3,5-Dimethyladamantan-1-amine Restores Short-term Synaptic Plasticity by Changing Function of Excitatory Amino Acid Transporters in Mouse Model of Spinocerebellar Ataxia Type 1

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Abstract

Introduction. *Memantine is an agent that used for treatment of Alzheimer's type dementia. Memantine considerably reduces the effects of neurodegeneration, may potentially slow down the neurodegenerative changes in the cerebellum and may act as treatment of choice for spinocerebellar ataxia type 1 (SCA 1).*

Our *objective was to study molecular mechanisms of the short-term synaptic plasticity improvement associated with long-term memantine use in SCA 1 transgenic mice.*

Materials and methods. *The experiments were performed on 12-week-old CD1 mice. We created a mouse model of cerebellar astrogliosis after expression of mutant ataxin-1 (ATXN1[Q85]) in the Bergmann glia (BG). To model the astrocyte-mediated neurodegeneration in the cerebellum, the mice were injected with LVV GFAP-Flag-ATXN1[Q85] lentiviral vector (LVV) constructs intracortically. Some of the mice received 0.35 mg/kg memantine dissolved in drink water once daily for 9 weeks. The control animals were administered LVV GFAP-ATXN1[Q2]-Flag. Changes of the excitatory postsynaptic currents amplitudes from Purkinje cells (PC) were recorded by patch clamp. Expression of anti-EAAT1 in the cerebellar cortex was assessed using immunohistochemistry.*

Results. *The reactive glia of the cerebellar cortex in SCA1 mice is characterized by a decrease in the immunoreactivity of anti-EAAT1, while chronic memantine use restores this capacity. The decay time of the excitatory postsynaptic current amplitude in the parallel fiber-Purkinje cell (PF-PC) synapses of the SCA1 mice is considerably longer, which indicates the slowing of glutamate reuptake and EAAT1 dysfunction. The prolonged presence of increased neurotransmitter levels in the synaptic cleft facilitates activation of the mGluR1 signaling and restoration of mGluR1-dependent synaptic plasticity in Purkinje cells of the SCA1 mice.*

Conclusions. *The slowing of neurotransmitter reuptake associated with long-term memantine treatment improves mGluR1-dependent short-term synaptic plasticity of the Purkinje cells in the SCA1 mice. Restoration of synaptic plasticity in these animals may underlie partial reduction of ataxic syndrome.*

Keywords*: short-term synaptic plasticity; astrogliosis; spinocerebellar ataxia type 1; glutamate reuptake*

Ethics approval. Authors confirm compliance with institutional and national standards for the use of laboratory animals in accordance with «Consensus Author Guidelines for Animal Use» (IAVES, 23 July 2010). The research protocol was approved by the Ethics Committee of Prof. V.F. Voino-YasenetskyKrasnoyarsk State Medical University (protocol No. 80/2017, December 27, 2017).

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3,5-диметил-адамантан-1-амин восстанавливает кратковременную синаптическую пластичность посредством изменения функции транспортёров возбуждающих аминокислот у модельных мышей со спиноцеребеллярной атаксией 1 типа

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Аннотация

Введение. *Мемантин – препарат для лечения деменции альцгеймерского типа, который значительно уменьшает явления нейродегенерации. Потенциально он может замедлить нейродегенеративные изменения в мозжечке и быть средством выбора в лечении спиноцеребеллярной атаксии 1 типа (СЦА1).*

Цель работы *– исследование молекулярных основ улучшения кратковременной синаптической пластичности при длительном потреблении мемантина модельными СЦА1-мышами.*

Материалы и методы. *Опыты проведены на 12-недельных мышах линии CD1. Мы создали модель астроглиоза мозжечка мыши после экспрессии мутантного атаксина 1 (ATXN1[Q85]) в глии Бергмана. Для моделирования астроцит-опосредованной нейродегенерации мозжечка данным мышам интракортикально в мозжечок вводили векторную конструкцию LVV GFAP-ATXN1[Q85]-Flag. Часть этих мышей получала мемантин в дозе 0,35 мг/кг в день, растворённой в питьевой воде, в течение 9 нед. Мышам контрольной группы вводили LVV GFAP-ATXN1[Q2]-Flag. Динамику амплитуд возбуждающих постсинаптических токов клеток Пуркинье регистрировали с помощью метода локальной фиксации потенциала. Экспрессию anti-EAAT1 в коре мозжечка изучали методом иммуногистохимии.*

