Eur Biophys J (2007) 36:95–105
DOI 10.1007/s00249-006-0109-z

ARTICLE

Modulation of channel activity and gadolinium block of MscL by static magnetic fields

Evgeny Petrov · Boris Martinac

Received: 17 July 2006 / Revised: 18 September 2006 / Accepted: 10 October 2006 / Published online: 7 November 2006

Abstract The magnetic field of the Earth has for long been known to influence the behaviour and orientation of a variety of living organisms. Experimental studies of the magnetic sense have, however, been impaired by the lack of a plausible cellular and/or molecular mechanism providing meaningful explanation for detection of magnetic fields by these organisms. Recently, mechanosensitive (MS) ion channels have been implied to play a role in magnetoreception. In this study we have investigated the effect of static magnetic fields (SMFs) of moderate intensity on the activity and gadolinium block of MscL, the bacterial MS channel of large conductance, which has served as a model channel to study the basic physical principles of mechanosensory transduction in living cells. In addition to showing that direct application of the magnetic field decreased the activity of the MscL channel, our study demonstrates for the first time that SMFs can reverse the effect of gadolinium, a well-known blocker of MS channels. The results of our study are consistent with a notion that (1) the effects of SMFs on the MscL channels may result from changes in physical properties of the lipid bilayer due to diamagnetic anisotropy of phospholipid molecules and consequently (2) cooperative superdiamagnetism of phospholipid molecules under influence of SMFs could cause displacement of Gd^{3+} ions from the membrane bilayer and thus remove the MscL channel block.

Keywords MscL · SMFs · Gadolinium · Patch-clamp · Bacteria

E. Petrov · B. Martinac (✉)
School of Biomedical Sciences, University of Queensland,
St Lucia, Brisbane, QLD 4072, Australia
e-mail: b.martinac@uq.edu.au

Abbreviations

MscL	Mechanosensitive channel of large conductance
SMFs	Static magnetic fields

Introduction

Behavioural experiments have established that diverse biological species are able to perceive magnetic fields as weak as the geomagnetic field of the Earth (Gould 1985). The Earth's magnetic field is known to influence the behaviour and orientation of a variety of organisms including magnetotactic bacteria (Blakemore 1975), marine molluscs (Lohmann and Willows 1987), honey bees (Walker and Bitterman 1985) and homing pigeons (Walcott and Green 1974), which indicates the existence of magnetoreception as another form of sensory transduction mechanism in living organisms.

Since biological tissues and organs are highly transparent to static magnetic fields (SMFs), a number of hypotheses have been considered suggesting that target(s) of SMFs could reside on subcellular, cellular, tissue or organ level (Azanza and del Moral 1994, 1996). One possibility is that SMFs may act upon cellular membranes—the main structure where external signals are received, processed and transmitted intracellularly. If biophysical properties of the cellular membrane can be affected by SMFs, then barrier or transport function of cell membranes will be up- or down-regulated under the influence of SMFs. Since phospholipid molecules possess diamagnetic anisotropy (a difference between parallel and perpendicular magnetic susceptibility) they align and reorient in the presence of SMFs (Braganza

et al. 1984). Consequently, as a major component of biological membranes the lipid bilayer presents a possible target of SMFs. Given that mechanosensitive (MS) ion channels are membrane proteins, whose function is closely related to the physical properties of the lipid bilayer (Hamill and Martinac 2001; Patel and Honoré 2001; Martinac 2004; Kung 2005) these channels are good candidate molecules to detect mechanical forces exerted onto biological membranes under the influence of SMFs (Kirschvink 1992). As a special class of ion channels MS channels are capable of transducing mechanical stimuli into electrical or biochemical signals, thus providing molecular basis of mechanosensory transduction in biological cells. They have been implicated to function as mechano-electrical switches in diverse physiological processes including touch, hearing, blood pressure regulation, mechanoelectric feedback in the heart, turgor control in plant cells and osmoregulation in bacteria (Hamill and Martinac 2001; Niu and Sachs 2003; Martinac 2004; Tan et al. 2004; Sukharev and Corey 2004). As indicated by this study and a previous study showing that application of SMFs could affect the open probability and gating of MscL reconstituted into liposomes (Hughes et al. 2005) MS channels could also play a role in magnetoreception.

In this study we show that SMFs not only affect the open probability of MscL reconstituted into artificial liposome bilayers of different thickness but also that the MscL block by gadolinium, a well-known blocker of MS channels (Hamill and McBride 1996), can be modulated by application of external SMFs.

Materials and methods

Liposome preparation and MscL protein reconstitution

The method used for the MscL protein purification and liposome reconstitution was as previously described (Häse et al. 1995; Hughes et al. 2005). Azolectin (soybean lecithin; Sigma P-3644) containing the carbon chains C16:0, C18:0, C18:2, and C18:3 (of which the 18:2 comprise 60%) with choline, ethanolamine and inositol as the main phospholipid head groups, 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (PC18) and 1,2-Dipalmitoleoyl-sn-Glycero-3-Phosphocholine (PC16) (Avanti Polar Lipids Inc., USA) were used to prepare liposomes suitable for patch-clamp recording from reconstituted MscL channels. Briefly, 2 mg of a phospholipid was dissolved in chloroform and dried in a glass tube by a nitrogen flow for 15 min until a thin lipid film is formed on the wall of the tube. A dehydration/rehydration (D/R) buffer (200 mM KCl, 5 mM HEPES, pH = 7.2 adjusted with 1 M KOH) of 2 ml was added to the tube. Resuspended lipids were vortexed for several minutes followed by bath sonication (Unisonics Pty Ltd, Australia) for approx. 10 min until clarity. MscL protein, isolated as a GST–MscL fusion protein using glutathione sepharose beads and purified by a thrombin cut (Häse et al. 1995) was dissolved in a phosphate buffer containing 1% octylglucoside (OG) and added at 1:4,000 protein:lipid (w/w) ratio to the liposome suspension. After 1 h incubation with liposomes BioBeads (Bio-Rad, USA) were added (approx 0.5 g) to remove the detergent and promote the MscL liposome reconstitution by leaving the proteoliposome suspension on a rocker for another 3 h at room temperature. After the BioBeads were left to settle on the bottom of the tube the proteoliposome suspension was centrifuged at 90,000 rpm for 30 min in a TL-100 desktop centrifuge (Beckman). The supernatant was discarded and the remaining pellet was resuspended in 40 µl of the D/R buffer. The resuspended pellet was spotted in 20 µl aliquots on a clean glass slide, which was put in a desiccator and left overnight at 4°C. Lipid spots were rehydrated next morning in 15 µl of D/R buffer for patch-clamp recording. If several slides were prepared simultaneously they were put in 25 ml flasks with a small quantity of desiccant and stored at –85°C. The frozen slides could be used in experiments for up to 2 months after defrosting them and rehydrating the lipid spots. After storage at –85°C the reconstituted MscL channels remained fully functional when examined by the patch clamp.

