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Neuroscience Letters 337 (2003) 163–166

Neuroscience Letters

www.elsevier.com/locate/neulet

Action potentials from human neuroblastoma cells in magnetic fields

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Received 16 August 2002; received in revised form 23 October 2002; accepted 14 November 2002

Abstract

The patch-clamp method was used to measure transmembrane Na^+ and K^+ currents of the action potential in SH-SY5Y neuroblastoma cells exposed to static magnetic fields of 1, 5, and 75 G, 60 Hz fields of 1 and 5 G, and to combined static and low-frequency fields tuned for resonance of Na^+ and K^+. The maximum currents and their inactivation rates, and the activation rate of the Na^+ current were measured. Application of the magnetic fields did not result in detectable changes in any of the parameters of the action potential chosen for study. The occurrence of effects due to the fields could be excluded down to at least one part in 1000. The results suggest that magnetic fields of the type studied do not affect the cellular mechanisms responsible for generating the action potential.

Keywords: Action potentials; Magnetic field; Patch clamp; Ion resonance; Transduction

Static low-frequency electromagnetic fields (EMFs), alone and in combination, produce a wide variety of biological effects [17], including effects on brain electrical activity [2,14]. The nature of the biophysical mechanism that mediates these effects and the process by which the EMF is converted into a biological signal are under investigation [5,9,10,22]. We previously presented indirect evidence that sensory transduction was an applicable model for the conversion process [1,2,13,14]. The prototypical change that occurs in sensory transduction is a stimulus-induced change of transmembrane ion conductance occurring in highly specialized receptor cells [20].

Alternative possibilities to explain EMF signal conversion are that the field alters the transmembrane ion conductances that establish the cell membrane potential, or that the field directly affects the action potential. We previously considered the possibility that EMFs might alter the ion conductances responsible for the resting potential in neuroblastoma cells, and excluded that possibility down to about one part in 2000 [19]. In the present study we considered the possibility that EMFs affected mechanisms responsible for generating the action potential. We show here that this possibility is also excluded to approximately the same extent.

The human post-ganglionic neuroblastoma cell line SH-SY5Y (5Y) (American Type Culture Collection, Manassas, VA) was used [3]. Cells were seeded into 35 mm petri dishes (10^4 cells) and grown at 37 °C, 5% CO_2 in F12 medium with 10% fetal calf serum (Gibco, Grand Island, NY). Dibutyryl cAMP (50 μm, Sigma, St. Louis, MO) was added to the growth medium to produce the electrically excitable phenotype [21].

The cells were studied using the patch-clamp technique in the whole-cell configuration [15]. Microelectrodes (7–9 MΩ in bath solution) were made from borosilicate glass capillaries (1 mm in diameter) that were pulled in two steps (PB-7, Narishige, Tokyo, Japan) and fire-polished in a microforge (MF-9, Narishige). Gigaseals (~10 GΩ) were formed under negative pressure (5–10 cm H_2O), typically within 0.5–5 min; the success rate was greater than 50%. After gigaseal formation, the negative pressure was removed, and the gigaseals usually remained stable for more than an hour. Electrophysiological measurements were made using a patch-clamp amplifier (Axopatch 200B, Axon, Foster City, CA) and computer interface (Digidata 1200, Axon). Commercial software (pClamp, Axon) was used to control the amplifier, and collect and analyze the data.

The composition of the pipette solution was (in mM) 155

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doi:10.1016/S0304-3940(02)01329-0

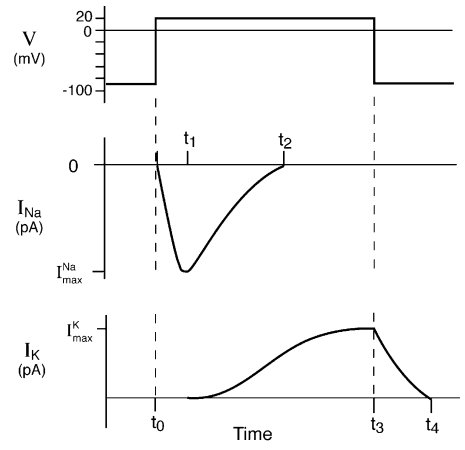

Fig. 1. Parameters measured in voltage-clamp experiments. The action potential triggered by the voltage step was decomposed pharmacologically into Na^+ and K^+ currents.

