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ARTICLE

Effect of 50 Hz magnetic field exposure on the adherent cell contacts of primary mouse Leydig cells in culture

Zsolt Forgács[1]*, Zoltán Somosy[2], Csaba Révész[1], Gábor Jánossy[3], György Thuróczy[3]

[1]Department of Molecular and Cell Biology, National Institute of Chemical Safety, Budapest, Hungary, [2]Department of Radiopathology, National Frederic Joliot-Curie Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary, [3]Department of Non-Ionizing Radiation, National Frederic Joliot-Curie Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

ABSTRACT Mouse primary testicular intersitital (Leydig) cells obtained from 30-35 g NMRI mice were exposed in a CO_2 incubator to a sinusoidal 50 Hz/100 μT magnetic field for 48 h. Non-exposed, human choriogonin stimulated (0.1 mIU/ml hCG) cells were also cultured as positive controls. Cells were grown as monolayer on cover slips posited on the bottom of the plastic 24-well culture plates. Following the incubation the cells were fixed and permeabilized with -20°C methanol for 2 hours. For immunocytochemical detection of cadherins, ß-catenin and tubulin, cells were incubated over 60 minutes at room temperature with (1:300 diluted) pan-cadherin, anti-ß-catenin or antitubulin. Anti-mouse FITC developed in rabbit was used as secondary antibody. Evaluating the samples by fluorescent microscopy, we found that the applied magnetic field exposure increased the amounts of cadherins and ß-catenin along the surface of the cell-to-cell contacts. The amount of microtubuli was also elevated and typical shape of cells was changed. The effects of magnetic field exposure were similar to those caused by hCG in the positive controls. Further investigations are required to clarify the subcellular action of applied magnetic field in Leydig cells. **Acta Biol Szeged 47(1-4):27-30 (2003)**

KEY WORDS

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With the increasing distribution and utilization of electrical machines and electronic equipment in our society, concern about the possible effect of the electromagnetic fields on our environment on our health is a topic of increasing interest. One of the most important field of research in this topic the investigation of the possible biological effects of power-line frequency (50/60 Hz) magnetic fields.

In our previous experiments we found that in vitro exposure to sinusoidal 50 Hz 100 μT magnetic fields was able to stimulate the testosterone (T) production of mouse Leydig cells in a 48-h primary culture (Forgács et al. 1998a). Similar response was detected following an in vivo 50 Hz, 100 μT magnetic field exposure performed over a period of 14 days (Forgács et al. 2001). In the last decade, several investigators reported that Leydig cells also expressed cell-to-cell adhesion molecules such as cadherins and catenins (Byers et al. 1994; Denduchis et al. 1996).

Hence, the main goal of this study was to evaluate whether the magnetic field-induced functional changes of Leydig cells were accompanied by some morphological alterations or not. In the experiments reported here, we investigated the possible effect(s) of the in vitro 50 Hz magnetic field exposure on the adherent cell contacts and microtubular system of mouse Leydig cells in primary 48-h culture.

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*Corresponding author. E-mail: forgacs@bigfoot.com

Materials and Methods

Animals

NMRI mice (Charles River, Hungary) weighing 30 to 35 g (8-9 week old) were kept in a room with a 12:12 light/dark photoperiod, temperature of 20-23 °C, and relative humidity of 50-60%. Animals were housed eight per cage and allowed free access to standard laboratory pellets and tap water. In each experiment 20 mice were anesthetized with 60 mg/kg ip. pentobarbital (Rhone-Poulenc Rorer, Vitry sur Seine, France) and testes were removed. After surgery, the animals were overdosed with pentobarbital. The mice were kept and handled according to the guidelines of the Hungarian Law of Animal Care.

Interstitial cell preparation and incubation

For isolation of interstitial (Leydig) cells by mechanical dissociation without enzyme treatment the method of Stoklosowa (1982) was used with some modifications, as described earlier (Forgács et al. 1998b). Four independent experiments were performed. In each experiment the cells (obtained from testicles of 20 mice) were isolated in one

