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Prolonged Ca^{2+} transients in ATP-stimulated endothelial cells exposed to 50 Hz electric fields

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Abstract

Human umbilical vein endothelial cells were exposed to sinusoidal electric fields of 0.3 or 30 kV/m, 50 Hz, for 24 h. Changes in intracellular calcium concentration ($[Ca^{2+}]_i$) induced by ATP-stimulation in the absence of extracellular Ca^{2+} were observed in individual cells. No differences were observed between the exposure and sham-exposure groups in $[Ca^{2+}]_i$ resting level before ATP-stimulation, or in the $[Ca^{2+}]_i$ peak levels induced by stimulation. However, the duration of the initial transients in $[Ca^{2+}]_i$ following an ATP stimulus was significantly prolonged by exposure to a 30 kV/m field. The inositol trisphosphate receptor inhibitor, xestospongin C, inhibited the ATP-induced elevation in $[Ca^{2+}]_i$ in both the exposure and sham-exposure groups. The ATP-receptor P2Y appeared to play an important role in the increase of $[Ca^{2+}]_i$. The present results suggest that an extremely low-frequency electric field affects the function of vascular endothelial cells by a mechanism involving activation of P2Y.

Keywords: Low frequency electric fields; Intracellular Ca^{2+}; Adenosine triphosphate (ATP); Endothelial cells; Fluo3

1. Introduction

Biological effects of electric fields in vitro have been reported by many investigators (ICNIRP, 1998; Misakian et al., 1993). However, few reports describe the effects of extremely low frequency (ELF) electric fields on vascular endothelial cells.

In a wide range of cell types, Ca^{2+} is an important messenger in signal transduction (Meyer, 1991; Rasmussen et al., 1984). Endothelial cells exhibit changes in $[Ca^{2+}]_i$ in response to vasoactive substances such as adenosine triphosphate (ATP) (Jacob, 1990; Yumoto et al., 1995). ATP receptors were considered in the 1990s to belong to two major families, the ligand-gated ion channel P2X and the G protein-coupled P2Y (Abbracchio and Burnstock, 1994). The amplitude of elevation by ATP-stimulation in pre-activated HL-60 cells was decreased after exposure to electric fields (Kim et al., 1998), suggesting that electric fields might affect the ATP receptors of the endothelium.

We have reported that the effect of an electric field on changes in $[Ca^{2+}]_i$ was agonist-specific (ATP) in confluent monolayers of human umbilical vein endothelial cells (HUVEC) (Takahashi et al., 2002). The area under the curve (AUC) of increased $[Ca^{2+}]_i$ after induction by 100 μM ATP in the absence of extracellular Ca^{2+} was significantly greater after exposure to a 30 kV/m field. However, it was not clear whether the electric field affected the $[Ca^{2+}]_i$ peak level or the duration of $[Ca^{2+}]_i$ elevation. If the peak level is increased, the field

Abbreviations: Ca^{2+}-free Tyrode's solution (FT); G protein-coupled ATP receptor (P2Y).

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may enhance the efficacy of ATP receptors in triggering the Ca^{2+} signal. However, if the duration is prolonged, the effect of the field may be associated with an increased period of intracellular signal transduction. In the present study, the effects of 50 Hz ELF electric fields on $[Ca^{2+}]_i$ peak levels and on the duration of Ca^{2+} transients were evaluated using Fluo3 fluorescence in individual HUVEC stimulated by ATP. To ascertain whether the influence of the electric fields on the Ca^{2+} signal was related to the release of Ca^{2+} from intracellular stores, the effects of an inositol phosphate receptor inhibitor were also studied.

2. Materials and methods

2.1. Electric fields

The apparatus for exposing cultured cells to electric fields, described elsewhere (Takahashi et al., 2002), is shown in Fig. 1. Briefly, an electric field was generated using an arrangement of parallel stainless-steel plates. Two units for exposure and sham-exposure were placed in the same incubator. The maximum applied voltage in this apparatus was 3 kV. The distance between electrodes was 10 cm. Voltages of 0.03 kV or 3 kV were applied sinusoidally (50 Hz), resulting in air fields of 0.3 or 30 kV/m, respectively. The current induced in the medium by a 30 kV/m air field was 0.28 μA, which corresponded to a current density of 0.42×10^{-3} A/m^2. The magnetic fields in the chambers were $\leq$0.2 μT. A second unit in which the plates were short-circuited was used for sham-exposure. Neither corona nor ozone was detected in the incubator.