Patch-clamp recording from proteoliposomes exposed to magnetic fields

All recordings were done several days after the liposome rehydration step, usually on a second day but no later than the sixth day. All experiments were done using an Axopatch 1D patch-clamp amplifier (Axon Corp., USA). Pipettes were pulled from borosilicate glass capillaries (Drummond Scientific Company, USA) on P87 pipette puller (Sutter Instruments, USA) and had a resistance of 3–5 MΩ. Recording solution contained 200 mM KCl, 40 mM $MgCl_2$ and 5 mM HEPES, pH = 7.2 adjusted with 1 M KOH. A small quantity of rehydrated proteoliposomes was put in a recording chamber and left for 15 min to settle to the bottom until pauci- or unilamellar blisters suitable for patch clamping were formed (Delcour et al. 1989). All recordings were obtained from excised liposome patches containing several MscL channels. Ion currents through MscL channels activated by applying negative

pressure to recording pipettes were digitized at 5 kHz and filtered at 2 kHz frequency. When liposome patches prepared from azolectin or PC18 lipids were examined, a potential of +30 mV was applied to a patch pipette. Liposome patches made of PC16 lipids were examined at a pipette potential of +10 mV. Pipette pressure was monitored by a piezoelectric pressure transducer (Omega Engineering, Stamford, USA) and recorded in mmHg. Acquisition and data analysis were done using pClamp 9.0 (Axon Corp., USA) and Origin 6.0 (OriginLab, USA) software. As a source of SMFs the rare-earth NdFeB magnet was used. The strength of SMFs was measured from poles using handheld Gauss meter (Model 4048, Omni Controls Inc, FL, USA). Magnet was attached to a stand allowing for its movement in any direction. SMFs were applied to a membrane patch as shown in Fig. 1.

Open probability of MscL channels (NP_o) was calculated using the equation:

$$\mathrm{NPo} = I/(i\Delta t) \quad (1)$$

where NP_o is the open probability of unknown number of channels in a liposome patch, I [ms·pA] is the total current recorded during the time interval Δt [ms], and i [pA] is the amplitude of the single MscL channel current. Statistical data analysis was done using Student's t test.

Results

Structural conformations of MscL and gramicidin channels were shown to be affected by the thickness of the lipid bilayer in which these channels were reconstituted (Kloda and Martinac 2001; Perozo et al. 2002a; Martinac and Hamill 2002) suggesting that hydrophobic mismatch is a physical parameter determining the gating in these MS channels. To test a hypothesis that hydrophobic mismatch could also play a role in the way SMFs affect activity of MS channels reconstituted into artificial bilayers we have examined the MscL channel activity in excised liposome patches made of azolectin, PC18 or PC16 lipids in the presence or absence of externally applied SMFs (Fig. 1a). Except for PC16 liposome patches and occasionally also PC18 patches in which MscL was spontaneously active, application of negative pressure to liposome patches made of azolectin or PC18 lipids was necessary to activate the channel. SMFs were applied by slowly moving the patch pipette towards the magnet to approx. a distance of 2 mm from the magnet (Fig. 1a) where SMFs of >400 mT were effective (Fig. 1b). In control experiments the channel activities were recorded in apparent absence of the magnetic fields by moving the patch pipette 20 mm away from the magnet where SMFs were reduced to <30 mT (Fig. 1b).

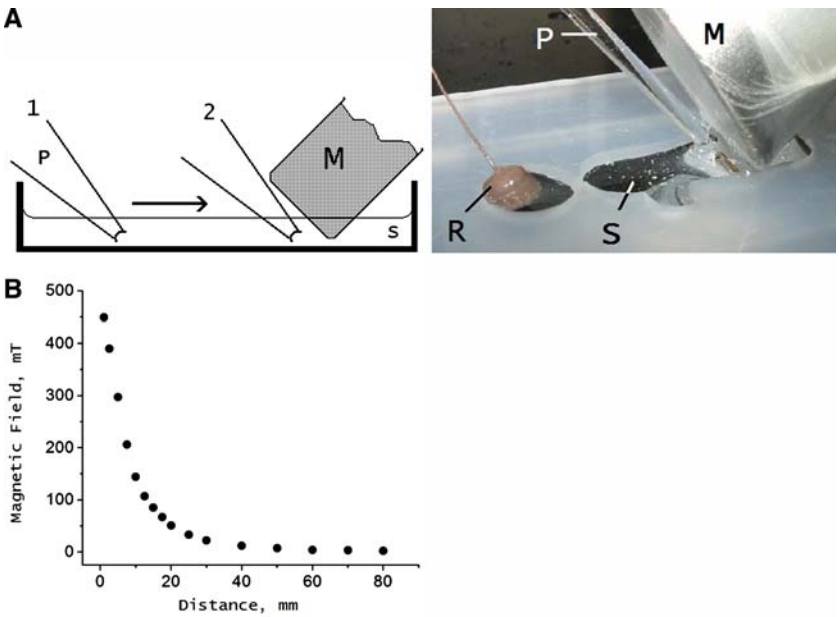

Fig. 1 Setup for application of SMFs to liposome membrane patches reconstituted with MscL. **a** *Left* cartoon and a photograph of the recording chamber (S), magnet (M), reference electrode (R) and patch pipette (P) before (*1*) and during (*2*) the SMF application. At position *1* the pipette is at a distance of 25 mm from magnet where the SMF is ~30 mT. At position *2* the pipette is about 2 mm close to the magnet where the SMF ≥400 mT. *Right* photograph showing the chamber, magnet and patch pipette during recording. **b** The SMF intensity measured as a function of the distance from the pole of the magnet

