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Threshold and limits of magnetic field action at the presynaptic membrane

Arthur D. Rosen *

Department of Neurology, School of Medicine, State University of New York at Stony Brook, Stony Brook, NY 11794-8121, USA

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Abstract

The relationship of field intensity and exposure duration to the inhibitory effect of static magnetic fields on presynaptic membrane function was examined in order to further define the mechanism of action of these fields. Miniature endplate potentials (MEPPs) were recorded from the isolated murine neuromuscular junction, maintained at a temperature of 35.5°C, during exposure to static magnetic fields of varying duration and intensity. Inhibition of MEPP generation correlated well with the product of the square of the flux density and exposure time. At lower product values the relationship was linear with an absolute flux density threshold of 37.9 mT. Higher product values were associated with deviation from linearity indicative of a limit on the extent of inhibition. These findings are consistent with the hypothesis that static magnetic fields induce a reorientation of diamagnetic molecular domains within the membrane but with a restriction on the degree of reorientation imposed by the membrane's cytoskeleton.

Key words: Static magnetic field; Diamagnetism; Neuromuscular junction; Magnetic field; Presynaptic membrane

1. Introduction

There is now substantial evidence that moderate intensity static magnetic fields can alter cellular function, with the plasma membrane being their most likely site of action. This evidence comes from several diverse phenomena, including changes in the blood–brain barrier [1], inhibition of spontaneous neuronal activity in the central nervous system [2], alteration in the function of membrane receptors [3], inhibition of the activity of a membrane-bound enzyme [4], and inhibition of membrane-mediated signals controlling intracellular protein synthesis [5]. Membrane conformational changes, due to the diamagnetic properties of phospholipid domains, have been shown to occur in the presence of static magnetic fields [6] and a recent report from this laboratory [7] provided strong support for the hypothesis that magnetically induced membrane deformation can reversibly alter the function of imbedded ion channels. This effect on membrane function has been shown to be dependent on both field strength and exposure duration [8]. The present study was undertaken in order to examine the relationship of these exposure parameters on one manifestation of magnetic field exposure, the release of acetylcholine at the neuromuscular junction. This information, taken in the context of some of the known physical properties of cellular structures, provides further insight into the mechanism of action of these fields.

2. Methods

Miniature endplate potentials (MEPPs) were recorded from the murine phrenic nerve-diaphragm preparation. The left hemidiaphragm of decapitated Swiss Webster mice (20–30 g) was rapidly excised and secured to the silicone rubber coated base of an 8-ml lucite chamber perfused with Krebs solution (mM: NaCl 135, KCl 5, $CaCl_2$, $MgCl_2$ 1, NaH_2PO_4 1, $NaHCO_3$ 15, glucose 11) bubbled with 95% O_2–5% CO_2. The perfusate had a pH of 7.4. Temperature was maintained at 35.5°C ± 0.05° by means of an in-line heater driven by a proportional temperature controller

* Corresponding author. Fax: + 1 (516) 444-1474. Email: arosen@neuro.som.sunysb.edu.

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with its sensing thermistor within the chamber. The chamber was centered between the poles of an electromagnet whose field was oriented horizontally, parallel to the plane of the tissue. That magnet consisted of a 2700 turn coil wound on a 2.4 cm^2 soft iron core with a pole separation of 4.4 cm. Power was provided by a computer-controlled regulated current source capable of providing up to 3.52 A. Induced currents were virtually abolished in the preparation and recording instrumentation by ramping the current on and off at 2.35 A/s. The maximum field produced by this magnet, measured midway between its poles, was 125 mT.

Recordings were always carried out at or very close to the geometric center of the magnetic field, where there was maximum flux homogeneity and no detectable field gradient. Using the course of the phrenic nerve as a guide, alignment of the tissue within the chamber was virtually the same in all experiments. Endplate regions were visually identified and both MEPPs and membrane resting potentials recorded with intracellular glass micropipettes filled with 3 M KCl and having impedances of 15–30 MΩ. Following amplification, MEPPs were displayed on an oscilloscope and on-line frequency and amplitude measurements carried out with a computer interfaced to the recording system. The bandpass of the system was 0.1 to 10 kHz with a RMS noise level typically less than 10 μV. Only electrode positions associated with MEPPs having rise times of less than 1 ms were considered true endplate recordings and accepted for analysis. Peak amplitudes were measured in selected 50-s epochs and sorted into five appropriately sized groups for evaluation of quantal size. Membrane resting potentials were continuously monitored with a DC milivoltmeter.

