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Decrease in glucose-stimulated insulin secretion following exposure to magnetic fields

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Abstract

We evaluated the effects of extremely low frequency magnetic field (ELFMF) on glucose-stimulated insulin secretion from HIT-T15 cells and investigated the mechanisms of these effects. We demonstrated that exposure to ELFMF at 5 mT decreased glucose-stimulated insulin secretion by preventing the increases in cellular adenosine 5′-triphosphate/adenosine 5′-diphosphate, membrane depolarization, and cytosolic free calcium ion concentration. The glucose-induced upregulation of insulin mRNA expression was also attenuated by exposure to ELFMF, although cell viability was not affected. These findings demonstrate the potential of exposure to ELFMF for clinical use as a novel inhibitory method of insulin secretion.

Keywords: 5 mT and 60 Hz; HIT-T15 cells; ATP/ADP; Membrane depolarization; Cytosolic free calcium ion concentration; Insulin mRNA expression

An extremely low frequency magnetic field (ELFMF) is defined as an alternating magnetic field at a frequency lower than 300 Hz. The various types of ELFMF (from the perspectives of frequency, intensity, and waveform) have been investigated in the treatment of osteoporosis [1], osteoarthritic knees [2], and Parkinson's disease [3] over the past two decades, and numerous studies have demonstrated their clinical efficacy. The advantages of clinical use of ELFMF are that it is painless, leaving no injury and no fatigue, the avoidance of hospitalization and side effects, and the potential for long term and local treatment.

Recently, we demonstrated that exposure to ELFMF decreased insulin secretion from RIN-m cells [4,5]. Insulin plays a vital role in regulating blood glucose levels, and insufficient release of insulin is the basis of various forms of diabetes. Although the incidence of diabetes mellitus has increased progressively, there have been few studies that have utilized advantages of ELFMF clinically in the treatment of diabetes.

In this study, we investigated the effects of ELFMF on glucose-induced insulin secretion from HIT-T15 cells that have reported to be responsive to glucose stimulation [6], to evaluate the potential of the use of ELFMF for the treatment of diabetes.

Materials and methods

ELFMF exposure unit. ELFMF exposure unit (at a frequency of 60 Hz) used in this study has been described previously [7]. The distribution of the magnetic flux density was measured using a Gauss Meter (Model 3251; Yokogawa Electrical, Tokyo, Japan).

Cell culture. HIT-T15 cells (Dainippon Pharmaceutical, Osaka, Japan) were cultured in RPMI-1640 containing 10% fetal bovine

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serum, penicillin (100 U/mL), streptomycin (100 μg/mL), amphotericin B (0.25 μg/mL, antibiotic–antimycotic; Invitrogen, Carlsbad, CA), and low concentration of glucose (1 g/L) to avoid reduction of the insulin content and glucose responsiveness of the HIT-T15 cells [8]. Cells were used from passage numbers 81 to 86.

Insulin secretion tests. To evaluate the effects of ELFMF on insulin secretion, insulin secretion tests were performed as follows: cells were plated on 24-well culture plates at a density of 1.21×10^5 cells/cm^2, grown to 80–90% confluence, and preincubated in 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (Hepes)-buffered Krebs–Ringer balanced buffer (KRBB; 119 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgSO_4$, 1.19 mM KH_2PO_4, and 25 mM $NaHCO_3$, pH 7.4) containing 0.2% bovine serum albumin (BSA, Nacalai Tesque, Kyoto, Japan) without glucose [KRBB (0)] for 30 min. After the preincubation, cells were divided into two groups: one was cultured under exposure to ELFMF during the stimulation period following the preincubation period, and the other was cultured under sham exposure conditions. During the stimulation period, cells were incubated in 0.4 mL KRBB (0) or Hepes-buffered KRBB containing 0.2% BSA and 200 mg/dL glucose for 2 h. The insulin concentration of the supernatant was measured by enzyme-linked immunosorbent assay (ELISA) kit (Sibayagi, Gunma, Japan) with rat insulin as a standard. Insulin secretion from the HIT-T15 cells was standardized to the cell number, which was counted by a cell and particle counter (COULTER Z1; Beckman Coulter, Fullerton, CA).

