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ORIGINAL ARTICLE

Mechanism of P2X7 receptor-dependent enhancement of neuromuscular transmission in pannexin 1 knockout mice

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Abstract

P2X7 receptors are present in presynaptic membranes of motor synapses, but their regulatory role in modulation of neurotransmitter release remains poorly understood. P2X7 receptors may interact with pannexin 1 channels to form a purinergic signaling unit. The potential mechanism of P2X7 receptor-dependent modulation of acetylcholine (ACh) release was investigated by recording miniature endplate potentials (MEPPs) and evoked endplate potentials (EPPs) in neuromuscular junctions of wild-type (WT) and pannexin 1 knockout ($Panx1^{-/-}$) mice. Modulation of P2X7 receptors with the selective inhibitor A740003 or the selective agonist BzATP did not alter the parameters of either spontaneous or evoked ACh release in WT mice. In $Panx1^{-/-}$ mice, BzATP-induced activation of P2X7 receptors resulted in a uniformly increased quantal content of EPPs during a short stimulation train. This effect was accompanied by an increase in the size of the readily releasable pool, while the release probability did not change. Inhibition of calmodulin by W-7 or of calcium/calmodulin-dependent kinase II (CaMKII) by KN-93 completely prevented the potentiating effect of BzATP on the EPP quantal content. The blockade of L-type calcium channels also prevented BzATP action on evoked synaptic activity. Thus, the activation of presynaptic P2X7 receptors in mice lacking pannexin 1 resulted in enhanced evoked ACh release. Such enhanced release was provoked by triggering the calmodulin- and CaMKII-dependent signaling pathway, followed by activation of presynaptic L-type calcium channels. We suggest that in WT mice, this pathway is downregulated due to pannexin 1-dependent tonic activation of inhibitory presynaptic purinergic receptors, which overcomes P2X7-mediated effects.

Keywords P2X7 receptors · Neuromuscular junction · Pannexin 1 · CaMKII · L-type calcium channels

Introduction

P2X7 receptors belong to the family of ATP-gated ionotropic P2X receptors. Unlike other P2 subtypes, these three-subunit ion channels are activated by high concentrations of extracellular ATP and highly permeable for calcium cations [1]. P2X7 receptors are widely present in both neurons and glial cells in the CNS [2–6], and their role in neurodegenerative processes is well described [1, 4, 7]. Moreover, P2X7 receptors were found in presynaptic terminals in different areas of the CNS where they supposed to modulate neurotransmitter release [3, 8]. Typically, this modulation potentiates synaptic transmission and is associated with an increase in the intraterminal calcium concentration [6, 9]. In addition to providing direct calcium influx into the presynaptic compartment, P2X7 receptors may initiate the activation of calcium-dependent intracellular signaling pathways due to the presence of cytoplasmic calmodulin-binding sites [4, 10].

At neuromuscular junctions (NMJs), the expression of P2X7 receptors at the presynaptic membrane has been demonstrated [11]. However, data concerning the role of these receptors in the regulation of acetylcholine release in motor synapses remain scarce, fragmented, and rather controversial. According to experiments with FM1-43 dye unloading by spontaneous exocytosis of cholinergic synaptic vesicles, tonic activation of P2X7 receptors by their agonist BzATP

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facilitates the rate of spontaneous ACh release [11]. However, in electrophysiological experiments performed in frog NMJs, BzATP induced no changes in the evoked secretion of ACh [12]. We assumed that the adequate assessment of P2X7 receptor activity in motor synapses would require taking into account their functional cross-talk with other types of purinoreceptors, which are localized at motor nerve terminals. At mammalian NMJs, endogenous ATP and various products of ATP hydrolysis (including adenosine) predominantly depress ACh release, acting via A_1 adenosine receptors and P2Y13 receptors during synaptic activity [13, 14]. We suggest that the pattern of P2X7 receptor activity and the functional consequences of their activation in NMJs might be more easily elucidated when the simultaneous activation of other purinoreceptors by endogenous purines is abolished.

Recently, we demonstrated that the suppression of evoked ACh release, mediated by presynaptic A_1 and P2Y13 receptors, depends strictly on the presence of the membrane channel protein pannexin 1 (Panx1), which acts as a synaptic ATP/adenosine supplier. At NMJs of $Panx1^{-/-}$ mice, which lack endogenous ATP/adenosine delivery to the synaptic cleft via Panx1 channels, the A_1- and P2Y13 receptor-mediated inhibition of evoked ACh release was completely abolished [15]. Possible alterations in neuromuscular synaptic transmission due to activation of P2X7 receptors under these conditions remain to be elucidated. In the present work, we sought to clarify the roles of P2X7 receptors in different patterns of purinergic signaling and functional states of mouse motor synapses. Our data showed that, in contrast to WT, stimulation of P2X7 receptors at NMJs of $Panx1^{-/-}$ mice resulted in enhancement of evoked ACh release via a presynaptic signaling mechanism that included activation of calmodulin, calcium/calmodulin-dependent kinase II, and L-type of voltage-dependent calcium channels (VDCCs).

Materials and methods

Animals

All experiments were performed on isolated hemidiaphragm neuromuscular preparations (*m. diaphragma–n. phrenicus*) of age-matched adult (7–8 weeks old, weighing 25–30 g) wild-type mice (strain C57BL/6, WT) or pannexin-1 knockout mice (Panx1−/−) of either sex. The Panx1−/− mouse strain was developed by V.I. Shestopalov and thoroughly analyzed as described previously [16]. All animal handling and experimental procedures were carried out in full accordance with the EC guidelines (Directive 86/609/EEC on the treatment of laboratory animals). Mice were housed under a 12-h light/dark cycle with free access to water and food. The experimental protocol was approved by the Bioethics committee of the MSU Biological department. Mice were sacrificed by quick decapitation.

Electrophysiology

The left hemidiaphragm with attached phrenic nerve was excised and stretched in a 3-mL chamber perfused by oxygenated (95% O_2, 5% CO_2) Liley solution (pH 7.2–7.4) containing the following (in mM): NaCl, 135; KCl, 4; NaH_2PO_4, 0.9; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 16.3; glucose, 11. Intact preparations were used when only spontaneous synaptic activity was studied. To record synaptic activity evoked by nerve stimulation, cut neuromuscular preparations were used to prevent contraction as well as to record both spontaneous and evoked endplate potentials from the same synapse [17]. Immediately after the transverse cutting of muscle fibers (before the recording of the synaptic activity), the preparation was thoroughly washed in a large volume (more than 150 mL) of Liley solution for more than 1 h to prevent the blockage of the action potential conduction. As a result, the recorded value of the resting membrane potential (RMP) was lower in cut fibers (≤ 50 mV) than in intact ones. Spontaneous miniature endplate potentials (MEPPs) and evoked endplate potentials (EPPs) were recorded using glass intracellular microelectrodes filled with 2.5 M KCl (tip resistance was 10–20 MΩ) and connected to a Neuroprobe Amplifier, model 1600 (A-M Systems, USA). The signals were digitized using an analog-digital converter E-154 (L-Card, Russia) with a PowerGraph 6.0 interface. To study evoked synaptic activity, the phrenic nerve was stimulated by short (1 s) high-frequency (50 Hz) trains of suprathreshold pulses (the duration of each pulse was 0.08–0.1 ms). To avoid fatigue of synapses and consequent amplitude decline and alteration of the EPP pattern not related to the applied drugs, pauses of at least 4 min were maintained between nerve stimulations. In each synapse before the nerve stimulation, MEPPs were recorded for 100 s (mean value of the MEPP amplitudes recorded within this period was used for calculation of the quantal content of the EPP). All experiments were carried out at room temperature (20–22 °C). Primary analysis was performed using MiniAnalysis software (Synaptosoft, USA). In control conditions, MEPPs and EPPs for at least five different synapses were recorded; after that, tested substances were added to the perfusion solution in the indicated order, and the activity of various synapses was recorded within 1 h of drug application. In each experimental series, no fewer than three neuromuscular preparations were used.

