Role of Modulation on the Effect of Microwaves on Ornithine Decarboxylase Activity in L929 Cells

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The effect of 835 MHz microwaves on the activity of ornithine decarboxylase (ODC) in L929 murine cells was investigated at an SAR of ~2.5 W/kg. The results depended upon the type of modulation employed. AM frequencies of 16 Hz and 60 Hz produced a transient increase in ODC activity that reached a peak at 8 h of exposure and returned to control levels after 24 h of exposure. In this case, ODC was increased by a maximum of 90% relative to control levels. A 40% increase in ODC activity was also observed after 8 h of exposure with a typical signal from a TDMA digital cellular telephone operating in the middle of its transmission frequency range (~840 MHz). This signal was burst modulated at 50 Hz, with approximately 30% duty cycle. By contrast, 8 h exposure with 835 MHz microwaves amplitude modulated with speech produced no significant change in ODC activity. Further investigations, with 8 h of exposure to AM microwaves, as a function of modulation frequency, revealed that the response is frequency dependent, decreasing sharply at 6 Hz and 600 Hz. Exposure with 835 MHz microwaves, frequency modulated with a 60 Hz sinusoid, yielded no significant enhancement in ODC activity for exposure times ranging between 2 and 24 h. Similarly, exposure with a typical signal from an AMPS analog cellular telephone, which uses a form of frequency modulation, produced no significant enhancement in ODC activity. Exposure with 835 MHz continuous wave microwaves produced no effects for exposure times between 2 and 24 h. except for a small but statistically significant enhancement in ODC activity after 6 h of exposure. Comparison of these results suggests that effects are much more robust when the modulation causes low-frequency periodic changes in the amplitude of the microwave carrier. *Bioelectromagnetics 18:132–141, 1997.*
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INTRODUCTION

Particular attention has been focused recently on the potential health effects of radio frequency (RF) and microwave fields, which are used extensively in telecommunications. The transmission of information via RF or microwave signals is accomplished by applying some form of modulation to a carrier wave, which changes some aspect of this wave as a function of the transmitted information. Basic modulation schemes modify the carrier wave's amplitude, frequency, or phase. However, more complex modulation schemes are often used to minimize transmission errors and increase bandwidth in telecommunications. For instance, in North America, digital cellular telephones transmit information in bursts, thereby introducing an amplitude modulation component onto the carrier. Clearly, a careful assessment of potential biological effects that might result from exposure to such fields must examine the role of modulation.

Because of the prevalence of cellular phone use, part of our investigation focuses on signals of the type used in cellular phone communications. Cellular phones may be broadly classified as analog or digital depending on the modulation scheme employed. Analog cellular phones generally use narrow band FM, which causes phase variations in the carrier with very little amplitude change. The analog standard most com-

monly used in the United States is the advanced mobile phone system (AMPS). We refer to fields generated using this standard as analog cellular fields. Digital cellular phones operate under various standards. GSM (global system for mobile communications), the pan-European digital system, has gained wide acceptance in Europe. DAMPS (digital AMPS) is still the most commonly used standard in the United States. DAMPS uses a type of modulation referred to as "time division multiple access" (TDMA), which quadruples the channel bandwidth by splitting the spectrum of each assigned analog channel [Boucher, 1992]. Under this scheme, cellular phones transmit encoded, digitized information using some form of phase or frequency modulation. Consequently, minimal or no fluctuations in amplitude occur when using this basic modulation scheme. However, transmission is generally implemented in burst mode, which introduces a periodic variation in the amplitude of the carrier. By one commonly used protocol, code bursts, approximately 7 ms in duration, are transmitted at a rate of 50 Hz. We refer to the fields generated by cellular phones operating in this fashion as digital cellular fields.

Previous investigations of biological effects from exposure to RF and microwave fields include a large number of both animal and in vitro studies. Included in the latter category are a number of experiments suggesting that, at SARs <5 W/kg, cellular effects occur primarily from exposure to microwaves that are amplitude modulated or pulse modulated at ELF frequencies. Reported effects include changes in calcium ion efflux [Bawin et al., 1975; Blackman et al., 1979, 1985; 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]. Some effects in in vitro preparations have also been observed with CW microwaves [Cleary et al., 1990; Krause et al., 1991; Saffer and Profenno, 1992; Garaj-Vrhovac et al., 1992]. However, all the latter studies used SARs greater than or equal to 10 W/kg. The evidence seems to indicate that modulation plays an important role in eliciting a biological response, particularly when exposing with weak (<5 W/kg) microwaves.

