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Bioeffects Induced by Exposure to Microwaves Are Mitigated by Superposition of ELF Noise

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We have previously demonstrated that microwave fields, amplitude modulated (AM) by an extremely low-frequency (ELF) sine wave, can induce a nearly twofold enhancement in the activity of ornithine decarboxylase (ODC) in L929 cells at SAR levels of the order of 2.5 W/kg. Similar, although less pronounced, effects were also observed from exposure to a typical digital cellular phone test signal of the same power level, burst modulated at 50 Hz. We have also shown that ODC enhancement in L929 cells produced by exposure to ELF fields can be inhibited by superposition of ELF noise. In the present study, we explore the possibility that similar inhibition techniques can be used to suppress the microwave response. We concurrently exposed L929 cells to 60 Hz AM microwave fields or a 50 Hz burst-modulated DAMPS (Digital Advanced Mobile Phone System) digital cellular phone field at levels known to produce ODC enhancement, together with band-limited 30–100 Hz ELF noise with root mean square amplitude of up to 10 μT. All exposures were carried out for 8 h, which was previously found to yield the peak microwave response. In both cases, the ODC enhancement was found to decrease exponentially as a function of the noise root mean square amplitude. With 60 Hz AM microwaves, complete inhibition was obtained with noise levels at or above 2 μT. With the DAMPS digital cellular phone signal, complete inhibition occurred with noise levels at or above 5 μT. These results suggest a possible practical means to inhibit biological effects from exposure to both ELF and microwave fields. *Bioelectromagnetics 18:422–430, 1997.* © 1997 Wiley-Liss, Inc.

Key words: cellular phones; EMFs; biological effects; amplitude modulation

INTRODUCTION

A wide range of biological effects from exposure to microwave fields, both in animals and in vitro, have been reported in the literature. In-vitro effects have been observed with CW microwaves at specific absorption rates (SARs) greater than or equal to 10 W/kg [Cleary et al., 1990; Krause et al., 1991; Saffer and Profenno, 1992; Garaj-Vrhovac et al., 1992]. Modulated microwaves (either amplitude or pulse) appear to induce cellular effects at substantially lower SARs (<5 W/kg). Reported effects in this latter case include changes in calcium ion efflux [Bawin et al., 1975; Blackman et al., 1979; Dutta et al., 1984, 1989], changes in enzymatic activity [Byus et al., 1984, 1988; Litovitz et al., 1993], and induction of cellular transformations [Balcer-Kubiczek and Harrison, 1985, 1989, 1991; Czerska et al., 1992]. These studies indicate that modulation plays an important role in eliciting biological responses with weak microwave fields.

The role of modulation was also examined in our laboratory by studying the effects of exposure with modulated microwaves on ornithine decarboxylase (ODC) activity in L929 cells. Experiments were conducted with microwaves modulated in various ways, including amplitude modulation, frequency modulation, square wave modulation, and analog and digital modulation schemes used in cellular phone communications [Penafiel et al., 1997]. It was found that ODC

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activity could be enhanced only when the amplitude of the carrier was varied periodically at extremely low frequencies (ELF). Modulation methods that did not change the amplitude of the carrier had little or no effect on ODC activity. These results corroborate the previous findings, cited above, that low frequency amplitude modulation is necessary for the induction of biological effects by microwaves.

The bioeffects resulting from exposure to amplitude-modulated microwaves are remarkably similar to those induced by ELF fields both in terms of the magnitude of the ODC response and the requirements for constancy of field parameters [Litovitz et al., 1993; Penafiel et al., 1997; Byus et al., 1987, 1988]. The constancy condition refers to the requirement that the exposure field parameters must be constant for some minimum time, τ_c, to obtain the maximum bioeffect [Litovitz et al., 1991, 1993]. The similarities between these ELF and microwave results led us to speculate that the superimposition of electromagnetic (EM) noise fields, which have been shown to inhibit biological responses due to ELF exposure [Litovitz et al., 1994a, 1994b], may also inhibit the effects of modulated microwaves. The purpose of the present study was to investigate this possibility by conducting experiments in which biological samples were exposed concurrently to AM microwaves and ELF noise fields.

