Effects of gadolinium and static magnetic fields on MscL channel activity

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All biological tissues are highly penetrable for static magnetic fields (SMF). There are a number of hypotheses concerning the cellular and/or subcellular target of these fields. One possibility is that they target the cell membrane. It was shown that applying a SMF of 80 mT affected the open probability (P_o) and gating of the bacterial **M**echano**s**ensitive **c**hannel of **L**arge conductance (MscL) reconstituted into liposomes (Hughes *et al.*, 2005). Since phospholipid molecules possess diamagnetic anisotropy (Rosen, 2003), the SMF effect on MscL could originate from the reorientation of the lipid molecules perpendicularly to the direction of the magnetic field. Taking into account that thousands of phospholipid molecules form well ordered arrays in the bilayer the effect of SMF thus becomes amplified affecting the embedded MscL protein. Another possible effect of SMF could be *via* membrane-bound ions, such as Ca^{2+} (Del Moral & Azanza, 1994). To test this hypothesis we examined if SMF could modulate the ability of Gd^{3+} ions (non-specific blocker of mechanosensitive channels (Hamill & McBride, 1996)) to inhibit MscL gating, since Gd^{3+} ions interact with phospholipid molecules in a similar way as Ca^{2+} ions (Ermakov *et al.*, 2001).

Single channel patch-clamp experiments were carried out using the MscL channels reconstituted into liposomes and effect of Gd^{3+} on MscL activity was recorded. The results showed that Gd^{3+}, in a dose-dependent manner, caused an increase in the negative pressure required to open the MscL channels. 50 μM Gd^{3+} in the bath partially blocked the MscL channel, whereas 400 μM Gd^{3+} blocked the channels completely. Gd^{3+} also prolonged the duration of the single channel openings by decreasing the frequency of the channel opening and reducing channel flickering.

Next we studied the effect of SMF on the MscL activity and MscL block by Gd^{3+}. Negative pressures of 40-50 mmHg were required to stretch liposome patches and activate the MscL channels. Only patches were examined which exhibited stable channel activity during the initial 5-7 minutes of an experiment. A rare-earth NdFeB magnet was positioned at a distance of 2 mm from the tip of the pipette. The estimated strength of SMF was 400 mT. Application of the SMF had a two-fold effect on the channel activity: (1) a decrease of the open probability NP_o (N, unknown number of channels in a patch) during application of the SMF to 70.6±8.3% (mean±S.E., n=10) of the initial steady-state level before the application of SMF; and (2) an increase of NP_o upon removal of the SMF to 119.0±10.8% (n=10). The effects of the SMF were slowly developing over approximately 10 minutes upon application /release of the SMF. The time-dependence of the SMF effect may be explained by formation and destruction of ordered phospholipid clusters in the bilayer. Variability in the extent of the observed effects in our experiments might be due to the fact that the patch membrane is not flat when suction is applied to the pipette (Sukharev *et al.*, 1999), so that the peripheral and central parts of a patch are at different angles to the SMF vector. In most of the examined patches a partial blockade of the MscL activity by 50 μM of Gd^{3+} increased in the presence of SMF. After removal of SMF the channel activity recovered to the previous level and often increased further regardless of the presence of Gd^{3+} ions. In some patches the channel activity did not increase after the removal of SMF, but had already done so in its presence. Our results suggest that ordering of phospholipid molecules in the bilayer by SMF could cause a displacement of Gd^{3+} ions bound to phospholipid molecules due to the electrostatic repulsion between the ions, which resulted in reduction of the MscL channel block by Gd^{3+}.

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