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ARTICLE

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The influence of static magnetic fields on mechanosensitive ion channel activity in artificial liposomes

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Abstract The influence of static magnetic fields (SMFs) on the activity of recombinant mechanosensitive ion channels (the bacterial mechanosensitive ion channel of large conductance—MscL) following reconstitution into artificial liposomes has been investigated. Preliminary findings suggest that exposure to 80-mT SMFs does not induce spontaneous MscL activation in the absence of mechanical stimulation. However, SMFs do appear to influence the open probability and single channel kinetics of MscL exposed to negative pipette pressure. Typical responses include an overall reduction in channel activity or an increased likelihood of channels becoming "trapped open" in sub-conducting states following exposure to SMFs. There is a delay in the onset of this effect and it is maintained throughout exposure. Generally, channel activity showed slow or limited recovery following removal of the magnetic field and responses to the magnetic were often reduced or abolished upon subsequent exposures. Pre-exposure of the liposomes to SMFs resulted in reduced sensitivity of MscL to negative pipette pressure, with higher pressures required to activate the channels. Although the mechanisms of this effect are not clear, our initial observations appear to support previous work showing that the effects of SMFs on ion channels may be mediated by changes in membrane properties due to anisotropic diamagnetism of lipid molecules.

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Introduction

For many years there has been interest in the effects of static magnetic fields (SMFs) on biological systems and human health. A number of studies have shown that moderate-strength SMFs (1 mT–1 T) can influence the activity of ion channels embedded in phospholipid membranes. Dobson et al. (2002a, 2002b) have shown that SMFs (1.35 mT) produced by an electromagnet can alter the gating kinetics/properties of the mechanosensitive (MS) ion channel of small conductance from *Escherichia coli* expressed in spheroplast preparations. In addition, it has previously been shown that both cloned sodium and calcium channels expressed in mammalian cell systems exhibit changes in firing patterns and activation kinetics during exposure to 125-mT SMFs, where the effect is temperature-dependent for both channels (Rosen 1996, 2003b). The same authors have previously suggested that ion channels may underlie the effect of moderate-strength SMFs on the swimming patterns of paramecium (Rosen and Rosen 1990) and changes in the frequency of miniature end-plate potentials recorded from the murine neuromuscular system (Rosen 1992, 1993a, 1993b).

The mechanisms by which moderate-strength SMFs influence ion channel activity (in vitro and in vivo) are not entirely clear. One explanation that has previously been proposed by Rosen (1993a, 1993b) is that the application of SMFs may induce magnetic re-orientation of membrane phospholipids (specifically the acyl chains of the molecules) via diamagnetic anisotropy effects (Rosen 2003a, 2003b).

Diamagnetism is a property of materials that have no intrinsic magnetic moment and are weakly repelled when exposed to a magnetic field. Though all biological molecules have some degree of diamagnetic susceptibility,

the majority of proteins have very weak susceptibility and thus very strong magnetic fields (greater than 1 T) are required to induce rotation or alignment of the molecules (Torbet and Ronziere 1984; Iwasaka et al. 1994; Bras et al. 1998). However, diamagnetic susceptibility is known to be additive for highly ordered, linked-parallel structures and thus it is possible that lower strength magnetic fields may exert an effect on highly ordered biological structures (Maret and Dransfeld 1977). It has been proposed that this is the case for phospholipid membranes (Rosen 2003a, 2003b).

It is worth noting that phospholipid molecules show clear levels of diamagnetic anisotropy and that orientation of phospholipid membranes is known to occur in the presence of strong and even moderate-strength magnetic fields (Boroske and Helfrich 1978; Scholz et al. 1984; Speyer et al. 1987; Sanders et al. 1994; Cardon et al. 2003). This is important, because the functioning of MS channels is closely related to their boundary lipids (Martinac 2004). Consequently, it is possible to theorize that ordering membrane phospholipids by magnetic fields may affect MS channel gating by perturbing the protein–bilayer interaction.

We conducted preliminary experiments to investigate whether or not SMFs (approximately 80 mT) produced by a permanent NdFeB magnet are able to influence the activity of recombinant MS ion channels of large conductance (MscL) following reconstitution into artificial liposomes.

Methods

Production of recombinant proteins

Recombinant glutathione S-transferase (GST)–MscL and C-terminal-6×His–MscL protein preparations were used in these experiments. Recombinant MscL fusion proteins were produced as described previously. Briefly, *E. coli* AW737 MscL-KO strain harbouring the plasmid pGEX1.1, which encodes a GST–MscL construct, was used for expression of the GST–MscL fusion protein. MscL was purified from detergent solubilized bacterial membranes, such that the solubilized GST–MscL protein was bound to glutathione-coated beads followed by thrombin cleavage of the fusion protein separating the recombinant MscL from GST (Häse et al. 1995). M15 *E. coli* cells were transformed by the MscL–pQE32 construct containing MscL with 6×His epitope at the C-terminus and used for expression and purification of the 6×His–MscL employing a Ni^{2+}-based metal–chelate chromatography resin (Perozo et al. 2002).