Результаты. *Для реактивной глии коры мозжечка у СЦА1-мышей характерно снижение иммунореактивности анти-EAAT1, хроническое потребление мемантина восстанавливает этот показатель. У СЦА1-мышей в синапсах параллельных волокон с клетками Пуркинье время спада амплитуд возбуждающих постсинаптических токов значительно увеличено, что свидетельствует о замедлении обратного захвата глутамата и нарушении функции EAAT1. Повышенное продолжительное нахождение нейромедиатора в синаптической щели способствует облегчению активации mGluR1-пути передачи сигналов и восстановлению mGluR1-зависимой синаптической пластичности в клетках Пуркинье СЦА1-мышей.*

Заключение. *Замедление обратного захвата нейромедиатора при длительном потреблении мемантина оказывает положительное влияние на mGluR1-зависимую кратковременную синаптическую пластичность в клетках Пуркинье СЦА1-мышей. Восстановление синаптической пластичности у данных животных может лежать в основе частичного уменьшения атаксического синдрома.*

Ключевые слова*: кратковременная синаптическая пластичность; астроглиоз; спиноцеребеллярная атаксия 1 типа; обратный захват глутамата*

Этическое утверждение. Все исследования выполняли с учётом принципов гуманного обращения с животными, протоколы были утверждены решением Локального этического комитета КрасГМУ им. проф. В.Ф. Войно-Ясенецкого (протокол № 80/2017 от 27.12.2017).

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Introduction

Spinocerebellar ataxia type 1(SCA1) belongs to the group of polyglutamine diseases caused by an increased number of CAG nucleotide repeats in the coding region of the ataxin-1 gene (*ATXN1*). SCA1 is characterized by progressive cerebellar ataxia followed by bulbar paralysis and death in 10–15 years after the onset [1]. The pathogenesis can be explained by the toxic effect of a protein encoded by a mutant *ATXN1* gene, which forms aggregates in cells [2–4]. Studies of various SCA1 models showed that the main targets of this toxic effect are the cerebellar Purkinje cells (PC) [5–7]. The same models also demonstrated impairment of the short-term and long-term synaptic plasticity [8].

Glutamate is the neurotransmitter which predominantly mediates excitatory synaptic activity in the central nervous system. The concentration of glutamate in the synaptic cleft is strictly controlled by the balance between its release and clearance. This function is performed by excitatory amino acid transporter EAAT1, which is Na^+-dependent glutamate transporter mainly expressed in glial cells of the cerebellum [9]. Astrocytic EAATs play an important role in modulation of glutamatergic excitation, allow glutamate reuptake from the synapse and thereby protect neurons [10].

Dysfunction in these processes results in extracellular glutamate accumulation leading to excitotoxicity and damage of neurons [11]. Glutamate spillover from the synaptic cleft may activate extrasynaptic of N-methyl-D-aspartate (NMDA) receptors. Excessive Ca^{2+} influx through extrasynaptic NMDA-receptors induces signaling pathways activating programmed cell death [12].

The use of NMDA-receptor antagonists in neuroprotective pharmacotherapy for various neurodegenerative diseases is promising therapeutic approach [13]. One of such agents is 3,5-dimethyladamantan-1-amine (memantine). Memantine has been approved by FDA for the treatment of Alzheimer's disease [14, 15]. The neuroprotective effect of memantine has been also studied in other pathological conditions, i.e. ischemia, migraine, depression-like behavior, etc. [16–18]. Potential effects of memantine on SCA1 treatment are still unknown.

Another important role of NMDA receptors is their involvement in synaptic plasticity, which underlies learning and memory formation.

Previously, we described a model based on chronic optogenetic activation of Bergmann glia with the light-sensitive cation channel rhodopsin-2 (ChR2), where it was demonstrated a crucial role of EAAT mechanism dysfunction and further excitotoxicity in the pathogenesis of the cerebellar neurodegeneration [6]. We also described the short-term synaptic plasticity impairment in this model [19].