Measurement of adenosine 5′-triphosphate and adenosine 5′-diphosphate. After the insulin secretion tests were performed with a stimulation period of 15 min, cellular adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP) content were measured, using CellTiter-Glo (Promega, Madison, WI) and luminometer (Lumi-Connter 2500; Microtech Nition; Chiba, Japan) as described previously [9].

Flow cytometric analysis. For membrane potential analysis, cells were suspended in KRBB (0) at a concentration of 1.5×10^6 cells/mL. After loading with propidium iodide (PI, 5 μg/mL final concentration; Nacalai Tesque), and 3,3′-dipentyloxacarbocyanine iodide [$DiOC_5(3)$, 20 nM final concentration; Molecular Probes, Eugene, OR; dissolved in dimethyl sulfoxide (DMSO)], by incubation at 37 °C for 5 min in the dark, flow cytometric analysis was carried out using FACSCalibur (Becton–Dickinson, Franklin Lakes, NJ), to record an initial intensity of $DiC_5(3)$ fluorescence (F_0). Immediately after flow cytometric analysis, an aliquot of cell suspension was collected, and glucose dissolved in KRBB (200 mg/dL final concentration) was added to the aliquot. After the aliquot was incubated at 37 °C for 10 min under ELFMF exposure or sham exposure conditions, flow cytometric analysis was again performed to record alterations of fluorescence intensity (F).

For analysis of cytosolic free calcium ion concentration ($[Ca^{2+}]_i$), cells were suspended in KRBB (0) at a concentration of 2.7×10^6 cells/mL. After loading with 1-[2-amino-5-(2,7-dichloro-6-hydroxy-3-oxo-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, pentaacetoxymethyl ester (Fluo-3 AM, 5 μg/mL final concentration; Dojindo, Kumamoto, Japan; in DMSO) by incubation at 37 °C for 30 min in the dark, excess Fluo-3 was removed by centrifugation, and the resulting cell pellets were gently resuspended in KRBB (0). After loading with PI (5 μg/mL final concentration), flow cytometric analysis was conducted to record the initial Fluo-3 fluorescence intensity (F_0) and alterations of Fluo-3 fluorescence intensity (F), as described in the membrane potential analysis. The data were analyzed using CellQuest software version 3.3.

Total RNA extraction and RT-PCR. After insulin secretion tests were performed, the cells were scraped and total RNA was isolated using ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. cDNA synthesis was performed using the Thermoscript RT-PCR system (Invitrogen) according to the manufacturer's instructions. PCRs were performed using Ex Taq polymerase (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. The primers were as follows: insulin: forward 5′-CCTGCCCAGGCTTTTGTCA-3′, reverse 5′-GGTGCAGCACTGATCCACAATG-3′ [8], β-actin: forward 5′-ATGGTGGGTATGGGTCAGAAGG-3′, reverse 5′-ACGCACGATTTCCCTCTCAGCT-3′. The PCR mixture was incubated in a thermal cycler (Program Temp control system PC-818A-02; Astec, Fukuoka, Japan) at 95 °C for 45 s, 58 °C for 45 s, and 72 °C for 90 s. β-Actin expression was used to standardize the input template cDNA in a semi-quantitative PCR. Serial half-dilution of cDNA was amplified to ensure analysis of products in the linear range of amplification. Each PCR product was analyzed on a 1.8% agarose gel with 0.1 μg/mL ethidium bromide, and quantification of the PCR products was performed by densitometry of the band intensity using a lumino capture system (AE-6950; Atto, Tokyo, Japan) and CS analyzer software version 1.0 (Atto).

Measurement of total protein content and insulin content. After insulin secretion tests were performed, cells were collected by trypsinization and centrifugation. Total protein was extracted using Cel-Lytic-M (Sigma, St. Louis, MO) according to the manufacturer's instructions. The extracted total protein concentration was measured using a BCA protein assay kit (Pierse, Rockford, IL). Cellular insulin was extracted using acidic aqueous ethanol, and the extracted insulin concentrations were measured by ELISA kit (Sibayagi).

