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Brief Communication

Magnetic Field Effects on Calcium Efflux and Insulin Secretion in Isolated Rabbit Islets of Langerhans

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Rabbit islets of Langerhans were exposed at 37 °C for 18 h to a low-frequency-pulsed magnetic field, generated in paired Helmholtz coils. Exposed islets showed a reduction of 26.1 ± 4.3% in $^{45}Ca^{2+}$ content ($P < .004$), a reduction of 25.1 ± 6.3% in $^{45}Ca^{2+}$ efflux ($P < .006$), and a reduction of 35.0 ± 8.7% ($P < .002$) in insulin released during glucose stimulation when compared with appropriate controls.

Key words: Calcium efflux, insulin, secretion, islets of Langerhans, rabbit

It has been generally agreed that a movement of Ca^{2+} into the β cell is required for glucose to stimulate the release of insulin [Hedeskov, 1980]. The exact role of the Ca^{2+} on secretion has been difficult to define. Calcium binds to many molecules, and hence studies on the site of action are difficult and extremely complex. The exact mechanism by which Ca^{2+} is involved in the discharge of insulin remains to be elucidated.

One attractive hypothesis of the mode of insulin release is that of exocytosis. This process involves several sequential steps. Malaisse et al [1971] have proposed that Ca^{2+} triggers movement of contractile proteins in the microtubular system. This movement could conceivably move insulin granules toward the plasma membrane. Dean [1975] has created a model in which Ca^{2+} functions electrostatically in coupling stimuli with secretion. The theory, briefly stated, proposes that once the insulin granule has been moved close to the plasma membrane, it will be held in an equilibrium position by van der Waals forces and electrostatic repulsion. A rise in cytosolic Ca^{2+} would break the equilibrium by altering the surface charge. Electrostatic repulsion would decrease, and the fusion of insulin granules with plasma membrane would result in exocytosis of insulin.

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Extremely low-frequency-pulsed electromagnetic fields induce weak extracellular electric gradients. It was postulated that these gradients modify cell membrane surface anionic binding sites normally occupied by Ca^{2+} [Bawin et al, 1978]. More recent studies strongly support this hypothesis. Cerebral synaptosome fractions exposed to 450-MHz fields sinusoidally modulated at 16 Hz increase $^{45}Ca^{2+}$ efflux by 38%. This change was distinguishable from $CaCl_2$ stimulated $^{45}Ca^{2+}$ efflux, which is most probably intracellularly derived [Lin-Liu and Adey, 1982]. Cultured pheochromocytoma cells exposed to a pulsed magnetic field similar to that in the present study increased noradrenalin secretion, but this response was inhibited by raised Mg^{2+} levels; a finding ascribed to antagonism of Ca^{2+} by Mg^{2+} at cationic binding sites on the membrane surface, with "subsequent cooperative changes in Ca^{2+} binding, modified membrane stability, promotion of Ca^{2+} entry, and vesicular release" [Dixey and Rein, 1982]. The pancreatic islet, therefore, offers a system for study of the role of calcium in glucose-stimulated islet secretion.

The present study was designed to test the effect of magnetically induced currents on isolated rabbit islets. The islets used in these studies were isolated by a nonenzymatic technique in order to preserve the β-cell membranes in the least altered condition possible. This research is part of a program of studies on biophysical and electrochemical mechanisms of tissue interactions with weak oscillating electromagnetic fields.

Pancreata were removed from New Zealand White or California rabbits using a sterile technique. The rabbits were euthanized by a lethal dose of pentobarbitol (70 mg/kg IV). The full details of the technique for isolation of islets are published elsewhere [Hinshaw et al, 1981], but essentially consisted of the following. The excised pancreata were carefully washed in chilled saline to remove most of the blood. The pancreata were then cut into small pieces and excess fluid decanted. The small pancreatic fragments were gently forced through a stainless-steel sieve with openings 200–280 μm. The collected sievate was kept in an ice bath for 4 min and centrifuged at 400 rpm for 5 min. The pellets were resuspended and the procedure repeated at centrifuge speed of 2000 rpm/5 min. The slow centrifugation removes the larger acinar and other unwanted masses. After washing, the pellet was suspended in 8–10 ml of culture media (RPMI 1640, contained 10% fetal calf serum, 11 mM glucose, and antibiotics). The islets were counted and viability ascertained by means of trypan blue dye exclusion technique. Most of the acinar cells seem to be dead at this stage.

Prelabeling of the islet cells with $^{45}Ca^{2+}$ was accomplished by incubating the islets with culture media containing 5 μCi, 25 mCi/mM, of $^{45}Ca^{2+}$ for 1 h at 37 °C. Aliquots were then placed in 75-ml culture flasks in equivalent humidified chambers containing 95% air and 5% Co_2 at 37 °C. The control flask was incubated in one culture chamber while the experimental flask was placed in the space between the two Helmholz coils in the magnetic field in a separate chamber.