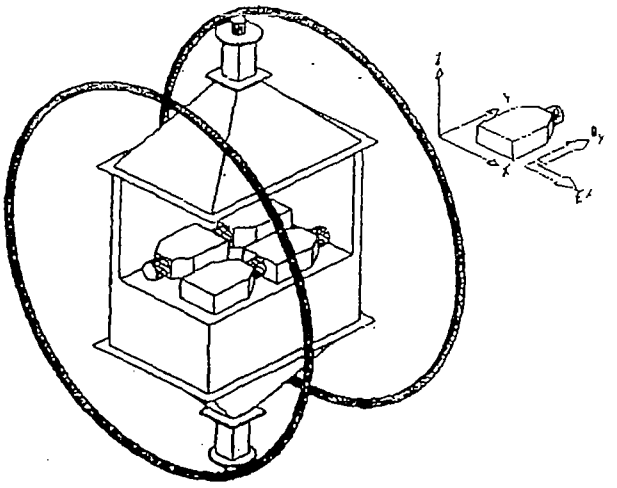


Fig. 1. Detail of the exposure chamber showing the placement of the sample flasks within the Crawford cell and the relative orientation of the flasks with respect to the Helmholtz coils. For ease of visualization, a section of the Crawford cell has been cut out and the center conductor is not shown. The samples are placed on nonconducting shelves located at a height of approximately 7 cm from the junction between the center rectangular section of the exposure chamber and the lower tapered end. In this exposure arrangement, the microwave electric field is perpendicular to the direction of microwave propagation, which is parallel to the long axis of the exposure chamber. The direction of the ELF magnetic field is parallel to the base of the flasks, so that the direction of the induced ELF electric field at the base of the flasks, where the cells are located, is parallel to the direction of the microwave electric field. The orientations of the ELF and microwave electric fields, Ex, and the ELF magnetic field, By, are shown in the inset in relation to the position of a sample flask.

MATERIALS AND METHODS

Exposure System

The exposure system consisted of a microwave exposure chamber positioned within a Helmholtz coil installed inside a water jacketed incubator. The incubator environment, which was air filled, was maintained at 37 °C. The microwave exposure chamber, a model CC110-SPEC Crawford cell, was mounted vertically on a rotary table and fitted with two access doors to allow easy loading of the sample flasks. The chamber was operated with amplitude-modulated microwaves, using either of two signal sources driving a 10 watt solid-state amplifier coupled to the Crawford cell with a double stub tuner. The detailed arrangement of this exposure system has been previously described in the literature [Litovitz et al., 1993].

Exposures were carried out either with 60 Hz amplitude-modulated 835 MHz microwaves or a digital cellular microwave signal. The sinusoidally modulated microwaves were obtained using a Hewlett Packard signal generator, model 8657B with RF plug-in 8352A, and a TENMA model 72-380 function generator. The digital cellular signal was obtained using a Motorola Micro T.A.C. Digital Personal Communicator set to transmit a test pattern in DAMPS (Digital Advanced Mobile Phone System) mode at approximately 840 MHz. DAMPS is a transmission protocol commonly used in the United States for digital cellular communications. The phone signal was coupled to the amplifier using a hands-free adaptor.

The Helmholtz coils for ELF magnetic field exposure were built around the Crawford cell with a nominal coil diameter of 0.46 m and nominal coil separation of 0.23 m. Each coil consisted of 10 turns of 26-gauge magnet wire wound on a support ring made of hard plastic tubing. The coils were assembled on a Plexiglas frame positioned around the Crawford cell as shown in Fig. 1. This ELF/microwave exposure system was designed to allow concurrent exposure to ELF and microwave fields. Because the Crawford cell is essentially a sealed metallic enclosure constructed of 0.089 cm aluminum, the question arises as to whether the ELF magnetic field reaches the samples inside the Crawford cell unaltered. Calculation of the attenuation factor due to eddy current formation of an equivalent cylindrical structure made of the same material as the Crawford cell yields a value of the order of 0.08 dB. Therefore,

the Crawford cell structure should be essentially transparent to the ELF magnetic field. The ELF noise magnetic field was generated using a custom made band-limited noise generator whose output was connected to the auxiliary input of a 35 watt audio amplifier (Realistic model MPA-45; Tandy Corporation, Fort Worth, TX). The Helmholtz coils, whose nominal resistance was of the order of 4Ω, were connected to the 4Ω speaker output of the amplifier.

Four 25 cm^2 tissue culture flasks were used for each exposure. The flasks, each containing L929 cells in 5 ml of culture medium, were placed as pairs, end-to-end, on either side of the center conductor of the Crawford cell. With this arrangement the base of each flask was perpendicular to the direction of propagation of the microwave field. Care was also taken to align the Crawford cell with the Helmholtz coils so that the ELF magnetic field was aligned with the long axis of the flasks. The SAR distribution for this configuration has been previously measured and reported [Litovitz et al., 1993]. In each pair of flasks placed to one side of the center conductor, the SAR peaks in a region shifted towards the center conductor and located towards the junction between flasks. In the rear flask, the SAR decreases towards the back and side edges to approximately 25% of maximum. In the front flask the SAR decreases towards the side edges to approximately 25% of maximum, and towards the front edge to approximately 75% of maximum. Because the configuration is symmetric about the center conductor, the nominal SAR was specified by taking a simple average of point measurements made on a pair of flasks placed to one side of the center conductor. The SAR calibration for this system, with the SAR calculated in this fashion, was determined to be 2.5 W/kg per watt of incident power. All experiments reported here were carried out at a nominal SAR of 2.5 W/kg. This SAR produced no measurable temperature increase within the samples over the 8-h exposure period used in all runs. The electric field associated with the corresponding input power was of the order of 1 V/cm [correction of our previous calculation of 0.7 V/cm, Litovitz et al., 1993].

To ensure a relatively uniform ELF magnetic field distribution at the sample locations, the Helmholtz coils were positioned so that sample flasks placed in the Crawford cell were contained within a 12.7-cm cylinder with axis parallel to the axis of the Helmholtz coils. Consistent alignment of the sample flasks with respect to ELF field exposure was ensured by always rotating the Crawford cell so that the access doors were on planes parallel to the planes of the Helmholtz coils. To confirm the specification of relative uniformity, the ELF magnetic field was measured in the sample area of the Crawford cell using a 1.27 cm, 400-turn pick up coil. The ELF magnetic field was found to vary in this region within less than ±2% of the nominal value.

