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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,