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Electric Field-Induced Changes in Agonist-Stimulated Calcium Fluxes of Human HL-60 Leukemia Cells

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The mechanism of biological effects of extremely-low-frequency electric and magnetic fields may involve induced changes of Ca^{2+} transport through plasma membrane ion channels. In this study we investigated the effects of externally applied, low-intensity 60 Hz electric (E) fields (0.5 V/m, current density 0.8 A/m^2) on the agonist-induced Ca^{2+} fluxes of HL-60 leukemia cells. The suspensions of HL-60 cells received E-field or sham exposure for 60 min and were simultaneously stimulated either by 1 μM ATP or by 100 μM histamine or were not stimulated at all. After E-field or sham exposure, the responses of the intracellular calcium levels of the cells to different concentrations of ATP (0.2–100 μM) were assessed. Compared with control cells, exposure of ATP-activated cells to an E-field resulted in a 20–30% decrease in the magnitude of $[Ca^{2+}]_i$ elevation induced by a low concentration of ATP (<1 μM). In contrast, exposure of histamine-activated HL-60 cells resulted in a 20–40% increase of ATP-induced elevation of $[Ca^{2+}]_i$. E-field exposure had no effect on non-activated cells. Kinetic analysis of concentration- response plots also showed that compared with control cells, exposure to the E-field resulted in increases of the Michaelis constant, K_m, value in ATP-treated cells and of the maximal $[Ca^{2+}]_i$ peak rise in histamine-treated HL-60 cells. The observed effects were reversible, indicating the absence of permanent structural damages induced by acute 60 min exposure to electric fields. These results demonstrate that low-intensity electric fields can alter calcium distribution in cells, most probably due to the effect on receptor-operated Ca^{2+} and/or ion channels. Bioelectromagnetics 19:366–376, 1998.

Key words: 60 Hz electric field; receptor-operated ion channels; intracellular free calcium concentration; purinergic receptor; histamine receptor

INTRODUCTION

Significant evidence has been reported in recent years indicating that extremely-low-frequency electric and magnetic fields (ELF EMF) of low intensity can change a variety of cell functions [Adey, 1992; Luben, 1991]. Although the basic mechanisms of interaction between EMF and cellular systems still remain unclear, there is a strong possibility that extracellular and/or intracellular electric currents induced by applied EMF are responsible for the observed cellular changes. Externally applied sinusoidal alternating electric fields (ac E-fields) have been shown to alter proliferation of bone cell cultures [Fitsimmons et al., 1989], protein synthesis in fibroblasts [McLeod et al., 1987], DNA synthesis in cartilage cells [Rodan et al., 1978], microfilament structure in human hepatoma cells [Cho et al., 1996] and changes of cell shape [Lee et al., 1993].

It seems unlikely that the applied electric field has directly stimulated these changes within the cells because of the high impedance of the cellular plasma membrane and the relatively low intensity of applied E-fields compared with plasma membrane potentials. Therefore, the effect of the AC E-field on cell surface components, such as membrane-receptor complexes, ion-transporting channels, and proteins has been widely

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discussed. Data supporting this hypothesis include alterations of the membrane Na^+/K^+- and Ca^{2+}-ATPase activities in vitro [Blank and Soo, 1993; Tsong, 1992], induction of the redistribution of cell surface receptors in fibrosarcoma cells [Cho et al., 1994], and changes in Ca^{2+} fluxes across the plasma membrane of lymphocytes [Liburdy, 1992]. Despite the wide range of electric field strength (from 10^{-5} to $2.0-2.5 \times 10^3$ V/m) used in these studies, the thermal effect of the applied AC electric field has been ruled out, either because of the frequency dependency of the observed effects or negligible heating of the samples during exposure (<0.05 °C).

Considering the strong dependence of numerous cellular processes on Ca^{2+} concentration, the reported changes of Ca^{2+} fluxes resulting from ELF EMF exposure are of special importance [Adey, 1992; Liburdy, 1992]. Several studies have shown that ELF EMF exposure can alter Ca^{2+} transport, probably through mitogen-induced Ca^{2+} channels and only in concanavalin A-activated thymocytes, without having any such effect on non-activated cells [Walleczek and Liburdy, 1990; Liburdy, 1992]. Even though the cause of this is yet unknown, such findings can provide the basis for understanding how and at what site EMF primarily interacts with cell systems. In this study we investigated whether Ca^{2+} fluxes through other receptor-operated ion channels can be altered during exposure to ELF E-fields. We used widely employed HL-60 human leukemia cells as a model system for the analysis of electric field effects on signal transduction processes. HL-60 cells express a number of relatively well-described receptors, including several subtypes of purinergic and histamine receptors [Sawutz et al., 1984; Klinker et al., 1996]. As in many other types of blood cells, an activation of HL-60 cells through these receptors is accompanied with a $[Ca^{2+}]_i$ rise due to Ca^{2+} release from the intracellular pools and increased ion fluxes through the specific receptor-operated ion channels [Dubyak et al., 1988; Stutchfield and Cockroft, 1990; Scharff and Foder, 1993].

In this study we found that a 60 min exposure of undifferentiated HL-60 cells to a low-intensity 60 Hz electric field changed the subsequent ATP-induced Ca^{2+} rise in the cytoplasm of HL-60 cells. The effect was observed only in agonist-activated cells. We did not find significant differences between exposed and sham-exposed non-activated cells. The direction of the changes depended on the nature of the agonist used for cell activation during exposure. The observed Ca^{2+} changes were reversible, indicating the absence of permanent structural damages to the cell components during a 60 min exposure.

MATERIALS AND METHODS

Cell Culture

Indo-1 acetoxymethyl ester was purchased from Molecular Probe (Eugene, OR). All other chemicals, including RPMI 1640, fetal bovine serum (FBS), HEPES, histamine, and ATP were obtained from Sigma (St. Louis, MO).

