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The Role of Coherence Time in the Effect of Microwaves on Ornithine Decarboxylase Activity

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Previously, we demonstrated the requirement for a minimum coherence time of an applied, small amplitude (10 μT) ELF magnetic field if the field were to produce an enhancement of ornithine decarboxylase activity in L929 fibroblasts. Further investigation has revealed a remarkably similar coherence time phenomenon for enhancement of ornithine decarboxylase activity by amplitude-modulated 915 MHz microwaves of large amplitude (SAR 2.5 W/kg). Microwave fields modulated at 55, 60, or 65 Hz approximately doubled ornithine decarboxylase activity after 8 h. Switching modulation frequencies from 55 to 65 Hz at coherence times of 1.0 s or less abolished enhancement, while times of 10 s or longer provided full enhancement. Our results show that the microwave coherence effects are remarkably similar to those observed with ELF fields. ©1993 Wiley-Liss, Inc.

Key words: coherence time, microwave, amplitude modulation, ornithine decarboxylase

INTRODUCTION

A considerable controversy has surrounded the question of biological response to weak electromagnetic fields. The mechanisms by which cells might detect and respond to these fields are unknown. It is argued [Adair, 1991] that a cellular response is impossible since the magnitudes of such fields are lower than those of the electrical noise fields produced by random thermal motion of ions in and about the cell. Nonetheless, a number of reports detailing responses to weak, extremely low frequency (ELF) fields [e.g., Goodman et al., 1989; Wei et al., 1990; Litovitz et al., 1991] and to amplitude-modulated microwave fields [e.g., Byus et al., 1988] are found in the literature.

In order to understand these results, Weaver and Astumian [1990] proposed that signal averaging over time might provide a mechanism for cellular response to weak fields. Unfortunately their calculations showed that the averaging times necessary were much longer than the exposure times found to be effective.

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Recently Litovitz et al. [1991] examined the hypothesis that cells do not respond to the local thermal noise fields because these fields are not temporally coherent, in contrast to the temporal coherence of the exogenous, applied fields used in the above experiments. They investigated the role of the coherence time of an exogenous ELF field in effecting a biological response. Ornithine decarboxylase (ODC) activity was selected as the biological marker for that study. Enhancement of ornithine decarboxylase specific activity following exposure of L929 cells to an ELF magnetic field of 10 μT rms (with an associated electric field of approximately 0.04 μV/cm) was found to depend upon the coherence time of the applied field. During a 4-h exposure the frequency of the field was shifted between 55 and 65 Hz at slightly random intervals termed coherence times (τ_{coh}). The coherence time here is loosely defined as the time interval over which one can reasonably predict the frequency, phase, and amplitude of the field. When (τ_{coh}) was 1 s or less no enhancement of ornithine decarboxylase activity resulted, while (τ_{coh}) of 10 s or greater yielded a significant enhancement of activity. The observed coherence times needed for full cellular response were too short to account for the signal averaging phenomenon hypothesized by Weaver and Astumian [1990]. However, the coherence time was revealed to be an important factor in determining the magnitude of the cellular response.

We report here the ornithine decarboxylase response of L929 cultures to microwave fields. Our results show that the microwave coherence effects are remarkably similar to those observed with ELF fields.

MATERIALS AND METHODS

Microwave Exposure System

Microwave exposure was accomplished using a Crawford cell placed within an incubator chamber maintained at 37 °C. A block diagram of the exposure system is shown in Figure 1. A model CC110 Crawford cell (Instruments for Industry, Farmingdale, NY) was vertically mounted on a rotary table and provided with an additional access door to facilitate sample insertion. The 915 MHz driving signal was produced by a signal generator (Hewlett Packard, 8657B) followed by a travelling wave tube amplifier (Amplifier Research #10W1000, Souderton, PA). A double stub tuner was used to match the impedance of the loaded Crawford cell for optimum power delivery to the sample.

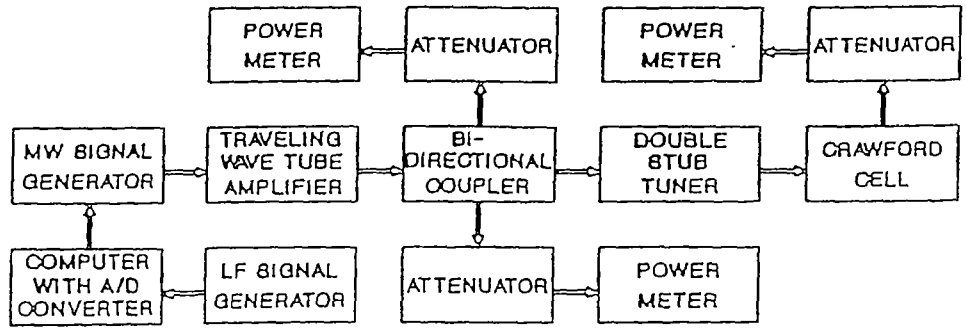

Fig. 1. Block diagram of the modulated microwave exposure system.

