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EFFECTS OF LOW-FREQUENCY ELECTRIC FIELDS ON THE INTRACELLULAR Ca^{2+} RESPONSE INDUCED IN HUMAN VASCULAR ENDOTHELIAL CELLS BY VASOACTIVE SUBSTANCES

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ABSTRACT

Human vascular endothelial cells (HUVEC) were exposed to air electric fields of 30 kV/m, 50 Hz for 24 hr. After exposure, the change in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by the vasoactive substances ATP and histamine were determined using confocal laser scanning microscopy. A statistically significant increase in $[Ca^{2+}]_i$ was observed following stimulation with 100 μM ATP, compared with the results in sham-exposed cells (22%, $p<0.05$, $n=20$). No changes in $[Ca^{2+}]_i$ due to the field were observed after stimulation with histamine, showing that the effect of the field on the change in $[Ca^{2+}]_i$ was agonist-specific. The Ca^{2+} concentration in the medium was low, suggesting that the ATP-induced increase in $[Ca^{2+}]_i$ in cells exposed to electric fields was due to the release of Ca^{2+} from intracellular stores. The results suggested that the applied low-frequency electric field affected the function of HUVEC via a change in $[Ca^{2+}]_i$.

Key Words: Low-frequency electric fields; Intracellular Ca^{2+} response; Human vascular endothelial cells

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INTRODUCTION

Biological effects of electric fields in vitro have been reported by many investigators.[1–3] However, there are few reports describing the effects of low-frequency electric fields on human vascular endothelial cells (HUVEC). Blood is a relatively high-conductivity component of the body. It has been estimated that the range of conductivity in human tissues is 0.019–0.86 S/m, and that the conductivity of the blood is 0.6 S/m.[4] Human vascular endothelial cells contact blood directly, and consequently, their activity may be influenced by electric fields induced in blood by fields applied to the body. Air electric fields produced using capacitor plates can induce fields and currents in cell-culture medium that directly expose cells to field levels comparable to those that occur in vivo when the body is exposed to an electric field.

In a wide range of cell types, Ca^{2+} is a key messenger in cell signal transduction.[5–8] Endothelial cells exhibit changes in $[Ca^{2+}]_i$ in response to vasoactive substances such as ATP and histamine.[9,10] We hypothesized that electric fields could affect endothelial cells by altering the agonist-induced change in $[Ca^{2+}]_i$. This hypothesis was tested by measuring the $[Ca^{2+}]_i$ changes induced in HUVEC by ATP and histamine, using confocal laser scanning microscopy.

MATERIALS AND METHODS

Electric Field

An electric field was generated using an arrangement of parallel stainless steel plates (Figure 1); the corners of the plates were rounded to avoid corona. A voltage of 3000 V, 50 Hz was applied, resulting in an air field of 30 kV/m. A second unit in which the plates were short-circuited was placed in the same incubator and used for sham exposure. The electric field in the sham unit was <1 V/m. The magnetic field at the location of the chambers was $\leq 2 \times 10^{-7}$ T (TMM-1, Denryokutec, Tokyo). Neither corona (Corona Scope, Shikoku Research Institute, Kouti) nor ozone (Ozone 18L, Gastec, Kanagawa) were detected in the incubator.

For determination of the induced current density in the culture medium, it was grounded and the resulting current was measured.

Cell Culture

Human vascular endothelial cells were isolated from human umbilical cord according to method of Jaffe et al.[11] The cells were exposed or sham-exposed in four-well plates (Lab-Tek, chambered cover glass, Nunc, New York) seeded with 1.2–1.5×10^5 cells/well from the sixth to ninth passage, and the cells were incubated for 2 days at 37°C in an atmosphere of 5% CO_2 and 95% air, using KFSM (Gibco BRL, New York) containing 10% fetal calf serum (Mitsubishi Chemical, Tokyo) and 10 ng/ml basic fibroblast growth factor (700 μL/well). When the HUVEC formed confluent monolayers, the medium was changed and the cells were incubated for 24 hr while being exposed continuously to either 30 kV/m, 50 Hz, or to sham conditions.

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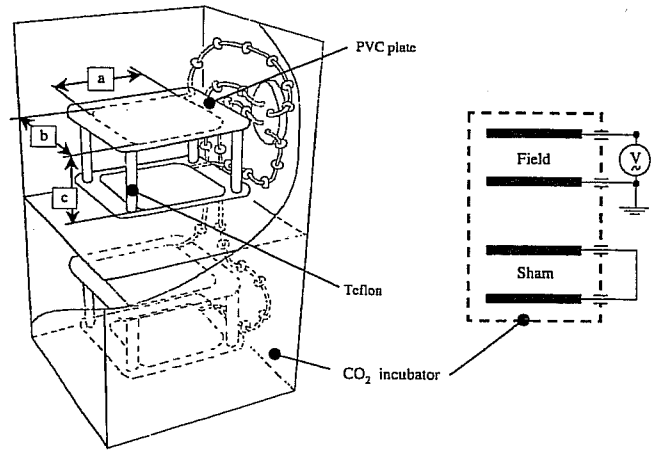

Figure 1. Apparatus for exposing cultured cells to electric fields. Two units for exposure and sham-exposure were placed in the same incubator. Stainless steel electrodes (1 mm thick) were insulated with polyvinyl chloride (PVC) and separated by Teflon insulation pillars. To produce the electric field, a voltage was applied to the upper electrode and the lower electrode was grounded. The electrodes in the sham-exposure unit were short-circuited. a = 370 mm, b = 290 mm, c = 100 mm.