In this study we used a SCA1 mouse model with selective expression of mutant ataxin-1 to study the effects of long-term memantine administration on short-term synaptic plasticity.

Our **objective** was to explore the molecular mechanism of the short-term synaptic plasticity improvement associated with long-term memantine use in the SCA1 mouse model.

Materials and methods

AVV and LVV production

In order to increase the LVV expression level we used a GFAP promoter.[20] Sequences of non-pathogenic *ATXN1*[Q2] (encoding human ataxin-1 with 2 glutamine repeats) or pathogenic ATXN1[Q85] (with 85 uninterrupted glutamine repeats) were fused in frame with the sequence encoding the FLAG tag at their 5'ends. After that, Flag-ATXN1[Q2] and Flag-ATXN1[Q85] constructs were transferred into the pTYF lentiviral shuttle vector, under the control of the enhanced GFAP promoter. The detailed procedure for viral vector production was described previously [21]. Titers of LVV-GFAP-Flag-ATXN1[Q2] LVV and LVV-GFAP-Flag-ATXN1[Q85] were 7×10^9 transducing units (TU) per 1 mL. LVV were stored at −80ºC and used within 6 months.

Neurodegeneration modeling

Three-week-old wild type mice (P21) were anesthetized by 50 mg/kg Zoletil (Virbac) intraperitoneally. Mice were kept warm by a heated pad during surgical interventions. 3 μL of LVV or phosphate-buffered saline (PBS) were slowly injected

into the cortex of the cerebellar vermis (lobule VI) using a 10 μL Hamilton syringe. Stereotaxic coordinates relative to bregma were: AP: –2.5 mm, ML: 0 mm, DV: 2 mm. Mice were used for further experiments 9 weeks after the injection when expression of transgenic ataxin-1 was prominent. Some SCA1 mice received memantine at a dose of 0.35 mg/kg per day, dissolved in drinking water, for 9 weeks [22].

Immunohistochemistry

For immunohistochemistry, mice were perfused transcardially with a paraformaldehyde, 4% in 0.1 M PBS after being anesthetized by Zoletil (50 mg/kg) intraperitoneally. The whole brain was removed and postfixed in the same fixative overnight. The cerebellar vermis was cut into 50 μm sagittal slices. The slices were treated with rabbit monoclonal anti-EAAT1 antibodies (1 : 500, Cloud Clone Corp.) and then visualized with Alexa Fluor 594-conjugated donkey anti-rabbit IgG (1 : 1000, Life Technologies). The antibodies were dissolved in a PBS solution containing 2% normal donkey serum, 0.1% Triton X-100, and 0.05% NaN_3. For comparison, confocal fluorescence images of the cerebellar slices from the corresponding region of the cerebellum were obtained using the FV10i microscope (Olympus). Images were recorded as Z-stacks using × 10 objective and 1024 × 1024 resolution. Microphotographs converted to black and white were analyzed using ImageJ software. To avoid false positive results, we used the anti-EAAT1 signal fluorescence filter at 30% of maximal fluorescence intensity. To measure the EAAT1-positive area, the images of more than 30 pixels were selected.

The patch clamp method

Once the mice were deeply anesthetized by Zoletil, they were decapitated. The whole brain was dissected out and quickly immersed in ice-cold Ringer's solution, oxygenated by 95% O_2 and 5% CO_2. Parasagittal slices (250 μm) of the cerebellar vermis were made using a vibratome Microtom CU65 (Thermo Scientific). The slices were cut in a Ringer solution containing (in mM): 234 sucrose, 26 $NaHCO_4$, 2.5 KCl, 1.25 NaH_2PO_4, 11 glucose, 10 $MgSO_4$, and 0.5 $CaCl_2$ at 4ºC with continuous oxygenation by a mixture of 95% O_2 and 5% CO_2 [6]. The slices were maintained in an extracellular solution containing (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 D-glucose, and 0.05–0.10 picrotoxin. This solution was oxygenated continuously with a mixture of 95% O_2 and 5% CO_2 at room temperature for 1 h before starting the electrophysiological experiments.

