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HOMOGENEOUS MAGNETIC FIELDS INFLUENCE PANCREATIC ISLET FUNCTION IN VITRO

Alberto Hayek, Carmela Guardian, Julian Guardian, and Gregory Obarski

Departments of Pediatrics, Medical School, and Physics,
University of New Mexico, Albuquerque, NM 87131

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SUMMARY: Pancreatic islets were isolated from newborn rats and exposed to homogeneous magnetic fields for 48 hours. Under these conditions insulin release, measured at low (5.6 mM) and high (16.7 mM) glucose concentrations, was significant and dose-dependent only at low glucose concentrations. High glucose and aminophylline (10 mM) inhibited insulin release. Thus, in the absence of stimulatory glucose concentrations, low-intensity magnetic fields (1 to 10 Gauss) significantly influence insulin discharge from rat islets in vitro.

The biological effects of magnetic fields have been a subject of much research but without reproducible results. Both strong (1) and low frequency magnetic fields have been reported to induce changes in growth or other physiological systems (2-5). Recently, Jolley et al (6) showed that Ca^{2+} content, Ca^{2+} efflux, and insulin released during glucose stimulation from rabbit islets were curtailed when exposed to low-frequency-pulsed magnetic fields. In this report under homogeneous magnetic fields, high glucose concentrations were also found to inhibit insulin release from islets isolated from newborn rats, but in the unstimulated state (low glucose concentration) insulin was significantly released in a dose-dependent fashion according to the intensity of the magnetic field.

MATERIALS AND METHODS

Magnetic Fields

Homogeneous magnetic fields were generated using an arrangement similar to a Helmholtz Coil configuration. If two induction coils of equal radii R and number of windings N are connected in series and aligned by a common axis perpendicular to their geometrical planes, a constant current flowing through them will induce an approximately constant magnetic flux

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density near the axis halfway between them. The magnitude of this flux density can be shown to be $8\upsilon NIR^2/(d^2 + 4R^2)^{3/2}$, where $\upsilon = 4\pi \times 10^{-7}$ is the permeability of free space in units of Webers/Amp-meter, I is the current flowing through the coils in Amperes, and d is the distance between the coils in meters. For our coils R=0.0665 meters, d=0.11 meters, and N=10,000 windings, so that the expression for the magnetic flux density reduces to B=0.0867I in units of Teslas, or B=867I in units of Gauss. Thus, a current of 1 Amp gives us a field of 867 Gauss.

Animals

Pregnant Sprague-Dawley rats were allowed to deliver. Their pups, 1 to 5 days old, were sacrificed before aseptic removal of the pancreas. Pancreatic islet isolation was carried out according to the tissue culture method, following collagenase digestion, described for the fetal rat pancreas by Hellerstrom et al (7).

The islets were incubated in a humidified atmosphere of 5% CO_2 and air, at 37° C in RPMI 1640 (Gibco) culture medium supplemented with 10% heat-inactivated fetal calf serum (Gibco) and antibiotics: penicillin 100 U/ml and streptomycin 0.1 mg/ml. After two weeks in culture, the islets were picked up under a stereomicroscope and transferred to MicroTest ™ (Falcon) plastic dishes, two islets per well. The incubator was fitted with the modified Helmholtz coils and the islets were placed between the coils on a shelf storage made of Plexiglas. The intensity of the field strength was measured with a transverse probe fitted to a Bell Gausmeter. Control islets were kept on a stage similar to the one used between the coils but in a separate incubator.

Twenty-four hours after placing the islets between the coils both exposed and non-exposed islets were viewed with an inverted microscope to determine attachment to the bottom of the well. After 48 hours insulin stimulations were done on 10 groups of experimental and equal number of control islets. For the stimulations the islets were rinsed once with modified RPMI 1640 (5.6mM glucose); then the wells, containing the islets,

were filled with the same media and incubated for one hour at the center of the Helmholtz coils. The control group was incubated separately. After one hour a 5 µl media sample was collected from each of the 10 wells with an adjustable 20 µl Pipetman and diluted to .5 ml with media for future measurement of insulin by radioimmunoassay (8). The islets were rinsed again and incubated with RPMI 1640 (16.7mM glucose + 10mM aminophylline) for one more hour. As before, samples were collected and stored at -20° C for future simultaneous radioimmunoassay for insulin. Photographs of the islets were also obtained after 48 hours of incubation.