In the work reported herein, we investigated the biological response of L929 murine fibroblasts to ELF-modulated and CW 835 MHz microwave fields. The 835 MHz frequency was chosen because it is within the range currently used in many wireless personal communication applications in North America and is therefore of practical relevance. Various modulation methods were examined, including sinusoidal AM and FM, speech AM, analog cellular, and digital cellular. The specific activity of ornithine decarboxylase (ODC), which performs a rate-limiting step in the synthesis

of polyamines [Hayashi and Murakami, 1995], was selected as the biological marker for this work. ODC activity has been shown to be a reliable indicator of EMF-induced cellular response [Litovitz et al., 1991]. Additionally, ODC is of interest because recent work has shown that overexpression of the ODC gene in cultured cells facilitates, and in some cases causes, cell transformation [Hibshoosh et al., 1990; Auvinen et al., 1992; Moshier et al., 1993; Holtta et al., 1994]. Furthermore, overexpression of ODC in transgenic mice enhances the tumor-promoting effects of PMA [Halmekyto et al., 1992]. Given these facts, it is conceivable that the enhancement of ODC activity as the result of EMF exposure is of relevance to questions of potential health risk posed by ambient EM fields.

MATERIALS AND METHODS

Exposure System

All exposures were carried out using a Crawford cell that was housed in a 37 °C, water-jacketed incubator. The Crawford cell, designed for operation between DC and 1,000 MHz (model CC110-SPEC; Instruments for Industry, Farmingdale, NY), was mounted vertically on a rotary table. This arrangement allowed easy access to both sample chambers, located at either side of the center conductor, through doors installed on opposite sides of the Crawford cell. A Hewlett Packard signal generator, model 8657B with RF plug-in 83522A, was used as the microwave signal source.

Amplitude and frequency modulation were accomplished by using the built-in AM and FM inputs of the signal generator. A function generator (TENMA model 72-380; MCM Electronics, Centerville, OH) was used as the signal source for sinusoidal modulation. Modulation with speech was implemented by using the signal available at the speaker output of a radio receiver tuned to a station broadcasting speech. Square wave modulation was implemented with a Hewlett Packard 8403A modulator to control a Hewlett Packard 8730B PIN modulator. Exposure with the cellular telephone signals was accomplished by using a hands-free adapter to couple the output from the telephone antenna to a coaxial line. The telephone was powered by a DC power supply (Hewlett Packard 6267B) to allow long-term operation. The modulated microwave signals (from the signal generator or the cellular telephones) were amplified to the required power level by using a 10 W solid-state microwave amplifier (model 10W1000; Amplifier Research, Souderton, PA). A double stub tuner was used to match the impedance of the loaded Crawford cell.

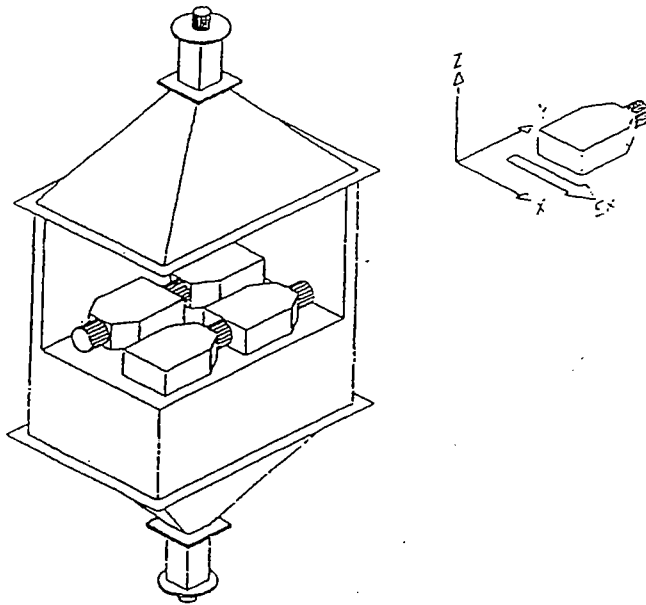All amplitude modulation experiments were car-

Fig. 1. Detail of the exposure chamber showing placement of the sample flasks. 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 electric field is perpendicular to the direction of wave propagation, which is parallel to the long axis of the exposure chamber. The orientation of the electric field is shown in the inset in relation to the position of a sample flask.

approximately 7 ms with a uniform repetition rate of 50 Hz. By contrast, the output signal from the analog phone was found to be constant (i.e., no amplitude modulation).

For each exposure, four 25 cm² flasks of L929 cells were used. The flasks, each containing 5 ml of culture medium, were placed as pairs, end to end, on either side of the center conductor (Fig. 1). This configuration ensured overall symmetry, if not complete uniformity, of the electric field distribution within the samples. The SAR distribution for this exposure arrangement has been previously reported [Litovitz et al., 1993]. To determine this distribution, measurements were made on two flasks located at one side of the center conductor. Because of symmetry, the SAR distribution within the other two flasks was assumed to be similar. The experimental SAR is specified as a simple average of the set of measurements within the two flasks, which were taken on a grid of 48 points within each flask. All experiments reported here were conducted with an input power of 0.96 W, which yielded an average SAR of 2.5 W/kg. The SAR distribution corresponding to this average SAR is shown in Figure 2. This SAR produced no measurable temperature increase within the samples.

