EFFECT OF MAGNETIC FIELDS ON DRUG INDUCED CONTRACTILITY AND MORTALITY IN SPIROSTOMUM

Angela RIPAMONTI, R.B. FRANKEL

Francis Bitter National Magnet Laboratory *, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

and

E.M. ETTIENNE **

University of Massachusetts Medical School, Worcester, MA 01600, USA

The influence of homogeneous magnetic fields up to 5.5 T on contractile frequency and mortality in the ciliate protozoan *spirostomum ambiguum* stimulated by 2,2' PDS is reported. Magnetic fields are observed to decrease contractile frequency and to significantly increase mortality.

Possibilities for installation of large scale magnetic devices for energy production have stimulated inquiries into the biological effects of magnetic fields [1,2]. Recently magnetic fields have been reported by Lucking et al. [3] to influence the force developed during contraction of isolated frog sartorius muscle. They observed that in homogeneous magnetic fields up to 5 T, the amplitude of isometric force development in isolated skeletal muscle is reduced by about 50%. The authors speculate, following Dudoladov and Trincher [4], that the structural state of intracellular water adjacent to protein or membrane surfaces can be altered by an external magnetic field.

We have investigated the effects of homogeneous magnetic fields on the mechanism of cellular contraction by studying the contraction frequency in cells following chemical stimulation with and without an applied magnetic field. In this study we used the contractile ciliate *spirostomum ambiguum* (Ward's Biological Supply, Rochester, NY), inducing contractions with 10 μ M of the thiol reagent 2,2'-dipyridyl-

disulfide (PDS) (Sigma Chemical Co., St. Louis, MO) in a medium consisting of 2 μ M NaCl, 0.5 μ M KCl; 0.05 μ M CaCl_2 ; 0.1 μ M KH_2PO_4 ; and 0.1 μ M KOH (pH 6.3). In all experiments, the temperature was monitored and held constant at 20 or 22°C during application of the field. PDS is a sulfhydryl oxidizing agent which, through action on the microsomal respiratory chain of cells [5] decreases cytoplasmic ratios of NADPH/NADP and GSH/GSSG (glutathione). These ratios have been implicated in the oscillatory regulation of ionized free calcium concentration in the cytoplasm over ranges which include threshold levels for contraction in *spirostomum* [6,7]. It has been demonstrated that contractility in *spirostomum* is fundamentally like that of striated muscle and that there is Ca^{2+} dependent regulation of contractile elements [8] with Ca^{2+} levels controlled through intracellular membranous compartments. Because its physiology has been elucidated in detail, we feel that *spirostomum* is an excellent organism for the study of the effects of magnetic fields on mechano-chemical activity.

Magnetic fields of 0.5 and 0.92 T were produced by a water-cooled electromagnet with flat, iron pole pieces and at 3.0 and 5.5 T by a water-cooled Bitter

Sponsored by the National Science Foundation
Work partially sponsored by the Muscular Dystrophy Association of America.

Fig. 1. Time course and magnitude of contractile response of *Spirostomum* to treatment with 10^{-5} M 2,2' PDS in zero field (●) and 0.92 T (○). Significant suppression of contractions are seen to occur at 7, 10, 13, 15 and 16 min.

solenoid. PDS induced contractions per minute were monitored over an interval of 20 min in populations of 15 or more cells exhibiting synchronous contractions. The results per time point of 10 to 30 experiments were analyzed statistically according to the Student 't' test [9]. The frequency of contractions as a function of time following stimulation with PDS

Fig. 2. Percentage survival as a function of time for cells stimulated with 2,2' PDS in homogeneous magnetic fields of 0 T (control), 0.5 T, 0.92 T, 3.0 T and 5.5 T. The data are also tabulated in table 1.

is shown in fig. 1 for magnetic field $H_0 = 0.92$ T and $H_0 = 0$ controls. At 7, 10, 13 and 16 min following incubation, peaks are observed in the contraction frequency for $H_0 = 0$ (see fig. 1) [6,7]. In the external magnetic field, the values for the peak contraction frequencies are depressed by 50% ($P < 0.001$), 37%

Table 1

Percentage survival as a function of time following simultaneous stimulation with PDS and exposure to 0.5, 0.92, 3 and 5.5 T magnetic fields

