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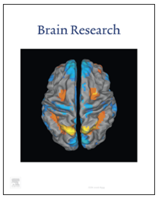

Research report

The influence of increased membrane conductance on response properties of spinal motoneurons

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ABSTRACT

During functional spinal neural network activity motoneurons receive massive synaptic excitation and inhibition, and their membrane conductance increases considerably – they are switched to a high-conductance state. High-conductance states can substantially alter response properties of motoneurons. In the present study we investigated how an increase in membrane conductance affects spike frequency adaptation, the gain (i.e., the slope of the frequency-current relationship) and the threshold for action potential generation. We used intracellular recordings from adult turtle motoneurons in spinal cord slices. Membrane conductance was increased pharmacologically by extracellular application of the $GABA_A$ receptor agonist muscimol. Our findings suggest that an increase in membrane conductance of about 40–50% increases the magnitude of spike frequency adaptation, but does not change the threshold for action potential generation. Increased conductance causes a subtractive rather than a divisive effect on the initial and the early frequency-current relationships and may have not only a subtractive but also a divisive effect on the steady-state frequency-current relationship.

1. Introduction

In vertebrates information processing involves activation of large-scale spatially distributed neural networks consisting of highly interconnected neurons. During functional activity in these networks neurons receive massive synaptic excitation and inhibition, and their membrane conductance increases considerably – they are switched to a high-conductance state (Alaburda et al., 2005; Destexhe et al., 2003). It was proposed that high-conductance states substantially alter integrative properties of cortical neurons and provide them with a number of computational advantages including gain modulation, increased temporal resolution and equalization of synaptic efficacies (Destexhe et al., 2003).

A number of membrane nonlinearities mediated by ion conductances influences signal processing, filtering and translation into the final motor commands in spinal motoneurons (Heckman et al., 2003; Llinás, 1988; Perrier and Hounsgaard, 2000; Rekling et al., 2000). These nonlinearities include spike frequency adaptation (Powers et al., 1999; Sawczuk et al., 1995), postspike afterhyperpolarization (Granit et al., 1963; Kernell, 1965a; Vervaeke et al., 2006; Vogalis et al., 2003) and persistent inward currents (Heckman et al., 2008; Perrier et al., 2002; Rekling et al., 2000; Schwindt, 1973). In motoneurons increased conductance suppresses the slow afterhyperpolarization and plateau potentials mediated by persistent inward currents (Alaburda et al., 2005). Spike frequency adaptation is enhanced by increased membrane conductance in pyramidal neurons (Fernandez and White, 2010; Prescott et al., 2006), however, it is not known how an increase in conductance would affect adaptation in motoneurons.

Spike frequency adaptation influences the gain (i.e. the slope of the frequency-current relationship) in motoneurons – the gain decreases from an initial maximum to a lower steady-state value during adaptation (Buisas et al., 2012; Granit et al., 1963; Kernell, 1965a, 1965b; Sawczuk et al., 1995). Spike frequency adaptation is caused by multiple ion conductances (Miles et al., 2005; Powers et al., 1999); therefore, shunting of a motoneuron may differently affect the gain during earlier and later phases of adaptation. For example, tonic somatic membrane conductance increase had a divisive effect on the steady-state gain in CA1 pyramidal cells without affecting the initial gain (Fernandez and White, 2010).

Spike frequency adaptation is also related to the threshold for action potential generation – during repetitive firing the threshold gradually depolarizes with time while firing frequency decreases (Miles et al., 2005; Powers et al., 1999). It was suggested that increased conductance depolarizes the threshold potential and that

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Abbreviations: CA1, cornu ammonis 1 region of the hippocampus; ChAT, choline acetyl transferase; f-I, frequency-current relationship; f-V, frequency-voltage relationship; I_{rh}, rheobase current; I-V, current-voltage relationship

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this might be the cause of the increased adaptation (Fernandez and White, 2010; Prescott et al., 2006). However, increased membrane conductance did not change the threshold of rat oculomotor nucleus motoneurons (Torres-Torrelo et al., 2014).

In the present study we investigated how pharmacologically increased membrane conductance affects the firing frequency adaptation, the gain and the threshold for action potential generation in turtle spinal motoneurons.

2. Results

2.1. Spinal motoneurons recorded with sharp and patch electrodes

Spinal motoneurons (n=33) recorded using sharp and patch microelectrodes were identified and accepted for analysis as described in Section 4. For histological verification a subset of motoneurons recorded using patch microelectrodes were filled with biocytin. 5 out of 5 tested cells were ChAT positive (Fig. 1A).

The discharge properties of spinal motoneurons are related to their input conductance (Torres-Torrelo et al., 2014; Turkin et al., 2010). In sharp-electrode recordings input conductance of spinal motoneurons was 110.6 ± 49.8 nS (ranging from 32 to 226 nS; n=25) while in patch-electrode recordings input conductance was 23.3 ± 11.0 nS (ranging from 15 to 48 nS; n=8) (Fig. 1B). The average membrane conductance of motoneurons recorded with sharp electrodes was significantly (about 5 times) larger than conductance of motoneurons recorded with patch electrodes. This difference, at least partially, can be explained by the shunt introduced by sharp electrodes (Li et al., 2004a; Svirskis et al., 1997). In sharp-electrode recordings rheobase (1.34 ± 0.71 nA; n=25) was significantly larger than in patch-electrode recordings (0.33 ± 0.26 nA; n=8) (Fig. 1C).

