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Fifty Hertz electromagnetic field exposure stimulates secretion of β-amyloid peptide in cultured human neuroglioma

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Abstract

Recent epidemiological studies raise the possibility that individuals with occupational exposure to low frequency (50–60 Hz) electromagnetic fields (LF-EMF), are at increased risk of Alzheimer's disease (AD). However, the mechanisms through which LF-EMF may affect AD pathology are unknown. We here tested the hypothesis that the exposure to LF-EMF may affect amyloidogenic processes. We examined the effect of exposure to 3.1 mT 50 Hz LF-EMF on Aβ secretion in H4 neuroglioma cells stably overexpressing human mutant amyloid precursor protein. We found that overnight exposure to LF-EMF induces a significant increase of amyloid-beta peptide (Aβ) secretion, including the isoform Aβ 1–42, without affecting cell survival. These findings show for the first time that exposure to LF-EMF stimulates Aβ secretion in vitro, thus alluding to a potential link between LF-EMF exposure and APP processing in the brain.

Keywords: Electromagnetic fields; Alzheimer's disease; Amyloid precursor protein; Neuron; Amyloidogenesis; Occupational risk

Exposure to low frequency (50–60 Hz) electromagnetic fields (LF-EMF) has been suggested to augment the risk of different human disorders, including not only disturbances in cardiac rhythm and tumors, particularly infantile leukaemia, but also neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases [5,6,8]. Indeed, recent epidemiological studies raise the possibility that individuals with occupational exposure to LF-EMF, are at increased risk of Alzheimer's disease (AD) [10,22,23,24]. However, the mechanisms through which EMF may affect AD are unknown.

One of the most striking neuropathological features of AD is the accumulation of the peptide amyloid β (Aβ) as fibrillar deposits in the brain parenchyma (senile plaques). The discovery that soluble Aβ is a constituent of cerebrospinal fluid [20,21] and is found in cultured cell media [9] indicates that Aβ is a normal product of cellular metabolism of amyloid precursor protein (APP). Aβ is generated from APP by a set of membrane-bound proteases, one at the amino-terminus referred to as β-secretase and one at the carboxy-terminus known as γ-secretase. The C-terminal length of Aβ generated by γ-secretase is heterogeneous, ranging from 37 to 43 amino acids. Aβ40 is the predominant species secreted from the cells, while Aβ42 is a relatively minor isoform [26] which, however, prominently accumulates in senile plaques. Pathogenic missense mutations associated with rare inherited cases of AD are found in APP itself or in presenilin (PS) type 1 and 2, which are essential components of the γ-secretase complex. The majority of these mutations result in an increased Aβ production [1,3,25], a phenomenon correlated with the rate of Aβ deposition [12].

We here questioned whether the exposure to LF-EMF affects amyloidogenic processes in vitro. To this end, human neuroglioma (H4) cells transfected with the human APP were exposed to alternated 50 Hz EMF. These cells are capable of proteolytically processing APP into its peptide fragments, including the Aβ peptides 1–40 and 1–42, and release these peptide fragments in the culture medium. We here provide evidence that

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exposure to LF-EMF stimulates Aβ secretion, including the Aβ 1–42 isoform, in human neuroglioma H4 cells stably transfected with human APP carrying the K670N, M671L Swedish double mutation (H4/APPswe).

H4/APPswe cells were routinely grown in Opti-MEM supplemented with 10% foetal bovine serum, hygromicin (0.1 mg/ml), penicillin 100 U and streptomycin (0.1 mg/ml) in 10 cm dishes. These cells were plated at the density of 10^5 cells/cm^2 in 48-well plates and allowed to grow to confluence. The medium was replaced with 0.5 ml fresh medium and culture plates, kept at $37.0 \pm 0.1\,^{\circ}C$ in an atmosphere of 5% CO_2, were exposed to a 50 Hz alternating magnetic field for 18 h.

LF-EMF was generated by a solenoid wound with a density of 10 turns/cm on a wood support with rectangular section (9.5 cm × 13.5 cm) and 20 cm length. The solenoid winding is directly connected to the secondary winding of a voltage transformer, developing an open-circuit electromotive force corresponding to 12 V_{eff}. The transformer primary winding is connected to the mains supply (220 V, 50 Hz). In these conditions, an ac current of 0.8 A_{eff} is flowing through the solenoid winding, generating an alternating magnetic field of 3.1 ± 0.2 mT along the solenoid axis. Sham-exposed cells were incubated inside the same incubator but outside the coil at a distance of about 40 cm. At such a distance the magnetic field was indistinguishable from the background values. During the experiments, temperature in the incubator was continuously monitored with thermocouple platinum probes (by Delta Ohm, Padova, Italy, featuring a sensitivity of 0.1 °C) in thermal contact with the plates. To avoid temperature increase in the volume surrounded by the solenoid winding (i.e. in the region of cell exposure), due to Joule effect in the current conducting wire, a ventilation system was installed to increase the gas flow through the inner volume of the solenoid support, and hence the heat exchange with the incubator thermal stabilizer.

