MOTOR NEURON FIRING IN A TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS: A SIMULATION STUDY

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease selectively affecting motor neurons functioning. Mutations of the Cu,Zn superoxide dismutase (SOD1) account for 20% of the familial cases of ALS. Previous studies investigated spinal motor neurons excitability in transgenic mice overexpressing the mutated (Gly93 → Ala) human SOD1 enzyme and showed that expression of the human G93A-mutant SOD1 gene induces a different excitability in motor neurons with respect to control and SOD1 motor neurons. We developed a mathematical model able to reproduce the firing properties of spinal motor neuron in non-transgenic mice. We performed a sensitivity analysis on the model to identify modifications in conductances and/or kinetics of the ionic currents that can be responsible of the observed alterations both in firing frequency and in AP duration in G93A motor neurons. We found that changes limited to ionic current conductances are not able to reproduce G93A firing alterations. We observed that the mutant motor neuron hyperexcitability can be reproduced by means of a faster kinetic of small conductance calcium-dependent potassium current. Thus, we propose this current mutation as a possible mechanism underlying ALS-associated alterations.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the progressive and selective degeneration of cortical and spinal motor neurons. Mutations in the gene coding for human Cu,Zn superoxide-dismutase (SOD1) have been reported for approximately 20% of all familial cases of ALS [1,2].

Transgenic mice overexpressing the mutated (Gly93 → Ala) human SOD1 enzyme develop phenotypic and pathological symptoms resembling ALS in humans, whereas SOD1 knockout mice do not [3,4].

The expression of the human G93A-mutant SOD1 gene induces excitability modifications in cultured mouse spinal motor neurons, such as an increased firing frequency and a shorter duration of the action potential (AP), with respect to control and SOD1 motor neurons [5,6]. Moreover, transcranial magnetic stimulation (TMS) studies have shown that, in ALS patients, increased excitability of corticomotoneurons contribute to motor cortex hyperexcitability [7]. The enhanced number of action potentials induced in mutant G93A motor neurons during activation, resulting from hyperexcitability, can contribute to pathological mechanisms suggested for motor neuron degeneration [8].

It is known that several distinct ion currents determine action potential properties and membrane excitability. The correlation between action potential waveform and ionic current changes in motor neurons has been analyzed in previous studies [9,10]. However, the modifications at the ion-channel level underlying the observed motor neurons hyperexcitability are still unknown and have to be deeply investigated.

In order to answer this question, mathematical modelling of neuronal excitability can strongly support the classical electrophysiological approach. Numerical simulation in fact allows to easily evaluate the effects produced by a ionic current alteration on firing properties, whereas studying in vitro the same problem is more difficult, due to complex interactions between ionic currents.

At this aim we developed a mathematical model based on a single-compartment Hodgkin-Huxley formulation [11]. The model was able to reproduce the essential features of non-transgenic mice spinal motor neurons firing.

In this work we present the results of a sensitivity analysis performed on the model through simulations. The target of this analysis was to characterize the possible ionic currents alterations able to produce the effects experimentally observed on motor neuron excitability.

Materials and Methods

The motor neuron excitability was simulated by means of a computer model implemented in Simulink (The MathWorks, Inc., Natick, MA; USA). This model includes a mathematical description of several ionic currents, pump currents and exchangers, mostly based on motor neuron electrophysiological data [11,12]. The model also includes a simplified description of an intracellular calmodulin calcium buffer. The firing

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behaviour was elicited in the model by simulating the application of a step of depolarizing current, in order to reproduce the current-clamp protocol used in [5].

Some parameters of the model were modified, with respect to the values indicated in [11], in order to better reproduce the qualitative features of the spike train. This was obtained by increasing fast sodium current conductance and calcium dependent potassium-current (large type) conductance, respectively to 130 nS and 180 nS. These changes did not alter the good fitting of quantitative features, like the relation between the firing frequency (F) and injected current (I), and AP waveform properties.

The experimental reference values, taken from [5], are summarized in Figure 1.