The effect of SMFs on MscL reconstituted in azolectin liposomes

In this set of experiments MscL protein was reconstituted into bilayers made of azolectin (soybean extract), which is a mixture of phospholipid molecules with different length of hydrocarbon chains. In terms of their lipid composition liposomes prepared from azolectin resemble somewhat the natural biomembranes (see Materials and methods). In these liposomes SMFs affected the MscL activity in three different ways (Fig. 2): (1) Open probability of *N* channels in the patch (NPo) remained unaffected in presence of the SMF, but it increased once the SMF was removed, (2) NPo decreased in presence of the SMF, but remained unchanged upon removal of the SMF, and (3) NPo decreased in presence of the SMF and subsequently increased when the SMF was removed. Common to all the observed effects of the SMF on the MscL activity was that the NP_o in presence of the SMF was on average lower compared to the NP_o recorded in absence of the SMF. There was always a delay in the onset of this effect and the channel activity exhibited slow recovery upon removal of the SMF. This finding is consistent with the results of a previous study showing that the overall effect of SMFs on the MscL channel activity consisted in reduction of the channel open probability (Hughes et al. 2005). The observed variability in our experiments might have been due to the fact that the orientation of liposome patches could

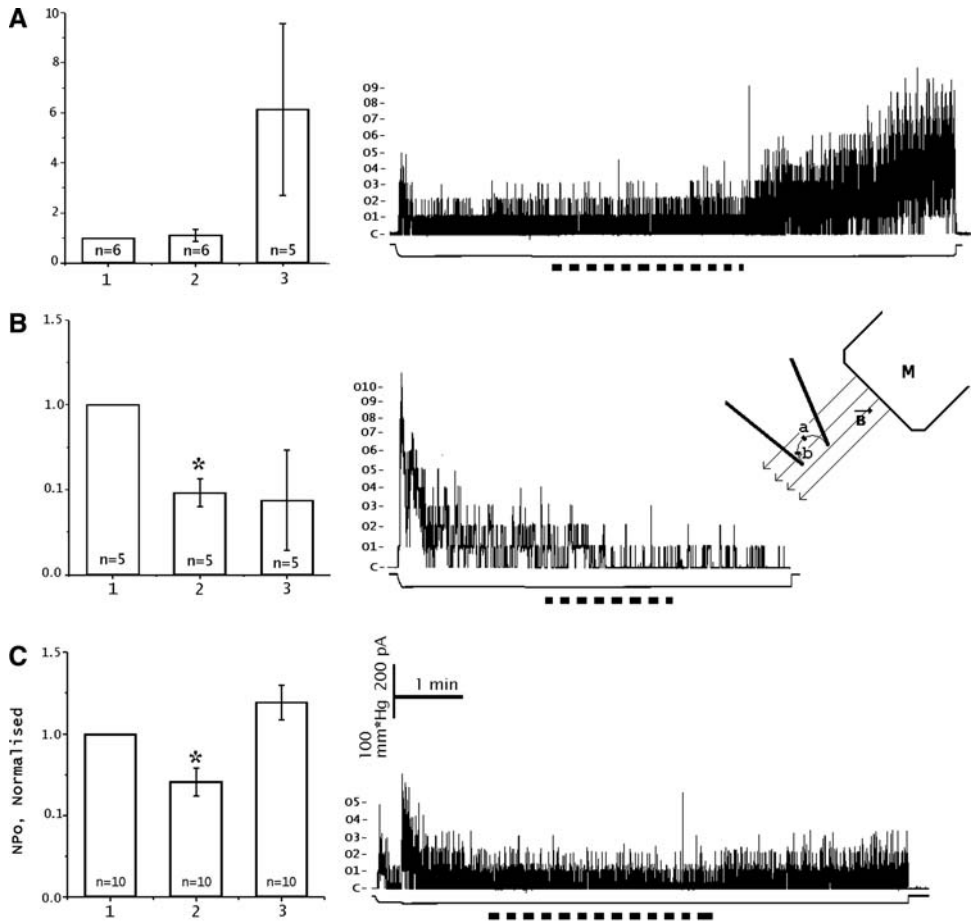

Fig. 2 Effect of SMFs on the MscL activity in azolectin liposomes. Activity of MscL was observed before (*1*), during (*2*) and after (*3*) the SMF application to liposome patches. *Left* a tree-fold effect was observed: **a** increase in the channel activity after removal of SMF, **b** decrease in the channel activity in the presence of SMF, and **c** decrease in the channel activity in the presence of SMF and increase after its removal. The *bars* indicate mean ± SE of *n* number of recordings. The data obtained in the presence of SMFs are statistically different from the control shown in **a** ($P \leq 0.05$). *Right* selected traces of current recordings of MscL reconstituted into azolectin liposomes. Negative pressure and duration of the SMF application (*dashed line*) to the liposome patches are shown *below* the current traces. *Inset* shows a patch configuration in which the central and peripheral parts of the patch membrane containing two channels (*a* and *b*) are at different angles to the SMF vector *B*. *M* symbolizes the magnet

differ from experiment to experiment, such that the peripheral and central parts of a patch are at different angles to the SMF vector as shown in the inset of Fig. 2 (Sukharev et al. 1999).

The effect of the SMF on the MscL block by gadolinium in azolectin liposomes

It has been suggested that SMFs could exert their effect on biological membranes by affecting membrane-bound ions, such as Ca^{2+} (Del Moral and Azanza 1992). Since Gd^{3+} ions interact with phospholipid molecules in a similar way as Ca^{2+}, to test this hypothesis we examined if SMFs could modulate the ability of Gd^{3+}, a non-specific blocker of MS channels (Hamill and McBride 1996), to block MscL. In this context it is important to mention that Gd^{3+} does not block MscL by affecting the channel protein directly but rather by modifying the mechanical properties of the lipid bilayer surrounding the MS channels (Ermakov et al. 2001). Also, worth keeping in mind is that Gd^{3+} is paramagnetic (Arajs and Colvin 1961).

The results shown in Fig. 3 demonstrate a significant decrease in open probability of MscL upon addition of 50 μM Gd^{3+}, which only partially blocked the channels. Nearly 400 μM Gd^{3+} was required to block the channels completely in our experiments (data not shown). We used a lower Gd^{3+} concentration, because it allowed us to observe the effects of the SMFs used in this study. Much stronger magnetic fields, which we presently do not have at our disposal, would have been required to modulate the MscL block at high Gd^{3+} concentrations. In most of the examined patches a partial blockade of the MscL activity by 50 μM Gd^{3+} increased further in the presence of the SMF. After removal of the SMF, however, the channel activity always recovered to the level before the application of the SMF and most often increased further regardless of the presence of Gd^{3+} ions (Fig. 3a). In some patches the channel activity started to increase even before the removal of the SMF (Fig. 3b).

