

Transmembrane calcium influx induced by ac electric fields

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ABSTRACT Exogenous electric fields induce cellular responses including redistribution of integral membrane proteins, reorganization of microfilament structures, and changes in intracellular calcium ion concentration ($[Ca^{2+}]_i$). Although increases in $[Ca^{2+}]_i$ caused by application of direct current electric fields have been documented, quantitative measurements of the effects of alternating current (ac) electric fields on $[Ca^{2+}]_i$ are lacking and the Ca^{2+} pathways that mediate such effects remain to be identified. Using epifluorescence microscopy, we have examined in a model cell type the $[Ca^{2+}]_i$ response to ac electric fields. Application of a 1 or 10 Hz electric field to human hepatoma (Hep3B) cells induces a fourfold increase in $[Ca^{2+}]_i$ (from 50 nM to 200 nM) within 30 min of continuous field exposure. Depletion of Ca^{2+} in the extracellular medium prevents the electric field-induced increase in $[Ca^{2+}]_i$, suggesting that Ca^{2+} influx across the plasma membrane is responsible for the $[Ca^{2+}]_i$ increase. Incubation of cells with the phospholipase C inhibitor U73122 does not inhibit ac electric field-induced increases in $[Ca^{2+}]_i$, suggesting that receptor-regulated release of intracellular Ca^{2+} is not important for this effect. Treatment of cells with either the stretch-activated cation channel inhibitor $GdCl_3$ or the nonspecific calcium channel blocker $CoCl_2$ partially inhibits the $[Ca^{2+}]_i$ increase induced by ac electric fields, and concomitant treatment with both $GdCl_3$ and $CoCl_2$ completely inhibits the field-induced $[Ca^{2+}]_i$ increase. Since neither Gd^{3+} nor Co^{2+} is efficiently transported across the plasma membrane, these data suggest that the increase in $[Ca^{2+}]_i$ induced by ac electric fields depends entirely on Ca^{2+} influx from the extracellular medium.—Cho, M. R., Thatte, H. S., Silvia, M. T., Golan, D. E. Transmembrane calcium influx induced by ac electric fields. *FASEB J.* 13, 677–683 (1999)

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EXOGENOUS ELECTROMAGNETIC FIELDS induce a variety of cellular responses, including cell surface

receptor redistribution (1–10), cytoskeletal reorganization (11–16), and changes in intracellular calcium ion concentration ($[Ca^{2+}]_i$)² (12, 17–20). Because $[Ca^{2+}]_i$ regulates numerous biological processes including signal transduction cascades, cytoskeletal reorganization, cell orientation and migration, and cell differentiation and proliferation, changes in $[Ca^{2+}]_i$ have been hypothesized to mediate cellular effects induced by exogenous electric fields. Although exogenous alternating current (ac) electric fields have been shown to affect intracellular calcium levels, quantitative measurements have not been performed at the single cell level and the mechanisms responsible for changes in $[Ca^{2+}]_i$ have not been elucidated.

Changes in $[Ca^{2+}]_i$ can be mediated by a variety of well-characterized mechanisms. First, membrane depolarization can activate voltage-gated Ca^{2+} channels (VGCCs) in electrically excitable and nonexcitable cell types, including myeloma cells, osteoclasts, astrocytes, and fibroblasts (21). These electrically operated channels are excellent candidates for mediating the Ca^{2+} influx across the cell membrane induced by direct current (dc) electric fields. To activate VGCCs, the electric field strength must be sufficiently large to induce a potential difference on the order of 100 mV. It is not clear whether ac electric fields can couple to VGCCs. The membrane potential in cells exposed to ac electric fields is likely to be periodically hyperpolarized and depolarized, and effects of oscillating membrane potential on Ca^{2+} flux across the plasma membrane have not been characterized. Second, increases in $[Ca^{2+}]_i$ can be mediated by activation of stretch-activated cation channels (SACCs), which, on opening, permit the

