Effects of a time-varying strong magnetic field on transient increase in cytosolic free Ca^{2+} induced by bradykinin in cultured bovine adrenal chromaffin cells

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Abstract We tested the effects of exposure to a time-varying magnetic field changing between 0.07 and 1.7 T at an interval of 3 s on transient increase in intracellular Ca^{2+} stimulated by bradykinin in bovine adrenal chromaffin cells. Addition of bradykinin induced the increase in intracellular Ca^{2+} within a few minutes. The exposure to the magnetic field perfectly suppressed the increase in intracellular Ca^{2+} in Ca^{2+}-free medium. The inhibition occurred for 15 min when the maximum magnetic flux density was more than 1.4 T. These results suggest that the exposure inhibits Ca^{2+} release from intracellular Ca^{2+} stores.

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Key words: Time-varying magnetic field; Bovine adrenal chromaffin cell; Ca^{2+} store; Bradykinin; Intracellular Ca^{2+}; Ca^{2+} influx

1. Introduction

Many investigators have reported the effects of ELF magnetic and electromagnetic fields on cellular mobilization of free Ca^{2+}. For example, exposure to the magnetic fields causes an increase in calcium influx of lymphocytes [1,2] and Ca^{2+} current of cultured chromaffin cells [3], and induces oscillations of cytosolic Ca^{2+} [4]. Combinations of AC/DC magnetic fields inhibit calcium influx of lymphocytes triggered by concanavalin A [5,6]. In contrast, exposure to an ELF magnetic field has not shown any significant influence on Ca^{2+} transport of clonal insulin-producing RINm5F cells observed by patch-clamp technique [7]. We have recently found that the same time-varying magnetic field as that used in the present study influences cellular Ca^{2+} mobilization of HeLa cells (in preparation for publication).

Changes in intracellular Ca^{2+} may influence some functions through signal transduction in different types of cells including neural cell lines. So, determination of free Ca^{2+} in neural cells mobilized by stimulants is important in testing the effects of the time-varying magnetic field on signal transduction in the cells. Adrenal chromaffin cells are a model of catecholamine (CA) containing neurons and are regarded to be suitable for studying the stimulus-secretion coupling [8]. For example, activation of nicotinic acetylcholine (ACh) receptors of the cells by cholinergic agents or high K^+-induced membrane depolarization is known to initiate transient rapid CA secretion accompanied by an increase in Ca^{2+} influx [8]. Bradykinin (BK), a non-cholinergic stimulant, also stimulates CA secretion through activation of the BK-B_2 receptor [9,10]. The stimulant increases the cytosolic levels of inositol 1,4,5-triphosphate (IP_3) and free Ca^{2+} [11], and stimulates Ca^{2+} efflux [12].

In the present study, we tested the effect of exposure to the time-varying strong magnetic field on BK-stimulated mobilization of cytosolic free Ca^{2+} in chromaffin cells. We found that the magnetic field perfectly inhibited the increase in intracellular Ca^{2+}, suggesting the effect of exposure on signal transduction or stimulus-secretion coupling in adrenal chromaffin cells.

2. Materials and method

2.1. Cell preparation and culture

Bovine adrenal chromaffin cells were dispersed enzymatically as reported by Oka et al. [8]. Briefly, the adrenal medulla was sliced with a hand slicer, and the slices were digested in a medium containing 0.1% collagenase, 0.01% soybean trypsin inhibitor, and 0.5% bovine serum albumin in the 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). Cells were plated on 13 mm diameter cover glasses (Matsunami Glass Ind., Ltd., Osaka, Japan) in 35 mm culture dishes at a density of 10^6 cells/dish for measuring intracellular Ca^{2+} concentration. After the cells were attached to the cover glasses, they were maintained for 2 or 3 days in 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 cystine arabinoside.

