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Introducing treatment strategy for cerebellar ataxia in mutant med mice: Combination of acetazolamide and 4-Aminopyridine

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ABSTRACT

Purkinje neurons are the sole output neuron of the cerebellar cortex, and they generate high-frequency action potentials. Electrophysiological dysfunction of Purkinje neurons causes cerebellar ataxia. Mutant med mice have the lack of expression of the Scn8a gene. This gene encodes the NaV1.6 protein. In med Purkinje neurons, regular high-frequency firing is slowed, and med mice are ataxic. The aim of this study was to propose the neuroprotective drugs which could be useful for ataxia treatment in med mice, and to investigate the neuroprotective effects of these drugs by simulation. Simulation results showed that Kv4 channel inhibitors and BK channel activators restored the normal electrophysiological properties of the med Purkinje neurons. 4-Aminopyridine (4-AP) and acetazolamide (ACTZ) were proposed as neuroprotective drugs for Kv4 channel inhibitor and BK channel activator, respectively.

1. Introduction

Electrophysiological properties of cerebellar Purkinje neurons play an important role in the normal function of the cerebellum, including fine-tuning movements, posture, coordination, and timing of motor behaviors. Cerebellar ataxia, a disease characterized by disturbance in coordination, instability of posture, gait abnormalities, and intention tremor, is the result of changes in the physiological function of cerebellar Purkinje neurons [1,2].

Khaliq et al. [3] mentioned that in mutant med mice which had the lack of expression of the Scn8a gene ataxia was observed. Scn8a gene encodes the NaV1.6 protein. In med Purkinje neurons, transient sodium current inactivated more rapidly than in normal neurons, and resurgent current was nearly abolished. Regular high-frequency firing was slowed; therefore, mutant med mice showed ataxia [3]. By restoring the Purkinje neuron output to the normal condition, ataxia could be reduced in mutant med mice.

Unfortunately, there is no specific treatment for ataxia, and treatments depend on the cause. Currently, one of the promising therapies for the neurodegenerative diseases, such as cerebellar ataxia, is the use of neuroprotective agents [1]. Previous studies suggested that applying ion channel modulators could improve or restore the intrinsic neuronal firing behavior altered in many neurological disorders, such as ataxia [1,4,5].

Therefore, this study investigated how the output of Purkinje neuron in mutant med mice restored to the normal conditions.

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The simulation environment makes it possible to change the properties of the specific ion channels as the possible mechanism of action of neuroprotective drugs. Also, it is possible to study the changes in the firing activity of Purkinje neurons.

To determine how the output of med Purkinje neuron restored to the normal conditions, computer simulations of the electrical behaviors of Purkinje neuron were performed. Manipulations of parameters in simulation were achieved based on the experimental evidences suggested in previous studies [6–8]. Neuroprotective drugs were proposed based on simulation results, which had been previously used for treatment of other types of ataxia in different experimental studies. In several experimental studies on neurodegenerative disease, it had proposed that a suitable therapeutic target for the treatment of neurodegenerative diseases might be the activation of BK [1,5,9] and inhibition of Kv4 channels [6,7,10]. Experimental studies also showed that inhibition of voltage gated Na+ channels [7,11] was beneficial in neurodegenerative diseases. But the results of our simulations indicated that inhibition of voltage gated Na+ channels in med Purkinje cells suppressed cell firing and did not change the firing activity of the cell toward normal activity. Therefore, we focused on BK and Kv4 channels.

Previous studies indicated that 4-aminopyridine (4-AP) was the inhibitor of Kv4 channels [6,7], and could act as a neuroprotective drug [12]. Experimental studies on animal models of 3-acetylpyridine-induced ataxia in rats [10] demonstrated the significant neuroprotective effect of 4-AP in cerebellar ataxia. Clinical studies reported that 4-AP produced clear neurological benefits in patients suffering from multiple sclerosis and episodic ataxia type 2 [13–16]. Acetazolamide (ACTZ), BK channel activator, is also the neuroprotective drug. Previous studies showed that episodic ataxia type 2 responded to ACTZ treatment [8], and the severity of cerebellar ataxia was reduced during the course of ACTZ administration [17].

