ORIGINAL

Effects of a 1.5 T time-varying magnetic field on cell volume regulation of bovine adrenal chromaffin cells in hyposmotic media

Hirotaka Nishisako[1], Toshitaka Ikehara[2], Tairoh Shiraishi[1], Yuki Minami[1], Hiromi Ichinose[4] (Sasaki), Hitoshi Houchi[4], Kazuyoshi Kawazoe[1], Kazuo Minakuchi[1], Mitsuo Kitamura[3], Masayuki Shono[3], Kazuo Yoshizaki[3], Yohsuke Kinouchi[5], and Hiroshi Miyamoto[3]

[1]*Division of Pharmacy, Tokushima University Hospital, Tokushima, Japan ;* [2]*Department of Human Welfare, Faculty of Health and Welfare, Tokushima Bunri University, Tokushima, Japan,* [3]*Departments of Physiology, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan ;* [4]*Department of Pharmacy, Kagawa University Hospital, Kagawa, Japan ; and* [5]*Graduate School of Advanced Technology and Science, the University of Tokushima, Tokushima, Japan*

Abstract : Effects of a time-varying magnetic field on cell volume regulation by hyposmotic stress in cultured bovine adrenal chromaffin cells were examined. Through regulatory volume decrease (RVD), cell volume of chromaffin cells that were incubated in a hypotonic medium initially increased, reached a peak and finally recovered to the initial value. Two hour exposure to a magnetic field and addition of cytochalasin D increased peak value and delayed return to initial value. Intracellular F-actin contents initially decreased but returned to normal levels after 10 sec. Two hour exposure to the magnetic field and addition of cytochalasin D continuously reduced the F-actin content. Results suggest that exposure to the magnetic field stimulated disruption of the actin cytoskeleton and that the disruption delayed the recovery to the volume prior to osmotic stress. J. Med. Invest. 58 : 95-105, February, 2011

Keywords **:** *eddy current, cytoskeletal protein, actin, hyposmotic stress, regulatory volume decrease*

INTRODUCTION

Many cultured cells show volume regulation in anisotonic media. Extracellular water osmotically penetrates cells incubated in hyposmotic media and produces swelling. When cells are incubated in hyposmotic media, initially extracellular water osmotically penetrates cells and they swell as a result. After the volume reaches a maximal value, intracellular water flows out from cells together with mainly K^+ or Cl^- through each ion channel (1, 2), and this is known as the volume regulatory decrease (RVD).

Pulsed magnetic fields can change the Ca^{2+} concentration ($[Ca^{2+}]i$) (3) and electric charges on cell membrane surfaces (4). Strong magnetic fields (1-2 T) are used in medical testing, treatment and

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Address correspondence and reprint requests to Dr. Toshitaka Ikehara, Department of Human Welfare, Faculty of Health and Welfare, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan and Fax : +81-88-602-8146.

analysis of molecular structure (5-9). Nuclear magnetic resonance (NMR) field increases Ca^{2+} uptake in rat thymocytes (10). Transcranial magnetic stimulation (1-3 T pulsed magnetic field) evokes neurons from rat hippocampi (11).

Actin cytoskeleton closely relates to volume regulation in Ehrlich cells (12), pulmonary artery smooth muscle cells (13) and HaCa T cells (14). Extremely low frequency (ELF) magnetic fields reorganize actin in AtT20D16V and A549 cells (15, 16). Strong magnetic fields (7 T and above) decrease F-actin (17). Magnetic flux density of more than 1.4 T strongly inhibits neurotransmitter induced Ca^{2+} release from intracellular Ca^{2+} stores (18).

The present study improves understanding of how a time-varying strong magnetic field of 1.5 T flux density and 2 hr exposure time affects RVD and how RVD relates to the actin cycle. Analysis of monolayer cultured adrenal chromaffin cells incubated with hypotonic stress shows how strong magnetic field exposure affects functions and morphology of the intracellular actin cytoskeleton during RVD.

MATERIALS AND METHODS

Cell preparation and culture

Bovine adrenal chromaffin cells were dispersed enzymatically as reported earlier (18, 19). Cells were plated on 35-mm culture dishes (Corning Inc., Corning, NY) at a density of 10^6 cells/dish. 13-mm diameter circular cover glasses were used for measuring Ca^{2+} concentration and 18-mm square cover glasses were used for staining with fluorescent dye. Cells that attached to the dishes or cover glasses were maintained for 2-5 days as monolayer cultures in Eagle's basal medium, supplemented with 5% heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml), gentamycin (40 μg/ml), fungizone (2.5 μg/ml) and 10 μM systine arabinoside.

Normal balanced salt solution (BSS : 135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose and 20 mM HEPES/NaOH, pH 7.4) was used to incubate cells during exposure to the magnetic field and to measure calcium concentration in cells. Ca^{2+}-free BSS in the presence of 1 mM EGTA was also used to measure that calcium concentration.

Exposure to a time-varying magnetic field

The magnetic field was produced by an electromagnet designed set up by Hitachi Metal Industrial Co. (Tokyo, Japan). Details of the electromagnet appear in Yamaguchi *et al*. (20) and Miyamoto *et al*. (21-23). The electromagnet has a pair of vertically placed coils and poles with round faces of 100 mm diameter attached to the coils. This system produces a maximal magnetic flux density of about 1.51 T in a gap between the poles that is slightly wider than 20 mm (Fig. 1A).