To investigate the role of the coherence time of the modulation, the signal was periodically switched between 55 Hz and 65 Hz. The time interval within which the signal was operated at each frequency was selected as a fixed period τ plus a randomly selected period $\Delta\tau$ of duration between 0 and 50 ms. The interval τ was varied between 0.1 and 50 s. The coherence time (τ_{coh}) is $\tau+\Delta\tau$. Since $\tau>>\Delta\tau$, (τ_{coh}) is closely equal to τ. The frequency of the sinusoidal output of the function generator was varied by a computer-generated signal to the voltage controlled oscillator (VCO) of the generator. The system was operated at 915 MHz with amplitude modulation at 55, 60, and 65 Hz. The modulation index was set at 0.23 and was calculated using the relation $P_t = P_c(1 + m^2/2)$, where P_t is the microwave power with modulation, P_c is the microwave power without modulation, and m is the modulation index.

Crawford cells are generally chosen for exposure of biological samples because, under special conditions, they allow uniform electric field exposure. To achieve uniform exposure the sample should be positioned perpendicular to the direction of wave propagation and parallel to the electric field. Additionally it should occupy no more than one-third of the distance between the center conductor and the outer plate. Due to constraints imposed by the availability of commercially fabricated tissue culture flasks, it was not possible to meet the requirements for the ideal exposure configuration. The flasks used in our experiments (25 cm^2, 50 ml) had a width approximately equal to 70% of the distance between the center conductor and the outer plate. Two flasks were placed end to end on both sides of the center conductor. This configuration insured overall symmetry, if not complete uniformity, of the electric field distribution within the samples.

To determine the uniformity of the field, SAR measurements were made within each tissue culture flask with the flasks filled with 5 ml of culture medium and positioned in the arrangement used for exposures. The SAR was determined by measuring the rate of temperature increase after switching on a high power field. The temperature was measured using a fiber optic thermometer (Luxtron model 3000) with two MPM 4 sensor probes. The temperature distribution at the base of the flask, where cells were located, was mapped at 7 mm intervals along the width of the flask and at 10 mm intervals along its length for a total of 48 points. Measurements were made on both flasks positioned on one side of the center conductor. Due to symmetry the SAR distribution on the other side was assumed to be similar. The optical probes were inserted into the flask through small holes made on the top of the flask. Each run of eight simultaneous measurements was carried out with an input power of 69 watts for a period of 20 s. This measuring interval allowed a 0.5–1 °C temperature rise from the starting temperature of 21 °C at the measuring site. To minimize the error due to heat loss the SAR was determined using the temperature vs. time data for the first 10 s after the power was turned on.

The SAR was calculated using the well-known relation $SAR = C_p\Delta T/\Delta t$, where C_p is the specific heat of the exposed sample medium and $\Delta T/\Delta t$ is the rate of temperature increase per unit time. For these calculations, C_p was assumed to be 3.95 J/°C/g. The results indicate that the SAR varied with an asymmetrical overall distribution if both flasks at either side of the center conductor were taken together. The SAR across the width of the flasks varied from low points (approximately 25% of maximum) at the edges to a maximum skewed toward the center conductor. Similarly, the SAR increased from the back end of the rear flask (approximately

25% of the maximum) to a maximum in the proximity of the junction between flasks and decreased again toward the front end of the front flask (approximately 75% of the maximum). An effective SAR of approximately 180 W/kg, corresponding to an input power of 69 watts, was calculated by performing a simple average over all the measured points.

In the cell culture exposures reported here, an input power of 0.96 watts was used. Accordingly, the corresponding average SAR was approximately 2.5 W/kg. With this input power no measurable temperature increase was expected within the samples. Measurements with a fiber optic thermometer demonstrated that within the resolution of the probe (±0.1 °C) no temperature change occurred at any location within the culture medium. The electric field within the Crawford cell, associated with an input power of 0.96 watts, was of the order of 0.7 V/cm. This field was calculated, assuming a uniform field, using the relation $E = (PZ_0/d^2)^{1/2}$, where $P = P_i/2$ is the power distributed to the Crawford cell at each side of the conductor, $Z_0 = 50\ \Omega$ is the characteristic impedance of the Crawford cell, and $d = 7$ cm is the distance between the center conductor and the outer plate (dimension slightly larger than the standard CC110).