Functional reconstitution of MscL proteins into liposomes

Recombinant MscL proteins were functionally reconstituted into phosphatidylcholine liposomes as described previously (Häse et al. 1995). Briefly, 2 mg phosphatidylcholine (Sigma) was dissolved in chloroform in a freshly cleaned glass tube. The chloroform was then evaporated and dried with a nitrogen stream for 15 min. The lipid was then resuspended in dehydration/rehydration (D/R) buffer [200 mM KCl, 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid, Hepes, KOH, pH 7.2] to a final concentration of 10 mg/ml. The solution was vortexed briefly and then sonicated for 5 min to produce a clear solution prior to the addition of recombinant MscL protein (in phosphae-buffered saline with 1% w/v octyl glucoside) at a protein-to-lipid ratio of 1:2,000. The solution was then placed on a platform rocker for 1 h at room temperature to allow protein–lipid interactions to occur before the addition of Bio-Beads SM-2 (Bio-Rad) for 4 h to remove residual detergent in the protein solution. The solution was then briefly centrifuged to remove the beads and then spun at 90,000 rpm at 4°C for 30 min (TL-100 ultracentrifuge, Beckman) to pellet the lipid. The lipid pellet was then resuspended in 40 µl D/R buffer and spotted onto ethanol-cleaned glass slides and dehydrated in a vacuum dessicator at 4°C for 6 h. Individual spots were then rehydrated with 20 µl D/R buffer and left overnight at 4°C to allow rehydration to occur.

Patch clamping of MscL expressed in liposomes

For patching experiments, 2–3 µl aliquots of the liposome preparation were placed into the recording chamber (approximately 1-ml volume) containing recording solution (200 mM KCl, 40 mM $MgCl_2$, 10 mM Hepes, adjusted to pH 7.2 with KOH). The recording chamber was mounted onto an inverted microscope placed inside a Faraday cage. Liposome blisters were patched 10 min after seeding of the liposome preparation.

Single-channel recordings were performed using non-filamented borosilicate glass micropipettes (Drummond Scientific, Broomall, PA, USA) pulled to a tip resistance of 6–8 MΩ using a Flaming/Brown micropipette puller (P-87 Sutter Instruments, CA, USA). Micropipettes were filled with recording solution and positioned onto the surface of liposome blisters via a micromanipulator (Ernst Leitz Wetzlar, Wetzlar, Germany). Patch current signals were amplified and filtered (1 kHz) with an Axopatch 1D (Axon Instruments) and digitized at 5 kHz with a PC using PCLAMP6 data acquisition software. Membrane patches were held at +30 mV during recordings in order to provide a driving force for ionic currents. Negative pipette pressure was applied to the membrane patch via a syringe-and-tube system. Suction applied to the patch was measured via a piezoelectric pressure transducer (Omega, Stamford, USA) and these data were also collected by the PC. Single-channel data were analysed using a combination of axoscope (Axon Instruments) and WinEDR software (John Dempster, Strathclyde University, UK).

Exposure to SMFs

SMFs were produced by a permanent rare-earth (NdFeB) cylindrical magnet (22 mm×25 mm) attached to a manoeuverable pole and micromanipulator (Narishige) in such a way that it could be moved into position with a high degree of precision. The orientation of the magnet with respect to the patch pipette is shown in Fig. 1. When in the "on" position, the magnet was approximately 15–20 mm from the pipette tip and produced a magnetic field of approximately 80 mT. In the "off" position the magnet was approximately 100–120 mm away from the pipette tip, reducing the field at the microscope stage back to the ambient field. Magnetic field strengths were measured via a handheld gaussmeter (Fig. 2).

Following formation of a giga seal and excision of the patch, negative pipette pressure was applied to pre-train the patches in an attempt to prevent "creeping" during experiments. Once stable channel activity had been observed, recordings were commenced. Typically, the channel activity in response to pressure alone was recorded for at least 30–60 s before the permanent magnet was moved into position close to the end of the pipette. The magnet was typically held in position for 30–60 s before it was removed for a further 30–60 s, before again being moved into position. The effect of SMF exposure on MscL activity was analysed by comparing channel-open probabilities and single-channel kinetics for periods of at least 30 s immediately prior to and then during exposure to the 80-mT SMF. For data analysis, the values shown are the mean ± the standard error. Statistical analysis was performed using Student's *t* test and Wilcoxon matched-pairs signed rank.