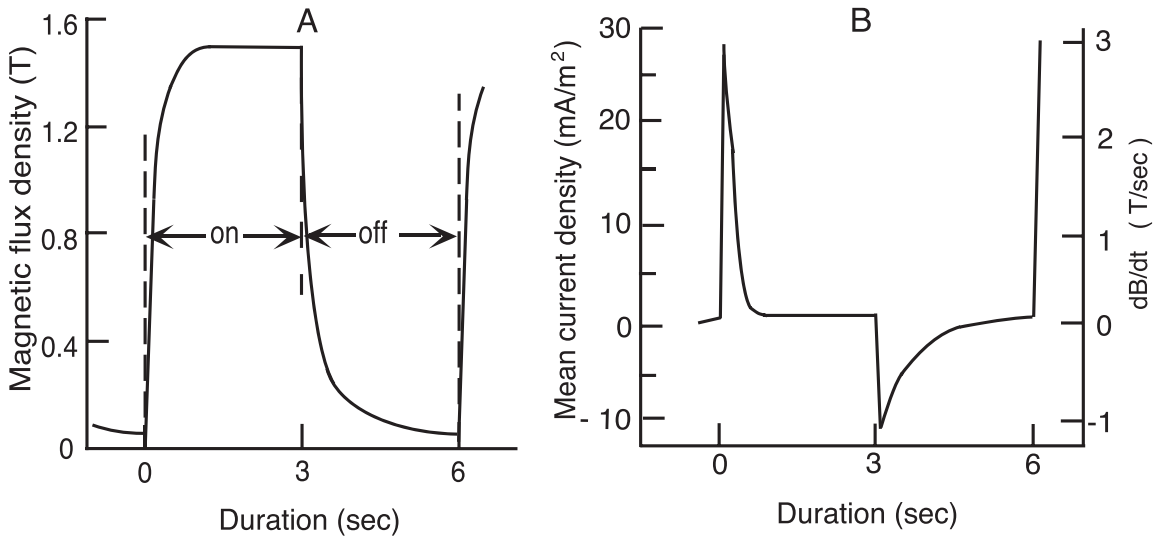

Fig. 1. Time-dependent changes in the magnetic flux density and calculated mean values of eddy currents induced in the medium. (A) Magnetic flux densities during and after a 3-sec pulse of a 1.5 T magnetic field. (B) Mean densities of eddy currents and values of *dB/dt* calculated from data in A. Illustrations are modified from Yamaguchi *et al*. (15).

The time-varying magnetic field was produced by changing the coil current automatically via an electronic switch. For simplicity, the duration of on/off times was set at 3 seconds. The peak mean densities of eddy currents induced in the culture medium (culture dish) were estimated to be 28 mA/m^2 (maximum) and -11 mA/m^2 (minimum) (Fig. 1B).

Temperature regulation of cultures

The experiment was done with specially designed incubators (129 mm diameter, 20 mm thick) that can contain four culture dishes in each incubator (20). Temperature was kept at 37°C. Normally, two similar incubators were used simultaneously, with one placed horizontally in the gap between the two poles of the electromagnet and the other placed outside the magnetic field as a control.

Measurement of cell volume

Measurement of cell volume was via a calcein fluorescent probe as described in Capó-Aponte *et al*. (24, 25). Fluorescence intensity was recorded with an ARGUS 20/CA system (Hamamatsu Photonics, Hamamatsu, Japan), at an excitation wavelength of 488 nm and an emission wavelength of 530 nm. Thirty minutes before cell volume measurement, cell monolayers cultured on cover glasses were preloaded with 5 μM calcein-AM, washed and incubated with isotonic BSS medium. Cultured chromaffin cells were placed in a specially designed chamber made by attaching glass to the bottom of a plastic culture dish in which a hole had been made. This chamber was inserted into a specially designed brass incubator (unlike the incubator used for exposure to the magnetic field) and fixed on the stage of a microscope (IX70) (Olympus Co. ltd, Tokyo, Japan). The brass incubator was kept at 37°C by circulation of warm water. After exposure to the magnetic field, cultures were washed with BSS to remove the probe. Within 2 min of washing, fluorescence intensity was measured in 0.4 ml BSS. Subsequently, the medium osmolarity was reduced by adding 0.6 ml (154 mOsM) or 0.4 ml (177 mOsM) of NaCl-free BSS and fluorescence intensity was continuously measured for 5-20 min. Errors due to decay or calcein leakage were corrected using data obtained via isosmotic medium.

Before measurement, cells were pre-incubated with actin polymerization inhibitors and ion membrane transporters. Cell volume was measured in BSS for 30 min in the presence of these inhibitors.

Measurement of intracellular calcium concentration with Fura-2

Intracellular Ca^{2+} concentration and distribution were determined with a permeable fluorescent probe, 1-(2-(5'-carboxyl-oxazol-2'-yl)-6-aminobenzofuran-5-oxy)-2-(2'amino-5'-methyl-phenoxy) ethene-N,N,N',N'-tetraacetic acid, penta-acetoxymethyl ester (Fura 2-AM, 6 μM) using an ARGUS-50/CA (Hamamatsu Photonics Co. Ltd., Hamamatsu, Japan) at excitation wavelengths of 340 and 380 nm and emission wavelength of 510 nm using a flow chamber. To preload, cells with the fluorescent probe were incubated and attached to cover glasses for 30 min at 37°C in the media containing the probe. Fluorescent dye was added to the medium 30 min before exposure ended. Cultures were washed with the same medium without the probe and fluorescence intensity was measured for 2 min. Fluorescence was assayed for 1-2 min in the BSS medium or Ca^{2+}-free BSS medium. Cells were then treated with hyposmotic media. Intracellular Ca^{2+} concentration was determined by the method of Grynkiewicz *et al*. (26).

