Protein Kinase C Activity in Rats Brain Exposed to Low Intensity 2.45 GHz Microwave Radiation

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Introduction

There has been a growing concern among the public regarding the potential human health hazard of exposure to radio frequency radiation by these appliances. Living beings are continuously under the influence of the magnetic fields on the earth's surface.
Studies on Central Nervous system (CNS) showed that the exposure to Electromagnetic field (EMF) resulted in depressed activities of different types of neurotransmitter systems. Some of which are acetylcholine esterase, acetylcholine, dopamine, serotonin and amino acids in the brain of animal (Kunjilwar and Behari, 1993; Hagino et al., 1992; Lai et al., 1993).
Zecca et al. (1998) reported that exposure to EMF field alters the neurotransmitter release in the brain cortex and pineal glands. In our previous experiment we found that certain enzymatic activity have been altered in the rats whole brain exposed to 2.45 GHz radiation (Paulraj and Behari 2002).
Protein kinase is an enzyme, which transfers phosphate groups from Adenosine tri-phosphate (ATP) to hydroxyl groups in the amino acid chains of acceptor proteins. The major sites of phosphorylation are specific serine and to a lesser extent the threonine residues. This enzyme plays a pivotal role in mediating cellular responses to extracellular stimuli involved in proliferation, differentiation, apoptosis and exocytotic release in a number of non-neuronal cells (Ohkusu, et al., 1995). The enzyme has also been implicated in phosphorylation of several neuronal proteins, which are thought to regulate neurotransmitter release and establish long-term proliferation in memory formation (Suzuki, 1994).

Several lines of evidence suggest that calcium dependent protein kinase (PKC) modulates ion conductance by phosphorylating membrane proteins such as channels, pumps and ion exchange proteins. It has been proposed that protein kinase C plays a role in extrusion of Ca^{2+} immediately after its mobilization into the cytosol and that Ca^{2+} transport. ATPase is a possible target of this protein kinase (Nishizuka, 1986). This protein kinase C is present in a wide variety of tissues and shows apparently neither tissue nor species specificity (Minakuchi et al., 1981). In the present study effect of 2.45 GHz radiation on PKC activity in the whole brain, hippocampus and whole brain with hippocampus removed was investigated.

MATERIALS AND METHODS

Materials

Radiolabeled ^{32}P ATP was procured from BRIT, Hyderabad India. Rest of the chemicals were procured from local companies.

Animals

Wistar rats of 30 days old (male, 60-65 gms) were obtained from Animal facility of Jawaharlal Nehru University, New Delhi. They were maintained in an air-conditioned room and were provided with standard food pellets (Hindustan Lever Ltd, India) and tap water *ad libitum.*
Exposure was given for 2 hrs /day for 35 days at power density of 0.344 mW/cm^2 in an anechoic chamber (Ray and Behari 1990). Six rats were kept simultaneously in a cage and placed inside the chamber. Control animals were kept in the same way without power input (sham irradiation).

Calcium dependent protein Kinase (PKC) assay

Immediately after the exposure period the animals were sacrificed and brain dissected out of cranial cavity. In these samples PKC was measured in (i) whole brain (ii) hippocampus, (iii) whole brain with hippocampus removed.

The tissue was homogenised in 40 volume of 1 mM sodium bicarbonate (pH 7.5). The homogenate was centrifuged at 600 g for 10 min at 4° C. The supernatant was taken out and centrifuged at 20,000 g for 30 min at 4° C. Supernatant was homogenated with sodium bicarbonate and again centrifuged at 20,000 g for 30 min. The pellet was resuspended in incubation medium (100 mM Hepes, 120 mM NaCl, 1.2 mM $MgSo_4$, 2.5 mM KCl, 15 mM $NaHCo_3$, 10 mM Glucose, 1 mM EDTA) (Havrankova et al., 1978). Protein concentration was measured by Lowry et al. (1951) method.

Protein kinase activity was assayed in a total volume of 0.5 ml incubation medium (50 mM Hepes (pH 7), 10 mM $MgCl_2$, 0.5 mM $CaCl_2$ and 0.2 mM EGTA (free calcium level of 0.1 mM). After addition of 100 μg protein the reaction was initiated by addition of P^{32} labeled ATP (specific activity 3000 Ci / m mole ATP). Incubations were carried out at 25° C. Samples of 50 μl were taken out at appropriate intervals (30 - 60 sec) and pipetted on to 3 mm filter discs which had been pretreated with 10% trichloroacetic acid (TCA), 20 mM sodium pyrophosphate, 10 mM EDTA. These filters were dropped into 500 ml of the TCA mixture and left overnight at 0° C. Filters were washed once in 5% TCA, heated to 90° C for 15 min in 10% TCA and a further 5% TCA wash were extracted in hot ethanol /ether (3:1 v/v) before drying. Radioactivity was measured by Beckman liquid scintillation β counter by dropping the filters in 5ml of Bray's solution (Hetherington and Trewavas, 1982).

RESULTS AND DISCUSSIONS

It is apparent that phenomenon is non thermal in nature. PKC activity (in whole brain) is reduced significantly ($p< 0.05$) in chronically exposed group as compared to their sham exposed counterpart. The PKC activity of exposed group animal was 3804.25 ± 471/ mg protein whereas for the control group, it was 6944.5 ± 193/mg protein (fig.1). In the hippocampus group the experimental results also show significantly different decline ($p< 0.05$). For the sham exposed group it was 7723.75 ± 256/ mg protein and exposed group it was 4390 ± 277/ mg protein (Fig 2).

