the nuclei of cerebellar Purkinje neurons of Wistar rats. The electrophysiological activity of LCC-channels was evaluated based on the currents passing through the channels, which were recorded with the patch-clamp technique in nucleus-attached configuration and voltage-clamp mode. We found that pilocarpine (1 mmol/l) applied to the bath does not affect the open-state probability (P_o) of LCC-channels, or the amplitude of the currents through them. In contrast, when applied via the patch pipette, pilocarpine, in addition to reducing P_o by 68.3% at -40 mV, decreases the amplitude of the ion currents at +40 mV by 13.9%. Platyphylline (1 mmol/l), applied to the bath solution, decreases both the amplitude of the currents (by 16.9% at -60 mV) and the P_o values (by 52.6% at -40 mV) of LCC-channels. However, when added to the patch pipette solution, this substance causes an increase in the amplitude of LCC-channels-mediated currents at negative applied potentials (by up to 20.9% at -40 mV), contrary to the impact observed for in-bath application. Moreover, platyphylline decreases P_o of LCC-channels at negative applied potentials (by up to 54.8% at -60 mV), and the amplitude of the currents at positive ones (by up to 22.2% at +40 mV). For both substances, variability in the effects depending on the application configuration may indicate distinct differences in the LCC-channels domains involved in interactions.

Key words: modulation; membrane conductivity; patch-clamp; ion channels; neurons; voltage-dependent currents; electrical activity; muscarinic acetylcholine receptor modulators.

INTRODUCTION

Large conductance cation channels (LCC-channels) were first discovered in the nuclear membrane of cerebellar Purkinje neurons by Marchenko et al. in 2005. They are described as channels that are weakly selective for K^+ and practically impermeable to Ca^{2+} and Ba^{2+} ions, with a conductance of 198 ± 27 pS in symmetric KCl solution, high open-state probability, and slow kinetics [1]. The density of these channels on the nuclear membrane of Purkinje neurons is high, with three to five channels typically present within a patch area (corresponding to approximately 7 channels per μm² [2]), whereas they were completely absent on the membrane of cerebellar granule neurons [1]. Later, LCC-channels were also discovered in the nuclear membrane of cardiomyocytes (with a conductance of 209 ± 13 pS) [3], hippocampal CA1 pyramidal neurons (248 ± 6 pS), CA3 pyramidal neurons (210 ± 6 pS), and dentate gyrus granule neurons (179 ± 15 pS). Nevertheless, in the CA3 area, LCC-channels were extremely rare [4]. It was also found that the density of LCC-channels correlates with the density of IP_3Rs, thus they are potentially functionally linked and coexpressed [2]. As suggested

by Marchenko [1], LCC-channels may be responsible for providing a counterflow of positively charged ions to compensate for electrochemical potential changes due to the release of Ca^{2+} from the endoplasmic reticulum.

Despite the possible involvement of these channels in Ca^{2+} signalling, their modulation and pharmacological sensitivity are scarcely studied. We showed, however, that certain acetylcholine receptor modulators can affect the amplitude of the currents passing through the channels and/or their open probability (P_o). The most pronounced effect was observed for nicotine, which decreased the amplitude of the currents by 50% at a concentration of 0.2 mmol/l. Less effective, but still able to inhibit LCC-channel activity, were pipecuronium bromide and rocuronium bromide, which, when applied at the same concentration, were shown to decrease the amplitude by 12% and 7%, respectively [5]. Intriguing results were obtained from the study of the effects of certain natural venoms on LCC-channels. Purified neurotoxin II, for example, which is typically extracted from the venom of *Naja oxiana*, and also acts as an inhibitor of nicotinic acetylcholine receptors, caused a decrease in the amplitude of the currents through LCC-channels by 13% at a concentration of 0.025 mmol/l. α-Cobratoxin, a component of the venom of *Naja kaouthia*, caused a two-fold decrease in P_o of these channels at concentrations of 1-2 mmol/l, and its effect was accompanied by increased channel flickering [6].

With the aforementioned findings in mind, we aim to extend the investigation of LCC-channel pharmacological sensitivity and research the effects caused by muscarinic acetylcholine receptor modulators, focusing on pilocarpine and platyphylline. Pilocarpine is naturally synthesised by only one plant species, *Pilocarpus microphyllus,* which is native to Brazil [7, 8]. This compound is an agonist of all five (M1-M5) muscarinic acetylcholine receptors [9], but in the case of M3 receptors, it can act as an antagonist as well, depending on the cell type [10]. In medical practice, pilocarpine is prescribed for the treatment of glaucoma and, in some cases, salivary gland hypofunction [11]. In low doses, it was also reported to mitigate the effects of presbyopia, improving near vision in affected individuals [12]. In addition to being an M-cholinergic receptor agonist, this compound increases the turnover rate of serotonin [13] and was also demonstrated to exhibit a degree of beta-adrenergic activity [9]. Platyphylline, meanwhile, is a muscarinic acetylcholine receptor antagonist. It was first isolated from *Senecio platyphyllus* [14] and is the only pyrrolizidine alkaloid that lacks hepatotoxicity and does not induce p53 reparative signaling [15]. Platyphylline was previously used to treat gastrointestinal hypermotility. It was also shown to abolish acetylcholine responses in the guinea-pig ileum [16].