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² Abbreviations: ac, alternating current; $[Ca^{2+}]_i$, intracellular calcium ion concentration; dc, direct current; HBSS, Hank's balanced salt solution; Hep3B cells, human hepatoma cells; PBS, phosphate-buffered saline; PLC, phospholipase C; SACCs, stretch-activated cation channels; VGCCs, voltage-gated calcium channels.

influx of cations including Ca^{2+} . SACCs have been identified in neuroblastoma cells (22), endothelial cells (23), *Xenopus* oocytes (24), skeletal muscle (25), cardiac myocytes (26), and hepatocytes (27–30). Third, $[\text{Ca}^{2+}]_i$ increases can be induced by activation of plasma membrane receptors that are coupled to phospholipase C (PLC). PLC activation causes production of inositol triphosphate, which binds to its own intracellular receptor and releases Ca^{2+} from the endoplasmic reticulum. This ‘trigger’ Ca^{2+} can then activate other ion channels in the plasma membrane that allow additional Ca^{2+} entry into the cytosol (31, 32). Ca^{2+} entry mediated by this pathway has been observed in hepatocytes, using extracellular ATP to activate purinergic receptors (28, 29). Finally, $[\text{Ca}^{2+}]_i$ increases can be mediated by changes in the rate of Ca^{2+} uptake by intracellular organelles (33–37).

$[\text{Ca}^{2+}]_i$ increases have been proposed to mediate electric field-induced microfilament reorganization. In fibroblasts, dc electric fields induce microfilament reorganization by causing rapid increases in $[\text{Ca}^{2+}]_i$ (12). Application of a 10 V/cm dc electric field, for example, imposes an induced voltage difference of 50 mV across a cell 50 μm in diameter. The magnitude of the induced voltage difference is sufficient to depolarize VGCCs, thus allowing rapid increases in $[\text{Ca}^{2+}]_i$. Because actin-binding proteins are sensitive to $[\text{Ca}^{2+}]_i$ (38, 39), dc electric field-induced $[\text{Ca}^{2+}]_i$ increases could also affect the binding of these proteins to microfilaments and thereby cause changes in microfilament structure. We have previously shown that ac electric fields induce microfilament reorganization in hepatocytes (13). Changes in microfilament structure were found to depend critically on the frequency of the applied field. The potential relationship between ac electric field-induced $[\text{Ca}^{2+}]_i$ increases and changes in microfilament organization remains to be elucidated.

In the present study, digitized fluorescence video microscopy is used to examine quantitatively the changes in single cell $[\text{Ca}^{2+}]_i$ induced by ac electric fields and to study the mechanisms responsible for these effects. AC electric fields in the 1–10 Hz frequency range are found to induce increases in $[\text{Ca}^{2+}]_i$ that are mediated entirely by Ca^{2+} influx across the plasma membrane. The time course of the increase is too slow to account for the previously observed microfilament reorganization by ac electric fields, however.

MATERIALS AND METHODS

Electric field exposure and fluorescence microscopy

The chamber used to expose cells to electric fields has been described (13). Sinusoidal signals were generated by a func-

tion generator (Model 19, Wavetek, San Diego, Calif.), fed into a 100 W amplifier (BOP100, Kepco, Flushing, N.Y.), and monitored by an oscilloscope (Model 2205, Tektronix, Beaverton, Oreg.). The computation of electric field strength followed Ohm’s law, $J = \sigma E$, where J was the electric current density and σ was the conductivity of the medium. Unless otherwise stated, the ac electric field strength represents the peak to peak value. All experiments were performed at room temperature. An electric field strength of 10 V/cm induced a maximum temperature rise of 3.5°C in our experimental apparatus (10), resulting in a maximum sample temperature of ~24°C. This degree of heating was independent of electric field frequency in the 1–10 Hz frequency range and did not cause a change in $[\text{Ca}^{2+}]_i$.