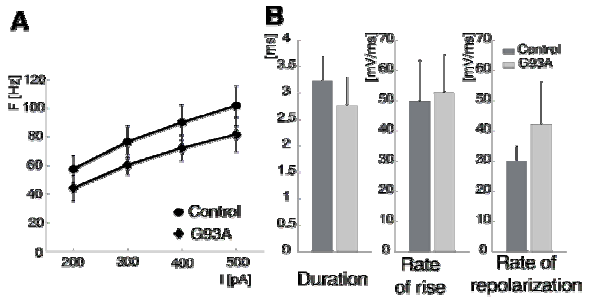

Figure 1: Firing properties in non transgenic (Control) and mutant (G93A) motor neurons (modified from Pieri et al., 2003 [5]). (A) Relation between firing frequency F and injected current I in Control and G93A cells. The firing frequency in G93A culture was significantly greater than that in the Control. (B) Comparison between AP properties in Control and G93A motor neurons in response to a +200 pA injected current. The AP rate of rise in the two cultures is comparable, whereas the AP rate of repolarization in the G93A culture was significantly greater compared to that of Control. The AP duration was shorter in the G93A cells than in the Control.

As a first step of our study, we analyzed the contribution of each ionic current present in the model to the slow repolarization phase between two action potentials. At this purpose we calculated the integral of each current during this period, corresponding to the total electric charge transported through the membrane by the considered ionic current.

The second step was a sensitivity analysis of the model, performed by applying small changes (±5%) to ionic currents conductances. We measured the firing frequencies (as the inverse of the first inter-spike intervals) elicited by a +200 pA and +500 pA injected current, and we compared them with the base values, in order to evaluate the effects of the alterations.

We then focused our attention on the small conductance calcium-dependent potassium current and we verified the effects of changes applied to its time constant.

Results

The value of the first inter-spike interval can be calculated from measured firing frequencies (22.8 ms in Control and 17.5 ms in G93A motor neurons, in response to a +200 pA injected current). The observed difference cannot be explained by the shortening in AP duration. Thus, the currents underlying the under-threshold phase of the action potential, must play a fundamental role in causing the hyperexcitability of G93A mutant cells.

For this reason, we focused our attention on the slow depolarization phase preceding the spike generation. We analyzed through simulation the ionic currents flowing through the membrane during this phase of the action potential. Time-course of all currents in the model, excluding pump currents and exchangers, is shown in Figure 2A.

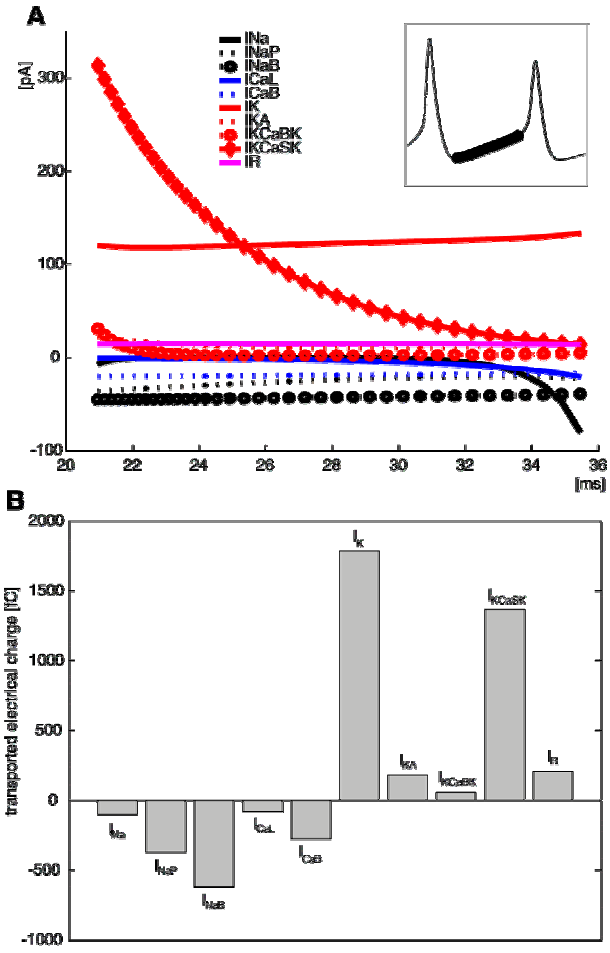

Figure 2: Ionic currents underlying the slow depolarization phase. (A) Time-course of voltage-dependent ionic currents during the slow depolarization phase (see the thickened line in the upper right inset). Outward currents (positive) hinder the depolarization, whereas inward currents (negative) facilitate it. (B) Total amount of electric charge transported by each current, calculated by numerical integration.

