

Effects of electromagnetic fields on membrane ion transport of cultured cells

Toshitaka Ikehara^{*}, Hisao Yamaguchi^{*}, and Hiroshi Miyamoto[†]

^{*}*Department of Physiology, The University of Tokushima School of Medicine, Tokushima, Japan ;*
and [†]*Department of Life, Environment and Information, Faculty of Domestic Science, Tokushima Bunri University, Tokushima, Japan*

Abstract : We have studied the mechanisms of ion transport mediated by Na⁺/K⁺-pump and Na⁺,K⁺,Cl⁻-cotransport pathway of HeLa cells using Rb⁺ as an analog for K⁺, and proposed models of binding of ions for the transport pathways. Also, we clarified the relation between ion and water movements in the cells. Based on these findings, we have studied the effects of homogeneous and time-varying magnetic fields on the ion transport activity. The research presented here covers (i) brief explanations of our kinetic studies on the ion transport pathways for promoting understanding of the effects of magnetic fields on the pathways, (ii) our and other reports of the effects of magnetic fields on ion transport systems. *J. Med. Invest.* 45 : 47-56 1998

Key words : magnetic field, Na⁺,K⁺ pump, K⁺ channel, Ca²⁺ movement, Na⁺,K⁺,Cl⁻-cotransport, volume regulation

INTRODUCTION

Cellular K⁺ transport takes place mediated by pathways in the cell membrane. In the present paper, we reviewed mechanisms of the major pathways of K⁺ transport in the cell membrane, and the biological effects of exposure to magnetic or electromagnetic fields on the functions of these pathways.

The pathways consist of Na⁺,K⁺ pump, Na⁺,K⁺, Cl⁻-cotransport pathway and K⁺ channels as well as other ion channels. In many cultured cells, K⁺ influx is chiefly mediated by the Na⁺,K⁺ pump (ouabain-sensitive), and so the pump activity strongly influences the intracellular concentrations of monovalent ions and membrane potential. The presence of Na⁺,K⁺,Cl⁻-cotransport pathway has been reported in a variety of cells, and the cotransport system is known to be specifically inhibited by certain diuretics such as bumetanide and furosemide. These agents are known to suppress Na⁺ reabsorption at the ascending limb of Henle's loop of the kidney. Ca²⁺

dependent K⁺ channels are present in various types of excitable and non-excitable cells. Activity of the K⁺ channels contributes to electrical characteristics and many functions of these cells. K⁺ flux through the cotransport pathway and K⁺ channel is known to be activated when many types of cells are incubated in hypotonic or high K⁺ medium.

There have been many reports on the biological effects of static or DC magnetic fields and time-varying magnetic or electromagnetic fields on membrane cation transport and related functions. These studies indicate that the effects of exposures to various magnetic fields are not identical. While some reports show an inhibitory effect by the fields, others show activation, and still others no significant influences on the cation transport. In recent years, many investigators have tested the effect of exposure to a magnetic field on Ca²⁺ influx across the cell membrane or intracellular Ca²⁺ movements.

The research reviewed here covers studies on the kinetics of membrane ion transport and the magnetic field exposure-induced effects on ion transport. We also discuss interactions of magnetic field-exposure with signal transduction events at the cell surface and in cytosol.

Received for publication July 17, 1998 ; accepted July 31, 1998.

¹ Address correspondence and reprint requests to Hisao Yamaguchi, Ph.D., Department of Physiology, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-886-33-9443.

METHODS

Electromagnet

The magnetic fields we used were produced by an electromagnet designed and set up by Hitachi Metal Indust. Co. (Tokyo, Japan). The apparatus consisted of a couple of vertically arranged coils attached with pole pieces of round polar faces with a large and a small diameter of 10 and 5 cm, respectively (Fig.1). In proportion to coil current, static magnetic fields of flux densities of 0.3 to 1.7 T were produced in the gap between the large polar faces. Similarly, a magnetic field of about 2 T at maximum was produced with the small polar faces.

Time-varying magnetic fields were produced by automatic switching of the coil current with an electronic device, which enabled us to change the magnetic flux density from 0.07 to 1.7 or 2 T in about 1 s (using the large or small polar faces). Time intervals of the changes were chosen to be 3 s or longer, since it took more than 2 s for eddy current to return to zero after switching off. The density of eddy current induced by switching on was 32 and 57 mA/m², the maximum value; and that for switching off was -13 and -17 mA/m² respectively, the minimum value, when the large polar faces were used (1).

Microfluorometry.

Changes in the electrical charge on the cell surface that occurred when cells were exposed to the time-varying magnetic fields were monitored with a fluorescent pH indicator, 4-heptadecyl-7-hydroxycoumarin, by a slightly modified method

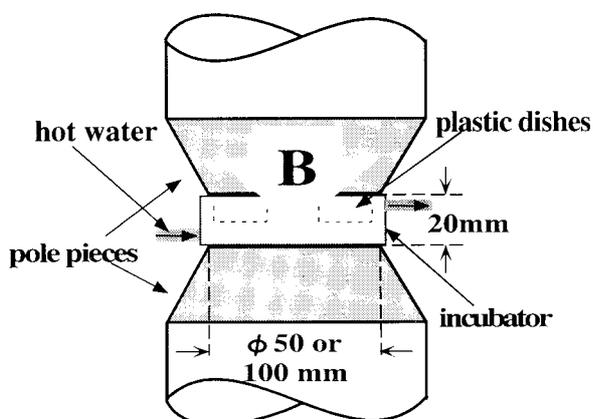


Fig.1. Electromagnet and special incubator. The electromagnet consisted of two vertically arranged coils. A pair of poles with round polar faces of small (50 mm) or large (100 mm) diameter are attached to the coils. A special incubator to contain four culture dishes was placed in the gap between the poles of the electromagnet. The temperature of the medium in the culture dishes was kept at 37°C in this incubator.

of Pal et al. (2). Membrane potential of cells was assayed with a fluorescent agent, 3,3'-dipropylthiadicarbocyanine iodide (diS-C3-(5)), by a modified method of Wright et al. (3). The intracellular Ca²⁺ concentration was determined and analyzed with a fluorescent probe, 1-(2-(5'-carboxyl-oxazol-2'-yl)-6-aminobenzofuran-5-oxo)-2-(2'-amino-5'-methylphenoxy)ethene-N,N,N',N'-tetraacetic acid, pentaacetoxymethyl ester (Fura 2-AM) by the method of Grynkiewicz et al. (4).

