Effect of Alternating Magnetic Field at Extremely Low Frequency on Cells: Secretion of Insulin by Beta Cells

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ABSTRUCT

The effect of alternating magnetic field at extremely low frequency on secretion of insulin from beta cells has been investigated *in vitro*. Beta cells from a hamster were exposed to the uniform magnetic field with the Helmholtz coil. After three days cultivation, insulin concentration in the medium was measured. The experimental results show that secretion of insulin by beta cells tend to decrease by exposure to alternating magnetic field.

Keywords: Bio-measurement, Magnetic field, Extremely low frequency, Cell-culture, Insulin, Beta cell, Helmholtz coil

1. INTRODUCTION

The effects of the magnetic field on a living body were discussed in many previous studies. Some epidemiological researches show possible effects of the magnetic field on human health. Electromagnetic effects are sometimes applied to accelerate wound healing. Some animal tests were also performed in the previous studies. The effect of magnetic field on cells had been measured in some previous studies [1-5]. Another experiments show that the molecules of collagen orient along the static magnetic field. Beta cells are included in the islets of Langerhans in the pancreas and secrete insulin to control the blood sugar levels.

In the present study, an experimental equipment has been manufactured with the Helmholtz coil to generate uniform alternating magnetic field at extremely low frequency, and the effect of alternating magnetic field at extremely low frequency on secretion of insulin from beta cells has been investigated *in vitro*.

2. METHODS

Helmholtz coil

An alternating magnetic field is generated around a coil with alternating electric current. To generate uniform magnetic field, Helmholtz coil was applied. The Helmholtz coil consists of two circular coils of which both radius and turns are equal to each other. In the Helmholtz coil, two coils are located in the parallel position with the common axis. The distance between two coils is equal to the radius of the coil.

The magnetic field generated between two coils is quasi-uniform, and the magnetic flux density between them is approximately constant [2].

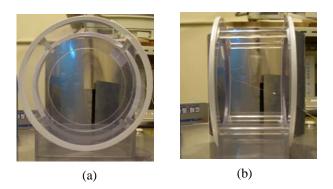


Fig. 1: Helmholtz coil: (a) front view, (b) side view.

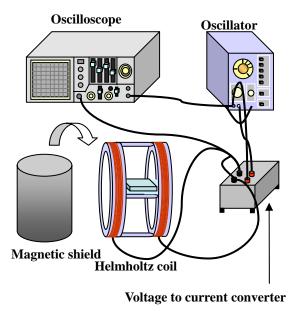


Fig. 2: Experimental system.

A uniform alternating magnetic field (50 Hz, 0.01 mT) was generated with the Helmholtz coil manufactured with radius of 80 mm (Fig. 1). The plate with 24 wells was placed in the magnetic field so that the culture plane of the dish is parallel to the magnetic field.

The Helmholtz coil was enclosed with the flexible sheet of metal to shield the experimental space from the surrounding magnetic field (Fig. 2). In the control group, the culture plate was also enclosed with the metal sheet without the coil to be shielded from magnet fields generated by surroundings.

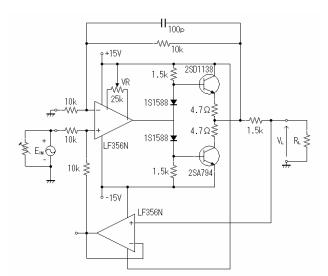


Fig. 3: Voltage to current converter.

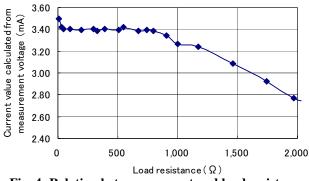


Fig. 4: Relation between current and load resistance.

Through the converter, the output electric current is controlled with the input voltage (Fig. 3). The current in the coil is able to be kept constant independent of load resistance (< 800 Ohm) of the coil (Fig. 4).

The input voltage generated with the oscillator is converted into the electric current, which flows in the Helmholtz coil. In the experiment, both the magnetic flux density and frequency were controlled with the oscillator. The wave-forms of input voltage and output current are measured with an oscilloscope. The generated magnetic flux density is measured with the gauss-meter.

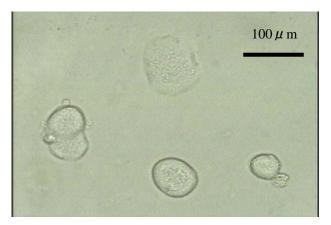


Fig. 5: Beta cells.

Cell culture

Pancreatic beta cells from a Syrian hamster (HIT-T15) were cultured in the F-12K medium (Sodium bicarbonate, L-glutamine) with dialyzed horse serum in 10 percent and fetal bovine serum in 2.5 percent at 37 degrees Celsius (Fig. 5). After exfoliation from a dish with trypsin solution of 2.5 percent and centrifugation at 100 G for one minute, the cells were seeded with density of 32 cells per micro-liter into wells. Two plates of 24 wells were used for cultivation: one is for exposure to the magnetic field, and the other is for control. The cells were cultivated in the uniform magnetic field for three days. The results were evaluated about multiplication rate of cells, and secretion of insulin.

The population of cells in each well was counted with a hemocytometer after tests.

After three days cultivation, insulin concentration in the medium of each well was measured. After reaction with antibody, insulin concentration was quantified with extinction intensity at wavelength of 492 nm. Data were compared between cells exposed to the magnetic field and those shielded from magnetic field (control).

3. RESULTS AND DISCUSSION

Magnetic field was kept constant regardless of the position in Helmholtz coil (Fig. 6).

