Biochemical Changes in Rat Brain Exposed to Low Level Microwave Radiation

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Abstract

Present investigation concerns with various biochemical changes in the developing rat brain exposed to 9.9 GHz (square wave modulated, 1 kHz) exposed for 2 h /d for 35 days. Thirty days old male wistar rats were used for the study. After the exposure biochemical assays such as assays such as calcium ion efflux, protein kinase C (PKC), and ornithine decarboxylase (ODC) were performed. Results of this study reveal that chronic exposure of rat to MW radiation may cause alteration in the brain enzymes. Since these enzymes are related to growth, any perturbation in these enzymes may lead to malfunction of the brain, which ultimately leads to physiological changes.

1. Introduction

Over the past three decades, there has been increasing interest in the biological effects and possible health outcomes of low level, low-frequency electric and magnetic fields. Epidemiological studies on magnetic fields and cancer, reproduction and neurobehavioural reactions have been presented. More recently, neurological, degenerative and heart diseases have also been reported to be related to such electromagnetic fields [1,2]. Furthermore, the increased use of mobile phones worldwide has focused interest on the possible effects of radio frequency fields of higher frequencies.

Interaction of non-ionizing electromagnetic radiations with biological system though non-thermal is now becoming a matter of increasing concern. Most of the studies done earlier with acute, high -level field exposure revealed detrimental effect, which result from thermal injury caused by these fields. Amplitude modulated (AM) radio frequency field has been reported to cause Ca^{2+} efflux from brain tissue *in vitro* [3].
These fields have been reported to affect the properties of the ionic channels, such as decrease in single channel formation and openings and increased rate of rapid burst like firing [4]. There are a number of reports suggesting that AM radio frequency radiation alters the Ca^{2+} binding in the membrane, Na^{+} K^{+}-ATPase activity [5], Ca^{2+}-ATPase activity, cell permeability and central cholinergic activity [6]. Calcium ions are essential in transductive coupling of a wide range of immunological, endocrinological and neurological events at the cell membrane surface. The typical extra cellular calcium concentration is around 2.0 mM, whereas concentrations in the general cytoplasm within cells are far lower around 10^{-7} M. Calcium is the most common signal transduction element in cells ranging from prokaryotes to well developed cells. Ca^{2+} is necessary for the growth and development and cell signalling. Earlier report from our laboratory reported an increased calcium ion efflux in the brain of exposed rats as compared to the control group [7].

Ornithine decarboxylase (ODC) performs a rate-limiting step in the synthesis of polyamines. Its activity has been shown to be a reliable indicator of EMF-induced cellular response [8]. ODC activity has been increased in the exposed rats brain as compared to the control group [9].

Protein kinase C (PKC) is a key enzyme involved in the transduction of signals conveyed from membrane receptors to the intra-cellular region of action of hormones, growth factors and cytokines. Decreased PKC level has been reported in cells exposed to radio frequency radiation [10,11].

2. Materials and Methods

Wistar rats of 30-35 days old (male 60-65 g) were obtained from animal facility of Jawaharlal Nehru University, New Delhi. They were maintained in an air-conditioned room at 30° C and were provided standard food pellets (Hindustan Lever Ltd., India) and tap water *ad libitum.*

At a time two rats were kept in a plexi glass cage (30x9x9cm), and was placed symmetrically along the midline of the pyramidal horn antenna aperture in an anechoic chamber [8]. Exposure was given for 2 h /d for 35 d at 9.9 GHz pulsed wave at power density 0.125 mW/cm^2 (SAR 0.1 W/kg). Sham exposure was similar kind of treatment as exposed group animals but the system was not energized.

2.1 Calcium ion efflux assay

Whole brain tissue of eight sham exposed and eight exposed animals were taken out from the cranial cavity and the assay was performed in each individual brain separately.
The assay was performed according to the method of Blackman et al. [12]. After the exposure period the forebrain was removed without damage from each rat, and each one was divided at the midline. Both the halves were placed in separate test tubes containing 2 ml of $^{45}Ca^{2+}$ (1 μ Ci, specific activity 130 mCi/g) labeled physiological medium These tubes were placed in a 37° C water bath and incubated for 30 min. radiactivty of was measured in a liquid scintillation counter.

2.2. Calcium dependent protein kinase (PKC) assay

Assay was performed in each individual brain separately as previously described by Paulraj and Behari [11]. Briefly each brain was homogenized separately and centrifuged at 20,000 g for 30 min at 4° C. PKC activity was assayed in a total volume of 0.5 ml incubation medium. After addition of 100 μg protein the reaction was initiated by addition of ^{32}P labeled ATP the incubation was carried out at 25° C. Samples of 50 μl were taken out at appropriate intervals (30 - 60 sec) and pipetted onto 3 mm filter discs. Radioactivity was measured by Beckman β counter [11].