WST-1 assay. After insulin secretion tests were performed, 40 μL of premix WST-1 (TaKaRa Bio) was added to each well, and all test plates were incubated for an additional 4 h, under non-ELFMF exposed conditions. The optical density of the supernatant was measured by a microplate reader (Ultrospec Visible Plate Reader II, Amersham Biosciences, Piscataway, NJ), at 450 nm wavelength using 96-well plates in duplicate.

Statistical analysis. Statistical comparisons were performed by Student's t tests or Welch's t tests according to the results of the F tests.

Results

The effects of ELFMF on insulin secretion from HIT-T15 cells

The HIT-T15 cells used in this experiment exhibit a relatively high responsiveness to glucose. The stimulation index, calculated by dividing the insulin secretion under the stimulation conditions (200 mg/dL glucose) by the insulin secretion without glucose stimulation, was 5.9 ± 0.7 (mean ± SE, $n = 3$). This relatively high responsiveness to glucose stimulation supports our use of the cells for evaluating the effects of ELFMF on insulin secreting cells in this experiment.

Fig. 1 represents the effects of ELFMF on insulin secretion at different magnetic flux densities (0.4, 1, and 5 mT) in the presence and absence of glucose stimulation (200 mg/dL glucose). A significant decrease in insulin secretion was observed following exposure to ELFMF at 5 mT under stimulation conditions ($89.0 \pm 1.5\%$ of sham exposure, mean ± SE, $n = 3$, $p = 0.018$). In the absence of glucose stimulation or under exposure to ELFMF at a lower magnetic density (1 or 0.4 mT), no significant differences were observed between insulin secretion under exposure to ELFMF and that under sham exposure conditions. The stimulation index was also attenuated by exposure to ELFMF at 5 mT ($89.1 \pm 0.7\%$ of sham exposure, mean ± SE, $n = 3$, $p = 0.004$), consistent with a reduction of the glucose-stimulated

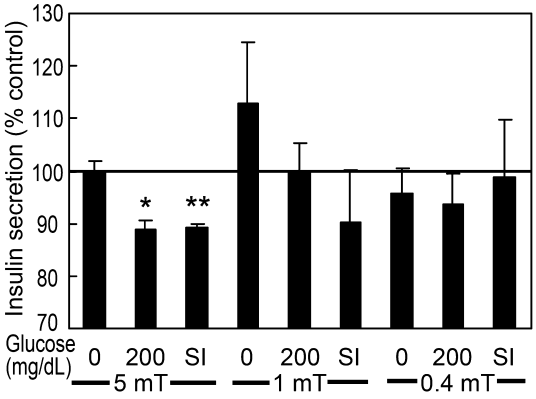

Fig. 1. The effects of ELFMF on insulin secretion at different magnetic flux densities (0.4, 1, and 5 mT) in the presence or absence of glucose stimulation (200 mg/dL glucose). The ratio of insulin secretion under ELFMF to that under sham exposure is shown. Data represent means ± SE ($n = 3$), and each experiment was performed in duplicate. SI, stimulation index, *$p < 0.05$, **$p < 0.01$.

insulin secretion and no change of basal insulin secretion following exposure to ELFMF.

Mechanistic analysis of the effects of ELFMF on insulin secretion

To evaluate the mechanisms of the attenuation of glucose-stimulated insulin secretion by exposure to ELFMF at 5 mT, we evaluated alterations in cellular ATP/ADP (Table 1), membrane potential, and $[Ca^{2+}]_i$ (Fig. 2).

The ATP/ADP levels were significantly increased by glucose stimulation under both ELFMF and sham exposure conditions ($p = 0.034$ and $p = 0.016$, respectively). Under both conditions, the increase of ATP/ADP by glucose stimulation was due to a significant decrease of ADP concentration ($p = 0.030$ and $p = 0.014$, respectively). Following exposure to ELFMF, the increase of ATP/ADP was significantly attenuated ($p = 0.046$).