Cell staining with fluorescent dye and fluorescent measurements by confocal microscopy

Chromaffin cells exposed to the magnetic field for 2 hours were fixed for 30 min in 2% paraformaldehyde dissolved in phosphate-buffered saline (PBS). Cells were made permeable by incubation for 1.5 min in a solution of 0.1% Triton X-100 in PBS. Blocking was with 1% BSA (bovine serum albumin fraction V) on ice. To quantify intracellular F-actin after the cells were made permeable and blocked, cells were incubated for 20-60 min in PBS containing 0.33 μM Alexa fluor 488 phalloidin (Molecular Probe).

Fluorescence observation was via a confocal laser scanning microscope, Leica TCS NT, (Heidelberg, Germany) equipped with a Kr-Ar laser. Markers were excited at 488 and 568 nm, with an emission wavelength of 530 (± 20) nm. Optical slice thickness was 0.33-0.4 μm and the middle 15 slices were chosen.

Quantification of intracellular F-actin

The method for quantifying F-actin was modified from Pederson *et al*. (12). The incubation medium was aspirated rapidly and cells were fixed for 30 min on ice with ice-cold 4% paraformaldehyde. Fixed cells were washed three times with ice-cold phosphate

buffered saline (PBS) and incubated for 1 hr on a rotator at room temperature in 0.33 μM rhodamine-phalloidin solution (10 mM MOPS, 5 mM EGTA, 20 mM K_2HPO_4 2 mM $MgSO_4$, 1% Triton X-100, pH 6.9). Cells were scraped off and washed twice by centrifugation using a MOPS buffer (10 mM MOPS, 5 mM EGTA, 20 mM K_2HPO_4, 2 mM $MgSO_4$, pH 6.9). Methanol was added to the suspended cells and those cells were incubated for 30 min on a rotator at room temperature. After centrifugation, supernatant rhodamine fluorescence was measured at 576 nm after excitation at 540 nm, using a fluorescence spectrophotometer (F-1200, Hitachi Co. Ltd., Tokyo, Japan).

Measurement of osmolarity

Solution osmolarity was determined by OSMOSTAT OM-6020 (Kyoto Daiichi-Kagaku).

Chemicals

Acetylcholine was from Nakarai Tesk (Osaka, Japan) and ethylene glycol bis(β-aminoethyl ester)-N,N,N',N'-tetraacetic acid (EGTA), Fura 2 and Fura 2-AM were from Dojindo Laboratories (Kumamoto, Japan). Other chemicals used were of commercial reagent grade. Rhodamine-Phalloidin was from Cytoskeleton Inc. Denver, CO. USA) and other fluorescent probes were from Molecular Probe, Inc. (Eugene OR, USA).

Statistical analyses

The Student's t-test was used to calculate *P* values to test statistical significance of differences.

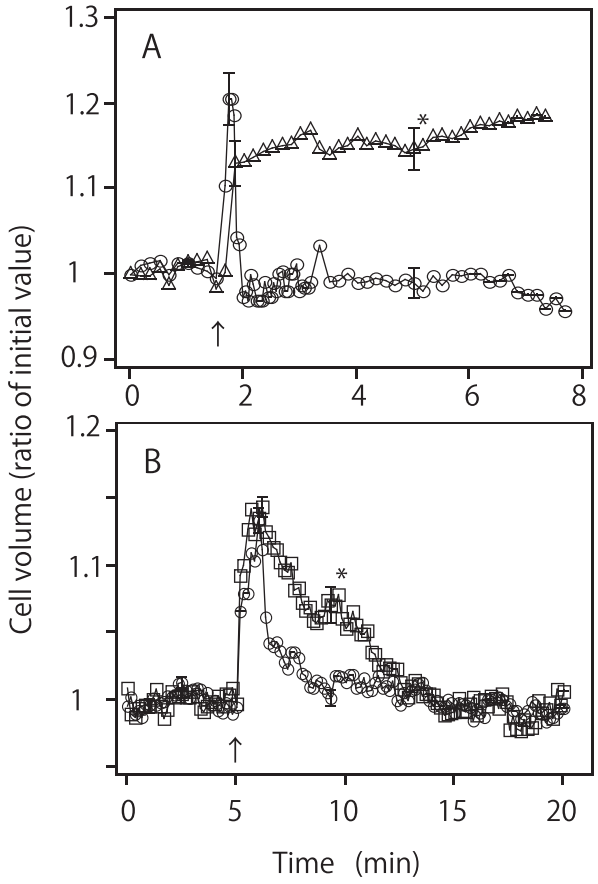

Fig. 2. Effects of ion channel inhibitors on time-dependent changes in cell volume due to hypotonic stress (177 mOsM). Cells were preincubated with the inhibitors for 30 min, and cell volume was determined in the presence of the inhibitors. A ; Open circles : control (n=20) ; open triangles : 5 mM $BaCl_2$ (n=11). B ; Open circles, control (n=19) ; open squares, 0.5 mM DIDS (n=11). Arrows show the time for reduction of medium osmolarity. Points and bars are mean and S.E. values. *, Significantly different from control at $P< 0.03$. The numbers in parentheses indicate the numbers of cells measured.