The effect of SMFs on MscL activity in liposomes made of PC18 and PC16 lipids

Since azolectin is a mixture of different phospholipids we hypothesized that SMF might have a more uniform effect on bilayers made of well-defined lipids. Therefore we reconstituted MscL protein into liposomes made of either PC18 or PC16 phospholipids.

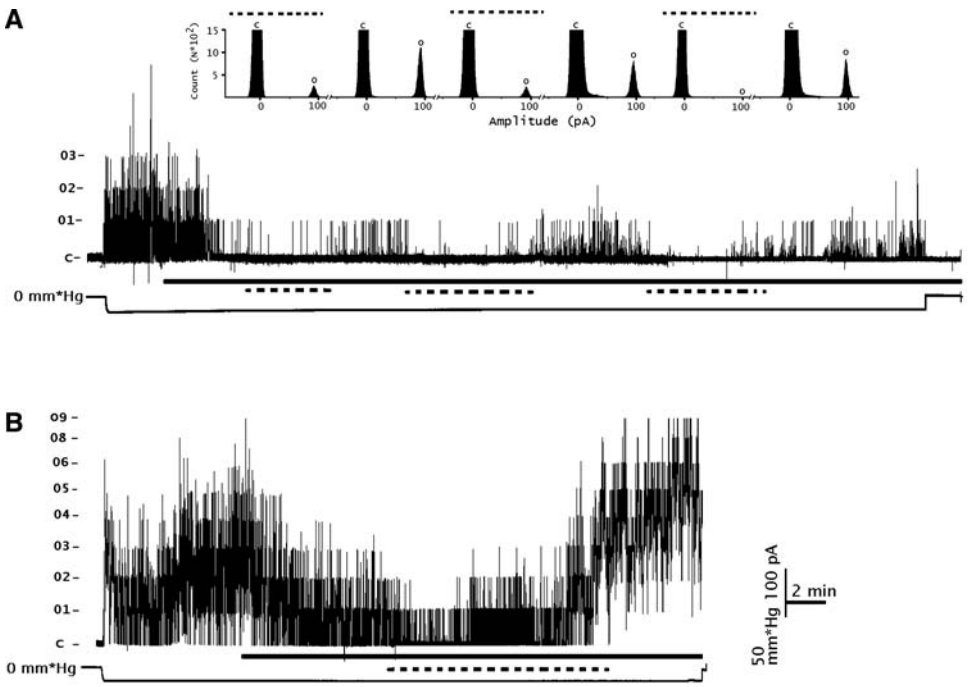

Fig. 3 Effect of SMFs on the MscL block by Gd^{3+}. SMFs can modulate blocking effect of Gd^{3+}. **a** Current recordings showing reduction of Gd^{3+} block on MscL activity after removal of SMFs. Similar results were obtained from seven different patches. *Inset* amplitude histograms of the corresponding segments of the current recordings shown below. **b** Reduction of Gd^{3+} block on MscL activity in the presence of SMFs. Similar results were obtained from two different patches. The *lines* indicate the presence of 50 μM Gd^{3+} in the bath solution (*bold*), application of SMFs (*dashed*) and pressure (*thin line*). Note that the MscL activity starts to increase in the presence of SMF and continues to increase further upon removal of the SMF

In addition, because MscL requires less tension for activation when reconstituted in liposomes made of a well-defined lipid composition and in particular if the liposomes are made of lipids having shorter acyl chains (Perozo et al. 2002a, b), we expected to see a more pronounced effect of SMFs on MscL in PC18 and PC16 bilayers. In liposome patches made of azolectin suction was always required to open the channels, whereas in 10 out of 16 liposome patches made of PC18 lipids MscL exhibited spontaneous activity (Fig. 4) and did not require additional negative pressure to activate the channel in these patches. Interestingly, the patches with spontaneously active MscL channels (Fig. 4b) responded to SMFs in a different way compared to the patches, for which suction was required to activate the channels (Fig. 4a). Open probability of channels exhibiting spontaneous activity was always reduced after the SMF was applied (Fig. 4b). In contrast in patches requiring suction for the channel activation the channels behaved similar to those reconstituted into azolectin liposomes by not always showing significant difference in activity before, during and after the SMF application (compare Figs. 4a, 3a). Furthermore, we have noticed another effect of the SMF. The channels had a tendency to gate more frequently at subconducting levels when the SMF was applied (Fig. 5).

We also reconstituted MscL into bilayers made of PC16 lipids, in which MscL was shown to be more readily activated by membrane tension as compared to bilayers made of lipids having longer acyl chains (Perozo et al. 2002a). Liposomes made of PC16 were on average much more fragile than those made of PC18 lipids or azolectin, and thus were more difficult to work with and obtain recordings lasting longer than 15 min. Nevertheless, we were able to record the effect of SMFs before and during the SMF application. In all recordings (n = 16) the channels were spontaneously active, probably because the residual tension in these liposome patches after giga seal formation was sufficient to activate the channels. Overall, although not always, application of the SMF most often caused a decrease in the channel activity (Fig. 6a, c).

Discussion

Our investigations present a follow-up study of previous findings showing that the activity of a bacterial mechanosensitive channel MscL reconstituted into artificial liposomes was affected by SMFs of moderate intensity (Hughes et al. 2005). Although the mechanisms by which moderate SMFs should affect activity of ion channels are not entirely clear one possible