MEPPs were counted continuously in 1-s intervals and, following a control period of 100 s, the magnetic field was activated for 150 s with an initial intensity of 125 mT. Counting continued for an additional 100 s after deactivation of the field. This sequence was repeated with consecutive 10 mT decrements in field strength down to a minimum intensity of 35 mT. The entire series was than repeated for field activation times of 100 and 75 s, but with these exposure intervals the minimum field intensity was only 65 mT, since below that level there was virtually no change in MEPP frequency. The mean MEPP frequency was calculated for successive 25-s intervals after which the maximum deviation of the 'field on' frequency from the control frequency was determined and examined as a function of field strength and exposure duration.

3. Results

MEPPs accepted for analysis had amplitudes of 1.0 to 1.5 mV but, for any endplate penetration, showed little amplitude variability (Fig. 1A). The rate of spontaneous MEPP generation ranged from 0.5 to 3.5 Hz though, for a given recording site, the rate was relatively constant. The preparation's stability was assessed by counting MEPPs for 10 min in the absence of the external magnetic field. Under these conditions, the mean MEPP frequency in successive 25-s intervals varied by less than 10%. Data collected in the field exposure sequences was considered valid only if this limit was not exceeded during the control period. Analysis was carried out only on those endplate recordings which remained stable for the full sequence of activation times and field intensities, i.e., 24 separate exposures in 126 min. In view of the recent finding that inhibition of MEPP generation is a function of exposure duration, but only for durations up to 150 s [8], longer exposures were not used in the present study. Activation of the magnet was not associated with any change in the system's noise level and, therefore, did not influence the accuracy of either the amplitude or frequency measurements.

Magnetic field exposure did not change the resting potential of the postsynaptic membrane nor were there any changes in MEPP amplitude as would be seen if there were alterations in quantal content of vesicular acetylcholine (Fig. 1B). There was, however, a consistent decline in MEPP frequency, typically beginning 50 to 75 s following activation of the field. This inhibitory effect of the magnetic field on MEPP generation was a function of both its strength and duration. The relationship of field strength to mean MEPP inhibition, for the three exposure durations studied, is shown in Fig. 2. The threshold for inhibition was considered to be that field strength associated with a MEPP frequency change just greater than the accepted 10% variability limit for the preparation. Based on this criteria, the mean 150 s exposure threshold was 44 mT, the mean 100 s exposure threshold was 67 mT, and the mean 75 s exposure threshold was 73 mT. Maximum inhibition occurred at and above 100 mT for 150 s exposures and at approximately 125 mT for 100 s exposures. Maxi-

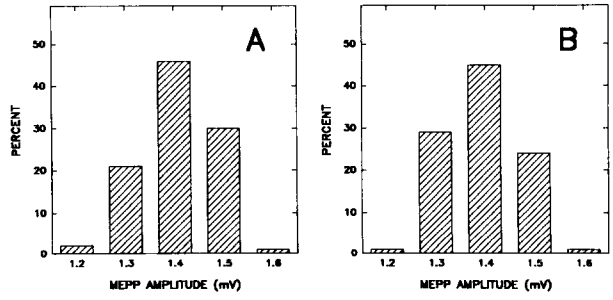

Fig. 1. MEPP amplitude distribution. (A) 50 s control epoch. Frequency 2.1 Hz, mean amplitude 1.41 mV. (B) 50 s epoch beginning 100 s after onset of 120 mT field. Frequency 1.4 Hz, mean amplitude 1.40 mV.