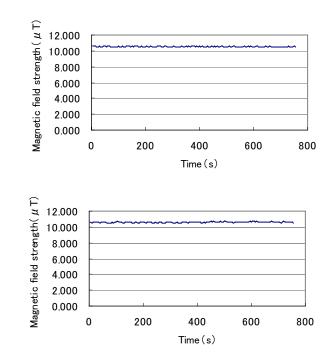


Fig. 6: Magnetic field intensity; at middle part, upper; at corner part, lower.

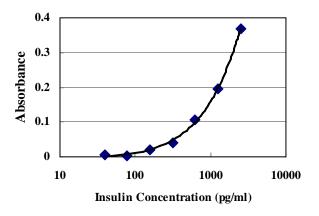


Fig. 7: Calibration for insulin concentration.

The population of cells increases exponentially with time. Table 1 shows cell population calculated from the cell density in each well after the test. The proliferation rate in the magnetic field was similar to that in control condition.

Table 1: Cell population after test; x10⁴

Exposure Cell	A 8		C 11	Mean 9.8
Control Cell	Е 6	_	G 10	 Mean 9.3

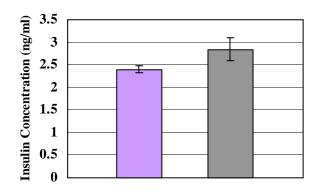


Fig. 8: Insulin concentration; exposure (left), control (right).

Figure 7 shows the calibration curve for insulin concentration. Figure 8 shows concentration of insulin after the test. Data show the mean value in four wells each: the exposure group and the control group. The mean value of insulin concentration in the medium exposed to the magnetic field was lower than that in control.

The intensity of surrounding magnetic fields sufficiently decreased with the flexible sheet of metal.

Experimental systems had been designed to improve magnetic conditions to study the effect of magnetic field on cell culture [6, 7]. The Helmholtz coil is effective to generate the parallel magnetic field. The uniform alternating magnetic field was controlled in the present experimental device with the Helmholtz coil and with the flexible sheet of micro-grain metal. The intensity level of 0.0005 mT occurs in the atmosphere of daily urban life.

Some previous studies show that the magnetic field attenuates insulin secretion from the insulinoma [8]. The magnetic field has possibility for noninvasive treatments for diabetes.

4. CONCLUSION

The experimental study shows that secretion of insulin by beta cells tends to decrease by exposure to alternating magnetic field.

5. ACKNOWLEDGMENT

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6. REFERENCES

- [1] Shigehiro Hashimoto, Hidekazu Tsuji, Chiaki Miyamoto, Atsushi Yamanaka, Yoshiaki Hirano, Naoki Ogawa, Tomohiro Sahara, Hajime Otani, Hiroji Imamura, Yusuke Morita, "Effect of magnetic field at low frequency on cells", *Proc. International Federation for Medical and Biological Engineering*, Vol.3, No.2, 2002, pp. 1402-1403.
- [2] Chiaki Miyamoto, Shigehiro Hashimoto, Yusuke Morita, Hidekazu Tsuji, Atsushi Yamanaka, Takayuki Sekiyama, Tomohiro Kenichi Sahara. Yamasaki, Teruvuki Yamanari, Yuuta Morioka, Yue Wu, Hajime Otani, Hiroji Imamura, "Effect of magnetic field at low frequency on cells arrangement", World Multiconference Proc. 7th on Systemics Cybernetics and Informatics, Vol.8, 2003, pp. 62-66.
- [3] Yuta Morioka, Shigehiro Hashimoto, Chiaki Miyamoto, Yusuke Morita, Takayuki Sekiyama, Tomohiro Sahara, Kenichi Yamasaki, Hideo Kondo, Teruyuki Yamanari, Yoshiaki Hirano, Hajime Otani, Hiroji Imamura, "Effect of Magnetic Field on Cells", *Proc. 8th World Multiconference on Systemics Cybernetics and Informatics*, Vol.7, 2004, pp. 177-182.

- [4] C.L.M. Baureus Koch, M. Sommarin, B.R.R. Persson, L.G. Salford, and J.L. Eberhardt, "Interaction between Weak Low Frequency Magnetic Fields and Membranes", *Bioelectromagnetics*, Vol. 24, 2003, pp. 395-402.
- [5] Jaroslaw Czyz, Teodora Nikolova, Jurgen Schuderer, Niels Kuster, Anna M. Wobus "Non-thermal Effects of Power-line Magnetic Field (50Hz) on Gene Expression Levels of Pluripotent Embryonic Stem Cells; the Role of Tumour Suppressor p53", *Mutation Research*, Vol. 557, 2004, pp. 63-74.
- [6] Jochen Ditterich and Thomas Eggert, "Improving the Homogeneity of the Magnetic Field in the Magnetic Search Coil Technique", *IEEE Transactions on Biomedical Engineering*, Vol. 48, No. 10, 2001, pp. 1178-1185.

- [7] Raj R. Rao and William S. Kisaalita, "A Single Magnetic Field Exposure System for Sequential Investigation of Real Time and Downstream Cellular Responses", *Bioelectromagnetics*, Vol. 25, 2004, pp. 27-32.
- [8] Tomonori Sakurai, Akira Satake, Shoichiro Sumi, Kazutomo Inoue, and Junji Miyakoshi, "An Extremely Low Frequency Magnetic Field Attenuates Insulin Secretion from the Insulinoma Cell Line, RIN-m", *Bioelectromagnetics*, Vol. 25, 2004, pp. 160-166.