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ORIGINAL ARTICLE

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Extracellular divalent ions modulate TREK-2-like channel conductance in prefrontal pyramidal neurons in rats

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ABSTRACT

Background: The aim of the study was to investigate, with the use of the patch clamp technique, the dependence of the conductance of the TREK-2-like potassium leak channel in the medial prefrontal cortex pyramidal neurons on the presence of extracellular magnesium and calcium ions. It is suspected that TREK-2 channels regulate mood and may be associated with the pathophysiology of depression. Since magnesium and calcium deficiency contribute to depressive symptoms, we investigated how TREK-2- like channel pore properties change in the absence of divalent cations.

Results: Single-channel currents were recorded in a cell-attached configuration in enzymatically dispersed pyramidal neurons of the prefrontal cortex in rats. Spontaneous TREK-2-like channel activity was recorded either in the presence or absence of magnesium and calcium ions in extracellular solution. A significant increase in the inward channel conductance was observed when divalent cations were removed from the extracellular solution. Inward rectification was also increased when the bath temperature was raised to 34-37°C.

Conclusions: The study confirmed that the activity of TREK-2-like channels is affected by the presence of magnesium and calcium ions in the extracellular solution. Therefore, *in vivo,* the TREK-2-like channel may possibly participate in the prefrontal cortex dysfunction associated with the deficiency of divalent cations.

Key words: magnesium and calcium homeostasis; depression; TREK-2 channels; leak potassium channels; patch-clamp

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Introduction

Dysfunction of the prefrontal cortex occurs in many neuropsychiatric diseases. Some of these, such as depression, schizophrenia, mania, or anxiety disorders, may accompany magnesium and calcium homeostasis dysregulation [1–5]. Neuronal excitability is controlled by the activity of ion channels. Therefore, understanding the impact extracellular ions have on the kinetics of ion channels in the prefrontal cortex pyramidal neurons may be important.

TREK-2 is a member of the two-pore domain channels that form a family of the leak (or background) K^+ channels. The main function of leak potassium channels is to stabilize the resting membrane potential, which affects the excitability of neurons. It was recently reported that TREK-2-like channels are present in pyramidal cells in the prefrontal cortex of rats [6]. Thus, the excitability of prefrontal pyramidal cells can depend on the activity of TREK-2-like channels, not only in physiological but also in pathophysiological conditions.

Although the TREK-2 channels are distinct from voltage-activated channels (Kv family) or inward rectifier channels (Kir family), to a certain degree their kinetics resemble those of Kv and Kir channels. The probability of opening (Po) of TREK-2 channels increases with membrane depolarization [6], and the inward conductance exceeds the outward conductance (the latter does not result from asymmetrical ion concentration) [7]. In our work, we examined how the rectifying properties of the TREK-2-like channel depend on the presence of divalent ions, such as Mg^{2+} and Ca^{2+}, in extracellular solution in temperature close to physiological.

Because it is believed that TREK channels family may be involved in the pathogenesis of depression [8–10], understanding the influence of Mg^{2+} and Ca^{2+} ions on the activity of TREK-2 channels may contribute to the understanding of the mechanisms

leading to depression in hypomagnesaemia and hypocalcaemia.

Methods

The experimental procedures used in this study conform to institutional and international guidelines for the ethical use of animals. The experiments were performed on young (20-days-old) male Wistar rats. Preparation of the slices and the procedure of enzymatic tissue dissociation were performed as described previously [6]. Voltage-clamp recordings were performed in the cell-attached configuration using an Axopatch 1D amplifier (Axon Instruments) and pClamp 9.0 software. Data were filtered at 5 kHz, digitized at 50 kHz and stored on a computer.

The cells were perfused with bath solution containing (in mM): KCl (145), $CaCl_2$ (2), $MgCl_2$ (2), glucose (10), $LaCl_3$ (0.0015) and HEPES (10). The pH of the solution was adjusted to 7.4 using NMDG, and the osmolality was adjusted to 330 mOsm using sucrose. The pipette solution contained (in mM): potassium acetate (130), HEPES (10), $MgCl_2$ (2), $CaCl_2$ (2), TTX (0.005), and $LaCl_3$ (0.0005). The pH of the solution was 7.4 and was adjusted using NMDG; its osmolality was 280–300 mOsm. In experiments with Mg^{2+}-free solution, the composition of the pipette solution was as follows: potassium acetate (122), HEPES (10), $CaCl_2$ (0.1), EGTA (0.1), KCl (8) and TTX (0.005), and $LaCl_3$ (0.0005).

The unitary current amplitudes were determined from all-point amplitude histograms fitted with Gaussian functions. Only full openings, i.e. openings with maximum amplitude, were included in the current analysis.

To evaluate statistical significance between four groups, we used one-way *ANOVA.* If not stated otherwise, p values refer to Tukey's post-hoc tests.