METHODS

The experiments were performed following the necessary bioethical regulations, including the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and the Regulations of the Bioethics Committee of the Bogomoletz Institute of Physiology (Protocol No. 9 dated 29.10.2025).

Electrophysiological studies of LCC-channels were conducted using the patch-clamp technique in a nucleus-attached configuration. Membrane voltage was consecutively clamped at potentials -40 mV, +40 mV, -60 mV, and +60 mV, and the currents through the nuclear membrane within the patch area were recorded. The Purkinje cell nuclei used for the experiments were obtained from the cerebella of Wistar rats. After decapitation, the cerebellum was dissected with a scalpel, cut into 0.4 mm-thick coronal slices, and placed into an Eppendorf microtube with a solution of the following composition (mmol/l): HEPES - 10, HEPES-K - 10, K-gluconate - 150, pH 7.2. All basic components of the solutions used during the experiments were manufactured by “Sigma-Aldrich” (Massachusetts, United

States). Protease inhibitors (cOmplete Protease Inhibitor Cocktail tablets, F. Hoffmann-La Roche AG, Basel, Switzerland) were added to the solution in advance according to the manufacturer's instructions. The cerebella slices were later frozen in the described solution until use. When a specific sample was needed for the experiment, it was carefully thawed and homogenized by passing through a 21-gauge syringe needle. The resulting suspension was centrifuged (2000g, 5 min) to separate the heavier nuclear fraction from the rest of the cellular organelles. The nuclei were then resuspended in a solution of KCl (mmol/l): KCl - 150, EGTA - 1, HEPES - 8, HEPES-K - 12, pH 7.2. This solution is hereafter referred to as the "control solution", as it was also used to fill the patch pipettes and the bath chamber mounted on the microscope stage ("Leica DM IRB, Leica Camera AG", Wetzlar, Germany), where the nuclei-containing suspension was then transferred. The microscope was used for the visual control during the patch-clamp process. The recording electrode was inserted into the patch pipette, while the reference electrode was connected to the bath with a sample via an agar bridge. The pipettes were made from borosilicate glass, and their resistance varied from 7 to 15 MΩ. The solutions of the studied substances were made using the KCl-based control solution as a medium and contained (mmol/l): pilocarpine or platyphylline - 1, KCl - 150, EGTA - 1, HEPES - 8, HEPES-K - 12, pH 7.2. Pilocarpine (Pil) was used in the form of pilocarpine hydrochloride, and platyphylline (Plt) was sourced as platyphylline hydrotartrate.

Given that the effects of the previously examined substances on LCC-channels depend on the side of the membrane they were applied to [17, 18], the currents through the LCC-channels were recorded in the following configurations:

1. In control conditions, with the control KCl-based solution present in both the bath and the patch pipette (marked in the Figures as Con/Con).

2. With the solution containing the examined substance (1 mmol/l) added to the bath, while the patch pipette still contained the control solution (referred to as Pil/Con and Plt/Con). In this configuration, the substances interact with the intranuclear side of the LCC-channels, and the perinuclear side of the membrane within the patch area is exposed to the control solution in the pipette.

3. With the test solutions filling the patch pipette, and the bath solution containing the control KCl-based solution. This configuration is indicated as Con/Pil and Con/Plt. In contrast to the previous one, this time the examined substance interacts only with the perinuclear side of LCC-channels, while the intranuclear side faces toward the control solution in the bath.

Additionally, we examined the lasting effects of platyphylline and pilocarpine on the electrophysiological activity of LCC-channels. For this purpose, after the currents through the channels with the test substance in the bath were recorded, this test solution was again replaced with the control solution, and a new series of recordings was performed. However, while allowing us to estimate the permanence of the effects of platyphylline and pilocarpine, the number of such recordings was substantially lower. The main reason for this is a mechanical sensitivity of the nucleus-attached patch contact, which often led to its spontaneous dissociation during the process of washing the test substance away. Thus, due to the low number of data points for this stage, statistical analysis was not always feasible. Nevertheless, in the cases when a general trend could be inferred, such results were mentioned in the text.