RESULTS

Cell volume regulation in the hyposmotic medium

Figure 2 shows two inhibitors of the Ca^{2+}-dependent ion channels that were added to chromaffin cells in the hypotonic medium. After adding hyposmotic medium, cell volume instantly and significantly increased, reached peak value and returned to initial value within about twenty seconds (Fig. 2A). Adding 5 mM $BaCl_2$ did not affect initial increase in cell volume but completely inhibited the recovery phase, with peak value maintained for about 10 min or longer. Adding DIDS to the medium did not affect initial increase in cell volume but significantly delayed the recovery phase (Fig. 2B). Such results suggest that Ca^{2+}-dependent K^+ or Cl^- channels are important in the RVD recovery phase of chromaffin cells in a hyposmotic medium.

Effects of the actin disruptor and exposure to a magnetic field

In chromaffin cells incubated in hypotonic medium (154 mOsM), cell volume instantly increased and peaked at 113± 2% (average± SE, n=14) of initial value within 1 min of the onset of hyposmotic stress (Fig. 3A). RVD then started to decrease, returning almost to initial value at about 2-3 min. Magnetic field exposure significantly increased peak value to 129± 2% (average± SE, n=19) of initial value and increased the time needed to reach peak value. Values returned to near original levels about 4-5 min after medium osmolarity reduced. Adding cytochalsin D increased peak value to 134± 3% (average± SE, n=13) and initial value returned 5-6 min after

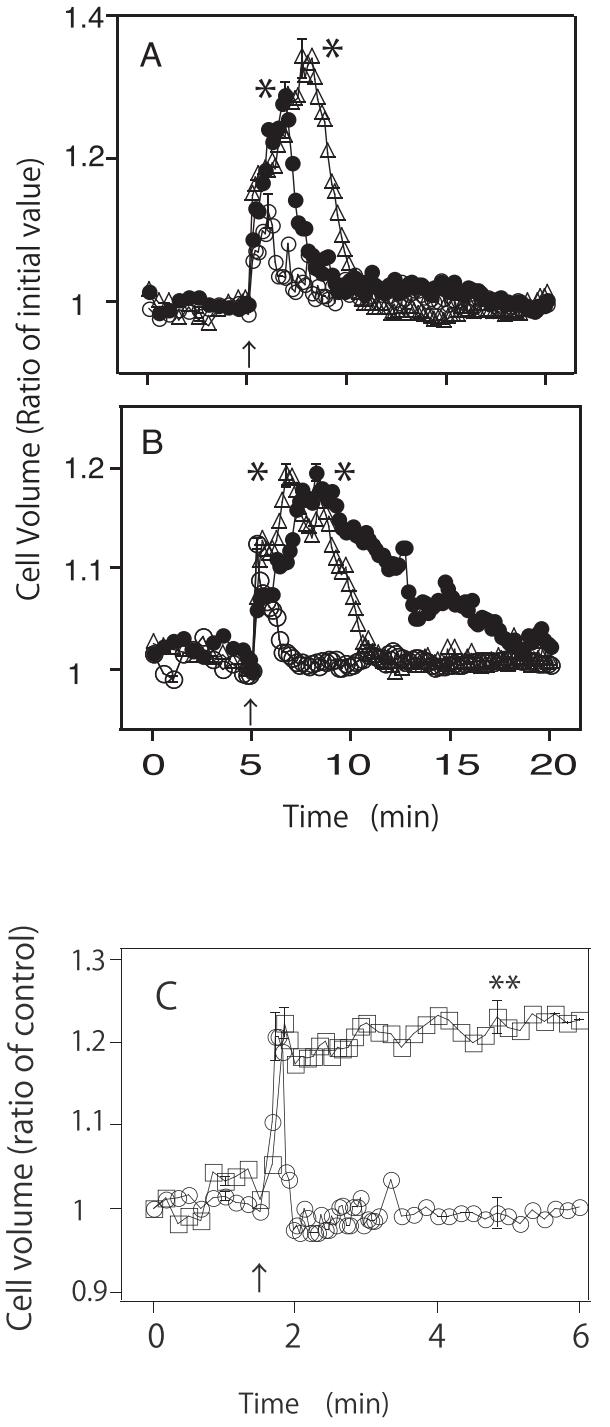

Fig. 3. Effects of actin disrupters and exposure to the time-varying magnetic field on the regulatory volume decrease due to hyposmotic stress. Cells were preincubated with the disruptors for 30 min and cell volume was determined in the presence or absence of the disruptors. Open circles, control (Co) ; open triangles, 2.5 μM cytochalasin D (Cy) ; open squares, 1 μM latrunculin B (La) ; closed circles, exposure (Ex). (A) 154 mOsM hypotonic medium (Co,14 ; Cy,13 ; Ex,19) ; (B) 177 mOsM (Co,34 ; Cy,18 ; Ex : 20). (C) 177 mOsM(C,20 ; La,29). *, Significantly different from control (peak value) at $P< 0.03$. **, significantly different from control at $P< 0.02$. Arrows show the time for medium osmolarity reduction. Points and bars are mean and S.E. values. Numbers in parentheses indicate the numbers of cells measured.

osmolarity reduced.