The average electric field within the sample can be calculated from the average SAR by using the equation SAR $= (\sigma/\rho)|E|^2$ [NCRP report No. 67, 1981],

ried out with a modulation index of 0.23, calculated by 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. When using speech as the modulating signal, P_t was set to the value needed for m = 0.23, on average. All frequency modulation experiments were carried out with the frequency deviation set to approximately ±60 kHz (3 mV signal at the FM input). The square wave modulation experiments were conducted at 50% duty cycle (i.e., the carrier amplitude was zero for 50% of the time during each cycle).

Experiments with cellular telephone signals were conducted using a Motorola Micro TAC Lite analog cellular telephone and a Motorola Digital Cellular Personal Communicator. The test signal was generated by placing the phone in test mode, selecting a transmission channel in the middle of the available range (approximately 840 MHz), selecting the transmission mode (AMPS for analog or TDMA for digital), and enabling continuous transmission of a pseudorandom test sequence. Examination of the output signal from the digital phone with a diode detector and oscilloscope revealed that transmission was executed in bursts lasting
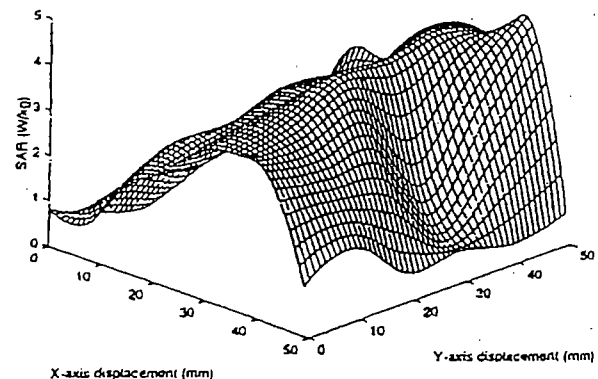

Fig. 2. SAR distribution measured inside the left front flask (see Fig. 1) over the 50 × 50 mm cell growth region located at the bottom of the flask. X and Y axis displacements are measured relative to the left front corner of the square region at the base of the flask. The region of maximal SAR is skewed towards the right back corner of the flask. The SAR decreases by as much as 25% of maximum across the width of the flask and by as much as 75% of maximum across the length of the flask. A somewhat similar distribution was measured inside the left rear flask. In this case, the region of maximal SAR is skewed towards the front right corner of the flask. In the rear flask, the SAR decreases by as much as 25% across both the width and the length of the flask. In both flasks, the regions of maximal SAR are located towards the junction between flasks.

where σ and ρ are, respectively, the conductivity and the density of the aqueous sample. For an SAR of 2.5 W/kg with $\sigma = 1.5$ S/m and $\rho = 1$ g/cm^3, the E-field is on the order of 0.6 V/cm. The field inside the Crawford cell can be calculated by using the relation $E = (PZ_o/d^2)^{1/2}$, where P is the input power, $Z_o = 50\Omega$ is the characteristic impedance of the Crawford cell, and $d = 7$ cm is the distance between the center conductor and the outer plate. For P = 0.96 W, the electric field within the Crawford cell is on the order of 1 V/cm [correction of our previous calculation of 0.7 V/cm, Litovitz et al., 1993]. The computations of the electric field, both inside the Crawford cell and within the aqueous samples, yield values of the same order of magnitude. This suggests that the SAR measurements are a reasonably good indicator of the electric field in the aqueous interface at the base of the flask, where the tangential components of the electric field must be continuous.

Cell Culture Preparation

Actively growing cultures of the murine L929 fibroblast cell line were maintained 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 prior to an experiment at a density (3 × 10^6 cells in 5 ml of culture medium per 25 cm^2 flask) to produce midlogarithmic phase growth by the time of use. Prior to exposure, cells were kept at 37 °C in a 95% air/5% CO$_2$ atmosphere. Microwave exposures were conducted without CO$_2$ flow; flasks were sealed for the duration of exposure. Experiments were conducted over approximately a 3 year period. To ensure uniformity of the cell cultures during this time, we maintained multiple ampules of our original L929 cell stocks in liquid nitrogen. New cultures were started from these frozen stocks approximately every 6 months.

Field Exposure Protocol

For each experimental run, four flasks of cells were placed into the Crawford cell for microwave exposure. An incubator shelf, cut to form a platform around the Crawford cell, provided for positioning of four control flasks within the same incubator chamber and at the same height as the flasks within the Crawford cell. Exposure times ranged between 2 and 24 h. Immediately after exposure, the cells in each flask were washed twice with 3 ml of ice-cold phosphate-buffered saline (PBS) and were then collected by gentle scraping in an additional 3 ml of PBS. To provide sufficient protein for the ODC assay, cells were pooled to provide one exposed and one control sample from each experimental run. Cells were pelleted for 5 min at 200g, and the resultant cell pellet was resuspended in 1 ml PBS

and centrifuged again for 5 min at 200g. After removal of the supernatant, the cell pellets were dried by briefly placing the inverted centrifuge tubes onto absorbent paper. These pellets were stored at −75 °C until assay (typically for 3–4 days).

