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Effects of adrenergic receptor modulators on nuclear LCC-channels in Purkinje neurons

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Large conductance cation channels (LCC-channels), located in the nuclear membrane, are potential mediators of potassium (K^+) countercurrent during calcium ion (Ca^{2+}) release from intracellular stores. This study examined the effects of epinephrine, norepinephrine, isoprenaline, and propranolol on the electrophysiological properties of these channels to evaluate their potential as blocking agents in future studies of LCC-channel involvement in Ca^{2+} release. The patch-clamp method, using voltage-clamp mode and a nucleus-attached configuration, was employed to record currents passing through LCC-channels in Purkinje cell nuclei. The effectiveness of Ca^{2+} blocking was estimated by analyzing changes in current amplitude and the probability of the channels being in an open state. All tested adrenergic receptor modulators decreased current amplitude through LCC-channels at negative membrane potentials to varying extents, while no changes in amplitude were found for positive applied potentials for any compound. Epinephrine, norepinephrine, and propranolol demonstrated blocking capabilities comparable to nicotinic acetylcholine receptor (nAChR) modulators under similar conditions. Notably, only norepinephrine significantly inhibited the open-state probability of LCC-channels, whereas isoprenaline increased this parameter and induced rapid flickering. Furthermore, isoprenaline produced a greater reduction in current amplitude through LCC-channels than the other compounds.

Key words: patch-clamp, ion channels, modulation, electrophysiology, neurons, nuclear membrane

INTRODUCTION

The nucleus, the largest cellular organelle in eukaryotic cells, plays a central role in regulating, storing, and transcribing genetic information. Within the liquid-like nucleoplasm, a chromatin network is tethered to the nuclear lamina at the nuclear periphery, and is primarily composed of deoxyribonucleic acid (DNA) molecules and histones (Nothof et al., 2022; Hertzog & Erdel, 2023). The nuclear envelope, also referred to as the nuclear membrane, surrounds the nucleus and separates nucleoplasm from the cytoplasm. This envelope is a complex, multilayered structure consisting of inner and outer nuclear membranes, with a perinuclear space between them (Fernández-Jiménez & Pradillo, 2020). Owing to its unique architecture, the nuclear envelope provides three principal pathways for ion currents (Matzke et al., 2010): through the nuclear pore complex (NPC) between the cytoplasm and the nucleoplasm; across the outer nuclear membrane between the cytoplasm and perinuclear space; and across the inner nuclear membrane between the nucleoplasm and perinuclear space. Nuclear pores facilitate exchange between the nucleoplasm and cytoplasm, while ion channels mediate ion transfer between the perinuclear space, cytoplasm, and nucleoplasm. Nuclear ion channels have recently emerged as promising molecular targets for drug discovery (Bkaily, 2009).

Among nuclear channels, large conductance cation channels (LCC-channels) are of particular inter-

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est due to their potential role in calcium ion (Ca^{2+}) release from intracellular stores (Marchenko et al., 2005). These channels exhibit high conductance (171-225 pS), slow kinetics, and voltage-dependence. At positive applied potentials, they are predominantly open, whereas a decrease in membrane potential reduces their functional activity (Marchenko et al., 2005; Kotyk et al., 2017). LCC-channels are selective for monovalent cations and impermeable to divalent cations (Fedorenko & Marchenko, 2014). Identifying molecules that may act as agonists or inhibitors of LCC-channels is essential for elucidating their physiological roles and structural characteristics.

Electrophysiological studies indicate that LCC-channels are insensitive to most currently known potassium channel inhibitors, such as tetraethylammonium (10 mmol/L) and 4-aminopyridine (2 mmol/L) (Fedorenko et al., 2010). To date, research on LCC-channels has primarily focused on their interactions with various modulators of nicotinic acetylcholine receptors (nAChRs), including mecamylamine (Nadtoka et al., 2025a), acetylcholine, carbachol (Kotyk et al., 2017; Nadtoka et al., 2025b), pipecuronium bromide, rocuronium bromide, nicotine, hexamethonium, methyllycaconitine, alpha-conotoxin PeIA (Kotliarova et al., 2019), as well as α-cobratoxin, neurotoxin II (Kotyk et al., 2019), ditiline, and atracurium (Kotyk et al., 2017). However, the effects of adrenergic receptor modulators on LCC-channels remain entirely unknown. Therefore, the present study aims to address this gap by investigating the electrophysiological properties of LCC-channels under the influence of adrenergic receptor modulators, specifically epinephrine, norepinephrine, isoprenaline, and propranolol.

Epinephrine (adrenaline) is a potent agonist of α1-, α2-, β1-, and β2-adrenergic receptors, produced and secreted by chromaffin cells in the adrenal glands (González-Santana et al., 2020; Skelding & Valverde, 2020). As a catecholamine, epinephrine is synthesized through tyrosine transformations (Dinu & Apetrei, 2020). Clinically, epinephrine is administered in emergencies to treat anaphylactic shock and cardiopulmonary arrest, although vasopressin has replaced it in some resuscitation protocols (Yan et al., 2023).

Norepinephrine (noradrenaline), a dopamine derivative, serves as the primary sympathetic neurotransmitter in the nervous system. Its effects are mediated mainly by α1-, α2-, and β-adrenoreceptors, where it acts as an agonist (Maletic et al., 2017). Norepinephrine also modulates attention and promotes arousal in the brain, with the locus coeruleus in the brainstem serving as its principal source (Kim, 2023).

Isoprenaline (isoproterenol) is a synthetic sympathomimetic drug derived from norepinephrine and is sometimes referred to as isopropylnoradrenaline (O'Shaughnessy, 2012). It functions as a non-selective agonist of β-adrenergic receptors (Motwani & Saunders, 2024). Currently, isoprenaline is primarily used in laboratory settings to induce acute myocardial infarction or cardiac fibrosis, depending on the administered dose (Bader Eddin et al., 2025).

Propranolol is a β-adrenergic receptor antagonist (Srinivasan, 2019) that was initially developed for the treatment of angina pectoris and is now frequently used as a first-line therapy for essential tremor (Frei & Truong, 2022). Its effects result from the blockade of β-1 and β-2 peripheral receptors in the sympathetic nervous system (Mingrui, 2024). Propranolol acts non-selectively on β-adrenergic receptors, interacting with both β1 and β2 subtypes, thereby preventing epinephrine and norepinephrine from binding to their specific sites (Srinivasan, 2019; Taha et al., 2025).