Drugs

The following drugs were used: *N*-[1-[[(cyanoamino)(5-quinolinylamino)methylene]amino]-2,2-dimethylpropyl]-3,4-dimethoxybenzeneacetamide (A740003); 2′(3′)-O-(4-benzoylbenzoyl)adenosine 5′-triphosphate triethylammonium salt

(BzATP); *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W-7); *N*-[2-[[[3-(4-Chlorophenyl)-2-propenyl]methylamino]methyl]phenyl]-*N*-(2-hydroxyethyl)-4-methoxybenzenesulfonamide (KN-93); 2-[*N*-(4′-methoxybenzenesulfonyl)] amino-*N*-(4′-chlorophenyl)-2-propenyl-*N*-methylbenzylamine phosphate (KN-92); 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine dicarboxylic acid ethyl methyl ester (nitrendipine). All drugs were purchased from Tocris Bioscience (Ellisville, MO, USA). BzATP and nitrendipine were dissolved in deionized water and ethanol, respectively. Stock solutions of all other drugs were prepared in DMSO (Helicon, Russia). The final concentrations of ethanol and DMSO in the working solution did not exceed 0.01% (*v*/*v*), and at this concentration, neither solvent affected the parameters of spontaneous and evoked activity in mouse motor synapses.

Data processing and statistical analysis

The values of muscle fiber resting RMP, the amplitude and time course of MEPPs and EPPs, and the MEPP frequency were estimated. The amplitudes of MEPPs and EPPs in cut preparations were first normalized to the membrane potential of − 50 mV to correct for the changes in the driving force caused by the voltage shift upon the changes in the RMP [18]. In the intact neuromuscular preparations, on which only spontaneous synaptic activity was studied, MEPP amplitudes were normalized to − 70 mV. The quantal content of EPPs was calculated as the ratio between the mean normalized EPP amplitude corrected for nonlinear summation [19] and the mean normalized amplitude of MEPPs. Estimation of the readily releasable pool (RRP) size was performed using a sequential model, which assumes that the initial fall in quantal content reflects the continuous depletion of RRP and that the plateau at the end of the EPP train originates from the coupling between the RRP depletion and the recruitment of vesicles [20, 21]. At the initial phase of the depression (the decline in the quantal content of EPPs in the trains), 6–10 EPPs were used to plot the intersection with the *x*-axis (linear regression). The first and several subsequent EPPs at beginning of the trains, where facilitation was present, were not taken into account for the calculation of RRP size [21, 22]. The probability of release was calculated by dividing the quantal content of the first EPP in the train by the RRP size [22–24].

Statistical analysis was performed using GraphPad Prism 6.0 software. All data in the text and figures except the original recordings are presented as the means ± standard error of the means; *n* corresponds to the number of synapses in the group. The normality of the parameter distribution was verified by the D'Agostino–Pearson normality test. If two groups were compared, the significance between them was estimated by unpaired Student's *t* test when the distributions were normal (amplitude and frequency of MEPPs, RRP, and release probability) and the Mann–Whitney rank sum test when the distribution was not normal (rise time and half decay of MEPPs). The significance between three groups was estimated by using one-way ANOVA with the post hoc Holm-Sidak's multiple comparisons test when the distributions were normal (amplitude and frequency of MEPPs) and Kruskal-Wallis test with post hoc Dunn's multiple comparison tests if any of the three distributions was not normal. For the analysis of EPP amplitude and quantal content, two-way ANOVA with the post hoc Bonferroni correction was used.

Values of $p < 0.05$ were considered statistically significant.

Results

To study the possible involvement of P2X7 receptor activity in the modulation of ACh release in WT mice, we applied 1 μM A740003 (P2X7 receptor antagonist) to determine whether any basal activity of P2X7 receptors is present during the short, rhythmical EPP trains (1 s, 50 Hz) (Fig. 1a). Such high-frequency stimulation resembles the motor neuron discharge pattern in vivo and is characterized by typical changes in EPP amplitude and quantal content during the train [25–27]. Within 1 h following the application of A740003, no changes in RMP, amplitude, and time course of MEPPs or their frequency were detected (Fig. 1b, c). Moreover, the blockage of P2X7 receptors did not affect the quantal content of EPPs in short trains. The mean quantal content of the first EPP in the train was 33.49 ± 2.60 in the control ($n = 15$), and in the presence of A740003, it was 35.65 ± 3.00 ($n = 16$, $p > 0.05$) (Fig. 1d).

In an attempt to selectively activate P2X7 receptors, we applied the agonist BzATP (30 μM). No changes were observed in RMP, MEPP frequency, their time parameters, and amplitude (Fig. 1f, g). Unexpectedly, BzATP did not cause any significant changes in evoked ACh release in NMJs from WT mice. The quantal content of the first EPP in the train was 26.92 ± 1.68 in the control ($n = 18$) and 26.62 ± 2.18 under BzATP ($n = 18$, $p > 0.05$) (Fig. 1h).

Next, we used intact neuromuscular preparations for a more detailed electrophysiological examination of the possible effect of BzATP on spontaneous ACh secretion in WT NMJs. In contrast to findings obtained by using vital fluorescent lipophilic dye unloading from the nerve terminal [2, 11], we did not find any significant changes in the parameters of spontaneous release. Within 1 h after the application of BzATP, the mean MEPP frequency (0.43 ± 0.03 Hz ($n = 20$)) remains unchanged relative to the control (0.45 ± 0.04 Hz ($n = 16$)). The amplitude and time parameters of MEPPs did not change. The mean amplitude of MEPPs was 1.65 ± 0.11 mV in control and 1.73 ± 0.09 mV under BzATP treatment ($p > 0.05$). MEPP rise time was 0.93 ± 0.06 ms in control and remained at the same level (0.93 ± 0.06 ms) during BzATP application. In control conditions, MEPP half-decay was 2.15 ± 0.11 ms and did not change significantly in the presence of BzATP—2.15 ± 0.10 ms ($p > 0.05$). Thus, neither