K⁺ transport measurement

First, the usefulness of Rb⁺ as an analog of K⁺ in membrane transport was tested. Extracellular K⁺ (K⁺e) was totally or partly replaced with Rb⁺, and movement of the cation across the plasma membrane was investigated in HeLa cells. Cells were proven to transport Rb⁺ to the same extent equally as they did K⁺ (5), and Rb⁺ and K⁺ uptakes were almost equally sensitive to various concentrations of ouabain (5) or furosemide (6). These cations were shown to bind to the external K⁺ site on the ouabain-sensitive pump with the same affinities (7), and a similar result was obtained for the furosemide-sensitive pathway (6). Based on the results, we used non-radioactive Rb⁺ as a tool to investigate membrane ion transport in HeLa cells. Fig.2 is a schematic presentation of the major pathways of K⁺ influx through the membrane of HeLa cells. About 50-70% of the total Rb⁺ uptake was inhibited by ouabain and about 70-80%

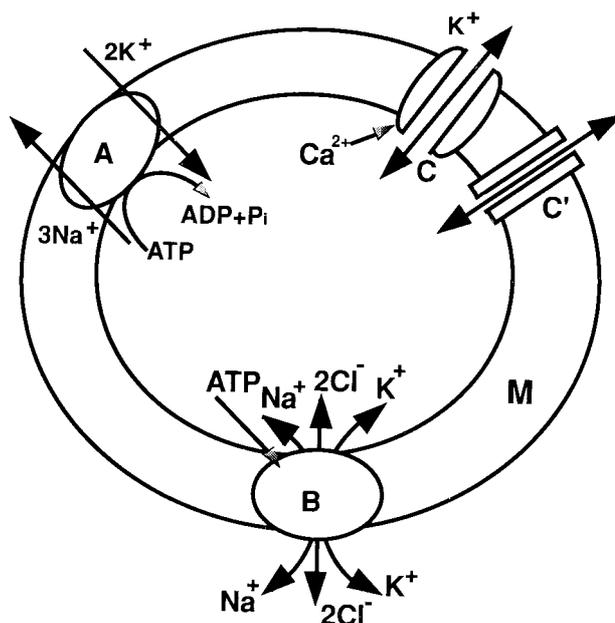


Fig. 2. Major K⁺ pathways of the membrane of HeLa cells. A, Na⁺,K⁺-pump (ouabain-sensitive); B, Na⁺/K⁺/2Cl⁻-cotransport (furosemide-sensitive); C, Ca²⁺-dependent K⁺ channel (quinine-sensitive); and C', Ca²⁺-independent ion channels. M: membrane.

of ouabain-insensitive Rb^+ uptake was inhibited by furosemide, indicating that about 50-70% of the Rb^+ uptake was mediated by the Na^+ -pump, and about 20-40% by the cotransport pathways. The fraction of the total K^+ influx mediated by K^+ channels was not determined, since K^+ permeability could be measured exactly only by the patch-clamp technique.

MECHANISMS OF ION TRANSPORT

Na⁺, K⁺ pump

Kinetic parameters and mechanism of the active cation transport in HeLa cells have been studied and the presence of two external K^+ sites per Na^+ / K^+ -pump demonstrated. The affinities of the K^+ sites for Rb^+ as a congener of K^+ are almost the same with that of K^+ . Na^+ e inhibits ouabain-sensitive Rb^+ influx competitively, whereas intracellular K^+ (K^+ c) is not inhibitory. Na^+ and K^+ are exchanged alternately through the pump by a binary mechanism (7). We have proposed a model system transporting the two solutes in consecutive sequence. Similarly, Sachs (8) has concluded that the kinetic behavior of the Na^+ pump considered as a bisubstrate reaction with Na^+ and K^+ as substrates can be adequately described by a consecutive model with the modification that the Na^+ binding sites can return from outside to inside even though no Na^+ is binding. The active Rb^+ influx can also be supported by oxidative metabolism and is regulated as a function of the cellular ATP content up to the control level, and 2 Rb^+ ions move concomitantly at the expense of one ATP molecule (9).

Na⁺, K⁺, Cl⁻-Cotransport

The Na^+ gradients generates the driving force for symports and antiports of many essential substances. One of the Na^+ -dependent symports is electrically silent Na^+ , K^+ , Cl^- cotransport known to be important for Na^+ reabsorption in Henle's loops of the renal tubules. This cotransport pathway is specially sensitive to loop diuretics such as furosemide, bumetanide, and piretanide. Furosemide slightly but definitely inhibited Rb^+ uptake in HeLa cells, but K^+ efflux was not influenced (5); showing asymmetry of the ion movements on cis and trans sides of the membrane, different from results reported for mouse 3T3 cells (10). Transmembrane gradients of the chemical potentials of Na^+ could be the primary driving force of the furosemide-sensitive Rb^+ influx (JRb) (11).