ODC Assay

ODC activity was determined through 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. Protein analysis was performed with the Bradford method by using a BioRad kit (BioRad Laboratories, Melville, NY). Each cell pellet was lysed in 140 µl of lysis buffer and centrifuged for 5 min at 13,000 rpm. One hundred microliters of the supernatant from each sample was added separately to 150 µl aliquots of the ^{14}C-labeled reaction mixture. ^{14}CO$_2$ generated by ODC activity from each sample was absorbed with 100 µl of 1.0 N NaOH. The reaction was allowed to proceed for 1 h with the samples placed in a shaker water bath at 37 °C. At the end of this period, 400 µl of 20% trichloroacetic acid (TCA) was added to each sample to terminate the enzymatic reactions. To measure the ^{14}C activity, each NaOH sample was transferred to a scintillation vial containing 7 µl of acetic acid and 10 ml of fluor. After 2 h, samples were counted in a scintillation counter. Background activity was determined by the use of samples in which ODC activity was eliminated by acid denaturation with TCA.

RESULTS

ODC activity is an effective marker for EM field-induced effects, provided that variations in ODC activity displayed by cell cultures established at different times are accounted for. To allow comparisons of results obtained on different days, we express our data as an "ODC activity ratio," obtained by dividing the mean activity of EMF-exposed samples from a given run by that of matched control samples. The validity of this approach was demonstrated in our previously published work [Litovitz et al., 1993, 1994]. Because some scientists are uncomfortable with the use of such ratios, the results of this work are also expressed in terms of the mean and standard deviation of the measured ODC activity for each exposure condition (see Tables 1–8). The standard deviation of the mean ODC activity data reflects the day-to-day variations in this parameter. Because these variations were often large, the analysis to determine whether the mean difference between exposed and control samples was statistically significant was performed on paired observations by using a standard two-tailed t test. The two-tailed test

was selected because there is no a priori knowledge of the direction of the differences between exposed and control samples.

Because of the large number of experiments performed and exposure conditions examined, the tabulated data summarize separately the results for each exposure condition. Included in the tables are the mean ODC activities of the control and exposed samples, the *P* value of the two-tailed t test, and the mean activity ratio. It should be stressed that the ODC activity ratio is not the ratio of mean E over mean C but rather the ratio of the mean activity of EMF-exposed samples over that of matched control samples.

Exposure With CW Microwaves

We have previously reported that 8 h of exposure with CW microwaves (835 MHz, 8 h, 2.5 W/kg) yielded no measurable changes in ODC activity [Litovitz et al., 1993]. Because AM-induced biological effects were shown to be transient, we decided that a more complete time course of CW exposure should be examined. To this end, experiments were carried out with 835 MHz CW microwaves for exposure times in the range of 2 to 24 h. Table 1 shows the results of these experiments. Exposures of 2, 4, 8, 12, 16, and 24 h yielded no measurable effects and confirmed previous results. However, a statistically significant effect was obtained after 6 h of exposure, which yielded an ODC activity ratio of 1.3.

Exposure With AM Microwave Fields

The ODC response of L929 cells exposed to AM, 835 MHz microwaves was examined as a function of exposure time (2−24 h) at two frequencies, 16 Hz and 60 Hz, and as a function of frequency in the range of 6−600 Hz for the exposure time that produced the most robust response in the time course experiments (8 h). In all cases, the modulation amplitude was adjusted to give a modulation index of 23%.

Dependence on time. Exposure with either 16 Hz or 60 Hz AM microwaves produced a transient enhancement in ODC activity that peaked after 8 h and returned to control levels by 24 h of continuous exposure. Table 2 shows the results of exposure with 16 Hz AM microwaves. Continuous 6 and 8 h exposures of cells produced enhancements in ODC activity that were statistically significant relative to control levels. The other exposure times tested did not induce statistically significant changes in ODC activity. Table 3 shows the results of exposure with 60 Hz AM microwaves. Statistically significant effects were observed after 6, 8, 12, and 16 hours of continuous exposure, but no statistically significant effects were seen after 2, 4, and 24 h

of exposure. The peak field-induced ODC activity ratios were 1.5 for 16 Hz AM and 1.9 for 60 Hz AM.

Dependence on frequency. Having determined that an exposure time of 8 h produced a peak in the ODC response at two AM frequencies, we examined the variation of the response for this exposure time as a function of frequency in the range of 6−600 Hz. Table 4 shows the results of these experiments. Statistically significant enhancements of ODC activity were obtained at frequencies in the range between 16 Hz and 65 Hz, whereas no significant effects were obtained at either 6 Hz or 600 Hz. The field-induced response peaked in the 60 Hz range, at which the ODC activity ratio approximately doubled. Because no experimental points were obtained between 65 Hz and 600 Hz, these results provide only a general idea of the variation of the frequency response.