Epifluorescence video microscopy was used to obtain digitized fluorescence images. Cells were observed using a Zeiss Axioskop microscope (Carl Zeiss Inc., Thornwood, N.Y.). The illumination source was a 100 W mercury arc lamp. Illuminating light was passed through a dichroic filter and focused on the sample through a 25x/0.8 NA oil immersion objective. Fluorescence emission was imaged by using a cooled CCD camera (Photometrics, Tucson, Ariz.) and processed by an image processor (Metamorph, Universal Imaging, West Chester, Pa.). Background intensity was subtracted from each image. All operations were controlled by a computer.

Cell culture

Human hepatoma (Hep3B) cells were grown in α minimal essential medium supplemented with 10% fetal calf serum (Sigma, St. Louis, Mo.), 100 U/ml penicillin-100 $\mu\text{g}/\text{ml}$ streptomycin and 100 mM L-glutamine (Sigma) at 37°C in a 5% CO_2 humidified incubator. Cells were subcultured at 60–70% confluence onto 35 \times 50 mm No. 2 coverslips 48 to 60 h before each experiment to ensure log phase of growth.

Calcium dye loading and fluorescence imaging

The Ca^{2+} -sensitive fluorescent dye Fluo-3 AM ester (Fluo-3, Molecular Probes, Eugene, Oreg.) was dissolved in DMSO to make a 1 mM stock solution, then dissolved at 10 μM final concentration in Hank’s balanced salt solution (HBSS). About 1 ml of 10 μM Fluo-3 solution in HBSS was carefully layered on top of the coverslip bearing cells, then incubated for 60 min in the dark at room temperature. Coverslips were washed twice with HBSS and used immediately in quantitative fluorescence microscopy experiments. In some experiments Ca^{2+} -free conditions were ensured by washing and incubating cells in modified HBSS containing 0 mM CaCl_2 , 2 mM MgCl_2 , and 1 mM EGTA.

Fluorescence images of Hep3B cells loaded with Fluo-3 were recorded in real time before and after electric field application. Typically, 8 to 12 cells were identified in a field of view, and changes in fluorescence intensity in each of the cells were monitored. Cell boundaries were drawn by using the image processor, and fluorescence intensity was integrated over all pixels within the boundary of each individual cell. Because the size and shape of Hep3B cells were variable, and to eliminate effects due to variation in Fluo-3 dye loading, the fluorescence intensities from each image were normalized by those from a reference image recorded before application of an electric field.

$[\text{Ca}^{2+}]_i$ calibration

$[\text{Ca}^{2+}]_i$ was estimated from the fluorescence intensity of Fluo-3 by using the equation, $[\text{Ca}^{2+}]_i = K_d(F - F_{\min}) / (F_{\max} - F)$, where K_d is 400 nM, and F_{\max} and F_{\min} are the

maximum and minimum fluorescence intensities determined according to a previously described method (40). In resting Hep3B cells loaded with Fluo-3, $[Ca^{2+}]_i$ was determined to be 50 nM. This calculation is consistent with the previously reported finding that in resting hepatocytes the baseline $[Ca^{2+}]_i$ is 40 to 70 nM (28, 29).

Cell treatment with U73122 and U73343

Hep3B cells were treated with either U73122 (a selective phospholipase C inhibitor) or an inactive analog of U73122 (U73343, Calbiochem, La Jolla, Calif.). Cells were incubated with U73122 (25 μ M) or U73343 (25 μ M) for 15 min at 37°C in an incubator, washed twice in HBSS, loaded with Fluo-3, washed twice, and mounted on the chamber for quantitative fluorescence microscopy experiments.