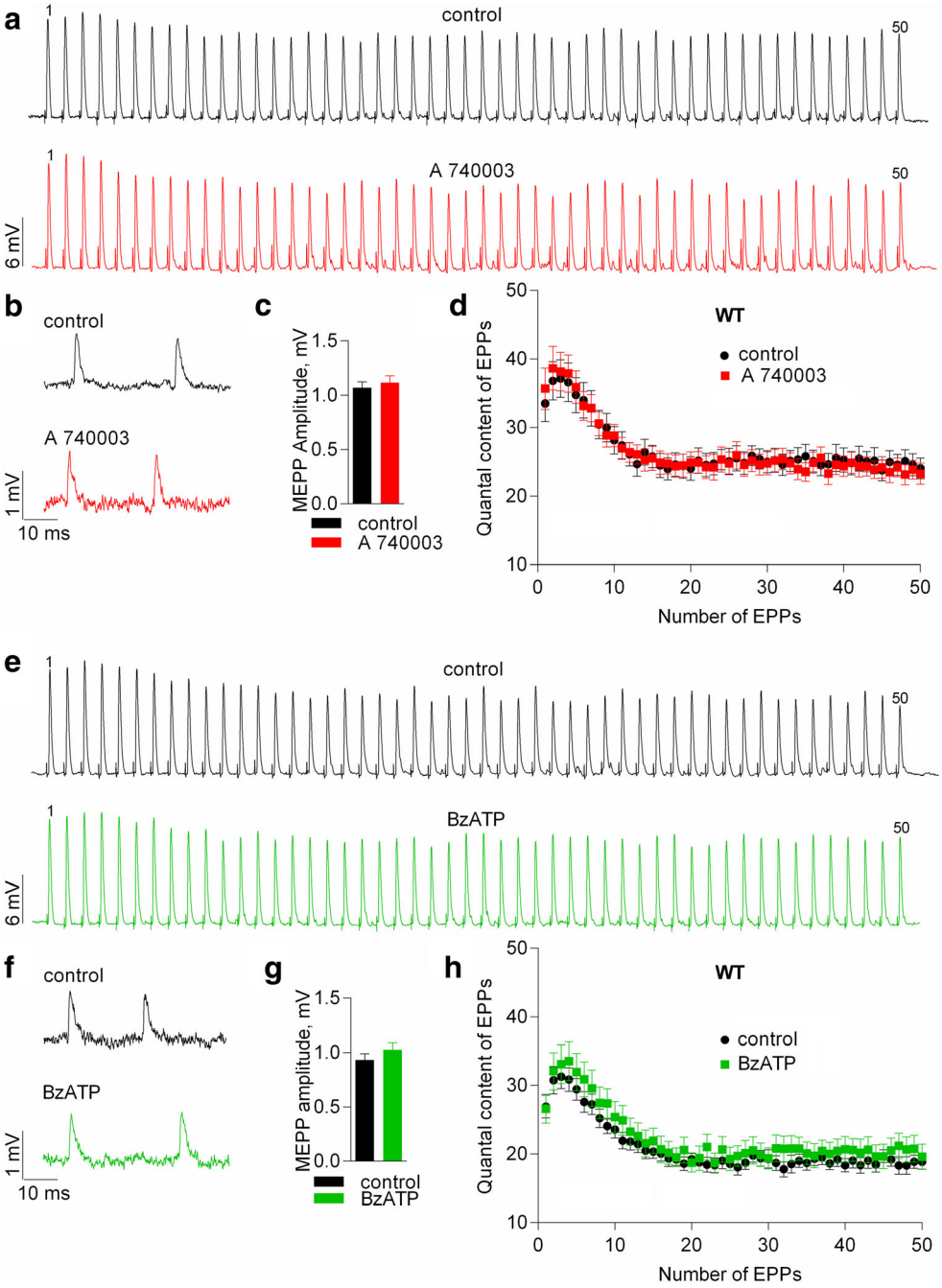

Fig. 1 WT mice do not show any signs of P2X7 receptor-mediated modulation of both spontaneous and evoked neuromuscular transmission during a short (1 s) high-frequency (50 Hz) train. **a** Representative recordings of EPPs in control (above) and upon application of P2X7 receptor antagonist A740003 (1 μM) (below). **b** Representative recordings of MEPPs in control (above) and upon application of A740003 (below). **c** Mean amplitude of MEPPs in control ($n = 15$) and in the presence of A740003 ($n = 16$). **d** Changes in the EPP quantal content in control and upon application of A740003. **e** Representative recordings of EPPs in control (above) and upon application of P2X7 receptor agonist BzATP (30 μM) (below). **f** Representative recordings of MEPPs in control (above) and upon application of BzATP (below). **g** Mean amplitude of MEPPs in control ($n = 18$) and in the presence of BzATP ($n = 18$). **h** Changes in the EPP quantal content in control ($n = 18$) and upon application of BzATP ($n = 18$). Symbols, histograms, and error bars represent the mean ± SEM

inhibition nor activation of P2X7 receptors affected either spontaneous or rhythmically evoked synaptic transmission in NMJs of WT mice. We hypothesized that P2X7-mediated modulation of ACh release during a short train might require some special conditions. In an attempt to define these conditions, we studied evoked synaptic transmission at NMJs from Panx1$^{-/-}$ mice. We reasoned that the lack of Panx1 may induce changes in the concentrations of ATP and its derivatives in the synaptic cleft [28], which in turn may help with evaluating P2X7-induced effects on synaptic transmission. In support of this hypothesis, we have recently found that knockout of Panx1 does not induce any changes in quantal ACh release by itself, but it alters its purinergic modulation in NMJs [15].

Similar to that in WT mice, P2X7 receptor blockade with A740003 did not change both spontaneous and evoked release in NMJs of Panx1$^{-/-}$ animals (Fig. 2a–c). The mean values of the first EPP quantal content in the control and in the presence of A740003 were 31.14 ± 2.03 ($n = 19$) and 30.80 ± 1.47 ($n = 22$, $p > 0.05$), respectively (Fig. 2d). Application of BzATP to the neuromuscular preparations of Panx1$^{-/-}$ mice did not induce any significant changes in MEPP parameters. However, in contrast to the lack of any modulatory effects on synaptic