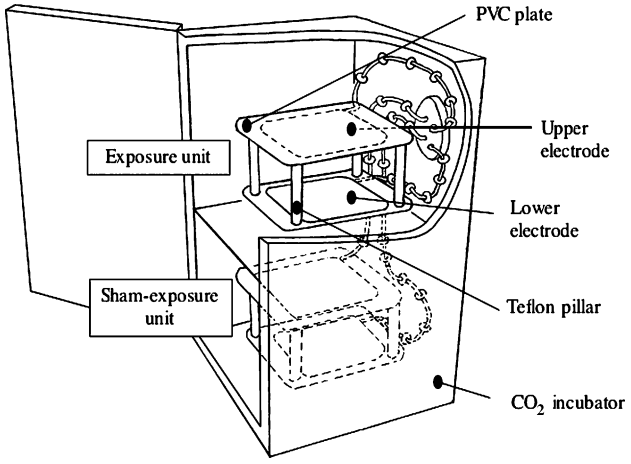

Fig. 1. Apparatus for exposing cultured cells to electric fields. Stainless-steel electrodes (1 mm thickness, 290 mm width, 370 mm depth) were insulated with polyvinyl chloride (PVC) and separated by Teflon insulation pillars. To produce the electric field, a voltage was applied to the upper electrode via a constant-voltage power supply; the lower electrode was grounded. The electrodes in the sham-exposure unit were short-circuited. The culture chamber was put on the midsection over 100 mm away from the edge of the lower electrode.

2.2. Cell culture

HUVEC were isolated from human umbilical cord according to method of Jaffe et al. (1973). The cells were seeded in type IV collagen-coated 4-well plates (Lab-Tek, chambered cover glass, Nunc, New York, USA) at 5×10^4 cells/well from the 7th to the 9th passage, and were incubated for 2 days at 37 °C in an atmosphere of 5% CO_2 and 95% air, using 700 μl/well KFSM (Gibco BRL, New York, USA) containing 10% newborn calf serum (Mitsubishi Chemical Corp., Tokyo, Japan) and 10 ng/ml basic fibroblast growth factor (Strathmann Biotec AG, Hamburg, Germany). The medium was changed and the cells were incubated for 24 h while being exposed continuously to electric fields or to sham conditions.

2.3. Fluorescence measurements

Fluo3-AM (Dojindo Labratories, Kumamoto, Japan) was used as fluorescent dye for $[Ca^{2+}]_i$. Fluorescence images of HUVEC loaded with Fluo3-AM were recorded using a laser scanning microscope (LSM 410, Carl Zeiss Jena GmbH, Jena, Germany). After electric field exposure, each chamber was washed with culture medium and then incubated in 500 μl of medium containing 5 mM Fluo3-AM for 1 h. The chambers were washed with Ca^{2+}-free Tyrode's solution (FT) (NaCl 139 mM, KCl 5.4 mM, $MgCl_2$ 1 mM, EGTA 1 mM, HEPES 5 mM, glucose 10 mM, buffered by NaOH to pH 7.4) and filled with 300 μl of FT. ATP-stimulation was performed as previously described (Takahashi et al., 2002) with the following modification: 300 μl of 200 μM ATP in FT was added into each well (100 μM, 600 μl/well). Fluorescence images were obtained every 1.5 s during ATP-stimulation using a laser scanning microscope at 488 nm excitation and 515 nm emission. Changes in fluorescence intensity in each cell were evaluated using an image processing program (Image J version 1.30, National Institute of Health, Maryland, USA).

2.4. Xestospongin C load

After loading with Fluo3-AM the chambers were washed with FT and filled with 300 μl of FT containing 10 μM xestospongin C (Wako Pure Chemical, Ohsaka, Japan) to inhibit the inositol 1,4,5-trisphosphate receptor, and then incubated for 10 min. ATP-stimulation was carried out as described above using ATP-solution containing 10 μM xestospongin C.