For electrophysiological whole-cell recordings we used intracellular solution containing (in mM): 140 Cs-gluconate, 8 KCl, 10 HEPES, 1 $MgCl_2$, 2 MgATP, 0.4 NaGTP, 0.2 EGTA (pH 7.3). Electrophysiological data were analyzed using pClamp10 (Molecular Devices), Patchmaster (HEKA), and Clampfit 10.5 (Axon Instruments) software. Voltage of the PC membrane was clamped at –70 mV. To record the excitatory postsynaptic currents (EPSCs) during the stimulation of the parallel fibers (PF), the stimulating electrode was placed into the molecular layer of the cerebellar cortex. The assessment of the EPSC decay time constant (characteristic decay time τ) was performed in Clampfit by approximating the EPSC curve with an exponential function from the peak value (A) to the end of the signal recording.

For the short-term synaptic plasticity analysis (synaptically evoked suppression of excitation, SSE), the PC membrane voltage was clamped at –70 mV. The control PF-EPSC recording was made at 0.2 Hz during 40 sec. To evoke SSE, we applied high frequency PF stimulation (15 impulses at 100 Hz) in order to activate mGluR-mediated signaling pathway in PCs. The averaged PF-EPSC amplitudes over 10 s were normalized to the baseline values equal to the mean values prior to the SSE evoking. PF-EPSCs were then recorded for 100 s after the stimulation.

Statistical methods and data processing

The data were expressed as mean values ± standard error of the mean (*M* ± *SEM*) with 95% confidence interval. Statistical analysis was performed using basic statistical functions from the R open-source statistical software. Differences between the individual groups were analyzed using ANOVA and Tukey–Kramer test, which allows to correct *p* values when sample sizes are unequal. The differences were considered as significant at $p < 0.05$.

Results

Long-term memantine administration affects EAAT1 expression.

Changes in the cerebellar cortex caused by target expression of mutant ataxin-1 in the Bergmann glia were described in detail previously [23]. In this study, SCA1 mice were administered 0.35 mg/kg memantine for 9 weeks starting from postnatal day 21 to block neurodegenerative process.

Reactivation of the Bergmann glia with mutant ataxin-1 affected EAAT1 expression in mice. The mice with ATXN1[Q85] showed a decrease in expression of this gene: the area of anti-EAAT1 positive signal blot relative to the total area of the image was 15.2 ± 0.5% (9 areas studied in 3 mice – area/number (*a/n*) = 9/3 versus 17.0 ± 0.3% (*a/n* = 8/3) in mice expressing ATXN1[Q2] ($p = 0.007$; Fig. 1, *A*, *B*). Chronic memantine administration increased the area of anti-EAAT1 positive signal up to 17.5 ± 0.1% (*a/n* = 11/3) compared with mice without chronic memantine administration in mice expressing ATXN1[Q85] ($p = 0.002$).

An increased number of expressed EAAT1 positive spots in SCA1 mice after long-term memantine administration