Although the adrenergic receptor agonists and antagonists discussed above have not been previously studied as potential regulators of nuclear LCC-channels, most cholinergic receptor modulators evaluated in this context were examined using isolated nuclei from cardiomyocytes (Kotyk et al., 2017) or cerebellar Purkinje neurons (Nadtoka et al., 2025a). This experimental model was selected to test the hypothesis regarding the role of LCC-channels in Ca^{2+} release from intracellular stores, a process essential for the function of excitable cells such as myocytes and neurons. Cerebellar Purkinje cells are particularly suitable due to the large size of their nuclei, which facilitates isolation and visual identification. Furthermore, Purkinje cells were the first cell type in which nuclear LCC-channels were described (Marchenko et al., 2005). To ensure comparability with previous findings, Purkinje neuron nuclei were used in the present study to assess the effects of adrenergic receptor modulators on LCC-channels.

METHODS

The experiments were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Kilkenny et al., 2010), and the guidelines of the Bioethics Committee of the Bogomoletz Institute of Physiology, National Academy of Sciences of Ukraine (Protocol No 8/25).

Preparation of Biological Samples

Nuclei of Purkinje neurons were isolated from male Wistar rats aged 3 to 4 weeks. The cerebella were rapidly excised and placed in a low-temperature (1-4°C) solution containing 150 mmol/L NaCl, 10 mmol/L HEPES, and 1 mmol/L EDTA (pH 7.4). Coronal cerebellar slices (~400 μm) were prepared and transferred to a solution containing 150 mmol/L K-gluconate, 10 mmol/L HEPES, and 10 mmol/L HEPES-K (pH 7.2), with a protease inhibitor cocktail (Roche Diagnostics, Germany) added according to the manufacturer's instructions. The slices were homogenized by passage through a 0.8 mm needle. The resulting homogenate was centrifuged at 5500 rpm for 5 minutes to separate the nuclear fraction. After removal of the supernatant, the nuclei-containing pellet was resuspended in a solution containing 150 mmol/L KCl, 8 mmol/L HEPES, 12 mmol/L HEPES-K, and 1 mmol/L EGTA (pH 7.2), hereafter referred to as the control solution. Approximately 50 μL of the suspension was placed into the chamber of a Leica DMIRB inverted microscope (Leica Microsystems, Germany). After the nuclei attached to the glass bottom of the chamber, 10 ml of the control solution was used to wash away residual cell fragments.

Electrophysiological Recording of Nuclear Currents

Currents passing through LCC-channels in the nuclear membrane of cerebellar Purkinje neurons were recorded in the presence of various adrenergic receptor modulators. The methodology was based on approaches devised by Marchenko, who first identified LCC-channels (Marchenko et al., 2005; Fedorenko et al., 2010), and is described in detail in our previous research (Nadtoka et al., 2025a). Currents were recorded using the patch-clamp technique in the nucleus-attached configuration and voltage-clamp mode. In this method, the voltage across the nuclear membrane is fixed, and the currents passing through the membrane patch, isolated by a micropipette, are recorded. The characteristics of these currents reflect the activity of the ion channels within the patch, making patch-clamp studies suitable for assessing channel activity. Current amplitude is typically measured at different membrane potentials, enabling the construction of current-voltage curves and the estimation of channel functional parameters under various conditions.

The nuclear membrane voltage was sequentially set at -40 mV, +40 mV, -60 mV, and +60 mV, and ion currents through the channels were recorded at each potential. Signals were filtered using a low-pass Bessel filter at 1 kHz, digitized at 5 kHz, and stored digitally. The reference electrode was connected to the bath chamber *via* an agar bridge. The recording electrode was placed in a micropipette fabricated from borosilicate glass capillaries and filled with the same KCl-based solution as the bath. Micropipette resistance ranged from 8 to 15 MΩ. The bath solution potential was set to 0 mV for all recordings. After the micropipette was attached to the nuclear membrane and a gigaohm seal was established, a control period of LCC-channel activity was recorded. Subsequently, the control KCl-containing solution in the bath was replaced by perfusion with a 1 mmol/L solution of one of the test substances (epinephrine, norepinephrine, isoprenaline, or propranolol) in KCl-containing medium, as described previously.

After recording the currents through the nuclear channels, the test solution was washed out and replaced with the control solution. A final series of recordings was then performed to assess the permanence of any changes caused by the tested substance. For the compounds exhibiting promising or unexpected effects, additional experiments were conducted to examine the concentration dependence of their actions. In these experiments, solutions were applied to the bath at progressively increasing concentrations, with the nuclear membrane potential fixed at -40 mV. The concentrations used (0.1 mmol/L; 0.2 mmol/L; 0.5 mmol/L; 1 mmol/L; 2 mmol/L; 10 mmol/L) were selected empirically, as pharmacological applications and kinetic analyses were beyond the scope of this study.

Kinetics studies and inhibition constant (K_i) evaluations are planned for future investigations of substances exhibiting promising antagonistic effects. The present study focused on the initial estimation of the blocking capabilities of the aforementioned substances. It is important to note that all test solutions were introduced to the bath after the gigaohm seal was established, ensuring interaction exclusively with the intranuclear portion of the LCC-channels, while the perinuclear side remained exposed to the KCl-based solution within the micropipette.

Data Processing and Representation

Data analysis was performed using Clampfit 10.7 (Axon Instruments, USA) and Origin 2018 (64-bit; OriginLab Corporation, USA). The primary electrophysiological parameters evaluated were the probability of the channels being in the open state (P_o) and

the amplitude of the current through the channels. Calculations were conducted separately for each voltage. Amplitude was estimated using built-in software tools as the difference between the mean current values in open and closed channel states. Calculation of P_o values required preliminary processing of the recordings, as described in previous works (Nadtoka et al., 2025a). Key steps included reducing flickering and excluding short-lived channels from the analysis. After processing, P_o was evaluated using the following criteria: a change in channel state was recognized when the amplitude change was at least 50% of the intrinsic amplitude for these channels at the specific membrane potential and concentration, and the event lasted at least 10 ms. Amplitude histograms were constructed to depict general patterns of amplitude distribution for representative recordings, with the y-axis representing the total number of data points ("Count") and the x-axis representing amplitude values.