RESULTS

Viewed under the phase contrast microscope, 24 hours after exposure to varying intensity magnetic fields, all the neonatal islets had attached to the bottom of the well in the tissue culture plate. By comparison, during the same time only 20-25% of the control islets had attached. Furthermore, cell migration (Fig. 1) was also evident in all exposed islets after 48 hours but in only 25% of the control islets.

Table 1 shows the insulin released by the neonatal islets placed in the magnetic fields, as a function of field strength, both during basal and stimulated conditions. It is clear that under basal conditions at relatively low glucose concentration (5.6 mM) there is a progressive increase in the amount of insulin released, in direct proportion to the intensity of the field (Fig. 2). In contrast, under conditions which normally stimulate insulin release by islets in vitro, such as the combination of high glucose and aminophylline (8), insulin release is blunted at all levels of field exposure.

DISCUSSION

Isolated pancreatic islets, maintained in culture with glucose media concentration not exceeding 5 mM, usually do not adhere to the dishes and only a few form monolayers. If, however, the islets are exposed to phosphodiesterase inhibitors such as 3-isobutyl-1-methylxanthine or to high glucose concentrations or both, adherence and monolayer formation exceed

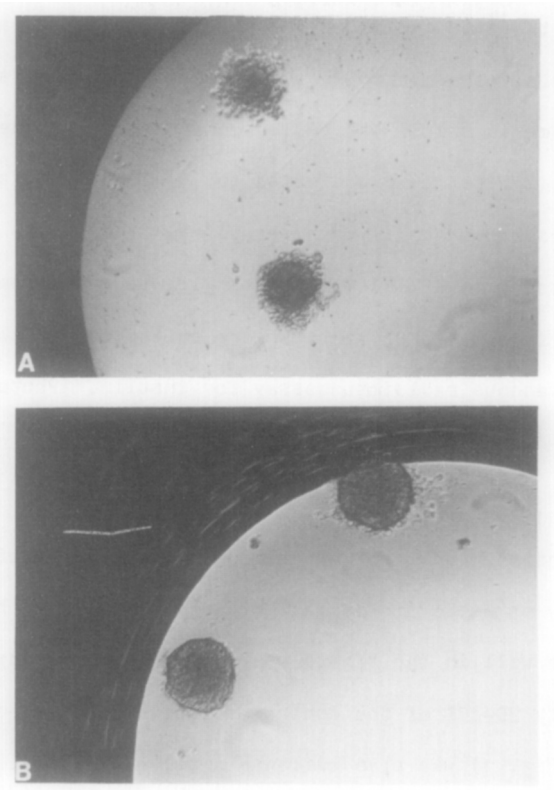

Fig. 1. Neonatal pancreatic islets following exposure to a magnetic field of 5 Gauss. In A there are 2 attached islets per well showing evidence of cell migration in the periphery. In B, 2 control islets, the upper islet also shows evidence of cell migration while the lower islet retains its capsule and appears quiescent (x100).

Table 1. Insulin release (μU/islet/hr) during baseline (glucose 5.6 mM) and stimulated (glucose 16.7 mM + 10 mM aminophylline) conditions

	Field Strength (Gauss)		
Controls	5	7.5	10
	Baseline		
1. 4.3 ± 0.7	9.6 ± 1.9	20.7 ± 2.6	28.8 ± 3.4
2. 3.6 (2.2-10.1)	7.9 (4.1-22.0)	19.8 (8.6-34.1)	26.9 (11.5-45.2)
	Stimulation		
1. 11.8 + 1.1	11.0 + 2.1	16.4 + 2.3	19.2 + 3.1
2. 11.2 (6.1-16.7)	9.6 (4.4-25.2)	16.3 (5.7-29.5)	16.7 (7.5-43.7)
p <.001	N.S.	N.S.	p <.05