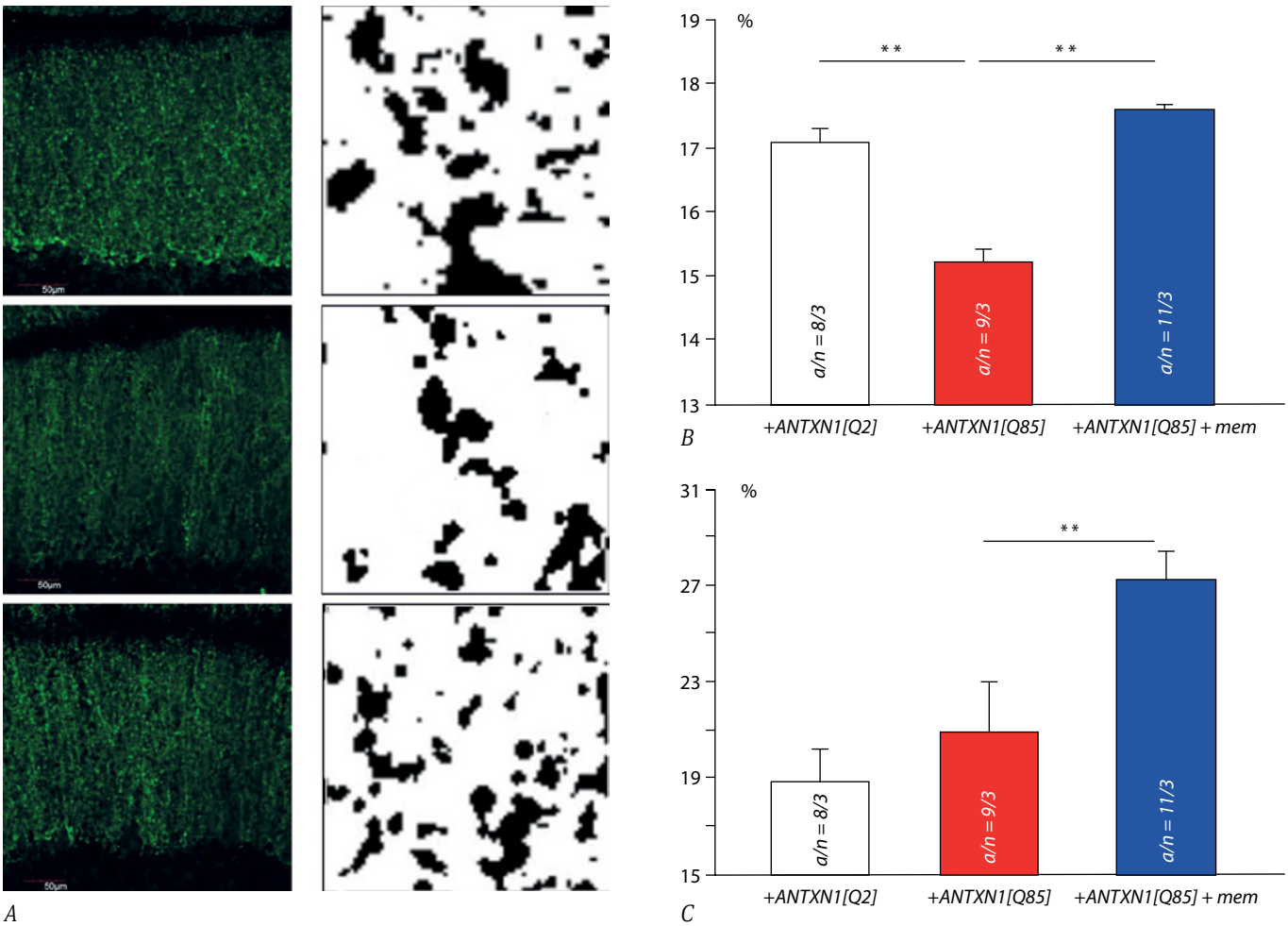

Fig. 1. EAAT1 expression in animals receiving and not receiving memantine.
A – fluorescent microphotographs of the cerebellar cortex slices labeled with anti-EAAT1 (left panel). The images processed with ImageJ software (right panel). Chart scales are 50 and 5 μm respectively. *B* – proportion of anti-EAAT1 positive signal area. *C* – total amount of anti-EAAT1 positive spots. *a/n* – number of examined areas/animals. $^{**}p < 0.01$.

turned out to be a more significant parameter: 27.1 ± 1.3 vs ATXN1[Q85] (21.0 ± 2.1) and ATXN1[Q2] (18.7 ± 1.7) in mice receiving vehicle ($p = 0.02$ and $p = 0.0001$ respectively; Fig. 1, *A*, *C*). These data indicate that memantine alters EAAT1 expression level by increasing the area and the number of transporters on the Bergmann glia cell membrane in the cerebellar cortex.

Long-term memantine administration affects synaptic transmission in PF-PC synapses

Altered EAAT1 expression levels change glutamate uptake from the synaptic cleft, which, in its turn, affects the synaptic transmission. To assess the effects of long-term memantine administration on synaptic transmission and plasticity, we studied electrophysiological characteristics of the PCs.

Decay time constant (τ) for PF-EPSC amplitudes recorded in PCs in SCA1 mice that did not receive memantine was not statistically different from the controls and was 14.5 ± 1.0 ms–1 (18 cells studied in 4 mice – cells/number (*c/n*) = 18/4) in ATXN1[Q2] mice and 15.1 ± 1.5 мс$^{-1}$ in ATXN1[Q85] mice (*c/n* = 19/4; $p = 0.75$; unpaired t-test). Long-term memantine administration increased τ-value for PF-EPSC amplitudes in SCA1 mice up to 21.0 ± 2.3 ms^{-1} (*c/n* = 14/4; $p = 0.048$; Fig. 2).