The recordings of the ion currents through LCC-channels at different membrane potentials were further used to assess the two main characteristics that represent the channels' functional activity: the mean difference in the amplitude of the currents between the open and closed states of the channels, and the probability of the channels being in an open state (P_o). Calculation of these parameters for each of the mentioned configurations was conducted

with the semi-automatic built-in tools in Clampfit 10.7 ("Molecular Devices", USA) and described in detail in our previous papers [17]. As the number of channels within a recording was usually greater than one, the evaluated NP_o was later divided by the number of channels in the patch, yielding an average probability for a single channel to be open. The number of channels present was assessed 100 seconds after the start of the recording. Statistical analysis of the data was performed in *Prism 8* ("GraphPad Software", USA) and Origin 2018 ("OriginLab Corporation", USA). Considering that the data followed a normal distribution within each group, a paired t-test was used to estimate the statistical significance of the intergroup differences when the test solution was applied to the bath with the sample, and the results were obtained as repeated measurements of the same parameter. However, during the studies of pilocarpine and platyphylline applied via a patch pipette, control values were taken from the previous stage, before the in-bath application of the substances. Considering that the control and experimental data in this case were obtained from different sets of nuclei, paired statistical tests were inappropriate, and an independent-samples t-test was employed instead. ANOVA with Sidak post hoc test was used to evaluate the statistical significance of intergroup differences when the effects of pilocarpine applied at various concentrations (0.1, 0.2, 0.5, 1, 2, 10 mmol/l) were studied. The final results are presented in Mean ± SEM format, n_1 indicates the sample size in the control, n_2 – in the test group, and a single n value is used for paired comparisons due to the equal number of measurements for both groups.

RESULTS

In the first stage of the study, the effects of pilocarpine were examined. It was found that this compound does not decrease the open probability of LCC-channels when applied to the bath solution at a concentration of 1 mmol/l. Despite initial observation of P_o reduction at the potentials of -60 mV and -40 mV, the statistical significance of such a change was found to be borderline and insufficient to conclude that the effect was present. Typical fragments of recordings and amplitude histograms are presented in Fig. 1A and 1B, respectively. Pilocarpine, when applied to the bath, was also ineffective in the context of modulation of the currents' amplitude, as depicted in Fig. 1C. At the potential of -60 mV, the currents' amplitude values were -12.26 ± 0.67 pA, and at +40 mV and +60 mV, we registered the amplitude values of 8.11 ± 0.25 pA and 12.38 ± 0.49 pA, respectively. These results did not differ between the control group and pilocarpine-treated samples. It must be mentioned, however, that the initial comparison between the control group and 1 mmol/l pilocarpine applied at the potential of -40 mV using the t-test signified that the null hypothesis should be rejected ($P = 0.0482$), but later evaluation of the effects of this substance, depending on its applied concentration, with ANOVA revealed no statistically significant difference. Taking into account that applications of multiple concentrations necessitate the usage of ANOVA as the main method of analysis, we conclude that pilocarpine at -40 mV does not affect the current amplitude, and the results in Fig. 1C are depicted accordingly. We also found that pilocarpine did not significantly change P_o even at a concentration of 10 mmol/l. Thus, it can be suggested that in this configuration, pilocarpine affects neither the amplitude of the currents through LCC-channels, nor their open-state probability (presented in Fig. 1D).

When applied to the patch pipette at the same concentration (1 mmol/l), pilocarpine exhibited some capacity to decrease the current amplitude, with this effect being pronounced at positive applied potentials. At +40 mV, the mean amplitude values dropped from 8.11 ± 0.25 pA in control to 6.98 ± 0.21 pA (13.9% change, $P < 0.01$, $n_1 = 16$, $n_2 = 3$), while at +60 mV a similar tendency was observed, with the amplitude of the currents decreasing

from 12.38 ± 0.49 pA to 11.01 ± 0.20 pA with pilocarpine in the patch pipette (11.1% change), but due to the small sample size (n_2 = 2) the statistical significance of the difference could not be evaluated. Contrary to the results obtained in the presence of pilocarpine in the bath solution, we also found a significant decrease in the open-state probability of LCC-channels at -40 mV, with P_o values decreasing from 0.41 ± 0.04 to 0.13 ± 0.05 (68.3% change, $P < 0.01$, n_1 = 16, n_2 = 4). A similar trend was seen at -60 mV as well, but the number of samples was insufficient to draw a reliable conclusion.