We have examined JRb into HeLa cells as functions of extracellular Rb^+ , Na^+ and Cl^- concentrations. Rate equations and kinetic parameters, including the apparent maximum JRb, the apparent values of K_m for the three ions and the apparent K_i for K^+ , were derived. We proposed a general and a specific furosemide-sensitive cotransport model. According to the model, one Na^+ and one Cl^- bind concurrently to their sites followed by one K^+ (Rb^+) and another Cl^- . After completion of the ion bindings, Na^+ , K^+ (Rb^+) and Cl^- show synchronous transmembrane movement in a ratio of 1 : 1 : 2. One unit molecule of this furosemide-sensitive cotransport system has one Na^+ , one K^+ and two Cl^- sites with different affinities (6). In different types of cells, Na^+ , K^+ and Cl^- are also transported across the cell membrane at a stoichiometry of 1 : 1 : 2 (12-14), and some kinetic parameters for the diuretic-sensitive cotransport in relation to extracellular ion have been estimated (15, 16).

The cotransport of HeLa cells has also been studied by measuring JRb as a function of the cellular ATP and the external Rb^+ and Na^+ concentrations. ATP stimulates the cotransport by increasing the affinity of the cotransport pathways, and two ATP molecules are needed for uptake of one Rb^+ by the cotransport pathway. Again, we have proposed a general and a specific model with respect to cellular ATP, and stated that cyclic AMP has no effect on the cotransport in HeLa cells (17).

The effects of K^+ c and Na^+ c on the cotransport pathways were also studied. Extracellular K^+ stimulates and Na^+ inhibits JRb, without any change in the affinities of the pathway for Rb^+ e and Na^+ e (18). Rb^+ uptake occurs when Rb^+ binds to the external K^+ site of the pathway after binding of internal K^+ to its regulatory site. In contrast to HeLa cells, an increase in Na^+ c or decrease in K^+ c stimulates JK (furosemide-sensitive K^+ influx) in the absence of external Na^+ in human erythrocytes (15). Furosemide-sensitive $^{86}Rb^+$ influx into human erythrocytes in vivo is negatively correlated with the concentration of external Na^+ e (19).

Cell Volume Regulation in Relation to K⁺ Channel

Addition of nystatin (non-voltage dependent channel forming ionophore) causes reversible changes in the cellular Na^+ and K^+ contents and cell volume of HeLa cells (20). These changes are inhibited by sucrose, and Donnan and osmotic equilibria are attained. The K^+ content, the cellular free- Ca^{2+} concentration ($[Ca^{2+}]_i$) and the cell volume of HeLa cells

increase after a latent period following change to an isosmotic Na⁺-free, high K⁺ medium (21). When [Ca²⁺]_i was extremely reduced by preloading BAPTA-AM, the K⁺ uptake decreased to the level reduced in the presence of quinine. The quinine-sensitive pathway depends on cellular Ca²⁺ and its major component is Cl⁻-dependent, whereas the quinine-insensitive pathway is independent of the Ca²⁺ and unselective for anions (22).

When medium osmolality is decreased, suspended HeLa cells instantly swell and then K⁺ loss and regulatory volume decrease (RVD) occurs, while attached HeLa cells instantly swell and then rapidly lose water and K⁺, before slowly gaining them again (23). Hyposmotic stress induces net water loss from attached cells, associated with K⁺ release through the Ca²⁺-dependent K⁺ channel. The initial increase in K⁺_c is a phenomenon related to osmotic water movement toward Donnan equilibrium, whereas the regulatory K⁺ decrease is caused by K⁺ efflux through Ca²⁺-dependent K⁺ channels.

We have demonstrated by patch clamp technique that the K⁺ channel of HeLa cells has characteristics of strong inward rectification, small conductance, and dependence on intracellular Ca²⁺ (24, 25). The inward K⁺ currents through the channel at fixed membrane potentials are highest in early G₁ and then decrease with time to a minimum in the S phase, increasing again in the M phase. The capacitance and the permeability change with progression of the cell cycle. We did not show the presence of other types of K⁺ channels in HeLa cells.

EFFECTS OF MAGNETIC FIELDS ON ION TRANSPORT

Effects of magnetic fields on Na⁺ pump.

Based on the characteristics of the membrane of HeLa cells stated above, we tested the effects of different magnetic fields on cation transport of the cells. However, no significant effects on ouabain-sensitive (active) and-insensitive (passive) Rb⁺ influx into HeLa cells on exposure to homogeneous static magnetic fields of flux density 0 to 1.6 T were detected at 37°C (26). There were no significant effects on either Rb⁺ influxes of the static magnetic field of 1.5 and 2T at different temperatures from 15 to 42°C, and so evidence for the membrane phase transition was not found in this temperature range (26, 27). The Arrhenius plot of the data showed no significant influence of the exposures to the activation energy (28).

The mean activity of Na⁺, K⁺ ATPase and Ca²⁺ ATPase of rat diaphragm muscle homogenates is reported to be significantly higher in the static magnetic field (0.02T)-exposed group, but that of Mg²⁺ ATPase is not significantly changed (29).

Next, we tested the effect on the ouabain-sensitive Rb⁺ influx of exposure to the strong time-varying magnetic fields of 1.7T maximum. When durations of the periods of "switching-on" and "switching-off" of coil current were limited to 1 min or longer, there was no significant effect of the magnetic field (1). However, when these times were shortened to 3 sec, the Rb⁺ influx was suppressed significantly. The inhibition of the active Rb⁺ influx increased with time during exposure for 2 hr. It is likely that the inhibition of Na pump by exposure to the time-varying magnetic field has a close relation to change in the surface properties of the cell membrane.

With respect to the effect of time-varying magnetic fields on active cation transport, ouabain-sensitive Na⁺ fluxes across stripped rabbit colon epithelium have not significantly been influenced by pulsed magnetic fields (30).

Na⁺, K⁺, Cl⁻-Cotransport

We could not show any significant effect of the static magnetic field at less than 2T on ouabain-insensitive Rb⁺ influx, suggesting no significant effect of the magnetic field on Na⁺, K⁺, Cl⁻-cotransport (1). No significant influence of pulsed magnetic field on the furosemide-sensitive ⁸⁶Rb⁺ influx into human erythrocytes has been reported (30).

