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(02)

Effects of time-variant extremely low-frequency (ELF) electromagnetic fields (EMF) on cholinesterase activity in *Dictyostelium discoideum* (Protista)

Andrea Amaroli[a], Francesca Trielli[a], Bruno Bianco[b], Stefano Giordano[b], Elsa Moggia[b], Maria U. Delmonte Corrado[a,*]

[a] *Department for the Study of the Territory and its Resources, Corso Europa 26, I-16132 Genoa, Italy*
[b] *Department of Biophysical and Electronic Engineering, Via all'Opera Pia 11a, University of Genoa, I-16145 Genoa, Italy*

Abstract

Recently, we detected propionylcholinesterase (PrChE) activity in single-cell amoebae of *Dictyostelium discoideum* using cytochemical, electrophoretic, and spectrophotometric methods. The involvement of this enzyme activity in cell–cell and cell–environment interactions was suggested. In this work, we found that exposure of single-cell amoebae to an extremely low-frequency electromagnetic fields (ELF-EMF) of 300 μT, 50 Hz, from 1 h up to 48 h at 21 ± 1 °C affected PrChE activity.

Keywords: Cholinesterase; Electromagnetic field; Protista; *Dictyostelium discoideum*

* Corresponding author. Tel.: +39 010 3538031; fax: +39 010 3538209.
E-mail address: corrado@dipteris.unige.it (M.U.D. Corrado).

There is increasing experimental evidence that several biological systems are affected by weak, extremely low-frequency (ELF) electromagnetic fields (EMF). However, as far as protists are concerned, at present there is little information in literature. Recently, we showed the presence of a propionylcholinesterase (PrChE) activity in single-cell amoebae of *Dictyostelium discoideum* [1]. This enzyme activity was suggested to be involved in cell–cell and cell–environment interactions, as its inhibition by xenobiotic compounds affects cell multiplication and aggregation [2]. In this work, we exposed single-cell amoebae to an ELF-EMF of 300 μT (50 Hz), from 1 up to 48 h, and checked its effects on PrChE activity.

The vegetative cycle of *D. discoideum* was axenically induced as previously described [2]. Single-cell amoebae, growing into a 50 ml-sterile Falcon flask containing 15 ml of Ax-2 axenic medium, were exposed to the ELF-EMF for 1 h up to 48 h (1-, 3, 4, 5, 24, and 48 h-exposure), at 21 ± 1 °C under shaking conditions. The 24 h-exposed samples were also observed after 24 h from their transfer to standard conditions (24 h-non-exposure). The control samples were kept into a non-working ELF-EMF generator (1, 3, 4, 5, 24, and 48 h-control). For the spectrophotometric evaluation of PrChE activity, the samples were centrifuged at 3000 rpm for 10 min and resuspended in 400 μl of 0.2 M phosphate buffer, pH 8.0. PrChE activity was recorded at λ = 412 nm by means of a Uvikon 930 spectrophotometer (Kontron Instruments), for the first 10 min after exposure to propionyl thiocholine iodide (PrTChI), used as a substrate, according to Ellman et al. modified method [3]. The total protein content (TP) was evaluated spectrophotometrically using the Biorad system (Biorad Microscience, Cambridge, MA, USA), according to the manufacturer's instructions. The PrChE activity was expressed as rate, that is the average rate of hydrolysis of nmoles of substrate per min per g of TP, at pH 8 and 25 °C [3]. All experiments were carried out in triplicate. Student's *t*-test was used to compare the mean values of the experimental samples and the controls (significance level $P < 0.001$).

The PrChE activity was inhibited significantly by 1 and 3 h-exposure to ELF-EMF, whereas it was similar to the control value after a 4 h-exposure. A significant increase in the enzyme activity was found after 5 and 24 h-exposures, while a decrease in PrChE activity appeared in 48-exposed samples (Fig. 1). The increased PrChE activity detected in 24 h-exposed cells returned to the control value, 24 h after transferring the amoebae to standard conditions (Fig. 2). The trend of PrChE activity values shown at increasing exposure times to the ELF-EMF could be a result of the adaptative process to these experimental conditions. In the attempt to

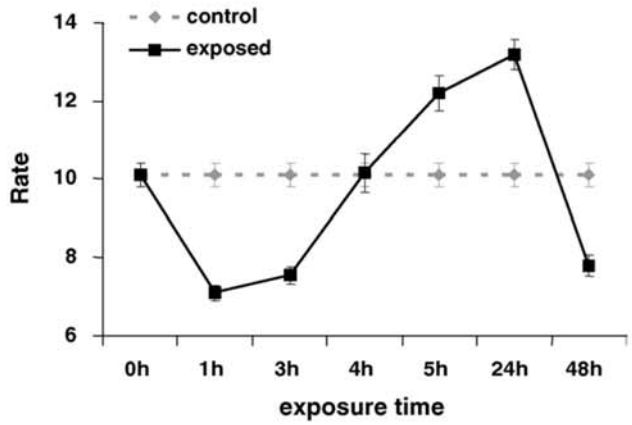

Fig. 1. Effect of exposure to ELF-EMF on PrChE activity.

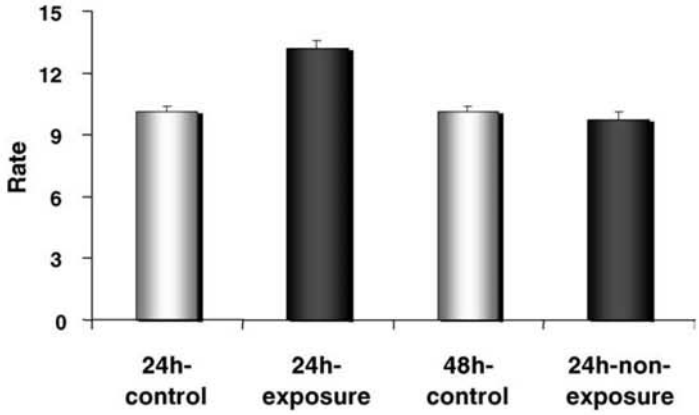

Fig. 2. Effect of a 24 h-exposure to ELF-EMF, followed by a 24 h-non-exposure, on PrChE activity.

restore homeostasis, the cells would be involved in an overcompensation mechanism, a phenomenon similar to hormesis described in higher organisms [4,5].

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