However in rest of the brain the experimental data do not show a significant difference. Sham exposed group it was 4048.5 ± 947/ mg protein whereas for the exposed group it was 3916 ±505 / mg protein (fig 3). This is suggestive that the hippocampus is probably a preferential site for EMF-biointeraction (Paulraj and Behari 2004). This is in line with many other reports that a chronic exposure of electromagnetic radiation affects learning and memory functions (Rodnight et al., 1982).
Butler et al. (1991) 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 control (Butler et al., 1991).

Byus et al. (1984) 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, amplitude modulated at 15, 40 and 60 Hz. Earlier study from our laboratory also suggest that there was a decrease in the activity of PKC in rats exposed to 147 MHz amplitude modulated at 16 Hz as compared to their control counterpart.

The important role of PKC is the transduction for the activation of many cellular functions and control of cell proliferation. Cells, which are subjected to prolonged exposure to tumor promoter phorbol esters, showed depletion in PKC level. Our results are agreement with these studies suggesting that this field cause decrease in activity of this enzyme. 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.

Furthermore, tumor promoters such as TPA have a membrane receptor in the membrane of all cells. This receptor is considered to be calcium phospholipid-dependent protein kinase (Protein kinase C). It has been involved in the regulation of a variety of cellular events including modulation of receptor functions for major hormones and certain enzymes such as adenylate cyclase and ornithine decarboxylase. It is suggested that protein kinase in the membrane may be a target for low level electromagnetic fields, which leads sequentially to a variety of altered intracellular events in the cells (Byus et al., 1988).

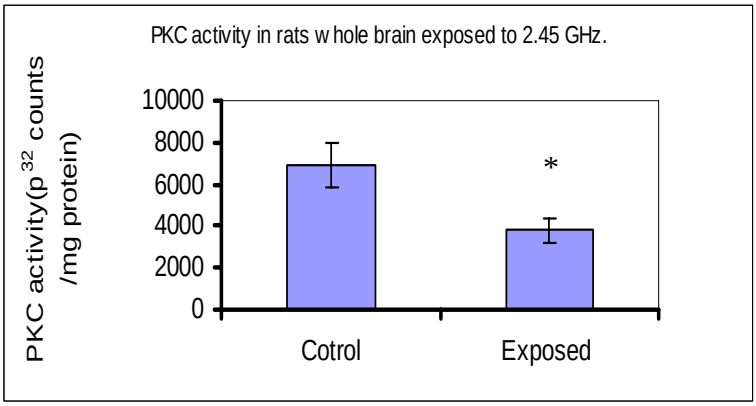

Fig.1 Effect of chronic exposure of 2 .45 GHz Microwave Radiation on protein kinase C activity in rats' whole brain

* Significant ($p<0.05$)

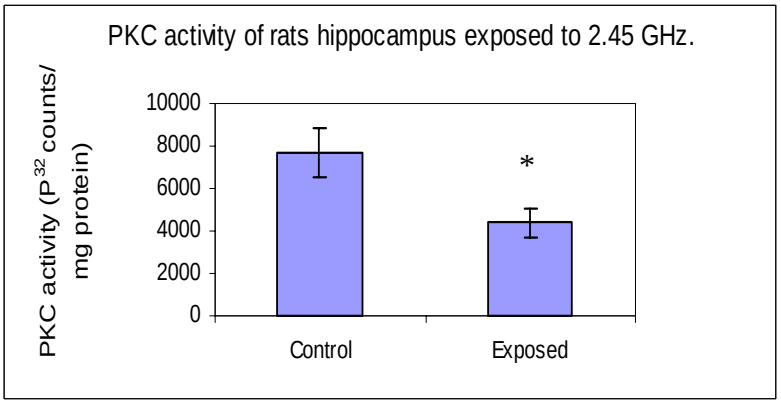

Fig.2 Effect of chronic exposure of 2 .45 GHz Microwave Radiation on protein kinase C activity in rats' hippocampus.

* Significant ($p<0.05$)

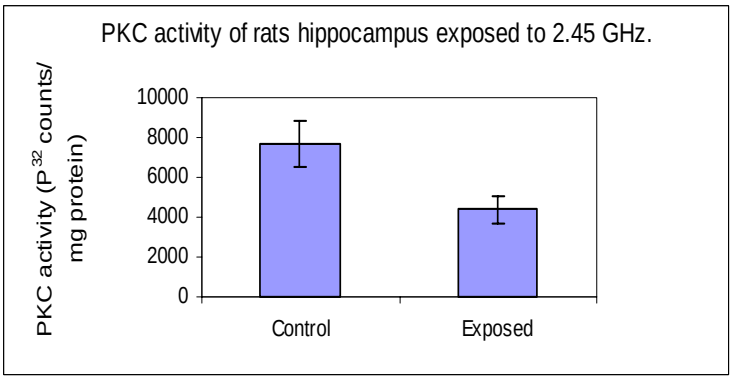

Fig.3 Effect of chronic exposure of 2 .45 GHz Microwave Radiation on protein kinase C activity in whole brain with hippocampus removed

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