1. :Mean ± SEM. 2. :Median (Range) from 10 separate experiments. Each experiment was carried out after 48 hours of incubation under the different magnetic fields shown above. p-value for Mann-Whitney one-tail test

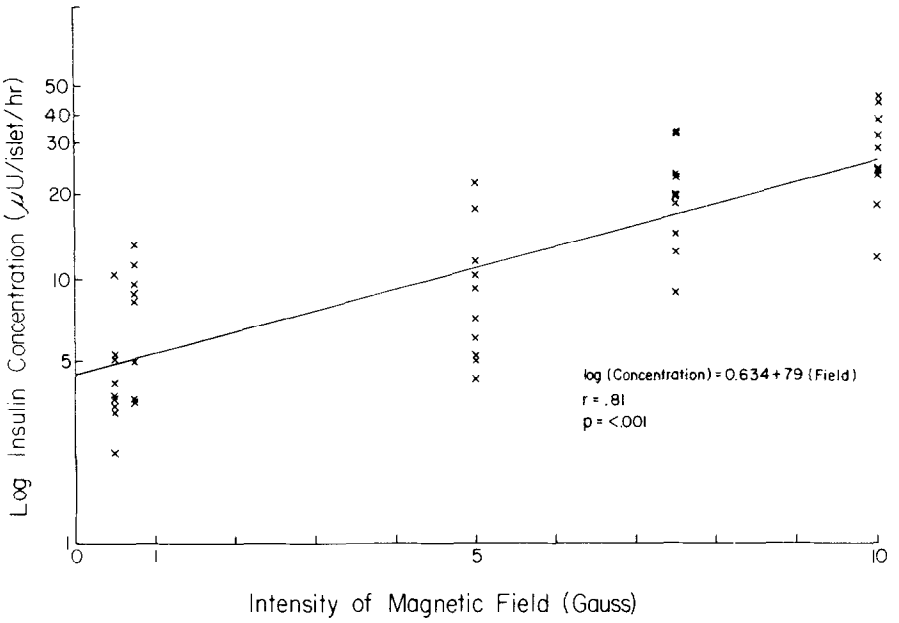

Fig. 2. Regression of the log insulin concentration vs. the strength of the magnetic field. By using the logarithm of the insulin concentration as the dependent variable, the variance was stabilized resulting in a straight line.

80% of the total islets in culture (9). Thus, these findings parallel the results obtained in our studies since most islets exposed to magnetic fields between 5 and 10 Gauss attached and showed evidence of cell migration. Although this cell migration may indicate monolayer formation further experiments are needed if one is to equate cell migration to monolayer formation.

Our observations support the data reported by Jolley et al (6) on the impaired insulin release in isolated rabit islets exposed to high glucose under low-frequency-pulsed magnetic fields. In addition, as seen in Fig. 2, we have shown the opposite effect on insulin release from islets maintained in low glucose concentration under similar conditions. This clearly stimulatory effect on insulin release was dose-dependent on the intensity of the magnetic field.

Insulin release from isolated islets is intimately related to Ca^{2+} movement across the β-cell in the presence of stimulatory glucose concentrations (10, 11). This Ca^{2+} movement is related to the observed

electrical activity in the β-cell (12) recently confirmed by Beigelman (13) in response to high glucose concentrations. In addition to the stimulated entry on Ca^{2+} through potential-dependent channels there is also active Ca^{2+} sequestration in cellular organelles (12).

The findings of Jolley et al (6) showing reduced $^{45}Ca^{2+}$ accumulation, reduced $^{45}Ca^{2+}$ efflux, and decreased insulin release during glucose stimulation under low-frequency-pulsed-magnetic fields in rabbit islets, along with our findings on rat islets showing an increased insulin release at low glucose concentrations under homogenous magnetic fields support their contention that the effects of magnetic fields on membrane function may open a new approach to the study of hormone secretion in endocrine cells.

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