Cell volume in control cells temporarily increased to about 113± 1% (average± SE, n=34) of initial volume and then decreased to initial value about 2-3 minutes after osmolarity reduced to 177 mOsM (Fig. 3B. In exposed cells, peak value was higher than in control cells (120± 1% [average± SE, n=20]) of initial value), prolonging the time needed to reach peak value and returning to almost the original value about 13 min after osmolarity reduced. Adding actin disruptor cytochalasin D increased the peak to 119± 1% (average± SE, n=18) of initial value, which was similar to the level in cells exposed to the magnetic field. Return to initial value was delayed until about 6 min after the reduction, which was longer than in the control but shorter than in exposed cells. Adding F-actin fiber disruptor latrunculin B made initial increase in cell volume the same as in the control but regulatory volume decrease was strongly inhibited and the volume was unchanged up to at least 10 min after the peak (Fig. 3C).

Influence of the magnetic field on the morphology of intracellular F-actin protein

There was microscopic imaging of intracellular F-actin stained with fluorescent-labeled phalloidin after osmolarity reduced (Fig. 4). Cells were exposed to the magnetic field for 2 hr or preincubated with 1 μM cytochalasin D or 0.1 μM latrunculin B for 30 min. Most F-actin fibers were long and straight in control cells (Fig. 4A), but fibers were short and in peripheral areas of the cytoplasm after exposure to the magnetic field (Fig. 4D). Adding cytochalasin D (Fig. 4G) or latrunculin B (Fig. 4J) decreased F-actin density and morphologically collapsed the fibers. Images showed that control cells appeared tightly attached to the surface of the cover glass through extension of actin filaments in a straight line. Exposed cells and inhibitor treated cells appeared to be partially detached from the cell surface.

Most F-actin fibers in control cells were temporally smaller at 10 sec after hypotonic medium change (Fig. 4B) and then the temporarily reduced fibers tended to increase in density again at 15 min (Fig. 4C). In cells exposed to the magnetic field, F-actin further decreased after replacement of the hyposmotic medium (Fig. 4E), but those fibers had not recovered well at 15 min (Fig. 4F). Remaining F-actin showed no clearly identifiable change in images of cytochalasin D or latrunculin B-treated cells (Fig. 4H, I, K, and L).

