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Catecholamine release from cultured bovine adrenal medullary chromaffin cells in the presence of 60-Hz magnetic fields

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Abstract

Effects of powerline frequency (50/60 Hz) electric and magnetic fields on the central nervous system may involve altered neurotransmitter release. This possibility was addressed by determining whether 60-Hz linearly polarized sinusoidal magnetic fields (MFs) alter the release of catecholamines from cultured bovine adrenal chromaffin cells, a well-characterized model of neural-type cells. Dishes of cells were placed in the center of each of two four-coil Merritt exposure systems that were enclosed within mu-metal chambers in matched incubators for simultaneous sham and MF exposure. Following 15-min MF exposure of the cells to flux densities of 0.01, 0.1, 1.0 or 2 mT, norepinephrine and epinephrine release were quantified by high-performance liquid chromatography (HPLC) coupled with electrochemical detection. No significant differences in the release of either norepinephrine or epinephrine were detected between sham-exposed cells and cells exposed to MFs in either the absence or presence of Bay K-8644 (2 μM) or dimethylphenylpiperazinium (DMPP, 10 μM). Consistent with these null findings is the lack of effect of MF exposure on calcium influx. We conclude that catecholamine release from chromaffin cells is not sensitive to 60-Hz MFs at magnetic flux densities in the 0.01–2 mT range.
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Keywords: Neural-type cells; Neurosecretion; Powerline frequency magnetic fields; Norepinephrine; Epinephrine; Calcium influx

1. Introduction

Exposure to powerline frequency (50/60 Hz) electric and magnetic fields (EMFs) has been reported to influence central nervous system function, such as causing a reduction in the analgesic effects of opiates [1], altering sleep patterns [2], eliciting behavioral effects in animals [3–7] and affecting human performance [8–11]. In addition, other research has demonstrated specific neurochemical changes linked to 50/60-Hz MF exposure that include decreases in choline uptake in the frontal cortex and hippocampus [12], decreases in the cerebrospinal fluid content of the dopamine metabolite, homovanillic acid, and the serotonin metabolite, 5-hydroxyindoleacetic acid [13] and alterations in the circadian pattern of serum melatonin levels both in the rat [14–17] and in humans [18].

Although an understanding of the mechanism underlying powerline frequency EMF effects on the central nervous system would be greatly aided by laboratory investigations conducted at the cellular level on isolated neural preparations, only a few such studies have provided direct evidence of alterations in neuronal activity or neurotransmitter systems after acute, short-duration exposure to 50/60-Hz MFs. In one study, 50-Hz MFs were found to mimic the effects of glutamate and caffeine on the spiking activity of snail neurons [19] and in another, to cause desensitization of serotonin 5-HT-1B receptors in rat brain membrane preparations [20]. A study conducted using PC12 cells, a catecholamine-producing cell line derived from a rat pheochromocytoma, reported that exposure of the cells to a 60-Hz MF reduced intracellular dopamine levels [21].

The goal of the present study was to use a neural-type cell preparation to explore the possibility that reported effects of powerline frequency MFs on central nervous system function may involve a direct effect of MF exposure on neurotransmitter release. We utilized primary cultures of bovine adrenal medullary chromaffin cells, a well-characterized, nontransformed model of neural-type catecholamine-producing cells, to determine whether 60-Hz MFs,

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at magnetic flux densities (0.01–2 mT range) reportedly causing effects on neural tissue both in vivo and in vitro, alter catecholamine release. MF effects on catecholamine release from nonstimulated cells as well as cells incubated in the presence of the calcium channel activator Bay K-8644 or stimulated with the nicotinic receptor agonist dimethylphenylpiperazinium (DMPP) were evaluated relative to similarly treated sham-exposed cells. The analyses included measurement of the release of both the catecholamines norepinephrine (NE) and epinephrine (EPI) that represents secretion from two distinct types of catecholamine-producing chromaffin cells.