Statistical Analysis

Given the normal distribution of data within the two groups (control group with KCl in the bath and test group with a test substance at a concentration of 1 mmol/L) and the repeated measurements of the same parameters under different conditions, a paired two-tailed Student's t-test was used to assess the statistical significance. When comparisons involved values from different sets of nuclei, an unpaired (independent) two-tailed Student's t-test was used. For multi-group comparisons, such as concentration dependency studies, analysis of variance (ANOVA) with Sidak's *post hoc* test was conducted. Results in text and figures are presented as mean ± standard error of the mean (SEM). Statistically significant findings are described with the test name, subscript degrees of freedom, and the confidence level for Type I error. Differences were considered significant at $P<0.05$. * indicates $P<0.05$, ** indicates $P<0.01$, and *** indicates $P<0.001$.

RESULTS

Applying 1 mmol/L norepinephrine resulted in a significant reduction in the amplitude of currents through LCC-channels at membrane potentials of -60 mV (t_6=7.49, $P<0.001$) and -40 mV (t_6=2.46, $P<0.05$). At -60 mV, mean amplitude values decreased from -14.93 ± 0.34 pA to -13.41 ± 0.43 pA, representing a 10% reduction. At -40 mV, values decreased from -9.72 ± 0.39 pA to -9.07 ± 0.41 pA, corresponding to a 7% reduction. Representative recording fragments obtained in a control KCl solution of with norepinephrine for these and other applied potentials are shown in Fig. 1A. The amplitude histograms of the corresponding recordings are presented in Fig. 1B. After replacing the tested solution in the bath with the control solution, current amplitudes tended to revert to baseline values, measuring -13.87 ± 0.46 pA at -60 mV and -9.68 ± 0.38 pA at -40 mV. The mean amplitude of a single channel opening event at different experiment stages is shown in Fig. 1C.

Norepinephrine substantially decreased the probability that LCC-channels were open at negative applied potentials. Specifically, 1 mmol/L norepinephrine reduced P_o values from 0.39 ± 0.01 to 0.18 ± 0.02 (54% decrease, t_5=6.26, $P<0.01$) at -40 mV, and from 0.25 ± 0.03 to 0.16 ± 0.02 at -60 mV (36% decrease, t_5=3.84, $P<0.05$). No significant changes in P_o were observed at the positive potentials.

Recording fragments under in control conditions and with 1 mmol/L epinephrine applied are shown in Fig. 2A, and the amplitude histograms are depicted in Fig. 2B. Epinephrine decreased the mean current amplitude through LCC-channels at -40 mV, as shown in Fig. 2C. Adding 1 mmol/L epinephrine to the sample reduced the current amplitude from -7.52 ± 0.15 pA to -6.51 ± 0.37 pA at -40 mV, representing a 13% reduction (t_7=4.18, $P<0.01$). After washing epinephrine from the sample, the amplitude partially recovered to that of the control (-6.83 ± 0.16 pA). Additionally, the current amplitude tended to decrease at -60 mV, but the difference was not statistically significant.

Increasingly negative applied potentials led to greater differences in current amplitude between control conditions and those with epinephrine. For example, at -80 mV, amplitude values decreased from -15.09 pA to -8.87 pA. However, the primary focus was on applied potentials of ±40 mV and ±60 mV to ensure comparability with our previous studies on these channels. The number of data points obtained for ±80 mV was insufficient for statistical confirmation.

Further research on the P_o of LCC-channels under the effect of epinephrine showed that epinephrine did not affect the P_o of these channels at any of the applied potentials. The P_o values obtained on this stage are presented in Fig. 2D.

The minimal concentration of epinephrine that produced a statistically significant effect on current amplitude through LCC-channels at -40 mV was 1 mmol/L. Increasing the concentration to 2 mmol/L further decreased the amplitude to -4.53 ± 0.68 pA (40% reduction compared to control, t_5=5.50, $P<0.05$). A similar trend was observed at -60 mV, where current

amplitude decreased from -11.22 ± 0.29 pA to -3.97 ± 0.47 pA (65% change, t_2=23.62, P<0.01) with 2 mmol/L epinephrine. However, effects at these higher concentrations are likely nonspecific and may reflect structural blockade of the channel pore rather than specific modulation. Increasing epinephrine concentration did not significantly alter its effect on the P_o of the channels.

In the subsequent phase, the effects of propranolol on nuclear LCC-channels were examined. Representative recording fragments and amplitude histograms are presented in Fig. 3A and 3B, respectively. When added to the bath, propranolol at 1 mmol/L significantly decreased the amplitude of channel currents at negative membrane potentials. Specifically, at -60 mV, the amplitude decreased from -11.46 ± 0.49 pA in the control solution to -8.83 ± 0.44 pA with propranolol (23% change, t_2=6.72, P<0.05), and at -40 mV, amplitude values decreased from -7.32 ± 0.13 pA to -6.62 ± 0.26 pA (10% reduction, t_7=2.84, P<0.05). Similar to other adrenergic receptor modulators described in this study, propranolol did not affect the amplitude of currents at positive membrane potentials. The graph of the mean amplitude of currents through LCC-channels, depending on the substance applied to the nuclear membrane, is presented in Fig. 3C.