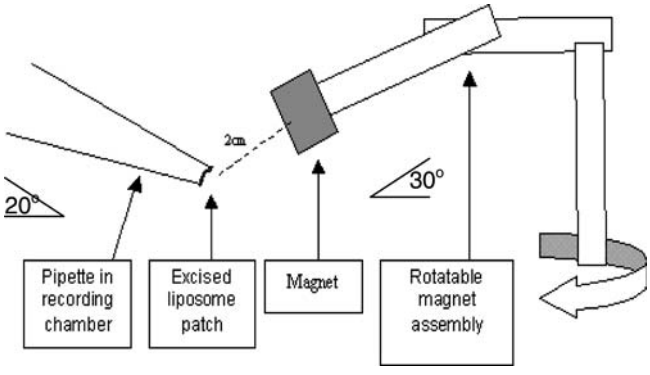

Fig. 1 Schematic representation of the magnet arm and proximity to the pipette tip during static magnetic field (*SMF*) exposure. The pipette and the magnet were inclined by approximately 20 and 30° from the horizontal plane, respectively

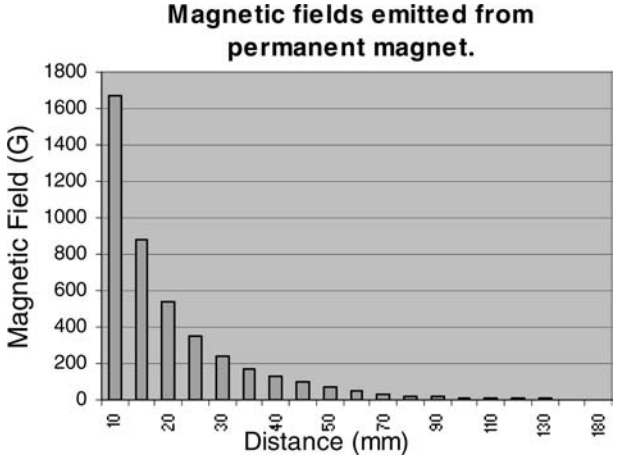

Fig. 2 Field strength vs distance from the magnet surface as measured by a gaussmeter

Results

Confirmation of functional channels—comparison of different preparations

Following reconstitution into phosphatidylcholine membranes, both GST-MscL and 6×His-MscL proteins demonstrated normal channel activity in response to negative pipette pressure. Both proteins produced currents with normal characteristics with regard to single-channel conductance and pressure activation thresholds (Fig. 3). In general, the activity of these channels was consistent with those described previously for MscL (Häse et al. 1995).

The effect of SMFs on spontaneous MscL activity

Following the formation of a giga seal and excision of a membrane patch the effect of SMF application on the recorded baseline current was investigated.

The application of an 80-mT SMF was found not to induce any spontaneous MscL activity in the absence of applied pipette pressure. In addition, there was no effect of the magnetic field on the recorded baseline current in the absence of applied pressure ($n = 5$ patches) (Fig. 4).

The effect of SMFs on pressure-induced MscL activity

Negative pipette pressure was applied to excised membrane patches to investigate the effect of SMFs on pressure-induced MscL activity. Comparisons were made of channel activity before, during and after exposure to the 80-mT SMF.

Initial investigations (using GST–MscL protein) showed that the application of the magnetic field does have an effect on MscL activity recorded in the presence of activating levels of negative pipette pressure ($n = 5$ suitable for single-channel analysis, $n = 8$ in total). In general, these experiments demonstrated that the application of an 80-mT field typically resulted in a decrease in MscL activity. The onset of the effect was typically delayed from the onset of magnetic field exposure

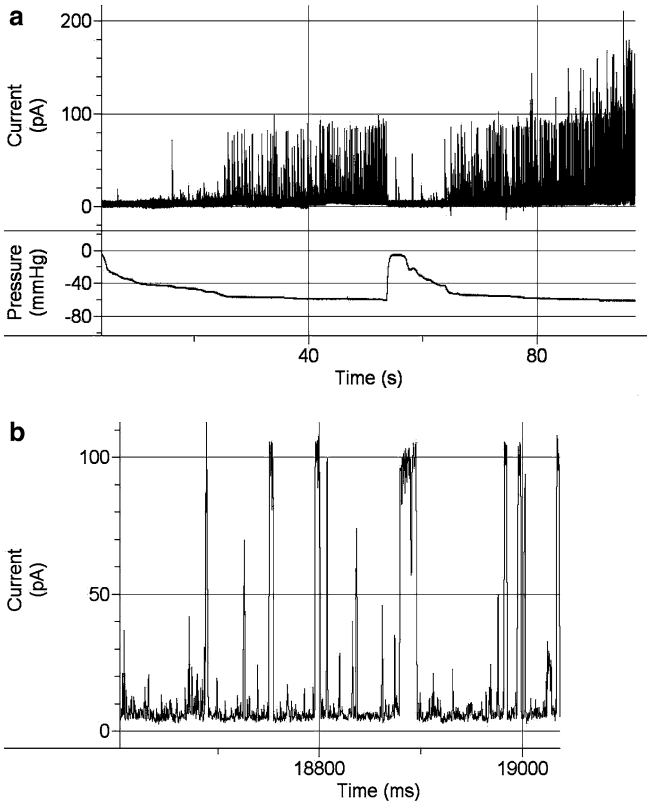

Fig. 3 **a** Single-channel recording of glutathione S-transferase (*GST*)–mechanosensitive ion channels of large conductance (*MscL*) in response to changing levels of negative pipette pressure. Note the effect of "creeping". Holding voltage +30 mV. **b** Close-up of single-channel recording of GST–MscL. The trace reveals the presence of "normal" open states (approximately 100 pA) and sub-conducting channel states, as would be expected. Holding voltage +30 mV

