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The effects of low level microwaves on the fluidity of photoreceptor cell membrane

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Abstract

Due to the extensive use of electromagnetic fields in everyday life, more information is required for the detection of mechanisms of interaction and the possible side effects of electromagnetic radiation on the structure and function of the organism. In this paper, we study the effects of low-power microwaves (2.45 GHz) on the membrane fluidity of rod photoreceptor cells. The retina is expected to be very sensitive to microwave irradiation due to the polar character of the photoreceptor cells [Biochim. Biophys. Acta 1273 (1995) 217] as well as to its high water content [Stud. Biophys. 81 (1981) 39].

Keywords: Athermal microwave effects; Photoreceptors; Membrane fluidity

1. Introduction

Previously reported experimental studies on the irradiation of living cells with athermal microwaves have produced conflicting results [1–3].

No information is available concerning the effects of low level microwaves on retinal cells.

In our experiments, we study the effects of low-power microwaves (2.45 GHz) on rod photoreceptor cell membrane.

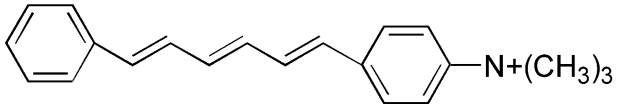
It was found that microwave-induced modifications on membrane fluidity of photoreceptor cells are strongly dependent on the power density of incident radiation:

- fluorescence anisotropy decreases in rod suspensions irradiated at 15 $\mu W/cm^2$ (1 h irradiation time);
- fluorescence anisotropy increases in rod suspensions irradiated at 5, 6 and 7 mW/cm^2 (1–2 h irradiation time);
- no microwave effects were observed at 1, 3, 8 and 9 mW/cm^2.

2. Experimental

2.1. Membrane fluidity measurements by fluorescent depolarization

The membrane fluidity of irradiated and control photoreceptor cells were monitored by fluorescence anisotropy measurements of TMA-DPH labeled photoreceptor cells [4]. TMA-DPH binds on the hydrophilic external surface of the cell membrane.



1-[4-(trimethylammonium) phenyl]-1,3,5-hexatriene (TMA - DPH)

Upon excitation of a molecule with polarized light, the molecule will emit a fluorescent radiation, more or less polarized, depending on the degrees of freedom of the molecule during the lifetime of the excited state. Depolarization degree of fluorescent light is a measure of the fluorophore freedom and therefore it is a measure of fluidity of the surrounding medium.

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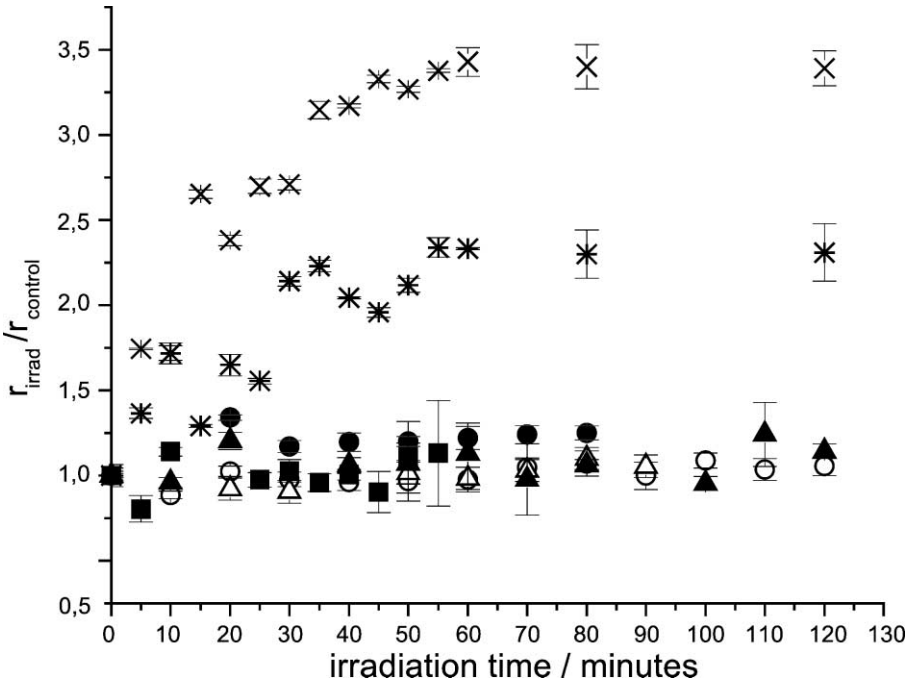

Fig. 1. Time dependence of fluorescence anisotropy for different microwaves irradiation power densities (○—1 mW/cm^2; △—3 mW/cm^2; ×—5 mW/cm^2; ●—6 mW/cm^2; ∗—7 mW/cm^2; ▲—8 mW/cm^2; ■—9 mW/cm^2)/fluorescence anisotropy control values. In one experiment, every value of the parameters I_{VV}, I_{VH}, I_{HV}, I_{HH} is the average of 150 different values. For every power density, five experiments were performed.