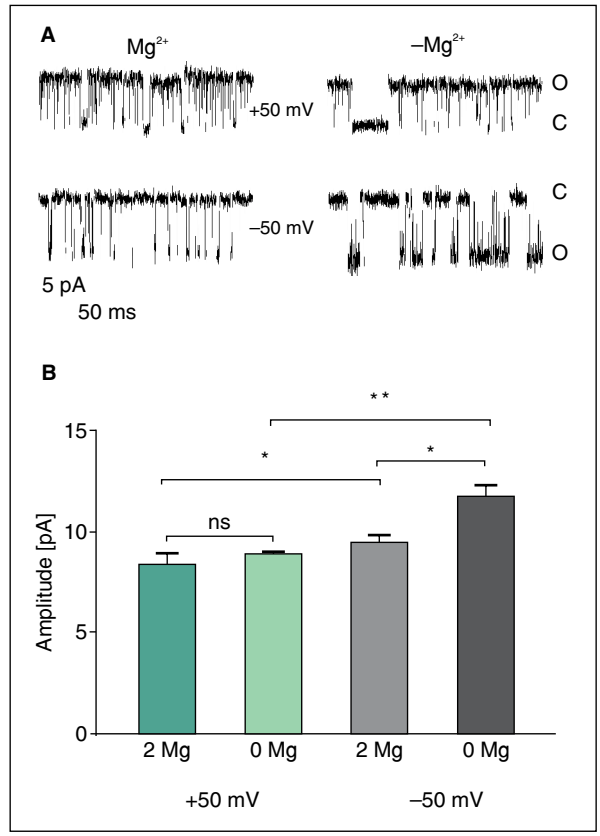

Figure 1. Effect of extracellular Mg^{2+} on potassium TREK-2 like currents.
A) Single channel recordings of TREK-2 like currents with 2 mM or without Mg^{2+} in the pipette solution at two membrane potentials. Positive current represents outward potassium current, negative — inward current; c — closed channel, o — open channel; high pass filters set at 5 kHz. **B)** The mean unitary current (absolute value) measured at depolarization (+50 mV) and hyperpolarization (-50 mV) of cell membrane with 2 mM or without Mg^{2+} in the pipette solution; *p < 0.02, **p < 0.01, ns p > 0.88.

Results

In the study presented here, we recorded single-channel potassium currents in the pyramidal prefrontal cortex of rats. Recordings were carried out in high extracellular potassium concentration in cell-attached configuration. In the study, we investigated the effect of divalent ions and temperature on the amplitude of TREK-2-like currents that appeared spontaneously in membrane depolarization and hyperpolarization. Identification of the channel as a TREK-2-like channel in its non-canonical isoform (b or c) has been described previously [6] and was performed on the basis of pharmacological and biophysical properties and with the use of immunofluorescence together with confocal microscopy. Non-canonical isoforms result from alternative splicing [7].

Channel rectifying properties in the presence (2 mM Mg^{2+} and 2 mM Ca^{2+}) and the absence of divalent ions (0 Mg^{2+}, 0.1 mM Ca^{2+}, 0.1 mM EGTA, a trace quantity of calcium ions were present) at room temperature are shown in Fig.1. A voltage of +50 mV (depolarization) or -50 mV (hyperpolarization) was applied to cell membranes and single-channel currents were recorded at room temperature. In both variants, the amplitude of the inward current evoked by negative potential was higher that of the outward current. Single channel conductance values *S* were as follows (subscribe D — depolarization, H — hyperpolarization, MgCa — presence of divalent ions, EGTA — 0 mM Mg^{2+}):

$$S_{D,MgCa} = 167.2 \text{ pS} \pm 10.5 \text{ pS} \ (n = 7)$$
$$S_{H,MgCa} = 188.1 \text{ pS} \pm 8.2 \text{ pS} \ (n = 7),$$
$$S_{D,EGTA} = 177.2 \text{ pS} \pm 1.9 \text{ pS} \ (n = 4),$$
$$S_{H,EGTA} = 234.4 \text{ pS} \pm 1.1 \text{ pS} \ (n = 4).$$

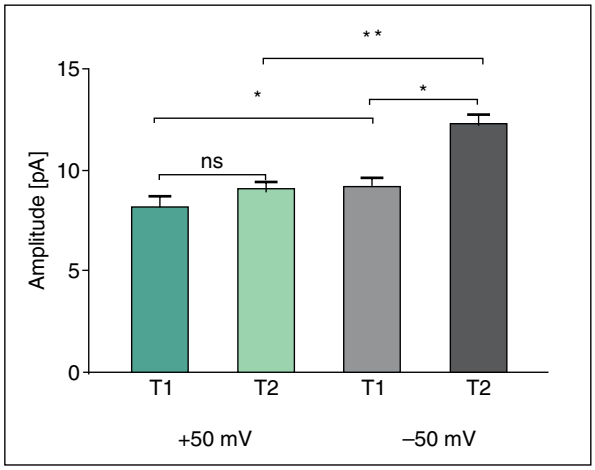

Figure 2. Effect of temperature on potassium TREK-2 like currents in the presence of 2 mM Mg^{2+} in the pipette solution. T1 21–25°C, T2 34–37°C. *p < 0.02, **p < 0.004, ns p > 0.036

The obtained results indicate that the studied channel had rectifying properties, i.e. outward conductance $S_{D\,MgCa}$ was significantly lower than inward conductance $S_{H,MgCa}$ (p = 0.0037) and that properties of inward conductance S_H were dependent on presence of divalent cations ($S_{H,MgCa}$ vs $S_{H,EGTA}$, p = 0.008; $S_{D,\mathrm{MgCa}}$ vs $S_{D,EGTA}$, non-significant).