Cell viability

The viability of cells exposed to exogenous electric fields or treated with U73122 was measured by using a cell viability assay (Molecular Probes). Cells plated at 60–70% confluence were either exposed to an electric field or treated with 25 μ M U73122, as described above. Control cells were neither exposed to an electric field nor treated with U73122. Cells were then washed twice in phosphate-buffered saline (PBS) and assayed for viability. Briefly, 500 μ l of reagent (2 μ M calcein-AM, 4 μ M ethidium homodimer in Dulbecco's PBS) was added to the coverslip; cells were incubated for 60 min at room temperature, mounted on a slide, and observed using fluorescence microscopy. Green fluorescence indicated living cells, because calcein-AM was hydrolyzed and retained by living cells. Red fluorescence indicated dead cells, because ethidium homodimer was membrane permeant only in dead cells.

RESULTS

Time dependence of $[Ca^{2+}]_i$ increase induced by ac electric fields

AC electric fields were found to induce significant increases in $[Ca^{2+}]_i$. **Figure 1** represents the kinetics of the $[Ca^{2+}]_i$ increase. Control cells showed no significant changes in $[Ca^{2+}]_i$ over 30 min of observation. In contrast, exposure of cells to a 1 Hz, 10 V/cm electric field induced a fourfold rise in $[Ca^{2+}]_i$. The increase in $[Ca^{2+}]_i$ was monotonic for 30 min of exposure to the field and then saturated. No additional increase in fluorescence intensity was observed over an additional 30 min of electric field exposure (data not shown). Exposure of cells to a 10 Hz, 10 V/cm electric field also induced a fourfold increase in $[Ca^{2+}]_i$ (data not shown).

To investigate the possibility that ac electric fields could induce sharp $[Ca^{2+}]_i$ transients in the early phase of an electric field application (i.e., within 3 min of field exposure), fluorescence images of Fluo-3 loaded Hep3B cells were recorded at 10 s intervals after application of a 1 Hz, 10 V/cm electric field. No significant $[Ca^{2+}]_i$ transient was observed

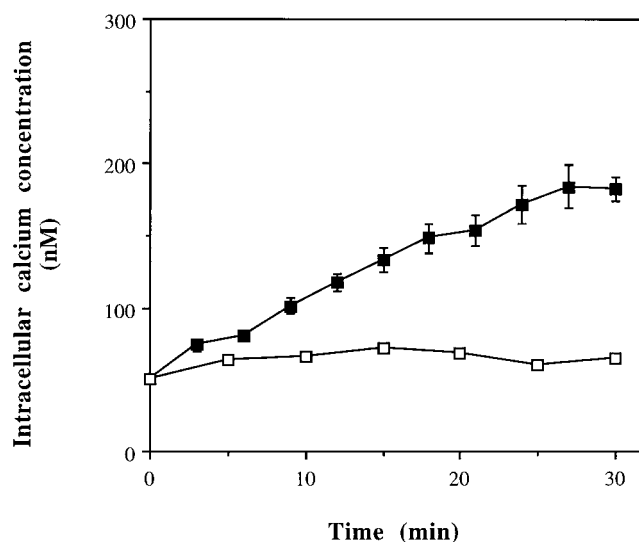


Figure 1. Time-dependent $[Ca^{2+}]_i$ increase induced by ac electric field. Hep3B cells were loaded with Fluo-3 and exposed to a 1 Hz, 10V/cm electric field for 30 min at room temperature (filled squares). Control cells (open squares) were treated identically, except these cells were not exposed to an electric field. The integrated fluorescence intensity of each cell was monitored and recorded at 3 or 5 min intervals and normalized against the fluorescence intensity of that cell at time $t = 0$. Each data point represents the mean \pm SEM of 4–9 independent experiments from 40–90 individual cells. Error bars for the control experiments were smaller than the symbols used to represent the values.

during the first 3 min of electric field application (data not shown).