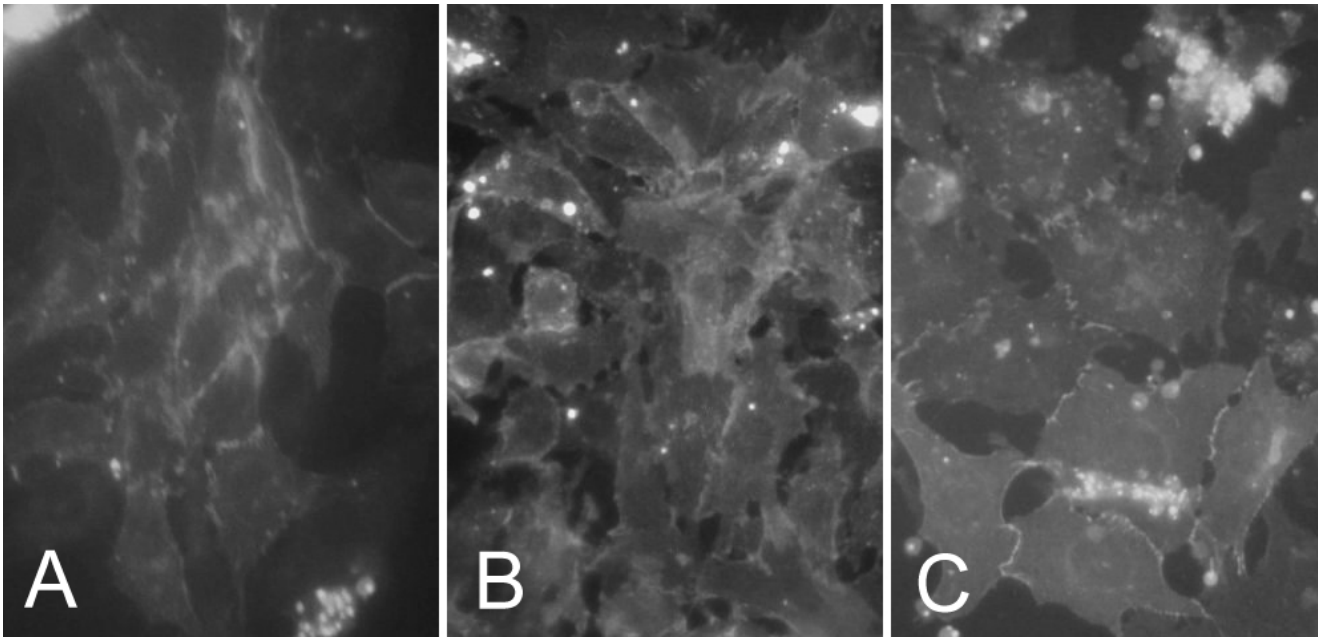

Figure 1. Localization of cadherins in 48-h mouse primary Leydig cell culture detected by pan-cadherin antibody. (A) Non-exposed (controls); (B) non-exposed + human choriogonin-stimulated (0.1 mIU/ml hCG; positive controls); (C) 50 Hz/100 μT magnetic field-exposed cells. Pan-cadherin antibody sdilution was 300:1. Photomicrograph was taken by a fluorescent light microscope (Zeiss Axioskope), with magnification of 400x.