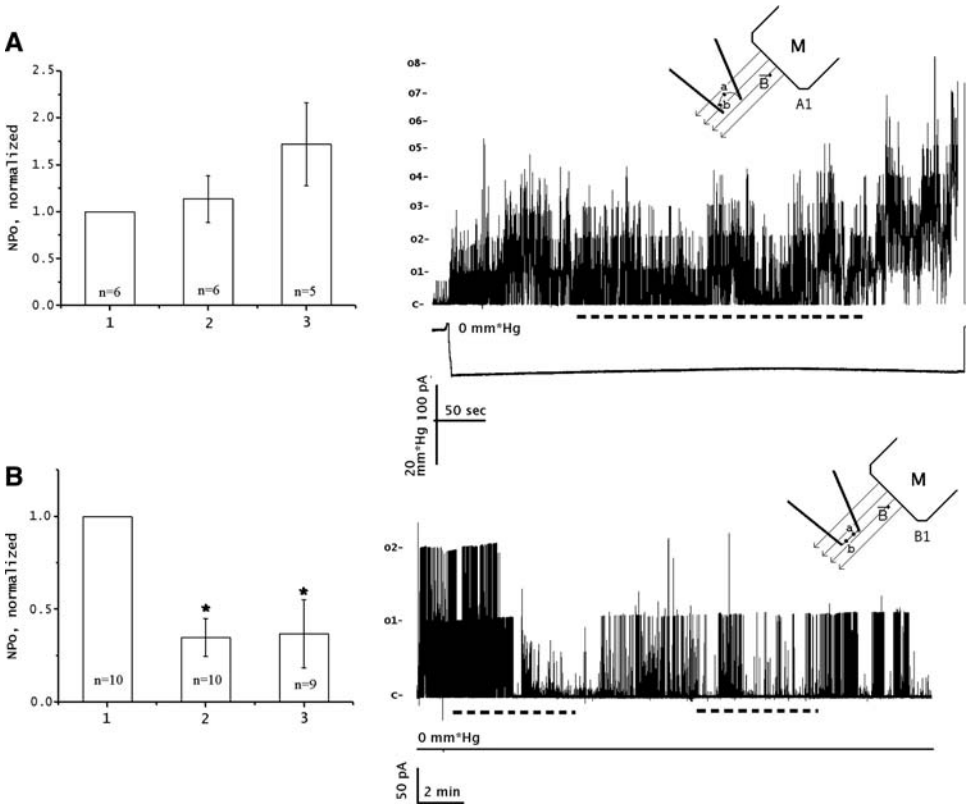

Fig. 4 Effect of SMFs on the activity of MscL reconstituted into bilayers made of PC18 lipids. **a** Patches requiring suction for the channel activation. **b** Patches containing spontaneously active channels. NPo was calculated before (*1*), during (*2*) and after (*3*) the SMF application. *Insets A1* and *B1* show possible patch configuration and orientation to the magnetic field. Other denotations are as in Fig. 3

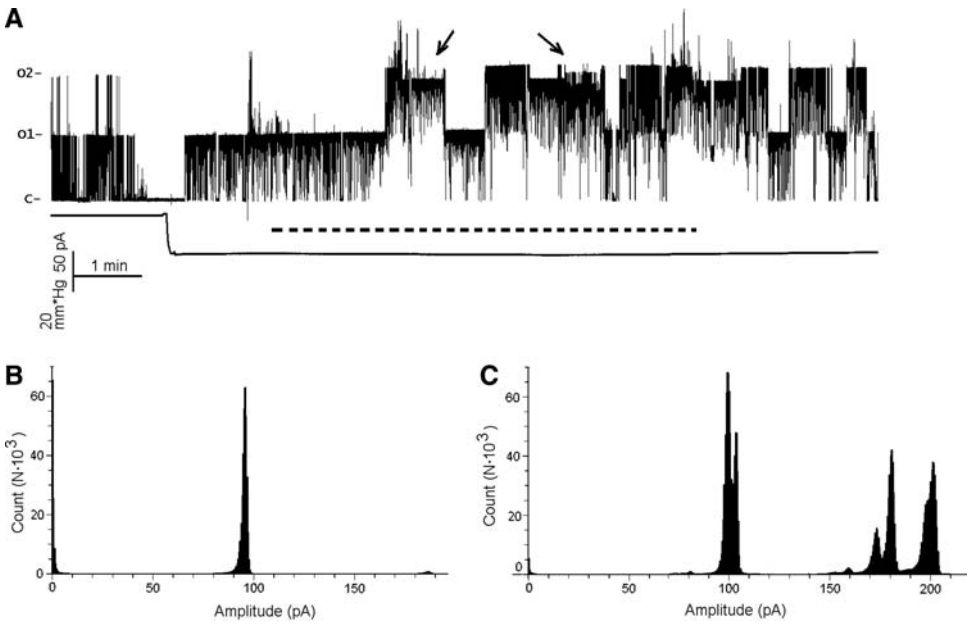

Fig. 5 SMFs cause MscL channels to gate at subconducting levels. **a** A current trace showing MscL activity before and during application of the SMF applied (*dashed line*). MscL channels were reconstituted into bilayers made up using PC18 lipids. Two channels were active in the particular patch. Note frequent channel gating at subconducting levels (*arrows*) during the application of the SMF. Pressure line is depicted below (*solid line*). **b** Amplitude histogram of the recording in **a** before the SMF application showing two peaks at 95 and 195 pA. **c** Amplitude histogram of the recording in **a** during the SMF application showing additional peaks at 170 and 180 pA indicating presence of subconducting levels

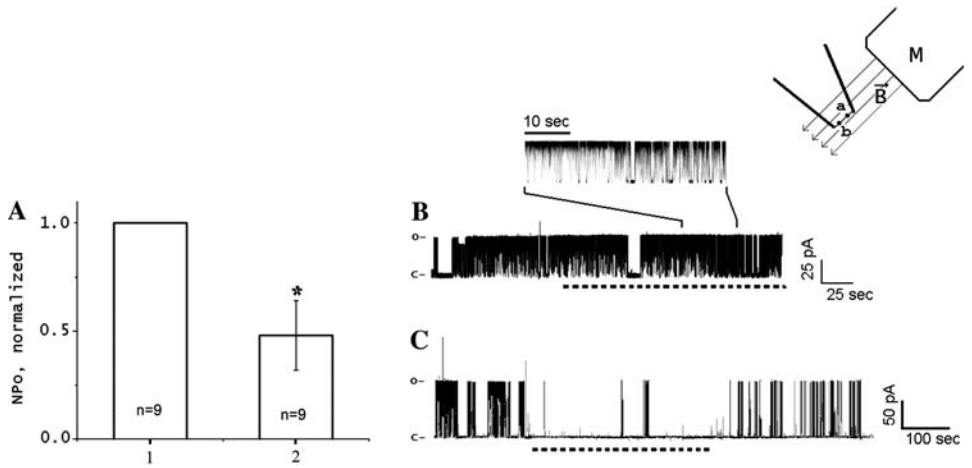

Fig. 6 Effect of SMF on MscL channels activity reconstituted in liposomes made of PC16 lipids. **a** Channel activity was recorded before (*1*) and during the SMF application (*2*). **b** If the channel was open most of the time, the SMF had a weak effect on the activity of such channels. As shown on an expanded scale the channels gated more frequently between the closed and fully open state in the presence of the SMF. **c** In most patches the SMF caused a decrease in open probability of spontaneously active channels. *Inset* shows the orientation of a liposome patch relative to the SMF, which would allow for a maximal effect of the SMF on phospholipids in the bilayer. Other denotations are as in Fig. 3

