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Blastogenesis of human peripheral blood lymphocytes stimulated *in vitro* by non-specific mitogens (PHA, ConA, PWM) upon exposure to extremely low frequency EMF has been studied. Different frequencies of square waveforms have been used. PHA-stimulation resulted in strong inhibitions as measured by [³H]thymidine incorporation. A frequency window (3–50 Hz) within which ConA-induced blastogenesis was significantly inhibited has been individuated. The mitogenic effect of PWM was significantly affected only at 3 Hz.

Lymphocyte blastogenesis Mitogenic activity Electromagnetic field

1. INTRODUCTION

Several investigators have reported effects, both inhibitory and stimulatory, produced by exposure to EMF on various parameters of cultured cells [1,2]. [³H]Noradrenaline release from PC12 cells is stimulated by an inductively coupled 500-Hz EMF with a magnitude comparable with certain cholinergic stimuli in this system [3]. Effects of low EMF in cultures have been studied to test the theories on EMF electrochemical action [4]. Their main experimental model has been the frog red blood cell. Waveform and frequency-dependent effects have been observed including a window from 40–70 Hz within which cell de-differentiation was enhanced and outside which it was inhibited. In [5], the responses were examined of cultured bone and bone cells to hormones that either do or do not appear to act primarily via plasma membrane receptors in the presence of EMF. The specific inhibition of collagen synthesis by parathyroid hormone was blocked by treatment

of these cells cultured *in vitro* with an EMF. However, the fields did not block the effects on collagen synthesis of 1,25-dihydroxy vitamin D₃, a hormone that apparently acts via a cytoplasmic rather than a membrane receptor. The effects of 60-Hz electric field on specific humoral and cellular components of the immune system *in vivo* exhibited no significant difference from controls [6].

Stimulated by these findings and the wide variety of effects reported in other biological systems [7,10], we have tried to establish a model system for studying the effects of low EMFs. Assuming EMF effects on ionic fluxes [8], and considering the prominent role of calcium in lymphocyte proliferation, we have studied the influence of EMF at different frequencies on blastogenesis of human peripheral blood lymphocytes stimulated *in vitro* by mitogenic plant lectins.

2. MATERIALS AND METHODS

2.1. Preparation of lymphocyte cultures

Human peripheral blood lymphocytes were purified from heparinized venous blood of healthy young adult donors taking no medications for at

Abbreviations: PHA, phytohaemagglutinin; ConA, concanavalin A; PWM, pokeweed mitogen; EMF, electromagnetic field

least 2 weeks, by density gradient centrifugation on Ficoll-Hypaque as in [9]. The cells were washed 3 times and suspended in RPMI 1640 supplemented with 25 IU/ml penicillin, 0.25 mg/ml streptomycin, 2 mM L-glutamine, and 10% foetal bovine serum. The cells were cultured at 2×10^5 cells/0.2 ml in quintuplicate in microtiter plates (Falcon) in the presence or absence of mitogen (PHA-P, Difco, 20 μ g/ml; ConA, Calbiochem, 5 μ g/ml; PWM, Calbiochem, 1:256 dilution of stock solution).

2.2. Exposure to EMF

The EMF was generated by passing a current through a pair of concentric 966-turn coils, 10 cm radius, separated by 2 cm. The device in question was wired in parallel to a pulse generator which gave train of pulses of variable form and intensity.

In this set of experiments square pulses with frequencies of 1, 3, 50 and 200 Hz were used. The generated field had intensities of 23–65 G. The waveform of current passing in the coils was only weakly smoothed due to the inductance of the two coil system (249 mH). The calculated eddy current densities were $\approx 10 \text{ mA} \cdot \text{m}^{-2}$ inducing an electric field of $0.1\text{--}1 \text{ V} \cdot \text{m}^{-1}$. The magnitude of the EMF was checked by using a Hall-effect probe and an associated Gauss meter. The temperature between the coils was measured with a digital thermometer. The coils were placed in a tissue culture incubator held at 37°C while the pulse generator unit was outside the incubator. The cells were exposed to EMF in the wells of a microtiter plate. Only those wells were used which showed an EMF homogeneity better than 1% when placed in the central space between the coils. Some experiments were con-

