Studie}s on the E{ff{ect of Static Magnetic Fields on Biological Systems}

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Abstract — Considerable evidence now exists relating exposure to static magnetic fields (SMFs) to changes in a number of biological systems, particularly those whose function is linked to the properties of membrane ion channels. Most of the reported effects of moderate SMF may be explained on the basis of alterations in membrane calcium ion flux as well as intracellular calcium availability. The mechanism suggested to explain these effects is based on the diamagnetic anisotropic properties of membrane phospholipids. It is proposed that reorientation of these molecules during SMF exposure will result in the deformation of imbedded ion channels, thereby altering their activation kinetics. Channel inactivation kinetics would not be expected to be influenced by these fields because the mechanism for inactivation is not located within the intramembraneous portion of the channel. Patch-clamp studies of calcium channels have provided support for this hypothesis, as well as demonstrating a temperature dependency that is understandable on the basis of the membrane thermotropic phase transition.

1. INTRODUCTION
The influence of static magnetic fields (SMFs) on biological systems has been an area of considerable interest for many years. These fields, unlike time-varying (electromagnetic) fields, are not associated with induced electric currents except during activation and deactivation or when there is movement within the field. Our interest has been primarily on moderate intensity fields, those with strengths of 1 mT to 1 T. Many of the initial reports on the bioeffects of these fields were phenomenological descriptions without attempts to identify a mechanism of action [1–5]. The present report describes the development of a hypothesis sufficient to explain the action of moderate SMFs on biological systems. This proposed mechanism involves the effect of SMFs on the molecular structure of excitable membranes, an effect sufficient to modify the function of imbedded ion-specific channels. This hypothesis would explain virtually all of the bioeffects attributed to these fields and is testable using several different neurophysiological techniques.

2. EXPERIMENTAL STUDIES
An important clue to understanding the mechanism of SMF influence on excitable membranes was the time course of this action. In examining the effect of a 120 mT SMF on the evoked potential in cats [6], a decrease in amplitude was first seen 50–95 seconds after the field was turned on and persisted for several minutes after it was turned off. If the field exerted its influence on the central nervous system simply by altering ionic current flow in excitable membranes, the effect should have been immediate. The amplitude of the evoked potential has been shown [7] to be largely a function of the activity of those cells in the lateral geniculate body that project to visual cortex. The spontaneous discharge frequency of these cells was found [8] to significantly decrease during and immediately following exposure to the 120 mT SMF. The time course was exactly as that previously observed for the evoked visual potential. In addition, the latency of the response of these cells to optic tract stimulation was not altered by exposure to a SMF. That latency, reflecting axonal conduction time, would be prolonged if the magnetic field were associated with sufficient Lorentz force to influence local ionic currents. The failure to delay central conduction is consistent with theoretical studies [9] that predicted that fields of at least 24 T would be required to slow axonal conduction. The slow change in cellular response to SMFs suggests a chemically mediated effect at the synapse, one that might influence neurotransmitter release.

A useful system for the study of synaptic neurotransmitter release is the murine neuromuscular junction. This preparation allows one to precisely control the temperature and ionic environment at the recording site, something that is virtually impossible when dealing with intact animals. Even in the absence of a presynaptic action potential, small quantities of acetylcholine are spontaneously released from presynaptic nerve terminals and trigger brief but partial depolarization of the postsynaptic membrane. These depolarizations, called miniature endplate potentials (mepps), have been
shown to be dependent on the movement of Ca$^{2+}$ through the presynaptic membrane [10]. Using an excised murine phrenic nerve-diaphragm preparation perfused with Tyrode’s solution, exposure to a 120 mT SMF resulted in a temperature dependent change in mepp frequency [11]. At and below 34°C, minimal changes in mepp frequency were noted. Above 34.5°C, a prominent decrease in mepp frequency was observed. The onset of this change was relatively slow, reaching maximum 50–100 seconds after the field was turned on. The response was reversible, with return to baseline within 50 seconds after the field was turned off. When Ca$^{2+}$ was eliminated from the perfusate, while retaining its osmolality, the change in mepp frequency during exposure to the SMF was abolished, regardless of temperature. When exposure duration was examined [12] it was found that with a 120 mT field, a minimum exposure of 50 seconds was necessary for mepp inhibition. The efficacy of the field in inducing further inhibition was a function of its duration, but only for periods up to 150 seconds. At and above this limit, recovery time remained constant at 135 seconds. The threshold for mepp inhibition was found to be 38 mT [13].