The mutant ataxin-1 expression in the Bergmann glia selectively affects SSE levels

Slow decay of PF-EPSC amplitudes may indicate a long-term effect of glutamate on postsynaptic receptors due to its accumulation caused by uptake dysfunction. This may create conditions for glutamate release from the synaptic cleft and for activation of perisynaptic receptors, such as mGluR1. Thus, we studied a certain type of short-term synaptic plasticity associated with activation of mGluR1 signaling in the PCs. Tetanic PF stimulation results in activation of mGluR1

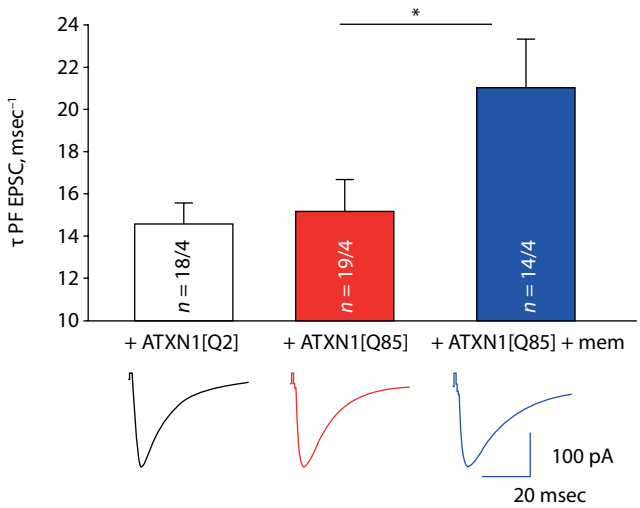

Fig. 2. Memantine increases the constant decay time (τ) of PF-EPSC amplitude in the PC of SCA1 mice.
Summary diagram of the PF-EPSCs mean constant decay time (τ). Representative curves are presented on the right panel. *c/n* is the number of cells/animals (*$p < 0.05$).

associated with a local increase in Ca^{2+} concentration in the PCs. Ca^{2+} influx triggers the synthesis of endocannabinoids, which signal retrogradely to inhibit the release of glutamate from the presynaptic PF terminals (SSE) [24–27].

In the presence of 25 μm CPCCOEt, an mGluR1-specific blocker, the amplitude PF-EPSC began to rise immediately after the tetanic stimulation: from 37.6 ± 5.9 to 63.5 ± 5.0% (*c/n* = 7/3; $p = 0.026$, the paired t-test; Fig. 3).

After the tetanic stimulation in ATXN1[Q85] mice the PF-EPSC amplitude increased (116.1 ± 8.9% [*c/n* = 8/3]). The amplitude inhibition was not detected, while in ATXN1[Q2] mice the PF-EPSC amplitude dropped after the stimulation and stayed reduced during the whole period of recording (79.1 ± 14.1%; *c/n* = 8/3; $p < 0.01$; Fig. 4). Long-term memantine administration restored the SSE level: the amplitude after the stimulation has dropped (44.9 ± 8.5%; *c/n* = 9/3; $p < 0.001$ versus the mice without memantine consumption); the changes of amplitude restoration were similar to those in ATXN1[Q2] mice (Fig. 4).

Discussion

We used SCA1 mouse model with LVV GFAP-ATXN1[Q85]-Flag target expression in the Bergmann glia [23] to assess the effects of memantine on the processes involved in short-term synaptic plasticity. Memantine was administered in drinking water for 9 weeks at 0.35 mg/kg.

Previously, we showed a decrease in expression and in the function of excitatory amino acid transporters EAAT1 in the optogenetic model of cerebellar neurodegeneration [6]. These changes are associated with dysfunction in astrocyte glutamate reuptake from the synaptic cleft and are well documented for various neurodegenerative conditions [13, 14, 16].