Next, we measured the conductance of the channel at room temperature T1 (~25°C) and at higher temperature T2 (temperature 34°–37°C) in the presence of divalent ions. Unfortunately, the instability of the seal at higher temperature in the absence of divalent cations made recording quality under these conditions insufficient for analysis. Single-channel conductances S were as follows (subscribe D — depolarization, H — hyperpolarization, T1 — room temperature, T2 — high temperature):

$$S_{D,T1} = 167.2\ \mathrm{pS} \pm 10.5\ \mathrm{pS}\ (n = 7),$$
$$S_{H,T1} = 188.1\ \mathrm{pS} \pm 8.2\ \mathrm{pS}\ (n = 7),$$
$$S_{D,T2} = 186.4\ \mathrm{pS} \pm 8.3\ \mathrm{pS}\ (n = 7),$$
$$S_{H,T2} = 250.5\ \mathrm{pS} \pm 10.6\ \mathrm{pS}\ (n = 7).$$

The conductances marked with the symbols $S_{D,T1}$ and $S_{H,T1}$ are the same as conductances $S_{D,MgCa}$ and $S_{H,MgCa}$ respectively. The results indicate that the increase in temperature from T1 to T2 increased the inward conductance by 62 pS ($S_{H,T1}$ vs. $S_{H,T2}$, p < 0.006), while the increase of the outward conductance was statistically insignificant ($S_{D,T1}$ vs. $S_{D,T2}$, p = 0.36). This means that the temperature has a significant influence on the inward conductance (Fig. 2).

Discussion

In the presented work, we measured single-channel potassium currents with a large conductance (> 150 pS) in rat pyramidal prefrontal cortex neurons. These currents have been previously identified as conducted by TREK-2-like channels in its non-canonical form b or c [6]. We performed experiments with two different solutions in a pipette (pipette solution corresponds to the extracellular environment). One solution contained divalent ions at a concentration of 2mM (2mM Mg^{2+}, 2mM Ca^{2+}), the other solution was Mg^{2+} -free and contained a trace concentration of Ca^{2+} ions (0.1 mM Ca, 0.1 mM EGTA).

Our most important result is the confirmation of the effect of extracellular divalent ions on the inward conductance of the recorded channels and presenting that the inward rectification depends on temperature: the difference between outward and inward conductance is bigger in higher temperature (Fig. 1). The effect of removing extracellular Mg^{2+} is similar to the effect of increasing temperature. In the absence of divalent ions in the extracellular solution, the amplitude of the inward current and thus the inward conductance of the channel increased (Fig. 1). The influence of extracellular magnesium ions on channel conductance can be analogous to the blocking mechanism of Kir channels by intracellular magnesium ions [11, 12]. Most likely, magnesium ions from the extracellular side penetrate into the entrance region of the channel pore and block the potassium current. That penetration can be impaired at higher temperature. Publications indicate that extracellular Ca^{2+} does not usually block potassium channels. An exception is the HERG channel [13]. The mechanism of action of divalent ions was not, however, the subject of the work presented here.

Our research suggests that the level of divalent ions (Mg^{2+}, Ca^{2+}) in extracellular solution may have a significant effect on the functioning of TREK-2-like potassium channels *in vivo*. Because these channels are expressed in the prefrontal pyramidal cells, the TREK-2-like channels possibly affect the functions of the prefrontal cortex in a manner dependent on the concentration of Mg^{2+} or Mg^{2+} and Ca^{2+}.

There are studies suggesting that the activity of TREK-1 and TREK-2 ion channels can affect mood regulation and showing that selective serotonin reuptake inhibitors affect the activity of TREK-1 and TREK-2 channels [8–10,14–16]. These channels are therefore a promising goal of drug action in new therapies for the treatment of depressive disorders [17].

A variety of neuromuscular and psychiatric symptoms, including depression, was reported in dysregulation of magnesium homeostasis [2, 3, 5]. Although the antidepressant activity of magnesium is mostly ascribed to the activity of the NMDA receptor, the mechanism of the antidepressant effect of magnesium is not yet fully understood [2]. Our work suggests that in the conditions of disturbed homeostasis of divalent ions, TREK-2 channels can interfere with the functioning of the cortex

pyramidal cells. Therefore, it would be important to examine the role of TREK-2 channels in depression accompanying hypomagnesemia in future studies.

Conclusions

The activity of TREK-2-like channels is affected by the presence of magnesium and calcium ions in the extracellular solution. *In vivo* the TREK-2-like channel may possibly participate in dysfunctions associated with the deficiency of divalent cations like depression, schizophrenia, anxiety.

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