We used two different kinds of media for incubating cells during exposure to the magnetic field: one was normal BSS containing Ca^{2+} and the other was the BSS without Ca^{2+}. When cells were incubated in Ca^{2+}-free BSS during exposure to the magnetic field, the BSS was used either in the presence or the absence of 1 mM EGTA for measuring the intracellular Ca^{2+} concentration. When BSS contained

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Ca^{2+}, the media used for both exposure to the magnetic field and Ca^{2+} measurement were the same.

2.2. Exposure to magnetic field

Magnetic field was produced by an electromagnet designed and set up by Hitachi Metal indust. Co. (Tokyo, Japan). Details of the electromagnet were described in a paper [13]. The apparatus consisted of a pair of vertically placed coils and poles with round polar faces of 100 mm diameter attached to the coils. This system produced maximal magnetic flux density of about 1.7 T in the gap between the poles. Intermittent magnetic field was produced by changing coil current automatically with an electronic switch. For simplicity, the durations of the switching on and off time were set to be equal (3 s). The peak mean densities of eddy current induced in the culture medium were estimated to be 32 mA/m^2 and 13 mA/m^2 corresponding to the rise and fall of the magnetic field (Fig. 1). When the time of exposure to the magnetic field was limited to be less than 3 h, the cells were preliminarily placed outside the magnetic field after medium change and then exposed for the requested periods (Figs. 4 and 5), so that the total incubation time was fixed at 3 h.

2.3. Temperature regulation of cultures

We used specially designed incubators (129 mm diameter, 20 mm thick) described in the previous paper [13], which contained four culture dishes in each and their temperature was kept at 37°C. The incubators made the temperature differences among the culture dishes less than 0.2°C as determined with thermistor sensors (1 mm diameter) of a thermometer (D117, Takara Indust. Co., Tokyo, Japan). Normally, two similar incubators were used simultaneously: one was placed horizontally in the gap between two poles of the electromagnet and the other was placed outside the magnetic field as a control.

2.4. Measurement of intracellular calcium concentration with Fura 2

The concentration and distribution of intracellular Ca^{2+} were determined and analyzed 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, pentaacetoxymethyl ester (Fura 2-AM, 6 μM) by ARGUS-50/CA (Hamamatsu Photonics Co. Ltd., Hamamatsu, Japan) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm, using a flow chamber. For preloading the cells with the fluorescent probe, we incubated the cells attached to cover slips for 30 min at 37°C in either of the media containing the probe. Then, the cultures were washed with the same medium without the probe, and fluorescence intensity was started to assay in 2 min. After the assay for 1 min in the normal BSS, cells were treated with 1 μM BK. We used Fura 2 and a calibration kit (Molecular Probes Inc., Eugene, USA) for calibrating the Ca^{2+} concentration. The intracellular Ca^{2+} concentration was determined using the equation described by Grynkiewicz et al. [14].

2.5. Chemicals

Bradykinin was purchased from Wako Pure Chemical Indust. (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.

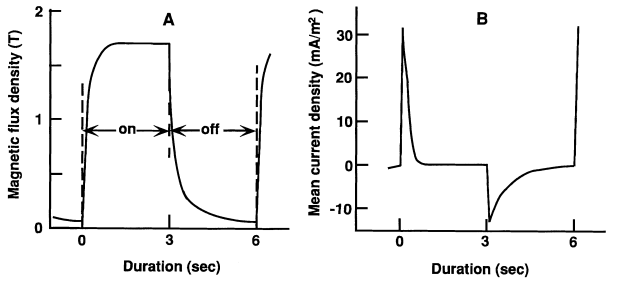

Fig. 1. Changes in the magnetic flux density and the density of the eddy current induced in the culture medium (modified from Fig. 2E and F of [13]). A: Magnetic flux density produced with the polar face of 100 mm diameter. B: Mean values of eddy current corresponding to changes in the magnetic field in A.