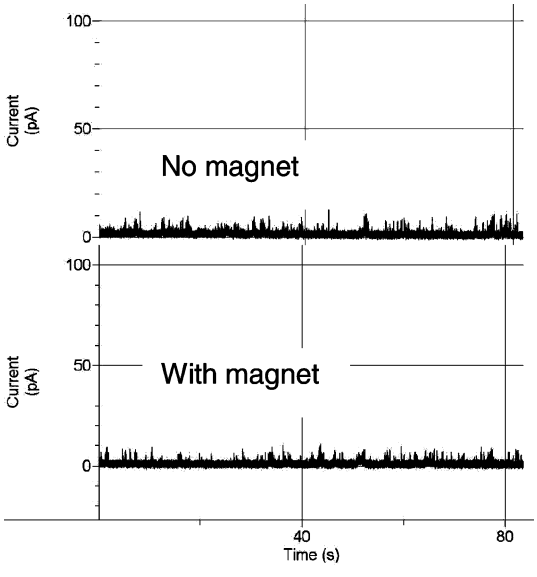

Fig. 4 Single-channel recordings showing the effect of SMFs on spontaneous MscL activity and baseline current recorded form excised patches in the absence of applied pipette pressure ($n = 5$). Pipette voltage +30 mV

(5–10 s) and was maintained throughout exposure to the field. In the majority of examples the channel activity showed slow or limited recovery following removal of the magnetic field and responses were often reduced or abolished upon subsequent exposures (Figs. 5a, 6).

Figure 5a shows a single-channel recording from a patch containing GST-MscL. Stable levels of channel activity were observed for approximately 60 s prior to

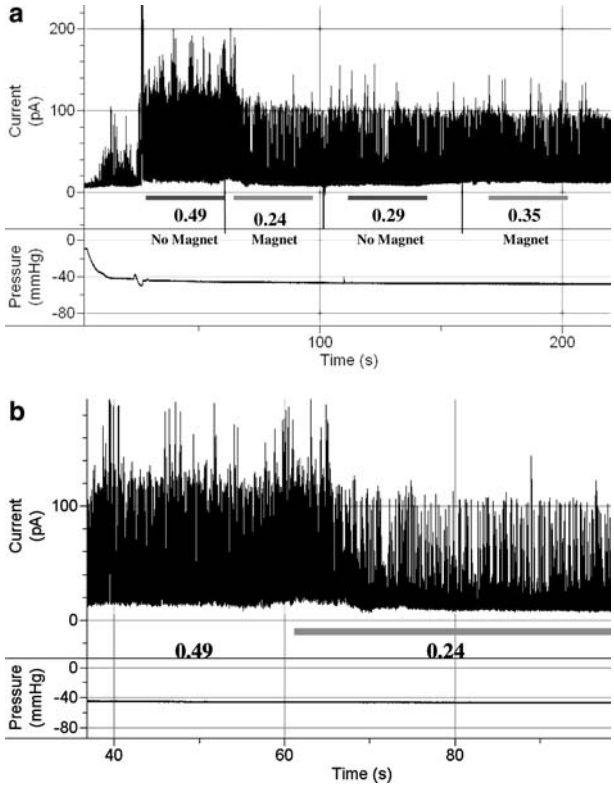

Fig. 5 **a** The effect of 800 G SMF on GST–MscL activity. The values shown are open probability (*NPo*) values calculated over 30-s periods, as indicated by *horizontal bars*. *Horizontal lines* denote magnet on/off transitions. **b** Close-up of the first "no magnet" to "magnet" transition shown in **a**

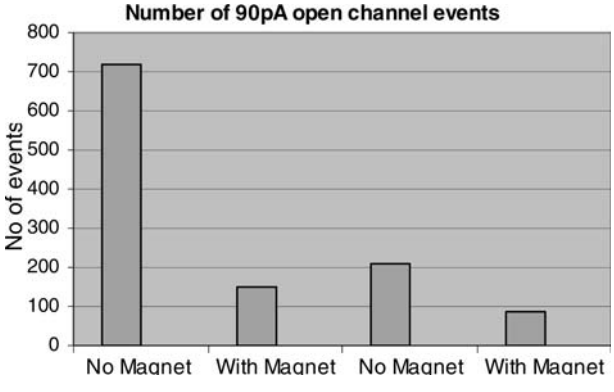

Fig. 6 Comparison of the number of "full" channel-open events before and during repeated magnetic field exposure for the recording shown in Fig. 5a

the application of the magnetic field. Following the application of the magnetic field, channel activity was markedly reduced. This can be seen clearly from visual inspection of the trace (Fig. 5) and by comparison of the channel-open probabilities during "on"/"off" periods. The effect was delayed by approximately 5–10 s following application of the magnetic field (Fig. 5b) and there was only limited recovery of channel activity following removal of the field. The effects of the second application of the magnetic field are far less obvious.