Measurement and Calibration of Superimposed ELF Fields

ELF band-limited noise with nominal bandwidth between 30 and 100 Hz was used as the superimposed ELF magnetic field. This field is an AC field characterized by random amplitude variations in time. One method to obtain a meaningful measure of such a field is by calculating its root mean square (rms) amplitude. To obtain a repeatable measure, the integration time for the rms calculation must be long enough to produce a constant value. In the experiments described here, the noise field level was set up by measuring the rms value of the noise magnetic field at a fixed location within the uniform magnetic field region of the Helmholtz coils. The applied noise magnetic field was measured using a 10.2 cm, 200-turn pick-up coil. Because the output of such a coil is the derivative of the magnetic field that passes through it, this signal was integrated (using an integrator made by Integrity Design and Research, Buffalo, NY) to produce the instantaneous noise magnetic field. The rms value of the noise field was determined from the instantaneous magnetic field using a custom made AC to DC converter with integration time of approximately 60 s. The integration time was selected to produce a stable reading of the rms noise field. The output of the AC to DC converter was calibrated using a 60 Hz magnetic field of known amplitude, which was itself calibrated using an IDR-109 60 Hz Magnetic Field Dosimeter (Integrity Design and Research, Buffalo, NY).

Amplitude Modulation Procedure

All experiments using 60 Hz amplitude modulation were carried out with a modulation index of 0.23. This value, which was chosen arbitrarily, 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. To obtain the nominal SAR of 2.5 W/kg the incident power (P_t) was set to 1 W.

Digital Modulation Procedure

Experiments with the digital cellular phone microwaves were conducted using the test mode feature of the Motorola Micro T.A.C. Personal Digital Communicator. While in test mode, the phone was set to transmit continuously a pseudorandom test sequence in TDMA (Time Division Multiple Access) mode at a carrier frequency approximately in the middle of the available range (channel 333, approximately 840 MHz). This signal is transmitted in bursts lasting approximately 7 ms with a uniform repetition rate of

50 Hz, corresponding to a duty cycle of approximately 33%. To obtain the nominal SAR of 2.5 W/kg, the incident power was set to 1 W average. The measured peak power corresponding to this average incident power was 3.8 W. Because the duty cycle is 33%, the value of the peak power indicates that the power level is not uniform during the on portion of the cycle.

Field Exposure Protocol

Each experiment was carried out by placing four sample flasks into the Crawford cell as described above. All exposures were carried out for 8 h. In previously reported work, control samples were placed outside the Crawford cell within the same incubator. This placement was not possible with the present arrangement because the Crawford cell was located within a Helmholtz coil for the purpose of superimposing the ELF noise field. Therefore, control samples were run concurrently inside a separate incubator maintained at 37 °C. The four control flasks were stacked one on top of another and covered with a 12.7 cm × 7.6 cm × 8.9 cm mil-annealed mu-metal enclosure. Because the enclosure could not be fully sealed against its base, it was only partially effective as a magnetic field shield.

The background ELF fields at the location of the samples were measured in both the control and the exposed incubators using an IDR-109 60 Hz magnetic field dosimeter, positioned to detect in turn the X, Y, and Z components of the field. To obtain a worst case situation, all measurements were made with the incubator heaters on. All 60 Hz magnetic field components were below 0.04 μT within the mu-metal enclosure loaded with the control samples and below 0.16 μT within the Crawford cell at the level of the exposed samples.

Cell Culture Preparation

Cultures of the murine L929 fibroblast cell line (NCTC clone 929; American Type Culture Collection, Rockville, MD) were maintained in active growth in Eagle's minimum essential medium supplemented as previously reported [Litovitz et al., 1991]. Cell cultures to be used for exposures were initiated approximately 20 h before an experiment. Each 25-cm^2 sample flask was given approximately 3×10^6 cells in 5 ml of culture medium, taken from a stock culture grown to about 70% confluency. These conditions ensured that the cells were at mid-log phase at the onset of an experiment. Before exposure, cells were kept at 37 °C in a 95% air, 5% CO_2 atmosphere by loosening the caps of the sample flasks. During exposure, the cells were also maintained at 37 °C but the flasks were sealed. Because the nominal exposure time was 8 h, the by-products of cellular growth and reproduction were not expected to change the atmosphere in the sealed flasks significantly.

ODC Assay

Sample preparation for the ODC assay was performed as previously described [Litovitz et al., 1994a]. The cells from each set of four flasks of an exposure condition were combined into single samples. One ODC measurement was obtained from each of these samples. ODC activity was determined by minor modifications of the method of Seely and Pegg [1983] as previously reported [Litovitz et al., 1991]. Units of ODC activity were expressed as pmol $^{14}CO_2$ generated/30 min/mg protein at 37 °C. This method measures ODC specific activity by the release of $^{14}CO_2$ from ^{14}C-labelled *l*-ornithine. Protein analysis was performed by the Bio-Rad modification of the Bradford method using a Bio-Rad protein test kit.