A tendency for the P_o of LCC-channels to decrease at membrane potentials of -40 mV and +40 mV was observed when propranolol at a concentration of 1 mmol/L was applied. This trend is evident in both the graph of mean P_o values at different membrane potentials and the amplitude histograms of represen-

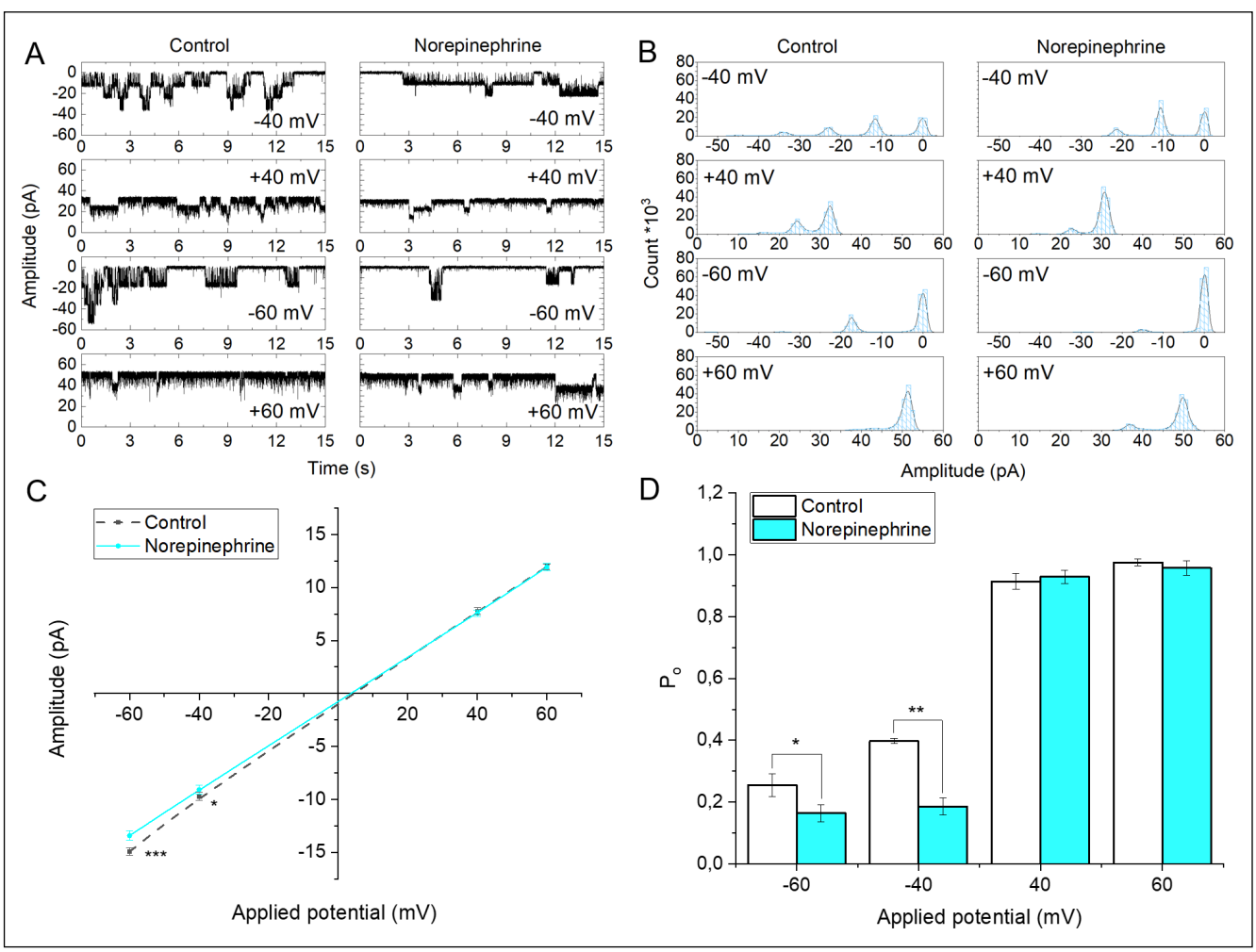

Fig. 1. Effects of norepinephrine at 1 mmol/L on the electrophysiological properties of LCC-channels. The "Control" condition refers to a solution containing (mmol/L): KCl 150, HEPES 8, HEPES-K 12, EGTA 1, pH 7.2. The "Norepinephrine" condition includes the same solution with norepinephrine at 1 mmol/L. P_o indicates the probability that the channels are open. (A) Representative recording fragments; (B) amplitude histograms derived from these recordings; (C) current-voltage (I-V) relationships for LCC-channels under norepinephrine and control conditions; (D) mean P_o as a function of membrane potential. * P<0.05, ** P<0.01, *** P<0.001 *versus* control.

tative recordings (Fig. 3D and 3B, respectively). However, statistical analysis did not confirm this observation. Furthermore, high variability between nuclei and a low number of data points (n=3) may have interfered with the estimation of P_o changes at -60 mV.

The properties of nuclear LCC-channels were subsequently examined under the influence of isoprenaline. Application of isoprenaline at 1 mmol/L to the bath produced a substantial decrease in current amplitude through the LCC-channels at negative membrane potentials. At -40 mV, the amplitude decreased from -7.58 ± 0.13 pA in the control to -5.64 ± 0.30 pA with isoprenaline (26% change, t_9=5.25, P<0.001). At -60 mV, the mean amplitude was reduced from -11.59 ± 0.31 pA to -5.48 ± 0.48 pA (53% decrease, t_4=31.90, P<0.001). Isoprenaline also induced pronounced flickering of the LCC-channels, particularly at negative applied potentials, as shown in Fig. 4A. This effect was reflected in the amplitude histograms of representative recordings (Fig. 4B). Notably, the amplitude inhibition caused by isoprenaline may be reversible; in one recording, the current amplitude returned close to initial values after washing the sample with control solution (-12.36 pA for -60 mV and -7.98 pA for -40 mV).