Table 1

[^3H]Thymidine incorporation [(cpm \pm SD) $\times 10^{-3}$] in human lymphocytes stimulated by PHA, ConA, or PWM after exposure to EMF at different frequencies during the whole incubation time (72 h)

Exp. no.	Mitogen	EMF (1 Hz)		EMF (3 Hz)		EMF (50 Hz)		EMF (200 Hz)	
		-	+	-	+	-	+	-	+
1		0.9 \pm 0.4	1.7 \pm 0.3	1.4 \pm 0.3	1.2 \pm 0.5	1.7 \pm 0.5	1.0 \pm 0.2	1.3 \pm 0.3	0.9 \pm 0.6
2		1.2 \pm 0.3	1.6 \pm 0.5	4.2 \pm 0.2	3.5 \pm 1.5	1.1 \pm 0.2	1.1 \pm 0.3	3.6 \pm 0.9	4.4 \pm 1.1
3	-	5.3 \pm 1.0	5.6 \pm 0.9	3.5 \pm 0.8	0.7 \pm 0.0	1.0 \pm 0.3	0.8 \pm 0.1	3.1 \pm 1.1	2.9 \pm 1.1
4				1.5 \pm 0.6	0.6 \pm 0.1	0.7 \pm 0.2	0.2 \pm 0.1		
5				2.2 \pm 0.7	1.8 \pm 0.6				
1		153.7 \pm 6.9	73.4 \pm 9.4	149.3 \pm 8.7	59.5 \pm 12.9	101.6 \pm 4.0	85.6 \pm 6.8	133.6 \pm 6.6	63.9 \pm 9.8
2		149.7 \pm 13.7	77.9 \pm 15.4	132.4 \pm 12.7	42.9 \pm 10.9	169.7 \pm 11.2	107.3 \pm 7.7	150.1 \pm 14.9	115.5 \pm 9.2
3	PHA	167.3 \pm 14.3	61.9 \pm 6.2	114.6 \pm 3.5	43.6 \pm 6.7	81.8 \pm 10.0	30.1 \pm 7.0	154.2 \pm 11.1	112.7 \pm 13.2
4				119.9 \pm 15.9	58.9 \pm 18.5	97.3 \pm 11.0	47.7 \pm 6.4		
5				129.0 \pm 13.5	77.4 \pm 12.1				
1		106.1 \pm 16.9	91.4 \pm 13.6	132.3 \pm 19.7	72.2 \pm 13.4	84.9 \pm 7.7	45.9 \pm 9.6	60.3 \pm 7.2	56.5 \pm 3.4
2		86.0 \pm 6.2	77.9 \pm 15.4	147.7 \pm 2.5	66.2 \pm 13.9	122.5 \pm 10.6	89.0 \pm 8.3	129.7 \pm 9.3	119.1 \pm 9.9
3	ConA	182.3 \pm 13.8	171.4 \pm 14.2	96.3 \pm 13.7	54.6 \pm 10.9	69.3 \pm 10.1	44.0 \pm 3.1	121.5 \pm 15.6	115.2 \pm 8.9
4				83.0 \pm 9.3	55.5 \pm 11.6	84.4 \pm 5.9	45.3 \pm 1.1		
5				61.3 \pm 7.7	25.1 \pm 9.4				
1		97.9 \pm 16.7	87.9 \pm 5.2	103.4 \pm 4.0	55.9 \pm 14.7	67.1 \pm 1.4	64.3 \pm 2.9	75.1 \pm 13.7	68.2 \pm 9.7
2		107.9 \pm 9.9	91.4 \pm 12.5	84.3 \pm 8.9	61.8 \pm 9.3	66.9 \pm 12.6	69.1 \pm 12.2	100.8 \pm 9.5	85.3 \pm 9.6
3	PWM	127.7 \pm 12.2	130.4 \pm 11.2	132.2 \pm 16.4	41.5 \pm 17.7	55.7 \pm 18.4	48.5 \pm 5.2	81.6 \pm 14.2	77.3 \pm 7.7
4				121.6 \pm 13.2	72.4 \pm 11.1	62.4 \pm 5.9	50.1 \pm 9.8		
5				109.7 \pm 9.3	77.4 \pm 12.1				

-, no field; +, field

ducted by exposing the lymphocyte cultures to EMF during the whole incubation time (72 h). Other experiments were instead performed by exposing the cells to EMF at different times of the incubation. In all experiments, [^3H]thymidine (25 Ci/mmol) was added to a final $2\ \mu\text{Ci/ml}$, 6 h before the end of the incubation. At the end of the culture the cell viability was evaluated by trypan blue exclusion and it was always over 90% both field off and field on. No appreciable difference in pH between the cultures incubated without or with field was detectable as measured with a digital pH-meter. At the end of incubation the cells were harvested with glass fiber filters using a semiautomatic multiple sample precipitator, air dried and the radioactivity was determined with a β -counter. Significance of the results was analyzed by Student's *t*-test.