The effects of ELFMF on the alterations of membrane potential were measured using the fluorescent dye, $DiOC_5(3)$, and flow cytometry (Fig. 2A). The mean fluorescence intensity of $DiOC_5(3)$ was decreased by glucose stimulation ($(F - F_0)/F_0 = -0.27 \pm 0.04$, mean ± SE, $n = 4$). The value of decrease in mean fluorescence intensity ($|(F - F_0)/F_0| = |\Delta F|/F_0$) was attenuated by exposure to ELFMF under glucose stimulation (88.7 ± 1.3% of sham exposure, mean ± SE, $n = 3$, $p = 0.014$); although it was not attenuated in the absence of glucose stimulation (101.3 ± 15.3% of sham exposure, mean ± SE, $n = 3$, $p = 0.94$). Since the $DiOC_5(3)$ fluorescence intensity decreased as the membrane depolarized, the results demonstrated that the degree of depolarization was attenuated by exposure to ELFMF.

Table 1
Alterations of ATP, ADP content, and ATP/ADP in the presence of glucose stimulation, compared with those in the absence of glucose stimulation

	% of without glucose stimulation	
	Sham	ELFMF
ATP	97.9 ± 8.9	82.8 ± 4.9
ADP	65.0 ± 4.1*	66.4 ± 5.9*
ATP/ADP	146.9 ± 5.9*,**	125.2 ± 4.7*

Data represent means ± SE ($n = 3$), and each experiment was performed in duplicate.
* $p < 0.05$ to the response without glucose stimulation.
** $p < 0.05$ to exposure to ELFMF.

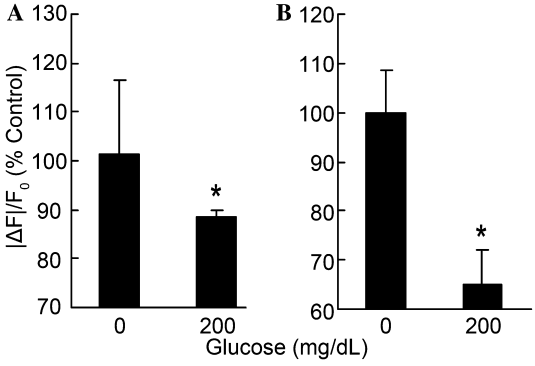

Fig. 2. The effects of ELFMF on alterations ($|\Delta F|/F_0$) of membrane potential (A) and $[Ca^{2+}]_i$ (B). The ratio of $|\Delta F|/F_0$ under ELFMF to under sham exposure is shown. Data represent means ± SE ($n = 3$–6). *$p < 0.05$.

Alterations of $[Ca^{2+}]_i$ were evaluated, relatively, using the Ca^{2+}-sensitive fluorescence dye, Fluo-3 AM, and flow cytometry (Fig. 2B). The mean fluorescence intensity of Fluo-3 increased following glucose stimulation ($(F - F_0)/F_0 = 0.14 \pm 0.03$, mean ± SE, $n = 6$). The increase of fluorescence intensity of Fluo-3 decreased following exposure to ELFMF under glucose stimulation (65.2 ± 6.8% of sham exposure, mean ± SE, $n = 6$, $p = 0.018$); although it was not altered in the absence of glucose stimulation (100.0 ± 8.5% of sham exposure, mean ± SE, $n = 3$, $p = 1.00$). Since the Fluo-3 fluorescence intensity increased in proportion to $[Ca^{2+}]_i$, these results demonstrated that the increase of $[Ca^{2+}]_i$ was attenuated by exposure to ELFMF.

The effects of ELFMF on insulin mRNA expression

The alterations of insulin mRNA expression were evaluated by semi-quantitative RT-PCR (Table 2). The

Table 2
Insulin mRNA expression

	Without exposure to ELFMF (insulin mRNA/β-actin mRNA)	% of sham exposure
0 mg/dL glucose	0.35 ± 0.13	105.6 ± 11.1
200 mg/dL glucose	0.55 ± 0.05	86.2 ± 9.5*

Data represent means ± SE ($n = 3$).
* $p < 0.05$.