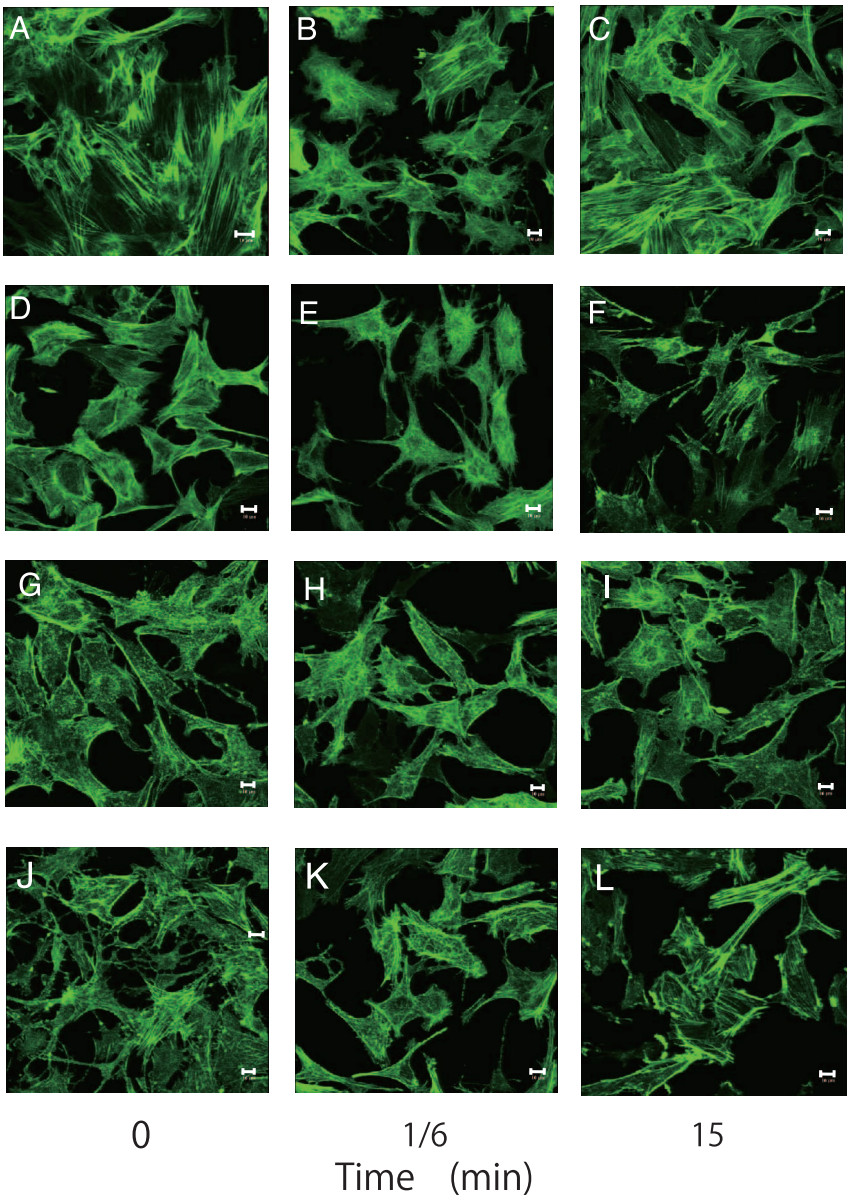

Fig. 4. Effects of exposure to the time-varying 1.5 T magnetic field for 2 hr on the F-actin in chromaffin cells. Cells were preincubated with disruptors for 30 min. At zero time, cells were diluted under hypotonic condition (177 mOsM) in the presence or absence of the disruptors. F-actin fibers were stained with alexa Fluor 488 phalloidin, and observed by confocal laser scanning microscopy. A-C, control ; D-F, 2 hr-exposure ; G-I, 1 μM cytochalasin D ; J-L, 0.1 μM latrunculin B ; (A,D,G,J), zero time ; (B,E,H,K), 10 sec after medium change ; (C,F,I,L), 15 min after medium change. Bars, 10 μm.

Effects of the magnetic field exposure on the F-actin content

Quantification of intracellular F-actin contents was via a rhodamine-phalloidin method (see Materials and Methods) in Fig. 5. Two hour-exposure to the magnetic field and addition of cytochalasin D or latrunculin B affected morphology (Fig. 4) and reduced F-actin content at zero time (Fig. 5). In an unpublished study, Western blotting showed total actin content was constant, but magnetic exposure increased G-actin content to a level of about 12% more than in control cells. Replacing isotonic medium with hyposmotic medium caused significant rapid decrease in F-actin content in the control at 10 sec. This F-actin content tended to increase 15 min after the medium was changed, but the increase was not significant. In exposed and cytochalasin D-treated cells, F-actin content was significantly decreased at 10 sec and the content further decreased up to 15 min. Adding latrunculin B markedly reduced F-actin content and an extremely low level was maintained for 15 min after hypotonic stress. The data suggest that initial decrease corresponded

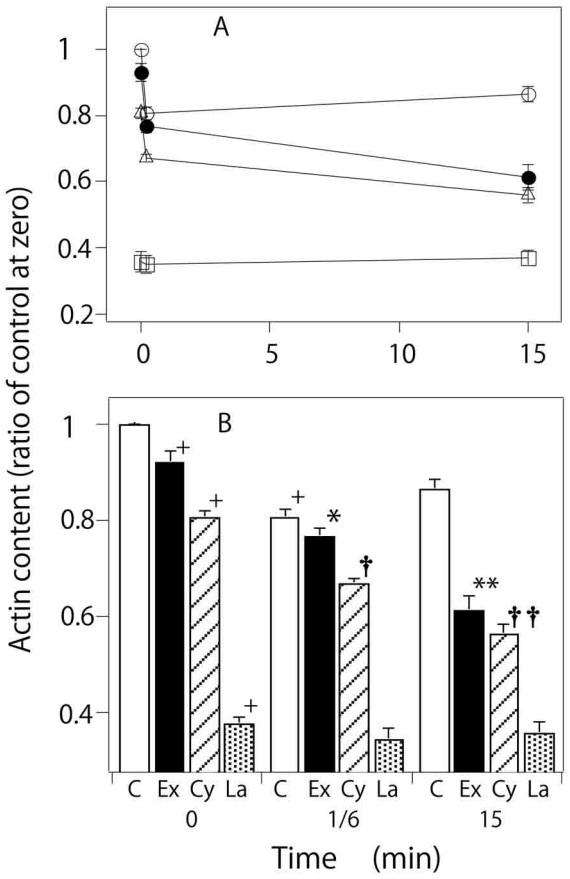

Fig. 5. Effects of exposure to the time-varying magnetic field and actin disrupters on the intracellular F-actin contents in a hyposmotic medium (177 mOsM). After cells were preincubated with the disruptors for 30 min, the medium was diluted at time zero to a hyposmotic condition in the presence or absence of disruptors. A. Time-dependent changes in intracellular F-actin content. B. The F-actin content replotted from data in A. (A) open circles, control ; open triangles, 5 μM cytochalasin D ; open squares, 1 μM latrunculin B ; closed circles, exposure. (B) C, control ; Ex, exposure ; Cy, 5 μM cytochalasin D ; La, latrunculin B. +, significantly different from control at 0 min at $P< 0.05$. *, significantly different from exposure at 0 min at $P< 0.02$. **, significantly different from the exposure at 10 sec at $P< 0.01$. †, significantly different from cytochalasin D at 0 min at $P< 0.01$. ††, significantly different from cytochalasin D at 10 sec at $P< 0.05$. Points and bars are means and S.E. values for four culture dishes.

to increase in cell volume and that subsequent changes in F-actin content related to return to initial value in hyposmotic medium. In particular, decrease in F-actin content after 10 sec with both magnetic field exposure and addition of cytochalasin D indicated delay in return to or lack of recovery to initial value.