Dependence on coherence. We previously demonstrated that the enhancement of ODC activity by AM microwaves requires a minimum coherence time of the modulating signal [Litovitz et al., 1993]. Optimal enhancement was obtained when the coherence time was 10 s or greater, whereas no enhancement resulted when the coherence time was 1 s or less. A case of some practical interest is that of RF or microwave signals amplitude modulated with speech. Because the coherence time of speech is less than 1 s, we predicted, based on our earlier work, that no effect on ODC activity would be elicited by exposure to such signals. The experimental data confirmed this prediction (see Table 8). Eight hour exposures with microwaves amplitude modulated with speech yielded no statistically significant effects as measured using a paired t test. Whereas the coherence time is an accurate predictor of the biological response in some cases, further research, to be reported elsewhere, has led us to conclude that the ability of an electromagnetic field to induce biological effects is best characterized in terms of a "constancy" interval, defined as the time interval over which the field parameters (e.g., amplitude, frequency) remain constant.

Exposure With FM Microwave Fields

The effects of frequency modulation with a 60 Hz sinusoid were examined as a function of exposure time in the range of 2−24 h. The modulation frequency, ω_m, and exposure times were selected to allow direct comparison to similar experiments using amplitude modulation. The maximum deviation, $\Delta\omega$, of the FM signal was set to 60 kHz to correspond approximately to the maximum deviation of commercial FM (75 kHz). The corresponding modulation index, $\beta = \Delta\omega/\omega_m$, was on the order of 1,000, which defines this signal as

wideband FM. Table 5 summarizes the results of exposure with FM microwaves. No statistically significant changes in ODC activity were induced by exposure of cultures to this signal.

Exposure With Cellular Phone-Type Signals

The results of the AM and FM experiments described above suggest that biological effects can be induced when the modulation causes periodic changes in the amplitude of the carrier, as is the case in sinusoidal AM. FM, which causes changes in phase but minimal changes in amplitude, appears to produce no measurable effect. A situation of practical interest is that of cellular phone transmissions. If amplitude modulation of the carrier is a significant factor in determining a biological response, then the extent to which cellular phones can induce a response would depend on whether the modulation schemes used for transmission impart a periodic ELF modulation component onto the carrier. Digital phones, which operate in burst mode, have periodic fluctuations of the carrier amplitude in the ELF range. Analog phones, which do not operate in burst mode, have relatively constant carrier amplitude (assuming no changes in reception between the cellular phone and the nearest cell). A series of exposures was conducted to determine whether either of these cellular phone signals produce enhancement of ODC activity in exposed cells.

Exposures were carried out with both the analog and the digital cellular fields and also with an 835 MHz carrier amplitude modulated with a 50 Hz square wave. This latter condition was intended to simulate the low-frequency burst modulation of the digital cellular field. Exposures with the analog cellular field produced no statistically significant enhancement in ODC activity for exposure times between 4 and 10 h (Table 6). Exposures with the digital cellular field produced statisti-

cally significant enhancements in ODC activity for 6, 8, and 10 h of exposure. The other two exposure times tested, 4 and 16 h, produced no statistically significant enhancement in ODC activity (Table 7). The square wave modulated signal was tested only after 8 h of exposure, yielding results similar to those for the corresponding condition with the digital cellular phone signal.

Table 8 and Fig. 3 summarize the results of 8 h exposures with the various signals tested. We note from this table that, in all cases tested with this exposure time, exposures with modulated microwaves produced statistically significant enhancements in ODC activity only when the modulation introduced low-frequency periodic changes in the amplitude of the carrier.

DISCUSSION

The ODC activity of L929 fibroblasts was transiently enhanced by exposure to some, but not all, of the modulated 835 MHz microwave fields we examined. Exposure to an unmodulated, CW field also produced a response. These results are consistent with other reports that demonstrate enhanced ODC activity after EMF exposure. EMF-induced changes in ODC activity have been documented for cultured cells exposed to 60 Hz electric or magnetic fields [Byus et al., 1987; Litovitz et al., 1991, 1994; Mullins et al., 1993], for chicken embryos exposed to 60 Hz magnetic fields [Farrel et al., 1993], and for cultured cells exposed to amplitude-modulated microwave fields [Byus et al., 1988; Litovitz et al., 1993]. Thus, ODC activity appears to provide a consistent and reliable measure of cellular response to both ELF and RF EM fields. As such, it represents one of the few replicated examples of a bioeffect being induced by a weak electromagnetic field.

Microwave-Induced ODC Response: The Role of Modulation

Whether a given microwave field induces an ODC response seems to be dependent upon the modulation scheme employed. Enhancements in ODC activity were observed for L929 cells exposed to 835 MHz fields that were amplitude modulated with sinusoidal 16 and 60 Hz signals or with a 50 Hz square wave signal. Use of digital cellular signals burst modulated at 50 Hz, which produces a pattern of amplitude modulation very similar to that of the 50 Hz square wave, also induced increases in ODC activity.