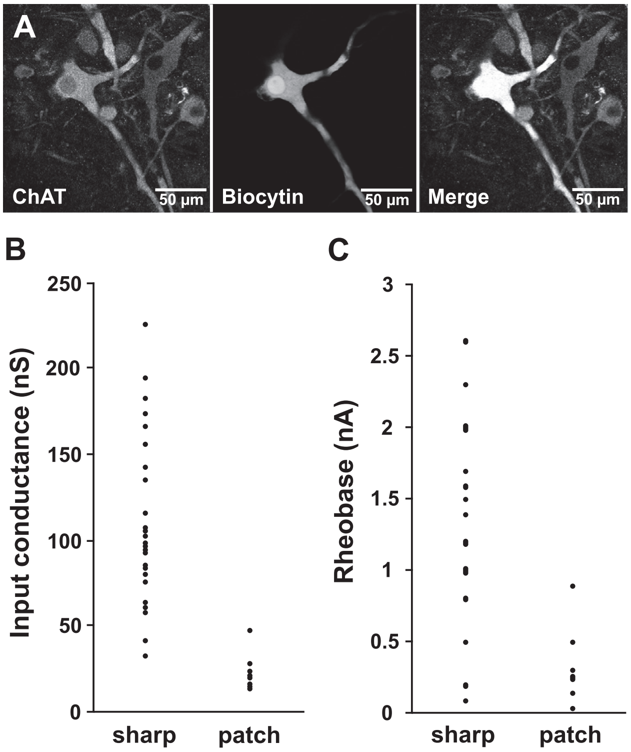

Fig. 1. Spinal motoneurons recorded with sharp and patch electrodes. (A) An example of histological verification showing that recorded neuron filled with biocytin is ChAT positive. Input conductance (B) and rheobase (C) values of motoneurons recorded using sharp and patch electrodes.

2.2. Muscimol increases membrane conductance and rheobase of motoneurons

The influence of increased membrane conductance on intrinsic response properties of motoneurons was tested using the extracellular application of the GABA_A receptor agonist muscimol. Muscimol caused a decrease in the amplitude of voltage responses to intracellularly injected current pulses (Fig. 2A). When motoneurons were stimulated with the same amplitude sustained suprathreshold current pulses, application of muscimol decreased firing frequency (Fig. 2B).

Muscimol significantly increased input conductance of motoneurons recorded using sharp electrodes by 40.6 ± 21.7% from 110.6 ± 49.8 nS to 152.8 ± 66.8 nS (n=25) (Fig. 2Ca) and of motoneurons recorded using patch electrodes by 49.9 ± 17.0% from 23.3 ± 11.0 nS to 35.1 ± 17.6 nS (n=8) (Fig. 2Cb). The resting membrane potential (−64.1 ± 3.5 mV) did not change significantly after the increase in membrane conductance (−64.7 ± 4.8 mV) in motoneurons recorded with sharp electrodes (n=25). In motoneurons recorded using patch electrodes increased conductance significantly hyperpolarized the resting potential from −60.1 ± 1.8 mV to −63.0 ± 3.4 mV (n=8). Thus, the activation of GABA_A receptors mainly increased membrane conductance without changing the resting potential substantially.

In sharp-electrode recordings increased membrane conductance caused a significant increase in rheobase from 1.34 ± 0.71 nA in control conditions to 2.10 ± 0.93 nA in the presence of muscimol (n=25) (Fig. 2Da). In patch-electrode recordings after the increase in membrane conductance rheobase significantly increased from 0.33 ± 0.26 nA to 0.87 ± 0.78 nA (n=8) (Fig. 2Db).

2.3. Increased membrane conductance increases the magnitude of spike frequency adaptation

Spike frequency adaptation is a well described property of motoneurons (Granit et al., 1963; Hounsgaard et al., 1988; Kernell, 1965a; Kernell and Monster, 1982; Sawczuk et al., 1995), caused by multiple ion conductances (Miles et al., 2005; Powers et al., 1999). An increase in membrane conductance can shunt some of the intrinsic conductances and therefore affect adaptation. To test the effect of increased conductance we evaluated the adaptation ratio before and after application of muscimol. In motoneurons recorded with sharp electrodes increased membrane conductance significantly increased the adaptation ratio from 3.6 ± 1.0 to 5.6 ± 2.2 (n=13) when motoneurons were stimulated with the same amplitude current pulse in control conditions and in the presence of muscimol (Fig. 3Ab). After the increase in conductance, the initial firing frequency was reduced by 17.1 ± 12.3% (from 122.8 ± 35.3 Hz to 104.5 ± 40.5 Hz) and the steady-state firing frequency was reduced by 43.6 ± 18.2% (from 36.1 ± 14.3 Hz to 20.9 ± 11.6 Hz). This indicates that increased membrane conductance decreases the steady-state firing frequency more than the initial frequency.

Spike frequency adaptation can depend on firing rate (Sawczuk et al., 1995). Increased membrane conductance reduced the firing rate (example in Fig. 2B) and the decrease in firing rate could be reflected in the modification of the adaptation ratio. To test if the increase in the adaptation ratio was caused by muscimol induced decrease in firing rate, we compared the adaptation ratio when firing rate was similar before and after application of muscimol. At first we compared the adaptation ratio when the initial frequency was similar before and after muscimol (104.3 ± 40.6 Hz and 104.5 ± 40.5 Hz, respectively; $P > 0.8$; n=13). In this case the amplitude of current pulse stimuli in control conditions was 0.5 ± 0.3 nA smaller than after application of muscimol. The

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