INTRODUCTION

Signal generation, propagation and transmission between cells are mediated by Voltage-Gated ion Channels, a class of transmembrane proteins that are activated by changes in electrical membrane potential. Among VGC, Kv family is one of the most studied and its direct involvement in action potential generation and propagation is well recognized. Membrane depolarization promotes structural re-orientation of the Voltage-Sensor Domains enabling gate displacement and channel opening. Physiologically, ionic currents flowing through the cellular membrane change the membrane potential and modify Kv opening.

In this work, we assess the effect of a weak and low frequency magnetic fields on VGCs dynamics. The effects of the exposure to the magnetic field on the dynamics of Kv1.3 channels expressed in mammalian transfected CHO-K1 cells were evaluated by electrophysiological measurement of the channel conductance. In particular, we attempted to discriminate between two possible mechanisms: a direct alteration in the dynamics of ion fluxes (instantaneous) or an indirect effect due to a change in the metabolic system of the cell (much slower, i.e. occurring in the seconds to minutes time range).

MATERIALS AND METHODS

CHO-K1 cells (ATCC, USA) were maintained in F-12 Nutrient Mixture -Ham- supplemented with 10% (v/v) heat-inactivated FBS, 10 μg/ml penicillin and 10 μg/ml streptomycin, in a humidified atmosphere at constant temperature (37° C) and CO2 concentration (5% v/v). About 2 × 10^3 cells/cm^2 were plated onto standard petri dishes. Two days after plating, two plasmids (2 μg each), one coding for the Kv1.3 channel and one for EGFP (used as a reporter), were transfected with Lipofectamine™ 2000 (Life Technologies).

The extracellular solution was composed by (in mM): 135.0 NaCl, 5.4 KCl, 1.0 MgCl2, 1.8 CaCl2, 10.0 glucose, 5.0 HEPES. Cells were immersed in this solution after being extracted from the incubator. The intracellular solution was composed by (in mM): 140.0 KCl, 2 MgCl2, 5.0 EGTA, 5.0 HEPES. Solutions were buffered with 1 N NaOH or 1N KOH if required, to reach a pH of 7.4 or 7.3 respectively.

Micropipettes were pulled from glass borosilicate capillaries and coated with Sylgard® to reduce pipette capacitance. The resistance of the tip ranged from 2.8 MΩ to 3.8 MΩ. Ag/AgCl electrodes were used for the pipette and bath ground reference.

Experiments were performed under a fluorescence microscope (cf. Fig. 3) to visualize transfected cells and whole-cell potassium conductance was measured 24h-48h after transfection using an Axopatch 200B amplifier (Molecular Devices, USA). Once the cell was patched, the holding potential was set to be V_h = −90 mV (close to the Nerst potential of K+ ions) to ensure a full removal of channels inactivation. Voltage steps (±100 mV in amplitude and 200 ms duration) were applied as a test pulse to induce Kv1.3 activation, while measuring the corresponding whole-cell current under magnetic field (B) exposure.

B (f = 20 Hz, intensity 268 ± 3 μT) was applied through a solenoid wound on a plastic support (8 cm diameter) having vertical axis, perpendicular to the adhesion substrate (i.e. the bottom of the petri dish). A commercial pulse generator was used to provide a current to the solenoid and the resulting magnetic field was measured by a Hall-effect magnetic field meter (Gauss meter Model 907, Magnetic Instrumentations Inc.).

Recordings were performed before the application of the B field, after 10”, 1’ and 3’ from the start of exposure and after 10”, 1’ and 3’ from the field removal. A leak-current subtraction was applied to raw traces using WinWCP (Strathclyde Electrophysiology Software, Glasgow, UK) and the average value of K+ currents was computed at the steady-state. Additional measures were performed, as a control, without exposing the cells to the magnetic field but following the same experimental procedure (all the recording steps were maintained but the solenoid was placed away from the petri dish, with d > 50 cm).

RESULTS AND DISCUSSION

Signals from a total of 58 cells were collected: of these, 38 cells were exposed to the B field whereas the other 20 cells were used as a control. Fig. 1 reports the whole-cell K+ current traces in the absence, during and after B exposure. From those exposed to the field, 9 cells (24%) did not show significant changes on their membrane conductance g_k after the application of the field; 11 cells (29%) showed a maximum positive change on their membrane conductance after 1’ from B application; 3 cells (8%) presented a peak of conductance after 10” and 4
(10%) after 3’. The remaining 11 cells (29%) showed a significant increment in $g_K$ only after the field removal (3 cells after 10”, 3 cells after 1’ and 5 cells after 3’). From these findings it seems clear that the B field produces noticeable effects on the membrane conductance only after some time (ranging from 30” to 1’) from its application, with effects persisting also for minutes after its removal.

Some experiments were performed to analyze a possible “acute” (i.e. instantaneous) effect of B, but no significant effects were found within the first 15” of exposure (not shown). Thus, overall, the observed responses were apparently not compatible with a direct effect of the magnetic field on ion fluxes across the potassium channel, for example due to modifications of electrostatic ion-protein interactions or of the motion of ions in solution [1]. On the contrary, a rearrangement of surface charges on the cell membrane following the exposure to the field seems more plausible [2].

As suggested by Tonini et al. [3], this effect would involve a B-dependent activation of complex biochemical pathways inside the cells, causing also an enhancement of inward Ca$^{2+}$ currents through Ca$^{2+}$ channels.

The two distributions of the maximum changes in $g_K$ of cells exposed and not exposed to the B field are shown in figure 2: in both cases, most of the cells showed percentage changes in membrane conductance between -15% and 10% and a few (6 cells, 16% of the total) exhibited large variations in $g_K$ (>15%). Intriguingly, only cells exposed to the magnetic field were displaying a tendency to a $g_K$ increase, with a subpopulation of four cells (11% of a total) showing an enhancement of conductance in the order of 35% or higher. Additional experiments will be required for a thorough statistical evaluation of this observation.

CONCLUSIONS

The results presented in this work suggest that weak and low-frequency magnetic fields can affect K$_v$ channels conductance, causing a persistent increase within about one minute from exposure. Further experiments may help to understand whether the distribution of affected cells is “bimodal”, with a Gaussian distribution centered on a mean of about 5% conductance change (cf. Fig. 2) and a second subpopulation very responsive to the B field.

Moreover, further cell imaging experiments (e.g. with calcium) will help to shed light on the possible involvement of intracellular signaling and metabolic events in K$_v$ channels modulation by weak magnetic fields.

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