Fluorescence Measurements

Real-time measurements of $[Ca^{2+}]_i$ were made using the method of Yumoto et. al.,[10] as modified. Briefly, after electric field exposure, each chamber was washed with Ca^{2+}-free Tyrode's solution (TS) (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 then incubated in 500 μL of medium containing 5 mM Fluo3-AM (Dojindo Labratories, Kumamoto) for 1.5 hr. The chambers were then washed with TS and filled with 400 mL of TS. Fluorescence intensity was evaluated using a confocal laser scanning microscope (LSM 410, Carl Zeiss Jena, Jena) at 488 nm excitation and 515 nm emission; the fluorescence from a 1.29×1.29 mm area was measured. After measurement of baseline fluorescence level (50 sec), 250 μL of 2.6 μM ATP in TS solution was added (1 μM, 650 μL/well). After the initial peak subsided (approximately 100–200 sec), 250 μL of 360 μM ATP solution was added (100 μM, 900 μL/well). The same method was used in experiments with histamine (Wako Pure Chemical, Osaka). The data were evaluated using the Mann–Whitney *U*-test at $p < 0.05$.

RESULTS

The average induced current in the medium was 0.28 μA, which corresponded to a current density of 0.42×10^{-3} A/m^2. Following exposure to the electric field, no abnormal cells were seen using phase microscopy (Figure 2).

Typical results for the fluorescence peak induced by ATP are shown in Figure 3; the magnitude of the change in $[Ca^{2+}]_i$ depended on agonist concentration.

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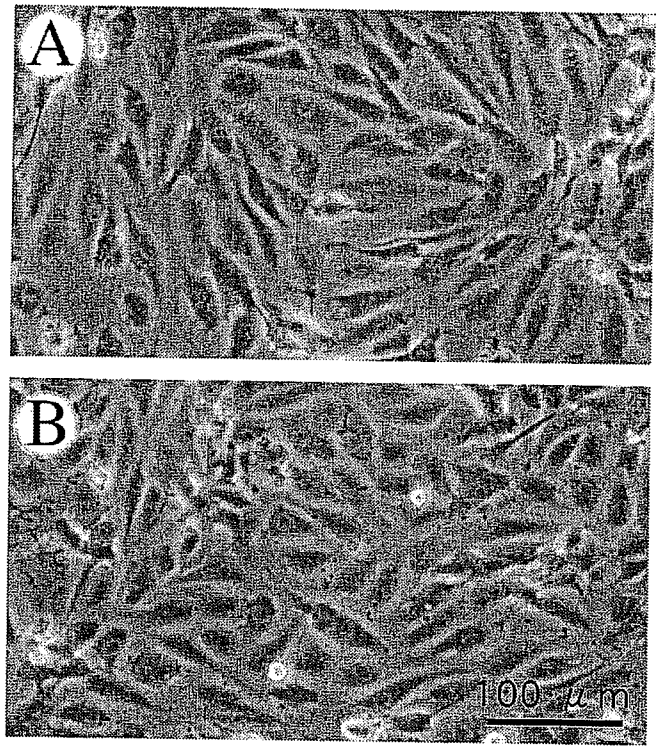

Figure 2. Phase contrast microphotograph of HUVEC. (**A**) Electric field. (**B**) Sham.

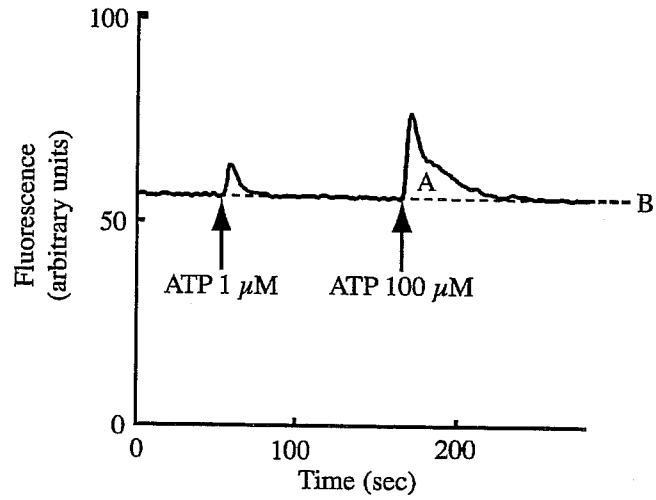

Figure 3. Typical change of Fluo3 fluorescent intensity after addition of ATP to HUVEC. The ratio of the area under the curve (**A**) to the baseline fluorescence (**B**) was used for the comparison between study groups.