The monitored parameter is the fluorescence anisotropy r, defined by:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}.$$

I_{VV}—recorded intensity of vertically polarized fluorescence light when excitation light is vertically polarized.

I_{VH}—recorded intensity of horizontally polarized fluorescence light when excitation light is vertically polarized.

G—correction factor depending on the fluorometer used for measurements; it is due to the imperfection of the detection system and of monochromators.

$$G = \frac{I_{HV}}{I_{HH}}$$

I_{HV}—recorded intensity of vertically polarized fluorescence light when excitation light is horizontally polarized.

I_{HH}—recorded intensity of horizontally polarized fluorescence light when excitation light is horizontally polarized.

The excitation and emission wavelengths for TMA-DPH are:

$$\lambda_{\text{excitation}} = 356 \text{ nm and } \lambda_{\text{emission}} = 429 \text{ nm}.$$

2.2. Preparation of rod cell suspensions

Frogs (*Rana ridibunda*) were dark-adapted for 24 h; their retinas were dissected from the eyecup and pigment epithelium in Ringer's solution (NaCl—111 mM; KCl—2.5 mM; $CaCl_2$—1 mM; $MgCl_2$—1.6 mM; Hepes—3 mM; EDTA—0.01 mM, glucose—1 mM; buffered to pH = 7.7–7.8 with NaOH).

The rods were isolated by gentle brushing of 12 retinas in 1 ml of Ringer's solution. All operations were performed in dim red light.

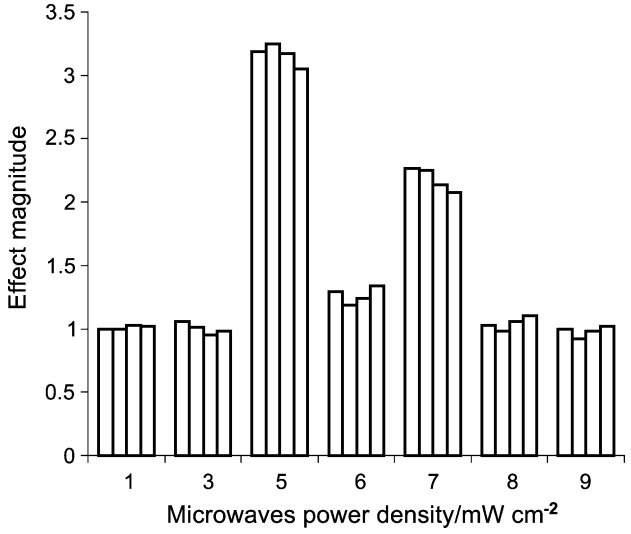

Fig. 2. The power density "window" observed in the range 5–7 mW/cm^2. The effect magnitude is represented by the ratio: average value of the *plateau* membrane fluidity/*control* membrane fluidity. The four bars at each power density correspond to different experiments.

2.3. Labeling the rods membranes with TMA-DPH

Five microliters of TMA-DPH stock solution (0.5 mM TMA-DPH in dimethylformamide-DMF) and 1 ml of rod suspensions were added to 1 ml Ringer's solution in a fluorometer cuvette. TMA-DPH was incubated with rod membranes for 2–3 min.

2.4. Rod irradiation with microwaves

The irradiation of photoreceptor suspensions was performed in the cuvette holder of the spectrofluorometer using a coaxial cable, built so that a uniform power density was obtained in the probe; the cellular suspension was continuously stirred by a magnetic stirrer.

The irradiation parameters were: microwave frequency, 2.45 GHz; irradiation times below 2 h; power densities below 10 mW/cm^2.

Fluorescence anisotropy was measured before, during and after microwave exposure of rod suspensions.

3. Results and discussion

Our results show that low level microwaves have small but significant effects on photoreceptor cell membranes.

A decrease in fluorescence anisotropy was observed in the rod suspensions irradiated at 15 μW/cm^2 (1 h irradiation); for irradiation at 5, 6, and 7 mW/cm^2 (1–2 h irradiation) fluorescence anisotropy of rod cells increases (Fig. 1).

The increase of fluorescence anisotropy is very sharp during the first hour of irradiation, proceeding thereafter to a plateau (5 and 7 mW/cm^2); for 6 mW/cm^2, the rate of increase is much smaller (Fig. 1). No microwave effects were observed at 1, 3, 8 and 9 mW/cm^2 (Fig. 1). The observed effects show a power density "window" in the power range 5–7 mW/cm^2 (Fig. 2).

These effects are not due to the photoreceptor cells ageing or to the internalization of TMA-DPH, as was shown by measurements on controls labeled with TMA-DPH.

The mechanisms of interaction between photoreceptor cell membranes and low-power microwaves can not be described at this moment. To understand this kind of phenomena it is necessary to perform more experiments using different microwave frequencies, irradiation times and power ranges in order to reveal other power densities and/or frequency "windows."

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