More detailed analysis of this trace revealed that there was no difference in the single-channel conductance of a fully open channel, yet there was a clear reduction in the number of full channel-open events following magnetic field exposure (the threshold used for event detection was 90 pA and therefore sub-conducting states were removed from the analysis). The number rises slightly when the magnetic field is removed and is again reduced on the second application of the magnetic field and thus shows a clear correlation between the presence of the magnetic field and the number of channel openings.

Comparisons between the initial off and on phases were used for detailed single-channel analysis of five replicant experiments. Of the five patches analysed in detail, four showed overall reductions in channel-open probabilities over a 30-s period following the initial application of the magnetic field, on the basis of channel-open probabilities. However, all five showed a decrease in the number of channel-opening events (threshold of 90 pA used for event detection analysis) over the same 30-s period (Fig. 7). Although the data sets are too small for robust statistical analysis, the changes in the channel-open probabilities and the number of channel openings were found to be statistically significant (using Student's t test for paired data) with a mean reduction in channel-open probability of 24.9% ($\pm 9.11\%$, $n=5$, $p=0.047$) and a mean reduction in the number of channel-opening events of 43.2% ($\pm 12.2\%$, $n=5$, $p=0.003$). Comparison of data sets using Wilcoxon matched-pairs signed rank, assuming samples are not equal produces p values of 0.1250 and 0.0625, respectively, although the data sets are too small to attain p values of less than 0.05. No further trends could be established between these five experiments with regard to changes in the mean open time or the mean closed time (data not shown). Analysis of individual traces does, however, suggest that there are also changes in the levels of sub-conducting channel activity seen during exposure to magnetic fields.

Observed reductions of MscL activity in response to magnetic field exposures were found to be reduced, if not completely absent, from similar experiments conducted on liposomes or individual patches with previous exposure to the magnetic field (i.e., second experiment on the same patch or a new patch from a bath that had previously been exposed to the magnetic field). The majority of these "pre-exposed" recordings (using GST–

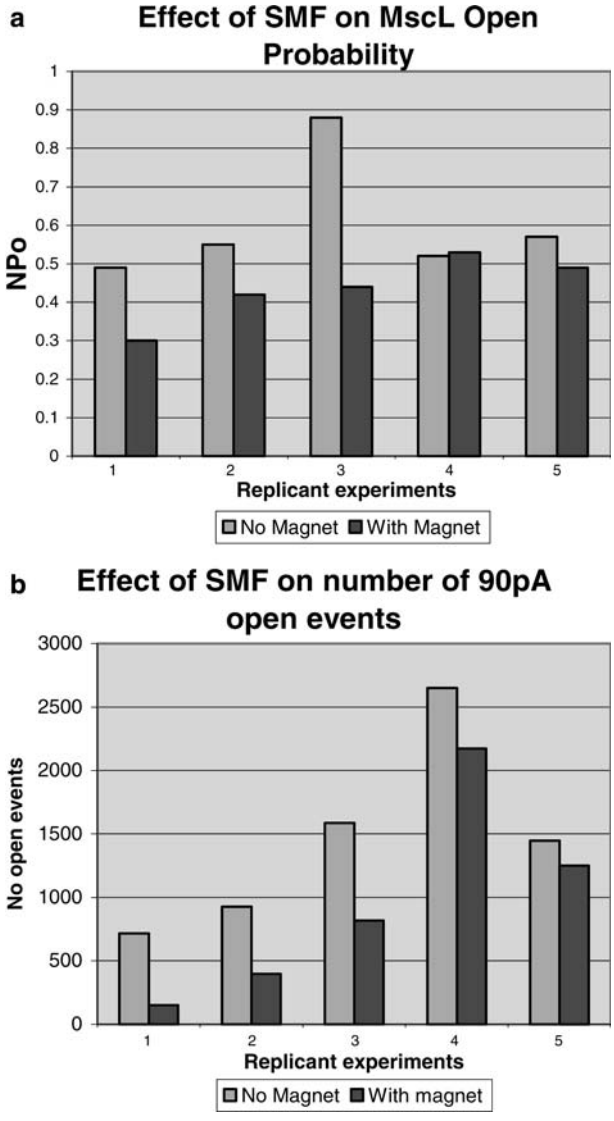

Fig. 7 **a** The effect of SMF exposure on MscL NPo for five replicant experiments. The mean reduction in NPo is 24.9% ($\pm 9.11\%$, $p=0.047$ using Student's t test, $p=0.1250$ using Wilcoxon matched-pairs signed rank). **b** The effect of SMF exposure on the number of 90-pA channel-open events for five replicant experiments. The number of events was counted over 30-s periods. The mean reduction is 43.2% ($\pm 12.2\%$, $p=0.003$ using Student's t test, $p=0.0625$ using Wilcoxon matched-pairs signed rank)

MscL protein) remained relatively constant and steady throughout the experiments (Fig. 8). There were no statistical differences in any aspect of channel activity following magnetic field exposures when data were compared for a similar series of replicant experiments ($n=5$) (Fig. 9).