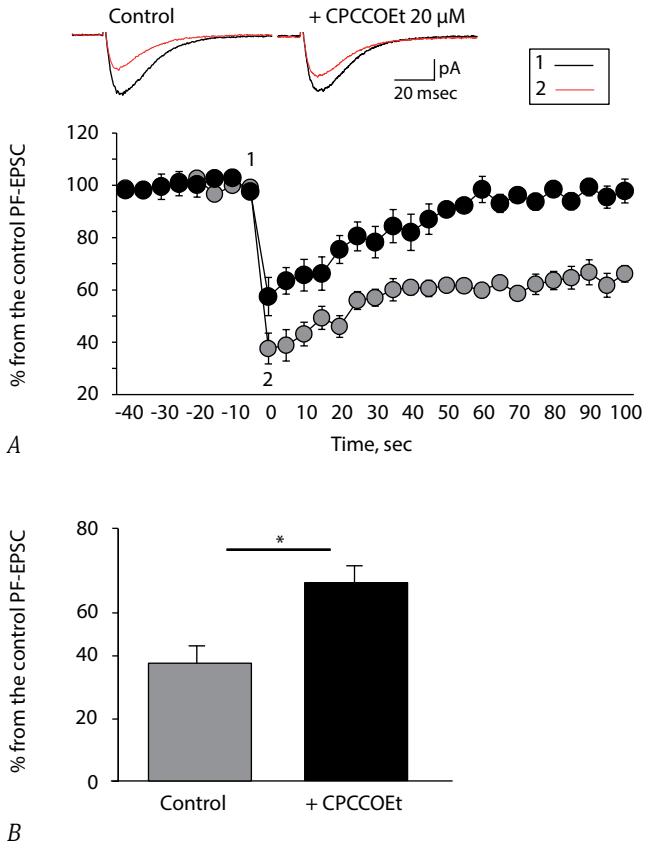

Fig. 3. The SSE impairment after the inhibition of mGluR1-dependent signaling pathway in the presence of CPCCOEt.
A – changes of PF-EPSC amplitudes after tetanic PF stimulation. Representative PF-EPSC curves above the chart: recorded immediately before the stimulation (point 1, 10 sec on the time axis) and after the stimulation (point 2, 0 sec on the time axis). *B* – amplitudes normalized to the pre-stimulation level immediately after the stimulation (point 2). *c/n* is the number of cells/animals. *$p < 0.05$.

In our SCA1 mouse model, a similar effect was observed: we found a decrease in EAAT1 expression in the cerebellar cortex. Memantine restored the expression levels up to the control values in both neurodegeneration models [6]; Fig. 1, *A, B*. There are data indicating that EAAT1 protein expression is activated by exogenous glutamate [10, 28]. S. Duan et al. have discovered that this glutamate-mediated mechanism of the augmentation in EAAT1 function is induced by the EAAT1 surface expression in cultured murine astrocytes without altering expression level of a membrane transport protein [29]. We speculate that such mechanism prorects neurons against excitotoxicity. We showed increased anti-EAAT1 positive blots in SCA1 model mice after memantine consumption (Fig. 1, *A, C*). It may indicate to altered clasterisation or transportation of these transmitters to cell membrane depending on glutamate presence in the synaptic cleft. It is important to continue studies to prove this hypothesis.