2. Materials and methods

2.1. Cell culture

Chromaffin cells were prepared from fresh bovine adrenal medullae and maintained in suspension culture in 100-mm plastic Petri dishes at a cell density of 1×10^5 cells/ml as previously described [22]. Dishes of cells were always placed on the same two shelves in a tissue culture incubator where ambient AC magnetic flux densities and static magnetic flux densities were determined to be the lowest (≤ 1 and 75 μT, respectively). In addition, ambient 60-Hz EMFs were mapped, monitored and recorded at all sites within the laboratory where cell handling occurred so that exposure of the cells to incidental EMFs could be minimized [23].

2.2. MF exposure

On the day of an experiment, cells were transferred to conical glass tubes and pelleted by centrifugation at $20 \times g$ for 2 min at room temperature. Cells were gently resuspended in prewarmed (36 °C) balanced salt solution (BSS; 145 mM NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose, 0.5% bovine serum albumin and 15 mM HEPES, pH 7.40). After resuspension, cells were immediately dispensed into either six-well tissue culture plates (3.33×10^5 cells/35-mm diameter well in 2.5 ml BSS) or into both the inner well (1.33×10^5 cells in one ml BSS) and outer well (4×10^5 cells in 3 ml BSS) of 60-mm organ culture dishes for MF exposure. The cells were then placed into each of two identical MF exposure chambers and equilibrated for 1 h at 31–33 °C.

MF exposure chambers were comprised of double wound, four square coil Merritt systems (13.5 × 13.5 × 13 in.) placed within mu-metal enclosures (Magnetic Shield) that were in turn positioned within separate matched cell culture incubators. Each set of Merritt coils had 33 turns in the outer coils and 14 turns in the inner coils, and was supported by a plexiglass stand. The plates or dishes of cells were placed on a free-standing acrylic platform positioned between the inner coils. Individual coils were constructed on square plastic forms using 16 gauge twisted (pitch of 0.3–0.5 twists/cm) bifilar enameled copper wire mechanically bound using lucite glue and each wrapped with electrically grounded copper tape with a small circumferential gap to stop the flow of induced eddy currents. Coils were energized with an Elgar AC power source interfaced with a computer so that all aspects of field generation were completely automated. The direction of the currents through the two strands of bifilar wire was parallel for field exposure and antiparallel for sham exposure and each Merritt coil system was used interchangeably in either the sham-field or 60-Hz MF exposure modes. Insulating foam rubber placed under the coils minimized vibrations.

Cells were exposed to continuous 60 Hz linearly polarized sinusoidal MFs for 15 min at magnetic flux densities of 0.01, 0.1, 1.0 or 2 mT (checked at the start of each experiment using an EFM-131 magnetic field measurement system). Turn-on/turn-off transients were eliminated by ramping MFs up and down over a period of 5 s and the uniformity of the vertically applied MF was constant to within 4% in the region where the cells were placed. In some experiments, a pulsed MF mode was used that consisted of applying either 30 or 90 pulses each of 5-s duration, with a 5-s duration between pulses (pulse repetition time of 10 s) and a 2-s rise and fall time.

MF exposure was carried out on cells in the absence of any drugs as well as in the presence of the calcium channel activator Bay K-8644 at 2 μM that slightly increases calcium uptake but does not stimulate secretion, or the nicotinic receptor agonist DMPP at 10 μM that causes a robust secretory response. Bay K-8644 addition was 5 min prior to initiation of MF exposure and DMPP addition immediately preceded the applied MF. During MF exposure, RMS current amplitude through the coils was continuously monitored using a multimeter and the waveform was continuously monitored using an oscilloscope.