Effects of magnetic fields on K⁺ channel and Ca²⁺ influx.

K⁺ channel activity plays an important role in producing the resting membrane potentials. We tested the effects of the strong time-varying magnetic field on K⁺ uptake of HeLa cells incubated in an isosmotic high K⁺ medium. The uptake was demonstrated to take place mainly mediated by Ca²⁺-dependent K⁺ channel, since specific inhibitors of the channel such as quinine, charybotoxin and iberitoxin significantly suppressed the uptake (31). It is known that cells are depolarized in the high K⁺ medium, and the intracellular Ca²⁺ concentration ([Ca²⁺]_c) is increased. However, the exposure to the magnetic field significantly reduced cellular K⁺ uptake and completely suppressed the increase in cell Ca²⁺ in the high K⁺-medium. The inhibition of K⁺ uptake was not restored by addition of Ca²⁺ ionophore ionomycin. These results suggest that exposure-induced inhibition of the K⁺ channel activity was related to inhi-

bition of intracellular Ca^{2+} mobilization. Furthermore, the results suggest that cell swelling in the high K^+ medium can be significantly suppressed by the exposure. This is the first study to report the effects of magnetic fields on K^+ channel activity.

Effects of Magnetic Fields on Molecular Events during Receptor-Mediated Signal Transduction including Ca^{2+} Uptake.

Ca^{2+} signaling is well documented in many types of cells as a part of the second messenger system. Recently, the number of reports on the effects of magnetic fields on Ca^{2+} signal transduction has increased. For example, ELF magnetic field exposures have no effect on concanavalin A-stimulated calcium uptake in rat thymocytes (32). There is no biologically significant effect of weak combined dc-ac magnetic fields on the binding of calcium to calmodulin and MLCK (myosin light chain kinase) activity (33). But, other reports show the influences of exposures to various magnetic fields. During exposure to an ELF field (50 Hz, 0.1 or 0.15 mT) an acute response is observed with oscillatory increases in intracellular Ca^{2+} and inositol 1,4,5-triphosphate (IP_3) levels in T cells or Jarkat cell line, which subsides when the magnetic field is turned off (34-36). Similarly, $^{45}\text{Ca}^{2+}$ uptake by lymphocytes increases during exposure to another ELF field (16 or 60Hz) (37,38). Low amplitude combined magnetic field is capable of increasing net Ca^{2+} flux in human osteoblast-like cells and the increase is frequency-dependent (39). In lymphocytes, an application of a low frequency electric field exerts little influence on the early release of Ca^{2+} from intracellular stores in Ca^{2+} -free buffer, but enhances Ca^{2+} influx through the plasma membrane in the presence of external Ca^{2+} (40). The magnetic field affects membrane depolarization, increases action potential discharge and reduces uptake of Ca^{2+} into ganglia and isolated neurons of *Helix pomatia* (41).

Effects of Magnetic Fields on Membrane Electrical Properties.

Membrane electrical properties such as membrane surface charge, membrane potential and so on may be directly influenced by eddy current induced by changing the flux density of magnetic field. There have been studies testing the effects of static or dc magnetic fields on some of the electric properties. Rat diaphragm has shown less negative resting potential, and the amplitude and overshoot of action potential is significantly decreased upon exposure

to a static magnetic field (29). Exposure to another static magnetic field of magnetic gradient of about 1 mT/mm blocks more than 70% of the action potential of sensory neurons in culture (42). When a mammalian cell line U937 is exposed to a pulsed magnetic field of peak flux density of 0.63mT, cells exhibit increased negative surface charge, whereas membrane hydrophobicity is not significantly altered (43). In our study, microfluorometry of HeLa cells loaded with a fluorescent pH indicator 4-heptadecyl-7-hydroxycoumarin (Fig.3) and a membrane potential indicator diS-C3-(5) (Fig.4) revealed increases in the negative surface charge during exposure to the strong time-varying magnetic fields. Simultaneously observed change in the K^+ (Rb^+) fluxes would closely relate to the change in the membrane electric properties (1). In contrast, myogenic cells exposed to a static magnetic field show no changes in membrane electrical parameters such as conductivity and permittivity (44).

Other Results related to the Effects of Magnetic Fields.

Static magnetic fields have been reported to affect the diffusion of biological particles in solutions by inducing Lorentz force or Maxwell stress. Lorentz force would influence the diffusion of charged particles

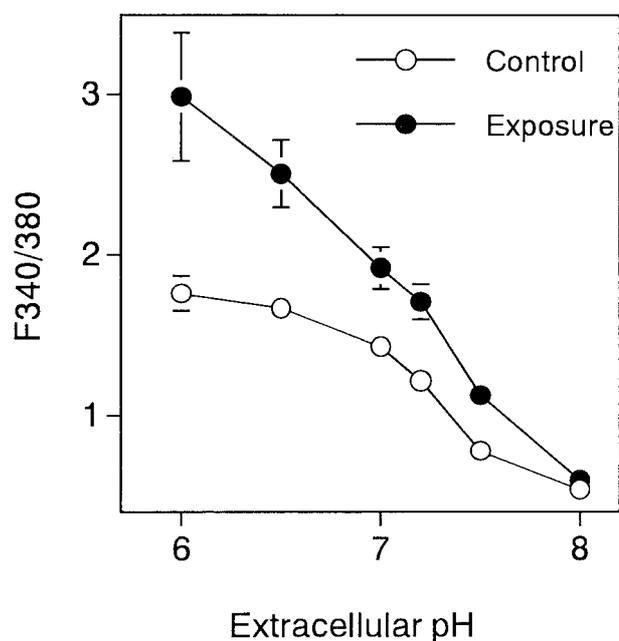


Fig.3. Effects of 2hr exposure to the time-varying strong magnetic field on fluorescence emission of HeLa cells loaded with a pH indicator 4-heptadecyl-7-hydroxycoumarin. Ratios of emissions determined at excitation wavelengths of 340 and 380 nm are shown at different medium pH (6-8). The "on-time" and "off-time" obtained by automatic switchings of coil currents were the same, i.e.,3sec. Values are means \pm SD for at least 30 cells. The differences of the ratios of emissions between control and exposed cells were significant in the pH range ($p < 0.01$).