Role of extracellular Ca^{2+} and internal Ca^{2+} stores

There are two general pathways by which $[Ca^{2+}]_i$ can be increased. First, a $[Ca^{2+}]_i$ increase can be mediated by Ca^{2+} influx across the plasma membrane. To test for the involvement of Ca^{2+} influx pathways, the effect of ac electric fields on $[Ca^{2+}]_i$ was examined in the absence of extracellular Ca^{2+} . Application of a 1 Hz, 10 V/cm electric field in Ca^{2+} -free medium did not induce an increase in $[Ca^{2+}]_i$ (**Fig. 2**), indicating that Ca^{2+} influx across the plasma membrane was required for the ac electric field-induced $[Ca^{2+}]_i$ increase. Second, a $[Ca^{2+}]_i$ increase can be mediated by activation of internal Ca^{2+} stores. This pathway is typically initiated by PLC activation at the plasma membrane (21, 29, 32). A PLC inhibitor (U73122) was therefore used to test the hypothesis that increases in $[Ca^{2+}]_i$ were mediated by ac electric field-induced PLC activation. Cells were incubated with 25 μ M U73122, an effective inhibitor of all PLC activities in hepatocytes (41). Cells treated with either 25 μ M U73122 or 25 μ M U73343 (an inactive analog of U73122) before exposure to a 1 Hz, 10 V/cm electric field showed increases in $[Ca^{2+}]_i$ similar to those in untreated cells (**Fig. 2**), indicating

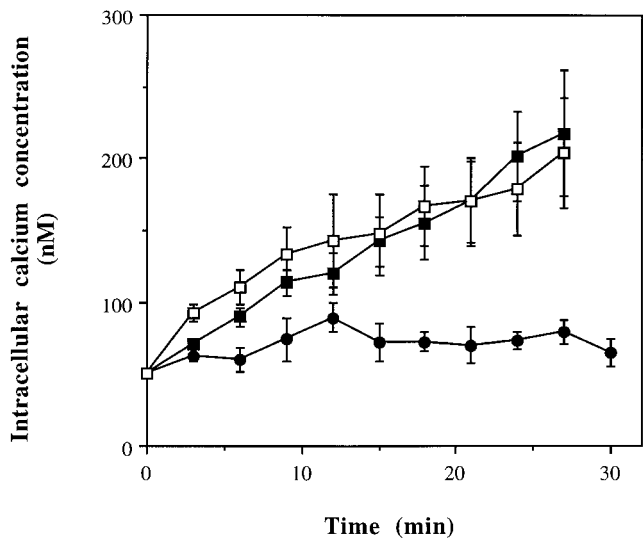


Figure 2. Requirement for extracellular Ca²⁺ for ac electric field-induced [Ca²⁺]_i increase. Cells were loaded with Fluo-3 and exposed to a 1 Hz, 10 V/cm electric field in the absence of Ca²⁺ in the extracellular medium. The ac electric field-induced [Ca²⁺]_i increase was inhibited by this treatment (filled circles). Prior to electric field exposure, some cells were incubated with 25 μM U73122 (filled squares) or U73343 (open squares) and exposed to a 1 Hz, 10 V/cm electric field at room temperature. Neither U73122 treatment nor U73343 treatment affected the ac electric field-induced [Ca²⁺]_i increase (compare with control curve in Fig. 1). Data points represent the mean ± SEM of 3–6 independent experiments from 30 to 54 individual cells.

that PLC activation is not involved in the [Ca²⁺]_i increase induced by ac electric fields.

Role of stretch-activated cation channels (SACCs) and Co²⁺-sensitive Ca²⁺ channels

Based on the observation that the ac electric field-induced [Ca²⁺]_i increase is mediated by Ca²⁺ influx across the plasma membrane, the potential role of several plasma membrane Ca²⁺ channels was examined. The involvement of VGCCs was ruled out because hepatocytes do not possess VGCCs in the plasma membrane (28, 42). To verify the lack of VGCC expression in the Hep3B cell plasma membrane, cells were incubated with fluorescently conjugated monoclonal antibodies directed against VGCCs (Affinity BioReagents Inc., Golden, Colo.). The lack of any specific staining confirmed that VGCCs are not found in the Hep3B cell plasma membrane (fluorescence images not shown).