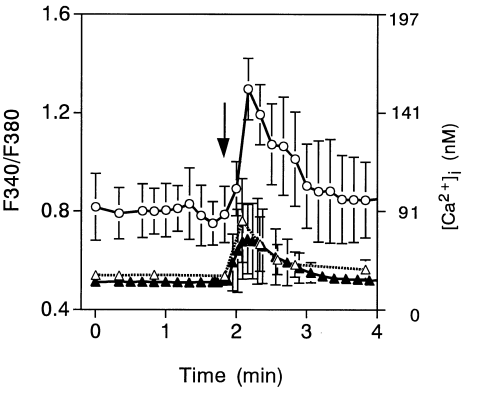

Fig. 2. Changes in $[Ca^{2+}]_i$ of bovine chromaffin cells after addition of BK in the different media. This and following figures show the intracellular Ca^{2+} concentration plotted as fluorescent signal ratio (F340 nm/F380 nm). The arrow shows addition of 1 μM BK. ○, normal BSS (7 cells). Cells were preincubated in BSS and $[Ca^{2+}]_i$ was measured in BSS. △, Ca^{2+}-free medium (18 cells). Cells were preincubated in Ca^{2+}-free BSS and $[Ca^{2+}]_i$ was measured in the same medium. ▲, Ca^{2+}-free medium plus 1 mM EGTA (16 cells). Cells were preincubated in Ca^{2+}-free BSS and $[Ca^{2+}]_i$ was measured in the Ca^{2+}-free BSS in the presence of 1 mM EGTA. Preincubation time was fixed at 3 h. Points and bars are means and S.D. values.

3. Results and discussion

Fig. 2 shows time-dependent changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) after addition of 1 μM BK. $[Ca^{2+}]_i$ increased instantly from the resting level (93 ± 18 nM; $n = 7$) on addition of BK, reaching a maximal value (153 ± 16 nM) within 15 s in normal BSS and then decreasing with time. BK stimulates not only intracellular release of Ca^{2+} but also Ca^{2+} efflux via Na^+/Ca^{2+} exchange in chromaffin cells [12], suggesting that the activated Ca^{2+} efflux is closely related to the decreasing phase. Addition of 1 mM EGTA to the Ca^{2+}-free BSS decreased the resting level to 35 ± 3 nM ($n = 16$), and the peak value (81 ± 18 nM) was only half that in normal BSS after addition of BK. Then, $[Ca^{2+}]_i$ decreased and reached the resting level in 2 min. Addition of 1 mM EGTA slightly decreased the resting and peak levels than those observed on only omission of Ca^{2+} from the BSS. But, the differences of the resting and peak levels between the two groups were not significant. These results indicate that the increase in $[Ca^{2+}]_i$ by BK takes place through two different pathways: (1) Ca^{2+} influx via plasma membrane and (2) Ca^{2+} release from intracellular stores. These pathways are estimated to mediate about 23 and 77% of total Ca^{2+} flux, respectively.

Next, we examined the effects of exposure to the time-varying magnetic field on increase in $[Ca^{2+}]_i$ by addition of BK in normal BSS. Immediate increase in $[Ca^{2+}]_i$ (164 ± 30, $n = 12$) by addition of BK was similar to that in normal BSS in Fig. 2 (Fig. 3). Exposure to the magnetic field for 3 h significantly inhibited the increase in $[Ca^{2+}]_i$ (127 ± 27, $n = 15$). Comparison of the components of $[Ca^{2+}]_i$ stimulated by BK between control and exposed cells showed about 23% inhibition by the exposure, and exposed cells needed more time to reach the maximum value. Times required for $[Ca^{2+}]_i$ to reach the peak values after addition of BK were less than 0.17 min in all control cells, but those in exposed cells were diverse; e.g. less than 0.17 ($n = 2$), 0.33 ($n = 5$), 0.5 ($n = 5$), 0.67 ($n = 1$),

