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An upper limit for the effect of low frequency magnetic fields on ATP-sensitive potassium channels

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Abstract

Currents have been recorded for ATP-sensitive potassium channels in excised patches of membranes from an insulin secreting cell line, CRI-G1. The multi-channel records have been analyzed to reveal the single-channel conductance, the frequency and duration of bursts and the frequency of flickers (with periods between 0.5 and 5 ms). Control records in the absence of applied magnetic fields are similar to those reported by others. Patches have been exposed to parallel static and low frequency magnetic fields including a combination satisfying the 'cyclotron resonance' condition. The fields were applied for 30 s periods interleaved with 30 s controls. No significant differences in channel properties were observed between the control and field exposed periods. The largest change in position of the peak of the distribution of opening and closing transitions was 3%.

Keywords: Magnetic field; Potassium ion channel, ATP-sensitive; Single-channel conductance

1. Introduction

Based on experiments on whole cells and tissues, there have been several suggestions that low-amplitude, low-frequency magnetic fields can exert effects on the transport of ions across cell membranes [1–4]. A recurrent feature has been that combinations of static and low-frequency fields are effective when the frequency, f_0 satisfies the cyclotron resonance condition,

$$f_0 = \frac{1}{2\pi}\frac{q}{m}B_{DC}$$

where q/m is the ratio of charge to mass for the ion, and B_{DC} is the static magnetic field intensity. Clearly before a resonance effect, or indeed any effect on ion transport per se, can be accepted as established, there must be support from experiments on the actual ion transport processes concerned. Unfortunately no one knows which transport system is the 'correct' system to investigate. We have therefore chosen to investigate systems which are amenable to careful study.

Perhaps the simplest system which might display such effects is provided by the pores formed in lipid bilayer membranes by gramicidin A. Two independent groups have now found no detectable effect of magnetic fields with strengths from 50 μT to 5 mT on potassium and hydrogen ion transport through these channels [5–7]. However, observing no effect with one type of channel does not prove that effects cannot exist for another. We have chosen ATP-sensitive potassium channels for further study because they display a wide range of kinetic behaviour and are relatively easy to study in cultured cells well suited for patch-clamp measurements. This paper reports initial studies on the effects of magnetic fields on single-channel conductance, burst frequency and duration, and flicker frequency.

2. Methods

2.1. Cell culture

CRI-G1 cells, an insulin producing cell line, were grown in Dulbecco's Modified Eagle's medium (ICN Flow, High Wycombe, UK) containing 10% foetal calf serum with

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added penicillin (50000 units/ml) and streptomycin (50 mg/ml) (ICN Flow) in accordance with the method described by Carrington et al. 1986 [8]. Cells were passaged at weekly intervals and the culture medium was replaced every 48–72 h between passages. In preparation for each patch clamp experiment, cells were grown as a monolayer on the base of 3.5 cm plastic culture dishes (Falcon 3001; Becton Dickinson, Plymouth, UK) at a temperature of 37°C in a humidified atmosphere of 95% O_2/5% CO_2. Cells were used 2 to 6 days after plating.

2.2. Single-channel recording in the presence of magnetic fields

The apparatus used to produce the fields and to record currents across small patches of membrane is described elsewhere [7]. In this study recording was conducted using inside-out excised patches [9]. Cells attached to the bottom of a culture dish were viewed from below with phase contrast optics using a 40× objective with adjustable cover slip correction (Plan 40/0.55 Ph3DL 160/0-2.5 ELWD, Nikon Corporation, Tokyo, Japan). A mechanical micromanipulator (MX-1, Narishige Scientific Instrument Laboratory, Tokyo, Japan) was tilted to 45° to allow use of one of the fine horizontal adjustments for final positioning of the pipette. Patch pipettes were made from 1.2 mm o.d. filamented borosilicate glass capillaries (GC120F-10; Clark Electromedical Instruments, Pangbourne Reading, UK) using a Narishige PP-83 puller (Narishige Scientific Instrument Laboratory, Tokyo, Japan) to give a final resistance of 12–20 MΩ. The pipettes were heat polished by bringing the pipette tip close to a ca. 0.4 mm diameter glass bead on a glowing 0.1 mm platinum wire and bent to an angle of 45° by heating the shank in the flame of a methanol burner. In final position the axis of the pipette tip was nearly vertical. When it was intended to filter the trace at 5 kHz rather than 1 kHz, the pipette taper was coated with Sylguard (184 Elastomer, Dow Corning, Seneffe, Belgium).