mechanism that has been proposed, could be via the lipid bilayer of cellular membranes. Since all biological molecules including membrane phospholipids are diamagnetic (Rosen 2003a) and diamagnetic susceptibility is known to be additive for highly ordered structures, such as phospholipid bilayers of biological membranes (Maret and Dransfeld 1977), lipid bilayers have superdiamagnetic properties enabling moderate-strength magnetic fields to exert an effect upon membranes of living cells. This is important because it has previously

been shown that activity of sodium and calcium channels (Rosen 2003b) as well as bacterial mechanosensitive channels (Dobson et al. 2002; Hughes et al. 2005) were affected by SMFs, and theoretical calculations have indicated that SMFs of moderate intensity are too weak to affect directly conformational changes of ion channels due to Lorenz forces (St Pierre and Dobson 2000). Moreover, the functioning of MS class of ion channels is closely dependent on the properties of lipid bilayers (Hamill and Martinac 2001; Martinac 2004; Kung 2005) suggesting that the effects of SMFs on MS channels are indirect and most likely a consequence of the SMFs effects on the lipid bilayer. Therefore, the experimental strategy used in our study was to investigate the effect of SMFs on the MscL channels reconstituted into liposomes made of different type of phospholipids, apply the SMFs and observe their effect on the MscL activity. The novelty in the approach used in this study compared to investigations reported previously (Hughes et al. 2005) was to examine the effect of SMFs of ≥400 mT on (i) the activity of MscL reconstituted into bilayers of different thickness, and (ii) the block of MscL by gadolinium ions Gd^{3+}.

Although our experimental results do not provide any direct evidence for the moderate-strength SMFs exerting their effect on the MscL activity through the surrounding phospholipid molecules the diamagnetic phospholipid reorientation under the influence of the SMFs presents the simplest explanation of the effects observed in this study. This is because phospholipid molecules rotate and orient with their long axis perpendicularly relatively to the SMF vector (Braganza et al. 1984; Seelig et al. 1985; Higashi et al. 1997; Picard et al. 1999) (Fig. 7). Consequently, phospholipid molecules are expected to be more tightly packed in the bilayer with its plane parallel to the SMF vector (i.e. individual phospholipid molecules are perpendicularly oriented to the SMF vector). Ordering of phospholipid molecules by magnetic fields could affect the lateral expansion of an opening MscL channel, because (1) hydrophobic mismatch due to very small changes in bilayer thickness has been shown to stabilize closed or open conformations of MscL (Perozo et al. 2002a) and (2) an open MscL channel occupies a larger membrane area than a closed channel, since it has been shown that the diameter of MscL increases by 16 Å upon the channel opening (Perozo et al. 2002b; Corry et al. 2005). Furthermore, we have observed that MscL was frequently gating at subconducting levels in the presence of SMFs (Fig. 5), which may reflect a reduction in the channel open probability in an ordered and thicker bilayer (Fig. 8). This observation is also consistent with the finding that an increase in the order of the lipid bilayer due to a lateral compression of phospholipid molecules by high hydrostatic pressures favoured closing or frequent gating at subconducting levels of MscS, the bacterial mechanosensitive channel of small conductance (Macdonald and Martinac 2005). Although the overall effect of the SMFs consisted in an overall reduction of the MscL activity in the presence of SMFs, which could result from an increased order of phospholipid molecules as well as an increase in the thickness of the bilayer, in some patches the channels failed to decrease their activity. Possibly, different regions of liposome patches could be oriented at a different angle to the SMF vector resulting in non-uniformity of the bilayer thickness in different areas of the curved liposome patches (Fig. 2, inset), since the bilayer regions perpendicular to the direction of the SMF vector should be thinner compared to the regions parallel to the SMF vector (del Moral and Azanza 1992). This may also explain why in some patches the channels not only recovered, but also increased their activity upon removal of SMFs. Deformations and restructuring of the bilayer could have also assisted in opening channels which where silent before the application of the SMFs.

When PC18 lipids were used to prepare liposomes, in about half of the patches examined the channels were spontaneously active without suction being

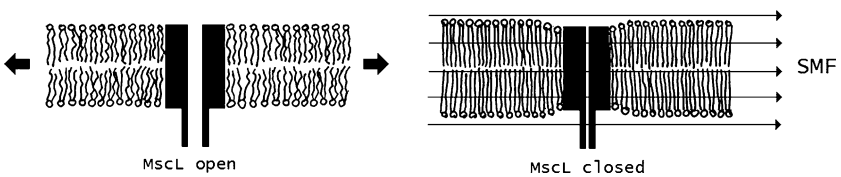

Fig. 7 Hypothetical effect of SMFs on the lipid bilayer and MscL activity. A *cartoon* depicting a reduction in flexibility of the phospholipid acyl chains and consequent stiffening of phospholipid molecules in the presence of SMFs, which could lead to an increase in lateral compression and thickening of the bilayer that reduce the freedom of movement of the MscL channel subunits. Consequently, the MscL channel open probability decreases. The *thick arrows* (*left*) symbolize stretch exerted on the liposome membrane, whereas the *thin arrows* (*right*) indicate the orientation of the SMF vector having maximum effect on the phospholipids