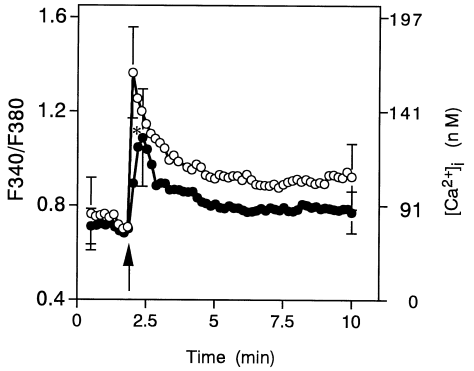

Fig. 3. Effects on $[Ca^{2+}]_i$ of bovine chromaffin cells in normal BSS (containing 2.2 mM $CaCl_2$) of exposure to the time-varying magnetic field shown in Fig. 1A for 3 h. BK (1 μM) was added at the time point shown by arrow. ○, Control (12 cells); ●, exposure (15 cells). *, the peak value of $[Ca^{2+}]_i$ of exposed cells was significantly different from that of control cells at $P < 0.025$. Points and bars are means and S.D. values.

1 ($n = 1$) and 1.33 min ($n = 1$). Average of the peak values for exposed 15 cells was 133 ± 25 nM ($n = 15$), which was significantly different from the average of control cells at $P < 0.01$. These results indicate that the exposure to the magnetic field inhibits the transient increase in $[Ca^{2+}]_i$ by about 81% and delayed the increase.

Cultured chromaffin cells were exposed for 2 h to the magnetic fields of various maximal flux densities of less than 1.7 T in the absence of medium Ca^{2+} (Fig. 4). The resting levels of $[Ca^{2+}]_i$ at the different maximal flux densities were similar, but the peak values of $[Ca^{2+}]_i$ after addition of BK significantly decreased at the flux densities of more than 1.4 T. There was no significant difference between the peak and the resting levels when exposed to 1.7 T, suggesting perfect inhibition of the transient increase in $[Ca^{2+}]_i$.

To examine the influence of exposure time to the magnetic field on the BK-induced Ca^{2+} release from intracellular stores, cells were exposed for different periods in the Ca^{2+}-free BSS added with 1 mM EGTA (Fig. 5). The maximal magnetic flux density was fixed at 1.7 T. The resting level of $[Ca^{2+}]_i$ was almost unchanged by exposure to the magnetic field, but the BK-induced increase in $[Ca^{2+}]_i$ was strongly suppressed for 15 min and the suppression continued for 2 h. The peak value of $[Ca^{2+}]_i$ decreased strongly, so that $[Ca^{2+}]_i$ was not significantly different from the resting level when exposed for 2 h. The results shown in Figs. 4 and 5 support that the BK-induced increase in $[Ca^{2+}]_i$ through Ca^{2+} release from intracellular stores is almost perfectly suppressed by the exposure, but the inhibition of Ca^{2+} influx through plasma membrane is partial.

The inhibitory effect of exposure to the magnetic field on the BK-induced increase in cytosolic Ca^{2+} could occur by affecting either of (1) BK-B_2 receptor of plasma membrane, (2) process of IP_3 production, (3) store membrane containing IP_3 receptor or (4) Ca^{2+} uptake. It has been reported that exposure to ELF magnetic fields affects free intracellular Ca^{2+} through Ca^{2+} influx across the cell membrane [2] and increases the IP_3 levels of lymphocytes [15]. However, the possibility of exposure effects on Ca^{2+} influx would be small in the present study. Because, an increase in $[Ca^{2+}]_i$ in Ca^{2+} containing medium by stimulation of BK was not strongly inhibited, while the increase was perfectly suppressed by the exposure in Ca^{2+}-free medium. On the other hand, Polk [16] has assumed that biological effects of time-varying magnetic fields are due to induced electric currents and that their influences will be exerted at the cell surface. Exposure to a 60 Hz electric field or a magnetically induced electric field of the identical strength has been shown to activate or inhibit Ca^{2+} transport [2,17]. We have pointed out changes in electric properties of the membrane surface of HeLa cells possibly caused by eddy current induced by the strong time-varying magnetic field [13]. Also, there is a report showing the influence of ELF magnetic field to membrane fluidity [18]. These results suggest that magnetic fields affect BK-B_2 receptors of plasma membrane by inhibiting BK binding to the receptor or by changing the affinity of the receptor to BK. Similarly,