Effects of the magnetic field on the intracellular Ca^{2+} concentration

Study of the effect of magnetic field-exposure on Ca^{2+}-dependent ion channel activity involves preventing increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). In control cells, $[Ca^{2+}]i$ increased instantly after medium osmolarity reduced and then decreased with time. This temporary increase in $[Ca^{2+}]i$ affected volume changes in the hyposmotic medium via activating Ca^{2+}-dependent channels, but magnetic field exposure did not significantly affect changes in $[Ca^{2+}]i$ (Fig. 6A). When Ca^{2+}-free medium changed to calcium free hypotonic medium, $[Ca^{2+}]i$ increased only slightly but concentration was not influenced by magnetic field exposure (Fig. 6B). The same RVD also occurred in a Ca^{2+}-omitted hyposmotic medium such as a Ca^{2+} containing medium (data not shown). Such results suggest that magnetic field exposure did not affect intracellular Ca^{2+}-dependent K^+ or Cl^- channel activity via inhibiting $[Ca^{2+}]i$ increase. $[Ca^{2+}]i$ increase due to hyposmotic stress was probably due to Ca^{2+} influx via a Ca^{2+} channel rather than due to intracellular Ca^{2+} stores.

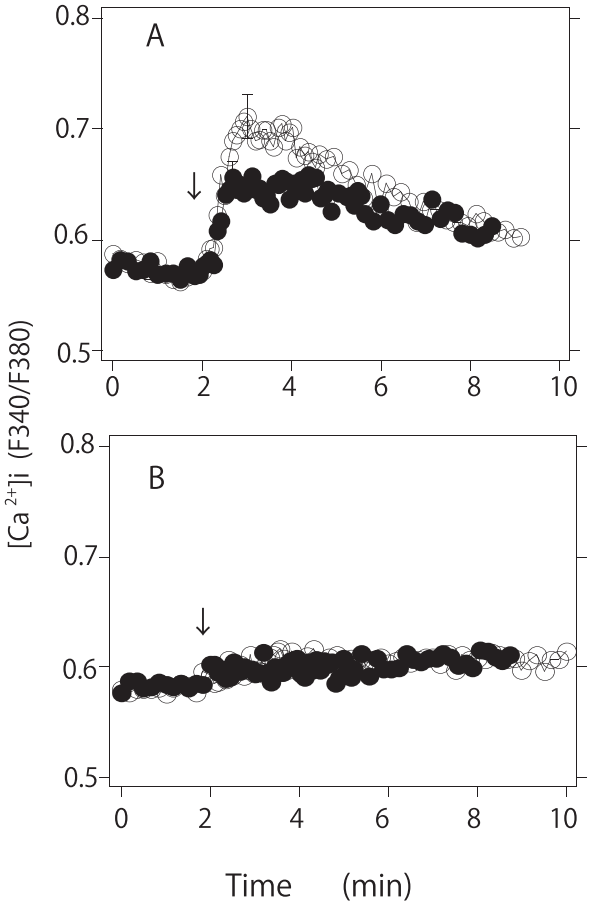

Fig. 6. Effects of exposure to the time-varying 1.5 T magnetic field on the hypotonic stress (177 mOsM)-induced transient increase in the $[Ca^{2+}]i$ of chromaffin cells. (A) Ca^{2+} containing medium. Open circles, control (n=11) ; closed circles, exposure (n=20). (B) Ca^{2+}-free medium plus 1 mM EGTA. Open circles, control (n=19) ; closed circles, the exposure (n=10). Points and bars are means and S.E.. The numbers in parentheses indicate the numbers of cells measured. Arrows show the dilution time.

DISCUSSION

Exposure to a time-varying magnetic field affected intracellular Ca^{2+} release from ER (endoplasmic reticulum) that results from adding neurotransmitters or other stimulators (27, 28). Exposure inhibited functions of ER, mitochondria and actin protein [unpublished data] and this inhibition was because of actin protein and not because of influences on mitochondria. Cytoskeletal proteins (especially F-actin) closely relate to volume regulation of cultured cells in hyposmotic media and in hypertonic media (12). Hyposmotic media for chromaffin cells were replaced to try to confirm if exposure affected volume regulation via influence on cytoskeletal proteins in those media.

Actin proteins affect volume regulation in anisotonic media. Channel activation and volume control employ some signal factors that depend on an intact actin skeleton (29). The transient receptor potential vaniloid 4 (TRPV4) channel is a non-selective cation channel and is a sensor of flow and osmolarity that seems to link F-actin and RVD in HaCaT keratinocytes (30). Intracellular cytoskeletal protein contains actin, tubulin and intermediate filaments, but some reportsshow actin link to RVD instead of microtubules. F-actin depolymerization due to cytochalasin inhibits RVD response by hypotonicity, but microtubule disassembly by colchicine does not affect RVD (1, 31). Normal RVD response requires an intact microfilament network, but microtubules do not significantly affect RVD in Ehrlich cells (32). In leukocytes, microtubule disruptors inhibit RVD but actin disruptors do not affect RVD (33). Such evidence suggests that microtubules or actin filaments are important in RVD after normal medium replaces with hypotonic medium. In fact, hypotonic challenge is associated with decreased F-actin content and hypertonic challenge is associated with increased F-actin content. These contents did not recover to initial level before medium change in Ehrlich cells or HEK 293 cells (2, 12). After hyposmotic challenge, F-actin decrease returned to the baseline in anulus fibrosus cells (34).