After overnight incubation at $37.0 \pm 0.1\,^{\circ}C$ in an atmosphere of 5% CO_2, the culture medium of H4/APPswe cells was collected from LF-EMF as well as sham-exposed cells, cleared by centrifugation (12,000 × g for 5 min at 4 °C) and immediately analysed for Aβ by sandwich ELISA. Sandwich ELISA for total Aβ was conducted employing the monoclonal 6E10 (5 μg/ml) against Aβ aminoacids 1–17 as the capturing antibody and biotinylated 4G8 against Aβ aminoacids 17–24 (0.2 μg/ml) as the detecting antibody, whilst Aβ 1–42 levels were determined employing commercial ELISA kits (Genetics Company, Zurich, Switzerland) according to the manufacturer's instructions. In both cases, streptoavidin conjugated with horse radish peroxidase and 3,3′,5,5′-tetramethylbenzidine as substrate was employed as detection system. All ELISA's detections were conducted in duplicate. The amount of Aβ released was expressed as percentage of control values (sham-exposed cells, corresponding to 100%).

After the exposure to the LF-EMF, cell viability was measured by the tetrazolium salt-extraction method which is based on the observation that viable cells with active mitochondria cleave the tetrazolium ring into a visible dark-blue formazan reaction product. 3(4,5-dimethyldiazol-2yl)2,5-diphenyl-tetrazolium bromide (MTT) was added to the culture medium at a final concentration of 0.5 mg/ml and incubated at 37 °C for 45 min. The reaction product of MTT was extracted in dimethylsulfoxide (DMSO) and the optical density (OD) was spectrophotometrically measured at 570 nm with DMSO as a blank. Viability of the cells was expressed as percentage of the control values (sham-exposed cells, corresponding to 100%).

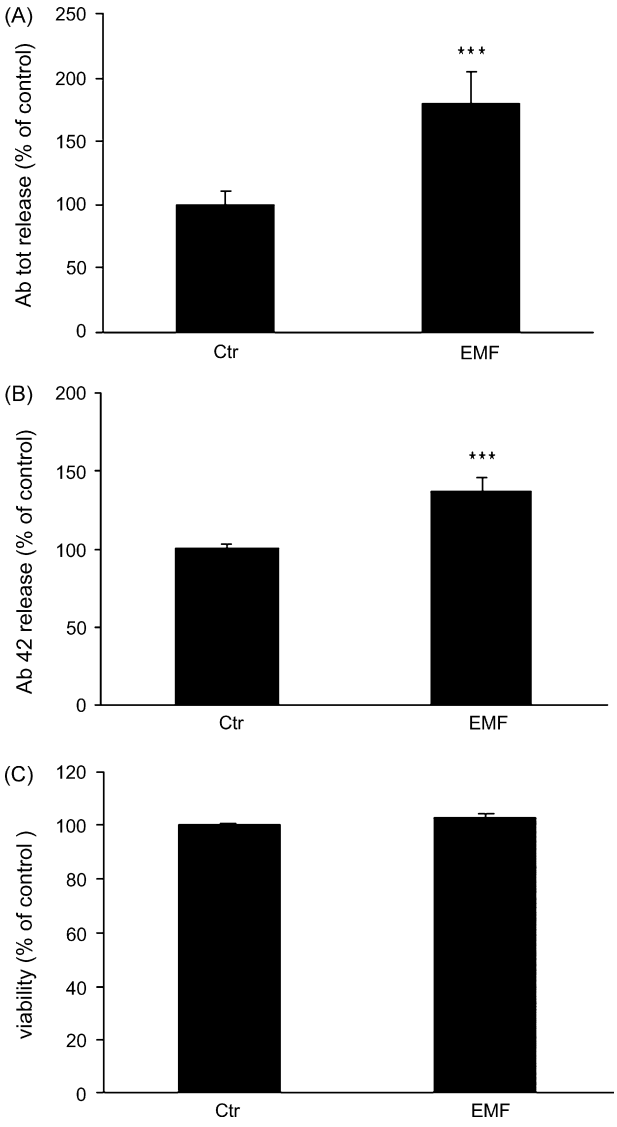

Fig. 1. Effects of LF-EMF on Aβ secretion of H4/APPswe. H4/APPswe cells were exposed (EMF) or sham-exposed (control) to a 50 Hz EMF of 3.1 mT for 18 h. The levels of Aβtot (A) and Aβ42 (B) were measured in the culture medium by ELISA. Cell viability was determined by MTT assay (C). Data, expressed as percentage of sham-exposed cells (control), are represented as mean ± S.E.M. of value from at least 3 independent experiments with $n \geq 5$. Significance of difference between groups were assessed by Student T test ($^{***}p < 0.001$ vs. control).

As shown in Fig. 1A and B, the exposure of H4/APPswe cells to LF-EMF for 18 h resulted in a significant increase of both total (3786 ± 516 pM versus 2098 ± 238 pM) and 1–42 Aβ (365 ± 13 pM versus 278 ± 9 pM) levels in the medium of the H4/APPswe cultures. Moreover, we also observed that LF-EMF does not modify the cell viability of H4/APPswe cells