Because hepatocytes have been shown to express SACCs (27–29), we also tested the hypothesis that ac electric field-induced increases in [Ca²⁺]_i are mediated by SACCs. In control experiments, cell swelling was used to activate SACCs (28). Briefly, cells were incubated in hypotonic HBSS buffer (60% HBSS and 40% water) and [Ca²⁺]_i measurements were performed in real time. Cell swelling induced a 30%

increase in [Ca²⁺]_i within 1 min after adding the hypotonic solution. This swelling-induced increase in [Ca²⁺]_i was completely inhibited by 50 μM GdCl₃, which is considered to be the most potent and specific SACC blocker (24). Our results using the cell swelling method are consistent with those of Bear and Li (28). The effect of Gd³⁺ on the increases in [Ca²⁺]_i induced by a 1 Hz, 10 V/cm electric field is shown in Fig. 3. Incubation of cells with 50 μM GdCl₃ for 20 min prior to electric field exposure reduced the field-induced increase in [Ca²⁺]_i by 40%, suggesting that activation of SACCs was partially responsible for the [Ca²⁺]_i increase induced by ac electric fields.

Finally, we studied the effect of the nonspecific calcium channel blocker Co²⁺ on the ac electric field-induced [Ca²⁺]_i increase. Incubation of cells with 1 mM CoCl₂ prior to electric field exposure reduced the field-induced increase in [Ca²⁺]_i by 40% (Fig. 3), suggesting that a portion of plasma membrane Ca²⁺ channels responsible for ac electric field-induced increases in [Ca²⁺]_i was sensitive to inhibition by Co²⁺. To test whether Co²⁺ and Gd³⁺ were blocking the same or different populations of channels, cells were incubated concomitantly with 1 mM CoCl₂ and 50 μM GdCl₃, and then exposed to ac electric fields. These cells showed no increase in [Ca²⁺]_i induced by ac electric fields (Fig. 3), suggesting that Co²⁺ and Gd³⁺ were inhibiting different populations of channels. Together, Co²⁺ and Gd³⁺

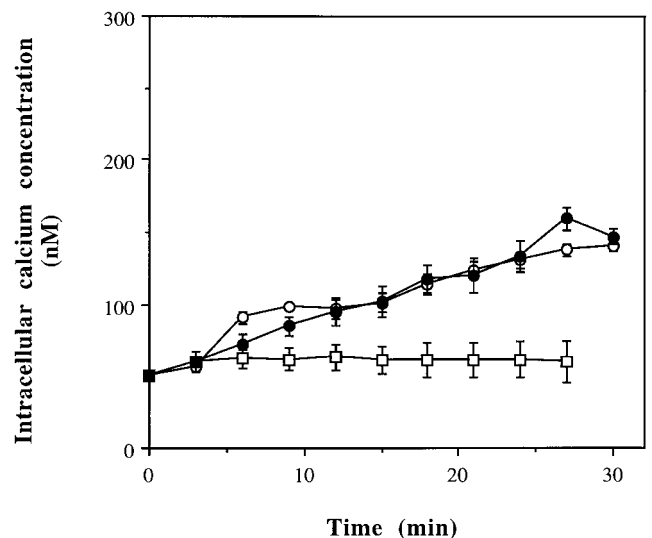


Figure 3. Effects of Gd³⁺ and Co²⁺ on ac electric field-induced [Ca²⁺]_i increase. After incubation with 50 μM GdCl₃ or 1 mM CoCl₂, cells were exposed to a 1 Hz, 10 V/cm electric field for 30 min at room temperature. Cells treated with GdCl₃ (open circles) or CoCl₂ (filled circles) showed a 40% reduction in the ac electric field-induced [Ca²⁺]_i increase (compare with control curve in Fig. 1). Cells incubated with both GdCl₃ and CoCl₂ (open squares) showed no ac electric field-induced [Ca²⁺]_i increase. Each data point represents the mean ± SEM of 3–6 independent experiments from 30–66 individual cells.