Table 3
Cell viability analysis

	% of sham exposure
Cell number	110.3 ± 5.6 ($n = 3$, $p = 0.21$)
Total protein content	98.1 ± 1.3 ($n = 3$, $p = 0.28$)
Cellular insulin content	103.0 ± 7.2 ($n = 4$, $p = 0.71$)
WST-1 cell viability assay	94.6 ± 6.2 ($n = 3$, $p = 0.48$)

Data represent means ± SE. Each experiment was performed in duplicate or triplicate.

fragments amplified for insulin and β-actin had the expected sizes (230 and 501 base pairs, respectively), and PCR products were undetectable when reverse transcription was performed without reverse transcriptase. Semi-quantitative RT-PCR was performed under conditions where the amplification reaction for the PCR products was within the linear range. Insulin gene expression was increased 1.6-fold by glucose stimulation. Under glucose stimulation, insulin gene expression was decreased by exposure to ELFMF ($p = 0.049$).

The effects of ELFMF on cell viability

To evaluate the effects of ELFMF on cell viability, we compared the cell counts, total protein content, insulin content, and WST-1 assay under exposure to ELFMF to those under sham exposure conditions in the presence of 200 mg/dL glucose (Table 3).

All of these parameters of cell viability under exposure to ELFMF were not significantly different from those under sham exposure conditions.

Discussion

In this study, we demonstrated that glucose-stimulated insulin secretion was decreased by exposure to ELFMF at 60 Hz and 5 mT. In the absence of glucose stimulation, or under exposure to lower magnetic flux density (less than 1 mT), insulin secretion was not altered by exposure to ELFMF (Fig. 1). These glucose dependences of ELFMF effects on insulin secretion are consistent with Hayek's report, although they used a static magnetic field [10]. The results that the effects of ELFMF were dependent on a magnetic flux density are similar to our previous investigations, using other cell lines [11–13].

The mechanisms of glucose-stimulated insulin secretion are as follows: enhanced glucose metabolism increases intracellular ATP/ADP and closes ATP-dependent potassium (K_{ATP}) channels. Simultaneously, membrane depolarization occurs, which induces opening of voltage-dependent calcium channels to increase $[Ca^{2+}]_i$ and stimulates insulin secretion (K_{ATP}-dependent pathway). Although the existence of another glucose signaling pathway (K_{ATP}-independent pathway) has been reported [14], HIT-T15 cells do not express the K_{ATP}-independent pathway [15]. Therefore, we evaluated the effects of ELFMF on the K_{ATP}-dependent pathway to clarify the mechanisms of attenuation of insulin secretion.

The time course of the increase in ATP/ADP [16,17], membrane potential [18], and $[Ca^{2+}]_i$ [16] has been reported previously. In these reports, ATP/ADP increased rapidly (within 20 s after glucose stimulation), and the increase continued for at least 15 min. The increase in $[Ca^{2+}]_i$ was slow (114 s after glucose stimulation) and continued for at least 10 min. The previous report demonstrated that glucose-stimulated insulin secretion from HIT-T15 cells reached a maximum after 6 min of stimulation, and that more than 60% of total insulin secretion was secreted during the first 20 min after stimulation [15]. From these observations, we evaluated the alterations of ATP/ADP, membrane potential, and $[Ca^{2+}]_i$ 5–15 min after stimulation.

In the presence of glucose stimulation and under exposure to ELFMF at 5 mT, we observed attenuation of the increase of ATP/ADP, membrane depolarization, and $[Ca^{2+}]_i$ (Table 1 and Fig. 2). Recently, we reported that exposure to ELFMF attenuated KCl-stimulated insulin secretion, but did not affect Bay K8644-induced insulin secretion [5]. KCl stimulation bypassed the K_{ATP} channels, and Bay K8644 induced the increase of $[Ca^{2+}]_i$. From these observations, we contend that ELFMF influenced the increase in ATP/ADP and membrane depolarization, and that the observed attenuation of the increase of $[Ca^{2+}]_i$ was due to a decrease in the degree of depolarization.