Temperature in each chamber was continuously monitored to within 0.1 °C by a thermistor probe attached to the inside edge of one of the wells of the dishes. For individual experiments, temperature typically ranged from 28 to 32 °C and data were collected only when interchamber temperature differences did not exceed 2 °C. As determined in preliminary experiments, basal and stimulated release of NE and EPI were indistinguishable between chambers that varied in temperature up to 2 °C. To minimize artefactual effects due to unintentional differences in cell handling between chambers, all operations such as opening and closing of chamber doors, adding drugs to the cells and terminating catecholamine release were performed synchronously for sham- and MF-exposed samples.

2.3. Measurement of NE and EPI secretion

Immediately after MF exposure, the dishes of cells were placed on ice. Cells were then transferred to chilled centrifuge tubes and pelleted by centrifugation at $850 \times g$ at 4 °C for 5 min. A fraction of the BSS supernatant fraction was

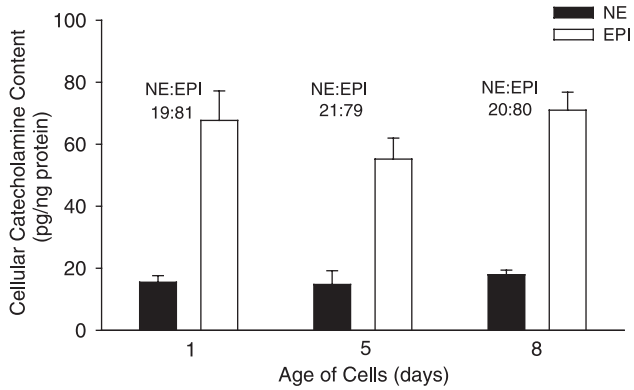

Fig. 1. Intracellular NE and EPI content over time in culture. Cells from a single chromaffin cell preparation were analyzed for NE and EPI content on the days indicated. Each value represents the mean ± S.D. of triplicate samples. The experiment was repeated once on a different cell preparation with similar results.

acidified with perchloric acid (to a concentration of 0.1 N perchloric acid) for measurement of the amount of NE and EPI released, and cell pellets were sonicated in 0.1 N perchloric acid for measurement of total cellular NE and EPI content. Aliquots of each were subjected to high-performance liquid chromatography (HPLC; Waters System) coupled with electrochemical detection (Coulochem II detector, ESA). Separation was performed on an HR-80 column (ESA) using a solvent consisting of 50 mM NaH_2PO_4, 0.24 mM Na_2EDTA, 1.36 mM L-heptanesulfonic acid and 3% methanol at pH 2.6, pumped isocratically at a flow rate of 1 ml/min [24]. 3,4-Dihydrobenzylamine (DHBA) was included in each sample as an internal standard to monitor recovery and catecholamine standards were run with each set of samples. Chromatographic data were acquired and processed using Millennium software (Waters) and the amount of NE and EPI released into the BSS was normalized both to the NE and EPI content and to the protein content of the corresponding cell pellet. Protein was determined using the bicinchoninic acid method of Smith et al. [25].

2.4. Measurement of ^{45}calcium influx

^{45}Calcium uptake studies were carried out using a modification of the method of Gandia et al. [26]. Chromaffin cells were plated at a density of 6.33×10^5 cells/well in six-well collagen-coated tissue culture plates. For the experiments, culture medium was removed, the cells washed with prewarmed (36 °C) BSS ($CaCl_2$ at 1 mM) and 1 ml of the BSS added to each well. Cells were equilibrated in the exposure chambers for 40 min at 31–33 °C. Immediately before MF exposure, each well received 6 μCi of ^{45}calcium chloride (8 mCi/mg calcium). Following MF exposure, the plates of cells were immediately placed on ice and 1.5 ml of calcium-free BSS containing 1 mM $LaCl_3$ added to each well. The ^{45}calcium medium was then aspirated and the cells washed with ice-cold $LaCl_3$–BSS. The cells were lysed in 10% trichloroacetic acid and radioactivity in the lysate was determined by liquid scintillation spectroscopy. An aliquot of the lysate was used to determine protein content.