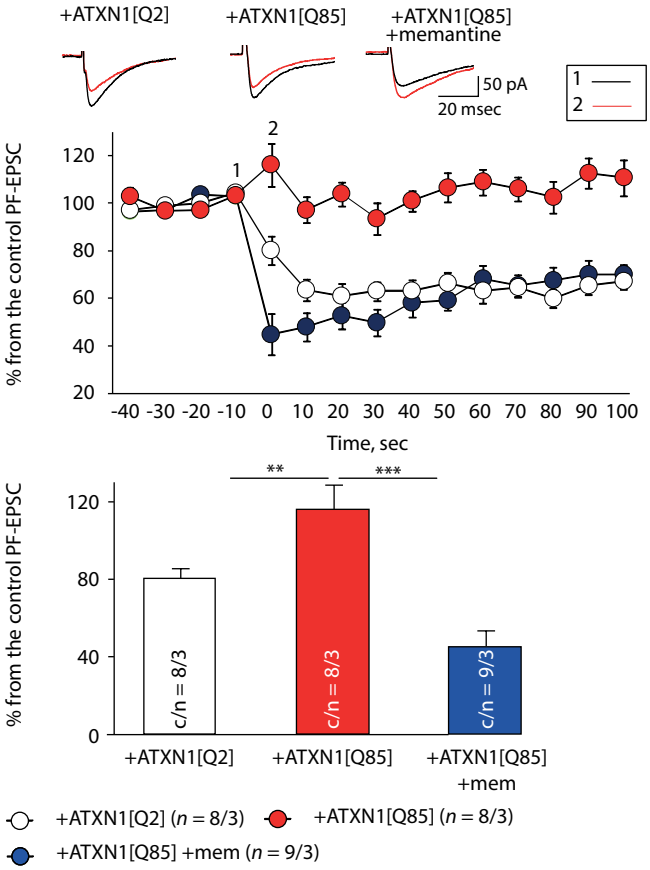

Fig. 4. SSE restoration in SCA1 mice after long-term memantine administration (mem).
A – changes of PF-EPSC amplitudes after tetanic PF stimulation. Representative PF-EPSC curves above the chart: recorded immediately before the stimulation (point 1, 10 s on the time axis) and after the stimulation (point 2, 0 s on the time axis).
B – amplitudes normalized to the pre-stimulation level immediately after the stimulation (point 2).
c/n is the number of cells/animals.
$^{**}p < 0.01$; $^{***}p < 0.001$.

At the same time, it was shown that memantine administration decrease glutamate uptake activity, both in the frontoparietal cortex and in the hyppocampus, with no effect on expression levels of the excitatory amino acid transporters [30].

The observed increase in EAAT1 expression might be a compensatory mechanism activated by decline in EAAT1 function. The EAAT1 dysfunction is also due to the increased τ-values for PF-EPSC observed in SCA1 mice receiving long-term memantine administration (Fig. 2).

One of the manifestations of the altered levels of gene expression and a result of astroglia reactivation in SCA1 mice is impaired glutamate signal transmission. There is a decrease of mGluR1 levels on the PC membranes, as well as the levels of glutamate symporters EAAT4 and of glutamate and aspartate transporter EAAT1 in the Bergmann glia [31–34], resulted in a number of electrophysiological PC dysfunctions, which compromises motor learning and synaptic plasticity [33, 34].

The most studied types of synaptic plasticity in PF-PC synapses are the paired pulse facilitation, impulse suppression after depolarization, SSE and long-term depression (LTD). Among these types, SSE and LTD are mGluR-dependent ones, but LTD is triggered by a combination of PF stimulation (mGluR activation) and Purkinje cell depolarization [35]. For this reason, LTD is not able to show selective changes of mGluR signaling in PCs. LTD induction is blocked not only in the presence of mGluR-specific blockers, but also in the absence of membrane depolarization [36]. So, the SSE was studied as a process completely dependent on mGluR activation [25]. The range of an increase in mGluR-signaling may be indirectly, but quite precisely determined based on restoration of EPSC curve after tetanic stimulation. With long-term memantine administration, an increase in neurotransmitter levels in the synaptic cleft allows glutamate accumulation, which causes mGluR1 activation and thereby contributes to SSE restoration (Fig. 4).

The decreased glutamate uptake from the synaptic cleft by PF-PCs is induced by long-term effects of memantine administration. This mechanism causes no neurodegradation, because NMDA receptors remain blocked by memantine. However, increased levels of glutamate in the synaptic cleft allow this neurotransmitter to reach the perisynaptic mGluR1 and induce synaptic plasticity, such as SSE (Fig. 4). Understanding this process would help to predict the effects of prescribed drugs on the glutamatergic system.

Achieving a balance between the release and clearance of glutamate may be the key to treating many neurodegenerative diseases. Understanding these mechanisms is of paramount importance for the planning of future clinical studies.

Conclusion

In neurodegenerative diseases affecting the cerebellum, such as SCA1, the impairment of SSE type of short-term synaptic plasticity is associated with mGluR degradation on the dendritic spines. In our study we demonstrated that memantine induced a decrease in neurotransmitter uptake by modulating EAAT1 function and an increase in mGluR signaling within PCs. Our research contributes to the picture of the impaired mechanisms of synaptic plasticity in the neuronal cells of the cerebellum, the understanding of which is a necessary element of the treatment strategy for neurodegenerative various conditions.

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