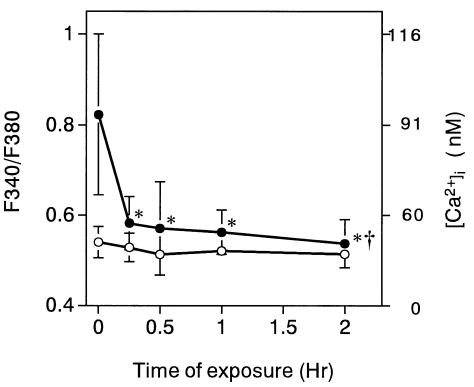

Fig. 5. Effects of exposure to the time-varying magnetic field for various periods of time on the increase in $[Ca^{2+}]_i$ of bovine chromaffin cells on addition of BK in Ca^{2+}-free BSS added with 1 mM EGTA. The maximal magnetic flux density was fixed at 1.7 T. Cells were placed outside the magnetic field after medium change to Ca^{2+}-free BSS, and then they were exposed for various periods, but the total incubation time was kept constant at 3 h. $[Ca^{2+}]_i$ was measured in Ca^{2+}-free BSS added with 1 mM EGTA. ●, peak values of $[Ca^{2+}]_i$; ○, resting values of $[Ca^{2+}]_i$. *, significantly different from the value of non-exposed cells at $P < 0.02$. †, not significantly different from the value of resting cells ($P > 0.05$). Points and bars are means and S.D. values for at least 18 cells.

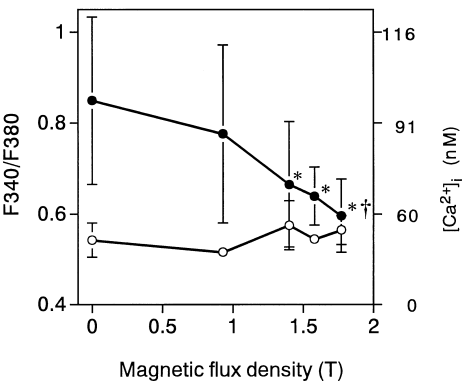

Fig. 4. Effects on the BK-induced increase in $[Ca^{2+}]_i$ of bovine chromaffin cells in Ca^{2+}-free BSS of the time-varying magnetic field at various maximal magnetic flux densities. Cells were placed outside the magnetic field for 1 h after medium change to Ca^{2+}-free BSS, and then they were exposed for 2 h. ●, peak values of $[Ca^{2+}]_i$; ○, resting values of $[Ca^{2+}]_i$. Points and bars are means and S.D. values. *, significantly different from the value of non-exposed cells at $P < 0.02$. †, not significantly different from the resting value ($P > 0.05$). Points and bars are means and S.D. values for at least 11 cells.

production of IP_3 derived from membrane phospholipids may be affected by magnetic fields.

Since lipid bilayer of the cell membrane acts as an electrical insulator, we basically agree that eddy currents induced by the time-varying magnetic fields do not easily penetrate the cell membrane. Though the magnetic field would induce eddy current in the cells, it is estimated to be about three order of magnitude lower than that in the medium. The tiny eddy current induced in the cells cannot cause significant inhibitory effect. For this reason, exposure to the magnetic fields would not directly influence internal cell structures [2], but influence indirectly through its effect on electrical properties of the cell surface. Exposure to ELF magnetic field enhances or inhibits Ca^{2+} influx [2,17], but exerts little influence to release of calcium from intracellular stores [2]. The difference of the effects between ELF and our magnetic field would be due to differences of wave forms and frequencies of changes between these magnetic fields.

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