HL-60 human promyelocytic leukemia cells from American Type Culture Collection (Rockville, MD) were grown in suspension culture in modified RPMI 1640 medium supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml streptomycin, in a humidified atmosphere with 5% CO_2 at 37 °C. Cells were subcultured every 4–5 days to maintain a culture density of $5-10 \times 10^5$ cells/ml.

Electric Field Exposure

Freshly isolated HL-60 cells were evenly divided into two groups for exposure and control samples and thereafter received identical treatment. Both groups of cells were simultaneously exposed or sham-exposed to a sinusoidal 60 Hz electric field in a rectangular, four-well Nunclon plastic multidish (Nunc, Naperville, IL) (Fig. 1). The size of each well (w × l × d) was $3.0 \times 1.9 \times 1.3$ cm. Current was applied through 1.5% (w/v) agarose salt bridges connected to platinum wire electrodes via 10 ml HEPES buffered salt solution (HBSS) reservoirs using a function generator (Wavetek, model 182, San Diego, CA or Hewlett-Packard, model 8904A, Santa Clara, CA) (Fig. 1).

The actual voltage across the wells with the cell suspension was measured using a multimeter (Fluke, model 8060A, Everett, WA). The root mean square electric field strengths (E_{max}) used for cell exposure were 0.1 and 0.5 V/m. The current density (J_{max}) in the cell suspension was determined to be 0.16 and 0.80 A/m^2, respectively, using the relationship $J_{max} = \sigma E_{max}$. The measured conductivity (σ) of the cell suspension was 1.6 S/m (YSI, model 33, Antioch, OH). The E- field exposures of HL-60 cells were conducted in a cell culture incubator at 37 ± 0.2 °C. The ambient 60 Hz AC magnetic field at the location of the culture dish was within the 0.1–2.0 μT range. The estimated electric field induced by such a background AC magnetic field in the exposure dishes was at least 10^4 less than the applied electric fields (0.1 and 0.5 V/m) used in the studies and, therefore, had negligible effect on the experimental outcome. The measured DC magnetic field was within the 6–25 μT range. The control (sham-exposed) cells were kept under the same conditions in parallel with the exposed cells, but without any attachments to the source of the E-field.

The agonists (1 μM ATP or 100 μM histamine)

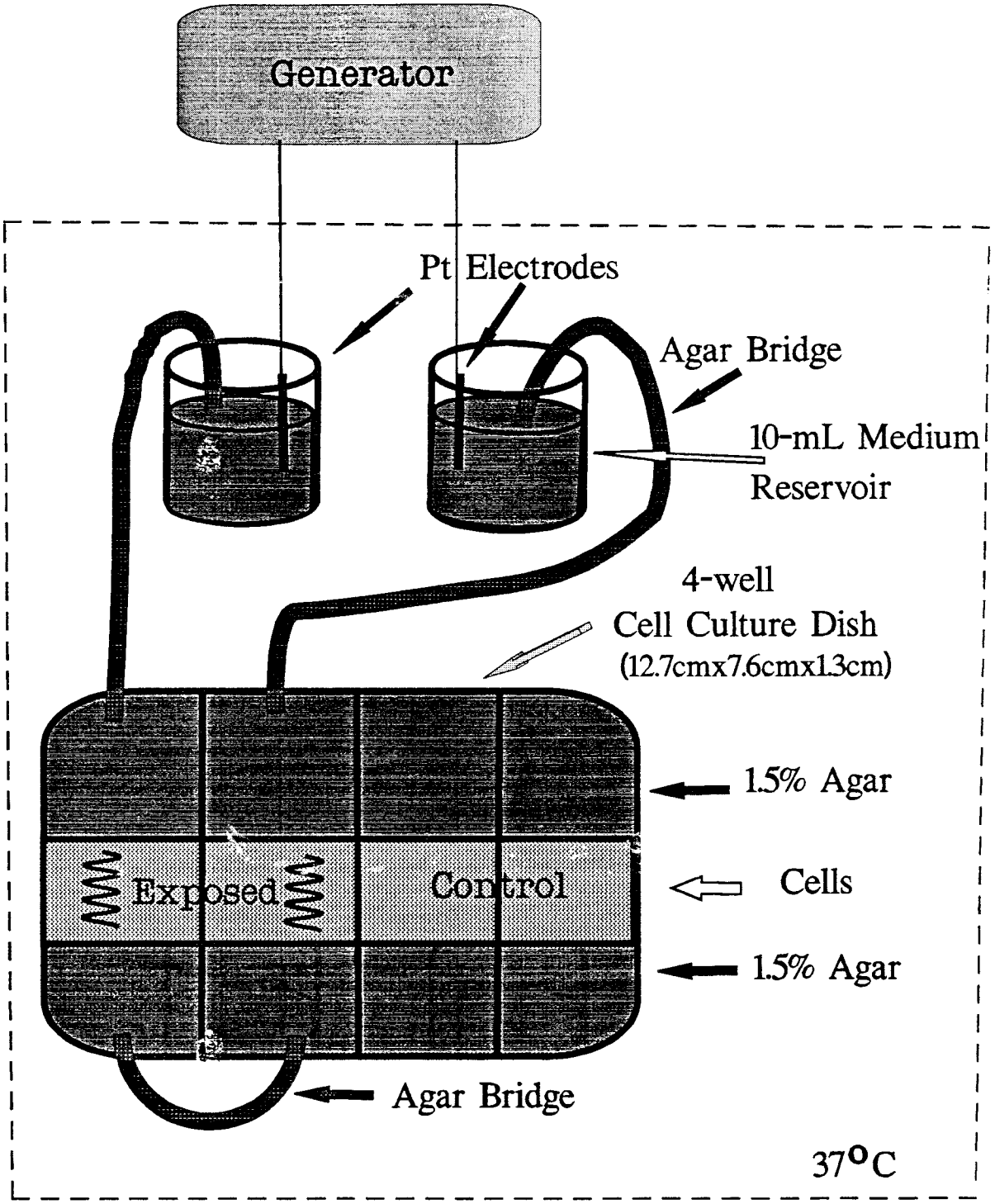

Fig. 1. Schematic representation of the electric field exposure system. All components (not drawn to scale) excluding a generator were placed inside the cell incubator at 37 °C.