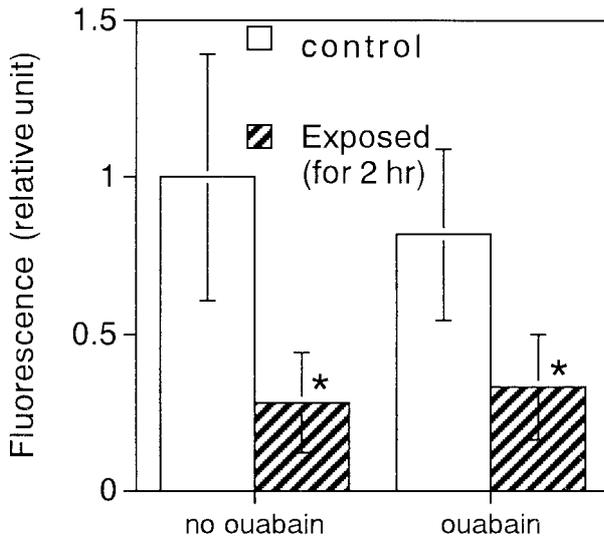


Fig.4. Effects of 2hr exposure to the time-varying magnetic field on fluorescence emission of HeLa cells loaded with a membrane potential indicator diS-C3-(5). The "on-time" and "off-time" were the same as those shown in Fig.3. Values are means \pm SD for at least 30 cells. The differences of the emission between exposed and control cells were significant ($p < 0.01$).

such as various ions including plasma proteins (45). In fact, it has been reported that changes in electrical conductivity of CaCl_2 solution are caused by exposure to static magnetic fields (2.3-350mT) (41).

Recently, we showed that ELF magnetic field (80 mT and 50 Hz) affected the molecular structure of membrane protein in HeLa cells, as determined by Fourier transform infrared spectroscopy (46). The exposure-induced modification of membrane protein structure may be related to the change in membrane electrical properties. Santoro et al. have reported a decrease in membrane fluidity and re-organized cytoskeletal components on exposure to ELF magnetic field in human B lymphoid cells (Raji) (47). Therefore, ELF magnetic fields would influence the structure of protein molecules composing the biomembrane.

Positive and negative data have been published on the effects of various magnetic fields on ion transports or membrane properties related to the transports. Liburdy has shown that the effects of ELF magnetic fields are due to induced electric fields rather than flux density (40). We basically agree with this. So, electric field or current induced by time-varying magnetic field could affect more strongly ion transports in the magnetic field. Strong eddy current in the medium induced by changes in the flux density could affect electric properties of the cell surface, but the current would not penetrate into the cells since the cell bilayer act as an electrical insulator.

A low frequency magnetic field in combination with a static magnetic field increases the number of receptors of the type II insulin-like growth factor (IGF-II) on the membrane of osteosarcoma cells(48). This result supports our hypothesis.

On the other hand, according to Faraday's law, the current induced by the magnetic fields inside the cells is far weaker than the one induced in the extracellular space. But, the possibility of a direct effect of eddy current in the cells cannot be totally excluded. Because, we found that the increase in $[\text{Ca}^{2+}]_i$ is strongly inhibited in cells incubated in a Ca^{2+} -free medium by the time-varying magnetic field.

Liburdy (49) has detected an increase in calcium uptake into mitogen-stimulated rat thymocytes (mature) and human lymphocytes during exposure to 60 Hz ELF field or high-field NMR (Nuclear Magnetic Resonance). As NMR fields contain a time-varying magnetic field, this result implies that the time-varying field of NMR is an operative component responsible for the effect on calcium transport.

Next, we concerned ourselves with a steady component of magnetic fields of NMR and MRI (NMR imaging system) clinically applied. The maximum flux density of the steady component of high-field MRI is about 2T, similar to that produced by our electromagnet. The static magnetic field was shown not to affect either ouabain-sensitive or-insensitive K^+ uptake in HeLa cells. Many investigators have also shown insignificant influences on various cellular functions of exposure to a similar magnitude static or DC magnetic field. However, MRI magnetic fields contain a non-homogeneous (gradient) component in addition to homogeneous static and time-varying components. Further investigation is needed on the effects on cellular functions of the gradient field that exerts Maxwell stress.

CONCLUSION

Figure 5 depicts the molecular events during receptor-mediated signal transduction in cultured cells, and also indicates the possible places that could be affected when these cells are exposed to magnetic fields. We propose these sites based on our data and those of others and describe them in detail in the legend to Fig.5.