To better understand those events occurring at the membrane in response to SMFs, direct measurement of membrane calcium currents were made using the whole-cell patch clamp technique in cultured GH3 cells [14]. Current-voltage relationships, activation kinetics, and inactivation kinetics of voltage activated calcium channels were examined before, during, and following exposure to a 120 mT SMF. The most significant change during SMF exposure was an increase in the activation time constant, evident one minute after exposure onset. There was no change in the inactivation time constant. Increase in the activation time constant was a temperature dependent phenomenon, present only above 27°C. All of the observed changes were reversible, with return to preexposure values within 3 minutes after the field was turned off.

In order to evaluate the possible effect of long term exposure to SMFs on basic cellular functions, we examined the effect of exposure of GH3 cells to a 0.5 T field, for periods of up to 5 weeks [15]. Following a 1 week exposure, cell growth declined by 22% and returned to control levels in 1 week. Although this was not statistically significant, a 4 week exposure was associated with a statistically significant decline in growth of 51% with return to control levels only after 4 weeks. Cell diameter, on the other hand, significantly increased following 3 weeks of exposure and did not return to control levels for 3 weeks after termination of exposure.

3. DISCUSSION

The one property of a biomembrane’s physical structure that has the potential to be influenced by moderate intensity SMFs is its diamagnetic anisotropy. Diamagnetic anisotropic molecules will rotate in a homogeneous magnetic field and ultimately achieve an equilibrium orientation, representing the minimum free-energy state. Weakly diamagnetic molecules will exhibit a preferred orientation in the presence of a sufficiently intense SMF. In a moderate intensity magnetic field the actual degree of orientation will be quite small for single molecules, even if they are strongly anisotropic. However, for molecules aligned parallel to one another and functionally linked, individual anisotropies summate [16]. Molecular orientation in a magnetic field is opposed by the randomizing effect of thermal energy. For a domain containing N cylindrical diamagnetic anisotropic molecules with axial symmetry and a volume V, in a homogeneous magnetic field H, the degree of orientation is given by β, the ratio of magnetic to thermal energy,

\[
\beta = \frac{-NH^2V(\chi_r + \Delta \cos^2 \phi)}{2k_B T}
\]

where $\chi_r$ is the radial magnetic susceptibility vector, $\Delta$ is the diamagnetic anisotropy, $\theta$ is the angle between the symmetry axis and the field direction, $k_B$ is Boltzmann’s constant and $T$ is absolute temperature.