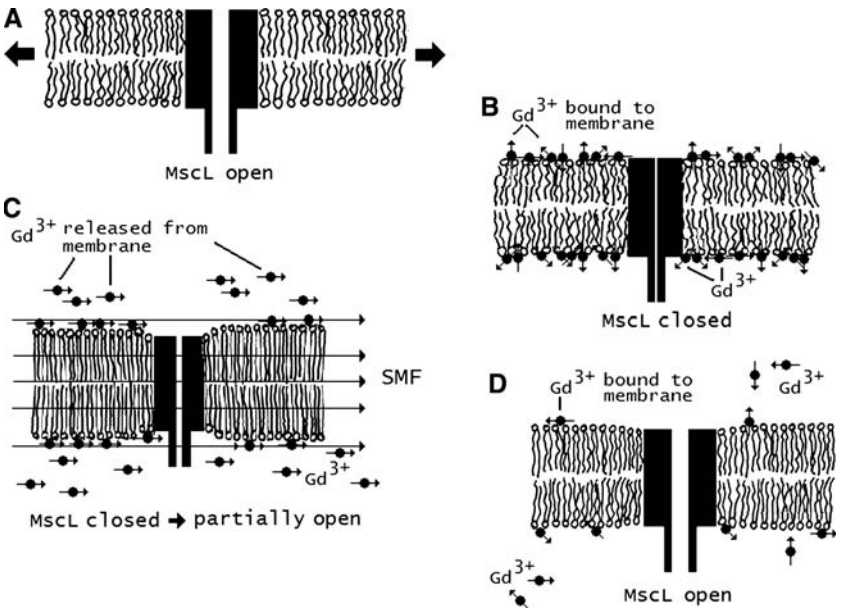

Fig. 8 Possible mechanism of the SMFs influence on gadolinium block of MscL channels. **a** Stretching the bilayer (indicated by *arrows*) opens the MscL channel. **b** Gd^{3+} ions bind to phospholipid heads, which causes the bilayer to stiffen and consequently the channels to close. **c** In the presence of SMFs phospholipid acyl chains reorient and become less flexible due to cooperative superdiamagnetism of the lipid bilayer. As a consequence the thickness of the bilayer increases causing a negative hydrophobic mismatch between the bilayer and the channel protein, which leads to further reduction in the channel activity. Paramagnetic Gd^{3+} ions (shown as *spheres with tiny arrows*) align in the magnetic field possibly leading to a further increase in order of phospholipids in the bilayer. Due to their closer proximity in the ordered bilayer Gd^{3+} ions experience repulsive electrostatic forces causing them to become partially displaced from the phospholipid heads. **d** Upon removal of SMFs lesser number of Gd^{3+} ions is bound to the bilayer because of possible hysteresis in physical properties of the lipid bilayer after exposure to the SMF. The bilayer partially restores its initial flexibility after the SMF has been removed allowing the MscL channels to recover their initial activity

applied to patch pipettes. Liposome patches with spontaneously active channels were more sensitive to SMFs. The NPo of MscL channels in these patches always decreased in the presence of SMFs. In contrast, in PC18 patches which required suction to activate the channels, SMFs were less efficient to decrease the channels activity (Fig. 4). A simple explanation for this discrepancy is the difference in geometry of the spontaneously active patches compared to patches requiring suction for the channel activation. Spontaneously active patches are flat, whereas patches requiring suction become spherically curved when suction is applied (Sukharev et al. 1999). The influence of SMFs on phospholipid molecules in flat liposome patches should be more uniform than on phospholipids in curved patches, because the peripheral and central parts of a curved patch are at different angles to a direction of the SMF vector (Fig. 2b).

Similar results were obtained when PC16 was used to prepare liposomes (Fig. 6). It has been shown that the energy for activation of MscL in PC16 liposomes was significantly reduced compared to MscL reconstituted in bilayers made of lipids having longer acyl chains (Perozo et al. 2002a). This is because the hydrophobic mismatch between MscL and PC16 phospholipids favour channel conformations, which require less tension to open the channel. In almost all liposome patches made of PC16 phospholipids examined in this study the MscL channels exhibited spontaneous activity similar to previously published results (Perozo et al. 2002a). Since the PC16 patches were very fragile a typical successful recording lasted only several minutes. Nevertheless, it was possible to obtain recordings of the MscL activity in these patches before and during the application of the SMFs. Although application of the SMFs always caused a decrease in the channel activity this effect was less obvious with patches containing very active channels (Fig. 6b). This finding seems to be consistent with a notion that hydrophobic mismatch between PC16 lipids and MscL favour opening of MscL to a larger extent than it is the case with PC18 lipids.

Further evidence for cooperative diamagnetism and ordering of phospholipids in the liposome bilayers under the influence of SMFs comes from the experiments in which we examined the effect of SMFs on the

MscL block by gadolinium. Del Moral and Azanza (1992) proposed an electrostatic repulsion model to explain the effect of SMFs on neuronal membranes. The model suggests that SMFs could affect membrane-bound ions, such as Ca^{2+}, to become displaced from the head groups of the membrane phospholipids through electrostatic repulsion upon reorientation of phospholipid molecules by SMFs (del Moral et al. 2002).

Our investigations of the effects of SMFs on the MscL block by gadolinium seem to support the idea of cooperative diamagnetism and electrostatic repulsion model. In the absence of SMFs Gd^{3+} inhibited MscL channels activity, but in the presence of SMFs the channel activity partially recovered reaching levels higher than when only Gd^{3+} was present (Fig. 3). Since Gd^{3+} has been reported to block MscL not by direct binding to the channel protein but by modifying mechanical properties of the lipid bilayer (Ermakov et al. 2001), the observed reduction of the Gd^{3+} block by SMFs appears consistent with the idea of reorientation of phospholipid molecules (cooperative superdiamagnetism) in the presence of SMFs. The reorientation and ordering of phospholipids might even be enhanced by the membrane bound Gd^{3+}, which should align in the magnetic field, because of its paramagnetic properties (Fig. 8). This could lead to ''Coulomb explosion'' between Gd^{3+} ions to displace them from the liposome membrane in a similar manner as it was speculated for Ca^{2+} ions in neurons (del Moral and Azanza 1992).

In summary, our study demonstrates that (1) SMFs of moderate intensity could affect the thickness of the lipid bilayer by causing reorientation and packing of phospholipid molecules due to superdiamagentic properties of the bilayer, (2) the change in packing of phospholipids in the bilayer could up- or down-regulate the open probability of mechanosensitive channels, such as MscL, whose activity is strongly dependent on the physical properties of the bilayer, and (3) reduction of the Gd^{3+} block of MscL by the SMFs can in this context be explained by displacement of Gd^{3+} ions due to electrostatic ion repulsion from the phospholipid head groups, which is consistent with the notion of SMFs influencing the orientation and packing of phospholipids in lipid bilayers due to their diamagnetic properties.

The Earth's magnetic field is about 1,000 times weaker than the SMFs used in our study and therefore would not be expected to exert an effect on the orientation of phospholipids in the bilayer sufficient to affect the function of MscL. Other structures, such as membrane bound magnetosomes, may be required for the Earth's magnetic field to exert an effect on mechanosensensitive ion channels. Nevertheless, since man-made magnetic fields are at least of the order or bigger than the SMF used in our study we believe that the results of this study may not only contribute to a better understanding of magnetoreception but they may also help to elucidate the effects of magnetic fields on human health.

Acknowledgments This project has been supported by the Australian Research Council.