Incubation in an isotonic high K^+ medium depolarized the membrane of HeLa cells, and the depolarization would increase the intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ by Ca^{2+} influx through Ca^{2+} channels of the

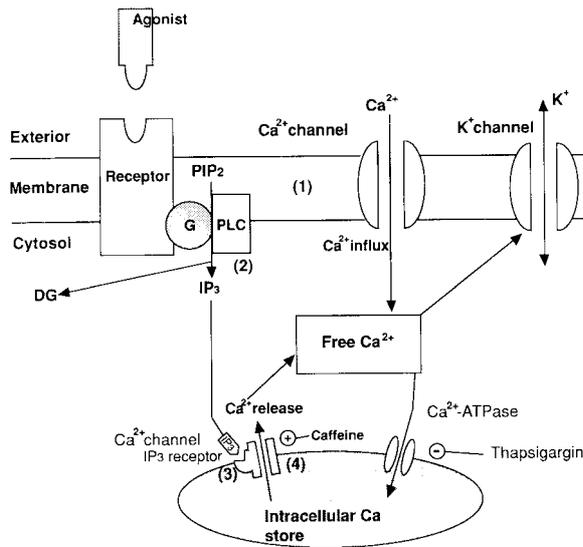


Fig.5. Typical molecular events of signal transduction in a cultured cell. Intracellular free Ca²⁺ increases by both Ca²⁺ influx through potential-dependent Ca²⁺ channels and Ca²⁺ release from intracellular Ca²⁺ stores. In the latter case, agonist binding to its receptor of the cell membrane initiates events mediated by the phosphatidylinositol pathway. Phospholipase C (PLC) is activated via G-protein complex and hydrolyzes PIP₂ to generate IP₃, which binds to the receptor of the membrane of intracellular Ca²⁺ store. Then, Ca²⁺ is released from the Ca²⁺ stores into the cytosol. On the other hand, Ca²⁺ influx through Ca²⁺ channels of the cell membrane is activated by membrane depolarization. The increase in cytosolic free Ca²⁺ activates Ca²⁺-dependent K⁺ channels of the cell membrane. Thapsigargin inhibits IP₃-sensitive Ca²⁺ accumulation by inhibiting Ca²⁺-ATPase (50, 51) of the membrane of intracellular Ca²⁺ stores. Opening of Ca²⁺ channels is stimulated by caffeine (52). The numbers (1)-(4) shown in this figure represent possible places where exposure to the magnetic field could affect: (1) cell membrane including receptors and transporters, (2) G-protein and PLC, (3) IP₃ receptor of Ca²⁺ channel of the membrane of Ca²⁺ store, and (4) Ca²⁺ release through the channel.

membrane or Ca²⁺ release from intracellular Ca²⁺ stores. Exposure to the time-varying magnetic field significantly suppressed the increase in [Ca²⁺]_i and partly inhibited the K⁺ influx through Ca²⁺-dependent K⁺ channels, suggesting inhibition of Ca²⁺ influx and/or Ca²⁺ release by the exposure. However, the inhibition of K⁺ influx would be due to a direct effect of the exposure on K⁺ channels rather than suppression of the increase in [Ca²⁺]_i (31). Liburdy et al. (40) have reported that ELF magnetic field affects Ca²⁺ uptake across the cell membrane, but Ca²⁺ release from the Ca²⁺ stores is not influenced. This is because electric field or eddy current induced by the magnetic field does not effectively penetrate the outer cell membrane. Since the cell membrane bilayer would act as an electric insulator. The ELF magnetic field will induce only a small eddy current inside the cells. Therefore, the eddy current induced in the medium could affect the surface electrical prop-

erties of the cell membrane. Virtually, Santoro, *et al.* (47) and Ikehara *et al.* (46) have shown that ELF magnetic field decreases membrane fluidity and changes the protein structure of the cell membrane, respectively. These reports suggest changes in the structure of the cell membrane, the number of receptors, Ca²⁺ influx via Ca²⁺ channels and K⁺ influx via K⁺ channels by exposure to time-varying magnetic fields. For the same reason, the activity of phospholipase C (PLC) for splitting phosphatidylinositol 4,5-bisphosphate [PI(4,5)P₂] in the cell membrane bilayer to yield IP₃ could be influenced by the magnetic fields. Recently, Santoro *et al.* (47) have pointed out that the magnetic field can also interfere with intracellular events such as protein phosphorylation in Raji cells. Also, we have reported that Ca²⁺ release from intracellular stores of bovine adrenal chromaffin cells in the absence of medium Ca²⁺ is perfectly inhibited by exposure to strong time-varying magnetic field (in preparation for publication). These events would involve IP₃ binding to its receptor, Ca²⁺ release from Ca²⁺ stores, etc. The exposure-induced change in electrical properties of the cell surface would influence signal transduction through G-protein related cascade, resulting in an inhibition of Ca²⁺ release from Ca²⁺ stores. However, it still cannot be ignored that the eddy current induced in cells by the magnetic field directly affects some intracellular event(s) of the signal transduction.

REFERENCES

1. Yamaguchi H, Ikehara T, Hosokawa K, Soda A, Shono M, Miyamoto H, Kinouchi Y, Tasaka T: Effects of time-varying electromagnetic fields on K⁺ (Rb⁺) fluxes and surface charge of HeLa cells. *Jpn J Physiol* 42 : 929-943, 1992
2. Pal R, Petri WA Jr, Barenolz Y, Wagner RR: Lipid and protein contributions to the membrane surface potential of vesicular stomatitis virus probed by a fluorescent pH indicator, 4-heptadecyl-7-hydroxycoumarin. *Biochim Biophys Acta* 729 : 185-192, 1983
3. Wright SH, Krasne S, Kippen I, Wright EM: Na⁺-dependent transport of tricarboxylic acid cycle intermediates by renal brush border membranes. Effects on fluorescence of a potential-sensitive cyanine dye. *Biochim Biophys Acta* 640 : 767-778, 1981
4. Grynkiewicz G, Poenie M, Tsien RY: A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J Biol Chem*