were added to control and exposed cells 10 min after starting the E-field exposure, and the field exposure was continued for the next 50 min to give, as in case of non-activated cells, a total exposure time of 60 min. Three additional experiments were conducted to determine if the E-field caused permanent structural damage

to cell components. HL-60 cells were additionally incubated in the same medium for a short (2–5 min) period immediately following electric-field or sham exposure. After that the cells were subjected to the standard procedures for intracellular [Ca^{2+}] measurements.

Measurement of Cytosolic [Ca^{2+}]

Undifferentiated, log-phase HL-60 cells were collected by centrifugation and washed in HBSS containing 120 mM NaCl, 4.2 mM KCl, 1 mM Na_2HPO_4, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 15 mM D- glucose, and 15 mM Na-Hepes; pH 7.4. The cells were resuspended in 8 ml of HBSS (final cell density $2-4 \times 10^6$ /ml) containing 1 μM of cell-permeant Ca^{2+}-sensitive probe Indo-1/AM. The HL-60 cells were normally loaded with Indo-1 during the 60 min E-field exposure. For comparison, the loading procedure was conducted several times during the 30 min prior to the E-field exposure. Such pre-loading with the dye had no significant effect on the experimental outcome.

After Indo-1 loading and E-field exposure, the cells were rapidly washed twice with a cold (~10 °C) nominally calcium-free HBSS ([Ca^{2+}] < 2 μM) and immediately used for further testing. Subsequent Ca^{2+} measurements were started within 10 min and were finished within 60–120 min after E-field exposure; during this period the cells were kept at ~10 °C and at a cell density of $5-10 \times 10^6$/ml.

After E-field exposure fluorescence measurements of the exposed and control cells were made with Perkin-Elmer spectrofluorometers (Perkin-Elmer Cetus Instruments, models MPF-66 and LS-5B, Norwalk, CT) equipped with a standard, continuous magnetic stirrer and a thermostated cuvette holder. Excitation and emission wavelengths were 331 (5 nm slit) and 405 nm (10 nm slit), respectively, essentially as previously described [Dubyak et al, 1988; Grynkiewicz et al., 1985; Luckoff, 1986]. During fluorescence measurements different ATP (0.2–100 μM) and histamine (100 μM) concentrations were added and Ca^{2+} fluxes in Indo-1-loaded cells were measured in 2 ml calcium-free HBSS with a final cell density of $2-4 \times 10^5$/ml at 35 °C.

At the end of each measurement, calibration of Indo-1 fluorescence vs. [Ca^{2+}] was done by adding 25 μM digitonin and 1.5 mM $CaCl_2$ to obtain the fluorescence intensity of Ca^{2+}-saturated dye (F_{max}) and 10 mM Tris-EGTA at pH > 8.0 to obtain the fluorescence intensity of Ca^{2+}-free Indo-1 (F_{min}). Cytosolic free calcium concentration was calculated using the equation: $[Ca^{2+}]_i = K_d\ (F\text{-}F_{min})/(F_{max}\text{-}F)$, with the dissociation constant, K_d, for the Indo-1-Ca^{2+} complex taken as 250 nM [Grynkiewicz et al., 1985].

Data Analysis

Data from exposed cells were normalized against the corresponding data of sham-exposed cells as the percentage of the response to a given concentration of the stimulant (ATP). Pooled data from 5–11 independent experiments were subjected to a single-factor analysis of variance (ANOVA). Values are given as means ± SEM. Differences were considered as statistically significant if the P value was <0.05.

For kinetic analysis, the concentration-response curves of four to six separate cell preparations were fitted using the computer software Data Analysis and Graphics Program (Erithacus Software, UK). The maximal peak $[Ca^{2+}]_i$ rise, $[Ca^{2+}]_{max}$, Hill coefficient, n_{Hill}, and Michaelis constant, K_m, were derived from the curves and then were statistically analyzed.

RESULTS

Ca^{2+} Responses of HL-60 Cells to ATP and Histamine

Addition of micromolar concentrations of the extracellular ATP to undifferentiated HL-60 cells caused transient elevation of cytosolic $[Ca^{2+}]_i$, mostly due to Ca^{2+} release from intracellular stores (Fig. 2a). The amplitude of the response increased with increasing ATP concentration and reached a maximum at ~100 μM ATP. In the low-calcium medium (<2 μM) the ATP-induced $[Ca^{2+}]_i$ rise rapidly returned to the basal level. In the medium containing 1 mM Ca^{2+} a more sustained level of $[Ca^{2+}]_i$ was observed due to the influx of the extracellular Ca^{2+} through agonist-induced Ca^{2+} channels. Characteristically, the $[Ca^{2+}]_i$ transient induced by low ATP (<5 μM) had at least three phases, which included a very fast release and removal of Ca^{2+} at the beginning, followed by a much slower phase of gradual decrease of $[Ca^{2+}]_i$ to the pretreatment levels. For HL-60 cells treated with a higher ATP concentration (>5 μM) only a fast release and gradual Ca^{2+} removal from the cytoplasm was observed (see, for example, Fig. 3).

