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Membrane response to static magnetic fields: effect of exposure duration

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The time-course for the reversible alteration in presynaptic membrane function associated with exposure to a 123 mT static magnetic field was examined in an attempt to help define the mechanism whereby these fields influence biomembranes. Miniature endplate potentials (MEPPs) were recorded in the isolated murine neuromuscular junction preparation, maintained at a temperature of 35.5°C. A minimum field duration of 50 s was found to be necessary for MEPP inhibition, with the efficacy of the field in inducing further inhibition being a function of its duration, but only for periods up to 150 s. Longer durations were not associated with additional inhibition. The time required for MEPP frequency to return to baseline, following deactivation of the field, was found to be linear for field durations up to 150 s. At and above this limit, recovery time remained constant at 135 s. These findings are consistent with the slow reorientation of diamagnetic molecular domains within the membrane and suggest tight coupling to the mechanism responsible for neurotransmitter release. The limits on this effect are compatible with the mechanical constraints imposed by the membrane's cytoskeleton.

Introduction

In a recent report from this laboratory [1] the influence of moderately intense static magnetic fields on presynaptic membrane function was described. In that study, changes in the frequency of miniature endplate potentials (MEPPs), recorded from the murine phrenic nerve-diaphragm preparation, were examined during and following exposure to a 123 mT static magnetic field. A temperature-dependent inhibition of MEPP generation was found which could be abolished by removing calcium from the tissue perfusate. This reversible alteration in synaptic function may be explained on the basis of the diamagnetic properties of membrane phospholipids. It has been proposed that the partial reorientation of molecular domains deforms the presynaptic membrane sufficiently to induce functional impairment in contiguous ion channels. The change in diamagnetic anisotropy known to be associated with the membrane thermotropic phase transition [2] would explain the temperature dependence of this phenomenon. Reorientation of diamagnetic molecules is an inherently slow process which, in theory, should be limited by the physical constraints of the membrane. Although neither the rate nor the limits of this molecular reorientation have been defined, a reasonable approximation should be possible by analysis of the time course of the membrane's functional changes. In addition, it is uncertain whether greatest functional changes occur coincidentally with maximal molecular reorientation or with maximal channel perturbation at a time when further molecular reorientation is still possible. The present study was undertaken in an attempt to resolve these issues in order to further define the mechanism by which static magnetic fields influence biomembranes.

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Materials and Methods

Miniature endplate potentials were recorded from the murine phrenic nerve-diaphragm preparation. The left hemidiaphragm of decapitated Swiss Webster mice (20–30 g) was excised and secured to the silicone rubber coated floor of an 8 ml lucite chamber perfused with Krebs solution (in mM: NaCl 135; KCl 5; $CaCl_2$ 1; $MgCl_2$ 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 ± 0.05°C by means of an in-line heater driven by a proportional temperature controller with its sensing thermistor within the chamber. The chamber was centered between the poles of an electromagnet with a horizon-

tally oriented field, 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. It was energized with 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 123 mT.

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. The recording site was always 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. MEPPs were counted continuously, with the first 100 s serving as a control after which the field was activated for 25 s. Counting continued for 100 to 300 s following deactivation of the field. This sequence was repeated with field activation times of 50, 100, 150, 200 and 250 s.

The low MEPP frequency (0.5 to 3.5 Hz) necessitated counting MEPPs in successive 25-s intervals and then calculating the mean frequency for each interval. For each sequence, the maximum deviation of the 'field on' MEPP frequency from the control frequency was determined and plotted as a function of field duration. In addition, the time required for MEPP frequency to return to baseline following deactivation of the field was examined as a function of field duration.

Results

In order to assess the stability of the preparation, MEPPs were counted for periods of up to 10 min in the absence of the magnetic field. Under these conditions, the mean MEPP frequency in successive 25 s intervals was found to vary by less than 10%. Data gathered in the field exposure sequences were considered valid only if this limit was not exceeded during the control period. Since our previous observations [1] demonstrated maximum inhibition of MEPP generation at 35.5°C, the present study was restricted to that temperature as well as to a field strength of 123 mT. Only the duration of the field was varied.

Magnetic field exposure was not associated with any change in the postsynaptic membrane resting potential. There was no observed change in MEPP amplitude as would be seen if there were an alteration in vesicular quantal content [3]. There was, however, a consistent decline in MEPP frequency when the field duration was 50 s or greater. This phenomenon had a delayed onset but typically began 50–75 s following activation of the field. The efficacy of the field in inhibiting MEPP generation was a function of its duration, but only for periods up to 150 s. Longer durations were not associated with additional inhibition. These relationships are shown in Fig. 1.