Present study examined the effect of applying 4-AP, ACTZ, and their combination on treatment of ataxia in med mice.

The outline of this article is as follows: in next section the materials and methods used in the present study are described then, the simulation results are shown. Finally, discussion and limitation of this study and conclusion is presented.

2. Materials and methods

Models of normal [18] and med Purkinje neurons [3] were used to study the altered firing behavior of Purkinje neurons in a rat model of med, and to analyze the neuroprotection effects of 4-AP and ACTZ.

The basic computational model of Purkinje neurons detailed by Akemann and Knopfel [18] was used to simulate the tonic firing activity of normal Purkinje neurons. Their model was the modified version of the model detailed by Khaliq et al. [3] for normal neurons. Both models included only the soma. Briefly, normal neuron model was simulated with a single compartment cylindrical model of length 20 μm and radius 10 μm, and consisted of eight types of ion channels (i.e. resurgent Na^+, non-resurgent Na^+, BK, Kv1, Kv3, Kv4, Ih, P-type Ca^{2+} channels, and leak channel). Sodium currents, resurgent and non-resurgent, were modeled using a kinetic scheme based on the model of Raman and Bean [19]. This model is shown below (Fig. 1), where C, I, O, and OB denote closed, inactivated, open and block states, respectively [3,19], and α, β, ζ, δ, ε, γ, C_{on}, C_{off}, O_{on}, and O_{off} are rate constants [3]. Where $a = (O_{on}/C_{on})^{1/4}$ and $b = (O_{off}/C_{off})^{1/4}$ [3].

The sodium reversal potential was set to 60 mV; the maximum conductance of resurgent and non-resurgent sodium current was set to 16 and 14 mS/cm^2, respectively.

Kv3 current was simulated with a binary model. Voltage dependence of activation of bKv3 was modeled as a step function with an activation threshold V_{th} set to −10 mV. The maximum conductance and reversal potential of this channel was set to 1.6 mS/cm^2 and −88 mV, respectively.

All other currents were represented using Hodgkin and Huxley type models [20]. The parameters used in this model for these channels are shown in Table 1.

For all ionic currents but calcium, current was computed from Ohm's law. Calcium current was computed using the Goldman–Hodgkin–Katz current equation [3].

To simulate med Purkinje neurons, the med Purkinje neuron model which was detailed by Khaliq et al. [3] was used.

For the purpose of this study, modifications described by Khaliq et al. [3] for med neuron, were applied to the normal model [18]. These modifications were as follows:

- Since resurgent current was absent in model of med Purkinje neurons, the rate constant ε was reduced from 1.75 to 1×10^{-12}/ms. This would eliminate entry into the blocked state. The rate constant O_{on} was increased from 0.75/ms to 2.3/ms in order to produce the faster decay of the transient current in med neurons.
- The resurgent sodium current was reduced to 90% compared to the normal conditions.
- Leak current was reduced to 70% compared to the normal conditions.
- An 8 mV positive shift in the half-activation voltage of Kv1 channel was made.

Simulations were performed in the NEURON environment (Version 7.1) [21], and run with a time step of 25 μs. At first, normal Purkinje cell was simulated; the simulations with the normal model qualitatively imitated the experimental recordings from normal Purkinje neurons. Then, aforementioned modifications were applied to the normal model, and med Purkinje neuron was simulated. Finally, the effects of ion channel modulators on med Purkinje cell output were investigated. To examine the effects of ion channel activators and inhibitors, the maximum conductance of different ion channels was changed as the action of channel activators and inhibitors, and their effects on firing activity of the Purkinje neuron were studied.