K-aspartate (monopotassium salt), 4 NaCl, 1 EGTA, 10 *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES)-KOH, pH 7.2. The composition of the bath solution was (in mM) 145 NaCl, 5.4 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-NaOH, 5 glucose, pH 7.3. All electrical measurements were made in bath solution at 25 °C. In particular experiments the K^+ channel blocker tetraethyl-ammonium chloride (TEA; 100 mM) (Sigma, St. Louis, MO) or the Na^+ channel blocker tetrodotoxin (TTX; 100 μM) (Calbiochem, San Diego, CA) was added to the bath solution.

Vertical magnetic fields were produced using a coil (average diameter, 19 cm) made from 907 turns of 18-gauge magnet wire. The coil was positioned on the microscope stage concentric with the petri dish containing the cells under study. The coil current was obtained from a wave generator (Wavetek, San Diego, CA) and power amplifier (Kron-Hite, Avon, MA), and was controlled using pClamp. The magnetic fields were measured using magnetometers whose sensing elements were about 1 cm^3 (Bartington MAG-03, GMW, Redwood, CA). The vertical and horizontal components of the geomagnetic field at the location of the cells were 280 and 286 mG, respectively. The geomagnetic field was not compensated, and therefore was present at all times in all experiments. The vertical component of the geomagnetic field was augmented for the resonance experiments (see below). Unless noted otherwise, all reported fields were measured at the location of the cells in the direction perpendicular to the plane containing them. The root mean square value of the applied time-varying fields is listed. The uniformity of the magnetic fields across the 35 mm petri dish was estimated to be ±2%, using commercial software (MF3D, ERM Inc., Pittsburg, PA).

Cells were clamped at zero current and stimulated with successive current steps from 20 to 120 pA in increments of 10 pA to verify excitability. Cells that responded with a fast membrane potential above 0 mV were used to study the effect of magnetic fields on the Na^+ and K^+ currents of the action potential, using TEA and TTX, respectively, in the bath solution to study the currents in isolation. The cell

Table 1
Effect of magnetic fields on the Na^+ current of the action potential in 5Y neuroblastoma cells[a]

	T_1 (ms)		I_{max}^{Na} (pA)		T_2 (ms)	
	Exp.	Control	Exp.	Control	Exp.	Control
1G AC	3.057 ± 0.203	3.050 ± 0.193	−263.1 ± 20.4	−262.6 ± 21.8	5.063 ± 0.905	5.072 ± 0.321
	4.111 ± 0.113	4.107 ± 0.129	−343.3 ± 17.0	−343.2 ± 17.4	6.747 ± 0.165	6.758 ± 0.195
	4.467 ± 0.117	4.463 ± 0.117	−320.0 ± 16.0	−320.6 ± 15.8	7.440 ± 0.194	7.427 ± 0.185
5G AC	2.897 ± 0.050	2.897 ± 0.053	−625.3 ± 56.3	−628.6 ± 57.1	4.475 ± 0.114	4.472 ± 0.116
	3.031 ± 0.004	3.031 ± 0.008	−554.2 ± 3.9	−554.7 ± 3.0	4.728 ± 0.013	4.730 ± 0.007
	3.418 ± 0.016	3.418 ± 0.013	−407.6 ± 10.6	−406.8 ± 10.2	5.410 ± 0.013	5.410 ± 0.014
1G DC	3.141 ± 0.019	3.133 ± 0.018	−298.1 ± 11.8	−299.6 ± 11.2	5.133 ± 0.010	5.124 ± 0.020
	3.172 ± 0.055	3.164 ± 0.050	−296.2 ± 20.7	−296.4 ± 23.6	5.150 ± 0.035	5.166 ± 0.067
	3.850 ± 0.101	3.846 ± 0.092	−389.0 ± 25.3	−390.5 ± 23.1	6.326 ± 0.163	6.337 ± 0.147
5G DC	3.183 ± 0.026	3.189 ± 0.031	−310.0 ± 17.5	−309.4 ± 17.3	5.196 ± 0.039	5.205 ± 0.045
	3.223 ± 0.020	3.223 ± 0.024	−288.6 ± 10.1	−286.9 ± 11.4	5.260 ± 0.020	5.250 ± 0.019
	2.747 ± 0.026	2.751 ± 0.031	−701.1 ± 12.1	−696.9 ± 12.4	4.238 ± 0.028	4.244 ± 0.025
75G DC	2.810 ± 0.007	2.811 ± 0.011	−439.1 ± 3.9	−440.3 ± 4.6	4.663 ± 0.008	4.664 ± 0.010
	3.163 ± 0.007	3.164 ± 0.009	−348.7 ± 3.5	−348.7 ± 4.8	4.976 ± 0.010	4.977 ± 0.014
	3.434 ± 0.008	3.436 ± 0.010	−303.2 ± 4.0	−304.4 ± 4.6	5.326 ± 0.006	5.327 ± 0.009
R−K^+	4.394 ± 0.010	4.394 ± 0.012	−249.7 ± 5.4	−250.2 ± 5.1	7.349 ± 0.003	7.352 ± 0.008
	4.617 ± 0.011	4.118 ± 0.009	−281.1 ± 7.1	−281.5 ± 4.1	6.548 ± 0.007	6.547 ± 0.004
	3.264 ± 0.006	3.262 ± 0.003	−552.7 ± 7.6	−553.3 ± 8.2	5.486 ± 0.007	5.485 ± 0.005
R−Na^+	3.332 ± 0.009	3.333 ± 0.015	−243.1 ± 5.4	−244.8 ± 5.7	5.289 ± 0.005	5.291 ± 0.008
	2.939 ± 0.009	2.939 ± 0.013	−443.3 ± 5.2	−444.4 ± 5.9	4.54 ± 0.010	4.523 ± 0.009
	3.746 ± 0.005	3.750 ± 0.012	−279.9 ± 3.4	−280.3 ± 6.0	5.468 ± 0.012	5.468 ± 0.010