Field	Control (0 T) $n^* = 15$	0.5 T $n^* = 15$		0.92 T $n^* = 15$	
Time (min)	$\bar{x} \pm \text{sd}$	$\bar{x} \pm \text{sd}$	S^+	$\bar{x} \pm \text{sd}$	S^+
10	99. $\pm$ 3.22	91.6 $\pm$ 9.96	$P < 0.01$	89.6 $\pm$ 12.2	$P < 0.001$
20	93.7 $\pm$ 9.6	76.12 $\pm$ 5.92	$P < 0.01$	66.3 $\pm$ 13.6	$P < 0.001$
30	74.1 $\pm$ 10.2	44.9 $\pm$ 15.3	$P < 0.0005$	33.1 $\pm$ 16	$P < 0.0005$
40	44.2 $\pm$ 27.8	7.56 $\pm$ 8.5	$P < 0.0005$	4.02 $\pm$ 6.3	$P < 0.0005$
Field	Control (0 T) $n^* = 15$	3.0 T $n^* = 10$		5.5 T $n^* = 10$	
Time (min)	$\bar{x} \pm \text{sd}$	$\bar{x} \pm \text{sd}$	S^+	$\bar{x} \pm \text{sd}$	S^+
10	99. $\pm$ 3.22	74.7 $\pm$ 4.5	$P < 0.001$	52.2 $\pm$ 7.4	$P < 0.001$
20	93.7 $\pm$ 9.6	41.09 $\pm$ 8.2	$P < 0.0005$	26.8 $\pm$ 9.3	$P < 0.0005$
30	74.1 $\pm$ 10.2	44.3 $\pm$ 9.5	$P < 0.0005$	3.3 $\pm$ 5.9	$P < 0.0005$
40	44.2 $\pm$ 27.8	2.8 $\pm$ 5.2	$P < 0.0005$	0 $\pm$ 0	$P < 0.0005$

n^* : Number of experiments with at least 15 cells per sample.

S^+ : Significance compared to control.

($P < 0.001$), 33% ($P < 0.001$), and 33% ($P < 0.001$), respectively. The duration of the relaxation phase in magnetic fields was qualitatively observed to be extended over controls.

In addition to the observed effects on contraction frequency, there are also very marked effects on cell mortality. In fig. 2, the percentages of survivors in 12 experiments with >15 cells each at 10, 20, 30 and 40 min after incubation are plotted as a function of time for magnetic fields $H_0 = 0$ (control), 0.50 T, 0.92 T, 3.0 T and 5.5 T. In table 1 we present percentage survival and statistical significance compared to controls over the given time intervals for the four magnetic field values. At 30 min, the effects of 0.50 T, 0.92 T, 3.0 T and 5.5 T on survival were all significant to $P < 0.0005$. We note that in the absence of PDS, there were no observed effects of exposure to magnetic fields up to 5.5 T for 30 min on cell viability.

Experiments were also conducted in which samples of spirostomum (15 cells per sample) were exposed to 5.5 T for 30 min in the absence of the drug, and then removed from the field. Immediately following field exposure, PDS was administered and the mortality in zero field was monitored as a function of time and compared with controls which had not been exposed to the external field. The results, presented in table 2 and fig. 3, show that at 10, 20, 30 and 40 min experimental values are 83% ($P < 0.001$), 55% ($P < 0.0005$), 33% ($P < 0.0005$) and 15% ($P < 0.0005$) of controls, respectively. These results imply that the effect of the magnetic field on mortality cannot be attributed solely to the direct effect

Table 2
Percentage survival as a function of time after PDS stimulation, following 30 min exposure to 5.5 T magnetic field

	Time (min)	$\bar{x} \pm \text{sd}$	
Control (0 T) $n^* = 10$	10	98.03 $\pm$ 3.2	
	20	94.1 $\pm$ 5.9	
	30	65.6 $\pm$ 7.8	
	40	46.2 $\pm$ 22.5	
5.5 T $n^* = 10$	10	81.4 $\pm$ 10.5	$P < 0.001$
	20	51.8 $\pm$ 16.1	$P < 0.0005$
	30	22.2 $\pm$ 15.2	$P < 0.0005$
	40	7.1 $\pm$ 8.3	$P < 0.0005$

n^* : number of experiments with at least 15 cells per sample.
 P : significance compared to control.

Fig. 3. Percentage survival as a function of time for cells exposed for 30 min to 0 T (controls) and 5.5 T, followed by removal of the field and stimulation with 2,2' PDS. The abscissa correspond to time following stimulation with 2,2' PDS. The data are also tabulated in table 2.

of the field on PDS, and indicate significant long lasting effects of the field on the organism.

We have seen that the magnetic field acts to decrease contraction frequency and increase mortality in response to PDS. Based on the known physiology of spirostomum [5,8] the observations can be interpreted as reflecting a decrease in the enzymatic transport of Ca^{2+} out of the cytoplasm following contraction in the magnetic field. Reduced Ca^{2+} transport might occur as a result of an interaction between the magnetic field and the intracellular membranous compartments. This hypothesis could be verified by observation of prolongation of Ca^{2+} transients in vivo. Alternatively, Ca^{2+} in transport vesicles isolated from cells exposed to magnetic fields could be measured in vitro with ^{45}Ca . These experiments are presently being pursued. Information from these experiments will enable us to ascertain whether the locus of the magnetic field effect is associated with the Ca^{2+} transport membranes.

Acknowledgements

We are pleased to acknowledge the continuing interest and support of D.B. Montgomery and P. Marston, and thank Y. Shapira for a careful reading of the manuscript.

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