RESULTS

Because ODC is a highly inducible enzyme, its activity can vary significantly between cell samples prepared at different times. In our hands, variations in the ODC activity of control samples occurred despite efforts to assure constant culture parameters. To minimize the effect of these day-to-day variations, the data were examined by considering concurrent paired observations of control and exposed conditions. The difference and the ratio of ODC activities of the exposed (E) and control (C) samples were computed for each observation. A standard double-sided paired t test was used to determine whether each ensemble of N observations ($E - C$) and (E/C) of a given exposure condition was statistically significant. A log transformation was performed on the ratio data before applying this test. With this transformation, the observable quantity becomes $\log(E) - \log(C)$. In both cases the statistical test was formulated to test the null hypothesis, that is, $E - C = 0$ or $\log(E) - \log(C) = 0$. The statistical analysis was carried out using INSTAT, a statistics program distributed by GraphPad Software, Inc. (San Diego, CA).

The data presented in Tables 1 and 2 and discussed below, summarize the results for each exposure condition as a function of the amplitude of the superimposed ELF noise. Included in the tables are the mean differences [mean ($E - C$) ± SD], the mean ratios [mean (E/C) ± SD] and the corresponding P values of the t test [$P_{(E-C)}$ and $P_{\log(E/C)}$]. $P_{(E-C)}$ is the probability that the mean of the observed differences ($E - C$) is due to chance. $P_{\log(E/C)}$ is the probability that the mean of the observed log-transformed ratios [$\log(E/C)$] is due to chance. The magnitude of the standard deviations of the difference data are an indication of the

TABLE 1. Results of Concurrent Exposures with 835 MHz Microwaves Sinusoidally Amplitude Modulated (23%) at 60 Hz and Band-Limited ELF Noise (30–100 Hz)*

ELF B field (μT)	N	Mean ($E - C$)	$P_{(E-C)}$	Mean (E/C)	$P_{\log(E/C)}$
0	22	16.0 ± 16.0	<0.0001	1.86 ± 0.43	<0.0001
0.25	6	21.5 ± 6.3	<0.0004	1.89 ± 0.14	<0.0001
0.5	6	29.9 ± 7.9	<0.0002	1.86 ± 0.22	<0.0001
1	6	5.1 ± 7.2	>0.14	1.20 ± 0.24	>0.17
2	6	1.6 ± 4.7	>0.44	1.04 ± 0.14	>0.44
4	12	2.1 ± 3.6	>0.038	1.11 ± 0.14	>0.072

*Mean ($E - C$) is the average difference in ODC activities (pmol $^{14}CO_2$ generated/30 min/mg protein) between the N paired exposed (E) and control (C) samples of each exposure condition. $P_{(E-C)}$ is the corresponding probability that the observed difference is due to chance. Mean (E/C) is the average ratio of ODC activities of the N paired exposed and control samples of each exposure condition. $P_{\log(E/C)}$ is the corresponding probability that the observed log transformed ratio is due to chance. (See text for further explanation of entries.)

TABLE 2. Results of Concurrent Exposures with Burst Modulated DAMPS Digital Cellular Phone Microwaves and Band-Limited ELF Noise (30–100 Hz)*

ELF B field (μT)	N	Mean ($E - C$)	$P_{(E-C)}$	Mean (E/C)	$P_{\log(E/C)}$
0	10	8.6 ± 4.0	<0.0001	1.38 ± 0.14	<0.0001
1	6	13.4 ± 3.7	<0.0003	1.25 ± 0.05	<0.0001
2	15	8.2 ± 8.7	<0.003	1.21 ± 0.17	<0.0004
5	15	3.5 ± 4.3	<0.007	0.95 ± 0.14	>0.10
10	7	0.3 ± 6.3	>0.91	1.00 ± 0.11	>0.87

*Column headings are as defined in Table 1.

variability of the ODC response amongst samples subjected to the same condition on different days. Although these fluctuations are large, their effect on the determination of $P_{(E-C)}$ is minimized by performing the t test on paired observations. Alternatively, the day-to-day ODC fluctuations can be factored out by computing the E/C ratio, which normalizes the data against corresponding controls. We have previously shown that this metric allows reliable comparison amongst experiments with a given exposure condition, even if their ODC activities are widely different [Litovitz et al., 1993]. Therefore, we use the ratio data for the graphical presentation of our results (Figs. 2 and 3).

Exposure with Sinusoidal AM Microwaves and Superimposed ELF Noise

The effect of superimposed ELF noise on the ODC response induced by 60 Hz AM 835 MHz microwaves was examined as a function of the rms amplitude of the ELF noise. Experiments were conducted with noise levels between 0 and 4 μT. The results are shown in Table 1 and Figure 2. Statistically significant microwave-induced enhancements of ODC activity were observed as long as the noise level was below 0.5 μT. When the superimposed ELF noise was above 0.5 μT, inhibition of the microwave-induced ODC effects occurred. Figure 2 shows that the microwave-induced ODC enhancement decreases monotonically as a function of the rms amplitude of the ELF noise. It is apparent from the results shown in Table 1 and Figure 2 that full inhibition of the ODC effect induced by the 2.5 W/kg microwaves, amplitude modulated at 60 Hz with modulation index of 23%, can be achieved with noise fields above approximately 2 μT.