2.5. Statistical analyses

All experiments were conducted using triplicate cell samples for each variable and results expressed as the mean ± standard deviation. Student's *t*-test was used to

Table 1
NE and EPI release from chromaffin cells placed in parallel chambers during simultaneous sham–MF exposure

Flux density	No drug		Bay K-8644		DMPP	
	Sham	MF	Sham	MF	Sham	MF
Norepinephrine (%)[a]						
0.01 mT	4.4 ± 0.2	4.9 ± 1.0	5.6 ± 0.4	4.7 ± 0.6	18.7 ± 2.0	13.5 ± 6.5
0.10 mT	3.9 ± 1.4	3.4 ± 0.5	4.2 ± 0.5	4.1 ± 0.3	27.8 ± 6.2	27.1 ± 2.7
1.00 mT	4.3 ± 0.2	3.8 ± 0.4	10.0 ± 1.5	7.0 ± 0.4[b]	48.9 ± 10.0	50.4 ± 10.3
2.00 mT	2.2 ± 0.7	2.5 ± 0.5	3.2 ± 0.5	3.1 ± 0.6	20.2 ± 13.5	34.2 ± 8.0
2.00 mT	3.1 ± 0.4	2.4 ± 0.4	6.2 ± 0.7	5.7 ± 0.8	26.4 ± 7.0	24.9 ± 1.2
2.00 mT	3.5 ± 0.3	2.9 ± 0.4	4.0 ± 0.4	3.6 ± 0.4	24.4 ± 4.6	20.9 ± 1.9
2.00 mT_{pulsed}	4.7 ± 0.7	6.2 ± 2.8	6.6 ± 0.6	5.1 ± 1.0	36.9 ± 5.0	47.8 ± 10.1
Epinephrine (%)[a]						
0.01 mT	8.8 ± 0.3	10.3 ± 1.4	9.8 ± 0.6	8.8 ± 1.1	15.9 ± 0.8	12.1 ± 3.4
0.10 mT	7.6 ± 2.5	8.1 ± 1.6	8.5 ± 0.6	8.0 ± 0.4	23.5 ± 6.9	25.4 ± 2.1
1.00 mT	2.1 ± 0.1	2.2 ± 0.2	2.7 ± 0.2	2.3 ± 0.2	32.5 ± 4.7	32.7 ± 5.3
2.00 mT	5.4 ± 0.9	6.7 ± 0.7	6.0 ± 1.0	5.9 ± 0.7	11.5 ± 2.7	15.3 ± 2.5
2.00 mT	2.4 ± 0.2	1.7 ± 0.3	2.5 ± 0.1	2.1 ± 0.2	14.4 ± 3.0	13.5 ± 0.5
2.00 mT	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.0	1.3 ± 0.3	11.7 ± 1.4	11.2 ± 1.0
2.00 mT_{pulsed}	5.0 ± 0.5	5.6 ± 1.5	4.6 ± 0.7	3.7 ± 0.6	15.5 ± 1.2	18.2 ± 3.1

[a] Data are expressed as the percentage of NE and EPI released into the BSS relative to the total cellular catecholamine content of each cell sample. Each value represents the mean ± S.D. of triplicate samples.

[b] $p \leq 0.05$ for MF vs. sham-exposed samples.

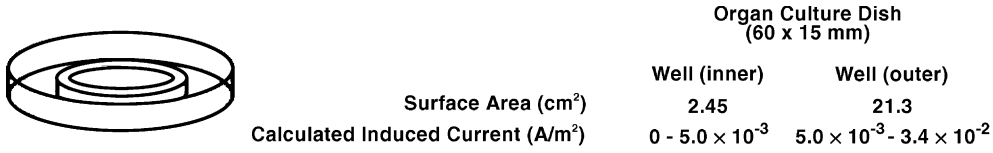

Fig. 2. Schematic of the organ culture dish and calculated induced current densities.

assess the statistical significance between two means and analysis of variance (one-way ANOVA) for comparisons involving multiple means. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Stability of cellular NE and EPI levels

The data in Fig. 1 show that the total cellular content of both NE and EPI remain constant over an 8-day culture period. Based on these data, only cells up to 8 days in culture were used in experiments so that MF effects on catecholamine release were assessed over a period in which cells exhibited a stable catecholaminergic profile. Relative to sham-exposed cells, the cellular content of either NE or EPI was not affected by any of the magnetic flux densities used in this study (data not shown).