The electrophysiological characteristics of the simulated neurons were assessed in two minutes interval. The specifications of the repetitive action potentials were expressed as mean ± standard deviation (SD). Statistical analyses were accomplished by the Student's t test, and differences were considered significant if $p < 0.05$. The electrophysiological characteristics which were used to compare the firing activity of the simulated normal, med and treated Purkinje neurons,

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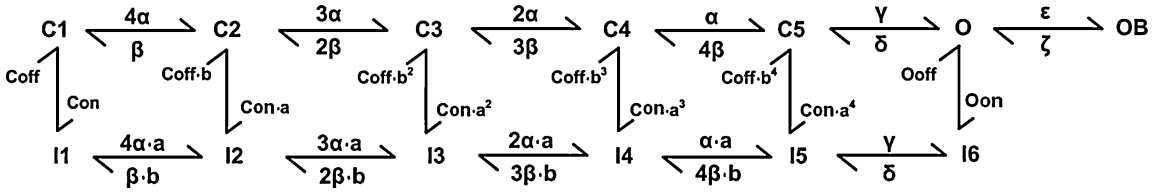

Fig. 1 – Model of sodium currents [3].

Table 1 – Model parameters for the ion channels of normal cell simulated with Hodgkin–Huxley formalism.

Channel	Reversal potential (mV)	Conductance	Maximum conductance (G_{max}) (mS/cm^2)
BK	−88	$G = G_{max}\, m^3\, z^2\, h$	14
Kv1	−88	$G = G_{max}\, m^4$	11
Kv4	−88	$G = G_{max}\, m^4\, h$	3.9
Ih	−30	$G = G_{max}\, m$	0.2
Leak	−61	$G = G_{max}$	0.09
P-type Ca^{2+}	−88	$G = P\, m$	Permeability (P) 6×10^{-5} cm/s

are as follows: The firing rate of the neuron, amplitude of the action potentials (APs), duration of the action potentials, amplitude of the after-hyperpolarization (AHP), the coefficient of variation (CV), and the baseline. The baseline was calculated as the mean of the Purkinje cell output. The amplitude of action potentials and AHP was measured from the baseline to the peak and to the negative peak of the APs, respectively. Firing rate was calculated as the inverse of average interspike intervals (ISIs) between spikes. Action potential duration was calculated as the duration measured at the half amplitude. In order to assess the regularity of firing, CV was calculated as the ratio of the standard deviation to the mean of ISIs between spikes. These characteristics were used in different experimental studies on Purkinje neurons [1,3,4,18,22]. Among these characteristics the firing rate and CV was more substantial in motor control [3,23–26].

3. Results

Normal Purkinje neuron generated regular spontaneous action potentials. Simulations indicated that normal Purkinje neuron somata produced action potentials spontaneously, with mean firing rate of 35.4 ± 4.31 spikes/s, while the baseline was about −56.68 mV (Fig. 2).

The mean amplitude, duration, and AHP amplitude of regularly spaced action potentials were 78.79 ± 1.07 mV, 0.7 ± 0.01 ms, and −4.17 ± 0.21 mV, respectively. Regularly spaced peaks of action potentials were observed for tonically firing normal neuron, which was associated with low CV (0.15).

Simulations showed that the spontaneous activity reduced in med neuron; firing was generally more irregular and slower than in the normal neurons, with CV of 0.41 and mean firing rate of 16.5 ± 2.12 spikes/s, and the baseline was about −51.08 mV (Fig. 3).

The AHP amplitude in med Purkinje neuron was higher than in the normal Purkinje neuron (−4.17 ± 0.21 mV in normal versus −8.41 ± 0 mV in med Purkinje neuron). The action potential amplitude decreased (78.79 ± 1.07 mV in normal versus 56.95 ± 4.06 mV in med neuron), and duration of action potentials increased (0.7 ± 0.01 ms in normal versus 1 ± 0.042 ms in med neuron).

In order to explore how ion channel modulators affect the firing activity of med Purkinje neuron, their effects were examined in med model as changes in conductance of different channels. Then, the firing activities of Purkinje neuron were compared with normal neuron.

The conductance of individual channels was changed in steps of 10% related to the original value in the model, and changes in the firing activity of med Purkinje neuron model were evaluated.

