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CHANGES IN CARDIAC-CELL MEMBRANE NOISE DURING MICROWAVE EXPOSURE

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Summary

The microscopic voltage fluctuations (noise) of a biological membrane result from electrical and chemical processes within the membrane. Analysis of spontaneously-occurring noise can reveal changes in ion channel kinetics which are not readily apparent from other types of analysis as well as information about membrane electrical properties. We report here the results of preliminary studies in which 2450-MHz CW energy was applied to aggregates of embryonic heart cells in culture with an open-ended coaxial exposure device. Irradiation at Specific Absorption Rates (SARs) of 122 to 237 mW/g caused a bulk temperature rise of no more than 0.8°C, but increased the power ratio of the membrane noise by 1.94±3.81 dB. Because of the large variability, this increase was not significant at the 5% level.

Background

The question of possible effects of microwave radiation on excitable nerve and muscle cells has received considerable attention. It has long been recognized that induced heating will affect these excitable tissues in a manner predictable from known thermal sensitivities. However, since normal functions of nerve and muscle involve ionic currents and electric fields, there is some basis for considering direct electromagnetic field interactions. Several experimental observations have also suggested that non-thermal microwave effects may occur. In turtle and rat hearts, low levels of microwave radiation caused a slowing of heart rate while high levels and warming caused the expected thermal response of a faster heart rate.[1,2] In snail pacemaker neurons, early changes in firing rate at the beginning of microwave irradiation were opposite from the slower thermal response.[3]

Recent theoretical work has examined possible non-thermal mechanisms at the molecular level. It has been predicted that effects on individual membrane particles are more likely than gross rectification at microwave frequencies.[4] Effects of oscillating fields on the voltage-sensitive parameters of the classical Hodgkin-Huxley equations for an excitable membrane have been studied.[5] The known voltage-sensitivity of membrane ion channels has also been used to study effects of oscillating fields on the probability that channels are in the open state.[6]

Measurement of membrane voltage noise can provide information about ion channels and other membrane electrical properties.[7] The cardiac-cell aggregate provides a source of membrane voltage noise in a well-characterized excitable membrane.[8] In the present work we have studied the membrane voltage noise of cardiac-cell aggregates during microwave irradiation as a probe of the molecular mechanism of interaction between electromagnetic energy and an excitable membrane.

Methods

Spheroidal aggregates of cardiac cells were formed during 48- to 72-hr gyration culture of cells prepared using procedures previously described in detail.[9,10] Cardiac cells were derived from embryos of white Leghorn chickens by dissociation with trypsin. The bathing medium contained 4% horse and 8% fetal bovine sera to improve microelectrode penetrations. Tetrodotoxin (1 μg/ml) was added just before an experiment to block fast sodium channels. Concentrations of the major cations in the medium (in mM) were: potassium, 3.3 (4.5 in one experiment); calcium, 3.6; and sodium, 136. Under these conditions, long periods of membrane voltage noise could be recorded without action potentials. In the same medium but without tetrodotoxin, action potentials occurred at a rate of 1 to 2 per second at 37°C.

A 35 mm culture dish containing aggregates in 4 ml of medium was positioned on an open-ended coaxial exposure device connected to a source of 2450 MHz CW power. The Specific Absorption Rate (SAR) of microwave energy deposition by this device had been determined previously as a function of position in the dish and of net input power to the exposure device.[11] Over the coaxial aperture, SAR decreased with radial distance from the center from 371 to 56 mW/g. Aggregates in these experiments were allowed to adhere to the bottom of a dish and were irradiated at 122-237 mW/g. Bulk medium temperature was maintained at 37°± 0.2°C while the maximum local temperature increase experienced by aggregates during irradiation was 0.8°C. Evaporation, gaseous atmosphere, and pH were controlled and mechanical vibration minimized.

The membrane potentials of two widely separated cells in an aggregate were recorded simultaneously with separate glass microelectrodes. Each microelectrode signal was stored on FM magnetic tape at gains of 10X and 1000X for later analysis. An HP5420A Digital Signal Analyzer was used to calculate the cross spectrum of the high-gain signals for different experimental conditions. The uncorrelated microelectrode and amplifier voltage noise did not contribute to the cross spectrum. The cross spectrum thus represented the membrane voltage spectral density, $S_V(f)$, with units of volts squared per Hertz (V^2/Hz). Spectral densities from 0.01 to 10.1 Hz were derived from 95-second epochs of data before, at the beginning of, at the end of, and after microwave exposures of 1-26 minutes. Within each epoch, as many spectral densities as it was possible to obtain were averaged (range: 16 to 50).