[a] T_1, activation rate (t_1-t_0). T_2, inactivation rate (t_2-t_1). I_{max}^{Na}, maximum Na^+ current (see Fig. 1). Mean ± SD. R−Na^+, R−K^+, field resonance conditions for Na^+ and K^+, respectively.

was clamped at 90 mV and stimulated using a voltage step to +20 mV for 50 ms. The parameters measured were the maximum Na^+ and K^+ currents and their inactivation rates, and the activation rate of the Na^+ current (Fig. 1). A series of ten consecutive voltage steps constituted a cell-exposure epoch (*E*), and the time average of the measured parameter was used to represent the response of the ion channels to the field. A cell-control epoch (*C*) was similarly constituted and averaged except that no field was applied. A pair of *E* and *C* epochs comprised a trial, and each cell was subjected to ten trials and the data was evaluated using the *t*-test at $P < 0.05$. Three experiments were performed for each field condition studied (each experiment on a different cell). All experiments were performed on isolated cells to avoid possible complications due to formation of gap junctions [19].

In some experiments the order of the epochs was reversed (*C* and *E* instead of *E* and *C*). The change had no effect on the results, and only those obtained using *E* and *C* are reported here.

The maximum Na^+ current of the action potential and its activation and inactivation rates were measured during and after application of 60 Hz magnetic fields of 1 and 5 G, in six independent experiments; no significant differences were seen in any experiment between the action potentials triggered during presentation of the field and those triggered in its absence (Table 1). A second set of nine experiments was performed using 1, 5, and 75 G, DC, with similar results (Table 1).

A third set of experiments using combined time-varying and DC fields was conducted to determine whether the Na^+ currents in the action potential was affected by ionic resonant frequencies defined by $2\pi f = qB_0/m$, where B_0 is a static field, f is the frequency of a time-varying field having a root mean square value approximately equal to B_0, and q/m is the ionic charge-to-mass ratio [11,12]. B_0 was chosen to be 1 G and was obtained by applying a DC field of 720 mG from the coil in an additive fashion with the vertical component of the geomagnetic field (280 mG). Possible resonances of Na^+ and K^+ were considered, corresponding to field frequencies (determined by the ionic q/m values) of 66.6 and 39 Hz. During the field epoch, the time-varying field (1 G in all cases) was applied, collinearly with the static field. For both ions, application of the combined time-varying and DC fields had no effect on the Na^+ current of the action potential in six independent experiments (Table 1).

All of the studies were repeated under conditions appropriate to observe the effect of alternating, direct, and combined fields on the maximum K^+ current of the action potential, but no effects were observed (Table 2).

Several cogent considerations suggested it was unlikely that the mechanism responsible for generating the action potential would be sensitive to the applied fields. First, the Na^+ and K^+ channels that produce the action potential are regulated by the electric field present in the plasma membrane, and the effect of EMFs of the type considered here on the membrane field is negligible [16], indicating that the dynamical activity of the ion channels would not likely be altered by the applied EMFs. Second, EMFs did not affect the kinetics of ion channels in natural or artificial cell membranes [7,8]. Third, in animals that have structures

Table 2
Effect of magnetic fields on the K^+ current of the action potential in 5Y neuroblastoma cells[a]