Calcium-activated potassium channels and Kv4 channels were more involved in many animal models of cerebellar ataxia [2,4,27]. Therefore, the main focus of this research was the study of these channels in order to propose treatment for ataxia in med mice.

As shown in Fig. 4a, the increment of 10% in the conductance of BK channel in med Purkinje neuron increased the regularity (CV = 0.1) and firing rate (21.3 ± 4.92 spikes/s) of med Purkinje neuron. Further increment (20% and more) in the BK channel conductance generated few action potentials, then suppressed the neuron firing, and the membrane potential rested at −62.58 mV (Fig. 4b).

Inhibition of Kv4 channel increased the firing rate and regularity of med neuron model and decreased the AHP and action potential amplitude. Fig. 5a and b shows the firing activity of med Purkinje neuron following 10% (firing rate and CV was about 27.9 ± 4.41 and 0.12, respectively) and 20% reduction in the Kv4 channel conductance, respectively.

The simulation results showed that changes in the conductance of just one channel enhanced the regularity and firing rate of med Purkinje neuron, but could not restore all electrophysiological characteristics of med Purkinje neuron to the normal conditions; however, changes in conductance of two ion channels may be useful.

As shown in Fig. 6, 10% reduction in the conductance of Kv4 channel along with 50% increase in the conductance of BK channel restored the firing activity of the med Purkinje neuron model to the normal conditions. The firing pattern of the Purkinje neuron became regular (CV = 0.09) and exhibited

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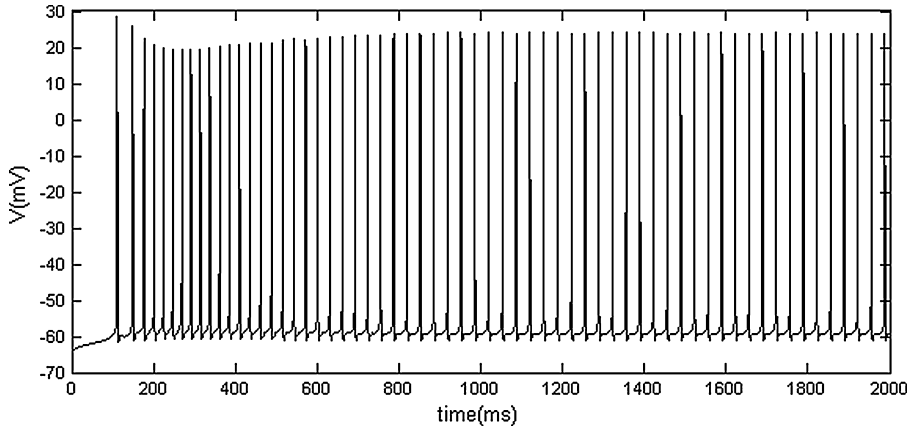

Fig. 2 – Spontaneous firing of normal Purkinje neuron.

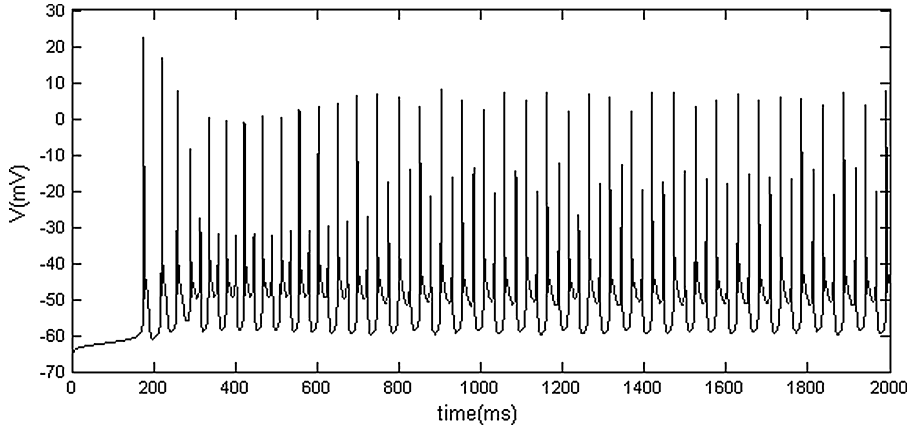

Fig. 3 – Spontaneous firing of med Purkinje neuron.

