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

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INTRODUCTION

Exposure to low frequency (50–60 Hz) electromagnetic fields (LF-EMF) has been suggested to increase the risk of several human disorders, including not only disturbances in cardiac rhythm and tumors, but also neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases [1-3]. Indeed, recent epidemiological studies raise the possibility that individuals with occupational exposure to LF-EMF, are at increased risk of Alzheimer's disease (AD). 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 and is found in cultured cell media indicates that Aβ is a normal product of cellular metabolism of amyloid precursor protein (APP).

In the present work effects of LF-EMF exposure on amyloidogenic processes in vitro were studied [4] and the results are presented.

MATERIALS AND METHODS

Human neuroglioma (H4) cells transfected with the human APP were exposed to alternate 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 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±0.1 °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 was directly connected to the secondary winding of a voltage transformer, developing an open-circuit electromotive force of 12V r.m.s. In these conditions, an ac current of 0.8A r.m.s. was flowing through the solenoid winding, generating an alternating magnetic field of 3.1±0.2 mT r.m.s. 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.

RESULTS AND DISCUSSION

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 LFEMF does not modify the cell viability of H4/APPswe cells. Although important epidemiological data indicate exposure to electromagnetic field as a risk factor for Alzheimer's disease , there is no experimental evidence directly linking LF-EMF with cellular and molecular mechanisms relevant to AD pathogenesis. As a first step in investigating the possibility of an association between the LF-EMF exposure and AD at the cellular level, here we employed the H4/APPswe neuroblastoma, a cell line secreting in the extracellular medium easily detectable quantities of Aβ. In this study H4/APPswe cells were exposed to a LF-EMF intensity of 3.1mT, which is higher than the magnetic field measured, for instance, beneath energized power transmission lines, generally ranging between 1 and 10 μT. We chose a relatively high LF-EMF intensity since occupational exposure, which usually results in a lower intensity, is much more prolonged than the one described in our experimental setting. We found that exposure of H4/APPswe cells to a 50 Hz EMF at 3.1 mT for a period of 18 h resulted in a significant increase of total Aβ release in the culture medium including the amyloidogenic 1–42 isoform.

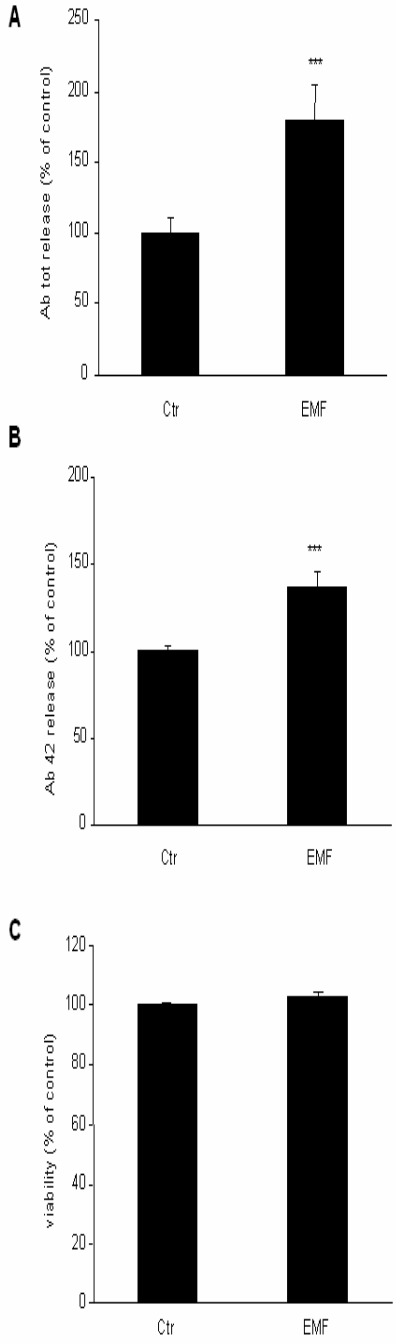

FIG. 1*: Effects of LF-EMF on Aβ secretion of H4/APPswe. H4/APPswe cells were exposed (EMF) or sham-exposed (control) to a 50Hz EMF of 3.1 mT for 18 h. The levels of Aβtot (A) and Aβ42 (B) were measured in the culture medium by ELISA. Cell viability was determined by MTT assay (C). Data, expressed as percentage of sham-exposed cells (control), are represented as mean±S.E.M. of value from at least 3 independent experiments with n≥5.*

We assessed that LF-EMF exposure adopted in this study did not cause altered vitality of H4/APPswe cells nor temperature changes which could potentially affect Aβ metabolism.

The mechanism through which LF-EMF exposure influences Aβ secretion remains to be established. One possibility is that LF-EMF may affect gene expression at the transcription levels. However, it has been reported that 60 Hz electromagnetic field exposure does not affect APP695 transcription in differentiating human neuroblastoma cells. As Aβ peptides 1–40 and 1–42 are 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, it cannot be excluded that LF-EMF may affect the function and/or expression of genes/proteins involved in the regulation of APP metabolism, thus affecting Aβ production.

CONCLUSIONS

This study provides the first experimental evidence that LF-EMF may directly affect processing of APP in neuronal cells. However, while this finding alludes to a potential link between LF-EMF exposure and Aβ production in the brain, further studies are nevertheless warranted to shed light on the relevance of this biological effect of LF-EMF exposure as a potential hazardous factor for AD in occupational environments.

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