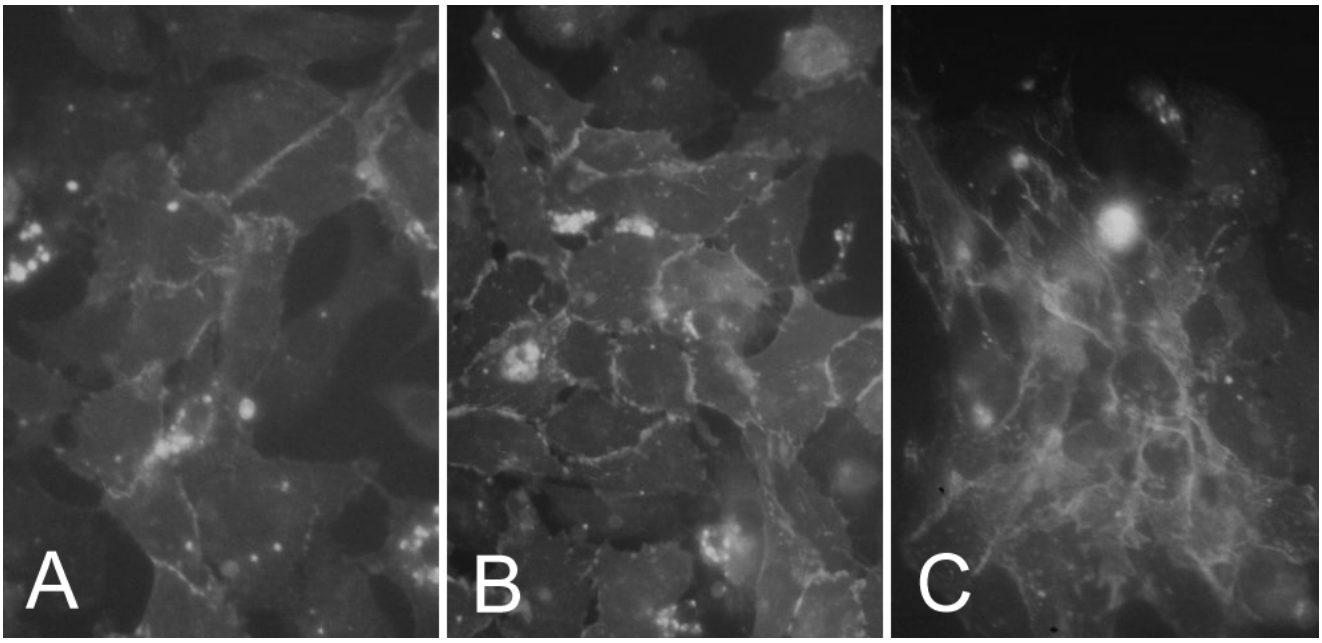

Figure 2. Localization of β-catenin in 48-h mouse primary Leydig cell culture detected by anti-β-catenin antibody. (A) Non-exposed (controls); (B) non-exposed + human choriogonin-stimulated (0.1 mIU/ml hCG; positive controls); (C) 50 Hz/100 μT magnetic field exposed cells. The anti-β-catenin antibody dilution was 300:1. Photomicrograph was taken by a fluorescent light microscope (Zeiss Axioskope), with magnification of 400x.

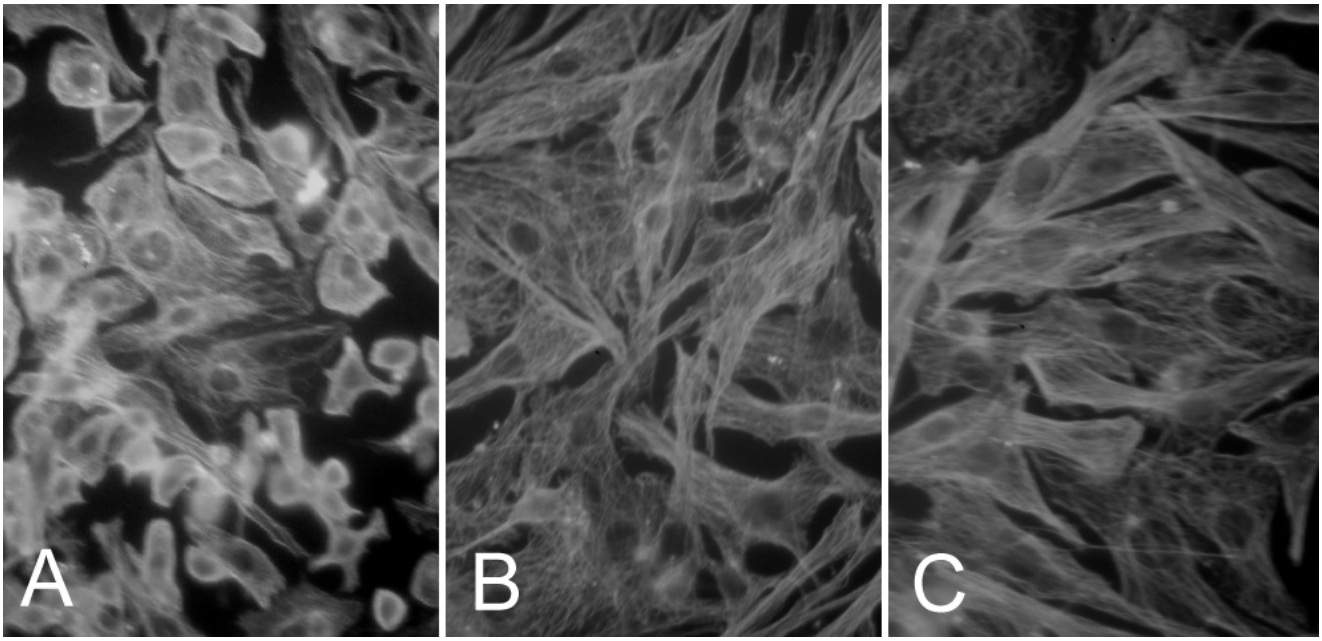

Figure 3. The microtubular structure of 48-h mouse primary Leydig cell culture detected by antitubulin antibody. (A) Non-exposed (controls); (B) non-exposed + human choriogonin stimulated (0.1 mIU/ml hCG; positive controls); (C) 50 Hz / 100 μT magnetic field exposed cells. The antitubulin antibody was purchased from Sigma, the dilution was 300:1. Photomicrograph was taken by a fluorescent light microscope (Zeiss Axioskope), with magnification of 400x.

block. The interstitial cell suspension was diluted to 10^6 cells/ml in (Sigma Chemical Co., St.Louis, MO, USA) supplemented with 10% fetal bovine serum (Sigma), 100 IU/ml penicillin and 100 μg/ml streptomycin (Sigma). The cell suspension was plated (with final volume of 500 μl/well) into sterile 24-well plates (Corning Glassworks, Corning, NY, USA). Cells were grown as monolayer on cover slips posited on the bottom of culture plates.

