Volume 162, number 1 FEBS 0850 October 1983

Reduced mitogenic stimulation of human lymphocytes by extremely low frequency electromagnetic fields

Pio Conti, Giovanni Ettore Gigante, Maria Grazia Cifone, Edoardo Alesse, Gianfranco Ianni, Marcella Reale and Pietro Ubaldo Angeletti

Biological Department, School of Medicine, University of L'Aquila, Collemaggio, 67100 L'Aquila, Italy

Received 1 August 1983

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 [^{3}H]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]. [^{3}H]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 D_3, 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.

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

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

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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 μg/ml; ConA, Calbiochem, 5 μg/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 $\simeq$10 mA.m^{-2} inducing an electric field of 0.1–1 V.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

[^{3}H]Thymidine incorporation [(cpm ± SD) × 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± 0.4	1.7± 0.3	1.4± 0.3	1.2± 0.5	1.7± 0.5	1.0± 0.2	1.3± 0.3	0.9± 0.6
2		1.2± 0.3	1.6± 0.5	4.2± 0.2	3.5± 1.5	1.1± 0.2	1.1± 0.3	3.6± 0.9	4.4± 1.1
3	−	5.3± 1.0	5.6± 0.9	3.5± 0.8	0.7± 0.0	1.0± 0.3	0.8± 0.1	3.1± 1.1	2.9± 1.1
4				1.5± 0.6	0.6± 0.1	0.7± 0.2	0.2± 0.1		
5				2.2± 0.7	1.8± 0.6				
1		153.7± 6.9	73.4± 9.4	149.3± 8.7	59.5±12.9	101.6± 4.0	65.6± 6.8	133.6± 6.6	63.9± 9.8
2		149.7±13.7	77.9±15.4	132.4±12.7	42.9±10.9	169.7±11.2	107.3± 7.7	150.1±14.9	115.5± 9.2
3	PHA	167.3±14.3	61.9± 6.2	114.6± 3.5	43.6± 6.7	81.8±10.0	30.1± 7.0	154.2±11.1	112.7±13.2
4				119.9±15.9	58.9±18.5	97.3±11.0	47.7± 6.4		
5				129.0±13.5	77.4±12.1				
1		106.1±16.9	91.4±13.6	132.3±19.7	72.2±13.4	84.9± 7.7	45.9± 9.6	60.3± 7.2	56.5± 3.4
2		86.0± 6.2	77.9±15.4	147.7± 2.5	66.2±13.9	122.5±10.6	89.0± 8.3	129.7± 9.3	119.1± 9.9
3	ConA	182.3±13.8	171.4±14.2	96.3±13.7	54.6±10.9	69.3±10.1	44.0± 3.1	121.5±15.6	115.2± 8.9
4				83.0± 9.3	55.5±11.6	84.4± 5.9	45.3± 1.1		
5				61.3± 7.7	25.1± 9.4				
1		97.9±16.7	87.9± 5.2	103.4± 4.0	55.9±14.7	67.1± 1.4	64.3± 2.9	75.1±13.7	68.2± 9.7
2		107.9± 9.9	91.4±12.5	84.3± 8.9	61.8± 9.3	66.9±12.6	69.1±12.2	100.8± 9.5	85.3± 9.6
3	PWM	127.7±12.2	130.4±11.2	132.2±16.4	41.5±17.7	55.7±18.4	48.5± 5.2	81.6±14.2	77.3± 7.7
4				121.6±13.2	72.4±11.1	62.4± 5.9	50.1± 9.8		
5				109.7± 9.3	77.4±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, [^{3}H]thymidine (25 Ci/mmol) was added to a final 2 μCi/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 ($p < 0.01$).