TABLE 1. Effect of ac electric fields and PLC effectors on Hep3B cell viability^a

| Electric field | Treatment | Live cells (%) | n |
|----------------|----------------|----------------|-----|
| None (control) | None | 91 ± 3 | 289 |
| 1 Hz | None | 92 ± 2 | 295 |
| None | U73122 (25 μM) | 94 ± 2 | 464 |
| None | U73343 (25 μM) | 91 ± 1 | 340 |

^a Cells were either exposed to a 10 V/cm electric field for 15 min or treated with U73122 or U73343 for 15 min. Control cells were incubated for 15 min in HBSS but not exposed to an electric field. Cell viability was determined as described in the text. These treatments did not cause cell death. Values represent the mean ± SEM for 2 to 4 independent experiments. n, number of cells.

were capable of completely inhibiting the Ca²⁺ influx across the plasma membrane induced by ac electric fields.

Controls

The viability of cells exposed to exogenous electric fields or treated with the PLC inhibitor U73122 was determined by using a cell viability assay. As shown in **Table 1**, ~92% of cells in the control experiment (no treatment, but 15 min incubation in HBSS) were live. Exposure of cells for 15 min to a 1 Hz, 10 V/cm electric field did not decrease the fraction of live cells. Similarly, treatment of cells with U73122 or U73343 did not affect the percentage of live cells. Thus, none of the treatments including ac electric fields, U73122, and U73343 caused cell death.

DISCUSSION

Results from the present study show that ac electric fields are capable of inducing increases in [Ca²⁺]_i. Application of a 1 or 10 Hz, 10 V/cm electric field causes a fourfold increase in [Ca²⁺]_i (from 50 nM to 200 nM) within 30 min of exposure to the electric field. Field exposure for longer than 30 min causes no additional [Ca²⁺]_i increase. Depletion of extracellular Ca²⁺ completely inhibits the field-induced [Ca²⁺]_i increase, as does concomitant treatment with the two plasma membrane channel blockers Co²⁺ and Gd³⁺. In contrast, treatment with the PLC inhibitor U73122 does not affect the field-induced [Ca²⁺]_i increase. Together, these data suggest that ac electric fields induce a [Ca²⁺]_i increase by activating Ca²⁺ influx pathways across the plasma membrane.

Increases in [Ca²⁺]_i can theoretically be mediated by Ca²⁺ influx across the plasma membrane and/or by Ca²⁺ release from internal stores. The observation that depleting Ca²⁺ in the extracellular medium completely inhibits the ac electric field-induced [Ca²⁺]_i increase provides strong evidence that Ca²⁺

influx across the plasma membrane is the mechanism responsible for the [Ca²⁺]_i increase. Based on theoretical considerations as well as experimental findings, direct activation of internal Ca²⁺ stores by an exogenous ac electric field is unlikely. Because the plasma membrane is highly resistive and the cytosol is conductive (4), ac electric fields of frequencies < 1 MHz do not penetrate inside the cell (43). Therefore, the ac electric field-induced [Ca²⁺]_i increase must be mediated by cellular events at the plasma membrane level. One such event could be the activation of PLC (31, 44, 45). Use of U73122 to inhibit PLC does not prevent the ac electric field-induced [Ca²⁺]_i increase, however, indicating that activation of an internal Ca²⁺ store via the PLC signal transduction pathway is unlikely. This result is consistent with the conclusion that the ac electric field-induced [Ca²⁺]_i increase is mediated by Ca²⁺ influx across the plasma membrane. Other potential mechanisms for [Ca²⁺]_i regulation include Ca²⁺ uptake into intracellular stores (often leading to Ca²⁺ oscillations) and electroconformational coupling of membrane proteins. Although agonist-induced Ca²⁺ oscillations have been observed in hepatocytes (33, 34), no oscillations in [Ca²⁺]_i are observed here on monitoring [Ca²⁺]_i at 10 s intervals after ac electric field application. Because proteins undergo structural changes that could lead to alterations in electrical properties (46), conformational transitions induced by exogenous electric fields cannot be ruled out. Although electric field-induced protein conformational changes have been observed (37, 46–49), it remains to be demonstrated whether such changes could be involved in the ac electric field-induced [Ca²⁺]_i increase.