The present study provided visual evidence that, after replacement with hypotonic medium, stained F-actin clearly decreased temporarily and then returned to normal in control cells (Fig. 4). Hypotonic stress initially decreased intracellular F-actin content in chromaffin cells and then caused content to increase, but not significantly (Fig. 5). Two hour exposure to the magnetic field before hypotonic challenge significantly decreased fibrous actin content compared to the control, but hypotonic challenge further reduced actin content and that decrease continued for 15 min after the challenge. Results in control cells confirm that initial decrease in F-actin content after hypotonic stress occurred in many types of cells according to some reports mentioned previously, but recovery to normal level was not always observable. In other words, recovery to normal volume does not correspond perfectly to F-actin content recovery.

Based on results related to exposed cells, hypotonic stress may further depolymerize F-actin that was depolymerized by exposure. Even if most F-actin was depolymerized by exposure or through adding a disruptor, volume recovery was not suppressed as in cells treated with an ion channel inhibitor. F-actin appeared to control changes in cell volume or morphology but did not closely related to recovery from swelling. One report suggests that F-actin distribution in cytoplasm relates to cell volume regulation and that activating volume sensitive anion channels during RVD requires distribution of peripheral F-actin but not distribution of perinuclear F-actin (13). Intracellular F-actin distribution change may be a reason why exposure to a magnetic field affects RVD in chromaffin cells.

Cytochalasin D does not affect basal Cl^- conductance under isotonic conditions but attenuates Cl^- current caused by hypotonicity in bronchial epithelial cells and HSG cells (35, 36). Such evidence indicates that actin protein modulate ion channel activity caused by hypotonic stress. But actin protein does not perfectly modulate ion channel activity caused under hypotonic condition because RVD is not perfectly inhibited by cytochalasin D as in media containing channel inhibitors. The magnetic field probably affects F-actin fibers mainly because effect of the magnetic field on RVD is very similar to that of cytochalasin D on the volume regulation.

There are reports that some magnetic fields affect actin protein-related functions, organelles, etc. A 50 or 60 Hz ELF magnetic field causes morphological changes in F-actin filaments and mitochondria in adenocarcinoma cells (2 mT) (16), causes morphological changes in cell shape and F-actin fibers (production of large stress fibers) in keratinocytes (2 mT) (37), and rearranges actin microfilaments in 78% of all cells (2 mT) (38). A weak static magnetic field (6 mT) modifies cell shape and actin filaments of U937 cells (39). Such studies indicate that exposure to low magnetic fields in the order of 1-6 mT

also affect actin protein related to RVD.

There are reports regarding exposure to a strong magnetic field of more than 0.7 T (a level similar to that used in the present experiment) on physiological functions of nerve or other cultured cells. Central nervous system neurons are evoked by repetitive transcranial magnetic stimulation (1-3 T) (11). Motor cortex functions can be investigated by simultaneous recording of this evoked potential (40). These studies show that exposure to magnetic field at high magnetic flux density depolarizes nerves or nerve cells. Symptoms of cell death due to stress are observed in exposure to a 5 T static magnetic field in rat cortical neurons (41). Quantitative analysis of cytoskeletal protein reveals significant decrease in actin stress fibers following exposure to a static magnetic field at 7 T (17). Such reports support the idea that strong or time-varying magnetic field disrupts the actin cytoskeleton and that the disruption delays the volume recovery in cultured chromaffin cells. Human glioblastoma cells embedded in collagen gel have been oriented perpendicular to the direction of a 10 T static magnetic field (42). But, at the level of 1.5 T, magnetic orientation is not observable in chromaffin cells that are already attached to a culture dish (Fig. 4).

$[Ca^{2+}]i$ is a regulating factor of ion channel activity or cell volume. Osmality reduction leads to RVD and increases $[Ca^{2+}]i$ via Ca^{2+} influx (43). However, exposure to the magnetic field did not affect Ca^{2+} influx $[Ca^{2+}]i$ in the presence or absence of medium Ca^{2+}(Fig. 6). Data suggest that temporary Ca^{2+} increase due to hypotonic stress was due mainly to Ca^{2+} influx via Ca^{2+} channels during RVD. If magnetic field exposure indeed affected Ca^{2+}, RVD would not occur in hyposmotic stress conditions.

A time varying magnetic field induces eddy currents and electric fields in a culture medium and in the cytoplasm of cultured cells. Electric fields, currents and surface charge distribution induced by time-varying magnetic fields appear to have biological effects (44). Currents induced in cells are estimated to be much lower than currents induced in the medium because of cell diameters. Inhibition of cell volume regulation due to exposure could relate to eddy currents induced in the culture medium. However, eddy currents cannot directly cause inhibition of volume regulation because the currents cannot easily penetrate the cell membrane. Apparently, eddy currents induced in the medium change certain properties of the cell membrane and finally affect cell volume regulation by hyposmotic stress through changes in actin protein.

Exposure to a time-varying magnetic field increased peak value after reduction of medium osmolarity and delayed the time needed for return to initial value. Exposure to the magnetic field did not affect transient increase in $[Ca^{2+}]i$ caused by hypotonic stress. Magnetic field exposure and addition of cytochalasin D greatly affected RVD, but unlike $BaCl_2$, there was imperfect inhibition of return to initial cell volume. Results suggest that 2 hr-exposure to a magnetic field inhibited the RVD induced by hypotonic stress via stimulation of F-actin disruption in adrenal chromaffin cells.

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