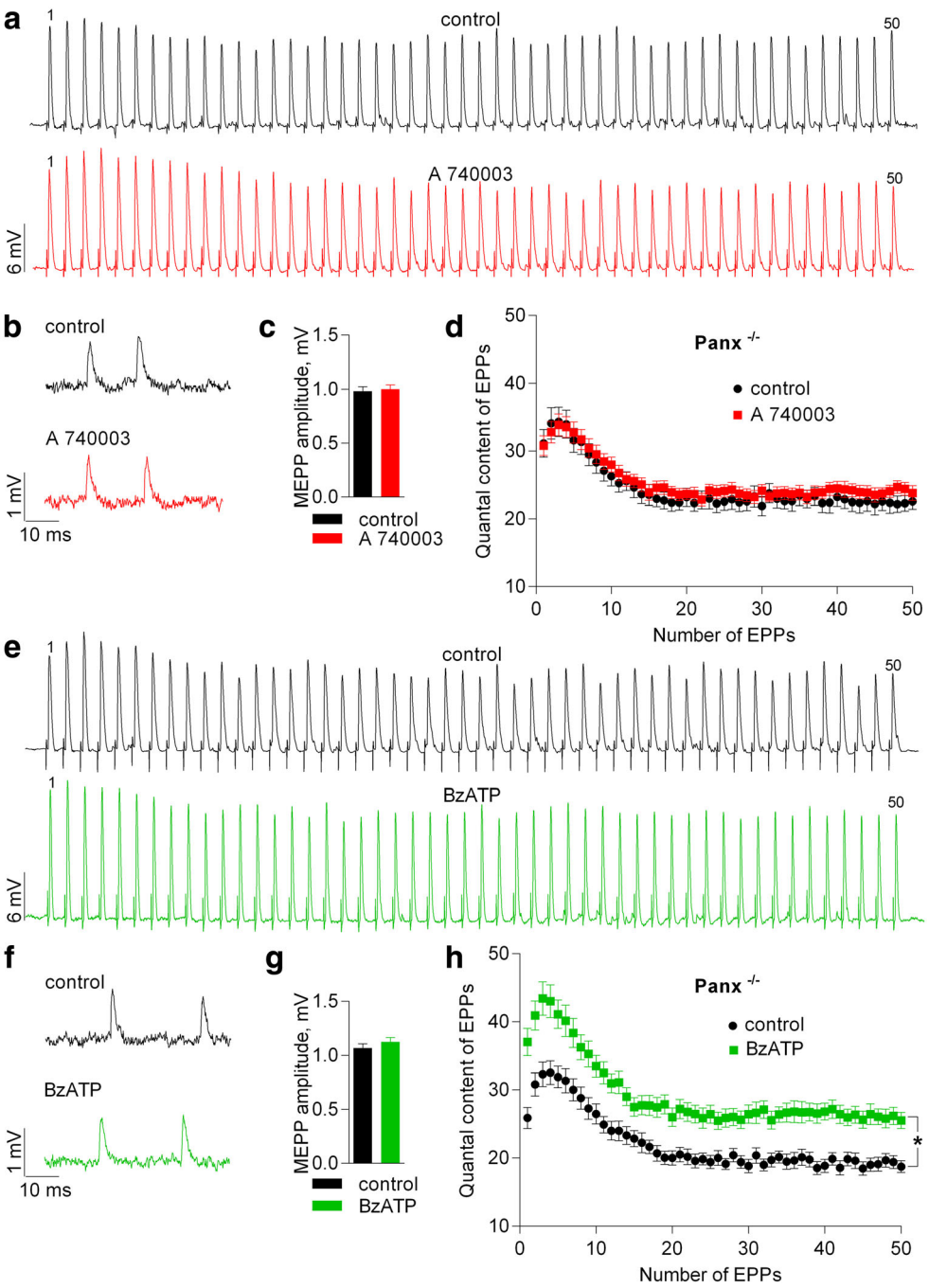

Fig. 2 P2X7 receptor-mediated upregulation of evoked neuromuscular transmission during short (1 s) high-frequency (50 Hz) trains in Panx1$^{-/-}$ mice. **a** Representative recordings of EPPs in control (above) and upon application of P2X7 receptor antagonist A740003 (1 μM) (below). **b** Representative recordings of MEPPs in control (above) and upon application of A740003 (below). **c** Mean amplitude of MEPPs in control ($n = 19$) and in the presence of A740003 ($n = 22$). **d** Changes in the EPP quantal content in control and upon application of A740003. **e** Representative recordings of EPPs in control (above) and upon application of P2X7 receptor agonist BzATP (30 μM) (below). **f** Representative recordings of MEPPs in control (above) and upon application of BzATP (below). **g** Mean amplitude of MEPPs in control ($n = 24$) and in the presence of BzATP ($n = 26$). **h** Changes in the EPP quantal content in control and upon application of BzATP. Symbols, histograms, and error bars represent the mean ± SEM. *$p < 0.05$ compared to control

transmission when BzATP was applied to WT NMJs, the activation of P2X7 receptors by BzATP caused a uniform increase in quantal content throughout the EPP train in Panx1$^{-/-}$ mice (for the first EPP in the train, from 25.88 ± 1.55 ($n = 24$) in control to 37.02 ± 2.01 ($n = 26$, $p < 0.05$) under BzATP) (Fig. 2e–h).

Next, we studied whether the potentiating effect of BzATP in NMJs of Panx1$^{-/-}$mice is mediated by its action on P2X7 receptors. In the presence of A740003 (1 μM), BzATP was unable to induce the increase in EPP amplitude and quantal content during the short train of EPPs (Fig. 3a). These data suggest that the applied concentration of A740003 is active and can effectively block P2X7 receptors thus preventing their activation by BzATP and abolish BzATP-mediated increase on EPP quantal content.

To study the mechanisms that determine the BzATP-induced increase in EPP quantal content in the train of EPPs in NMJs of mice lacking pannexin 1, we estimated the changes in the size of the readily releasable pool (RRP) of vesicles participating in evoked ACh release and the probability of release. We found that the stimulation of P2X7 receptors in Panx1$^{-/-}$ NMJs induced a 32% increase in RRP size—from 866.1 ± 60.8 in the control to 1198.0 ± 85.5 under BzATP (Fig. 3b, c). The

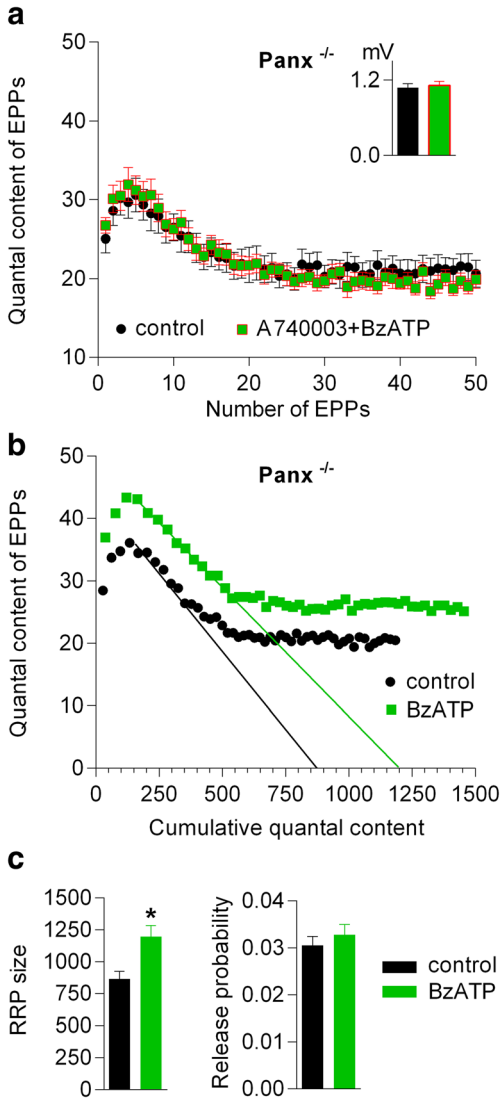

Fig. 3 **a** Changes in the EPP quantal content in control ($n = 20$) and upon application of BzATP (30 μM) in the presence of A740003 (1 μM) ($n = 25$). Inset shows MEPP amplitudes. **b** Quantal content of EPPs plotted against cumulative quantal content in the control and under application of BzATP. The x-intercept extrapolated from the initial phase of depression reflects the RRP size. **c** Activation of P2X7 receptors by BzATP increased the size of the RRP (left) without changing the probability of release (right). *n* for each group are the same as in Fig. 2g. Symbols, histograms, and error bars represent the mean ± SEM. *$p < 0.05$ compared to control

probability of release did not change significantly in the presence of BzATP (Fig. 3c).

In an additional series of experiments performed on intact neuromuscular preparations, we studied more thoroughly if activation of P2X7 receptors by BzATP may induce any changes in the parameters of spontaneous ACh release at NMJs of Panx1$^{-/-}$ mice. As in the case of WT mice, application of BzATP did not alter any parameters of MEPPs. Under control conditions, the mean MEPP frequency was 0.53 ± 0.05 Hz ($n = 22$) and did not change significantly in the presence of BzATP—0.53 ± 0.05 Hz ($n = 26$, $p > 0.05$). Likewise, the mean amplitude of MEPPs has almost the same value in control—1.52 ± 0.08 mV—and under BzATP treatment—1.51 ± 0.06 mV ($p > 0.05$). No changes were observed in the rise time of MEPPs (0.75 ± 0.04 ms in control and 0.74 ± 0.04 ms in the presence of BzATP ($p > 0.05$)), or in their half decay (1.76 ± 0.1 ms in control and 1.75 ± 0.06 ms in the presence of BzATP ($p > 0.05$)). Thus, our experiments in Panx1$^{-/-}$ mice have demonstrated for the first time a significant potentiation in the evoked but not in spontaneous ACh release upon activation of P2X7 receptors by an exogenous agonist.

In order to clarify the signaling mechanisms underlying the BzATP-induced increase in quantal content of EPPs and the increase in RRP size in NMJs of Panx1$^{-/-}$ mice, we further investigated probable intraterminal pathways that would lead to P2X7-mediated upregulation of synaptic transmission.

It is well known, that upon activation, the Ca^{2+}-permeable channels of P2X7 receptors might provide calcium influx into the nerve terminal. Such influx can trigger signaling pathways, which, as shown in some CNS synapses, include activation of calmodulin (CaM) and calcium/calmodulin-activated kinase II (CaMKII) [8].