A single coil on each axis (the smallest with 5 cm diameter) was used to produce the magnetic fields because this arrangement makes the patch experiments much easier and control of the magnetic fields is only required near the tip of the patch pipette, i.e., over a region less than 1 mm on a side. The ambient fields in all directions were nulled (to better than 5 μT) and further fields applied relative to this nulled state. In this study the applied fields indicated in Table 1 were applied vertically, i.e., parallel to the axis of the electrode.

Data, stored on DAT tape, were replayed through an 8 pole Bessel filter (902LPF2, Frequency Devices, Haverhill, MA), set at 1 or 5 kHz, sampled at twice this frequency and stored directly on hard disk using a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK) controlled using the Continuous Sampling module of the CED Patch 5.5 software.

2.3. Solutions and chemicals for single-channel recording

In all experiments, the recording electrode contained (in mmol/l): 140 KCl, 1 $MgCl_2$, 1 $CaCl_2$, and 10 Hepes (pH 7.4). The bath solution consisted of 140 KCl, 1 $MgCl_2$, 0.9 $CaCl_2$, 1 EGTA and 10 Hepes (pH 7.4). All chemicals used were analytical grade. Solutions were prepared using distilled water from a commercial still modified by replacing components containing plasticizers with PTFE.

3. Results and analysis of records

To allow comparison of channel properties with fields off or on it is necessary to record data for as many channels as possible in the shortest time possible which requires analysis of multi-channel data. Current vs. time records for these channels (Fig. 1) are very similar to those reported for ATP-sensitive K^+ channels by others (see, e.g., [10–15]). The records were analyzed off line in stages in a manner similar to that reported previously [7]. A

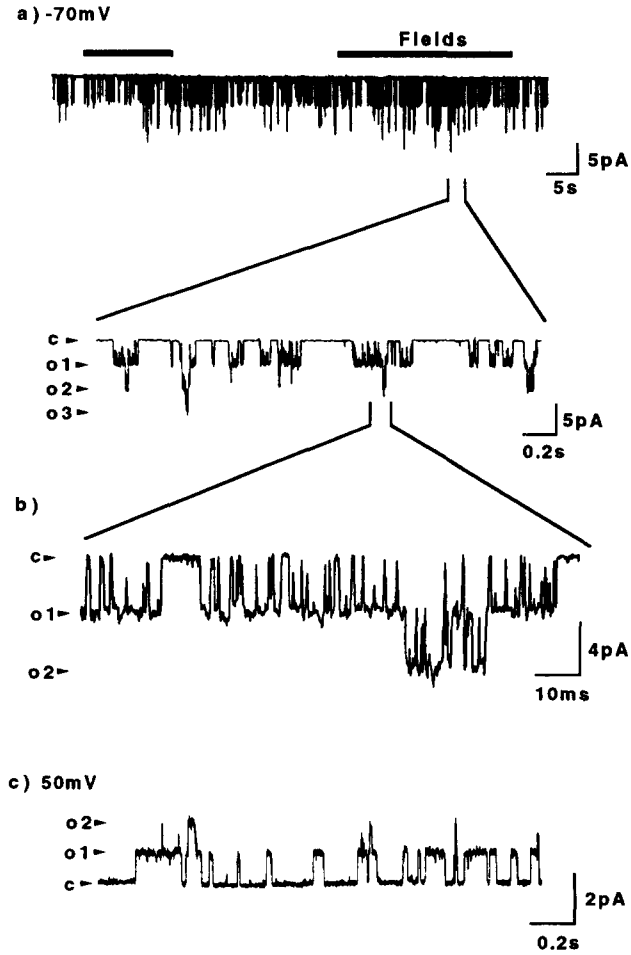

Fig. 1. Sample traces. (a) Playback from DAT tape Gould 2200S pen recorder (effective bandwidth ≈ 100 Hz). (b) and (c) Plot output from digitized data (filtered 5 kHz, sampled 10 kHz). The record (c) at +50 mV displays much less apparent flickering than the records at −70 mV.