Exposure with a DAMPS Signal and Superimposed ELF Noise

The effect of superimposed ELF noise on the ODC response induced by a DAMPS test signal from a Motorola Micro T.A.C. digital cellular phone was examined as a function of the rms amplitude of the ELF noise. Experiments were conducted with noise levels between 0 and 10 μT. The results are shown in Table 2 and Figure 3. Statistically significant enhancements of ODC activity were observed with noise levels of 2 μT and lower. When the noise level was above 2 μT, inhibition of the ODC effect induced by the DAMPS signal occurred. The enhancement of ODC activity induced by the DAMPS signal was smaller than that observed with the 60 Hz sinusoidally ampli-

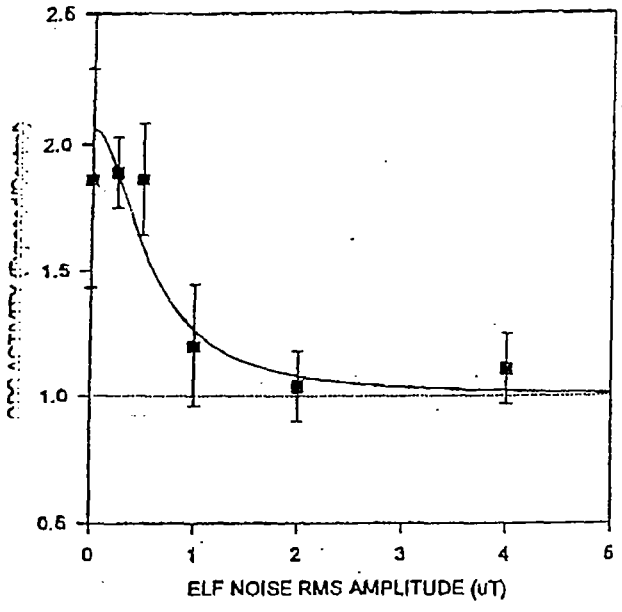


Fig. 2. Effect of superposition of a 30–100 Hz noise magnetic field on the enhancement of ODC activity induced by a 2.5 W/kg microwave field sinusoidally amplitude modulated at 60 Hz with modulation index of 23%. The continuous line is a fit to the data described by Eq. (1), in which S_{app} is the effective value of the microwave stimulus in equivalent units of ELF magnetic field (set to 5 μT), and N_{app} is the rms amplitude of the superimposed ELF noise.

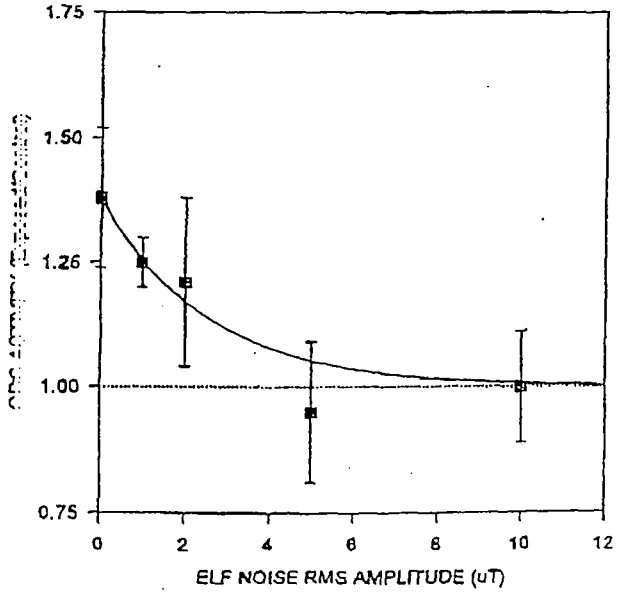


Fig. 3. Effect of superposition of a 30–100 Hz noise magnetic field on the enhancement of ODC activity induced by a 50 Hz burst-modulated DAMPS digital cellular phone microwave signal. The continuous line is a fit to the data with an arbitrary function of the rms amplitude of the noise.

TABLE 3. Comparison of the Magnitudes of the Electric Fields (μV/m) Induced by Externally Applied 5 μT ELF Noise and 60 Hz Magnetic Fields, and a 60 Hz AM Microwave Field (SAR = 2.5 W/kg), and the Effect of Various Exposure Combinations on the ODC Activity Ratio

External 60 Hz (B = 5 μT)	External noise (B = 5 μT)	Microwave 60 Hz AM (B ≈ 2.5 μT)	ODC activity
5	—	—	Increase
5	5	—	No change
—	—	10^4	Increase
—	5	10^4	No change

tude modulated microwave signal. Figure 3 shows the ODC activity ratio as a function of the rms amplitude of the ELF noise. As in the previous case, the ODC activity ratios decrease monotonically towards 1 with increasing amplitude of the noise. It is apparent from the results shown in Table 2 and Figure 3 that full inhibition of the effect induced by the 2.5 W/kg digital cellular phone microwave field can be achieved with noise fields above approximately 5 μT.