To test for the activation of CaM and its participation in the signaling triggered by BzATP-induced activation of P2X7 receptors in Panx1$^{-/-}$ mice, we used the CaM blocker W-7. When applied by itself, W-7 (10 μM) caused no significant effect on spontaneous or evoked ACh release: the mean first EPP quantal content was 35.35 ± 2.44 ($n = 15$) in the control and 36.05 ± 1.97 ($n = 18$, $p > 0.05$) in the presence of W-7. However, the blockade of CaM completely prevented the enhancing effect of BzATP on EPP quantal content: for the first EPP in the train, the mean value of this parameter was 32.92 ± 2.27 ($n = 19$, $p > 0.05$) (Fig. 4a). Next, we investigated whether CaMKII, one of the main targets of CaM, can mediate the P2X7-induced increase in evoked ACh release at NMJs of Panx1$^{-/-}$ mice. When applied alone, the CaMKII inhibitor KN-93 (3 μM) did not cause any significant changes in the parameters of spontaneous and evoked ACh release. However, in the presence of KN-93, BzATP lost its ability to increase the EPP quantal content. For the first EPP in the short train, the mean quantal content was 34.63 ± 2.42 in the control ($n = 22$), 32.73 ± 2.41 during the application of KN-93 alone ($n = 16$), and 34.15 ± 2.16 following the application of BzATP in the presence of KN-93 ($n = 17$, $p > 0.05$) (Fig. 4b). The specificity of KN-93 was tested using its inactive analog KN-92 (3 μM) as a negative control. The application of KN-92 was unable to prevent the increase in the EPP quantal content under the influence of BzATP (Fig. 4c). The mean quantal content in the first EPP in the train was 28.97 ± 2.71 in the control ($n = 22$) and 28.35 ± 2.01 in the presence of KN-92 ($n = 17$), and it increased to 40.76 ± 3.93 ($n = 15$, $p < 0.05$) following the application of BzATP in the presence of KN-92. These data suggest that the stimulation of presynaptic P2X7 receptors may trigger a signaling pathway involving sequential activation of CaM and CaMKII. We next

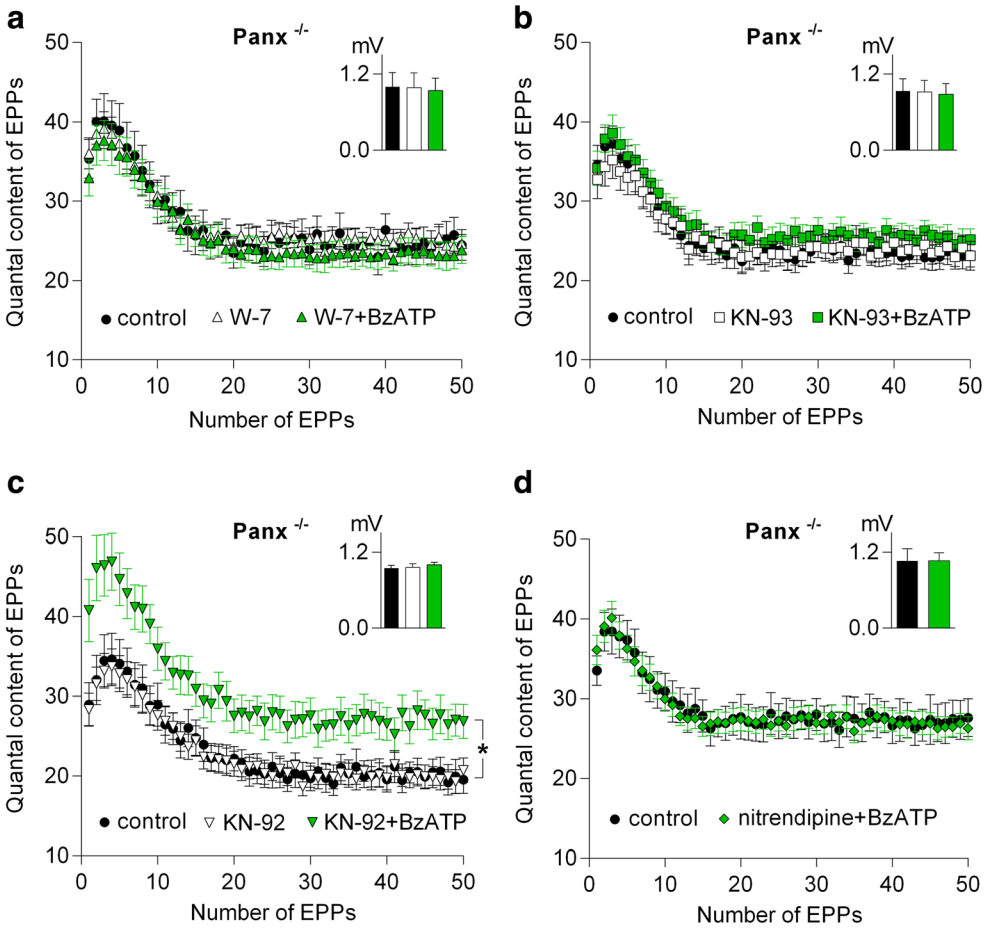

Fig. 4 Intracellular signaling mechanisms underlying P2X7 receptor-mediated upregulation of evoked neuromuscular transmission in Panx1$^{-/-}$ mice. **a** Changes in the EPP quantal content in the control ($n = 15$), after the CaM blocker W-7 (10 μM) was applied ($n = 18$) and after BzATP (30 μM) was added in the presence of preapplied W-7 ($n = 19$). **b** Changes in the EPP quantal content in the control ($n = 22$), upon application of the CaMKII blocker KN-93 (3 μM) ($n = 16$) and during application of BzATP (30 μM) in the presence of KN-93 ($n = 17$). **c** Changes in the EPP quantal content in the control ($n = 22$), during application of an inactive analog of KN-93 (KN-92, 3 μM) ($n = 17$) and upon application of BzATP (30 μM) in the presence of KN-92 ($n = 15$). **d** Changes in the EPP quantal content in the control ($n = 15$) and with application of BzATP (30 μM) in the presence of a blocker of L-type VDCCs (nitrendipine, 1 μM) ($n = 22$). Symbols and error bars represent the mean ± SEM. The insets show MEPP amplitude. *$p < 0.05$ compared to control

tried to evaluate possible targets of CaMKII activity, which may provide a uniform increase in quantal content of each EPP throughout the short, high-frequency train in the motor nerve terminals of Panx1$^{-/-}$ mice.

It was previously suggested that presynaptic L-type VDCCs may be possible targets of CaMKII action followed by disinhibition of these channels with subsequent increase in evoked ACh release at mammalian NMJs [29]. To detect whether presynaptic L-type VDCCs are upregulated as a result of activation of P2X7 receptors followed by CaMKII activation, we tested the effects of BzATP when L-type VDCCs were blocked. First, we examined the effect of the L-type VDCC blocker nitrendipine (1 μM) on neuromuscular transmission in NMJs of Panx1$^{-/-}$ mice. Nitrendipine alone did not alter both spontaneous and evoked ACh release during short trains. Under control conditions, the mean quantal content of the first EPP in the train was 25.30 ± 1.43 ($n = 19$) and remained at the same level—24.24 ± 1.22 ($n = 21$, $p > 0.05$)—within 1 h of nitrendipine application. In the next series of experiments, the activation of P2X7 receptors by BzATP in the presence of nitrendipine failed to increase EPP quantal content (Fig. 4d). These results suggest that L-type VDCCs became unmasked (disinhibited) in NMJs of Panx1$^{-/-}$ mice by stimulation of presynaptic P2X7 receptors and these L-type VDCCs became involved in the potentiation of evoked ACh release.

Discussion

Our studies have shown that the application of either an agonist or an antagonist of presynaptic P2X7 receptors does not change either spontaneous or evoked ACh release in motor synapses of WT mice. Consistent with these results, studies in frog NMJs have also shown that activation of P2X7 receptors

by BzATP did not produce any change in the amplitude of multiquantal endplate currents [12]. On the other hand, it has been reported that BzATP induced an increase in vital dye destaining from mouse motor nerve terminals as the result of P2X7-mediated potentiation of spontaneous release of cholinergic vesicles [2, 11]. The discrepancy between electrophysiology results and those obtained using fluorescent dye might be due to the differences in recording methods and experimental conditions when spontaneous ACh secretion was studied.