In the next stage of the research, the impact of platyphylline (1 mmol/l) on the nuclear LCC-channels was studied. We found that in the presence of this substance in the bath, the current amplitude reduced primarily at -60 mV. At this potential, the amplitude values dropped from -11.86 ± 0.60 pA in control conditions to -9.85 ± 0.55 pA with platyphylline (16.9% change, $P < 0.01$, n = 8). After replacing the platyphylline solution in the bath with the control one, the amplitude of the currents returned almost to the initial values (-11.28 ± 0.67 pA, n = 7). The decrease observed at -40 mV was

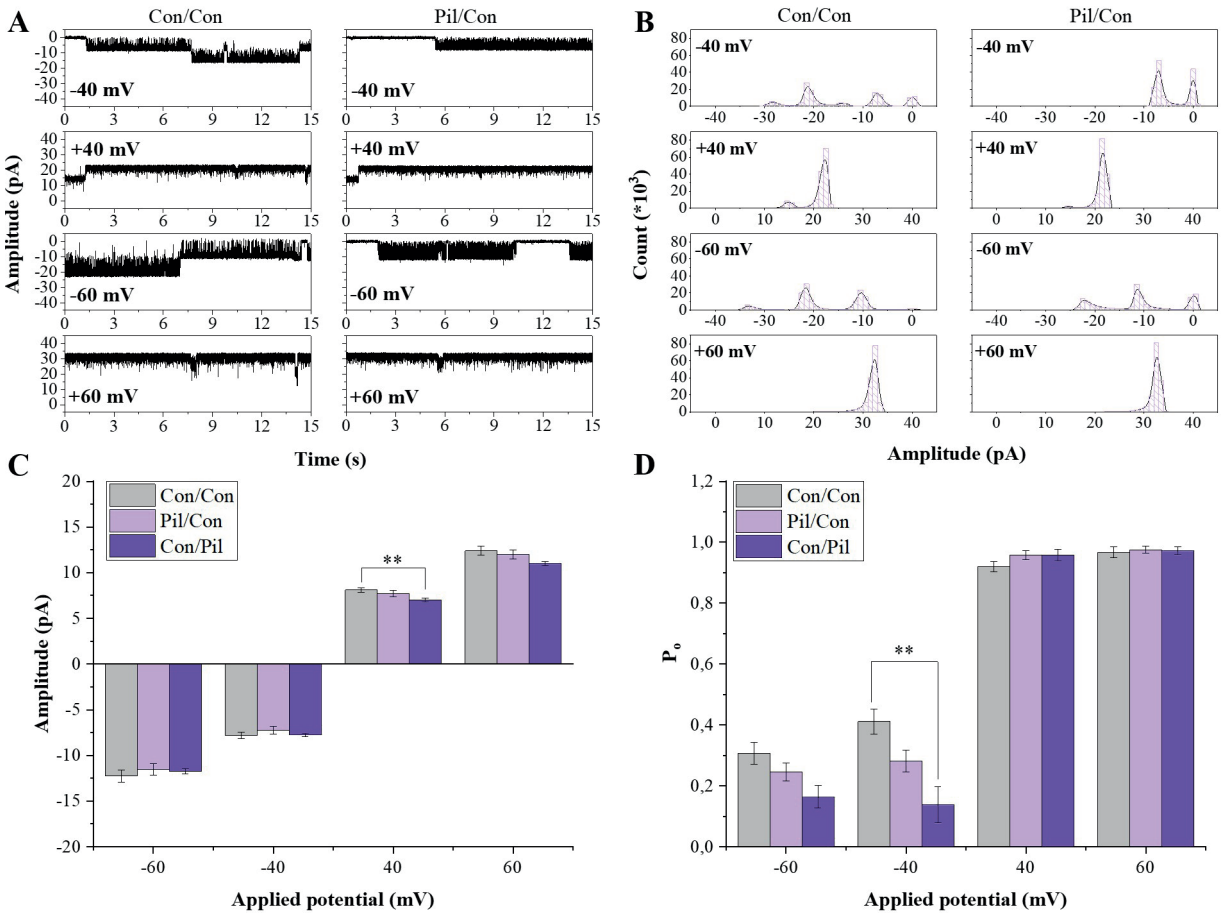

Fig. 1. Effects of pilocarpine (1 mmol/l) on LCC-channel properties. Con/Con designates that the control solution was both in the bath and in the patch pipette. Pil/Con indicates that the bath contained pilocarpine solution, while the pipette contained the control solution. Con/Pil corresponds to the control solution being in the bath, and the patch pipette filled with the pilocarpine solution. A – representative fragments of the recordings. B – amplitude histograms of the typical recordings. C – mean amplitude of the currents through LCC-channels depending on the applied potential. D – mean open-state probability of LCC-channels depending on the membrane potential. * $P < 0.05$, ** $P < 0.01$ compared to control

not statistically significant. However, at the potential of -40 mV, we registered a reduction in the open-state probability of LCC-channels, with its values changing from 0.57 ± 0.05 in control to 0.27 ± 0.06 under the effect of platyphylline (52.6% decrease, $P < 0.01$, $n = 9$). Replacing platyphylline with the control KCl-based solution led to the partial restoration of the open-state probability of LCC-channels, with P_o values of 0.42 ± 0.09 ($n = 8$). Overall, it can be inferred that the inhibitory effect of platyphylline in this configuration is reversible and is pronounced mostly at negative membrane potentials. The representative fragments of the recordings are depicted in Fig. 2A, and corresponding amplitude histograms are shown in Fig. 2B.