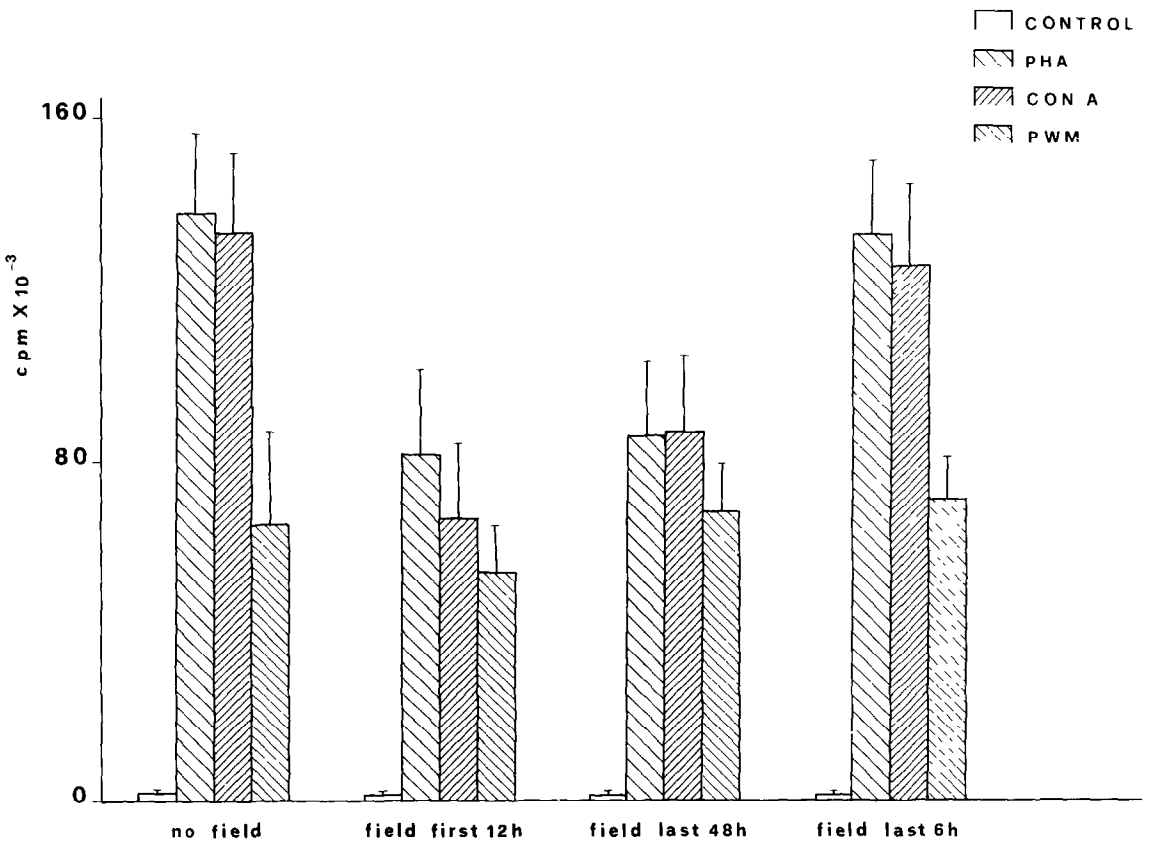

Fig.1. [^{3}H]Thymidine incorporation in human lymphocytes stimulated by PHA, ConA, or PWM after shorter exposure to 3 Hz-EMF.

3.2. *Shorter exposure to EMF*

In another set of experiments, the cell cultures were exposed to a 3 Hz-EMF during the first 12 h only. After this time the cultures were carried out without field for 60 h. The application of EMF even limited to this early time is able to significantly reduce ($p < 0.01$) the thymidine incorporation by lymphocytes stimulated by PHA or ConA (fig.1). However the inhibition of PWM-stimulation is instead not significant.

Three experiments were performed by incubating the cell cultures without EMF for the first 24 h and with EMF for the last 48 h. Upon these conditions the PHA- or ConA-stimulation results inhibited ($p < 0.01$) while the thymidine incorporation into PWM-stimulated cells is not influenced (fig.1).

To exclude the possible interference of EMF on thymidine incorporation, in another set of experiments the cultures were exposed to the field for only the last 6 h. The results (fig.1) indicate that no difference occurs between the values of isotope incorporation in the cells cultured without field or into the field.

4. DISCUSSION

The above data indicate an inhibitory effect of EMF on blastogenesis of mitogen-stimulated human lymphocytes. The most affected mitogen is PHA whose effect is reduced by all investigated frequencies. Instead, the stimulation by ConA or PWM seems to be affected in a frequency-dependent way. In fact, a frequency window within which ConA-induced blastogenesis can be reduced, has been found, and the stimulation by PWM appears inhibited only at 3 Hz-frequency. The different extent of inhibition for the 3 mitogens can be explained by the fact that after binding, every mitogen selectively triggers a subclass of lymphocytes [11]. Shorter exposure to EMF either for the first 12 h or for the last 48 h while affecting the blastogenesis induced by PHA and ConA does not significantly reduce the PWM-stimulation.

The reported results do not unequivocally indicate the mechanism by which lymphocytes respond to EMF. However, some considerations can be made. The results show that EMF does not interfere with the ability of the mitogens to bind to membrane receptors because the inhibitory effect of EMF was also evident when cultures were exposed for the last 48 h. It is in fact known that binding of lectin molecules to the cell membrane takes place in the early phase of the cellular response [12]. A direct interference of EMF on thymidine incorporation can be excluded because the field exposure for the last 6 h was without effect. The decrease in the isotope incorporation by stimulated lymphocytes upon exposure to EMF would thus represent a decrease in the rate of DNA synthesis in all cells and/or a reduction in the number of cells undergoing DNA replication.

Ca^{2+} are involved in the control of lymphocyte proliferation. In fact, mitogenic lectins produce a rapid, initial calcium influx and calcium is required for DNA synthesis some 18–72 h after the mitogenic stimulus [13–15].

Considering the theoretical model proposed by some authors [8,16] where the EMF action is mediated by an electrochemical mechanism, we think that an alteration of calcium fluxes by EMF may be the most realistic hypothesis to explain the observed inhibitory effect on human lymphocyte blastogenesis. Experiments are in progress to determine the EMF influence on calcium fluxes with radiolabeling and electrochemical methods.

ACKNOWLEDGEMENT

The authors thank Professor A. Finazzi-Agrò for helpful criticism of the manuscript.

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