Although isoprenaline (1 mmol/L) inhibited the amplitude of currents through LCC-channels, at -60 mV it caused a statistically significant increase in P_o values from 0.33 ± 0.05 in control to 0.57 ± 0.07 with isoprenaline (73% change, t_4=3.82, P<0.05). This finding suggests that the channel remained open for a longer duration than in control conditions. The

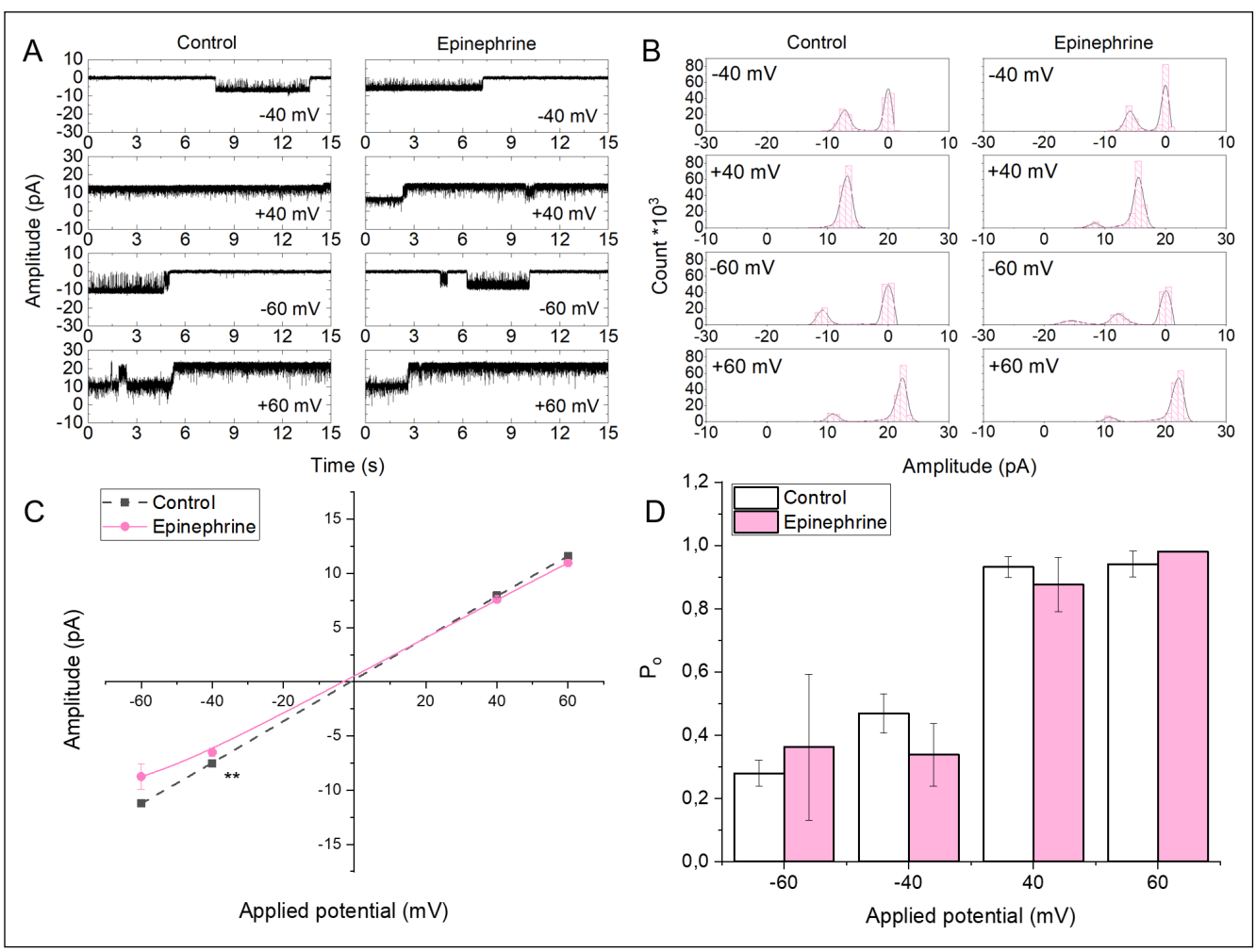

Fig. 2. Effects of 1 mmol/L epinephrine on LCC-channel activity. "Control" refers to the solution containing (mmol/L): KCl 150, HEPES 8, HEPES-K 12, EGTA 1, pH 7.2. "Epinephrine" indicates the same solution with 1 mmol/L epinephrine added. P_o represents the probability of channels being open. (A) Representative recording fragments; (B) amplitude histograms derived from the recordings in A; (C) current-voltage (I-V) relationships of LCC-channels under control and epinephrine conditions; (D) mean P_o values as a function of the potential applied to the nuclear membrane. **P<0.01 *versus* control.

graph of amplitude changes under isoprenaline is shown in Fig. 4C, and its effect on P_o of LCC-channels is shown in Fig. 4D.

To further investigate the effects of isoprenaline on LCC-channels, the concentration-dependence of its action was assessed. During these studies, the potential on the nuclear membrane was fixed at -40 mV. The minimal concentration of isoprenaline that produced a discernible amplitude decrease was 0.5 mmol/L, as amplitude values declined from -7.58 ± 0.13 pA in the control to -6.47 ± 0.15 pA with isoprenaline (15% decrease, t_5=6.36, P<0.01). The current amplitude continued to decrease, reaching a minimum at a concentration of 10 mmol/L. At this concentration, the mean amplitude was -1.83 ± 0.39 pA (76% decrease, t_3=18.53, P<0.01). However, the amplitude reduction observed at this concentration is unlikely to reflect specific modulation. The data from this stage are presented in Fig. 5A.

At a membrane potential of -40 mV, increasing isoprenaline concentration did not affect the probability of LCC-channels being open. Even at 10 mmol/L, there was no statistically significant difference between P_o in control (0.51 ± 0.03, n=11) and with isoprenaline (0.72 ± 0.11, n=4). Notably, during consecutive applications of isoprenaline solutions, only a few patch contacts remained stable at later experimental stages, limiting the sample size. The normalized P_o values for different isoprenaline concentrations are shown in Fig. 5B.