3.2. NE and EPI release during sham–sham exposures

Sham–sham exposure experiments (direction of current through the two strands of bifilar wire antiparallel for both exposure chambers) provided an indication of the inherent variability in measuring NE and EPI release between corresponding samples in each of the two exposure chambers. For cells in the absence of drugs, small interchamber variations in the amount of NE and EPI present in the BSS that reached statistical significance ($p < 0.05$) were observed in three out of three experiments. For cells incubated with Bay K-8644 at 2 μM, that does not enhance NE and EPI release to any great extent, and with DMPP at 10 μM, that robustly stimulates both NE and EPI release, variations between corresponding samples reached statistical significance ($p < 0.05$) in only one experiment out of three in each case. As determined by one-way ANOVA, differences in NE and EPI release were, overall, not significant between chambers (data not shown).

3.3. NE and EPI release during MF exposure

Table 1 summarizes the results of seven independent experiments in which NE and EPI release from cells exposed for 15 min to continuous 60-Hz MFs of either 0.01, 0.1, 1.0 or 2 mT or, to a pulsed 2-mT, 60-Hz MF, was compared to NE and EPI release measured for parallel sham-exposed cells. The data provide no evidence that basal release of NE or EPI, release of NE or EPI in the presence of Bay K-8644 or release of NE or EPI stimulated by DMPP, was affected by any of the magnetic flux densities applied to the cells. Only one paired sham-exposed vs. field-exposed sample showed a difference that reached statistical significance.

Table 2
NE and EPI release from chromaffin cells exposed to two different ranges of induced current density at 2 mT

Age of cells	Norepinephrine (%)[a]				Epinephrine (%)[a]			
	Inner well		Outer well		Inner well		Outer well	
	Sham	MF	Sham	MF	Sham	MF	Sham	MF
(1) No drug								
Day 1	2.4 ± 0.8	1.9 ± 0.2	4.1 ± 0.4	4.2 ± 0.6	3.2 ± 1.0	2.6 ± 0.1	4.1 ± 0.5	4.3 ± 0.4
Day 6	3.1 ± 0.7	2.5 ± 0.3	4.6 ± 0.9	4.1 ± 0.5	3.9 ± 0.9	3.2 ± 0.3	4.8 ± 0.7	4.5 ± 0.1
Day 8	10.5 ± 0.8	7.7 ± 0.8[b]	5.1 ± 1.7	3.2 ± 0.2	4.0 ± 0.6	3.0 ± 0.3	2.7 ± 0.7	1.8 ± 0.1
(2) Bay K-8644								
Day 1	5.2 ± 1.6	4.6 ± 0.6	4.2 ± 0.6	4.6 ± 0.6	5.1 ± 1.6	4.8 ± 0.1	4.4 ± 0.8	4.3 ± 0.7
Day 6	8.9 ± 4.4	5.1 ± 0.7	4.2 ± 0.7	4.8 ± 1.0	8.9 ± 4.7	4.5 ± 0.6	4.0 ± 0.8	4.4 ± 0.2
Day 8	15.9 ± 1.3	23.7 ± 10.7[b]	7.5 ± 1.6	6.9 ± 0.5	5.9 ± 0.7	13.6 ± 12.2	2.9 ± 0.2	2.3 ± 0.2
(3) DMPP								
Day 1	11.8 ± 1.6	14.9 ± 1.5	36.2 ± 3.9	32.2 ± 7.2	8.1 ± 1.0	9.7 ± 0.7[b]	22.3 ± 2.3	19.7 ± 4.4
Day 6	26.2 ± 4.1	37.4 ± 12.6	23.8 ± 7.9	18.3 ± 2.8	17.3 ± 2.3	18.4 ± 4.3	16.8 ± 6.7	12.1 ± 1.1
Day 8	22.5 ± 1.7	25.1 ± 3.9	32.2 ± 5.0	32.6 ± 4.2	17.6 ± 1.3	18.0 ± 1.9	29.4 ± 5.2	26.0 ± 4.3