A similar series of experiments ($n=8$ patches) was conducted using a C-terminal 6×His tagged MscL protein. The results of these experiments, although less consistent than those observed with GST-MscL, revealed similar trends. Once again the application of the SMF was found to influence channel activity. Two types of response were typically found. Firstly, a number of

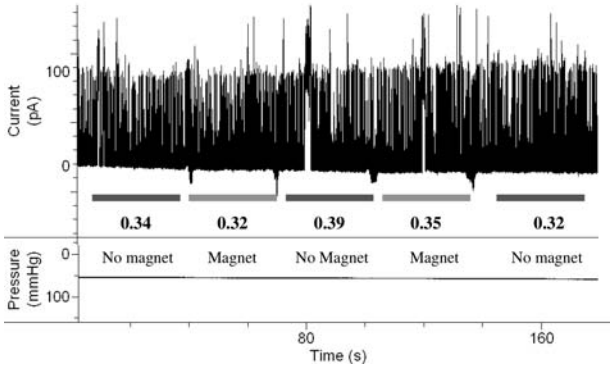

Fig. 8 Single-channel trace showing the effect of 800 G SMF on MscL activity recorded from a patch with previous exposure to the SMF. The values shown are NPo values calculated over 30-s periods

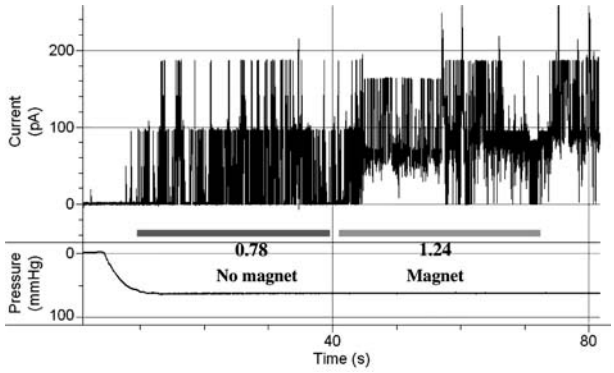

Fig. 10 Single-channel recording showing the effect of SMF exposure on 6×His–MscL. Note the distinct changes in channel gating and the prevalence of sub-conducting channel states following SMF exposure. The values shown are NPo values calculated over a 30-s period

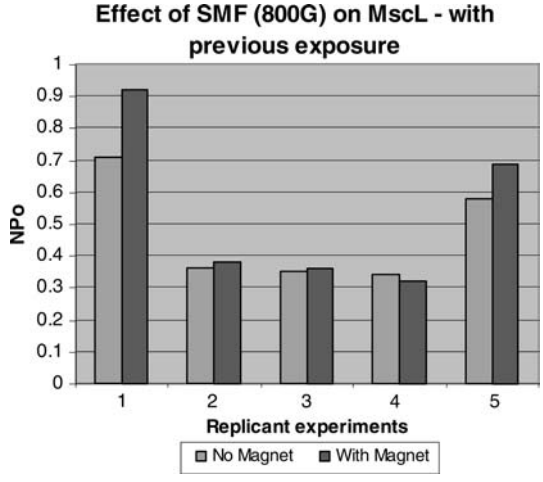

Fig. 9 The effect of initial SMF exposure on NPo values recorded from five replicant experiments conducted on patches with previous exposure to the SMF. The NPo values were calculated over 30-s periods. The mean change observed of +10.2% (±6.27%) was not statistically significant ($p = 0.9043$ using Student's t test, $p = 0.3125$ using Wilcoxon matched-pairs signed rank)

patches showed similar behaviour to that seen for GST–MscL, with clear reductions in channel events and channel-open probabilities during magnetic field exposure (data not shown). In addition, there was seemingly a greater propensity for C-terminal-6×His–MscL to become "trapped open" in response to the magnetic field, This response was seen in three of eight experiments and was characterized by increased levels of sub-conducting channel activity and increased values of mean current and open probability (as measured by channel-open probabilities) (Fig. 10).

Effects of magnetic field exposure on the pressure activation threshold of C-terminal-6His-MscL

In a related series of experiments, a liposome preparation was seeded into the recording chamber and a patch taken from a particular blister. Negative pipette pressure was applied and the pressure activation threshold observed during single-channel recordings. The same blister was then exposed to an 80-mT SMF for 30 min prior to excising a series of further patches from approximately the same region of the blister. Following excision, negative pipette pressure was again used to initiate channel activity and determine the pressure activation thresholds. The activation threshold values obtained before and after exposure were compared. In addition, before values were also compared with values obtained from similar unexposed preparations on different days. The values of the unexposed preparations show that the pressure activation threshold observed for MscL in normal untreated liposomes is relatively consistent and similar to what is expected from published reports concerning these channels (around 80 mmHg; Hamill and Martinac 2001). There was, however, a significant increase in the pressure required to activate MscL in the membrane patches excised from the blister following exposure to the SMF ($n = 4$ patches). The level was relatively consistent between the four post-exposed patches and was clearly above that seen for normal liposome patches (Fig. 11).