In contrast to these results, neither the 60 Hz sinusoidal frequency-modulated 835 MHz carrier nor the frequency-modulated microwave field produced by analog cellular telephones induced an ODC response in L929 cells. These frequency-modulation schemes
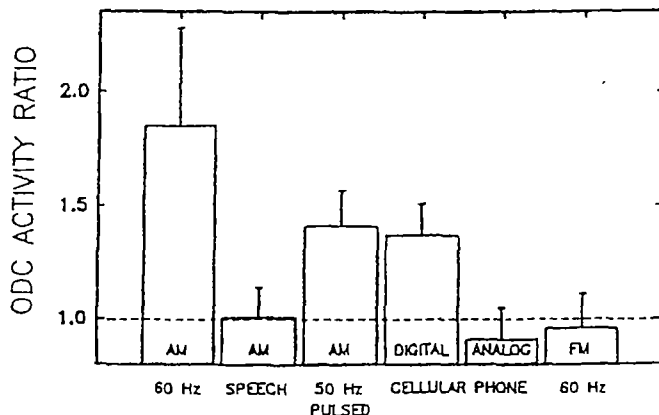

Fig. 3. ODC activity ratios for L929 murine cells exposed for 8 h with 835 MHz microwaves modulated by various methods.

TABLE 1. Results of Exposures With 835 MHz Continuous Wave Microwaves. The Mean E and Mean C Values Are the Average ODC Activities and Corresponding Standard Deviations. Expressed In Terms of pmol $^{14}CO_2$ Generated/30 Min/mg Protein, of the N Exposed (E) and (N Control (C) Samples of Each Exposure Condition. The P Value Is the Probability That the Observed Differences Between Control and Exposed Samples in Each Set of N Paired Observations Is Due to Chance. The ODC Activity Ratio Is the Mean Value of the Ratios of the ODC Activity in Exposed Samples to That of Corresponding Control Samples, Computed from N Paired Observations of Each Exposure Condition. The ODC Activity Ratio is Not the Ratio of Mean E Over Mean C

Exp time (hrs)	N	Mean C	Mean E	P	ODC activity ratio
2	5	13.8 ± 6.3	12.9 ± 6.4	>0.52	0.9 ± 0.2
4	6	9.2 ± 6.1	8.7 ± 5.2	>0.69	1.0 ± 0.2
6	11	13.3 ± 12.2	16.5 ± 13.7	<0.004	1.3 ± 0.2
8	16	16.8 ± 13.8	15.9 ± 15.2	>0.41	0.9 ± 0.2
12	8	8.9 ± 1.7	8.3 ± 2.2	>0.34	0.9 ± 0.2
16	10	5.3 ± 4.1	4.8 ± 3.3	>0.32	1.0 ± 0.3
24	9	5.4 ± 2.3	5.0 ± 2.2	>0.32	0.9 ± 0.2

TABLE 2. Results of Exposures With 835 MHz Microwaves Amplitude Modulated (23%) With 16 Hz Sinusoids. Column Headings Are as Defined in Table 1

Exp time (hrs)	N	Mean C	Mean E	P	ODC activity ratio
2	7	12.1 ± 4.1	11.5 ± 4.2	>0.77	1.0 ± 0.3
4	7	12.0 ± 7.9	13.2 ± 10.0	>0.38	1.1 ± 0.3
6	13	14.2 ± 18.9	15.8 ± 20.3	<0.045	1.2 ± 0.3
8	11	9.9 ± 8.5	13.6 ± 11.8	<0.012	1.5 ± 0.3
12	6	12.8 ± 7.4	10.5 ± 6.1	>0.17	0.8 ± 0.2
16	7	6.7 ± 3.7	7.2 ± 4.0	>0.49	1.1 ± 0.3
24	9	10.3 ± 11.2	10.3 ± 9.2	>0.97	1.1 ± 0.1

TABLE 3. Results of Exposure With 835 MHz Microwaves Amplitude Modulated (23%) With 60 Hz Sinusoids. Column Headings Are as Defined in Table 1

Exp time (hrs)	N	Mean C	Mean E	P	ODC activity ratio
2	8	8.4 ± 3.4	9.8 ± 4.6	>0.15	1.2 ± 0.3
4	9	16.3 ± 12.8	16.1 ± 10.9	>0.91	1.1 ± 0.5
6	13	7.2 ± 4.0	11.6 ± 5.3	<0.0001	1.7 ± 0.4
8	22	24.0 ± 32.7	40.0 ± 47.2	<0.0001	1.9 ± 0.4
12	9	7.4 ± 3.4	11.1 ± 4.9	<0.0017	1.5 ± 0.3
16	9	7.6 ± 2.0	9.5 ± 2.6	<0.0058	1.3 ± 0.2
24	9	7.4 ± 2.4	6.7 ± 2.3	>0.13	0.9 ± 0.2