3. RESULTS

3.1. Exposure to EMF for 72 h

Table 1 shows the values of thymidine incorporation into normal human lymphocytes stimulated by PHA, ConA, or PWM after an exposure for 72 h to EMF at different frequencies. Mitogenic effect of PHA is markedly reduced by exposure to 1 Hz-EMF ($p < 0.01$), while ConA- and PWM-action is not affected by this field frequency. It appears instead that lymphocyte blastogenesis induced by all 3 mitogens is strongly inhibited after exposure to 3 Hz-EMF ($p < 0.01$). A 50 Hz-EMF is able to decrease the lymphocyte mitogenesis induced by PHA or ConA, but not that induced by PWM. At 200 Hz-EMF only the stimulating effect of PHA appears significantly inhibited ($p < 0.01$).

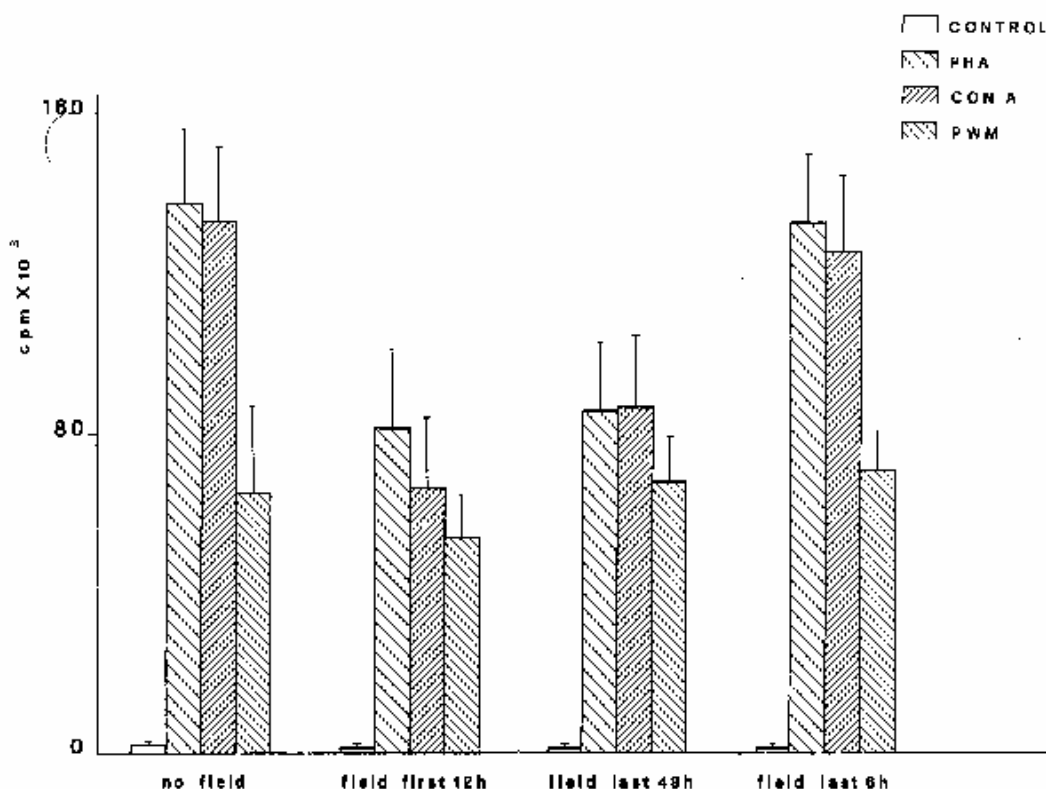


Fig.1. [^3H]Thymidine incorporation in human lymphocytes stimulated by PHA, ConA, or PWM after shorter exposure to 3 Hz-EMF.

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