Intriguing results were obtained when platyphylline (1 mmol/l) was applied via the patch pipette. In contrast to the reduction of the current amplitude, described for the in-bath configuration, this substance increased the mean amplitude values at both -60 mV and -40 mV when added to the pipette. At -60 mV, the current amplitude rose from -11.92 ± 0.53 pA in control to -13.56 ± 0.35 pA with the test substance (13.8% change, $P < 0.05$, $n_1 = 9$,

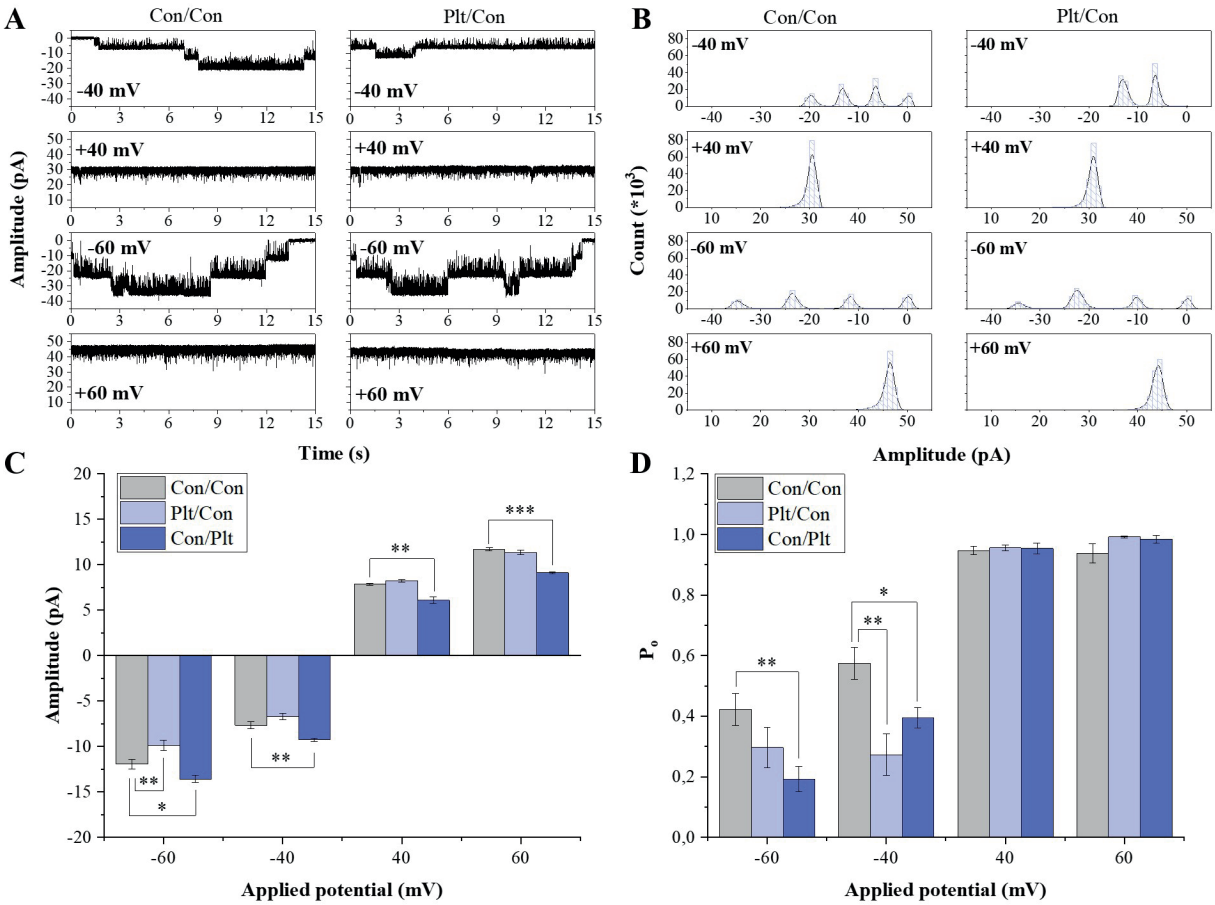

Fig. 2. Electrophysiological properties of LCC-channels in the presence of platyphylline (1 mmol/l). Con/Con indicates the presence of the control solution in both the bath and the patch pipette. Plt/Con specifies that the bath was filled with the platyphylline solution, while the pipette still contained the control KCl solution. Con/Plt means that the platyphylline solution was added to the patch pipette, but not to the bath. A – fragments of the typical recordings. B – amplitude histograms based on the recordings, depicted in A. C – graph of the mean amplitude of the currents at different applied potentials. D – mean probability of LCC-channels being in an open state at different membrane potentials. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control