In contrast to ATP receptors, histamine-induced elevation of $[Ca^{2+}]_i$ is mostly dependent on the influx of extracellular Ca^{2+} through receptor-stimulated ionic pores (Fig. 2b). In the absence of extracellular Ca^{2+}, the histamine-induced rise of $[Ca^{2+}]_i$ was practically undetectable. In addition, the shape of histamine-induced Ca^{2+} rise resembled the shape of a higher ATP-induced Ca^{2+} rise, but with significantly lower peak amplitudes (Fig. 2). The other important difference between these two receptor complexes was irreversible desensitization of the histamine receptors after stimulation by the agonist (data not shown), while sensitivity

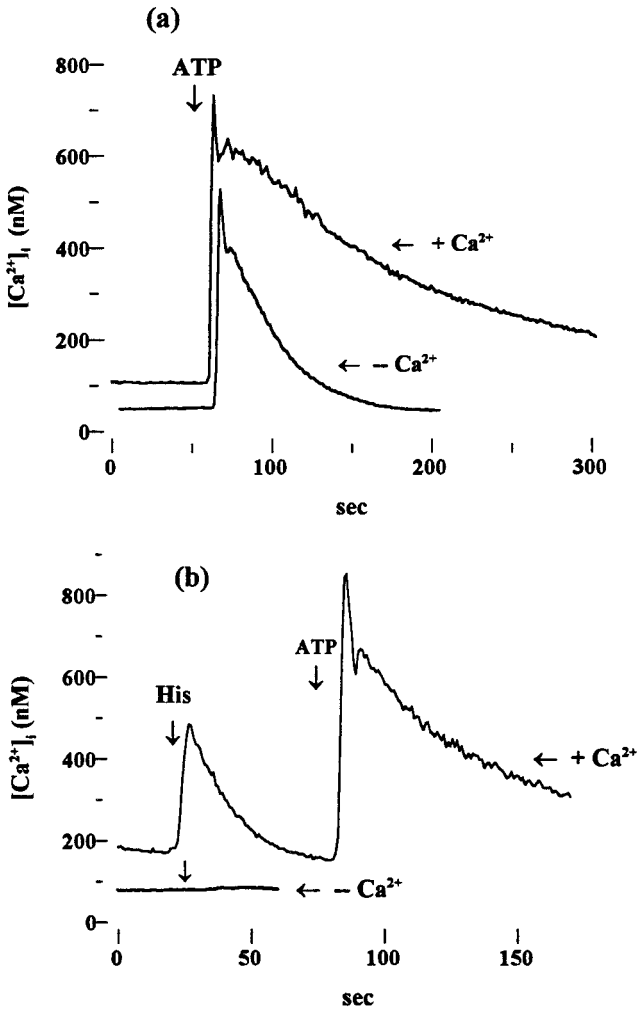

Fig. 2. Typical changes of $[Ca^{2+}]_i$ in undifferentiated HL-60 cells on addition of ATP and histamine. Arrows indicate treatment of the cells with (**a**) 1 μM ATP or (**b**) 100 μM histamine and 1 μM ATP either in Hepes-buffered salt solution containing 1 mM $CaCl_2$ ($+Ca^{2+}$) or in nominally Ca^{2+}-free buffer ($-Ca^{2+}$).

of the ATP receptor can be fully restored by washing out the previous addition of ATP.

The difference in Ca^{2+} responses of HL-60 cells to the addition of ATP and histamine, and the different Ca^{2+} systems involved during stimulation by these agonists, provided the opportunity to conduct a comparative analysis of how the application of a low-level, 60 Hz E-field would affect Ca^{2+} distribution in the agonist-activated cells.

Effects of 60 Hz Electric Fields on $[Ca^{2+}]_i$ Changes in HL-60 Cells

The $[Ca^{2+}]_i$ changes of the cells at various ATP concentrations were determined as the difference between the maximal ATP-induced $[Ca^{2+}]_i$ peak and basal $[Ca^{2+}]_i$. Figure 3 shows typical responses of exposed (dashed lines) and sham-exposed (solid lines) cells to low and high ATP concentrations. The summarized data of the E-field effect on $[Ca^{2+}]_i$ are presented in Table 1. The exposure of non-activated cells to E-fields did not result in statistically significant changes of ATP-induced maximal Ca^{2+} release at any ATP concentration, compared with control cells (Fig. 3a, Table 1). In contrast, the exposure of ATP-activated HL-60 cells resulted in a decrease of the maximal $[Ca^{2+}]_i$ peak amplitude in response to a low [ATP] (i.e., less than <1 μM; Fig. 3b, Table 1). In exposed cells the sharp $[Ca^{2+}]_i$ peak that usually was observed immediately after addition of a low [ATP] (Figs. 2, 3a) was much less evident. At higher [ATP] the E-field effect did not occur. For instance, at 0.5 μM ATP the mean peak $[Ca^{2+}]_i$ amplitude of exposed cells comprised 76 ± 7% (mean ± SEM, $P = 0.003$, n = 11) of control cells while at 100 μM the peak $[Ca^{2+}]_i$ amplitudes were almost identical. As with non-activated cells, an insignificant increase of basal $[Ca^{2+}]_i$ in exposed cells was observed (Table 1).

In contrast, the exposure of histamine-activated cells resulted in a 30% mean increase of peak $[Ca^{2+}]_i$ amplitude in exposed cells in comparison with control cells (Fig. 3c, Table 1). In addition, a small decrease of basal $[Ca^{2+}]_i$ occurred in exposed cells.

Finally, three independent experiments were conducted using agonist-activated or non-activated HL-60 cells exposed to a 0.1 V/m 60 Hz electric field. For these conditions, there was no detectable effect on peak $[Ca^{2+}]_i$ (data not shown).