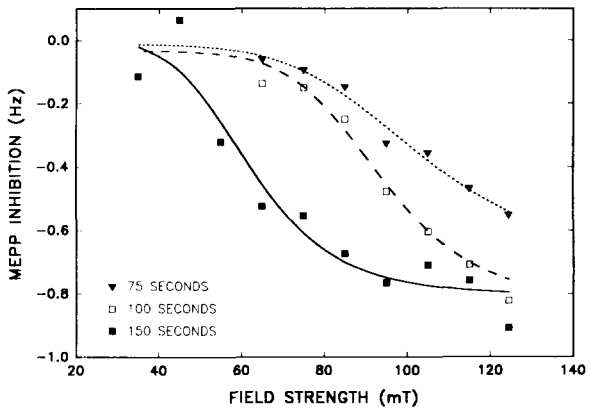

Fig. 2. MEPP inhibition as a function of magnetic field strength for exposure of 75, 100, and 150 s. Mean values for five endplate recordings which remained stable throughout entire exposure sequence, with best fit sigmoid functions.

mum inhibition for the 75 s exposures appeared to require a field strength greater than that available with the present electromagnet.

4. Discussion

Molecules with diamagnetic anisotropy will, in a homogeneous static magnetic field, align to an orientation representing the minimum free energy state. For individual molecules, the randomizing effect of thermal energy is more than adequate to prevent such reorientation. It has been shown [9] that for domains of interacting molecules, aligned along a common axis, the aggregate molecular anisotropy can be sufficient to overcome the effects of thermal energy. The likelihood of such a molecular domain rotating in a magnetic field may be expressed by the ratio, β, of magnetic energy to thermal energy.

$$\beta = \frac{-N\Delta_\chi H^2}{k_B T} \quad (1)$$

where N is the number of interacting molecules, each with a diamagnetic anisotropy of Δ_χ, H is flux density, k_B is Boltzmann's constant and T is absolute temperature. Molecular orientation will occur when $\beta > 1$.

The enhanced diamagnetic properties of biological membranes, with their highly ordered phospholipid bilayer structure, is well established [10–13]. The suggestion that magnetically induced reorientation of membrane phospholipids could result in a temporary membrane deformation sufficient to impair the function of imbedded ion channels [14] was supported by studies carried out on the murine neuromuscular junction [7]. In that system, the disruption of presynaptic calcium channels inhibits the release of acetylcholine and thereby MEPP generation. This phenomenon is highly temperature-dependent and appears to be related to the non-homogeneous nature of the membrane thermotropic phase transition. At the prephase transition temperature, domains of lipid molecules in the gel (L_β) phase exist within a more fluid liquid crystal (L_α) phase bilayer. These domains exhibit marked enhancement in diamagnetic anisotropy, termed superdiamagnetism [11], which is a function of their volume. With increasing temperature, the volume of individual L_β domains decreases until, just above the phase transition temperature, the entire bilayer is in the L_α phase. The temperature at which the present study was carried out was that which, based upon previous work [7], appears to be associated with the optimum L_β domain volume.

Reorientation of diamagnetic structures in a magnetic field is an inherently slow process which is a function of both their geometry and anisotropy, as well as of the field strength and the nature of the suspension medium. Rotatory motion of large ensembles of oriented diamagnetic molecules in a homogeneous magnetic field can be described by a non-linear differential equation [15]. From that equation, the time-course for rotation becomes

$$\ln \theta_1 - \ln \theta_0 = \frac{-N\Delta_\chi H^2}{\zeta} t \quad (2)$$

where θ is the angle between the molecular axial diamagnetic vector and the field direction and t is the time required for rotation from θ_0 to θ_1. ζ is the rotatory frictional coefficient, the value of which is established by the size and shape of the ensemble and by the viscosity of the suspension medium. For large diamagnetic ensembles the randomizing effect of thermal energy will be negligible, though this is not necessarily the case with small membrane domains where thermal energy may constitute a significant impediment to rotation. Superdiamagnetic domains have, however, a $\beta \gg 1$ and for these temperature-dependent membrane structures Eq. (2) is theoretically applicable. Specifically, that equation should provide a reasonable description of the relationship between exposure parameters and domain reorientation.