$n_2 = 4$), and at -40 mV, we registered an increase from -7.66 ± 0.37 pA to -9.26 ± 0.20 pA (20.9% change, $P < 0.01$, $n_1 = 9$, $n_2 = 8$). The graph of the mean amplitude changes is presented in Fig. 2C. At positive values of the membrane potential, the amplitude of the LCC-channel mediated currents decreased by 22.2% and 22.1% for +40 mV and +60 mV, respectively. At +40 mV, the amplitude values in the control KCl solution were measured as 7.85 ± 0.11 pA, and changed to 6.11 ± 0.34 pA with platyphylline applied ($P < 0.01$, $n_1 = 9$, $n_2 = 5$). Similarly, at +60 mV, the amplitude decreased from 11.69 ± 0.16 pA to 9.11 ± 0.11 pA ($P < 0.001$, $n_1 = 8$, $n_2 = 3$).

The probability of LCC-channels being in an open state also changed significantly under the effect of platyphylline applied to the patch pipette, as depicted in Fig. 2D. At -60 mV, P_o dropped from 0.42 ± 0.05 to 0.19 ± 0.04 (54.8% change, $P < 0.01$, $n_1 = 7$, $n_2 = 4$), and at -40 mV, P_o decreased from 0.57 ± 0.05 to 0.39 ± 0.03 (31.6% change, $P < 0.05$, $n_1 = 9$, $n_2 = 8$). However, no change in P_o values was registered at positive membrane potentials of +40 mV and +60 mV.

Both substances were found to exert suppressive effects on the electrophysiological properties of LCC-channels. In the case of pilocarpine, the inhibition was side-specific and pronounced when the substance was applied via the patch pipette and thus interacted with the perinuclear side of the channels. Due to the absence of an impact on the amplitude of the ion currents when applied to the bath, pilocarpine resembles the N-cholinergic receptor modulator carbachol, which also did not modulate the amplitude of currents through LCC-channels, while being able to decrease their P_o [18]. Switching the application configuration to in-pipette, however, enabled both pilocarpine and carbachol to modulate the current amplitude. Considering this, it can be assumed that these substances possess structural patterns specific to the perinuclear domains of LCC-channels. It must be noted that the amplitude decrease, caused by pilocarpine, was less pronounced compared to the one mediated by carbachol. When comparing pilocarpine to rocuronium bromide, another modulator of M-cholinergic receptors, it can be discerned that they share an intrinsic ability to significantly decrease P_o [5], which may reflect the existence of shared structural features involved in this inhibition, but this effect manifests in different application configurations for these substances.

Platyphylline also decreased the open probability of LCC-channels, but in both application modes. Unlike pilocarpine, it additionally reduced the amplitude of the ion currents through the studied channels when applied to the bath. However, this effect reached statistical significance only at -60 mV, and by its magnitude was not particularly dissimilar to the impact observed in the presence of pilocarpine in the patch pipette. In contrast, when platyphylline was applied to the micropipette, we observed an increase in the amplitude of LCC-mediated currents at both -60 and -40 mV. To date, no other substance has been capable of increasing the amplitude of the currents mediated by LCC-channels, with mecamylamine being the only reported exception [17]. But in the case of mecamylamine, this effect was much less pronounced, with a different substance application configuration and at positive membrane potentials. At the same time, platyphylline decreased the amplitude of the currents at these values of applied potentials, so it does not seem likely that the mechanisms of previously described amplitude increase are similar for these two compounds. It also must be highlighted that the same substance, platyphylline, was seen to have not just different, but opposite effects on the current amplitude at -60 mV. Considering the results discussed in the previous studies [17, 18], it can be suggested that modulation of LCC-channel electrophysiological activity is highly side-specific. This, in turn, suggests the presence of structural differences between intranuclear and perinuclear domains, which must be considered in future investigations.

CONCLUSIONS

Summarizing the key results of this research, the following conclusions can be drawn:

1. Pilocarpine at a concentration of 1 mmol/l decreases the open-state probability of LCC-channels (by 68.3%) and reduces the amplitude of the currents through the channels (by 13.9%), but only when applied via the patch pipette.