Discussion

The results of our preliminary investigations reveal that 80-mT SMFs produced by a permanent magnet are capable of influencing the activity of MscL reconstituted into artificial lipid membranes. Typically, the initial application of the magnetic field resulted in a decrease in the channel-open probability and a reduction in the number of channel-open events. This effect was usually delayed from the onset of the magnetic field, lasted throughout exposure and showed only partial recovery following removal of the field.

In addition, a number of experiments showed a clear tendency for channels to become trapped open in sub-

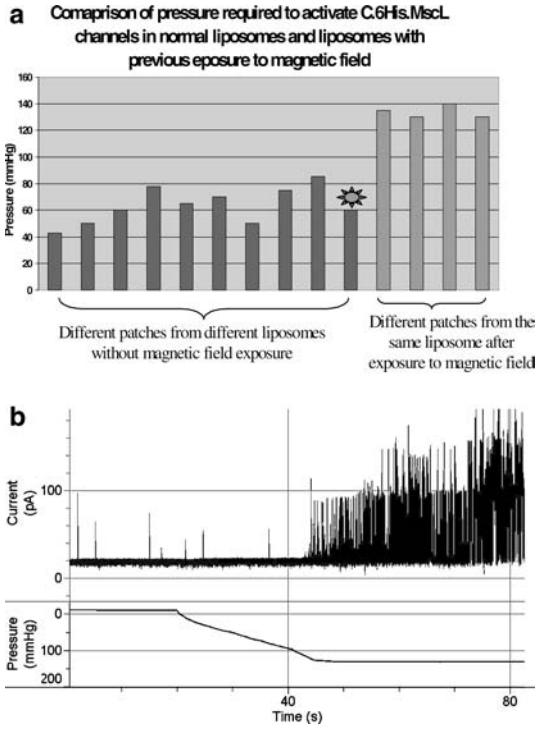

Fig. 11 **a** The effect of prolonged SMF exposure (30 min) on the pressure activation thresholds observed for MscL in normal patches and those excised from a blister following prolonged exposure to the SMF. The *highlighted bar* represents a patch excised from the same blister prior to SMF exposure. **b** Single-channel recording from a patch excised from a liposome blister following a 30-min exposure to the SMF. Note the pressure required to elicit channel activity (approximately 135 mmHg) is increased from that seen in normal unexposed patches

conducting states during SMF exposure. It would seem therefore from these experiments that the effect of the SMF on MscL activity is not entirely specific and that a number of responses can be seen.

Overall our experimental results would seem to support the findings of previous work (Rosen and Rosen 1990; Mclean et al. 1995; Rosen 1996, 2003a, 2003b; Dobson et al. 2002a, 2002b; Bareus et al. 2003) suggesting that moderate-strength SMFs are capable of influencing ion channel activity. However, the mechanisms underlying these observations are not clear.

Anisotropic diamagnetism has previously been suggested as a mechanism to explain the effect of moderate-strength SMFs on ion channels (Rosen 1993a, 1993b, 2003a, 2003b) and this may explain our results. It is proposed that the primary effect of magnetic fields is to induce rotation and reorientation of the individual lipid molecules that comprise the lipid membrane and is not therefore dependent on any effect of the magnetic fields on ion channels themselves. In fact, theoretical evaluations have shown that fields of this magnitude are too small to affect conformational changes in ion channels due to Lorenz forces (St. Pierre and Dobson 2000). This type of reorientation, however, could still result in indirect alterations to ion channel activity owing to changes to the mechanical properties of the membrane as a whole, potentially influencing factors such as levels of tension and flexibility.

If the results observed are due to membrane lipid orientation in the presence of the magnetic field, our results would seem to suggest that this alignment persists following the removal of the field with only limited levels of recovery observed within the time scale studied (typically 1–5 min). A possible explanation for this observation is that cell structures such as transmembrane proteins and the cytoskeleton, that are likely to both resist re-orientation and also provide a driving force to "correct" lipid membrane alignment following removal of the magnetic field (as suggested by Rosen 2003a, 2003b), are absent from the liposome system. It would appear that in this system there are few, if any, forces acting on the lipid membrane to drive re-orientation of lipid molecules back to the original state.

Our results also suggest that alignment of lipid molecules is not immediate, but instead there is a characteristic time delay between the onset of response and the application of the magnetic field. Similar time delays have been reported by other authors and have been explained in the context of anisotropic diamagnetism (Rosen 2003a, 2003b) with lipid orientation occurring relatively slowly over time until sufficient re-orientation has occurred to influence ion channel activity.