DISCUSSION

The work presented here yields the somewhat startling result that the biological effects associated with an ELF amplitude-modulated microwave can be inhibited by the superposition of an ELF noise field. This result becomes even more surprising if one assumes that the interaction occurs via the induced electric field. Listed in Table 3 are values of the relevant electric and magnetic fields for the various exposure conditions that we have investigated. In our experimental arrangement for ELF exposure, cells are subjected to induced electric fields of approximately 5 μV/m when exposed to a 5 μT field. In the case of the exposures reported here, the microwave electric field to which the cells are subjected is about 100 V/m, while the associated magnetic field is approximately 2.5 μT. These data suggest that the effects of a 100 V/m, 2.5 μT, 835 MHz microwave field can be inhibited by a 5 μT, 60 Hz magnetic field (with an associated 5 μV/m induced electric field). In terms of the process depicted in Figure 4, this implies that the 100 V/m (2.5 μT) microwave field produces an "effective" ELF stimulus approximately equivalent to that produced by a 5 μT, 60 Hz magnetic field, with an associated induced electric field of 5 μV/m.

Whereas the results reported here and in earlier work [Penafiel et al., 1997] indicate that microwaves modulated at ELF frequencies induce ELF-like effects, it is not known whether these effects are mediated via the magnetic field or the electric field. The ELF mag-

TABLE 4. Induction of ODC Activity in Equivalent Cell Cultures by Exposure to RF and ELF Electromagnetic Fields (L929 Murine Cells

Lab	Model	Frequency	ODC (Exp/Con)	Reference
VA Hospital, CA	Hepatoma cells	AM RF	1.3	Byus et al., 1988
		ELF	1.5	Byus et al., 1987
Catholic University	L929 Murine cells	AM RF	2.0	Penafiel et al., 1997
		ELF	1.9	Litovitz et al., 1991

1993, at Catholic University], (2) inhibition of EM field-enhanced gene transcription (c-*myc*) [Lin and Goodman, 1995, at Columbia University], (3) inhibition of EM field-induced alterations in neurotransmitter synthesis (dopamine) [Opler et al., 1997 at Columbia University], and (4) inhibition of EM field-induced cell proliferation in human amniocytes [Raskmark and Kvee, 1996, at the University of Aarhus, Denmark].

The in vivo systems in which EM noise was studied include (1) inhibition of enhancement in truncal neural tube abnormalities in chick embryos [Litovitz et al., 1994b, at Catholic University], (2) inhibition of modification of ODC enzyme activity in chick embryos [Farrell et al., 1997, at Catholic University], and (3) inhibition of the modification of 5′ nucleotidase activity in chick brains [Martin and Moses, 1995, at the University of Western Ontario]. In every system investigated, the superposition of an EM noise field inhibited the bioeffect associated with the coherent field alone. The inhibition effect of EM noise appears to be one of the most replicated results in ELF magnetic field studies.

ELF Noise Inhibits Microwave Effects in the Same Manner as It Inhibits ELF Induced Effects

In the present study of AM microwave-induced changes in ODC activity, the inhibitory effect of the superimposed ELF noise field increased as its amplitude increased. This general behavior has also been observed in the case of ELF noise inhibition of the bioeffects of an ELF signal [Litovitz et al., 1993]. In that study, the amplitude dependence of the inhibitory effects of an ELF noise field on a coherent ELF field was described by the empirical function

$$A_{ODC} = 1 + \frac{1.06}{1 + 76\left(\frac{N_{app}}{S_{app}}\right)^2} \qquad (1)$$

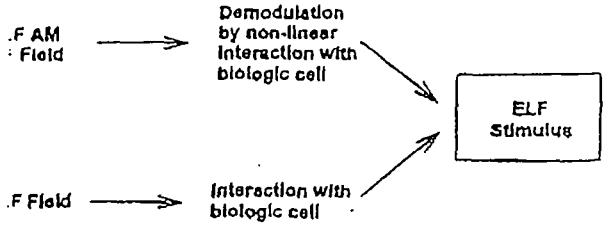
where A_{ODC} is the activity ratio of ODC resulting from exposure to the superposition of an applied noise field of amplitude, N_{app}, and an applied coherent ELF signal of amplitude, S_{app}.

To determine whether the functional dependence on noise amplitude given by Eq. (1) could be also be used to describe the results of the present experiment involving ELF noise and microwaves, we reasoned as follows: First, guided by our previous data on the role of modulation, we assumed that the cell somehow demodulates the microwave signal, creating an "effective" ELF stimulus (i.e., an "effective" S_{app}). In this demodulation concept (as diagramed in Fig. 4) similar responses will result from exposure to ELF or AM microwave signals as long as the "effective" ELF stimuli are equivalent. Second, we assumed that S_{app} in Eq. (1) could be replaced by an "effective" value S_{eff} when considering the microwave data reported here. Using this, it was found that (see the solid line in Fig. 2) Eq. (1) fit the microwave data reasonably well when S_{eff} was set equal to 5 μT. This similarity in the functional dependence on ELF noise amplitude is consistent with the other instances described above in which the effect of AM microwave fields was the same as that of the ELF fields alone.

The inhibitory effect of ELF noise on the ODC response to the DAMPS microwave signal cannot be analyzed in the same manner because data for the effect of the ELF burst signal alone are not available. However, because the frequency content of the modulating signal is broad, it not unreasonable to expect that the functional dependence with respect to the magnitude of the noise might be different. What is more important to note is that the general trend of the result, a steady decrease in the response with increasing noise level, is consistent with the results at 60 Hz.