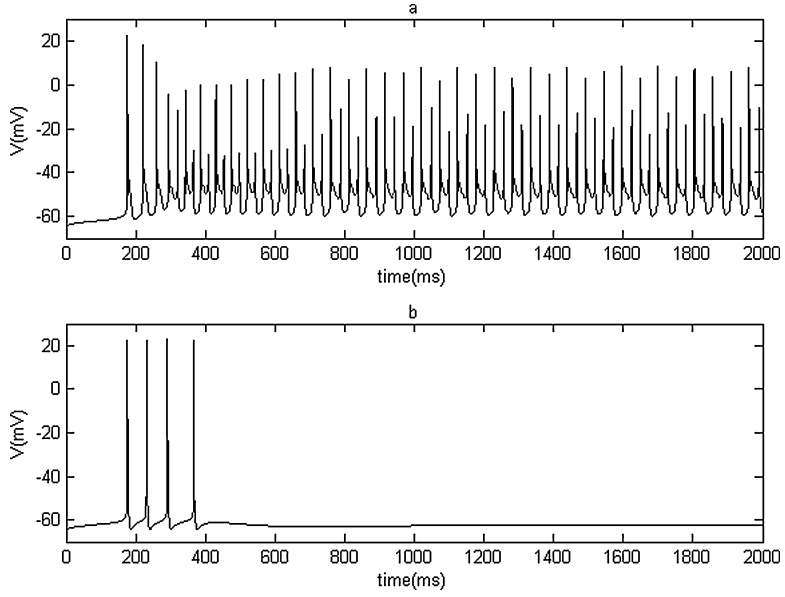

Fig. 4 – Spontaneous firing of med Purkinje neuron with increased BK channel conductance: (a) 10% increase and (b) 20% increase.

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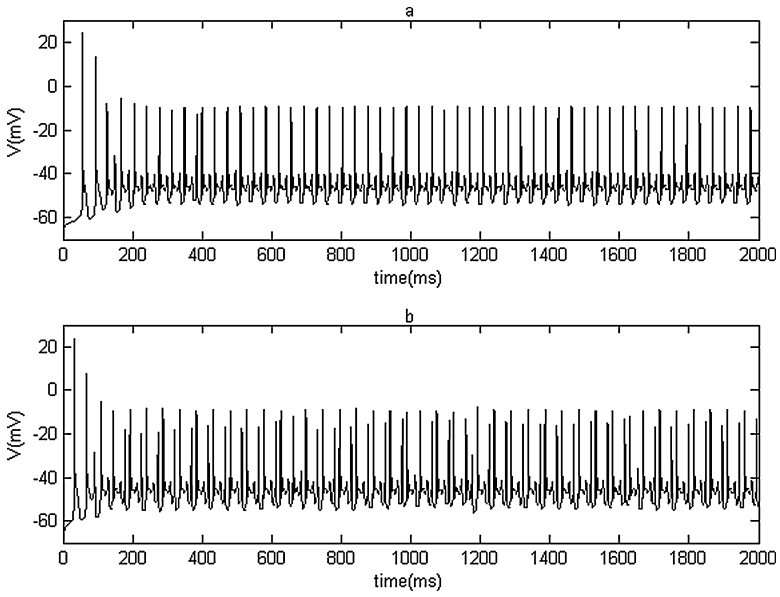

Fig. 5 – Spontaneous firing of med Purkinje neuron with reduction in the conductance of Kv4 channels: (a) 10% reduction and (b) 20% reduction.

spontaneous tonic firing at 25.1 ± 3.41 spikes/s. Amplitudes of the action potentials and AHP restored to the normal conditions (81.34 ± 0.99 and -4.61 ± 0.33 mV, respectively).