2. Platyphylline, when added to the bath solution at 1 mmol/l, reduces both the current amplitude (by 16.9%) as well as their P_o (by 52.6%). However, when applied to the patch pipette, this substance increases the current amplitude at negative membrane potentials (by up to 20.9%) and decreases it at positive ones (by up to 22.2%), while still reducing the P_o of LCC-channels (by up to 54.8%).

3. The ability of pilocarpine and platyphylline to decrease the open-state probability of LCC-channels is more pronounced than that of other cholinergic receptor modulators, such as carbachol. In contrast, their effects on the current amplitude are rather modest and, to a great extent, depend on the configuration of the application and the nuclear membrane potential.

4. The results obtained in the current study complement the understanding of the pharmacological sensitivity of LCC-channels and support the conclusion that the modulation of their electrophysiological activity is side-specific and voltage-dependent. These characteristics should be carefully considered in further analysis of their potential role in Ca^{2+} release and in the search for new drug targets for dysregulated Ca^{2+} signaling associated with pathological conditions.

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ВПЛИВ ПІЛОКАРПІНУ ТА ПЛАТИФІЛІНУ НА ВИСОКОПРОВІДНІ КАТІОННІ КАНАЛИ ЯДЕРНОЇ МЕМБРАНИ КЛІТИН ПУРКІНЬЄ МОЗОЧКА ЩУРА

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Високопровідні катіонні канали (LCC-канали) є численними на зовнішній і внутрішній ядерних мембранах, однак їх специфічний антагоніст досі не ідентифіковано. Враховуючи, що багато попередньо вивчених модуляторів цих каналів належить до агоністів та антагоністів N-холінорецепторів, метою цієї роботи було встановити здатність до блокування LCC-каналів модуляторами М-холінорецепторів, а саме пілокарпіном та платифіліном. Дослідження проводили на ядрах нейронів Пуркіньє мозочка щурів лінії *Вістар*. Електрофізіологічну активність LCC-каналів оцінювали на підставі реєстрацій іонного струму, які отримували методом patch-clamp у конфігурації nucleus-attached та режимі фіксації потенціалу. Встановлено, що пілокарпін (1 ммоль/л) при додаванні до ванночки зі зразком не впливав на ймовірність відкритого стану (P_o) LCC-каналів та амплітуду струму крізь них. Водночас при аплікації крізь patch-піпетку пілокарпін, окрім інгібування P_o на 68,3% при -40 мВ, також знижував амплітуду іонного струму при +40 мВ на 13,9%. Платифілін (1 ммоль/л), при додаванні до ванночки, знижував як амплітуду струму (на 16,9% при -60 мВ), так і значення P_o (на 52,6% при -40 мВ) LCC-каналів. Однак при додаванні у patch-піпетку він підвищував амплітуду струму при негативних потенціалах (на 20,9% при -40 мВ) на відміну від ефекту, який виявлено за умов аплікації платифіліну у ванночку. Також він знижував P_o LCC-каналів при негативних мембранних потенціалах (на 54,8% при -60 мВ) і амплітуду струму при позитивних (на 22,2% при +40 мВ). Відмінність дії залежно від конфігурації аплікації обох речовин може свідчити про різниці у

доменах LCC-каналів, залучених до взаємодії.

Ключові слова: модуляція; мембранна провідність; patch-clamp; іонні канали; нейрони; потенціалзалежний струм; електрична активність; модулятори мускаринових ацетилхолінових рецепторів.