[a] Same as for Table 1.
[b] $p \leq 0.05$ for MF vs. sham-exposed samples.

3.4. NE and EPI release during 2-mT MF exposure at two different ranges of induced current density

To determine whether the magnitude of the induced electric field, and hence the induced current density, was important for observing 60-Hz MF effects on NE and EPI release, chromaffin cells were placed into both the inner and outer wells of an organ culture dish for simultaneous exposure to two different ranges of induced current density. A schematic diagram of the organ culture dish and the induced current density calculated in the inner and outer wells at 2 mT, the field intensity that was used, are given in Fig. 2. Taken as a whole, the data for these experiments (Table 2) show that NE and EPI release from sham-exposed and corresponding MF-exposed cells placed either in the inner wells, which are analogous to the 35-mm wells of the tissue culture dishes used in the studies described previously, or in the outer wells that have induced current densities greater than the inner wells, are equivalent. Because these experiments used cells that were 1, 6 and 8 days in culture, the age of the cells does not appear to be a factor in whether MF effects on NE and EPI release can be detected.

3.5. ^{45}Calcium influx during 2-mT MF exposure

Because calcium influx is necessary for catcholamine release to be stimulated, the inability of 60-Hz MFs to enhance release of NE and EPI from chromaffin cells could mean that either MF exposure has no effect on calcium influx or it has only a slight effect, the magnitude of which is not sufficient to trigger a secretory response (similar to the case for Bay K-8644 at 2 μM). To distinguish between these possibilities, ^{45}calcium uptake into chromaffin cells was examined in the presence of 60-Hz MFs. The results of two representative experiments are given in Table 3. The data show that exposing chromaffin cells to either a continuous or pulsed 1-mT MF was without effect on ^{45}calcium uptake. In one of the experiments in which 2 μM Bay K 8644 had been added to some of the cells, resulting in a two-fold increase in ^{45}calcium uptake, there was no further increase in calcium influx in response to MF exposure. Thus, 60-Hz MFs neither increase calcium influx per se nor enhance calcium influx elicited by Bay K-8644-mediated calcium channel activation.

Table 3
^{45}Calcium uptake into chromaffin cells exposed to 1-mT MFs

	^{45}Calcium uptake (cpm)[a]
1 mT[b]	
Sham (no drug)	8557 ± 611
MF (no drug)	9051 ± 1775
1 mT_{pulsed}[c]	
Sham (no drug)	2444 ± 60
MF (no drug)	2521 ± 195
Sham (Bay K-8644)	5153 ± 124
MF (Bay K-8644)	5292 ± 369

[a] Each value represents the mean ± S.D. of triplicate samples.
[b] Exposure was for 15 min; a 45-s application of 10 μM DMPP increased ^{45}calcium uptake six-fold.
[c] Exposure was for 5 min and Bay K-8644 added 5 min prior to ^{45}calcium; a 45-s application of 10 μM DMPP increased ^{45}calcium uptake eight-fold.