The energetics for orientation in a magnetic field is favorable for structures made up of a large number of parallel molecules, characteristic of biomembranes. Moderate intensity SMFs have been shown to interfere with the mechanism for calcium channel activation. This activation is a function of the intramembranous portion of the channel, specifically it is the $\alpha_1$ subunit that is the primary voltage sensitive molecule in the calcium channel complex. This is the largest of the five proteins that make up the channel and is located almost entirely within the membrane. As such, it would be expected to be especially vulnerable to any membrane deformation induced by a SMF. Calcium channel inactivation was not influenced by moderate intensity SMFs since voltage dependent inactivation is linked to movement of a peptide moiety in the cell’s cytoplasmic domain and membrane deformation would have little effect on such a mechanism.
Most of the diamagnetic anisotropy of lipids is contributed by their acyl chains and biological membranes, with their highly ordered phospholipid bilayer structure, would be expected to exhibit substantial diamagnetic properties. The actual molecular reorientation within a phospholipid bilayer is the balance between the theoretical value of $\beta$ as given in Eq. (1) and those intermolecular forces which limit movement. At low temperatures, the membrane exists in a gel phase similar to that of a crystalline dehydrate and denoted as the $L_C$ phase. In this phase the hydrocarbon chains are packed tightly and rotation about their long axis is restricted. With increasing temperature, a thermally induced rotational excitation of the hydrocarbon chain occurs. This is at the subtransition temperature where the $L_C$ phase is converted to the laminar gel, $L_\beta$, phase. In this phase, the hydrocarbon chains exhibit limited rotational motion about their long axis. With further increase in temperature there is an abrupt rotameric disordering of the lipid acyl chain, marking the transition to the liquid-crystal, $L_\alpha$ phase. Although diamagnetic anisotropic molecules may assume a preferred orientation in the presence of an adequate SMF, this effect is enhanced when the membrane transitions to a less rigid phase.

Reorientation of diamagnetic structures in a magnetic field is an inherently slow process and 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 SMF has been described by a nonlinear differential equation [17]. From that equation, the time course for rotation becomes

$$\ln \theta_1 - \ln \theta_2 = -\frac{N\Delta_\chi H^2}{\zeta} t$$

where $\theta$ is the angle between the molecular axial diamagnetic vector and the field direction, and $t$ is the time required for rotation from $\theta_1$ and $\theta_2$. $\xi$ is the rotatory frictional coefficient, the value of which is determined by the size and shape of the ensemble and the viscosity of the suspension. For large diamagnetic ensembles, the randomizing effect of thermal energy is negligible. Eq. (2) provides a reasonable description of the relationship between exposure parameters and membrane molecular reorientation. At any given temperature, the size and diamagnetic anisotropy of a molecular ensemble will be constant as will the rotatory frictional coefficient. Therefore, the time course for rotation becomes a function of exposure time and the square of the flux density ($H^2t$). This correlates with the observed effects of SMFs at the neuromuscular junction [13]. In that study, Ca$^{2+}$ flux through the presynaptic membrane was a linear function of $H^2t$, with deviation from linearity only at the highest product values. That nonlinearity suggests a mechanical limit imposed on the free rotation of membrane phospholipids. Any structure capable of restricting such movement should be distributed uniformly in close proximity to the membrane and, in some manner, bound to it. Additionally, it would have to be less influenced by a SMF than the membrane itself. These conditions are met by the cellular cytoskeleton [18].

The effect on cellular growth and size during long term exposure to SMFs is believed to be the result of changes in cell division following mitosis (cytokinesis) and is the cell’s actin cytoskeleton that controls cytokinesis. Considering the low diamagnetic properties of proteins, it is unlikely that actin would be directly influenced by moderate intensity SMFs but an indirect of SMFs on the actin cytoskeleton is a possibility. This structure is not a static component of the cell but is involved in a process of continuous dynamic reorganization, modulated by intracellular Ca$^{2+}$. Although this is a relatively slow process, its disruption would be reflected not only in plasma membrane structure but in cytokinesis. Disruption of cytokinesis would explain the decreased rate of growth coupled with an increase in cell size seen with long term exposure to a 0.5 T SMF [15].

4. CONCLUSION

Moderate intensity SMFs influence a number of biological systems, notably those whose function is linked to transmembrane ion flux. These fields can result in a rotational displacement of the membrane’s phospholipid molecule by virtue of their collective diamagnetic properties. Evidence is presented indicating that molecular rotation within the membrane matrix will influence imbedded ion channels, most likely by producing some degree of deformity of their intramembranous segment, that part of the structure which is responsible for activation. Channel inactivation, a function of that portion of the channel not within the membrane per se, would not be expected to be altered by changes in the membrane’s matrix. It is the structural properties of biological membranes that allow for summation of individual molecular anisotropies and, therefore, rotation of those ensembles at moderate field intensities.
REFERENCES