Following deactivation of the field there was a slow return in MEPP frequency to preexposure levels. The time required for recovery to baseline was a function of

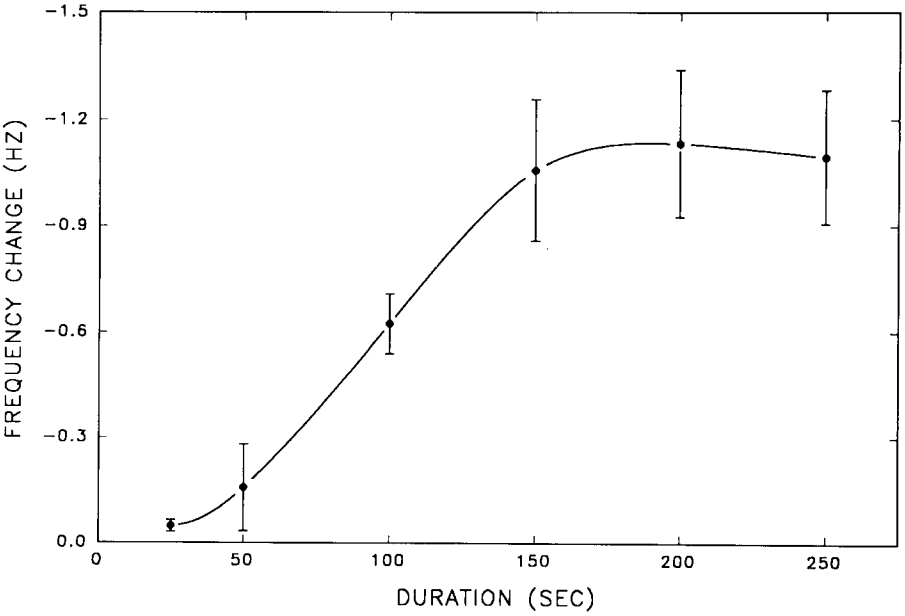

Fig. 1. Maximum observed inhibition of MEPP generation associated with exposure to a 123 mT magnetic field for periods of 50 to 250 s. Cumulative data for eight experiments with standard error bars.

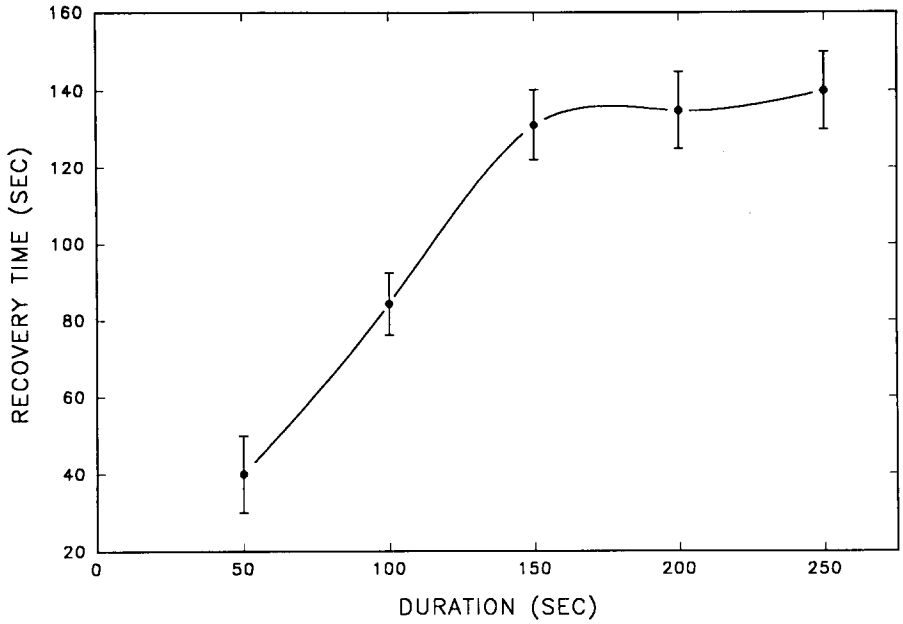

Fig. 2. Time required for return of MEPP generation to control values, following 50 to 250 s exposure to a 123 mT magnetic field. Cumulative data for eight experiments with standard error bars.

field duration. This was linear for durations up to 150 s. At and above this limit the recovery time remained constant at 135 s. This is illustrated in Fig. 2.

Discussion

Molecules with diamagnetic anisotropy will, in a homogeneous magnetic field, rotate to an equilibrium orientation representing the minimum free energy state. This orientation is opposed by the randomizing effect of thermal energy. For single molecules, even those that are highly diamagnetic, thermal energy is more than sufficient to prevent orientation. For domains of interacting molecules, however, individual anisotropies are additive and the summed anisotropy may be sufficient to overcome the disordering effects of thermal energy [4]. The ratio, β, of magnetic energy (E_M) to thermal energy, is a measure of the probability of a molecular ensemble rotating in a magnetic field.