In the artificial lipid membrane system, the entire membrane is composed mostly of one type of lipid molecule. It is therefore likely that the artificial membrane has a greater potential to adopt a highly ordered arrangement than does a native mammalian cell membrane which contains many different lipid and non-lipid molecules as well as transmembrane proteins. As a consequence of this difference in structure there is also a clear difference in the thermal properties of the two membranes, with the artificial lipid membrane existing in a more fluid state at room temperature than would a native mammalian cell membrane and thus the acyl chains of the lipid molecules may be able to rotate and move more freely.

These two facts support our results showing membrane re-orientation effects at lower temperatures and within quicker time frames than those shown by Rosen's work. In addition, it was suggested that the involvement of the cytoskeleton may be key for the recovery processes seen by Rosen (2003a, 2003b). However, as there is no cytoskeleton in the artificial membrane system there is no residual force acting on the membrane to either resist initial lipid movement or drive a return back to the original configuration following removal of the magnetic field, as is the proposed case for studies conducted in mammalian cells.

Although our experimental results show a clear effect of SMFs on MscL activity the data presented here do not provide any direct evidence to support the diamagnetic lipid reorientation hypothesis as a means for explaining the mechanism by which moderate SMFs influence ion channel activity. Nevertheless, ordering of

membrane phospholipids by magnetic fields could affect the lateral expansion of the MscL area as it adopts the open state, since minute changes in bilayer thickness (approximately 1 Å) have been shown to be sufficient to stabilize the closed or open conformation of MscL depending on the thickness of the bilayer (Hamill and Martinac 2001; Perozo et al. 2002). Further studies will be needed to address this; however, the artificial liposome technique appears ideally suited for this type of investigation. Only ion channels and lipid molecules are present and therefore there is no risk of contamination from any cell signalling processes or cell structures, such as enzymes, second messengers or the cytoskeleton. The liposome technique also offers the opportunity to control and adjust the composition of the lipid membrane itself. By choosing a range of different lipid molecules with known levels of diamagnetic anisotropy it should be possible to investigate any correlations between magnetic susceptibility of lipid membranes and changes in ion channel activity observed in response to SMF exposure.

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References

Baroske E, Helfrich W (1978) Magnetic anisotropy of egg lecithin membranes. Biophys J 24(3):863–868

Baureus Koch CL, Sommarin M, Perrson BR, Salford LG, Ebehardt JL (2003) Interaction between weal low frequency magnetic fields and cell membranes. Bioelectromagnetics 24(6):395–402

Bras W, Diakun GP, Diaz JF, Maret G, Krammer H, Bordas J, Medrano FJ (1998) The susceptibility of pure tubulin to high magnetic fields: A magnetic biofrigence and X-ray fibre diffraction study. Biophys J 74:1509–1521

Cardon TB, Tiburu EK, Lorigan GA (2003) Magnetically aligned phospholipid bilayers in weak magnetic fields: optimisation, mechanism, and advantages for X-band EPR studies. J Magn Reson 161:77–90

Dobson J, Stewart Z, Martinac B (2002a) Preliminary evidence for weak magnetic field effects on mechanosensitive ion channel sub-conducting states in *E. coli*. Electromagnetic Biol Med 21:89–95

Dobson J, Stewart Z, Martinac B (2002b) preliminary evidence for weak magnetic field effects on mechanosensitive ion channel sub-conducting states in *E. coli*–errata. Electromagnetic Biol Med 21:309–309

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

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

Iwasaka M, Ueno S, Tsuda H (1994) Effects of magnetic fields on fibrinolysis. J Appl Phys 75:105–107

Maret G, Dransfeld K (1997) Macromolecules and membranes in high magnetic fields. Physica 86b:1077–1083

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

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

Rosen AD (1992) Magnetic field influence on acetylcholine release at the neuromuscular junction. Am J Physiol 262(6:1):1418–1422

Rosen AD (1993a) A proposed mechanism for the action of strong static magnetic fields on biomembranes. Int J Neurosci 73 (1–2):115–119

Rosen AD (1993b) Membrane response to static magnetic fields: effect of exposure duration. Biochim Biophys Acta 1148(2):317–320

Rosen AD (1996) Inhibition of calcium channel activation in GH3 cells by static magnetic fields. Biochim Biophys Acta 1282(1):149–155

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

Rosen MS, Rosen AD (1990) Magnetic field influence on paramecium motility. Life Sci 46(21):1509–1515

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

Sanders CR, Hare BJ, Howard KP, Prestegard JH (1994) Magnetically orientated phospholipid micelles as a tool for the study of membrane associated molecules. Prog NMR Spectrosc 26:421–424

Scholz F, Boroske E, Helfrich W (1984) Magnetic anisotropy of lecithin membranes. A new anisotropy susceptometer. Biophys J 45(3):589–592

Speyer JB, Sripada PK, Das Gupta SK, Shipley GG (1987) Magnetic orientation of sphinogomyelin-lecithin bilayers. Biophys J 51:687–691

Torbet J, Ronziere M (1984) Magnetic alignment of collagen during self assembly. Biochem J 219:1057–1059