Results

Membrane voltage noise was recorded from 5 different aggregates in 5 experiments. Two aggregates were exposed to microwave radiation once, while the other three were exposed twice for a total of 8 exposures. Because electrode impalement was lost at the end of some experiments, data were not obtained for 3 periods at the end of exposure and for 2 periods after exposure.

All membrane voltage noise spectral densities or "spectra," declined with frequency (f) approximately as 1/f, with values between -70 and -110 dB (re 1 V^2/Hz). These values were well above the -137 dB level of thermal noise derived from a typical membrane resistance of 1 megohm. Fig. 1 shows the 4 spectra obtained for one exposure, in which the power (in dB re 1 V^2RMS) in each spectrum was derived using a calculating function of the Digital Signal Analyzer.

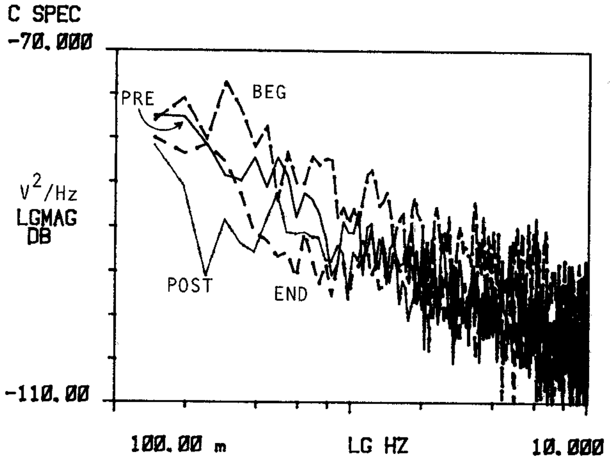

Fig. 1. Membrane voltage noise spectral density curves for a second exposure of a cardiac-cell aggregate at 122 mW/g at 2450 MHz. Curves for epochs before (PRE, solid), during the beginning of (BEG, long dashes), during the end of (END, short dashes), and after (POST, dash-dot) a 26-minute exposure. Numbers of individual spectra averaged were 41, 16, 50, and 50 for the respective epochs.

The average pre-exposure value of power for the 8 exposures was -83.34±3.12 dB (mean ± standard deviation); during the beginning of exposure, -82.36 ±4.25 dB. Because the high levels of radiation may have had a lasting effect on an aggregate, analysis was also done using only data from first exposures. When only the five first exposures were considered, the average powers were -83.39±3.87 dB and -81.44 ±2.90 dB for pre-exposure and beginning epochs, respectively. To analyze changes in power further, the pre-exposure power was subtracted from powers for subsequent epochs in each exposure. Over all 8 exposures, the average increase in power during beginning of exposure was 0.99±3.93 dB; for the 5 first exposures, 1.94±3.81 dB. Using powers from the 6 available post-exposure epochs, the average change was +0.25±4.54 dB.

The average increase of 1.94 dB at the beginning of the microwave exposures represents a rise of 56% in power ratio. However, the large variability (std. dev. = 3.81 dB) makes this result inconclusive. Indeed, comparing the mean powers before, and at the beginning of exposure with a paired t-test shows that with an N of 5, they do not differ significantly at the 5% level. Nonetheless, it is interesting that the pre- and post-exposure powers differ by only 0.035 dB, consistent with the possibility that a larger sample of data may confirm a statistically significant initial effect of microwave exposure.

The SAR of an aggregate was the same for the epochs at the beginning and end of irradiation period since net input power to the exposure device varied by less than 1% during an exposure. The temperature increased with a time constant of about 30 seconds to its steady-state value (maximum increase 0.8°C). Thus, a temperature effect is not a likely explanation for an increase in S_V (f) during irradiation since the average noise increase was larger during the first 60-90 seconds of irradiation, whereas the maximum temperature increase occurred later. This is also supported by results from separate warming experiments in which noise level failed to increase with temperature over the range of 36°-38°C.

Further experiments are currently in progress with modified design to reduce experimental variability and increase in sample size.

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