10% decrease in the conductance of Kv4 channel and 50% increase in the conductance of BK channel were the minimum amount of required changes for restoring all normal characteristics of firing of the Purkinje neuron model. Changes more than these minimums, resulted in slight changes in the response for some range of values, and produced quantitatively responses closer to the normal neuron.

Electrophysiological characteristics of simulated neurons in normal, med, and treatment conditions are summarized in Table 2.

4. Discussion

The cerebellum underlies the control of posture, balance and fine coordination of motor movements. Since Purkinje neurons establish the sole output of the cerebellar cortex [28], they play vital roles in maintaining balance and regulating movements by modulating the firing response of the deep cerebellar nuclei. Purkinje neuronal function impairment will cause ataxia. Therefore, rescue of Purkinje neurons can have significant therapeutic implications [1]. Despite major progress in understanding the genetic and molecular mechanisms of ataxia, the confirmed pharmacological treatment of cerebellar ataxia is still lacking [29]. Thus, the major challenges for future research are the identification of novel drug targets, development of novel therapeutic approaches, and initiation of clinical trials [29,30]. As a result of the diversity of ataxias, symptomatic therapeutic approaches that improve ataxia without interfering with underlying molecular mechanism must be seriously considered [30]. There is a medical need to develop effective therapies in ataxia disorders for which no cure is currently available. Furthermore, there is an obvious medical need to develop anti-ataxic drugs with proved efficacy. Such knowledge is required to develop potential therapeutic agents in order to prevent neuronal dysfunction and neurodegeneration [31]. With the discovery of ion

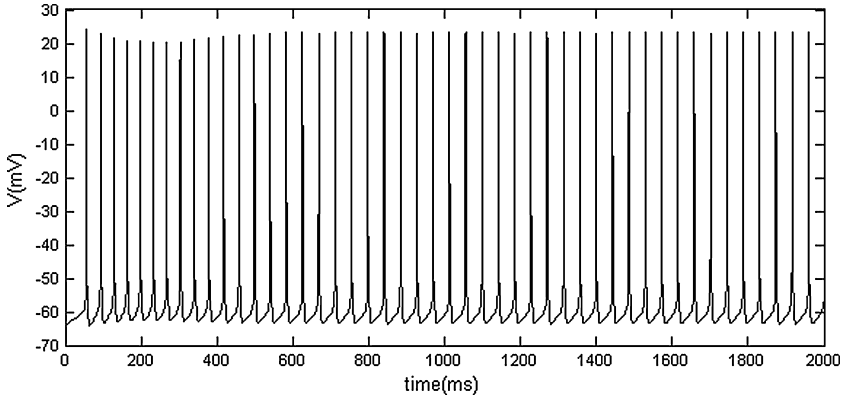

Fig. 6 – Spontaneous firing of med Purkinje neuron with 10% reduction in conductance of Kv4 channel along with 50% enhancement in conductance of BK channel.

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Table 2 – Electrophysiological characteristics of normal, med and treated Purkinje neuron.

Parameter	Normal neuron	Med neuron	Treated neuron
Firing rate (spikes/s)	35.4 ± 4.31	16.5 ± 2.12	25.1 ± 3.41
CV	0.15	0.41	0.09
AP amplitude (mV)	78.79 ± 1.07	56.95 ± 4.06	81.34 ± 0.99
AHP amplitude (mV)	−4.17 ± 0.21	−8.41 ± 0	−4.61 ± 0.33
AP duration (ms)	0.7 ± 0.01	1 ± 0.042	0.75 ± 0.04
Baseline (mV)	−56.68	−51.08	−58.66

channelopathies, the therapeutic value of many basic drugs targeting ion channels has been confirmed [9,32–38].

Purkinje neurons in mutant med mice with the lack of expression of the Scn8a gene, showed a reduction in the rate of spontaneous action potential firing; therefore, these mice were ataxic [3]. By restoring the Purkinje neuron output to the normal condition, ataxia could be reduced in mutant med mice. Therefore, we tried to restore electrophysiological characteristics of med Purkinje neuron to the normal conditions in simulations. The changes used in simulations were based on experimental evidence suggested in previous studies for other types of ataxia.