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We estimated the contribution of each current to the depolarization phase by numerical integration in the considered time period. The histogram in Figure 2B shows the calculated values, corresponding to the total amount of electric charge transported through the membrane. Outward currents, conventionally indicated as positive, transport a positive charge out of the cell, hindering the membrane depolarization. Inward currents, instead, facilitate the membrane depolarization transporting a positive charge into the cell.

A shortening of the slow depolarization phase can be obtained either by reducing an outward current or by enhancing an inward current. We then performed a first sensitivity analysis on the model, by applying small changes to ionic currents conductances. We applied a 5% increment to inward currents and a 5% decrement to outward currents. Table 1 shows the variations (expressed as percentages) of the firing frequency corresponding to each current variation. The frequency variation was calculated both for a +200 pA and a +500 pA stimulating current. Inward and outward currents are listed separately in the table, ordered by the total charge transported during the slow depolarization phase (decreasing from top to bottom).

Table 1: Results of the sensitivity analysis on ionic currents conductances

	ionic current	F variation (+200 pA)	F variation (+500 pA)
inward	I_{NaB}	+0,64%	+0,27%
	I_{NaP}	+0,45%	+0,28%
	I_{CaB}	+0,74%	+0,30%
	I_{Na}	-3,06%	-1,79%
	I_{CaL}	+2,82%	+0,20%
outward	I_{K}	+2,44%	+0,65%
	I_{KCaSK}	+0,91%	+1,28%
	I_{R}	+0,43%	+0,20%
	I_{KA}	+0,10%	+0,23%
	I_{KCaBK}	-2,79%	-2,60%

These results show that the model presents a low sensitivity to conductances variations. The frequency percentage increments were always lower than the change applied to the parameters. In two cases the variations did not produce the desired effect, and the firing frequency resulted decreased with respect to the base values.

We also tried to apply greater variations to the parameters (±10%). In most cases the model lost its ability to produce a sustained oscillation. In conclusion, we did not find any ionic conductance that could be modified to obtain firing frequency values comparable with those of G93A mutant cells.

Conductance is only one of the parameters characterizing ionic currents in the model. It is known that ionic channel kinetics are fundamental in determining current properties. We then tried to identify a current whose kinetic could be effective in enhancing firing frequency. Looking at the time-course of ionic currents during the slow depolarization (see Fig. 2A), it was evident that the greater part of the considered currents was almost constant in this phase.

Small conductance calcium-dependent potassium current seemed to be the only current which kinetic could be modified in order to reduce the flow of positive electric charges out of the membrane, and consequently shorten the duration of the depolarization phase. In fact, this current showed a decreasing time-course during the considered period.

We found that the reduction of the time constant of this current was effective in enhancing firing frequency and it did not affect the ability of the model to produce repetitive spikes. We then reduced the time constant to 2 ms, corresponding to half of its original value, and we adjusted the conductance of this current, by applying a 30% increase, in order to better sustain membrane potential oscillations in response to high stimulating currents. The new values of the parameters were chosen in order to reproduce the modifications observed in experimental F-I relation in G93A mutant cells.