Exposure Did Not Cause Irreversible Changes

In a series of three independent experiments the exposed and sham-exposed HL-60 cells were additionally incubated in the medium for a short (2–5 min) period immediately following electric field or sham exposure. After this preincubation of the cells in the medium containing 1 mM Ca^{2+} without an applied E-field, there were no significant differences between exposed and control cells either activated or non-activated by agonists (data not shown). These experiments indicated that the applied electric field interferes with Ca^{2+} transport systems only directly during the 60 min exposure of the agonist-activated cells. When electric field exposure stopped, HL-60 cells were able to recover and quickly restore the original status of intracellular Ca^{2+} pools.

Kinetic Analysis

To find out in more detail which parameter of ATP-induced Ca^{2+} release was most affected during the 60 min exposure to a 60 Hz electric field, we con-

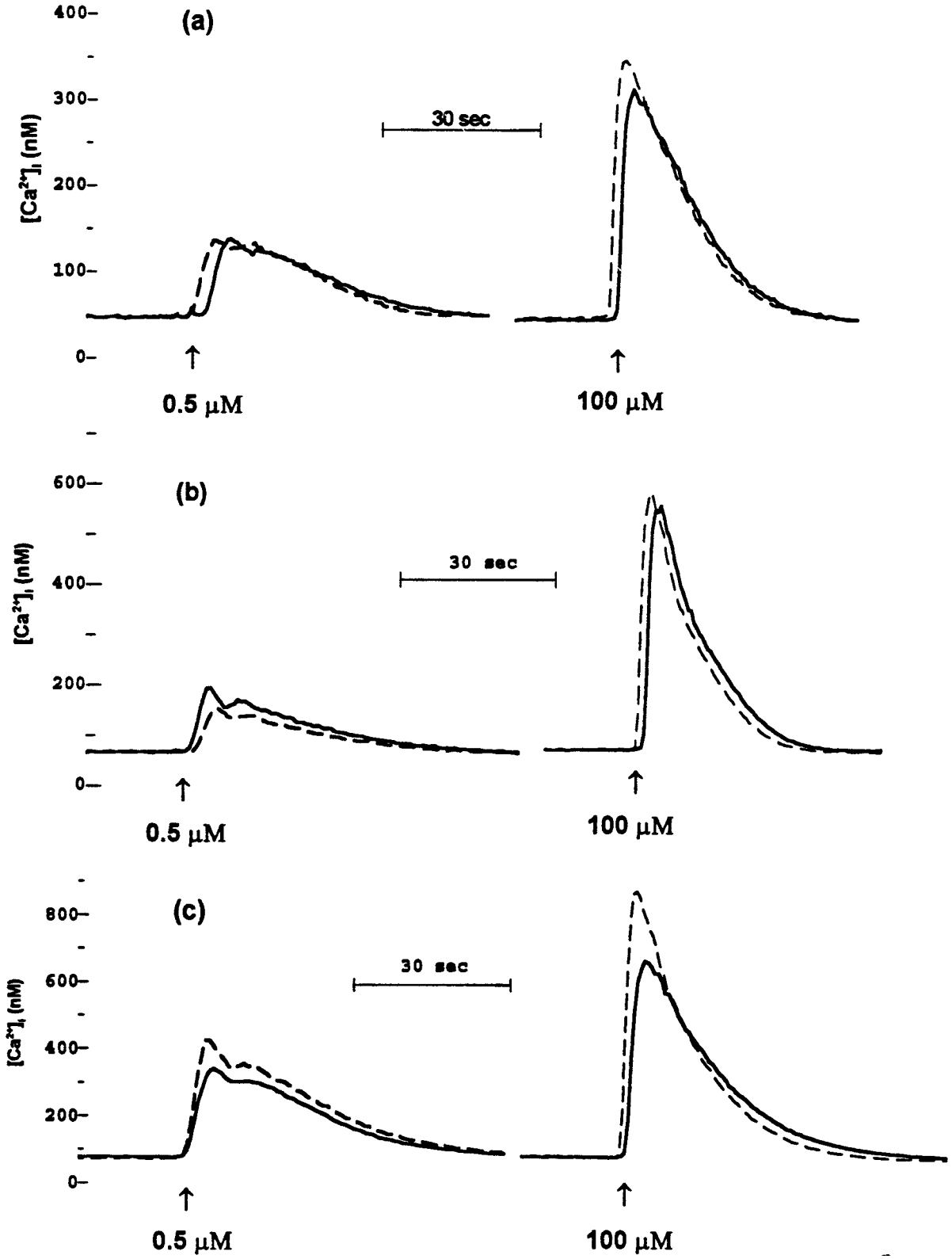

Fig. 3. Effect of 0.5 V/m, 60 Hz sinusoidal electric field exposure on ATP-induced $[Ca^{2+}]_i$ increases in HL-60 cells. Cells were exposed or sham-exposed to the E-field for 60 min in the absence of agonists (**a**), in the presence of 1 μM ATP (**b**) or 100 μM histamine (**c**). Representative curves of Ca^{2+} responses of exposed (dashed lines) or sham-exposed cells (solid lines) on addition of 0.5 or 100 μM ATP are presented.

TABLE 1. Effect of 1-h Exposure to 0.5 V/m, 60 Hz Electric Fields on $[Ca^{2+}]_i$ Increase in HL-60 Cells Treated During Exposure to ATP or Histamine[†]

	Increase in $[Ca^{2+}]_i$ (% of control)		
	No agonists	ATP (1 μM)	Histamine (100 μM)
Basal $[Ca^{2+}]_i$	111 ± 6	105 ± 4	90 ± 4*
ATP (μM)			
0.2	132 ± 15	67 ± 9***	138 ± 22
0.5	94 ± 9	76 ± 7***	139 ± 28
1.0	100 ± 4	80 ± 4***	124 ± 11*
10.0	112 ± 9	93 ± 7	110 ± 7
100.0	98 ± 9	98 ± 4	130 ± 10**