References

Arajs S, Colvin RV (1961) Paramagnetism of polycrystalline gadolinium, terbium, and dysprosium metals. J Appl Phys 32(3):S336–S337

Azanza MJ, del Moral A (1994) Cell membrane biochemistry and neurobiological approach to biomagnetism. Prog Neurobiol 44:517–601

Azanza MJ, del Moral A (1996) Isolated neuron amplitude spike decrease under static magnetic fields. J Magn Magn Mater 158:593–594

Blakemore C (1975) Magnetotactic bacteria. Science 190(4212):377–379

Braganza LF, Blott BH, Coe TJ, Melville D (1984) The superdiamagnetic effect of magnetic fields on one and two component multilamellar liposomes. Biochim Biophys Acta 801(1):66–75

Corry B, Rigby P, Martinac B (2005) Conformational changes involved in MscL channel gating measured using FRET spectroscopy. Biophys J 89:L49–L51

Delcour AH, Martinac B, Adler J, Kung C (1989) Modified reconstitution method used in patch-clamp studies of *Escherichia coli* ion channels. Biophys J 56(3):631–636

del Moral A, Azanza MJ (1992) Model for the effect of static magnetic fields on isolated neurons. J Magn Magn Mater 114(3):240–242

del Moral A, Azanza MJ, Calvo AC, Pérez-Bruzón RN (2002) Cooperative diamagnetism and Ca2+ liberation of plasma membrane molecules explains the neuron responses to applied static and extremely low frequency magnetic fields. In: Kostarakis P (ed) Biological effects of EMFs, vol 1. Demokritos Publishers, Rhodes, pp 298–308

Dobson J, Stewart Z, Martinac B (2002) Preliminary evidence for weak magnetic field effect on mechanosensitive ion channel subconducting states in *Escherichia coli*. Electromagn Biol Med 21(1):89–95

Ermakov YA, Averbakh AZ, Yusipovich AI, Sukharev SI (2001) Dipole potentials indicate restructuring of the membrane interface induced by gadolinium and beryllium ions. Biophys J 80:1851–1862

Gould JL (1985) Are animal maps magnetic?. In: Kirschvink JL, Jones DS, Mac Fadden BJ (eds) Magnetite biomineralisation and magnetoreception in organisms: a new magnetism. Plenum, New York, pp 257–267

Hamill OP, Martinac B (2001) Molecular basis of mechanotransduction in living cells. Physiol Rev 81:685–740

Hamill OP, McBride DW Jr (1996) The pharmacology of mechanogated membrane ion channels. Pharmacol Rev 48:231–252

Häse CC, Le Dain AC, Martinac B (1995) Purification and functional reconstitution of the recombinant large mechanosensitive ion channel (MscL) of *Escherichia coli*. J Biol Chem 270:18329–18334

Higashi T, Ashida N, Takeuchi T (1997) Orientation of blood cells in static magnetic field. Physica B 237–238:616–620

Hughes S, El Haj AJ, Dobson J, Martinac B (2005) The influence of static magnetic fields on mechanosensitive ion channel activity in artificial liposomes. Eur Biophys J 34(5):434–441

Kirschvink JL (1992) Comment on ''Constraints on biological effects of weak extremely-low-frequency electromagnetic fields''. Phys Rev A 46:2178–2184

Kloda A., Martinac B. (2001) Mechanosensitive channel in Thermoplasma a cell wall-less Archaea: cloning and molecular characterization. Cell Biochem Biophys 34:321–347

Kung C (2005) A possible unifying principle for mechanosensation. Nature 436(7051):647–654

Lohmann KJ, Willows AO (1987) Lunar-modulated geomagnetic orientation by a marine mollusk. Science 235(4786):331–334

Maret G, Dransfeld K (1977) Macromolecules and membranes in high magnetic fields. Physica 86b:1077–1083

Macdonald AG, Martinac B (2005) Effect of high hydrostatic pressure on the bacterial mechanosensitive channel MscS. Eur Biophys J 34:434–442

Martinac B (2004) Mechanosensitive ion channels: molecules of mechano-transduction. J Cell Sci 117:2449–2460

Martinac B, Hamill OP (2002) Gramicidin A channels switch between stretch activation and stretch inactivation depending on bilayer thickness. Proc Natl Acad Sci USA 99(7):4308–4312

Niu W, Sachs F (2003) Dynamic properties of stretch-activated K+ channels in adult rat atrial myocytes. Prog Biophys Mol Biol 82(1–3):121–135

Patel AJ, Honoré E (2001) Properties and modulation of mammalian 2P domain K^+ channels. Trends Neurosci 24:339–346

Perozo E, Kloda A, Marien Cortes D, Martinac B (2002a) Physical principles underlying the transduction of bilayer deformation forces during mechanosenstitive channel gating. Nat Struct Biol 9:696–703

Perozo E, Marien Cortes D, Sompornpisut P, Kloda A, Martinac B (2002b) Open channel structure of MscL and the gating mechanism of mechanosensitive channels. Nature 418:942–948

Picard F, Paquet M-J, Levesque J, Bélanger A, Auger M (1999) ^{31}P NMR first spectral moment study of the partial magnetic orientation of phospholipid membranes. Biophys J 77:888–902

Rosen AD (2003a) Mechanism of action of moderate-intensity static magnetic fields on biological systems. Cell Biochem Biophys 39:163–173

Rosen AD (2003b) Effect of a 125 mT static magnetic field on the kinetics of voltage activated Na^+ channels in GH3 cells. Bioelectromagnetics 24(7):517–523

Seelig J, Borle F, Cross TA (1985) Magnetic ordering of phospholipid membranes. Biochim Biophys Acta 814:195–198

St Pierre TG, Dobson J (2000) Theoretical evaluation of cell membrane ion channel activation by applied magnetic fields. Eur Biophys J 29:455–456

Sukharev S, Corey DP (2004) Mechanosensitive channels: multiplicity of families and gating paradigms. Sci STKE 2004(219):re4

Sukharev SI, Sigurdson WJ, Kung C, Sachs F (1999) Energetic and spatial parameters for gating of the bacterial large conductance mechanosensitive channel, MscL. J Gen Physiol 113:525–540

Tan J, Liu W, Saint D (2004) Differential expression of the mechanosensitive potassium channel TREK-1 in epicardial and endocardial myocytes in rat ventricle. Exp Physiol 89:237–242

Walcott C, Green RP (1974) Orientation of homing pigeons altered by a change in the direction of an applied magnetic field. Science 184(133):180–182

Walker MM, Bitterman ME (1985) Conditioned responding to magnetic fields by honeybees. J Comp Physiol A 157:67–71