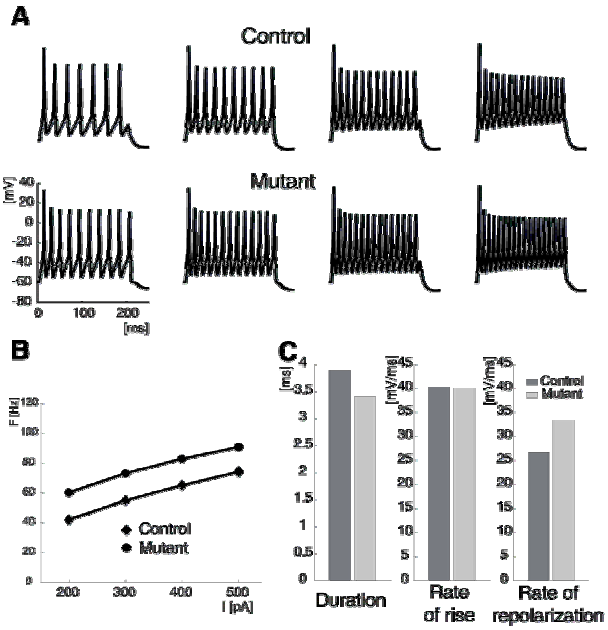

Figure 3: Simulated firing properties obtained with the base values of the parameters (Control) and after the modification of small conductance calcium-dependent potassium current (Mutant) (A) Membrane potential traces in response to +200, +300, +400, +500 pA depolarizing current step (from left to right). (B) Relation between firing frequency and injected current. After parameters modification motor neuron firing frequency is strongly enhanced. (C) AP properties in Control and Mutant simulations.

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Figure 3 shows the effects produced on firing properties by the described modifications. The results obtained with the base values of the parameters are indicated as Control, whereas the modified parameters are indicated as Mutant. Simulated membrane potential traces are reported in Figure 3A, in response to stimulating current steps of four different amplitudes (+200, +300, +400, +500 pA from left to right). Figure 3B shows the relation between firing frequency and injected current. The effect produced by the modification of small conductance calcium-dependent potassium current parameters is comparable with the alterations observed in G93A mutant cells (see Figure 1A).

We found that the parameter values chosen to fit firing frequency curve are effective also in reproducing the modifications observed in G93A mutant AP properties. After parameter modification, the simulated action potential had a shorter duration, comparable rate of rise, and increased rate of repolarization, as observed in motor neurons expressing G93A mutant human SOD1 gene. The comparison between action potential properties obtained with base and modified parameters is shown in Figure 3C.

Discussion

In this work we carried out a sensitivity analysis in a mathematical model of spinal motor neuron. The main purpose of this analysis was to identify parameters in the model able to reproduce the alterations in firing properties observed in G93A mutant cells.

Based on the results of our analysis, we exclude that hyperexcitability in G93A motor neurons could depend only of a variation in ionic channel conductances. We propose that small conductance calcium-activated potassium current could play an important role in modifying the mutant motor neuron firing properties. This hypothesis was suggested by the evidence that the simulated firing frequency is highly sensitive to this current kinetic. In particular, reducing the time constant driving this current causes an enhanced firing frequency of the simulated motor neuron.

The suggested hypothesis is derived from a simulation study and it obviously can not be considered as an exhaustive conclusion about ALS pathogenic mechanisms. However, there are some experimental evidences that could support this result. The increase of intracellular calcium concentration has been proposed as one of the mechanism by which a mutation of SOD1 may lead to motor neuron toxicity and degeneration [8,13,14,15]. Modifications in intracellular calcium concentration can be the cause of an alteration in calcium-dependent potassium currents. In our mathematical description of small conductance calcium-activated potassium current, the time constant was independent of both membrane potential and calcium concentration. A more accurate description of this current should be included in the model, in order to verify whether an increased intracellular calcium concentration could result in a modified kinetic of this current comparable with the proposed one.

Moreover, a recent study demonstrated that G93A motor neurons are characterized by a larger TTX-insensitive outward current, and proposed also small conductance calcium-activated potassium current as a good candidate for the observed changes [16].

For these reasons, we think that an electrophysiological characterization of small conductance calcium-activated potassium current should be highly suitable, in order to experimentally verify the simulation results.

Conclusions

The present analysis was aimed to investigate the role of different ionic current parameters in determining motor neuron firing properties, by using numerical simulation. Based on this analysis results, small conductance calcium-activated potassium current was proposed as mechanism responsible for the observed hyperexcitability in G93A mutant motor neurons.

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