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

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 absolute temperature. Molecular orientation will occur when $\beta > 1$. For the field strength and temperature used in this study, this condition will be satisfied, for phospholipid molecules, when $N > 10^6$. A value of N several orders of magnitude larger would be required for proteins, even for those with an α-helical structure where the value of Δ_χ is greater than that of β-pleated sheets or collagen structures [5].

The diamagnetic anisotropy of the phospholipid bilayer in the gel phase is much higher because of tight packing of the membrane lipid molecules. The rigidity of the membrane will, however, limit its ability to be deformed in a magnetic field. With increasing thermal energy, an abrupt rotameric disordering of the lipid acyl chains occurs, denoting the transition to the liquid-crystal phase. Although the liquid-crystal membrane will have less diamagnetic anisotropy [2], its fluidity makes it more susceptible to deformity by a magnetic field. Thermotropic phase transition in biomembranes is not an entirely uniform process and, at the prephase transition temperature, domains of gel-phase molecules exist within the more fluid liquid-crystal bilayer. These domains exhibit 'superdiamagnetic' properties [6] as a function of their volume. Previous studies with the murine neuromuscular preparation [1] suggests that the optimum domain volume occurs at 35.5°C.

The latency of 50–75 s between activation of the magnetic field and onset of measurable changes in presynaptic membrane function may be readily explained on the basis of the inherent slowness of rotation of diamagnetic structures within a fluid medium. Synthetic multilamellar liposomes have been shown to require 4 s for 90° rotation in a 2.5 T field [6]. Retinal rod outer segments rotate 88° in 4.1 s when subjected to a 1 T field [7] but 30 s are required to rotate these same structures only 60° when the field was 0.5 T [8]. Up to 100 s is required for total alignment of large

lecithin vesicles in a 1.5 T field [9]. The time required for rotation of a diamagnetic molecular cluster in a homogeneous magnetic field is a function of its size, shape and diamagnetic anisotropy, as well as the nature of the suspension medium and the field strength. Rotatory motion of such structures can be described by a nonlinear differential equation [7]. Discarding the small inertial term in that equation was shown to provide a good approximation. Therefore

$$\zeta \frac{d\theta}{dt} + \frac{N\Delta_\chi H^2}{2} \sin 2\theta = 0 \quad (2)$$

where ζ is the rotatory frictional coefficient and θ is the angle between the cluster's principal diamagnetic axis and the direction of the magnetic field. It follows from Eqn. 2 that the time-course for rotation is

$$\ln \theta_1 - \ln \theta_0 = \frac{-N\Delta_\chi H^2}{\zeta} t \quad (3)$$

where t is the time required for rotation from θ_0 to θ_1. Rotation time, therefore, is inversely proportional to the square of the field strength and directly proportional to ζ. The value of ζ is independent of the cluster's molecular nature but is defined by its geometry and the viscosity of the suspension medium.

Except at extremely high field strengths, it is unlikely that cluster rotation during a short field exposure would be of sufficient magnitude to perturb adjacent ion channels. The present study found that at least 50 s is required for a field of 123 mT to induce detectable changes in membrane function. A limit on these changes was seen for exposure durations above 150 s, presumably reflecting some superimposed restriction on cluster rotation. A likely candidate for such a restrictive mechanism is the network of proteins adjacent to the cytoplasmic side of the cell membrane, with its numerous filamentous attachments to intramembranous polypeptides. This complex structure makes up the membrane cytoskeleton [10], which has as one of its functions, the provision of a structural framework for the cell [11]. Since, for those reasons previously stated, protein structures are relatively insensitive to the effects of strong magnetic fields, the fixed cytoskeleton could function to limit the movement of clusters within the attached lipid bilayer membrane.

The possibility must be considered that although maximum channel perturbation appears to occur with exposures of 150 s, this does not preclude further cluster rotation with longer exposures. If this were the situation, recovery time following deactivation of the field should have two components; the time necessary for clusters to return to the orientation associated with maximum channel effect, and the time necessary for return from that orientation to the normal configuration. Recovery times for exposures longer than 150 s would therefore be greater than those seen with exposures of just 150 s. This was not the case and although recovery time did increase with exposure duration, it reached its maximum value for exposures of 150 s. The observation that the limit for both membrane effect and recovery time occur as a consequence of the same exposure duration, supports the hypothesis that ion channel functional disruption is tightly coupled to the degree of cluster reorientation in a magnetic field.

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