There are several alternative explanations for the lack of changes in the parameters of rhythmically evoked postsynaptic potentials during activation or inhibition of P2X7 receptors in the WT NMJs. First, P2X7 receptors have the lowest affinity for ATP among P2 receptors and, unlike other P2 receptors, are not capable of being activated by ATP derivatives (ADP and AMP) [30]. Under the conditions of rapid hydrolysis of endogenous ATP in the synaptic cleft [31], this low affinity prevents the effective activation of P2X7 receptors. Second, signaling cascades in motor nerve terminals are triggered by various presynaptic receptors during evoked synaptic activity. For this latter reason, the effects of activation of P2X7 receptors by endogenous ATP can be masked by concurrent inhibition of ACh release mediated by metabotropic A_1 and P2Y13 receptors [14, 15, 26, 29] or by muscarinic receptors [32]. To test this hypothesis, we used Panx1$^{-/-}$ NMJs in further experiments, since our recent results have shown that in the absence of Panx1, the activation of presynaptic A_1 and P2Y13 receptors by endogenous adenosine or ATP was abolished, leading to the disappearance of purinergic inhibition of synaptic transmission [15].

Our analysis revealed that, in contrast to WT NMJs, the application of a selective exogenous agonist of P2X7 receptors at Panx1$^{-/-}$ NMJs triggered an increase in the EPP quantal content during the entire short rhythmic train. It is reasonable to suggest that the effect observed in Panx1$^{-/-}$ mice was due to the unmasking of the P2X7-mediated facilitatory effect on synaptic transmission. Most likely, it is a consequence of the loss of activity of the inhibitory A_1 and P2Y13 receptors, which are deprived of Panx1-mediated release of their endogenous agonists into the synaptic cleft.

In addition to our data, the ability of BzATP as P2X7 receptor agonist to modulate neurotransmitter release was demonstrated in central and peripheral synapses, where the direct activation of presynaptic P2X7 receptors by BzATP was suggested [11, 33–37]. However, the presence of presynaptic P2X7 receptors at nerve terminals has been recently challenged and presynaptic effects of BzATP were considered as a result of activation of glial rather than neuronal P2X7 receptors, followed by release of gliotransmitters [38]. But along with this, there are convincing data demonstrating the localization of P2X7 receptor on nerve terminals of NMJs [2, 11], whereas the presence of P2X7 receptors on perisynaptic Schwann cells has not been demonstrated yet.

We have also established that the increase in EPP quantal content caused by the application of P2X7 receptor agonist may be prevented by blocking CaM, CaMKII, and L-type VDCCs in Panx1$^{-/-}$ mice. It should be noted that when applied alone at Panx1$^{-/-}$ NMJs, none of the blockers induced any changes in ACh release. It is known that P2X7 receptors are incapable of forming large pores in the membranes of motor nerve terminals [11]. Therefore, it is possible to exclude the influence of large molecules released via the P2X7 receptor channels upon their long-term activation by an exogenous agonist on ACh release. Thus, we assumed that the activation of P2X7 receptors triggers a signaling pathway in the motor nerve terminals that includes calcium entry via the P2X7 channels, followed by calcium-dependent sequential activation of CaM and CaMKII and potentiation of L-type VDCCs.

It has been shown that in cerebellar granule neurons, the stimulation of P2X7 receptors by exogenous agonists results in CaMKII activation [8]. Presynaptic CaM and CaMKII activation and effects of those proteins on evoked neurotransmitter release have been reported in both CNS and peripheral synapses. However, CaM- and CaMKII-dependent modulation of synaptic transmission resulted in different effects depending on distinct routes of calcium influx, patterns of synapse activation, and so on [39–44]. Recently, we have shown that in mouse NMJs, both spontaneous and evoked ACh release are insensitive to CaMKII blockers [44, 45]. In addition, we previously identified the conditions under which CaMKII may be activated and oppositely modulate synaptic ACh release. In particular, this occurs during selective activation of alpha7 nicotinic ACh receptors, when CaMKII mediates the inhibition of evoked ACh release [45]. It can also occur during the blockade of presynaptic phosphatase calcineurin, when the L-type VDCCs become disinhibited, leading to the activation of ryanodine receptors and CaMKII, which results in ACh release potentiation [44]. Here, our new data demonstrate that the activity of calcium-permeable P2X7 receptors may serve as an additional source for calcium-dependent CaMKII activation and its potentiating effect on ACh release.

L-type VDCCs are known to be present at motor nerve terminals [46], but they are usually silent and do not contribute to ACh release until they are unmasked. Numerous ways to unmask these channels have been described so far—via inhibition of BK channels [18, 47], activation of A_{2A} adenosine receptors [29], or via inhibition of phosphatases [48, 49]. All of these manipulations resulted in increased secretion of ACh. In the present study, we found that potentiation of presynaptic L-type calcium channels may also be achieved via activation of presynaptic P2X7 receptors. The exact mechanism of this potentiation of L-type VDCCs is not entirely understood. The ability of the α1C-subunit of neuronal L-type VDCCs to interact with CaMKII, leading to an increase in the opening probability of L-type calcium channels, has been shown in various studies at CNS [50–53]. It is possible that a similar

CaMKII-mediated facilitating effect on the activity of L-type VDCCs occurs in motor nerve terminals in response to CaMKII activation by calcium influx via P2X7 receptors. We suggest that P2X7- and CaMKII-mediated upregulation of L-type VDCCs activity provides calcium influx in response to each action potential, thus increasing the quantal content of each EPP from the very beginning of the short 50 Hz train. Unexpectedly, despite the increase in the quantal content of EPPs, we did not observe an increase in the release probability when L-type VDCCs became coupled to the ACh evoked release. An increase in the RRP size was found instead. RRP is defined in the literature as the number of docked synaptic vesicles that may be released in response to action potential. An increase in the RRP size in motor nerve terminals is usually associated with the calcium-dependent recruitment of additional pool of silent active zones and their vesicles into the RRP [21, 27, 42, 54]. We found for the first time that P2X7-induced upregulation of L-type VDCCs in Panx1$^{-/-}$ NMJs may selectively increase the size of RRP. We suggest that this effect is associated with the recruitment of previously silent active zones, thus increasing the RRP, in response to the appearance of the additional calcium entry via L-type VDCCs during evoked synaptic activity. However, this suggestion requires more detailed and fundamental proofs.

We have previously shown that the experimental approach utilizing Panx1$^{-/-}$ mice and short-term rhythmic stimulation of motor synapses makes it possible to exclude the activation of many (if not all) inhibitory ATP and adenosine receptors by their endogenous ligands [15]. Thus, in the absence of inhibitory A_1 and P2Y13 receptor activity, it became possible to observe (in pure form) the potentiation of ACh release caused by selective activation of P2X7 receptors by their exogenous agonist and to reveal the mechanism of P2X7-induced increase in synaptic transmission at motor synapses with the participation of activated CaM, CaMKII, and L-type VDCCs.

In summary, for the first time, we demonstrated that presynaptic P2X7 receptors at NMJs may serve as an additional source for calcium influx, which, however, does not directly affect the rate of calcium-dependent exocytosis of cholinergic vesicles but activates intraterminal calcium-dependent messengers involved in the modulation of ACh release. Moreover, it appears that activation of P2X7 receptors requires special conditions that are not observed during normal rhythmically evoked synaptic activity. It is well known that P2X7 receptors are activated by high concentration of extracellular ATP. Therefore, it is reasonable to suggest that activation of presynaptic P2X7 receptors in NMJs may occur upon stress, tissue damage, inflammation, ischemia, or glucose deprivation which are accompanied by a significant increase in extracellular ATP [7, 55]. It remains unclear whether an activation of presynaptic P2X7 receptors under these conditions may potentiate an evoked ACh release like in Panx1$^{-/-}$ mice. For the reasons given above, further detailed investigation would be essential to find the conditions under which the activity of P2X7 receptors may be triggered in NMJs of WT mice, leading to the modulation of ACh release, as well as the relative contribution of other presynaptic inhibitory purinergic receptors in the prevention of P2X7-induced potentiation of neurotransmitter release at NMJs.