CONCLUSIONS

The proliferation of personal communication devices that operate at microwave frequencies has raised concerns about their safety. The results of this study and previously reported experimental results seem to indicate that fields of the type radiated by these devices can indeed produce biological effects, at least as mea-



4. Schematic diagram outlining the generation of an ELF ulus either from exposure to ELF AM microwaves, or ELF s.

c noise field (~1 μT) is about the same magnitude he magnetic component of the AM microwave field 2.5 μT). If we assume that the interaction occurs a direct magnetic phenomenon (e.g., if there is gnetite present in the system) the inhibition effects ented are not obviously implausible. However, if assume that the interaction occurs via the induced tric field, then the observation that an electric field .00 V/m might be blocked by a noise field whose gnitude is only about 5 μV/m is at first glance dis-certing.

Nevertheless, such a result is plausible if one con-rs the electrical properties of the biological system which the interaction is occurring. The interaction biological system with an externally applied EM l is characterized by its dielectric response, that is, frequency-dependent variation in the permittivity conductivity of the system. At low frequencies primary field-induced effect is the displacement ounterions associated with the electrically charged logical cells, which gives rise to very large increases he relative permittivity (~10^6–10^7) [see, for exam-Chew and Sen, 1982]. The magnitude of the effect eases with increasing frequency and is referred to he alpha dispersion. Due to the frequency depen-e of the counterion displacement phenomenon, a h stronger (by many orders of magnitude) micro-e field is needed to produce the same displacement given ELF field. Thus, if the EMF interaction with cell which produces the ODC response is mediated counterion displacement, the experimental result the microwave field needs be many times larger the ELF to be equally effective is to be expected.

Although some plausible explanations of the inhi-n effect have been discussed, a full description of phenomenon is not possible since the mechanism nteraction is not understood. However, we believe a consistent picture emerges when our findings are ed in the light of relevant data in the literature. w we present a summary of these data, which we ve lend credence to the unusual result reported in present work.

ELF and AM Microwave Fields Modify ODC Activity in a Similar Manner

Table 4 shows the experiments performed by two different laboratories in which ELF and RF fields were found to enhance the activity of ODC in cultured cells. Both labs performed the exposures using 60 Hz ELF fields and 60 Hz AM microwave fields. The magnitude of the change in the ODC activity with both the ELF and RF fields is essentially the same in each cell system. Hepatoma cells experienced an increase of approximately 50% in ODC activity, whereas L929 murine cells showed an approximately doubling in ODC activity. The observed similarities suggest that the ELF and RF fields generate equivalent effective stimuli and therefore equivalent effects.

The Required Field Constancy Time, τ_c, Is the Same for ELF and Microwave Fields

To further explore the similarities between ELF EM field effects, and those due to ELF AM microwave fields, the field constancy time needed to produce biological effects were examined for both RF and ELF fields by Litovitz et al. [1991, 1993]. They found that an ELF field can induce a biological effect only if it is temporally constant, i.e., parameters such as frequency or amplitude are constant for some minimum "constancy" time, τ_c. In addition they showed that the required τ_c for ELF AM microwaves is the same as that for the ELF field alone. In both cases, if τ_c is less than 1 s, no enhancement in the biological response (ODC activity of murine L929 cells) was observed, whereas fields with constancy times of 10 s or more yielded the full biological response (nearly a doubling of ODC activity). In addition, the functional dependence on τ_c was found to be the same in the ELF and microwave experiments.

ELF Noise Inhibits Effects Induced by Coherent ELF Fields

Before accepting that ELF noise fields can inhibit microwave fields, it is reasonable to ask whether the concept that ELF noise can inhibit the bioeffects of coherent ELF fields is strongly supported in the literature. A thorough review of the pertinent publications reveals that the ability of an ELF noise field to block an ELF field has been broadly tested and verified. These studies have been conducted by several laboratories using a number of markers both in vivo and in vitro. The following is a summary of the systems in which the inhibiting effects of superimposed ELF EM noise have been demonstrated. The in vitro studies include (1) inhibition of EM-induced ODC activity in mouse fibroblasts [Litovitz et al., 1994a, at Catholic University] and in human lymphoma cells [Mullins et al.,

l in terms of changes in ODC activity. Whether
:t these bioeffects are related to adverse health
's is unknown and has not been addressed by our
However, because ODC activity plays a critical
:n cell transformation [Hibshoosh et al., 1990;
:ien et al., 1992], it is reasonable to assume that the
:nduced response may be physiologically relevant.
We have demonstrated that superimposed random
magnetic fields can be effectively used to inhibit
:DC response not only to ELF EM fields but also
:F amplitude modulated microwave fields. This
: offers a promising means to inhibit biological
is induced by exposure to low level (<5 W/kg)
:wave fields.

:RENCES

:n M, Paasinen A, Andersson LC, Hölttä E (1992): Ornithine decarboxylase activity is critical for cell transformation. Nature 360:355–358.

Kubiczek EK, Harrison GH (1985): Evidence for microwave carcinogenesis in vitro. Carcinogenesis 6:859–864.

Kubiczek EK, Harrison GH (1989): Induction of neoplastic transformations in C3H/T10½ cells by 2.45-GHz microwaves and phorbol ester. Radiat Res 117:531–537.