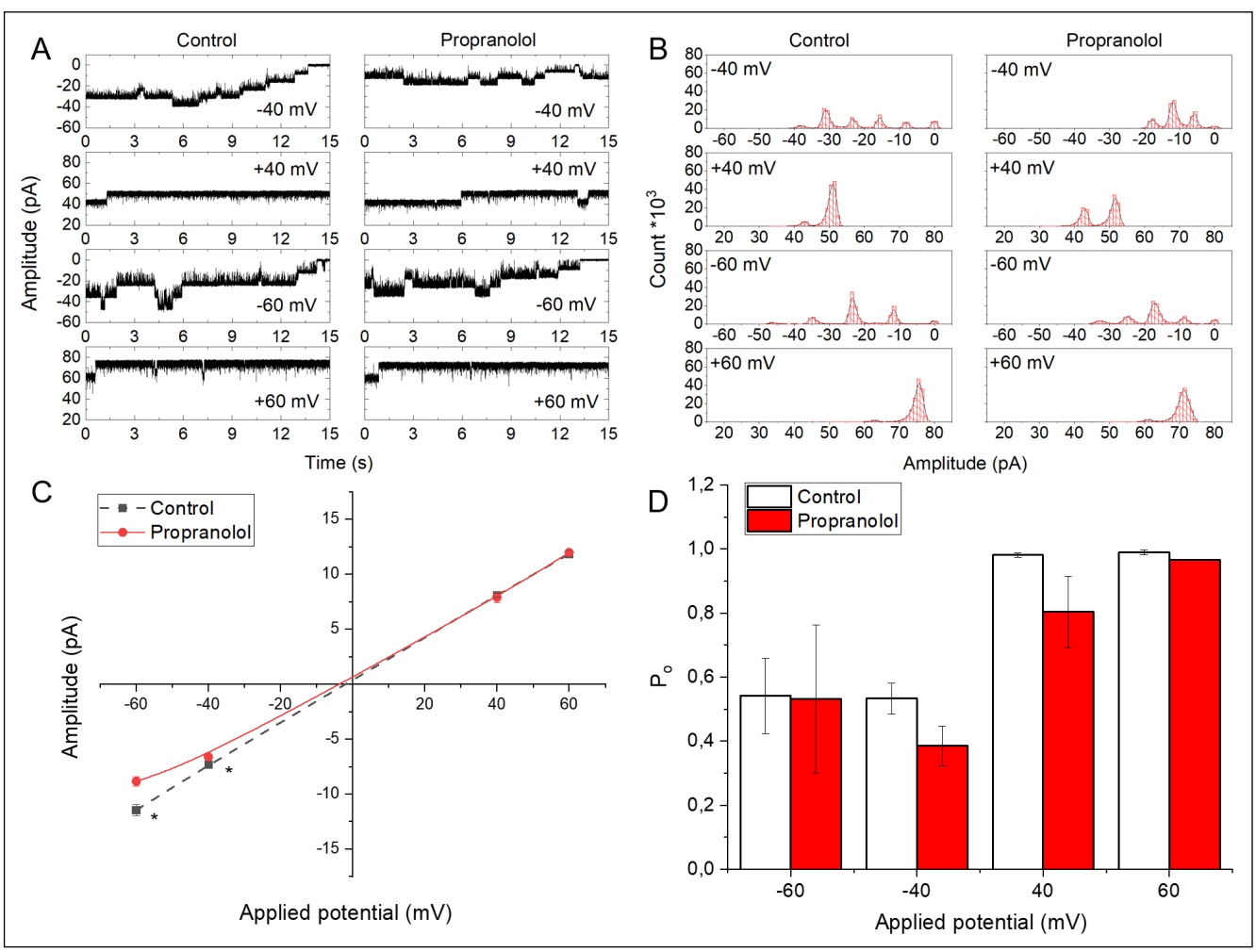

Fig. 3. Effect of 1 mmol/L propranolol on the electrophysiological properties of nuclear LCC-channels. "Control" refers to the solution containing (mmol/L): KCl 150, HEPES 8, HEPES-K 12, EGTA 1, pH 7.2. "Propranolol" refers to the same solution with the addition of 1 mmol/L propranolol. P_o denotes the open-state probability of the channels. (A) Representative recording fragments; (B) amplitude histograms derived from these recordings; (C) current-voltage (I-V) relationship for LCC-channels with and without propranolol; (D) mean open-state probability of LCC-channels as a function of membrane potential and bath solution composition. * P<0.05 *versus* control.