All incubations were carried out at 34°C under a humidified atmosphere of 95% air/5% CO_2. Non-exposed (controls), non-exposed + human choriogonin stimulated (0.1 mIU/ml hCG; positive controls) and magnetic field exposed cells were cultured.

Magnetic field exposure

The cell cultures were exposed in a CO_2 incubator to AC magnetic field vertically parallel to cylinder axis of a cylindrical recording chamber. Since the lenght of the 12.8 x 8.5 cm culture plates was larger than that of the diameter of coils, only the central 16 wells of the plates were used for cell culture. The frequency of AC magnetic field was 50 Hz with the flux density B(AC) = 100 μT (rms). The duration of exposure was 48 h. Two coils in Helmholtz arrangement were used to produce the vertical AC magnetic field. The inner diameter of coils was 12 cm, the number of turns was 85 for each coil, the diameter of copper wire was 0.3 mm. The coils were connected in a parallel mode (as a pair) with the resultant resistance of 4.2 Ohm (De Seze, 1994). The magnetic field was measured by three dimensional magnetic field meter (Fluxset 3C) and one dimensional Hall-probe connected to gaussmeter (Lakeshore Model 410) and Wandel & Goltermann (EFA-3) magnetic field measurement system (Vertessy 1994). The spectral component of the applied and background magnetic field was analyzed as well. The resultant earth's magnetic field was 46 μT at the National Institute of Chemical Safety (where experiments were conducted). The background 50 Hz magnetic field in the incubator was 40/300 nT (heating off/on).

The stray field of the applied 50 Hz magnetic field was 1-4 μT at the place of the unexposed controls, while the first (100 Hz) and second (150 Hz) harmonics were less than 8% and 3% respectively. The inhomogeneity of the applied 50 Hz magnetic field was better than ± 5% inside the chamber area where the cell culture measurements were performed.

The AC magnetic field was generated by power audio-complex generator (Type TR 0157; HT, Budapest, Hungary).

Immunocytochemistry

Following the incubation the aliquots of the culture medium were removed and the wells of the culture plates were twice washed with phosphate buffer saline (PBS). After washing, for the immunohistochemical detection of cadherins, ß-catenin and tubulin, cells were fixed and permeabilized with methanol at -20 °C for 2 hours. Pan-cadherin, anti-ß-catenin

or anti-tubulin obtained from Sigma (St. Louis, MO) were used in a 1:300 dilution for 60 minutes at room temperature. The FITC-labeled anti-mouse secondary antibody developed in rabbit was obtained from Sigma. Cells were then mounted in Vectashield mounting medium (Vector Lab. Inst. Burgilame, CA) and examined in Axioskope (Zeiss, Germany) fluorescent microscope. Pictures were taken on color slides (Fujichrome 200 or 400 ASA) and scanned in by a slide scanner (Minolta, Dimage Scan Elite II) with 2,800 dpi optical resolution.

Results and Discussion

We found that the applied magnetic field exposure increased the amounts of cadherins (Fig 1C) and ß-catenin (Fig 2C) along the surface of the cell-to-cell contacts detected by immunocytochemical methods. The amount of microtubuli was also elevated (Fig 3C) and the typical shape of cells was changed (Fig 1C, Fig 2C, Fig 3C). The effects of magnetic field exposure were similar to those that caused by hCG, which was used as positive control (Fig 1B, Fig 2B, Fig 3B).

The exact mechanism of action of the applied magnetic field on Leydig cell-to-cell contacts and morphology cannot discerned from the present results. A possible mechanism of action may be associated with the alterations in cAMP content, and intercellular communication may be induced by the applied field. Schimmelpfeng et al. (1995) found increased cAMP content and gap junction-mediated intercellular communication after 5 min of exposure to 50 Hz, 2 mT magnetic field in monolayers of SV40-Swiss-3T3 mouse fibroblasts at intermediate cell density. It is possible that in our prevoius experiments the testosterone production was increased due to an elevated cAMP level. It is known that cAMP is one of the most important second messengers in LH receptor-mediated steroidogenesis by Leydig cells. On the other hand, according to literature data, elevated level of cAMP has a stabilizing effect on cell contacts and increases the number of microtubuli.

Further investigations are required to clarify the subcellular action of applied magnetic field in Leydig cells, as well as to establish the biological significance of this phenomenon.

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