The present results suggest that at least two plasma membrane Ca²⁺ pathways are responsible for mediating the ac electric field-induced [Ca²⁺]_i increase. First, the finding that Gd³⁺, the most potent SACC blocker, inhibits the [Ca²⁺]_i increase by 40% suggests that exogenous electric fields are likely to activate SACCs. SACC activation could be mediated by direct coupling of the applied electric field to SACCs (e.g., by electroconformation) or by induced SACC clustering (e.g., by redistribution). Although electroconformation of transmembrane enzymes has been postulated (37, 46), we note that very large electric field strengths (~10 KV/cm) are required to induce this effect. In contrast, we have shown that cell surface receptors are induced to redistribute in response to ac electric fields of the field strengths used in this study (10). Because protein clustering can cause protein activation and transmembrane signaling (50–52), SACC activation could be mediated by induced SACC redistribution on the cell surface. Second, the observation that Co²⁺ reduces the ac electric field-induced [Ca²⁺]_i increase by 40%

suggests that some Ca^{2+} pathways activated by ac electric fields are sensitive to inhibition by Co^{2+} . Because VGCCs are not expressed in the Hep3B plasma membrane, Co^{2+} must act by inhibiting Ca^{2+} pathways other than VGCCs. Further, because Co^{2+} and Gd^{3+} act additively to inhibit all Ca^{2+} influx induced by ac electric fields, Co^{2+} cannot act by inhibiting SACCs. Identification of the Co^{2+} -sensitive Ca^{2+} pathways that couple to exogenous electric fields remains to be determined.

Considering the observations of the present study along with results of our previous work (13), it is likely that ac electric field-induced microfilament reorganization in Hep3B cells is independent of an increase in $[\text{Ca}^{2+}]_i$. Control Hep3B cells have cytoplasmic microfilaments aligned in cables along the cell axis as well as microfilaments associated with the plasma membrane (13). SACC activation by swelling of Hep3B cells does not alter microfilament structures even though this treatment causes a $[\text{Ca}^{2+}]_i$ increase (27; the present study), indicating that the $[\text{Ca}^{2+}]_i$ increase mediated by SACC activation is not associated with ac electric field-induced microfilament reorganization. More important, the characteristic half-time for microfilament reorganization in response to a 1 Hz, 10 V/cm electric field is ~ 5 min (13), whereas the $[\text{Ca}^{2+}]_i$ increase in response to the identical field is manifested as a slow, monotonic response that saturates only after 30 min of field exposure. Because the ac electric field-induced $[\text{Ca}^{2+}]_i$ increase lags significantly behind the field-induced microfilament reorganization, the latter response cannot be caused by the $[\text{Ca}^{2+}]_i$ increase. An alternative mechanism is proposed in which ac electric field-induced cell surface receptor redistribution (which also has a characteristic half-time in the 5 min range) is the primary stimulus to microfilament reorganization. Reorganized microfilament structures could then cause changes in cellular morphology, which could result in additional SACC activation. In this mechanism, the electric field-induced $[\text{Ca}^{2+}]_i$ increase could be a consequence of cytoskeletal reorganization, not the cause. Consistent with this hypothesis, the cytoskeletal rearrangements that accompany electric field-induced cell locomotion have been shown to be calcium independent but to involve cell surface receptor redistribution (53). Together with previously reported results, the present observations lead to the conclusion that the mechanisms responsible for electric field-induced $[\text{Ca}^{2+}]_i$ increases and microfilament reorganization depend on the particular cell type exposed to the field and on the mode (i.e., dc, ac, or pulsed) of exogenous electric field application. **FJ**

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