Previous experimental studies indicated that the regularity and frequency of Purkinje neuron spiking was relevant for motor control. Tottering mice showed impaired motor behavior and pronounced ataxia [26]. They suffered from disrupted regularities of their Purkinje neuron spiking, while the amplitude modulation of their spike rate during optokinetic stimulation was indistinguishable from that in wild-type littermates [24–26]. Moreover, the use of regular stimulation patterns [39] and pharmacological activation of calcium-activated potassium channels [25] rescued the ataxic motor behavior. A growing body of evidence indicated that potassium channel blockers and activators may exert an important neuroprotective effect in different diseases of central nervous system [15,40–43]. Blockade of BK channels disrupted the precision of firing and produced less regularity in firing [25,27,44]. Based on previous studies [44,45], it was possible that opening the Ca^{2+} dependent K^+ channels in riluzole treated rats may be involved in increasing the firing precision and regularity in cerebellar Purkinje neurons.

Mathematical modeling has a great role in elucidating medical knowledge. Computational models of neurons have become important tools for investigating different aspects of the complex behavior of the neurons. In the simulation environment it is possible to investigate how each specific ionic current can affect the electrophysiological properties of neurons. This is a good way to reproduce the response of neuron in the presence of channel blockers and activators. In addition, simulation is inexpensive and non-invasive.

In the present study we discussed results of using BK channel activator and Kv4 inhibitor on med Purkinje cell firing by simulation. We investigated the effect of each of these channels alone and combination of both. We observed that for small increase in conductance of BK channel the firing rate of med Purkinje neuron increased; also CV decreased which indicated increase in regularity. For larger increment in BK channel conductance the med Purkinje cell could not fire. By inhibiting of Kv4 channel, the firing rate and regularity of med neuron increased and the AHP and action potential amplitude decreased. Therefore, our simulation results indicated that activation of BK channels and inhibition of Kv4 channels alone could enhance the regularity and firing rate in med Purkinje neuron but could not restore the electrophysiological characteristic of Purkinje cell completely. Moreover, activation of BK channels along with inhibition of Kv4 channels could enhance the spontaneous activity in med Purkinje neuron and restore the electrophysiological characteristics of med Purkinje neuron to the normal condition. Based on these findings, we propose that combined treatment with BK channel activators and Kv4 channel inhibitors can reduce ataxia in med mice, and be useful for treatment of them.

One of the inhibitors of Kv4 channels is 4-aminopyridine (4-AP) [6,7]; earlier studies indicated that 4-AP could act as a neuroprotective drug [10,12–16,22]. Acetazolamide (ACTZ) is a BK channel activator; the previous studies showed that ACTZ could also act as a neuroprotective drug [8,17].

This study provided computational insight that 4-AP and ACTZ co-treatment (with appropriate dose) could prevent the ataxia in med mice, by restoring the intrinsic firing properties of med Purkinje neuron to the normal conditions.

Therefore, we propose that combination of 4-AP and ACTZ can be an effective treatment to reduce ataxia in med mice, though experimental research is needed to validate our hypothesis.

One factor may contribute to the shortcoming of this hypothesis. Kv4 current was not measured in the med neurons by Khaliq et al., if this current reduced in the med neurons, then only BK channel activator is sufficient to restore intrinsic firing properties of med Purkinje neuron to the normal conditions. If this current increased or did not altered in the med neurons, co-treatment with BK channel activator and Kv4 channel inhibitor is needed.

5. Conclusion

We offered a treatment for cerebellar ataxia in med mice based on simulation results. We simulated normal and med Purkinje cell and investigated the effects of ACTZ (BK channel opener) and 4-AP (Kv4 channel inhibitor) on med Purkinje cell. Simulation results indicated that these drugs could restore med Purkinje cell output characteristics to the normal condition.

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