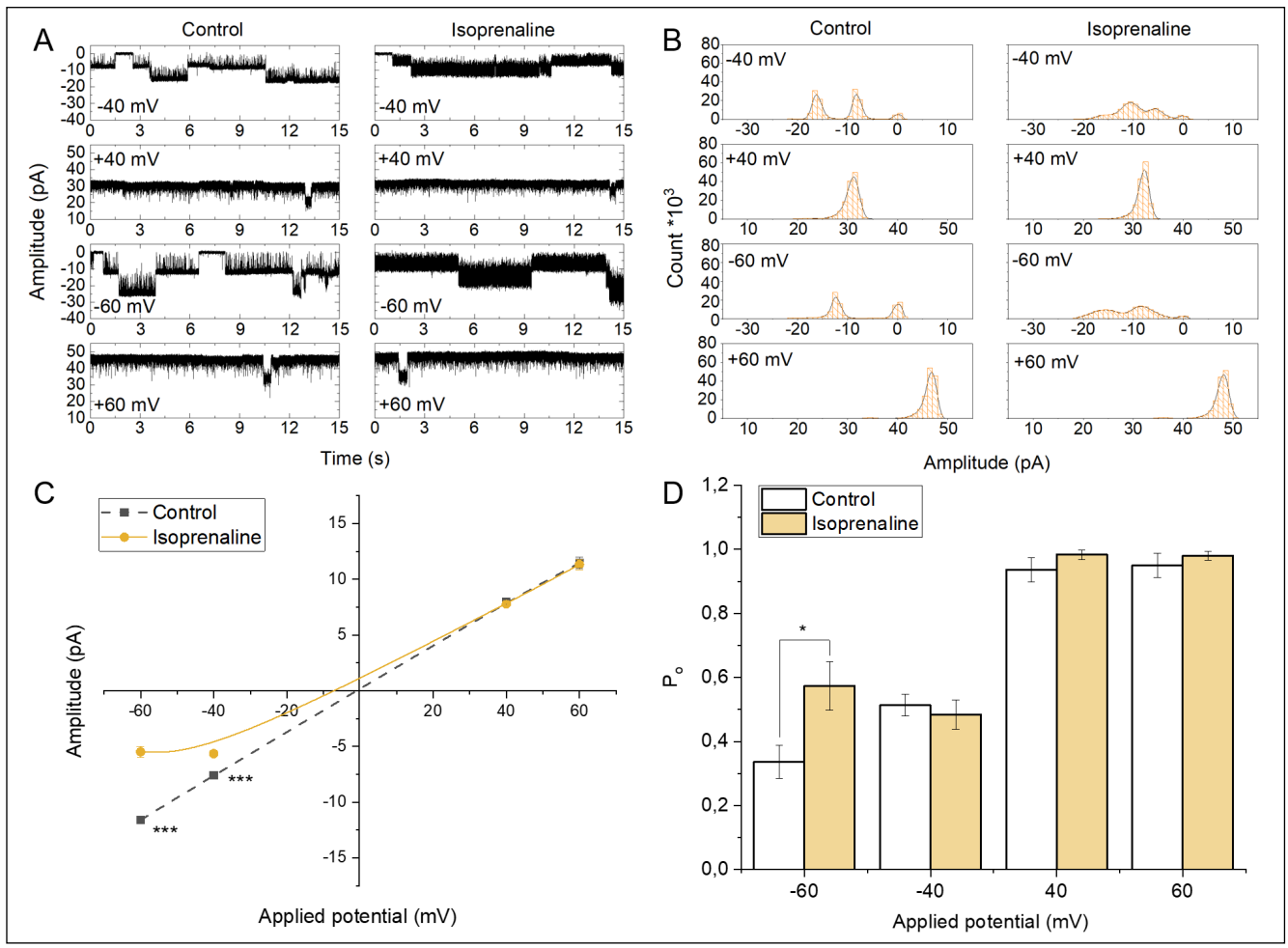

Fig. 4. Effects of isoprenaline at 1 mmol/L on LCC-channel function at various applied potentials. "Control" refers to the solution containing (mmol/L): KCl 150, HEPES 8, HEPES-K 12, EGTA 1, pH 7.2. "Isoprenaline" refers to the same solution with isoprenaline added (1 mmol/L). P_o denotes the probability that the channels are in the open state. (A) Representative recording fragments; (B) amplitude histograms derived from recordings in (A); (C) current-voltage (I-V) characteristics of LCC-channels under each solution; (D) mean open-state probability of LCC-channels at different applied potentials. * P<0.05, *** P<0.001 *versus* control.

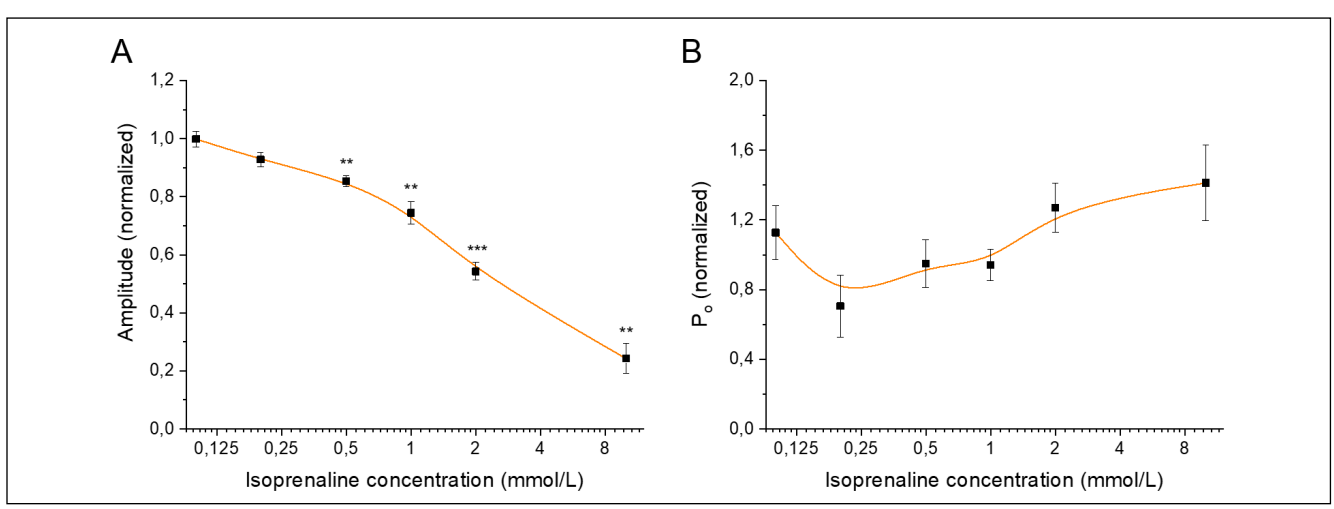

Fig. 5. Effects of isoprenaline on the electrophysiological properties of LCC-channels as a function of test substance concentration, with nuclear membrane potential maintained at -40 mV. P_o denotes the open-state probability of the channels. Isoprenaline was applied at concentrations of 0.1, 0.2, 0.5, 1, 2, and 10 mmol/L in a solution containing (mmol/L): KCl 150, HEPES 8, HEPES-K 12, EGTA 1, pH 7.2. (A) Normalized amplitude of currents through LCC-channels. (B) Normalized probability of LCC-channels being in the open state. ** P<0.01, *** P<0.001 *versus* control.

DISCUSSION

The findings of this study demonstrate that all examined adrenergic receptor modulators reduced the amplitude of currents through LCC-channels at negative membrane potentials. However, this effect was observed only at concentrations of 0.5–1 mmol/L, suggesting that the decrease may be nonspecific rather than a result of selective modulation. These initial results are valuable for screening the potential of adrenergic receptor modulators to alter the electrophysiological properties of LCC-channels. Further research at lower concentrations and determination of the minimum effective dose are necessary for substances that exhibited pronounced effects during initial testing. The effects of epinephrine and isoprenaline were concentration-dependent, with greater reductions observed at higher concentrations. Notably, for isoprenaline, a statistically significant reduction in amplitude was detected only at 0.5 mmol/L, whereas specific interactions with β-adrenergic receptors typically occur at nanomolar concentrations (Delpy et al., 1996). No significant changes in the P_o of LCC-channels were observed at -40 mV for any concentration of these substances. A summary of the changes in P_o and ion current amplitude under the influence of the studied compounds is provided in Table 1.