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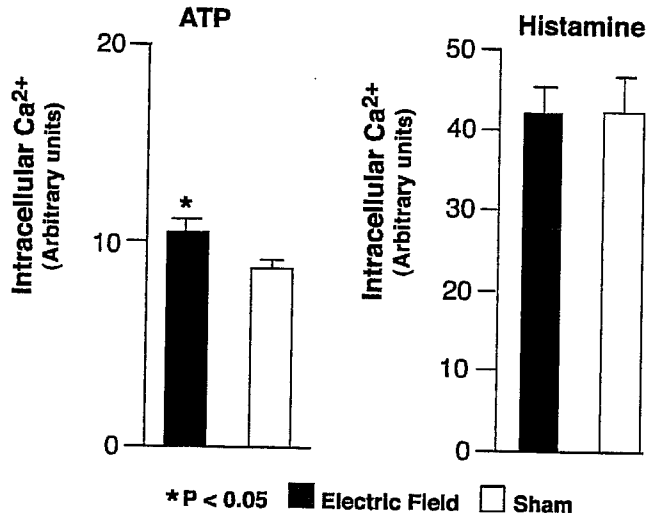

Figure 4. Effect of exposure for 24 hr of monolayer cultures of HUVEC to 30 kV/m, 50 Hz, on changes in $[Ca^{2+}]_i$ due to the addition of vasoactive substances (mean ± SE, $n = 20$ for each group).

Fluorescence was positively correlated with cell density. To avoid the influence of the distribution of cells in a monolayer, the area under the curve, A (which was directly proportional to the total change in $[Ca^{2+}]_i$) divided by the baseline fluorescence intensity, B, was used for comparison between the study groups.

Following stimulation with 1 μM ATP or histamine, no consistent fluorescence peak was found. However, fluorescence peaks due to 100 μM ATP or histamine were clearly identified in all measurements. For ATP, the average A/B for the exposed cells was significantly greater than that of the sham-exposed cells (Figure 4). For histamine, no differences were observed.

DISCUSSION

Stimulation of HUVEC with vasoactive substances was carried out after exposure of cell monolayers to 30 kV/m, 50 Hz. The induced current density in the cell medium was far below $10-100 \times 10^{-3}$ A/m^2, which is the range that produces irreversible changes.[12] A statistically significant increase of $[Ca^{2+}]_i$ was observed after addition of 100 μM ATP, compared with the response observed in sham-exposed cells. No difference was observed following histamine stimulation. The induced current density has been generally related to the biological effects of low-frequency electric fields. The electric field strength may, however, also contribute to these effects.

The cell membrane shields the cytoplasm from externally applied electric fields.[13] Changes of surface charge induced by applied electric and magnetic fields were observed in *Physarum polycephalum*.[14] The conformation changes of receptors may be associated with low-frequency fields due to modulation of the cell membrane charge.[15] Robertson and Astumian[16] analytically discussed electric field effects on a

conformational change of membrane enzymes in enzyme-catalyzed processes from a viewpoint of chemical kinetics and equilibrium, using the electroconformational coupling model of Tsong and Astumian[17] In the study of Kim et al.,[18] electric field exposure (0.5 V/m, 0.8 A/m^2) of ATP-activated HL-60 cells resulted in a decrease of $[Ca^{2+}]_i$ peak amplitudes, whereas exposure of histamine-stimulated cells caused the opposite effect. In the present study, exposure to the electric field potentiated the ATP-induced increase in $[Ca^{2+}]_i$ in HUVEC, but such a potentiation was not detected after the stimulation with histamine. If electric fields produce a conformational change of membrane proteins, it appears that the field has different effects on the tertiary structure of the receptors for ATP or histamine.

Ca^{2+}-free Tyrode's solution was used to study the effect of the agonist on $[Ca^{2+}]_i$. Therefore, the observed increase may be attributed to the release of Ca^{2+} from intracellular stores, and not to an influx from outside the cells. This agrees with the findings of Ohata et al.,[19] that ATP-induced $[Ca^{2+}]_i$ increase was caused by the release of Ca^{2+} from the intracellular store via activation of the P_{2y} purinoceptor, not by a P_{2x} purinoceptor cation-channel-mediated Ca^{2+} influx.

We speculate that the electric field affected the activity of P_{2y} purinoceptors, with the result that the ATP-induced change in $[Ca^{2+}]_i$ was potentiated. Since P_{2y} purinoceptors play an important role in endothelium-dependent relaxation of vascular smooth muscle,[20,21] a change of blood flow might represent a promising marker of effect of electric field in vivo. On the other hand, P_{2x} purinoceptors have been implicated in the pain pathway,[22] and have been associated with the reduced pain transduction in the spinal cord pain pathway.[23] If the electric field affects the activation of P_{2x} purinoceptors, for example, via conformational changes of membrane protein, pain-dependent processes may change simultaneously.

In summary, a statistically significant increase of ATP-induced $[Ca^{2+}]_i$ was observed in HUVEC after exposure to low-frequency air electric fields. The observed increase $[Ca^{2+}]_i$ was due to the release of Ca^{2+} from intracellular store. The results suggest that applied electric fields may affect the function of HUVEC via changes of $[Ca^{2+}]_i$.

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