Under conditions of fixed temperature, the number of molecules in each domain and their diamagnetic anisotropy will be constant. The rotatory frictional coefficient may be assumed to remain constant and domain reorientation will therefore be a function of the product of the square of the flux density and exposure time (H^2t). Since the manifestation of magnetic field exposure appears to be tightly coupled to domain reorientation [8], the correlation between this product and the observed MEPP frequency changes was examined. That relationship is shown in Fig. 3, where a regression curve fitted to the data had a

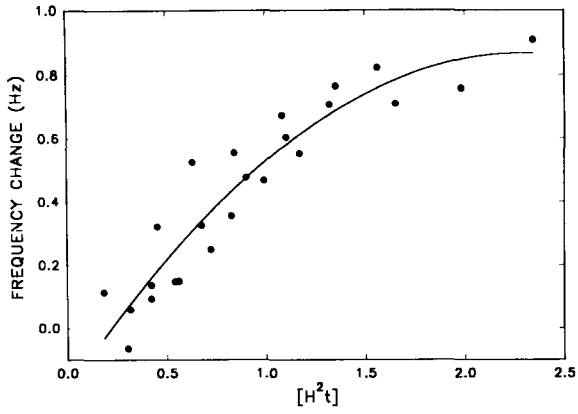

Fig. 3. MEPP frequency change as a function of the product of the square of the flux density and exposure time. Same data as from Fig. 1, with best fit regression curve. $r^2 = 0.8836$.

coefficient of determination of 0.8836. For values of H^2t below 1.55, a near linear relationship was evident and the interpolated threshold value for H^2t was 0.216. The corresponding field strength, for the previously established optimum exposure duration of 150 s, is 37.9 mT. This represents the absolute lower limit for magnetic field influence in this system and is comparable to the threshold found in two quite dissimilar systems. A 10 mT static magnetic field has been shown to reversibly block action potentials elicited from cultures of dorsal root ganglia [16], but only at temperatures greater than 32°C. A 30 mT static magnetic field has been shown to produce permeability changes in synthetic liposome vesicles [17], with optimum effects evident at the prephase transition temperature. The present study is consistent with these reports and suggests that temperature-dependent sensitivity to static magnetic fields may be a ubiquitous property of all phospholipid membranes.

Deviation from linearity when $H^2t > 1.55$ suggests a mechanical limit imposed on the free rotation of membrane diamagnetic domains. Any structure capable of restricting such movement should be distributed uniformly in close proximity to the membrane and, in some manner, mechanically bound to it. In addition it should be less influenced by a magnetic field than the membrane diamagnetic domains. These conditions are met by that protein network adjacent to the cytoplasmic side of the membrane which, along with numerous filamentous attachments to a variety of intramembranous polypeptides, constitutes the membrane cytoskeleton [18]. This subcellular structure's primary function is to provide a support framework for the less rigid plasma membrane. Since non-oriented proteins have a low diamagnetic anisotropy [19], the cytoskeleton will be relatively insensitive to the effects of magnetic fields. In the presence of a field sufficient to induce domain rotation, it is not difficult to envision this essentially immobile structure exerting a restrictive influence on such movement. Unlike viscous frictional forces which are present over the entire range of domain rotation, the forces exerted by the cytoskeleton first become manifest at some, as yet undefined, limit. This mechanism explains, at least qualitatively, the non-linearity seen in Fig. 3.

The changes observed in the frequency of spontaneously generated MEPPs would not be expected to produce any behavioral manifestations in the intact organism since, in order to do so, they would have to influence the synaptic potential. That potential requires the synchronous release of several hundred quanta in a 1–2 ms period. A change in the background release rate of less than 1 per second is not likely to alter the probability of a synaptic potential being generated. In addition, since the time-course of this phenomenon is a function of the square of the field strength, changes in background activity occur within seconds in the presence of sufficiently high fields. The magnitude of the change, however, remains limited and therefore the generation of synaptic potentials should not be influenced even by fields with intensities of several Tesla.

The present study defines both the threshold and limit of magnetic field interaction with a specialized type of membrane, at a specific temperature. The observed phenomena may be adequately explained on the basis of reorientation of diamagnetic domains within the membrane, though further studies are needed to establish the precise magnitude of such reorientation.

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