Although the reduction in current amplitude induced by epinephrine appeared greater than that of norepinephrine (13% *versus* 7% at -40 mV), the absence of confirmed changes at -60 mV for epinephrine does not substantiate this observation. Furthermore, unlike norepinephrine, epinephrine did not affect the P_o of LCC-channels at any tested potential, nor did propranolol. It is possible that a concentration of epinephrine above 1 mmol/L is required to significantly alter the P_o of these channels; however, using such concentrations raises concerns about off-target interactions. Notably, no confirmed effect of epinephrine on P_o values was observed even at 2 mmol/L. The dissociation between epinephrine's effect on current amplitude and its lack of impact on P_o suggests that these parameters are regulated by distinct mechanisms and may involve separate molecular pathways. Although epinephrine appears to possess structural features that allow it to inhibit current amplitude through LCC-channels in a concentration-dependent manner, the possibility of nonspecific interactions with the channel pore at the applied concentrations cannot be excluded.

None of the studied compounds affected the current amplitude at positive applied potentials. This finding distinguishes the effects of adrenergic receptor modulators investigated in this study from those of certain nicotinic acetylcholine receptor modulators, such as mecamylamine (Nadtoka et al., 2025a). In contrast, other nAChR modulators, specifically acetylcholine and carbachol, influenced the amplitude of currents through LCC-channels at positive membrane potentials only when applied *via* the patch pipette to the perinuclear side of the channels. Therefore, adrenergic receptor modulators may also exhibit similar effects if applied in an alternative configuration. However, when added to the bath with a sample, the effects of norepinephrine, epinephrine, and propranolol on the amplitude of LCC-channel mediated currents were comparable to those observed for acetylcholine, carbachol, and mecamylamine under the same conditions (Nadtoka et al., 2025a; 2025b).

Isoprenaline, however, exhibited a more pronounced effect than the three previously mentioned choline receptor modulators. In this study, isoprenaline at 1 mmol/L reduced the amplitude of LCC-channel currents by half, and at 10 mmol/L nearly eliminated the difference in current amplitude between open and closed channel states. Nevertheless, the effects observed at these concentrations are unlikely to reflect selective modulation, as some acetylcholine receptor modulators produced even greater effects at lower doses. For instance, nicotine reduced the amplitude of

Table 1. Summary of the effects of adrenergic receptor modulators at a concentration of 1 mmol/L on the amplitude of currents through LCC-channels (Amp) and their open-state probability (P_o) at various applied potentials. NoE indicates "no effect." * P<0.05, ** P<0.01, *** P<0.001 compared to control.

Substance applied	-60 mV		-40 mV		+40 mV		+60 mV	
	Amp	P_o	Amp	P_o	Amp	P_o	Amp	P_o
Norepinephrine	↓10% ***	↓36% *	↓7% *	↓54% **	NoE	NoE	NoE	NoE
Epinephrine	NoE	NoE	↓13% **	NoE	NoE	NoE	NoE	NoE
Propranolol	↓23% *	NoE	↓10% *	NoE	NoE	NoE	NoE	NoE
Isoprenaline	↓53% ***	↑73% *	↓26% ***	NoE	NoE	NoE	NoE	NoE

currents through these channels by half at 0.2 mmol/L (Kotliarova et al., 2019), and neurotoxin II caused a significant decrease in amplitude at 0.025 mmol/L (Kotyk et al., 2019). Additionally, unlike nicotine and neurotoxin II, isoprenaline's effects were accompanied by pronounced channel flickering, as shown in Fig. 4A. This phenomenon, previously reported for tubocurarine, tolperisone, α-cobratoxin, and ditiline, typically indicates mechanical blockage of the channel pore by the tested compound (Kotyk et al., 2019).

Rapid switching between open and closed channel states obscures the distinction between the peaks in Fig. 4B, which typically represent the most frequent amplitude values. This observation suggests that, at negative potentials, the channel rarely remains fully open to sustain a stable current amplitude. Notably, isoprenaline was the only substance observed to increase the P_o of LCC-channels, as demonstrated at a potential of -60 mV. This effect may result from the pronounced flickering described previously. When the LCC-channel pore is blocked by isoprenaline, it may be unable to fully close or open, which could be recorded as an increased P_o. However, this phenomenon was not observed at other membrane potentials or at any other isoprenaline concentrations, challenging this interpretation and indicating that the results at -60 mV may be incidental. Therefore, the effects of isoprenaline on the P_o of LCC-channels remain inconclusive.

Although preliminary observations were conducted with propranolol, norepinephrine was the only adrenoreceptor modulator shown to decrease the P_o of LCC-channels. The observed difference in P_o-modulating capabilities suggests that norepinephrine possesses unique molecular features responsible for this effect. Further investigation of these features could elucidate the fundamental mechanisms underlying the modulation of P_o in LCC-channels.

The findings of this study suggest that LCC-channels are unlikely to share structural patterns or regulatory sites with adrenergic receptors, as the effects of adrenergic receptor modulators on the amplitude of currents through LCC-channels were not observed at concentrations below 0.5 mmol/L and may be nonspecific. Further research is necessary to determine whether norepinephrine influences the P_o of these channels at lower concentrations and to identify the specific molecular mechanisms underlying this effect.

CONCLUSIONS

The results of this study indicate that none of the examined adrenergic receptor modulators (norepinephrine, epinephrine, propranolol, and isoprenaline) affected the amplitude of currents through LCC-channels at positive membrane potentials. However, all compounds produced a significant decrease in amplitude values at negative potentials. Non-target and mechanical interactions between the compounds and the channels should be considered as potential factors contributing to the observed reduction in current amplitude.

The effects of norepinephrine, epinephrine, and propranolol demonstrate that their ability to block the amplitude of currents through LCC-channels and reduce the P_o of these channels is comparable to that of the nAChR modulators previously discussed. Among the adrenergic receptor modulators examined, only norepinephrine decreased the probability of LCC-channels remaining in an open state. In contrast, isoprenaline significantly increased the P_o only at a membrane potential of -60 mV, while concurrently reducing the amplitude of currents through the LCC-channels. The action of isoprenaline on LCC-channels was also associated with pronounced channel flickering, which may account for the observed increase in P_o. Under the described experimental conditions, with all substances applied at a concentration of 1 mmol/L, the potency of amplitude reduction can be ranked as follows: isoprenaline > propranolol > norepinephrine ≈ epinephrine.

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