Authors' contributions A.S. Miteva, A.E. Gaydukov, and O.P. Balezina conceptualized ideas, designed the study, and wrote the manuscript. A.S. Miteva and A.E. Gaydukov performed the experiments and analyzed the data; V.I. Shestopalov assisted in the discussion of the results and crafting the manuscript.

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Compliance with ethical standards

Conflicts of interest Anna S. Miteva declares that she has no conflict of interest.

Alexander E. Gaydukov declares that he has no conflict of interest.

Valery I. Shestopalov declares that he has no conflict of interest.

Olga P. Balezina declares that she has no conflict of interest.

Ethical approval All experimental procedures in this study were approved by the Bioethics Committee of Moscow State University.

References

1. North RA (2002) Molecular physiology of P2X receptors. Physiol Rev 82:1013–1067. https://doi.org/10.1152/physrev.00015.2002
2. Deuchars SA, Atkinson L, Brooke RE, Musa H, Milligan CJ, Batten TFC, Buckley NJ, Parson SH, Deuchars J (2001) Neuronal P2X7 receptors are targeted to presynaptic terminals in the central and peripheral nervous systems. J Neurosci 21:7143–7152
3. Lemos JR, Custer EE, Ortiz-Miranda S (2018) Purinergic receptor types in the hypothalamic-neurohypophysial system. J Neuroendocrinol 30:e12588. https://doi.org/10.1111/jne.12588
4. Marín-García P, Sánchez-Nogueiro J, Gómez-Villafuertes R, León D, Miras-Portugal MT (2008) Synaptic terminals from mice midbrain exhibit functional P2X7 receptor. Neuroscience 151:361–373. https://doi.org/10.1016/j.neuroscience.2007.10.038
5. Sánchez-Nogueiro J, Marín-García P, Bustillo D, Olivos-Oré LA, Miras-Portugal MT, Artalejo AR (2014) Subcellular distribution and early signalling events of P2X7 receptors from mouse cerebellar granule neurons. Eur J Pharmacol 744:190–202. https://doi.org/10.1016/j.ejphar.2014.10.036
6. Sperlágh B, Köfalvi A, Deuchars J, Atkinson L, J. Milligan C, Buckley NJ, Vizi ES (2002) Involvement of P2X7 receptors in the regulation of neurotransmitter release in the rat hippocampus. J Neurochem 81:1196–1211
7. Vasileiou E, Montero RM, Turner CM, Vergoulas G (2010) P2X7 receptor at the heart of disease. Hippokratia 14:155–163
8. León D, Hervás C, Miras-Portugal MT (2006) P2Y1 and P2X7 receptors induce calcium/calmodulin-dependent protein kinase II phosphorylation in cerebellar granule neurons. Eur J Neurosci 23:2999–3013. https://doi.org/10.1111/j.1460-9568.2006.04832.x
9. Gutiérrez-Martín Y, Bustillo D, Gómez-Villafuertes R, Sánchez-Nogueiro J, Torregrosa-Hetland C, Binz T, Gutiérrez LM, Miras-