Although the increase of cellular ATP/ADP was due to a decrease in ADP (Table 1), which is consistent with a previous report [17], the ELFMF-induced decrease in ATP/ADP was caused mainly by a decrease in the cellular ATP content ($88.4 \pm 2.3\%$ of sham exposure, mean ± SE, $n = 3$, $p = 0.037$). The decrease in ATP production by exposure to ELFMF has been reported previously using bovine retina [19], which supports the current findings. It is considered likely that the cellular membrane or ion channels could be influenced by ELFMF via the magnetically induced currents and electric field [20,21]. Since the induced electric field is greater in proportion to the magnetic flux density [21], it was reasonable that we could detect the effects of ELFMF at only 5 mT. Inhibition of the increase in $[Ca^{2+}]_i$ by ELFMF, and its relation with signal transduction or stimulus-secretion coupling has also been reported [22], which is consistent with our findings.

The effects of ELFMF have also been detected at the level of mRNA expression. The expression of insulin mRNA was increased by glucose stimulation (Table 2), consistent with a previous report [23]. The increase in insulin mRNA expression was decreased by ELFMF at 5 mT in the presence of glucose stimulation (Table 2). It has also been reported that glucose metabolism and

the increase of $[Ca^{2+}]_i$ were considered to be involved in the glucose-dependent transcriptional control of the insulin gene [23]. These findings suggested that the observed attenuation of insulin mRNA expression exhibited a close relation to attenuation of the increase of ATP/ADP, $[Ca^{2+}]_i$, rather than a direct effect of ELFMF.

While glucose-stimulated insulin secretion and insulin mRNA were decreased, cell viability was not affected by exposure to ELFMF for 2 h. These results showed that exposure to ELFMF at strong magnetic flux density had the potential for clinical use as a novel inhibitory method against insulin secretion.

In conclusion, we investigated the effects of exposure to ELFMF on insulin secretion from HIT-T15 cells. Insulin secretion was decreased by exposure to ELFMF at 60 Hz and 5 mT under glucose stimulation. Cellular mechanistic analysis revealed that exposure to ELFMF prevented the increases of ATP/ADP, membrane depolarization, and $[Ca^{2+}]_i$. The glucose-induced upregulation of insulin mRNA expression was also attenuated, but cell viability was not affected. These findings demonstrate the potential of exposure to ELFMF at strong magnetic flux density for clinical use. Exposure to ELFMF might to be useful for treatment of type 2 diabetes with excessive insulin secretion.

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References

[1] N. Giordano, E. Battisti, S. Geraci, M. Fortunato, C. Santacroce, M. Rigato, L. Gennari, C. Gennari, Effects of electromagnetic fields on bone mineral density and biochemical markers of bone turnover in osteoporosis: a single-blind, randomized pilot study, Curr. Ther. Res. 62 (2001) 187–193.

[2] J.I. Jacobson, R. Gorman, W.S. Yamanashi, B.B. Saxena, L. Clayton, Low-amplitude, extremely low frequency magnetic fields for the treatment of osteoarthritic knees: a double-blind clinical study, Altern. Ther. Health Med. 7 (2001) 54–69.

[3] R. Sandyk, Reversal of an acute Parkinsonian syndrome associated with multiple sclerosis by application of weak electromagnetic fields, Int. J. Neurosci. 86 (1996) 33–45.

[4] T. Sakurai, A. Satake, S. Sumi, K. Inoue, J. Miyakoshi, An extremely low frequency magnetic field attenuates insulin secretion from the insulinoma cell line, RIN-m, Bioelectromagnetics 25 (2004) 160–166.

[5] T. Sakurai, S. Koyama, Y. Komatsubara, J. Wang, J. Miyakoshi, Decrease in KCl-stimulated insulin secretion induced by exposure to extremely low frequency magnetic fields, Eur. Biol. Bioelectromagnetics 1 (2005) in press.

[6] S.J.H. Ashcroft, P. Hammonds, D.E. Harrison, Insulin secretory responses of a clonal cell line of simian virus 40-transformed B cells, Diabetologia 29 (1986) 727–733.

[7] J. Miyakoshi, N. Yamagishi, S. Ohtsu, K. Mohri, H. Takebe, Increase in hypoxanthine–guanine phosphoribosyl transferase gene mutations by exposure to high-density 50-Hz magnetic fields, Mutat. Res. 349 (1996) 109–114.