[†]HL-60 cells were exposed to E-fields or sham-exposed in the presence of the indicated agonists, and ATP-induced $[Ca^{2+}]_i$ increase was immediately measured after exposure. Values represent percentage of control (sham-exposed cells) and are means ± SEM of 9–11 cell preparations for ATP and histamine and 5–7 cell preparations in the absence of agonists.
*$P < .05$.
**$P < .01$.
***$P < .005$ compared with sham-exposed cells.

ducted a comparative kinetic analysis of agonist- induced Ca^{2+} fluxes of exposed and sham-exposed HL-60 cells. For our conditions (low Ca^{2+}-HBBS), the unstimulated HL-60 cells had Hill plots with a maximal peak $[Ca^{2+}]_i$ rise of ~210 μM, a Michaelis constant, K_m, ~0.4 μM, and a Hill coefficient, n_{Hill}, ~2 (Fig. 4A, Table 2). Figure 4 and Table 2 also give the results for 60 Hz, 0.5 V/m E-field exposure and sham-exposure for six independent experiments with agonists (1 μM ATP and 100 μM histamine) and four independent experiments without agonists.

A subsequent statistical analysis (ANOVA) revealed two differences in kinetic parameters between exposed and sham-exposed cells after a 60 min exposure to E-fields. First, E-field exposure in the presence of 1 μM ATP resulted in a statistically significant increase of K_m values from 0.5 ± 0.3 μM in sham-exposed cells to 1 ± 0.6 μM (mean ± SEM, $P < 0.05$, n = 6). These data support our previous observation (Table 1, Fig. 2) that E-field exposure in the presence of 1 μM ATP resulted in a decreased Ca^{2+} content in the intracellular Ca^{2+} pool(s) that are sensitive to low ATP concentration. The maximal peak $[Ca^{2+}]_i$ rise in ATP-treated HL-60 cells remained unchanged, 400 ± 44.5 μM and 401 ± 32.8 μM (mean ± SEM, n = 6) in exposed and sham-exposed cells, correspondingly. There also was no statistically significant change in the Hill coefficient, n_{Hill}, in these experiments, 1.6 ± 1.0 and 2.4 ± 1.0 (mean ± SEM, $P > 0.05$, n = 6).

Secondly, a significant increase in the ATP-induced maximal peak $[Ca^{2+}]_i$ rise was observed between the cells exposed and sham-exposed to the E-field in the presence of 100 μM histamine, 411 ± 57.0 μM and 320 ± 19.2 μM (mean ± SEM, $P < 0.05$, n = 6), respectively. Changes in the Hill coefficient and Michaelis constant were not statistically significant in these series of experiments (Table 2).

Finally, in accordance with the data reported earlier, kinetic analysis of Hill plots did not reveal any significant changes in measured parameters between

TABLE 2. Effect of 1-h Exposure to 0.5 V/m, 60 Hz Electric Fields on Kinetic Parameters of Postexposure ATP-Induced $[Ca^{2+}]_i$ Increases in HL-60 Cells Activated or Non-activated During Exposure to ATP or Histamine[†]

	No agonists		+1 μM ATP		+100 μM histamine	
	Exp.	Sham	Exp.	Sham	Exp.	Sham
$[Ca^{2+}]_{max}$ (μM)	223 ± 30.8	211 ± 37.7	400 ± 44.5	401 ± 32.8	411 ± 57.0*	320 ± 19.2
K_m (μM)	0.5 ± 0.4	0.4 ± 0.5	1 ± 0.6*	0.5 ± 0.3	0.9 ± 0.4	0.6 ± 0.2
n_{Hill}	1.5 ± 1.0	1.7 ± 1.3	1.6 ± 1.0	2.4 ± 1.0	0.7 ± 0.4	1 ± 0.3

[†]Data are means ± SEM of four (no agonists) and six (+1 μM ATP and +100 μM histamine) independent cell preparations.
*$P < .05$.

Fig. 4. Concentration-response relationships characterizing ATP-induced changes in cytosolic $[Ca^{2+}]_i$ in HL-60 cells exposed to a 0.5 V/m, 60 Hz electric field. Cells were exposed or sham-exposed to E-field for 60 min in the absence of agonists (**A**), in the presence of 1 μM ATP (**B**) or 100 μM histamine (**C**). Peak changes of $[Ca^{2+}]_i$ were measured and plotted versus [ATP]. Each point represents the mean ± SEM of four (A) or six (B and C) separate experiments. Data of exposed (solid circles, dashed lines) and sham-exposed (open squares, solid lines) were fitted according to the Hill kinetic equation using commercially obtained software. From the resulting Hill plots, kinetic parameters such as maximal peak $[Ca^{2+}]_i$ increase, $[Ca^{2+}]_{max}$, the Michaelis constant, K_m, and the Hill coefficient, n_{Hill}, were derived for further comparative data analysis (see Table 2).