Portugal MT, Artalejo AR (2011) P2X7 receptors trigger ATP exocytosis and modify secretory vesicle dynamics in neuroblastoma cells. J Biol Chem 286:11370–11381. https://doi.org/10.1074/jbc.M110.139410
10. Roger S, Pelegrin P, Surprenant A (2008) Cellular/molecular facilitation of P2X7 receptor currents and membrane Blebbing via constitutive and dynamic calmodulin binding. J Neurosci 28:6393–6401. https://doi.org/10.1523/jneurosci.0696-08.2008
11. Moores TS, Hasdemir B, Vega-Riveroll L, Deuchars J, Parson SH (2005) Properties of presynaptic P2X7-like receptors at the neuromuscular junction. Brain Res 1034:40–50. https://doi.org/10.1016/j.brainres.2004.12.001
12. Sokolova E, Grishin S, Shakirzyanova A, Talantova M, Giniatullin R (2003) Distinct receptors and different transduction mechanisms for ATP and adenosine at the frog motor nerve endings. Eur J Neurosci 18:1254–1264. https://doi.org/10.1046/j.1460-9568.2003.02835.x
13. Correia-de-Sá P, Timóteo MA, Ribeiro JA (1996) Presynaptic A1 inhibitory/A2A facilitatory adenosine receptor activation balance depends on motor nerve stimulation paradigm at the rat hemidiaphragm. J Neurophysiol 76:3910–3919
14. Guarracino JF, Cinalli AR, Fernández V, Roquel LI, Losavio AS (2016) P2Y13 receptors mediate presynaptic inhibition of acetylcholine release induced by adenine nucleotides at the mouse neuromuscular junction. Neuroscience 326:31–44. https://doi.org/10.1016/j.neuroscience.2016.03.066
15. Miteva AS, Gaydukov AE, Shestopalov VI, Balezina OP (2017) The role of pannexin 1 in the purinergic regulation of synaptic transmission in mouse motor synapses. Biochem Moscow Suppl Ser 11:311–320. https://doi.org/10.1134/S1990747817040067
16. Dvoriantchikova G, Ivanov D, Barakat D, Grinberg A, Wen R, Slepak VZ, Shestopalov VI (2012) Genetic ablation of Pannexin1 protects retinal neurons from ischemic injury. PLoS One 7:e31991. https://doi.org/10.1371/journal.pone.0031991
17. Barstad JA, Lilleheil G (1968) Transversaly cut diaphragm preparation from rat. An adjuvant tool in the study of the physiology and pbarmacology of the myoneural junction. Arch Int Pharmacodyn Ther 175:373–390
18. Flink MT, Atchison WD (2003) Iberiotoxin-induced block of Ca2+−activated K+ channels induces dihydropyridine sensitivity of ACh release from mammalian motor nerve terminals. J Pharmacol Exp Ther 305:646–652. https://doi.org/10.1124/jpet.102.046102
19. McLachlan EM, Martin AR (1981) Non-linear summation of end-plate potentials in the frog and mouse. J Physiol 311:307–324
20. Elmqvist D, Quastel DM (1965) A quantitative study of end-plate potentials in isolated human muscle. J Physiol 178:505–529. https://doi.org/10.1113/jphysiol.1965.sp007639
21. Ruiz R, Cano R, Casanas JJ, Gaffield MA, Betz WJ, Tabares L (2011) Active zones and the readily releasable Pool of synaptic vesicles at the neuromuscular junction of the mouse. J Neurosci 31:2000–2008. https://doi.org/10.1523/jneurosci.4663-10.2011
22. Yang L, Wang B, Long C, Wu G, Zheng H (2007) Increased asynchronous release and aberrant calcium channel activation in amyloid precursor protein deficient neuromuscular synapses. Neuroscience 149:768–778. https://doi.org/10.1016/j.neuroscience.2007.08.025
23. Ling KKY, Lin M-Y, Zingg B, Feng Z, Ko CP (2010) Synaptic defects in the spinal and neuromuscular circuitry in a mouse model of spinal muscular atrophy. PLoS One 5:e15457. https://doi.org/10.1371/journal.pone.0015457
24. Cano R, Ruiz R, Shen C, Tabares L, Betz WJ (2012) The functional landscape of a presynaptic nerve terminal. Cell Calcium 43:2–7. https://doi.org/10.1016/j.ceca.2012.04.012
25. Moyer M, van Lunteren E (1999) Effect of phasic activation on endplate potential in rat diaphragm. J Neurophysiol 82:3030–3040
26. Perissinotti PP, Uchitel OD (2010) Adenosine drives recycled vesicles to a slow-release pool at the mouse neuromuscular junction. Eur J Neurosci 32:985–996. https://doi.org/10.1111/j.1460-9568.2010.07332.x
27. Slater CR (2015) The functional organization of motor nerve terminals. Prog Neurobiol 134:55–103. https://doi.org/10.1016/j.pneurobio.2015.09.004
28. Horton SM, Luna Lopez C, Blevins E, Howarth H, Weisberg J, Shestopalov VI, Makarenkova HP, Shah SB (2017) Pannexin 1 modulates axonal growth in mouse peripheral nerves. Front Cell Neurosci 11:365. https://doi.org/10.3389/fncel.2017.00365
29. Tarasova EO, Miteva AS, Gaidukov AE, Balezina OP (2015) The role of adenosine receptors and L-type calcium channels in the regulation of the mediator secretion in mouse motor synapses. Biochem Moscow Suppl Ser 9:318–328. https://doi.org/10.1134/S1990747815050141
30. Ralevic V, Burnstock G (1998) Receptors for purines and pyrimidines. Pharmacol Rev 50:413–492. https://doi.org/10.1007/978-3-642-28863-0_5
31. Smith DO (1991) Sources of adenosine released during neuromuscular transmission in the rat. J Physiol 432:343–354
32. Santafe MM, Priego M, Obis T, Garcia N, Tomàs M, Lanuza MA, Tomàs J (2015) Adenosine receptors and muscarinic receptors cooperate in acetylcholine release modulation in the neuromuscular synapse. Eur J Neurosci 42:1775–1787. https://doi.org/10.1111/ejn.12922
33. Ireland MF, Noakes PG, Bellingham MC (2004) P2X7-like receptor subunits enhance excitatory synaptic transmission at central synapses by presynaptic mechanisms. Neuroscience 128:269–280. https://doi.org/10.1016/j.neuroscience.2004.06.014
34. León D, Sánchez-Nogueiro J, Marín-García P, Miras-Portugal MT (2008) Glutamate release and synapsin-I phosphorylation induced by P2X7receptors activation in cerebellar granule neurons. Neurochem Int 52:1148–1159. https://doi.org/10.1016/j.neuint.2007.12.004
35. Cho J-H, Choi I-S, Jang I-S (2010) P2X7 receptors enhance glutamate release in hippocampal hilar neurons. Neuroreport 21:865–870. https://doi.org/10.1097/WNR.0b013e32833d9142
36. Cuadra AE, Custer EE, Bosworth EL, Lemos JR (2014) P2X7 receptors in neurohypophysial terminals: evidence for their role in arginine-vasopressin secretion. J Cell Physiol 229:333–342. https://doi.org/10.1002/jcp.24453
37. Miras-Portugal MT, Sebastián-Serrano Á, de Diego García L, Díaz-Hernández M (2017) Neuronal P2X7 receptor: involvement in neuronal physiology and pathology. J Neurosci 37:7063–7072. https://doi.org/10.1523/JNEUROSCI.3104-16.2017
38. Illes P, Khan TM, Rubini P (2017) Neuronal P2X7 receptors revisited: do they really exist? J Neurosci 37:7049–7062. https://doi.org/10.1523/JNEUROSCI.3103-16.2017
39. Jinnai K, Takahashi K, Fujita T (1986) Enhancement of spontaneous acetylcholine release from motor nerve terminal by calmodulin inhibitors. Eur J Pharmacol 130:197–201
40. Singh S, Prior C (1998) Prejunctional effects of the nicotinic ACh receptor agonist dimethylphenylpiperazinium at the rat neuromuscular junction. J Physiol 511(Pt 2):451–460
41. Brailoiu E, Miyamoto MD, Dun NJ (2002) Calmodulin increases transmitter release by mobilizing quanta at the frog motor nerve terminal. Br J Pharmacol 137:719–727. https://doi.org/10.1038/sj.bjp.0704923
42. Wang Z-W (2008) Regulation of synaptic transmission by presynaptic CaMKII and BK channels. Mol Neurobiol 38:153–166. https://doi.org/10.1007/s12035-008-8039-7
43. Mukhamedyarov MA, Kochunova JO, Yusupova ER, Haidarov BA, Zefirov AL, Palotás A (2010) The contribution of calcium/calmodulin-dependent protein-kinase II (CaMKII) to short-term plasticity at the neuromuscular junction. Brain Res Bull 81:613–616. https://doi.org/10.1016/J.BRAINRESBULL.2009.12.010

44. Tarasova EO, Gaydukov AE, Balezina OP (2015) Methods of activation and the role of calcium/calmodulin-dependent protein kinase II in the regulation of acetylcholine secretion in the motor synapses of mice. Neurochem J 9:101–107. https://doi.org/10.1134/S1819712415020099
45. Gaydukov AE, Balezina OP (2017) CaMKII is involved in the choline-induced downregulation of acetylcholine release in mouse motor synapses. Acta Nat 9:110–113
46. Pagani R, Song M, McEnery M, Qin N, Tsien RW, Toro L, Stefani E, Uchitel OD (2004) Differential expression of alpha 1 and beta subunits of voltage dependent Ca2+ channel at the neuromuscular junction of normal and P/Q Ca2+ channel knockout mouse. Neuroscience 123:75–85
47. Gaydukov AE, Melnikova SN, Balezina OP (2009) Facilitation of acetylcholine secretion in mouse motor synapses caused by calcium release from depots upon activation of L-type calcium channels. Bull Exp Biol Med 148:163–166
48. Urbano FJ, Uchitel OD (1999) L-type calcium channels unmasked by cell-permeant ca 2+ buffer at mouse motor nerve terminals. Pflügers Arch Eur J Physiol 437:523–528. https://doi.org/10.1007/s004240050813
49. Gaydukov AE, Tarasova EO, Balezina OP (2013) Calcium-dependent phosphatase calcineurin downregulates evoked neurotransmitter release in neuromuscular junctions of mice. Neurochem J 7:29–33. https://doi.org/10.1134/S1819712413010030
50. Hudmon A, Schulman H, Kim J, Maltez JM, Tsien RW, Pitt GS (2005) CaMKII tethers to L-type Ca2+ channels, establishing a local and dedicated integrator of Ca2+ signals for facilitation. J Cell Biol 171:537–547. https://doi.org/10.1083/jcb.200505155
51. Wheeler DG, Barrett CF, Groth RD, Safa P, Tsien RW (2008) CaMKII locally encodes L-type channel activity to signal to nuclear CREB in excitation-transcription coupling. J Cell Biol 183:849–863. https://doi.org/10.1083/jcb.200805048
52. Ma H, Cohen S, Li B, Tsien RW (2012) Exploring the dominant role of Cav1 channels in signalling to the nucleus. Biosci Rep 33:e00009. https://doi.org/10.1042/BSR20120099
53. Huang W-H, Chao H-W, Tsai L-Y, Chung MH, Huang YS (2014) Elevated activation of CaMKIIÎ± in the CPEB3-knockout hippocampus impairs a specific form of NMDAR-dependent synaptic depotentiation. Front Cell Neurosci 8:367. https://doi.org/10.3389/fncel.2014.00367
54. Dittrich M, Homan AE, Meriney SD (2018) Presynaptic mechanisms controlling calcium-triggered transmitter release at the neuromuscular junction. Curr Opin Physiol 04:15–24. https://doi.org/10.1016/j.cophys.2018.03.004
55. Balboa E, Saavedra-Leiva F, Cea LA, Vargas AA, Ramírez V, Escamilla R, Sáez JC, Regueira T (2018) Sepsis-induced Channelopathy in skeletal muscles is associated with expression of non-selective channels. Shock 49:221–228. https://doi.org/10.1097/SHK.0000000000000916