Cell Cultures

Actively growing cultures of the murine L929 fibroblast cell line (NCTC clone 929; American Type Culture Collection, Rockville, MD) were maintained in Eagle's minimum essential medium supplemented with 5% calf serum and 10 mM HEPES buffer. Cells were kept at 37 °C in a 95% air, 5% CO_2 atmosphere. Cultures to be used for exposure were initiated approximately 20 h prior to an experiment, at a density (3×10^6 cells in 5 ml of culture medium per 25 cm^2 flask) to produce mid-logarithmic phase growth by the time of use. To avoid large increases in ODC activity due to serum stimulation, the culture medium was not replaced prior to exposure.

Electromagnetic Field Exposure and Harvesting of Cells

From 5–12 separate experiments were conducted for each exposure condition (i.e., for each modulation frequency or coherence time). The order in which exposures were conducted was random with regard to the values of τ_{coh} used, so that different exposure conditions were intermixed in sequence during the approximately 12-month duration of the project.

For microwave exposure four, 25 cm^2 flasks were inserted into the Crawford cell as described above. Four 25 cm^2 flasks of cells, serving as control cultures, were placed in the same incubator chamber at the same height as the exposed cultures within the Crawford cell. In the experiments described below, an average SAR of 2.5 W/kg, modulation index of 0.23, and an 8 h exposure time were consistently employed.

Immediately following exposures the cells in each flask were washed twice with 5 ml ice-cold phosphate buffered saline (PBS) and then gently scraped from their growth surface in a third aliquot of cold PBS. In order to provide sufficient protein for ODC assay the cells from the four exposed or four control flasks were combined for a single determination of ODC activity. Released cells were washed with cold PBS, pelleted at 350g for 5 min, and the supernatant aspirated. Cell pellets were frozen and stored at -75 °C until assay.

Determination of ODC Activity

ODC activity was determined by the method of Seely and Pegg [1983] modified by the addition of 50 μM pyridoxal 5′-phosphate, 50 μg/ml leupeptin, and 0.2% Nonidet P-40 to the cell lysis buffer. Absorption of generated $^{14}CO_2$ was accomplished with 150 μl of 1.0 N NaOH, and addition of 400 μl 20% TCA was done to terminate reactions. Background counts were determined by counting preparations in which ODC activity was eliminated by acid denaturing. ODC activity units were expressed as picomoles $^{14}CO_2$ generated/30 min/mg protein. Protein concentrations were determined by the method of Bradford [1976].

Evaluation of Data

ODC activities of control L929 cultures assayed on different days varied over an approximately five fold range, from about 5 to 25 units. Most of this variation appeared to be inherent to the cell cultures. ODC activity is readily altered by changes in cell growth conditions [Heby et al., 1975; reviewed by Jänne et al., 1978], and despite our rigorous attempts to assure constant culture parameters, variations in control activities occurred. To deal with such day-to-day variations in control ODC activity and to allow ready comparisons among experiments, the results of each experiment were expressed as an ODC activity ratio, calculated by dividing the ODC activity of an exposed culture with that of its matched control. An activity ratio of 1.8 would thus indicate an ODC activity in an exposed culture that was 1.8 times that of the control.

Figure 2 provides a demonstration of the validity of the ODC activity ratio for assessing enhancement of ODC activity due to EM field exposure. Results are shown for a total of 59 separate experiments in which L929 cells were exposed to a 60 Hz, sinusoidally varied magnetic field of 10 μT rms amplitude. Time of exposure was 4 h, which was previously found [Litovitz et al., 1991] to induce a maximum ODC response to such a field. Mean activity ratios (and standard deviations) are plotted against 5 groups of control ODC activities spanning the range from 5–30 units. The ODC activity ratio for each group was approximately 2 (range 2.0 ± 0.2 to 2.1 ± 0.3; mean for all 59 exposures = 2.1 ± 0.2). Nearly identical activity ratios were obtained for cultures varying as much as five fold in control ODC specific activities. These values demonstrate the effectiveness of the ODC activity ratio for assessing EMF-induced cellular response.

RESULTS

Initially, a series of exposure times (2–24 h) was conducted using 60 Hz amplitude-modulated, 915 MHz microwaves, with modulation index of 0.23 and an average SAR of approximately 2.5.W/kg. These conditions produced a transient, approximately two-fold increase in ODC activity which peaked after 8 h of exposure. Using SARs as low as 0.5 W/kg or a modulation index of 0.60 also produced an approximate doubling of ODC specific activity after 8 h exposure.