The general characteristics of the fields have been described [Pilla, 1980]. The magnetic field generator (Electro-Biology, Inc., Fairfield, NJ) produced bursts of pulses at a 4-kHz rate, each burst lasting 5 ms and repeating at a 15-Hz rate. The initial deflection of each pulse lasted 200 μs, followed by a deflection of opposite polarity lasting 18.5 μs and limited in amplitude to 20% of the initial deflection. The initial pulses of positive current were applied to paired square Helmholtz coils 10 cm on a side, arranged coaxially and 6.3 cm apart. The characteristics of the field have been determined by Sheppard and by Adey, as described in the paper by Luben et al [1982], using search coil and Hall-effect probe methods to determine field intensity at the exposure distribution in the frequency domain. Rate of change of the rising phase of the initial pulse was measured

at 0.92 to 1.02 G/μs. Energy distribution was essentially uniform ($\pm$ 0.3 db) over the range 0.8–9.5 MHz, with a sharp cutoff around 11.0 MHz. These fields produced an electric gradient of approximately 1.0 mV/cm around a 1.0-cm loop in the spatially homogeneous portion of the field between the coils. Expected peak extracellular current density in homogeneous conducting electrolytes would be around 1.0 $\mu A/cm^2$. By reason of higher cell membrane impedance, transmembrane components of these fields would be substantially less.

The islets were cultured for 18 h, and then the islets were perifused in Millipore chambers with culture media containing 18 mM glucose using a perfusion technique [Lacy et al, 1976]. The culture media contained the following concentration of inorganic salts: NaCl, 104 mM; KCl, 5.36 mM; Ca $(NO_3)_2$, 2 mM; $MgSO_4$, 0.41 mM; $NaHCO_3$, 23.81 mM; and Na_2HPO_4, 5.65 mM. Parallel perifusions allowed comparison of insulin secretion and $^{45}Ca^{2+}$ efflux from the control and field-exposed islets.

The $^{45}Ca^{2+}$ determinations were made by counting the perifusate in Beta-Phase scintillation fluid (West. Chem. Prod., San Diego, CA)

Immunoreactive insulin (IRI) assays were performed with 100-μl samples from each fraction [Herbert et al, 1965]. Insulin content in the islet pellets was determined by acid ethanol extraction overnight and then assayed for IRI.

or triplicate determinations on islets from eight different rabbits showed the percentage reduction of $^{45}Ca^{2+}$ content of the field pellets was 26.3% $\pm$ 4.3 SEM; the $^{45}Ca^{2+}$ efflux during glucose stimulation was reduced 25.1% $\pm$ 6.3 SEM; insulin released during glucose stimulation was reduced 35.0% $\pm$ 8.7 SEM. Paired t statistical evaluation of the data showed all three values to be highly significant, $P < .004$, $P < .006$, and $P < .002$, respectively. (The reproducibility of insulin release by this perfusion technique using islet preparations from 25 different rabbits has been very good. 1 $\pm$ 0.19 μU/islet min.) An interesting observation immediately after exposure to the field was a rapid attachment of the islets to the plastic culture flask. This attachment was not seen in the controls and diminished rapidly after removal of the flask from the field. Although we have no direct proof, it appears that this arises because of some change in the islet cell membrane surface charge.

The use of 11 mM glucose in the culture media was important, since various workers have shown that β-cell electrical activity in islets in the presence of 11 mM glucose is characterized by a slow membrane depolarization followed by a rapid depolarization plateau. During the plateau phase, fast action potential spikes are elicited, following which repolarization occurs [Beigelman and Ribalet, 1980; Meissner, 1976; Ribalet and Beigelman, 1979]. Although no evidence is available from the present studies, it seems possible that the weak-pulsed magnetic fields might exert an influence in a very definitive manner during the period of rapid small voltage potential change. Dean and Matthews [1970] showed that β-cell electrical activity during glucose stimulation was due to movement of Ca^{2+} into the intracellular space. Meissner and Schmelz [1974] showed that Ca^2 withdrawal caused a cessation of glucose-induced electrical activity. Channels for Ca^{2+} entry into cells have been proposed [Matthews and Sakamoto, 1975; Dean et al, 1972; Hedeskov, 1980; Schafer and Klöppel, 1974]. These studies indicate that membrane depolarizations are voltage and Ca^{2+} dependent, similar to those shown in squid axon membrane [Baker et al, 1971]. The amount of field-induced current may not be large enough to detect impaired Ca^{2+} movement, unless prolonged exposures are used.

The findings of these studies in which islets have been exposed to weak-induced currents by means of a pulsed magnetic field have shown reduced $^{45}Ca^{2+}$ accumulation

in the islet, reduced $^{45}Ca^{2+}$ efflux during glucose stimulation, and decreased insulin release. It is felt that these electrical gradients have altered membrane charge which, in turn, may have altered the $^{45}Ca^{2+}$ flux and insulin release. The technique appears to have potential use as a probe of cell membrane function.

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