4. Discussion

Effects of 60-Hz MFs on the mammalian nervous system have been reported to include alterations in neurotransmitter/neurohormone turnover and release. The present study used a nontransformed model of electrically excitable catecholamine-producing cells, adrenal medullary chromaffin cells, to determine whether analogous effects can be demonstrated directly in vitro. Because in vitro studies examining responses of biological systems to MFs have described small, rather than robust effects, a great deal of attention was given to carefully controlling all aspects of our experimental procedures and eliminating extraneous stimuli during MF exposure. Our data suggest that acute, short-term exposure of either unstimulated or stimulated chromaffin cells to 60-Hz MFs in the 0.01–2 mT range has no effect on the intracellular level or release of catecholamines.

Cultured bovine adrenal chromaffin cells constitute a well-characterized model of neural-type cells that has been extensively used for studying fundamental mechanisms of neurosecretion. These cells therefore provide an ideal in vitro system for examining the effects of 60-Hz MFs on neurotransmitter release. Because two major types of catecholamine-producing chromaffin cells are present in adrenomedullary tissue, adrenal chromaffin cell cultures contain two distinct populations of secretory cells. One synthesizes, stores and releases NE and in the bovine species comprises 20–40% of the cells. The other synthesizes, stores and releases EPI and comprises the remaining 60–80% of cells. Evidence suggests that the intracellular pathways and/or exocytotic machinery subserving the release mechanisms for the two catecholamines are different [27,28].

Each chromaffin cell type can be readily distinguished by measuring intracellular catecholamine content. Thus, assuming cells contain approximately the same amount of catecholamine, the 20:80 ratio of cellular NE to cellular EPI measured for our cultures indicates that the chromaffin cell population used here contained approximately 20% NE-producing cells and 80% EPI-producing cells. Effects on the release of catecholamine from each cell type also can be distinguished by measuring the NE and EPI content of the medium surrounding the cells. In the absence of stimuli, chromaffin cells spontaneously release catecholamines. The mechanism for the release is unknown but is possibly the result of spontaneous action potentials (depolarizations) that are characteristic of these cells and that are mediated by inward sodium and calcium currents through voltage-sensitive ion channels [29,30]. As determined in the sham-

exposed cell samples, basal release under our experimental conditions was typically less than 10% of the total cellular catecholamine content for each cell type. Exposure of the cells to the specific magnetic flux densities and induced current densities employed in this study neither increased nor reduced the amount of NE and EPI present in the medium, an indication that the cellular mechanisms that contribute to the basal release of catecholamines from either cell type were unaffected by 60-Hz MFs.

As for neurotransmitter release from neurons, catecholamine release from chromaffin cells is stimulated in response to an influx of calcium. Therefore, further evidence that 60-Hz MFs do not stimulate catecholamine release was the lack of effect of 60-Hz MFs on ^{45}calcium uptake. Also, a previous study from this laboratory that evaluated real-time effects of 60-Hz MF exposure in the 0.01–2 mT range on intracellular calcium activity in chromaffin cells did not find evidence of MF-induced alterations on calcium level or spontaneous calcium transients [22]. Thus, our null findings are based on the examination of several biological endpoints.

A number of variables, most notably the state of the cells (i.e., unstimulated versus stimulated), have been proposed to be crucial for detecting 50/60-Hz MF effects in vitro [31]. To determine the relevance of this variable for observing MF effects on NE and EPI release from chromaffin cells, MF exposures were carried out on cells under two experimental conditions. In the first, cells were incubated in the presence of a low concentration of the L-type calcium channel agonist, Bay K-8644. As our data show, the concentration of Bay K-8644 used causes a modest influx of calcium but little or no stimulation of catecholamine release. Thus, cells are not stimulated per se. However, secretory responses evoked by submaximal concentrations of depolarizing stimuli can be enhanced under these conditions [32–34], raising the possibility that MFs may have effects on such "primed" cells. Our findings do not bear out this possibility since neither NE and EPI release, calcium uptake nor intracellular calcium level [22] were altered during MF exposure of Bay K-8644 treated cells.