An approximate doubling of ornithine decarboxylase activity (ODC activity ratio = 1.9 ± 0.3 (S.D.), n = 11) was obtained for L929 cultures exposed to the 60 Hz amplitude modulated microwave field for 8 h. However, no ornithine decarboxylase enhancement was observed for cultures exposed for 8 h to the unmodulated 915 MHz

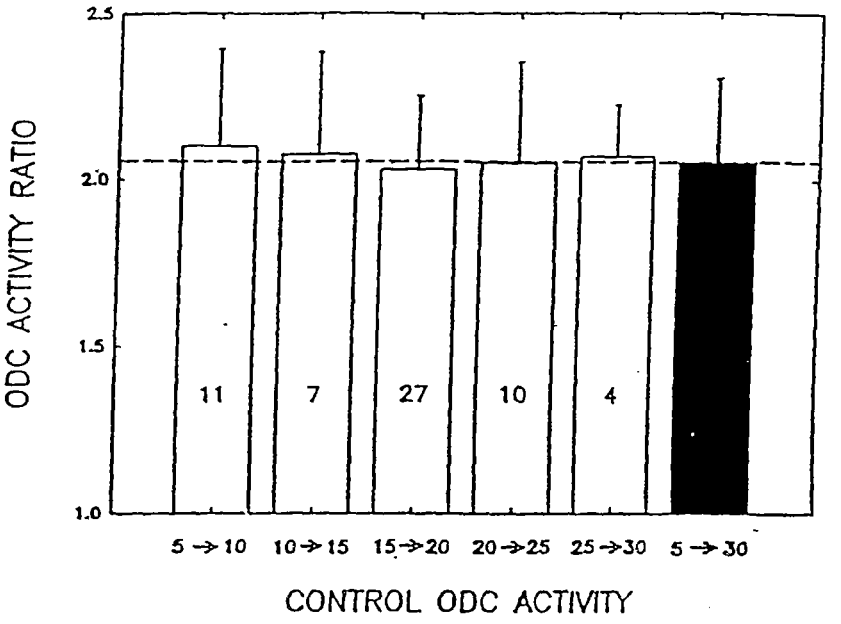

Fig. 2. Plot of ODC activity ratios (ODC activities of exposed cultures/ODC activities of matched controls) against control ODC specific activities for 59 experiments in which cultures were exposed for 4 h to a 60 Hz, 10 μT field. The number of experiments that fell in each specific activity range are indicated in the open bars. Mean ODC activity ratio for all 59 experiments is indicated by the filled bar and the dashed line.

microwave field (ODC activity ratio = 0.9 ± 0.1, n = 10). Thus, the extremely low frequency of the amplitude modulation, was the critical factor in eliciting cellular response. In order to examine the importance of the coherence time (τ_{coh}) for the microwave response, we also assessed the ornithine decarboxylase activities of cultures exposed for 8 h to microwave fields amplitude modulated at 55 or 65 Hz. Results, 1.9 ± 0.5 (n = 6) and 1.9 ± 0.3 (n = 5), respectively, were statistically indistinguishable from those obtained with 60 Hz modulation.

Results of the coherence time studies are plotted in Figure 3. When the modulation frequency was shifted with τ_{coh} of 0.1 or 1.0 s no significant enhancement of ornithine decarboxylase activity over control levels was observed. However, a τ_{coh} of 10 s yielded an enhancement of ornithine decarboxylase activity equivalent to that obtained with constant amplitude modulated frequencies of 55, 60, or 65 Hz. Increasing the τ_{coh} five fold to 50 s produced an enhancement equivalent to that obtained at 10 s. Thus, approximately two-fold increases in activity were obtained with values of τ_{coh} of 10.0 s or greater. A 5 s coherence time produced a level of enhancement that was intermediate between control values and those obtained with τ_{coh} of 10 s or longer. The differences in ODC activity ratios between τ_{coh} of 1 s or less, and τ_{coh} of 10 s or more are very significant. Analysis by independent t-test yielded p values $\leq 1.3 \times 10^{-4}$.