TABLE 4. Results of Exposure With 835 MHz Microwaves Amplitude Modulated (23%) With 6-600 Hz Sinusoids. Column Headings Are as Defined in Table 1

Freq (Hz)	N	Mean C	Mean E	P	ODC activity ratio
6	7	7.1 ± 5.0	6.6 ± 3.1	>0.61	1.1 ± 0.2
16	11	9.9 ± 8.5	13.6 ± 11.8	<0.012	1.5 ± 0.3
55	6	10.4 ± 4.8	18.5 ± 6.7	<0.009	1.9 ± 0.5
60	22	24.0 ± 32.7	40.0 ± 47.2	<0.0001	1.9 ± 0.4
65	6	10.0 ± 1.9	20.5 ± 4.7	<0.0011	2.1 ± 0.4
600	7	7.8 ± 5.3	9.4 ± 8.7	>0.37	1.3 ± 0.5

TABLE 5. Results of Exposure With 835 MHz Microwaves Frequency Modulated (60 kHz Deviation) With 60 Hz Sinusoids. Column Headings Are as Defined in Table 1

Exp time (hrs)	N	Mean C	Mean E	P	ODC activity ratio
2	9	35.9 ± 14.2	36.8 ± 14.5	>0.72	1.0 ± 0.1
4	6	17.7 ± 9.8	17.5 ± 7.7	>0.84	1.0 ± 0.1
6	8	22.0 ± 11.5	20.7 ± 11.5	>0.28	0.9 ± 0.1
8	7	18.9 ± 7.2	18.4 ± 7.7	>0.69	1.0 ± 0.2
12	6	13.0 ± 4.3	12.5 ± 2.9	>0.51	1.0 ± 0.1
16	7	12.4 ± 5.8	10.7 ± 5.6	>0.29	0.9 ± 0.2

TABLE 6. Results of Exposures With an AMPS Analog Cellular Phone Signal. Column Headings Are as Defined in Table 1

Exp time (hrs)	N	Mean C	Mean E	P	ODC activity ratio
4	6	28.9 ± 8.2	28.6 ± 8.6	>0.86	1.0 ± 0.1
6	6	33.4 ± 7.3	34.2 ± 11.7	>0.74	1.0 ± 0.1
8	6	17.5 ± 6.9	15.8 ± 6.7	>0.06	0.9 ± 0.1
10	6	33.9 ± 23.1	27.7 ± 16.1	>0.11	0.9 ± 0.2

TABLE 7. Results of Exposure With a DAMPS Digital Cellular Phone Signal. Column Headings Are as Defined In Table 1

Exp time (hrs)	N	Mean C	Mean E	P	ODC activity ratio
4	11	36.3 ± 20.1	40.0 ± 19.4	>0.07	1.2 ± 0.2
6	12	24.6 ± 12.5	29.1 ± 13.3	<0.0085	1.2 ± 0.2
8	9	26.6 ± 11.3	35.6 ± 13.4	<0.0002	1.4 ± 0.2
10	8	27.0 ± 8.8	31.4 ± 10.1	<0.0008	1.2 ± 0.1
16	3	8.9 ± 7.3	9.0 ± 5.9	>0.97	1.1 ± 0.1

TABLE 8. Results of 8 Hour Exposures to 835 MHz Microwaves Modulated by Various Methods. Column Headings Are as Defined in Table 1

Modulation type	N	Mean C	Mean E	P	ODC activity ratio
FM 60 Hz	7	18.9 ± 7.2	18.4 ± 7.7	>0.69	1.0 ± 0.2
AM speech	7	14.6 ± 11.7	14.1 ± 9.3	>0.69	1.0 ± 0.1
AM 60 Hz	22	24.0 ± 32.7	40.0 ± 47.2	<0.0001	1.9 ± 0.4
Sq wave 50 Hz	8	25.9 ± 6.6	36.4 ± 9.5	<0.0004	1.4 ± 0.2
Digital cellular	9	26.6 ± 11.3	35.6 ± 13.4	<0.0002	1.4 ± 0.2
Analog cellular	6	17.5 ± 6.9	15.8 ± 6.7	>0.06	0.9 ± 0.1

produce no measurable changes in carrier amplitude. The results suggest that, to induce a cellular response through microwave exposure, the microwave field must be modulated by a method that produces periodic alterations in the amplitude of the carrier wave. For example, the fact that the square wave and digital phone signals induced similar responses suggests that the cells responded to the 50 Hz pulsing of the carrier amplitude common to both signals. Thus, it appears that the cells did not respond to the very-high-frequency digitized information transmitted within the envelope of each pulse of the cellular signal.