2.3. Ornithine decarboxylase (ODC) assay

Method of Wu et al. [13] was followed. Each sample was treated separately in separate test tube. Whole brain was centrifuged at 20,000 g for 10 min at 4° C. Supernatant was centrifuged again at 45,000 g for 90 min at 4° C. Fifty μl of the sample was taken in a test tube containing the reaction mixture. The CO_2 was trapped in 0.2 ml benzothonium hydroxide contained in the center well, followed by injection of 0.5 ml of 5 N H_2SO_4 into the reaction mixture after the 60 min incubation to stop the reaction. Radioactivity was measured by β- counter.

3. Results

Results of our study show that there is an increase in the Ca^{2+} efflux in the brain of chronically as well as 20 min exposed rats' brain as compared to their sham exposed counterpart. The average value of calcium efflux of sham irradiated animals were 647 ± 152/g brain tissue, whereas for the animals exposed to 9.9 GHz 2h/d for 35 days the same was 1682 ± 1047/g brain tissue. The result showed a statistically significant difference as compared to the sham exposed group ($p< 0.05$).
Brain tissues were dissected from chronically exposed animals after the exposure and were further exposed for 20 minutes in the same field, showed a statistically insignificant increase. It was 1682 ± 1047 /g brain tissue for the chronically exposed group animals and 1789 ± 258 /g brain tissue for further exposure for 20 min.

When fresh brain tissue was dissected out and exposed to the above field for 20 min only the calcium efflux level increased as compared to the sham exposed counterpart. It was 647 ± 152 / g brain tissue for sham exposed group and 1605 ± 158/g brain tissue for exposed group. The difference was statistically significant ($p< 0.05$).

PKC activity was reduced significantly in rats chronically exposed to 9.9 GHz radiation as compared to their sham exposed counterpart. PKC activity of sham exposed group was 57083 ± 4970/ mg protein whereas in group exposed 2 hr/ day for 35 days the activity was 37221 ± 8740/ mg protein. The results were statistically significant ($p< 0.001$).

Results showed a significant increase in the activity of ODC of chronically exposed rats brain compared to the sham exposed group. The activity of ODC in the sham exposed group was 0.01298 ± 0.0126 pmole /h/mg

protein, whereas for exposed group the activity was 0.0194 ± 0.0089 pmole/h/mg protein. The increase is statistically very significant ($p < 0.001$) as compared to the sham exposed group.

4.Discussion

Most of the physiological effects triggered by EMF upon nervous tissue are mediated by Ca^{2+} ions and imply that the ions are liberated from their intracellular stores. In mammals inward calcium current during the action potential also induces a release of calcium from internal store.
Earlier reports of Bawin et al. [14] documented that cytochrome system was not involved in this process. Calcium release was measured as a function of time and found to increase more rapidly following exposure to 16 Hz modulated fields rather than following injection of $CaCl_2$ (which release calcium from the intracellular compartment). they suggested that intracellular calcium was not involved in the RF effect on calcium movement. Membrane bound calcium was the probable source of calcium released by these radiation.

The efflux is a whole exchange of Ca^{2+} between cells, tissues and the solutions in the extracellular space. The source of these exchanges can be (i) Ca^{2+} is being exchanged across the cell membrane between cytoplasm and the external solution. (ii) Ca^{2+} is being exchanged between binding sites on the external surface of the membrane and the external solution. In the present study, it is suggested that during the chronic exposure the calcium ions are released from the membrane. These calcium ions are the membrane bound calcium and when the field is applied they are released into the cytosol.

Earlier report showed that cells subjected to prolonged exposure to tumor promoter phorbol esters, resulted depletion in PKC level. Our results also suggest that this field causes decrease in the activity of this enzyme[11]. Since this enzyme plays a pivotal role in mediating cellular stimuli involved in proliferation, differentiation, apoptosis and exocytotic release in a number of neuronal and non-neuronal systems any alteration finally leads to affect the normal growth of the cells.

Byus et al., [15] showed that, there was a transient decrease in the activity of protein kinase C in lymphocytes after 60 min of exposure to a 450 MHz at 1.0 mW/cm^2 field amplitude modulated at 15, 40 and 60 Hz.. Protein kinase C is activated by diacylglycerol formed from membrane inositol phospholipid in response to ligand protein-receptor interactions, which in turn activate the phospholipase C. Butler et al. [16] reported that cells might be functionally depleted of protein kinase by prolonged exposure to biologically active phorbol esters. They reported that the activity was reduced to 92% as compared to sham exposed [16].

Earlier reports showed that both modulated EM field and tumor promoters act at the cell membrane. Several authors have reported similar results. ODC activity increased by 50% following one hour exposure to 450 MHz field, sinusoidally amplitude modulated at 16 Hz [10]. Another study showed an enhancement in ODC activity in L929 cells exposed to 835 MHz field amplitude modulated with sinusoidal 16 and 60 Hz [17]. Byus et al. [18] reported that the enhancement in ODC activity induced by this field exposure was transient. The cell demodulates the microwave signal and that the demodulated ELF stimulus is what affects the cell function [19].

It is suggestive that during the exposure period the proliferation rate is higher and hence the increased activity, leading to conclude that microwave radiation alter the cellular growth and its proliferation.

5. References

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