The ODC activity ratio, [ODC], was fit to the function

$$[ODC] = 1 + A\left(1 - e^{\frac{\tau_{coh}}{\tau_{cell}}}\right), \qquad (1)$$

where A is a constant associated with the magnitude of the enhancement and τ_{cell}

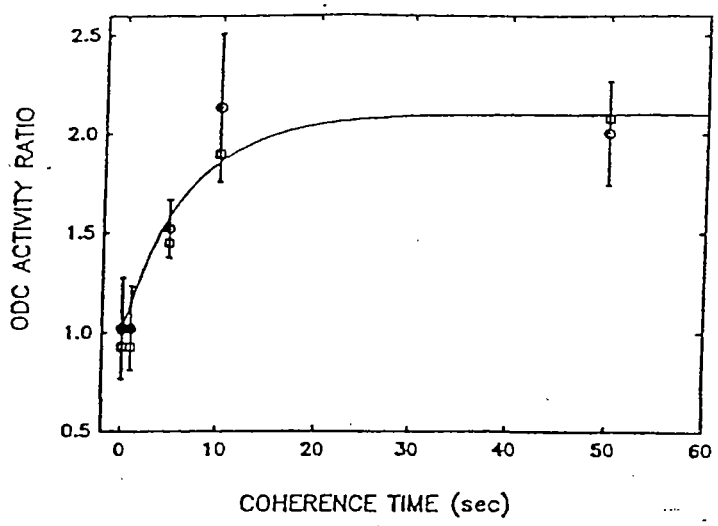

Fig. 3. ODC activity ratio is plotted against coherence time. Numbers of exposures used to determine each time point were as follows: 0.1 s, n = 5; 1.0 s, n = 8; 5.0 s, n = 6; 10.0 s, n = 12; 50.0 s, n = 6. Values from the previously reported ELF coherence time analysis [Litovitz et al., 1991] are plotted (open squares) for purposes of comparison. The solid line represents values calculated for τ_{coh} according to equation 1.

is a time constant apparently associated with the cell detection mechanism. The fit parameters are listed in Table 1.

DISCUSSION

Enhancement of ornithine decarboxylase activity by exposure to microwaves requires that the microwave carrier be amplitude modulated. It is thus the extremely low frequency of the modulating signal which is critical to producing the ornithine decarboxylase response. The mechanism by which the amplitude modulated signal affects the cell is not known. However, it does appear that the cell somehow demodulates the microwave signal and that the demodulated ELF stimulus is what affects cell function.

Previously we used a Helmholtz coil configuration for exposures at ELF frequencies [Litovitz et al., 1991]. The coils were driven by a computer-controlled function generator followed by an audio amplifier. Monolayer cultures of L929 cells,

TABLE 1. Comparison of Fit Parameters in the Microwave and ELF Experiments

Parameter	Microwave exposed	ELF exposed	Combined ELF and microwave data
A	1.08 ± .15	1.11 ± .15*	1.1 ± .16
τ_{cell} (s)	5.3 ± 2.2	8.2 ± 2.9	6.6 ± 1.6

*The originally published value of A (the constant in equation 1) for ELF-exposed cells (1.26 [Litovitz et al., 1991]) was calculated using a subset of the complete data and was published by mistake. The data given in the text and in Figure 1 of that report are, however, correct. The value of 1.11 for A, as given here, is correct.

oriented parallel to the direction of a 60 Hz, 10 μT rms magnetic field and experiencing an induced electric field of 0.04 μV/cm [Bassen et al., 1992; Wang et al., 1993], displayed a transient doubling of ODC activity that peaked at 4 h of exposure.

We have thus demonstrated that 60 Hz ELF fields [Litovitz et al., 1991] and 60 Hz amplitude-modulated 915 MHz microwave fields both produce a transient increase in the ornithine decarboxylase activity of L929 cells. The maximum value of this increase is, in both experiments, a factor of about 2. Data presented here show that the ELF and amplitude-modulated microwave effects share an additional and striking similarity in the requirement for maintenance of coherence over some minimum time interval.

In each case the use of a τ_{enh} of 1.0 s or less resulted in no ornithine decarboxylase enhancement; 5.0 s produced a value intermediate between control and the maximum electromagnetic field-induced values, and 10.0 s or longer yielded full enhancement. In Figure 3 the ODC activity ratios (ODC), for the ELF data are plotted. It can be seen by comparing the microwave and ELF data that the variation of [ODC] with τ_{enh} is quite similar.

In our earlier study on the role of coherence in ELF effects on ornithine decarboxylase activity [Litovitz et al., 1991] we were also able to fit the results to equation 1. The fit parameters are listed in Table 1. It can be seen that there is good agreement between the fit parameters for the two experiments. The values of τ_{cell} for the modulated microwave and ELF are the same to within the uncertainty of the fit. This is further indication that τ_{cell} is truly some fundamental time constant of the mammalian cell.

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