	T_3 (ms)		I^{Na}_{max} (pA)	
	Exp.	Control	Exp.	Control
1G AC	7.314 ± 0.008	7.317 ± 0.009 d	294.5 ± 10.5	295.1 ± 10.7
	6.772 ± 0.011	6.767 ± 0.010	365.8 ± 10.6	364.8 ± 10.0
	7.417 ± 0.017	7.416 ± 0.022	295.6 ± 8.8	294.5 ± 9.6
5G AC	6.145 ± 0.008	6.838 ± 0.010	540.2 ± 8.2	539.6 ± 7.5
	7.360 ± 0.008	7.360 ± 0.002	293.5 ± 5.0	292.9 ± 5.6
	7.202 ± 0.008	7.206 ± 0.010	324.6 ± 4.0	324.3 ± 7.0
1G DC	7.659 ± 0.005	7.661 ± 0.008	287.3 ± 5.9	286.9 ± 3.4
	7.894 ± 0.010	7.893 ± 0.009	268.0 ± 5.6	266.4 ± 5.2
	6.838 ± 0.012	6.838 ± 0.010	433.1 ± 6.0	432.0 ± 5.0
5G DC	6.515 ± 0.062	6.602 ± 0.034	380.5 ± 5.7	379.9 ± 6.1
	6.157 ± 0.458	5.931 ± 0.038	447.3 ± 3.9	446.9 ± 5.4
	7.036 ± 0.105	7.123 ± 0.038	333.5 ± 7.0	333.3 ± 5.9
75G DC	7.012 ± 0.003	7.011 ± 0.003	374.6 ± 8.0	373.8 ± 7.9
	7.740 ± 0.007	7.740 ± 0.006	316.5 ± 5.8	315.0 ± 3.2
	6.412 ± 0.004	6.413 ± 0.005	486.5 ± 4.5	486.1 ± 4.3
$R - K^+$	7.091 ± 0.029	7.081 ± 0.026	326.1 ± 17.3	326.7 ± 17.4
	5.750 ± 0.033	5.745 ± 0.029	569.7 ± 12.3	573.9 ± 12.2
	7.124 ± 0.022	7.124 ± 0.021	314.5 ± 11.4	316.3 ± 10.2
$R\text{-}Na^+$	6.884 ± 0.016	6.892 ± 0.020	420.3 ± 10.1	421.2 ± 10.5
	5.967 ± 0.047	5.965 ± 0.053	541.4 ± 57.1	540.2 ± 54.6
	6.300 ± 0.009	6.306 ± 0.003	568.3 ± 3.0	567.6 ± 3.9

[a] T_3, inactivation time ($t_4 - t_3$). I^{Na}_{max}, maximum K^+ current (see Fig. 1). Mean ± SD. $R - Na^+$, $R - K^+$, field resonance conditions for Na^+ and K^+, respectively.

known to mediate electrosensation [6,18], the process is carried out by highly specialized cells. It therefore seemed implausible that an electrosensory mechanism would exist in every cell capable of exhibiting an action potential, which would have been the inference if effects had been demonstrated in this study. Nevertheless, the effect of EMFs on the action potential had not previously been examined directly.

We studied the impact of seven different fields on the Na^+ and K^+ channels, with each experiment performed in triplicate (42 independent experiments). Application of the magnetic fields did not result in detectable changes in any characteristics of the action potential chosen for study (Tables 1 and 2). The characteristics chosen (Fig. 1) were arbitrary, but they are frequently used to quantitate the action potential, and it is reasonable to expect that one or more of them would have been altered in at least some of the experiments if the action potential were susceptible to EMFs.

In the experiments using combined time-varying and DC fields, the exposure conditions tested were those described by Liboff and colleagues [10,12]. Blackman and colleagues showed that orthogonal time-varying and static fields could give different results from parallel time-varying and static fields [4]. Further, still different results could be obtained when the static magnetic field had components simultaneously parallel and perpendicular to the time-varying component. Thus the conditions tested here were not those described in the model of Blanchard and Blackman [5].

We were able to measure current to about 0.2 pA, and time to at least 5 μs. Consequently, any effects on the measured parameters could be excluded down to at least one part in 1000, as assessed by comparing the measurement precision with the magnitude of the measured parameters (Tables 1 and 2).

Taken together with our previous study [19] the present results could mean that any transduction of EMFs by excitable cells involves cells more specialized than 5Y neuroblastoma cells. However, there are possibilities for transduction other than those tested here including alterations in intracellular signaling cascades, a requirement for a network of cells rather than isolated cells, and the need for specific agents to confer sensitivity on cells to EMFs.

Finally, the experiments reported here were performed at 25 °C, rather than 37 °C, which is a more optimal temperature for mammalian cells. It is possible that different results would be obtained under more physiological conditions.

Supported in part by a grant from the National Institute of Environmental Health Sciences.

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