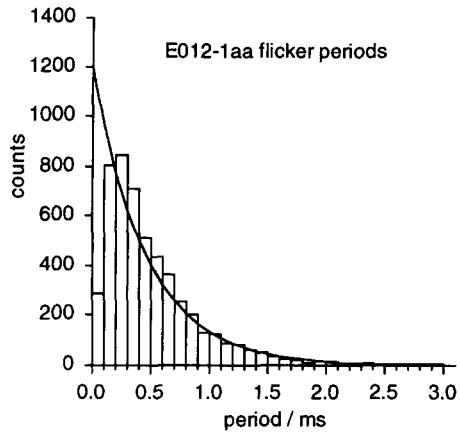

Fig. 2. Histogram of brief closed times. Data were collected for four periods, each of approx. 30 s. The curve, a single exponential with initial value 1556 and time constant 0.43 ms was fitted to the values in all except the first bin. Filtering prevents some of the fastest flickers from crossing a threshold. 'Off' periods, filtered at 5 kHz, sampled at 10 kHz.

results file is first created using the CED Patch 5.5 software. Transitions between levels are recognized as threshold crossings. The period of a result is the time between two successive crossings and its amplitude is the average of the current measurements during the period.

With 140 mM KCl and 1 mM $MgCl_2$ in the pipette and negative conventional membrane potentials (positive pipette potentials), channel activity is marked by rapid flickering between a high and low (possibly zero) conductance state (Fig. 1). As reported by previous workers, the current during the high conductance state varies linearly with the applied potential (data not shown). Fitting of exponentials to histograms of the durations of closed periods reveals at least three components. The fastest component, ca. 400 μs at this potential (see Fig. 2), is similar to that reported by Ashcroft et al. [13]. The longer closed times vary between patches and become longer with time after excision of a patch. For positive membrane potentials, flickers are much less evident, conductances are lower than at negative potentials and the current approaches a limit with increasing potential.

Some criterion for the end of a burst must be adopted to allow simple comparison of channel properties in the presence and absence of applied magnetic fields. Because the distribution of longer closed times could not be reproducibly characterized from our data (cf. discussion in [13]), 'flickers' were arbitrarily defined to include any decrease in level that was followed by an increase within 5 ms. (For consistency with the data sampled at 2 kHz, in the records sampled at 10 kHz only flickers lasting for at least five points have been counted). A burst, is then terminated by a decrease in level that persists for longer than 5 ms. An auxiliary program was used to process the results file to calculate the average period of a burst, the average amplitude of the high-conductance state during a burst, and the frequency of flickers during the burst. The program produces a new CFS (Cambridge File System) results file in which flickers are deleted by merging the levels before and after the flicker. The period of the merged level extends from the start of the first to the end of the second, while the amplitude is the average of the two levels, excluding the flicker. The result of this process can be inspected, edited, or further processed for the calculation of histograms using the CED software. The amplitudes correspond within the line width on the screen to the levels that would be drawn by eye through the 'tops' of the channels. Dwell time histograms for this result file reveal the presence of an excess number of brief openings represented by a single sample. Direct inspection of the data with the results file superimposed reveals that most of these are brief increases in conductance superimposed on top of a burst.

A second program using the results files as input, counts the number of channel openings, N, (ignoring those represented by a single point) and the number of flickers and calculates the average number of channels displaying bursts,

$$n = (\text{level} \times (\text{level period}))/T$$

where T is the total duration of the record. The average open time, τ, is then calculated using the relation

$$N \times \tau = n \times T$$

The flicker frequency reported in Table 1 is calculated as the number of flickers with periods between 0.5 and 5 ms divided by the average number of channels displaying bursts and the duration of the data sample. The program also calculates and tabulates the sizes of all transitions between levels (see Fig. 3). The position of the peak of these distributions and the average open-times are listed in Table 1.

In the presence of magnesium ions, ATP-sensitive K^+ channels in dialyzed whole cells and excised patches exposed to millimolar levels of magnesium are subject to

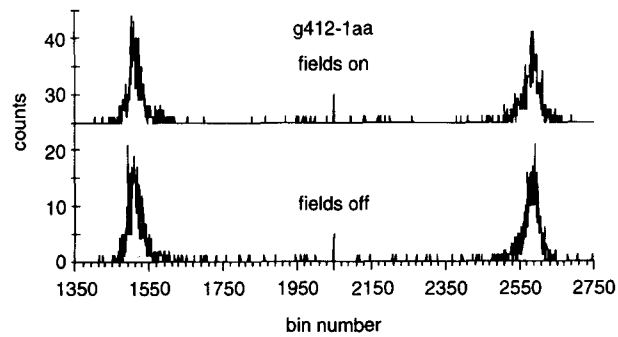

Fig. 3. Histograms of the amplitudes of channel openings and closings. For the purposes of comparing channel properties in the presence and absence of magnetic fields, a closing (end of a burst) is defined as a decrease in level that is not followed by a return to the original level within 5 ms. A marker has been added to the central bin (2049). Openings are tabulated to the right, closings to the left. The maximum change of 700 bins corresponds to 77 pS.