REFERENCES

1. Marchenko SM, Yarotskyy VV, Kovalenko TN, Kostyuk PG, Thomas RC. Spontaneously active and $InsP_3$-activated ion channels in cell nuclei from rat cerebellar Purkinje and granule neurons. J Physiol. 2005 Jun 15;565(3):897-910. doi: 10.1113/jphysiol.2004.081299.
2. Fedorenko O, Yarotskyy V, Duzhyy D, Marchenko S. The large-conductance ion channels in the nuclear envelope of central neurons. Pflüg Arch Eur J Physiol. 2010 Nov;460(6):1045-50. doi: 10.1007/s00424-010-0882-5.
3. Kotyk OA, Kotliarova AB, Polishchuk AO, Marchenko SM. Single-channel ion currents in the nuclear envelope of rat cardiomyocytes. Fiziol Zh. 2016 Dec 5;62(6):3-8. doi: 10.15407/fz62.06.003. [Ukrainian].
4. Fedorenko OA, Marchenko SM. Ion channels of the nuclear membrane of hippocampal neurons. Hippocampus. 2014 Jul;24(7):869-76. doi: 10.1002/hipo.22276
5. Kotliarova A, Kotyk O, Yuryshynets I, Marchenko S. The functioning of large conductance cationic channels in the nuclear membrane of cardiomyocytes and cerebellar Purkinje neurons under the influence of nicotinic cholinoreceptors modulators. Fiziol Zh. 2019 Dec 5;65(6):30-7. doi: 10.15407/fz65.06.030. [Ukrainian].
6. Kotyk O, Kotliarova A, Isaeva O, Marchenko S. The effect of some anesthetics and natural venoms on the LCC-channels functioning of the nuclear membrane of cardiomyocytes and cerebellum Purkinje neurons. Bull Taras Shevchenko Natl Univ Kyiv Ser Biol. 2019;79(3):43-8. [Ukrainian].
7. Caldeira CF, Giannini TC, Ramos SJ, Vasconcelos S, Mitre SK, Pires JPDA, Ferreira GC, Ohashi S, Mota JA, Castilho A, Siqueira JO, Furtini Neto AE. Sustainability of Jaborandi in the eastern Brazilian Amazon. Perspect Ecol Conserv. 2017 July;15(3):161-71. doi: 10.1016/j.pecon.2017.08.002.
8. Sobreiro MB, Soares-Souza GB, Magalhães L, De Morais Cordeiro D, Molina M, Vasconcelos S, Dias YN, Moreira-Oliveira RR, Gastauer M, Ramos S, Oliveira G, Caldeira CF, Vidal AF. Decoding pilocarpine biosynthesis and its roles in Pilocarpus microphyllus through a comparative transcriptomics approach. BMC Plant Biol. 2025 Aug 4;25(1):1024. doi: 10.1186/s12870-025-07087-4.
9. Kapourani A, Kontogiannopoulos KN, Barmpalexis P. A review on the role of pilocarpine on the management of xerostomia and the importance of the topical administration systems development. Pharmaceuticals. 2022 Jun 18;15(6):762. doi: 10.3390/ph15060762.
10. Pronin AN, Wang Q, Slepak VZ. Teaching an old drug new tricks: Agonism, antagonism, and biased signaling of pilocarpine through m3 muscarinic acetylcholine receptor. Mol Pharmacol. 2017 Nov;92(5):601–12. doi: 10.1124/mol.117.109678.
11. Aronson JK, editor. Pilocarpine. In: Meyler's Side Effects of Drugs. 16th ed. Oxford: Elsevier; 2016. p. 763-4. doi: 10.1016/B978-0-444-53717-1.01278-6
12. Holland E, Karpecki P, Fingeret M, Schaeffer J, Gupta P, Fram N, Smits G, Ignacio T, Lindstrom R. Efficacy and safety of CSF-1 (0.4% pilocarpine hydrochloride) in presbyopia: pooled results of the NEAR phase 3 randomized, clinical trials. Clin Ther. 2024 Feb;46(2):104-13. doi: 10.1016/j.clinthera.2023.12.005.
13. Haubrich DR, Reid WD. Effects of pilocarpine or arecoline administration on acetylcholine levels and serotonin turnover in rat brain. J Pharmacol Exp Ther. 1972 Apr;181(1):19-27. doi: 10.1016/S0022-3565(25)29171-6.
14. Sadgrove NJ. Comment on pyrrolizidine alkaloids and terpenes from senecio (asteraceae): chemistry and research gaps in Africa. Molecules. 2022 Dec 13;27(24):8868. doi: 10.3390/molecules27248868.
15. Ebmeyer J, Rasinger JD, Hengstler JG, Schaudien D, Creutzenberg O, Lampen A, Braeuning A, Hessel-Pras S. Hepatotoxic pyrrolizidine alkaloids induce DNA damage response in rat liver in a 28-day feeding study. Arch Toxicol. 2020 May;94(5):1739-51. doi: 10.1007/s00204-020-02779-2.
16. Pomeroy AR, Raper C. Pyrrolizidine alkaloids: actions on muscarinic receptors in the guinea-pig ileum. Br J Pharmacol. 1971 Apr;41(4):683-90. doi: 10.1111/j.1476-5381.1971.tb07076.x.
17. Nadtoka S, Kotyk O, Protsenko K, Kotliarova A. Effects of mecamylamine on the electrophysiological properties of LCC-channels in rat cerebellar Purkinje neurons. Fiziol Zh. 2025;71(5):22-30. doi: 10.15407/fz71.05.022.
18. Nadtoka S, Kotyk O, Tarnopolska O, Kotliarova A. Effects of acetylcholine and carbachol on nuclear large conductance cation channels in rat cerebellar Purkinje neurons. Fiziol Zh. 2025;71(6):67-77. doi: 10.15407/fz71.06.067.

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