The second approach to raise excitability was to incubate cells with the nicotinic acetylcholine receptor agonist, DMPP, causing a robust NE and EPI secretory response. Nicotinic acetylcholine receptors are the predominant neurotransmitter receptors responsible for stimulating catecholamine release from bovine chromaffin cells. Agonist binding to the receptor increases sodium and possibly calcium influx through the receptor ionophore. The resulting membrane depolarization causes calcium influx through voltage-sensitive calcium channels and the secretory process is stimulated [35]. As shown here and by others [27], there is a greater release of NE relative to EPI. Because studies have provided evidence that neurotransmitter receptors in the central nervous system appear to constitute molecular targets for 50/60-Hz MFs [1,20], we considered the possibility that the nicotinic receptor itself or the ion fluxes stimulated in response to nicotinic receptor activation could be targets of 60-Hz MFs, resulting in either enhanced or attenuated catecholamine release in response to DMPP. Moreover, previous data from our laboratory showed that a greater percentage of chromaffin cells (up to 20%) exhibited increases in intracellular calcium in response to DMPP after exposure to 1- and 2-mT, 60-Hz MFs for 10 min when compared to nonexposed and sham-exposed cells [22]. On this basis, catecholamine release stimulated by DMPP could be potentially greater during MF exposure since more cells would be responding to the nicotinic receptor stimulus. However, the present findings clearly show no effect of MF exposure on nicotinic receptor-mediated stimulation of catecholamine release. Thus, neither the nicotinic receptor itself nor processes stimulated by nicotinic receptor activation are affected by 60-Hz MF exposure. Furthermore, although our calcium-imaging experiments suggest subtle effects of MF exposure on nicotinic-receptor-mediated chromaffin cell responsiveness, the effects, if real, are not sufficient to translate into functional consequences of any significance.

PC12 cells are a clonal line of rat adrenal pheochromocytoma cells that synthesize and store the catecholamine dopamine. Opler et al. [21] reported that these cells undergo a 20% reduction in intracellular dopamine content during a 10–15-min exposure to a 60-Hz, 8-μT MF. The reduction correlated with an increase in the extracellular content of the dopamine metabolite dopac, indicating that MF exposure affected catecholamine metabolism in these cells. A 60-Hz MF-induced effect on catecholamine levels due to altered metabolism was not similarly observed in chromaffin cells since intracellular levels of NE and EPI were the same in the presence and absence of applied MFs. Differences in the cells (e.g., transformed versus nontransformed), exposure conditions or other experimental variables could underlie these contrasting findings.

In conclusion, adrenal medullary chromaffin cells are not sensitive to 60-Hz MFs in the 0.01–2 mT range. Thus, although these cells are electrically excitable, this property alone is not sufficient to render them susceptible to 60-Hz MFs. The present study leaves open the question of whether the MF exposure conditions that were used here may not have included specific field parameters that exert effects on chromaffin cells since there may be "window effects" [36,37]. We also cannot completely exclude the possibility of effects taking longer to develop and requiring longer exposure periods. For example, exposing neonatal rat adrenal chromaffin cells in vitro to a 60-Hz, 0.7-mT MF for 4 h/day for 7 days is reported to induce phenotypic changes in the cells that include neurite outgrowth [38] and significant increases in dopamine in the culture medium [39]. We tried to replicate these findings in bovine chromaffin cells by carrying out a similar exposure protocol on freshly isolated cells. Neurite outgrowth was only increased in response to staurosporine, an inhibitor of protein kinase C that has been shown to stimulate this process in this cell preparation [40],

not to 60-Hz MFs (data not shown). In addition, intracellular and culture medium levels of both NE and EPI were unchanged. Dopamine levels, constituting a negligible proportion of the total cellular catecholamine content of bovine chromaffin cells, were also unchanged. Taken together, these results suggest that 60-Hz MFs may affect only less-differentiated and not fully differentiated chromaffin cells.

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