- 260 : 3440-3450, 1985.
5. Miyamoto H, Sakai T, Ikehara T, Kaniike K : Effects of Rb⁺ substituted for K⁺ on HeLa cells: Cellular content and membrane transport of monovalent cations, and cell growth. *Cell Struct Funct* 3 : 313-324, 1978
 6. Miyamoto H, Ikehara T, Yamaguchi H, Hosokawa K, Yonezu T, Masuya T : Kinetic mechanism of Na⁺, K⁺, Cl⁻-cotransport as studied by Rb⁺ influx into HeLa cells : Effects of extracellular monovalent ions. *J Membrane Biol* 92 : 135-150, 1986
 7. Ikehara T, Yamaguchi H, Sakai T, Miyamoto H : Kinetic parameters and mechanism of active cation transport in HeLa cells as studied by Rb⁺ influx. *Biochim Biophys Acta* 775 : 297-307, 1984
 8. Sachs JR : A modified consecutive model for Na, K-pump. In : Skou JC, Nørby JG, eds. *Na, K-ATPase. Structure and kinetics*. Academic Press, New York, 1979, pp.463-473
 9. Ikehara T, Yamaguchi H, Hosokawa K, Sakai T, Miyamoto H : Rb⁺ influx in response to changes in energy generation : effect of the regulation of the ATP content of HeLa cells. *J Cell Physiol* 119 : 273-282, 1984
 10. Spaggiare S, Wallach MJ, Tupper JT : Potassium transport in normal and transformed mouse 3T3 cells. *J Cell Physiol* 89 : 403-416, 1976
 11. Ikehara T, Yamaguchi H, Hosokawa K, Miyamoto H : Effects of transmembrane concentration gradients of monovalent ions on furosemide-sensitive Rb⁺ influx into HeLa cells. *Biomed Res* 7 : 197-198, 1986
 12. McRoberts JA, Erlinger S, Rindler MJ, Saier MH, Jr : Furosemide-sensitive salt transport in the Madin-Darby canine kidney cell line. Evidence for the cotransport of Na⁺, K⁺, and Cl⁻. *J Biol Chem* 257 : 2260-2266, 1982
 13. Smith JB, Smith L : Na⁺/K⁺/Cl⁻ cotransport in cultured vascular smooth muscle cells : stimulation by angiotensin II and calcium ionophores, inhibition by cyclic AMP and calmodulin antagonists. *J Membr Biol* 99 : 51-63, 1987
 14. Kim HD, Tsai Y-S, Franklin CC, Turner JT : Characterization of Na⁺/K⁺/Cl⁻ cotransport in cultured HT29 human colonic adenocarcinoma cells. *Biochim Biophys Acta* 946 : 397-404, 1988
 15. Canessa M, Brugnara C, Cusi D, Tosteson DC : Modes of operation and variable stoichiometry of the furosemide-sensitive Na and K fluxes in human red cells. *J Gen Physiol* 87 : 113-142, 1986
 16. Tas PWL, Massa PT, Kress HG, Koschel K : Characterization of an Na⁺/K⁺/Cl⁻ cotransport in primary cultures of rat astrocytes. *Biochim Biophys Acta* 903 : 411-416, 1987
 17. Ikehara T, Yamaguchi H, Hosokawa K, Miyamoto H : Kinetic mechanism of ATP action in Na⁺-K⁺-Cl⁻-cotransport of HeLa cells determined by Rb⁺ influx studies. *Am J Physiol* 258 : C599-C609, 1990
 18. Ikehara T, Yamaguchi H, Hosokawa K, Takahashi A, Miyamoto H : Kinetic study on the effects of intracellular K⁺ and Na⁺ on Na⁺,K⁺,Cl⁻ cotransport of HeLa cells by Rb⁺ influx determination. *J Membrane Biol* 132 : 115-124, 1993
 19. Duhm J, Gobel BO : Role of the furosemide-sensitive Na⁺/K⁺ transport system in determining the steady-state Na⁺ and K⁺ content and volume of human erythrocytes in vitro and in vivo. *J Membrane Biol* 77 : 243-254, 1984
 20. Ikehara T, Yamaguchi H, Hosokawa K, Yonezu T, Miyamoto H : Effects of nystatin on intracellular contents and membrane transport of alkali cations, and cell volume in HeLa cells. *J Membrane Biol* 90 : 231-240, 1986
 21. Ikehara T, Takahashi A, Yamaguchi H, Hosokawa K, Masuya T, Miyamoto H : Regulatory changes in the K⁺, Cl⁻ and water contents of HeLa cells incubated in an isosmotic high K⁺-medium. *Biochim Biophys Acta* 1068 : 87-96, 1991
 22. Kaku M, Ikehara T, Yamaguchi H : Mechanism of cell swelling of HeLa cells in an isosmotic Na⁺-free, high K⁺ medium : study on the K⁺ transport pathways. *Tokushima J exp Med* 39: 45-53, 1992
 23. Ikehara T, Yamaguchi H, Hosokawa K, Takahashi A, Masuya T, Miyamoto H : Different patterns of cell volume regulation in hyposmotic media between attached and suspended HeLa cells. *Biochim Biophys Acta* 1111 : 151-158, 1992
 24. Takahashi A, Yamaguchi H, Miyamoto H : Change in K⁺ current of HeLa cells with progression of the cell cycle studied by patch-clamp technique. *Am J Physiol* 265 : C328-C336, 1993
 25. Takahashi A, Yamaguchi H, Miyamoto H : Change in density of K⁺ current of HeLa cells during the cell cycle. *Jpn J Physiol* 44 : S321-S324, 1994
 26. Miyamoto H, Yamaguchi H, Ikehara T, Kinouchi Y : Effects of electromagnetic fields on K⁺(Rb⁺) uptake by HeLa cells. In : Ueno S, ed. *Biological Effects of Magnetic and Electromagnetic Fields*.