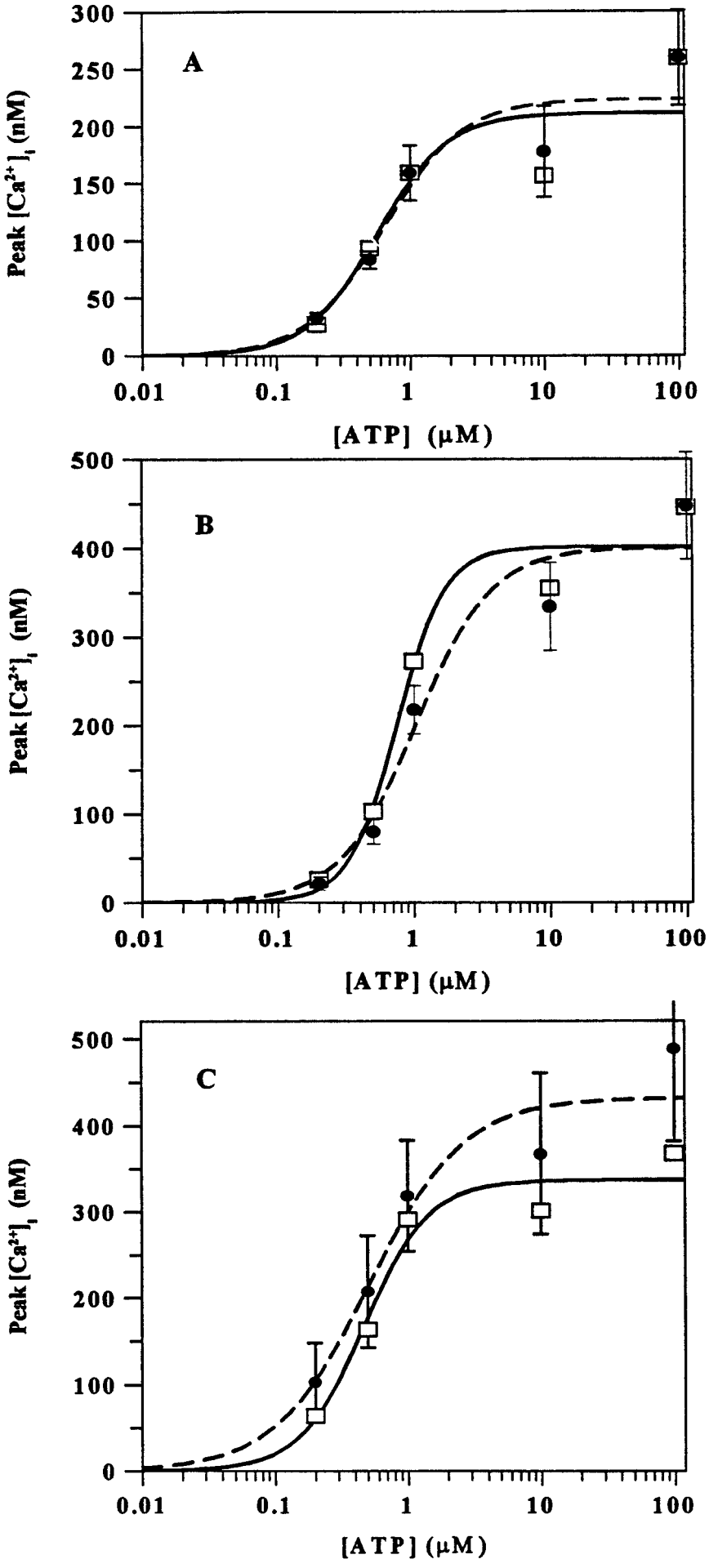
A
B
C
Peak [Ca2+]i (nM)
[ATP] (μM)
0
50
100
150
200
250
300
400
500
0.01
0.1
1
10
100

Fig. 4.

exposed and sham-exposed cells that were not treated with agonists (Table 2).

DISCUSSION

The results of this study indicate that exposure of human promyelocytic HL-60 leukemia cells to weak sinusoidal 60 Hz electric fields alters agonist-induced cytosolic [Ca^{2+}] changes. Sensitivity to E-fields depended on the activation status of the cells; non-activated cells were unaffected by E-fields. Electric field exposure of ATP-activated HL-60 cells resulted in a subsequent decrease of $[Ca^{2+}]_i$ peak amplitudes when low ATP concentrations were added, whereas exposure of histamine-stimulated cells caused the opposite effect. Because of the low intensity of the applied electric field (0.5 V/m) and the high impedance of the cellular membrane, it is highly unlikely that these effects were the result of E-field-induced heating of the cell suspension or of the direct influence of the applied E-field on the intracellular Ca^{2+} pools.

Therefore, the data collected suggest that the temporary application of a 60 Hz, sinusoidal E-field causes some dynamic changes in cell surface membrane components and/or within the vicinity of the cellular membrane resulting in reduced or enhanced Ca^{2+} influx, respectively, through ATP- or histamine-induced ion channels. The use of agarose salt bridges excluded direct contacts between the platinum electrodes and cell medium, therefore preventing possible toxic effects on cells from electrolysis products formed during electrochemical reactions on the electrode surface. In addition, because of the identical treatment of the exposed and control (sham-exposed) cells (including cell incubation and isolation, and calcium measurements), the possible interfering effects of surrounding magnetic fields, for instance from the magnetic stirrer of the spectrofluorometer or heating system of the cell incubator, have been totally ruled out.

The opposite direction of Ca^{2+} changes induced by ATP and histamine may be attributed to different properties of these channels. While the exact molecular structure of these particular receptors has not yet been reported, it is known that ATP induces opening of specific Ca^{2+} channels [Dubyak et al., 1988; Stutchfield and Cockroft, 1990], while histamine stimulates ion fluxes through nonspecific ion channel pores [Klinker et al., 1996]. Another characteristic difference between these receptor complexes is that histamine receptors become desensitized after the first application of histamine [Sawutz et al., 1984], while the sensitivity of ATP receptors to subsequent addition of ATP can readily be restored after washing [El-Moatassim et al., 1992]. These data reflect some differences in the chain of events occurring with the ATP and histamine receptors after their activation by the agonists. Even though the exact pathways of the signal transduction through both receptors have not yet been established, there are indications of an involvement of the different subtypes of G-proteins in the activation process of these receptors [Klinker et al., 1996].