The data obtained by using modulated microwaves are all consistent with the concept that the ELF amplitude modulation is critical in causing a biological effect. However, the results of our CW experiments

present an exception. Exposure of L929 cells to the 835 MHz CW field produced a statistically significant enhancement in ODC activity after 6 h of exposure. However, the time course of this response seemed unusual in that the increase in ODC activity was sharply demarcated in time, with no indication of rising or falling activity at the 4 or 8 h exposure time points. Nonetheless, we believe this effect to be real, having obtained the same result in two separate sets of experiments that were conducted more than 3 years apart, each set having independently yielded a statistically significant enhancement. How this CW effect relates to the ODC enhancements observed in cells exposed to AM fields is not clear. The obvious distinctions are that the enhancements induced by AM fields peaked 2 h later than those caused by the CW field and that the most robust response, that produced by the 60 Hz AM field, was significantly larger than the response produced by the CW field.

In each instance for which a time course was measured, the enhancement in ODC activity induced by AM microwave exposure was transient. ODC activity peaked after 8 h of exposure and then returned to control values despite continued exposure. Byus et al. [1988] also showed the transient enhancement of ODC activity in three different cultured cell lines exposed to AM microwaves. Approximately 15–60% increases in ODC activity were observed after 1 h exposures to 450 MHz microwaves sinusoidally amplitude modulated at 16 Hz. Under the exposure conditions used by Byus et al., 60 Hz amplitude modulation failed to elicit changes in ODC activity. However, direct comparisons to our results are not possible, because their carrier frequency, cell lines, exposure time, and modulation index differed from ours. For example, our data indicate that it is not until at least 6 h after onset of exposure to the 60 Hz AM microwave field that a clearly discernible effect on ODC activity is observed. The longest time that Byus et al. observed ODC activity was only 4 h after onset of exposure. It is possible that, had they waited longer, they would have observed an effect similar to that observed by us when using a 60 Hz AM exposure. Regardless, the observations of Byus et al. underscore the fact that exposure to a sinusoidal, amplitude-modulated microwave field can enhance ODC activity.

Microwave-Induced ODC Responses Resemble Those Induced by ELF Fields

The basic response to 60 Hz AM microwaves (i.e., a transient, approximately twofold increase in ODC activity) is similar to that observed after exposure of cells to a 60 Hz ELF magnetic field. The major distinction is that the timing of the two responses is different. The ELF field-induced ODC response peaks

at 4 h of exposure, with a return to control values by 8 h of exposure. The 60 Hz AM microwave response peaked at 8 h and returned to control levels ther approximately 24 h. Our results, considered with those of others, suggest that the responses induced by ELF and AM microwave fields are fundamentally similar and that it is the ELF modulation frequency of the microwave field that plays a critical role in determining the characteristics of the response.

For example, the ODC responses to ELF and AM microwave fields display similar requirements for temporal coherence of the stimulating field. frequency. Cells exposed to an ELF magnetic field for which the frequency was switched between 55 and 65 Hz at regular intervals yielded a twofold enhancement in ODC activity only when each frequency was maintained over intervals ≥ 10 s throughout exposure [Litovitz et al., 1991]. When the frequency was switched at intervals ≤ 1 s, ODC activities remained at control levels. This temporal requirement for frequency coherence was also demonstrated by the fact that L929 cells showed no enhancement of ODC activity after exposure to ELF random noise fields of amplitude comparable to that of the 60 Hz stimulating field [Litovitz et al., 1994]. A similar temporal coherence requirement determines the response of L929 cells exposed to AM microwaves [Litovitz et al. 1993]. If the modulation frequency is switched between 55 and 65 Hz at regular intervals throughout the 8 h exposure period, the ODC response is determined by the duration of the constant frequency interval. As with the ELF studies, switching at an interval ≥ 10 s produced an approximate doubling in ODC activity, but intervals ≤ 1 s produced no ODC response. This result is reinforced by the data presented herein, which demonstrate that amplitude modulation using speech (which has a coherence time of <1 s) produced no enhancements in ODC activity. However, in this case, the decrease in the response may also be attributed in part to the frequency spectral distribution of speech. Our data show that the ODC response to AM microwaves decreases as the modulation frequency increases (Table 3). Speech is generated mostly in the range between 50 Hz and 10 kHz, but the highest concentration of sounds is in the range 100–600 Hz [Denes and Pinson, 1963]. Consequently, regardless of other effects, a decreased response relative to 60 Hz AM would be expected.

SUMMARY AND CONCLUSIONS

Our results indicate that amplitude-modulated microwaves at an SAR of 2.5 W/kg, corresponding to a plane wave equivalent power density of approximately 1 mW/cm², are capable of altering biological activity in in vitro cell cultures. Frequency-modulated micro-

waves at this power level appear to have no effect at all. The radiation from TDMA digital cellular phones can cause significant changes in ODC activity, whereas that from analog phones does not (evidently because they are FM). The data suggest that the same coherence requirements necessary for ELF-induced bioeffects apply to the modulation of ELF amplitude-modulated microwaves. It is clear from this study that the use of SAR alone is inadequate for setting safety standards. The type of modulation must also be considered.

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