Table 1
Channel properties in the presence and absence of applied magnetic fields

Magnetic fields applied (μT)	Membrane potential (mV)	Amplitude (pA)	Burst duration (ms)	Burst frequency (s^{-1})	Flicker frequency (s^{-1} $burst^{-1}$) [a]
–	50	1.96	63.4	13.1	7.3
127	50	1.96	66.7	12.2	6.7
–	50	2.02	48.4	10.9	16.1
127	50	2.01	45.8	10.4	5.0
–	−50	2.71	20.4	3.02	221.5
127	−50	2.72	21.0	3.26	225.6
–	−70	4.0	20.4	6.58	184.7
127	−70	3.98	20.6	7.41	187.3
–	−40	2.07	31.5	6.53	126.5
254	−40	2.06	29.0	6.82	144.7
–	40	1.63	30.3	4.20	79.8
254	40	1.68	32.9	5.61	121.9
–	60	1.96	296	3.82	2.4
254	60	1.94	318	3.59	2.5
–	−50	2.48	24.5	4.98	152.3
254	−50	2.48	18.2	3.97	126.3
–	−50	2.43	9.1	3.38	120.4
254	−50	2.41	10.0	4.53	120.7
–	−30	1.57	13	4.29	111.7
508	−30	1.59	13.1	5.90	107.1
–	−40	2.16	12.8	9.38	125.5
508	−40	2.14	13.0	8.65	128.6
– [a]	−50	2.84	13.8	15.8	105.0
508 [a]	−50	2.84	16.4	19.9	104.0
– [a]	−70	4.09	32.0	4.31	156.0
508 [a]	−70	4.11	31.1	3.77	148.0
– [a]	−70	4.09	16.0	5.78	154.2
508 [a]	−70	4.11	16.1	6.20	155.0

A static field and a 50 Hz low frequency field of the same amplitude (mean to peak) were applied vertically.
[a] Filtered at 5 kHz, sampled at 10 kHz. Others filled at 1 kHz, sampled at 2 kHz.

run-down [15]. Under our conditions the long closed times between bursts increase, and the burst duration decreases over a period of 10–20 min. Therefore to allow comparison of channel properties in the presence and absence of the applied magnetic fields, on and off periods alternated at 30 s intervals. The data reported are the aggregates for the on or the off periods.

Examples of amplitude transition histograms with fields on (upper traces) and off (lower traces) are given in Fig. 3. In similar plots for all experiments the largest percentage difference ([on − off]/off) between the positions of the peaks with fields on or off was 3% (see Table 1). The mean ± 95% confidence limits for the 127, 254, and 508 μT data were $-0.16 \pm 0.67\%$, $0.15 \pm 2.1\%$, and $0.26 \pm 1.0\%$, respectively. There were also no significant changes in burst duration, the number of bursts per second, or the frequency of flickers. These results provide no evidence for an effect of the magnetic fields and demonstrate that any effect on the current through an open channel is very small.

4. Discussion

In previous studies of low frequency magnetic fields, no effect was found on the properties of gramicidin channels in lipid bilayers [6,7]. This precludes a general effect, present for all channels, of magnetic fields on ion permeation through pores. However, gramicidin channels are much smaller than the ion channels in biological membranes and they lack many of the gating properties which are important in the control of ion fluxes. Thus it may be argued that they also lack specific features needed to make a channel sensitive to the effects of magnetic fields. This paper reports investigations of a more complicated protein

channel that is well suited for careful quantitative investigations.

The multiple threshold feature of the CED Patch Continuous Analysis programme has been used to produce an idealized results trace from multi-channel records obtained for ATP-sensitive channels in excised patches from CRI-G1 cells. Subsequent analysis of the results trace provides values for the conductance of the open channel, the frequency and duration of bursts, and the frequency of flickers. Low-frequency magnetic fields applied so as to satisfy the 'cyclotron resonance' condition had no observable effect ($< 1\%$) on the current through the open channel. While there was considerably more scatter in the values (whether or not fields were applied), there was also no detectable change in the burst duration, burst frequency, or the flicker frequency. Further study will be required to investigate perpendicular combinations of fields and any interactions between the fields and the inhibition of channel activity by ATP. The present results and the observations that calcium currents across the membranes of a cell line are not affected by the presence of magnetic fields [16], provide no support for any effect of the fields under the conditions of the experiments. However, it remains a fundamental limitation of this type of study that demonstration of no effect, even for several types of channel under controlled conditions, provides only circumstantial evidence that effects of magnetic fields are absent for some as yet untested type of channel.

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