As in another study [Lyle et al., 1991], the effects of short-term E-field exposure on Ca^{2+} systems of HL-60 cells were transient and not detectable after a 2–5 min incubation of the cells in the absence of the applied electric field. This suggests that the electric field-induced changes in $[Ca^{2+}]_i$ responses are not the result of permanent changes in the molecular structure of the proteins involved in Ca^{2+} transport, but rather are the result of electric field effects on some kinetic parameters of the reactions directly involved in the signal transduction pathway(s). Therefore, it would be interesting to investigate whether the same type of E-field effects would be observed in the presence of various modificators of the purinergic and histamine receptors, such as ATPγS and GTP or dimarpit and cimetidine and some others, respectively. Even though the observed ELF electric field effects have been temporary, it is possible that chronic exposure to low-intensity E-fields could have longer lasting effects on cell physiology, for instance, through changes of Ca^{2+} distribution within the cells and therefore could affect the activity of numerous calcium-dependent cellular processes.

Our data are consistent with the report showing that 60 Hz electric field exposure can alter Ca^{2+} influx pathways of rat thymocytes [Liburdy, 1992]. In this report and one by Walleczek and Liburdy [1990], the authors showed that the effects of EMF were dependent on the presence of mitogen concanavalin A. Without the mitogen no significant effects of EMF exposure on cellular Ca^{2+} transport systems were observed. In addition, the direction of the EMF-induced changes of Ca^{2+} fluxes (enhancement or reduction) across the plasma membrane depended on the mitogen responsiveness of the thymocytes. The strength of the applied and induced E-fields in these studies was comparable with our study, 0.17 V/m and 0.50 V/m, respectively. These authors suggested that ELF EMF exposure can affect mitogen-induced calcium channels of thymocytes.

Interestingly, the same type of response has been observed during the studies of magnetic field effects on mitogen-induced calcium fluxes [Walleczek and Liburdy, 1990, Walleczek and Budinger, 1992, Liburdy et al., 1993]. These findings imply that the observed effects were due to electric fields induced by the applied AC magnetic fields [Liburdy, 1992]. However, a

direct effect of magnetic fields on cellular processes has also been suggested [Walleczek and Budinger, 1992]. In addition, it should be noted that the effect of the applied magnetic field on unstimulated leukemic Jurkat cells was also reported [Lindström et al., 1993].

The results of our study with purinergic and histamine receptors of HL-60 cells and the above findings with mitogen receptors of lymphocytes make the receptor-operated ion channels a likely primary target of ELF EMF cell effects. All these three receptors are related to the G-protein-coupled receptor family [Klinker et al., 1996]. Characteristic features of these receptors are that during agonist stimulation the signal transduction processes involve a mediation of the regulatory guanine- nucleotide binding proteins (G-proteins) and the opening of specific ion channels. Therefore, it is of interest to find out whether stimulation of other members of the G-protein-coupled receptors will be affected by the applied ELF electric fields and to compare the results with the responses of other types of receptors such as ligand-gated ones. The possible involvement of some temporary, conformational modifications of G-protein-linked receptors under the influence of 60 Hz EMFs has been extensively discussed by Luben [1991,1994]. In a series of experiments with bone and pineal cells, for instance, they showed that the application of 0.1 mT 60 Hz magnetic fields resulted in inhibition of cAMP production in cells activated by the agonists of G-protein-coupled receptors, such as parathyroid hormone and isoproterenol. In addition, it is important to note that as in our study these authors did not observe EMF effects on cells in the absence of the agonists or at high agonist concentrations. It seems quite possible that the same general mechanism of action was responsible for the observed effects of electric and magnetic fields.

The E-field-induced changes of the agonist-activated Ca^{2+} fluxes can be a cause of the observed changes in cell functions, such as DNA and protein synthesis and cell proliferation [Fitsimmons et al., 1989; Rodan et al., 1978; Schimmelpfeng and Dertinger, 1993]. The electric field intensities used in these studies varied significantly, from 10^{-5} V/m in case of increased proliferation of bone cells to 7 V/m in case of decreased DNA content and increased cyclic AMP content in cultured mouse fibroblasts. However, the E-field intensities reported in these studies were in the microvolt to millivolt per cm ranges and are still well below the plasma membrane potential of non-excitable, resting cells (10^6–10^7 V/m).

The molecular mechanism by which such weak electric fields can affect cellular systems in the presence of a large transmembrane potential with the background of thermal and electric noises still remains unclear [Astumian et al., 1995; Markin et al., 1992]. However, recent reports implying stochastic resonance or noise-induced enhancement as a mechanism of signal amplification across ion channels provide very promising clues for the problem of EMF interaction with cellular systems [Bezrukov and Vodyanoy, 1995; Bezrukov and Vodyanoy, 1997; Kruglikov and Dertinger, 1994]. Using mathematical and experimental model systems, Bezrukov and Vodyanoy [1995,1997] have clearly shown that the small input signal of a low-frequency, sinusoidal electric field can be amplified by voltage-dependent ion channels hundreds to thousands of times by utilizing ambient noise. Such amplification of the signal output was also accompanied by a significant improvement of the signal-to-noise ratio.

The receptor-operated ion channels may also exert the same type of signal amplification. Furthermore, the change of surface charge induced by the applied electric field that was observed in various cells [Marron et al., 1986] can also contribute to signal amplification by such channels because the significant part of such receptor complexes is located on the cell surface. Considering the fact that in many cases agonist stimulation induces a parallel opening of 10^2–10^4 receptor-operated channels per cell, the potential for additional amplification of the signal as a result of summation increases even more. In such a context, it is possible to understand why exposure of non- activated cells does not reveal significant differences with the control cells. In this case no differences are observed because the number of open channels-amplifiers is not enough for reliable detection of the small changes by routine methods.

CONCLUSIONS

The present results show that 60 min exposure of HL-60 cells to 60 Hz, sinusoidal electric fields changes calcium distribution in agonist-activated cells. We did not find significant differences between exposed and sham-exposed cells in the absence of the added agonists. These results are comparable with results reported by Walleczek and Liburdy [1990] for the mitogen-activated rat thymocytes. These data suggest that receptor-operated ion channels could be a primary target of ELF electric field effects.

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