Kubiczek EK, Harrison GH (1991): Neoplastic transformation of C3H/T10½ cells following exposure to 120 Hz modulated 2.45-GHz microwaves and phorbol ester tumor promotor. Radiat Res 126:65–72.

SM, Kaczmarek LK, Adey WR (1975): Effects of modulated VHF fields on the central nervous system. Ann NY Acad Sci 247:74–81.

:an CF, Elder JA, Weil CM, Benane SG, Eichinger DC, House DE (1979): Induction of calcium ion efflux from brain tissue by radio-frequency radiation: Effects of modulation frequency and field strength. Radio Sci 14 65:93–98.

V, Lundak RL, Fletcher RM, Adey WR (1984): Alterations in protein kinase activity following exposure of cultured human lymphocytes to modulated microwave fields. Bioelectromagnetics 5:341–351.

V, Pieper SE, Adey WR (1987): The effects of low-energy 60-Hz environmental fields upon the growth-related enzyme ornithine decarboxylase. Carcinogenesis (Lond) 8:1385–1389.

V, Karlun K, Pieper SE, Adey WR (1988): Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promotors. Cancer Res 48:4222–4226.

C, Sen PN (1982): Dielectric enhancement due to electrochemical double layer: Thin double layer approximation. J Chem Phys 77:4683–4693.

F, Liu LM, Merchant RE (1990): Glioma proliferation modulated in vitro by isothermal radiofrequency radiation exposure. Radiat Res 121:38–45.

EM, Elson EC, Davis CC, Swicord ML, Czerski P (1992): Effects of continuous and pulsed 2450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Bioelectromagnetics 13:247–259.

Dutta SK, Subramoniam A, Ghosh B, Parshad R (1984): Microwave radiation induced calcium ion efflux from human neuroblastoma cells in culture. Bioelectromagnetics 5:71–78.

Dutta SK, Gosh B, Blackman CF (1989): Radiofrequency radiation-induced calcium ion efflux enhancement from human and other neuroblastoma cells in culture. Bioelectromagnetics 10:197–202.

Farrell JM, Barber M, Krause D, Litovitz TA (1997): The superposition of a temporally incoherent magnetic field inhibits 60 Hz induced changes in the ornithine decarboxylase activity of developing chick embryos. (Submitted to Bioelectromagnetics).

Garaj-Vrhovac V, Fucic A, Horvat D (1992): The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. Mutat Res 281:182–186.

Hibshoosh H, Johnson M, Weinstein IB (1990): Effects of overexpression of ornithine decarboxylase (ODC) on growth control and oncogene-induced cell transformation. Oncogene 6:739–743.

Krause D, Mullins JM, Penafiel LM, Meister R, Nardone RM (1991): Microwave exposure alters the expression of 2-5A-dependent RNase. Radiat Res 127:164–170.

Lin H, Goodman R (1995): Electric and magnetic noise blocks the 60 Hz magnetic field enhancement of steady state c-myc transcript levels in human leukemia cells. Bioelectrochem Bioenerg 36:33–37.

Litovitz TA, Krause D, Mullins JM (1991): Effect of coherence time of the applied magnetic field on ornithine decarboxylase activity. Biochem Biophys Res Commun 178:862–865.

Litovitz TA, Krause D, Penafiel M, Elson EC, Mullins JM (1993): The role of coherence time in the effect of microwaves on ornithine decarboxylase activity. Bioelectromagnetics 14:395–403.

Litovitz TA, Krause D, Montrose CJ, Mullins JM (1994a): Temporally incoherent magnetic fields mitigate the response of biological systems to temporally coherent magnetic fields. Bioelectromagnetics 15:399–409.

Litovitz TA, Montrose CJ, Doynov P, Brown KM, Barber M (1994b): Superimposing spatially coherent electromagnetic noise inhibits field induced abnormality in developing chick embryos. Bioelectromagnetics 15:105–113.

Martin AH, Moses GC (1995): Effectiveness of noise in blocking electromagnetic effects on enzyme activity in the chick embryo. Biochem Mol Biol Int 36:87–94.

Mullins JM, Krause D, Litovitz TA (1993): Simultaneous application of a spatially coherent noise field blocks response of cell cultures to a 60 Hz EMF. In M Blank (ed): "Electricity and Magnetism in Biology and Medicine." San Francisco: San Francisco Press Inc, pp 345–346.

Opler M, Rukenstein A, Cole K, Goodman R (1997): Reduced dopamine levels in PC-12 cells exposed to low frequency electromagnetic fields. Bioelectrochem Bioenerg, in press.

Penafiel LM, Krause D, Desta A, Litovitz TA, Mullins JM (1997): Role of modulation on the effect of microwaves on ornithine decarboxylase activity in L929 cells. Bioelectromagnetics 18:132–141.

Raskmark P, Kwee S (1996): The minimizing effect of electromagnetic noise on the changes in cell proliferation caused by ELF magnetic fields. Bioelectrochem Bioenerg 40:193–196.

Saffer JD, Profenno LA (1992): Microwave-specific heating affects gene expression. Bioelectromagnetics 13:75–78.

Seely JE, Pegg AE (1983): Ornithine decarboxylase (mouse kidney). Methods Enzymol 94:158–161.