[8] Y. Shinozuka, M. Okada, T. Oki, K. Sagane, Y. Mizui, I. Tanaka, K. Katayama, K. Murakami-Murofushi, Altered expression of HES-1, BETA2/NeuroD, and PDX-1 is involved in impaired insulin synthesis induced by glucocorticoids in HIT-T15 cells, Biochem. Biophys. Res. Commun. 287 (2001) 229–235.

[9] V. Schultz, I. Sussman, K. Bokvist, K. Tornheim, Bioluminometric assay of ADP and ATP at high ATP/ADP ratios: assay of ADP after enzymatic removal of ATP, Ann. Biochem. 215 (1993) 302–304.

[10] A. Hayek, C. Guardian, J. Guardian, G. Obarski, Homogeneous magnetic fields influence pancreatic islet function in vitro, Biochem. Biophys. Res. Commun. 122 (1984) 191–196.

[11] H. Yaguchi, M. Yoshida, D.R. Ding, K. Shingu, J. Miyakoshi, Increased chromatid-type chromosomal aberrations in mouse m5S cells exposed to power-line frequency magnetic fields, Int. J. Radiat. Biol. 76 (2000) 1677–1684.

[12] J. Miyakoshi, M. Yoshida, K. Shibuya, M. Hiraoka, Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks, J. Radiat. Res. 41 (2000) 293–302.

[13] J. Miyakoshi, M. Yoshida, H. Yaguchi, D.R. Ding, Exposure to extremely low frequency magnetic fields suppresses X-ray-induced transformation in mouse C3H10T1/2 cells, Biochem. Biophys. Res. Commun. 271 (2000) 323–327.

[14] M. Gembal, P. Detimary, P. Gilon, Z.-Y. Gao, J.-C. Henquin, Mechanisms by which glucose can control insulin release independently from its action on adenosine triphosphate-sensitive K^+ channels in mouse B cells, J. Clin. Invest. 91 (1993) 871–880.

[15] S.G. Straub, G.W. Sharp, Glucose-stimulated signaling pathways in biphasic insulin secretion, Diabetes Metab. Res. Rev. 18 (2002) 451–463.

[16] V.N. Civelek, J.T. Deeney, K. Kubik, V. Schultz, K. Tornheim, B.E. Corkey, Temporal sequence of metabolic and ionic events in glucose-stimulated clonal pancreatic beta-cells (HIT), Biochem. J. 315 (1996) 1015–1019.

[17] M.D. Meglasson, K.M. Smith, D. Nelson, M. Erecinska, alpha-Glycerophosphate shuttle in a clonal beta-cell line, Am. J. Physiol. Endocrinol. Metab. 256 (1989) E173–E178.

[18] J.E. Meats, M.D. Tuersley, L. Best, A.M. Lynch, S. Tomlinson, Lactate alters plasma membrane potential, increases the concentration of cytosolic Ca^{2+} and stimulates the secretion of insulin by the hamster β-cell line HIT-T15, J. Mol. Endocrinol. 3 (1989) 121–128.

[19] S. Ravera, E. Repaci, A. Morelli, I.M. Pepe, R. Botter, D. Beruto, Effects of extremely low frequency electromagnetic fields on the adenylate kinase activity of rod outer segment of bovine retina, Bioelectromagnetics 25 (2004) 545–551.

[20] A. Mathie, L.E. Kennard, E.L. Veale, Neuronal ion channels and their sensitivity to extremely low frequency weak electric field effects, Radiat. Prot. Dosimetry 106 (2003) 311–315.

[21] K.R. Foster, Mechanisms of interaction of extremely low frequency electric fields and biological systems, Radiat. Prot. Dosimetry 106 (2003) 301–310.

[22] T. Ikehara, K.H. Park, H. Houchi, H. Yamaguchi, K. Hosokawa, M. Shono, K. Minakuchi, T. Tamaki, Y. Kinouchi, K. Yoshizaki, H. Miyamoto, 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, FEBS Lett. 435 (1998) 229–232.

[23] B. Leibiger, T. Moede, S. Uhles, P.-O. Berggren, I.B. Leibiger, Short-term regulation of insulin gene transcription, Biochem. Soc. Trans. 30 (2002) 312–317.