- Plenum Press, New York, 1996, pp.101-119
27. Ikehara T, Yamaguchi H, Hosokawa K, Takahashi A, Kinouchi Y, Miyamoto H : Effects of homogeneous magnetic fields on potassium (rubidium) ion transport of HeLa cells at different temperatures. *J J Mag Dent* 2 : 30-36, 1993
 28. Yamaguchi H, Ikehara T, Miyamoto H, Kinouchi Y, Tasaka T : K⁺ uptake of HeLa cells exposed to steady and time-varying strong magnetic fields. In : Blank M ed. *Electricity and Magnetism in Biology and Medicine*, San Francisco Press, San Francisco, 1993, pp.485-487
 29. Itegin M, Gunay I, Logoglu G, Isbir T : Effects of static magnetic field on specific adenosine-5' -triphosphatase activities and bioelectrical and biomechanical properties in the rat diaphragm muscle. *Bioelectromagnetics* 16 : 147-151, 1995
 30. Hinsenkamp M, Lheureux P, Martirls D : Transmembrane Na/K exchanges under electromagnetic field. Preliminary study on human erythrocytes. *Reconstr Surg Traumat* 19 : 63-69, 1985
 31. Ikehara T, Park K H, Kinouchi Y, Yamaguchi H, Yoshizaki K, Miyamoto H : Effects of strong magnetic fields on membrane ion transport of cultured cells. *Tech Rep IEICE. MBE* 97-40 : 7-12, 1997
 32. Doida Y, Miller MW, Brayman AA, Carstensen EL : A test of the hypothesis that ELF magnetic fields affect calcium uptake in rat thymocytes in vitro. *Biochem Biophys Res Commun* 227 : 834-838, 1996
 33. Hendee SP, Faour FA, Christensen DA, Patrick B, Durney CH, Blumenthal DK : The effects of weak extremely low frequency magnetic fields on calcium/calmodulin interactions. *Biophys J* 70 : 2915-2923, 1996
 34. Lindstrom E, Lindstrom P, Berglund A, Mild KH, Lundgren E : Intracellular calcium oscillations induced in a T-cell line by a weak 50 Hz magnetic field. *J Cell Physiol* 156 : 395-398, 1993
 35. Lindstrom E, Berglund A, Mild KH, Lindstrom P, Lundgren E : CD45 phosphatase in Jurkat cells is necessary for response to applied ELF magnetic fields. *FEBS Lett* 370 : 118-122, 1995
 36. Korzh-Sleptsova IL, Lindstrom E, Mild KH, Berglund A, Lundgren E : Low frequency MFs increased inositol 1,4,5-triphosphate levels in the Jurkat cell line. *FEBS Lett* 359 : 151-154, 1995
 37. Walleczek J, Liburdy RP : Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances ⁴⁵Ca²⁺ uptake in rat thymocytes : dependence on mitogen activation. *FEBS Lett* 271 : 157-160, 1990
 38. Lyle DB, Wang X, Ayotte RD, Sheppard AR, Adey WR : Calcium uptake by leukemic and normal T-lymphocytes exposed to low frequency magnetic fields. *Bioelectromagnetics* 12 : 145-156, 1991
 39. Fitzsimmons RJ, Ryaby JT, Magee FP, Baylink DJ : Combined magnetic fields increased net calcium flux in bone cells. *Calcif Tissue Int* 55 : 376-380, 1994
 40. Liburdy RP : Calcium signaling in lymphocytes and ELF magnetic fields. Evidence for an electric field metric and site of interaction involving the calcium ion channel. *FEBS Lett* 301 : 53-59, 1992
 41. Ayrapetyan SN, Grigorian KV, Avanesian AS, Stamboltsian KV : Magnetic fields alter electrical properties of solutions and their physiological effects. *Bioelectromagnetics* 15 : 133-142, 1994
 42. Cavopol AV, Wamil AW, Holcomb RR, McLean MJ : Measurement and analysis of static magnetic fields that block action potentials in cultured neurons. *Bioelectromagnetics* 16 : 197-206, 1995
 43. Smith OM, Goodman EM, Greenebaum B, Tipnis P : An increase in the negative surface charge of U937 cells exposed to a pulsed magnetic field. *Bioelectromagnetics* 12 : 197-202, 1991
 44. Santini MT, Cametti C, Straface E, Grandorfo M, Indovina PL : A static magnetic field does not affect the dielectric properties of chick embryo myoblast membranes. *Int J Radiat Biol* 65:277-284, 1994
 45. Kinouchi Y, Tanimoto S, Ushita T, Sato K, Yamaguchi H, Miyamoto H : Effects of static magnetic fields on diffusion in solutions. *Bioelectromagnetics* 9 : 159-166, 1988
 46. Ikehara T, Aizawa K, Hosokawa K, Yamaguchi H, Park KH, Yoshizaki K, Kinouchi Y, Miyamoto H : Analysis of magnetic field effects on Ca²⁺-dependent K⁺ uptake into HeLa cells using FT-IR spectra. *33th Int Congr physiol Sci Abstracts* : 33, 1997
 47. Santoro N, Lisi A, Pozzi D, Pasquali E, Serafino A, Grimaldi S : Effect of extremely low frequency (ELF) magnetic field exposure on morphological and biophysical properties of human lymphoid cell line (Raji). *Biochim Biophys Acta* 1357 : 281-290, 1997
 48. Fitzsimmons RJ, Ryaby JT, Magee FP, Baylink DJ : IGF-II receptor number is increased in TE-85 osteosarcoma cells by combined magnetic fields.

- J Bone Miner Res 10 : 812-819, 1995
49. Liburdy RP : Biological interactions of cellular systems with time-varying magnetic fields. Ann N Y Acad Sci 649 : 74-95, 1992
 50. Thastrup O, Cullen PJ, Drøbak BK, Hanley MR, Dawson AP : Thapsigargin, tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+} -ATPase. Proc Natl Acad Sci USA 87 : 2466-2470, 1990
 51. Casperson C, Treiman M : Thapsigargin discriminates strongly between Ca^{2+} -ATPase phosphorylated intermediates with different subcellular distributions in bovine adrenal chromaffin cells. FEBS Lett 377 : 31-36, 1995
 52. Liu PS, Lin YJ, Kao LS : Caffeine-sensitive calcium stores in bovine adrenal chromaffin cells. J Neurochem 56 : 172-177, 1991

