

POLY ADP RIBOSYLATION AS A POSSIBLE MECHANISM OF MICROWAVE - BIOINTERACTION

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Abstract: Electromagnetic fields (EMFs) affect the metabolism of the body including the nervous, endocrine, cardiovascular, hematological as well as the reproductive system. EMFs are environmental pollutants, thus posing a health hazard which can cause steric changes in the molecule located at the cell surface. Microwaves are known to cause chromosomal aberrations and act as tumor promoters. The process involves a stream of signals from cell membrane to nucleus and other organelles.

The present investigations aim to understand the mechanism of biological effects of microwaves (2.45 GHz). The effect was studied on poly ADP-ribosylation, which is a post translational modification of chromatin protein catalysed by the enzyme poly ADPR polymerase using NAD⁺ as the substrate. Poly ADP-ribosylation has been shown to be involved in several aspects of chromatin structure and function.

Twenty-three days old rats weighing 42-48 gms were exposed at a microwave dose level of 1.0 mW/cm². After exposure for sixty days the animals were sacrificed and an estimation of poly ADPR polymerase activity was undertaken in different organs of these animals. There was an increase of 20% in its activity in liver, 35% in testis, whereas brain showed a 53% decrease in diencephalon and 20% decrease in the cortex in the exposed animals as compared to their respective controls. There was no change in enzyme activity in spleen and kidney. This was accompanied by concomitant changes in NAD⁺ levels. The above results may be cited as important events in carcinogenesis and tumor promotion related to microwave exposure and the signal transduction mechanism involved. The goal is to shed light on complex ecogenetic interactions leading to cancer modulation of gene expression by epigenetic mechanism.

Key words: electromagnetic field tumour promoter poly ADPR
polymerase chromosomal aberration carcinogenesis

INTRODUCTION

Electromagnetic fields (EMFs) are rapidly intensifying ecological factors produced by high voltage

power lines and by some domestic and therapeutic devices. Electromagnetic fields [extremely low frequency (ELF) and microwaves (MW)] affect the metabolism of the body system including the nervous, endocrine,

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cardiovascular, haematological, immune and reproductive systems (1). There is a higher incidence of childhood leukemia, lymphoma, pancreatic cancer and brain tumor after exposure to ELF (2, 3). MW energy lies between the spectra of radiowaves and visible light and contains characteristics of both electrical and magnetic fields. The mechanism responsible for MW interaction with biological system is poorly understood. However, exposure of laboratory animals and tumors to MW radiation can evoke physiological and psychological responses in experimental subjects and can be harmful to cell membrane, its structural cytoplasm, its growth capacity and whole organism. There is experimental data (4) indicating that MW radiations are not carcinogenic *per se*, but may enhance the potency of certain carcinogens and possess a tumor promoting activity and that there is increased risk of neoplastic disease in human beings exposed to MW radiations. The present study was undertaken to observe the effect of post translational modification of chromatin proteins by poly ADP-ribosylation, an epigenetic mechanism of modulation of gene expression, in a well controlled *in vivo* system.

METHODS

Twenty three days old male Wistar rats, weighing about 42-48 gms, were exposed to MW (2.45 GHz) at a dose level of 1.0 mW/cm² in a microwave anechoic chamber for sixty days. A corresponding group of animals maintained similarly but to which no field was applied, served as controls. Food and water was given *ad libitum*. After the exposure, the animals were sacrificed, the various organs removed and processed accordingly.

Assay of poly ADPR polymerase: The assay system essentially contained 5×10^6 permeabilized cells, 40 mM Tris-HCl, pH 7.8, 0.6 mM EDTA, 1 mM NAD⁺ containing 0.4 μ Ci of [³H] NAD⁺. The radioactivity incorporated into TCA insoluble material was determined as described earlier (5).

NAD⁺ estimation: NAD⁺ and NADH were extracted with cold 0.1M NaOH containing 1mM nicotinamide and NAD⁺ estimated by the method of Jacobson et al (6). Protein was estimated by the method of Lowry et al (7).

SDS-PAGE gel: Electrophoresis of total crude homogenates of brain, testis, liver, and kidney was

carried out on 12.5% SDS polyacrylamide gels according to the method of Laemmli (8).

RESULTS

The effect of exposure to sublethal levels of microwaves (2.45 GHz) was studied on poly ADP-ribosylation in some organs of male Wistar rats. There was an increase of 20% in the basal level of poly ADPR polymerase enzyme activity in liver, 35% in testis, whereas there was a decrease of 53% in brain diencephalon and 20% in brain cortex in the MW exposed animals as compared to their respective unexposed controls. No change in enzyme activity was seen in spleen and kidney (Table I). The alterations in Poly ADPR Polymerase activity were accompanied by changes in NAD⁺ levels. There was a 15% drop in NAD⁺ levels in liver, 20% in testis, but brain diencephalon and cortex showed an increase of 72% and 54% respectively. No significant changes were observed in spleen and kidney (Table II). However, the alterations in NAD⁺ levels are not commensurate with alterations in poly ADPR polymerase, as NAD⁺ is continuously being resynthesized.

TABLE I: Effect of M.W. on poly ADPR polymerase activity.

Tissue	n	Poly ADPR polymerase activity [³ H] NAD incorporated cpm 5×10^6 cells	
		Control	Experimental
Liver	9	35555±8944	42666±8696
Kidney	7	45777±9779	46595±10006
Spleen	7	32936±7894	34964±8847
Testis	7	33297±8900	44950±9016
Brain cortex	9	34020±7902	27216±2322
Brain	9	44952±8878	20963±4326

n = Number of animals. The results are expressed as \pm S.D.

TABLE II: Effect M.W. on NAD⁺ levels.

Tissue	n	NAD ⁺ p moles/mg protein	
		Control	Experimental
Liver	9	1316±106	1119±113
Kidney	7	1710±271	1700±260
Spleen	7	1360±134	1370±163
Testis	7	1463±193	1170±118
Brain cortex	9	1233±163	1898±176
Brain diencephalon	9	1022±120	1757±157

n = Number of animals. The results are expressed as \pm S.D.

When whole cell homogenates from these individual organs were separated on 12.5% SDS-PAGE gels, no significant changes in the protein bands were discernable in MW exposed animals as compared to their respective controls (not shown).

DISCUSSION

Over the past few decades, there has been a drastic increase in the use of devices employing electromagnetic energy. Recent research has shown that ELF can modulate action of hormones, antibodies, neurotransmitters and cancer promoter molecules, at their cell surface receptor sites involving non-linear, non-equilibrium and highly cooperative processes that mediate a major amplification of the initial weak trigger by transmembrane signal coupling (9). Three groups of intracellular enzymes that respond to signals initiated at cell membrane as a response to ELF exposure are membrane bound adenylate cyclase, c-AMP independent protein kinases performing messenger function and ornithine decarboxylase essential for cell growth (9, 10, 11).

Poly ADP-ribose is a homopolymer found in all types of eukaryotic cells. It is synthesized by the nuclear enzyme poly ADPR polymerase which in turn utilizes NAD^+ as substrate. The importance of poly ADP-ribose lies in its role in several aspects of chromatin structure and function (12). Increased polymer levels are seen in malignant and premalignant changes (13).

The effect of MW on male rats was studied and compared for the following parameters - poly ADPR polymerase activity, NAD^+ levels and alteration of protein patterns. MW stimulated poly ADPR polymerase activity in liver and testis to varying extents but decreased the enzyme activity in the brain. This could be because

of inherent differences in cell types of these organs. It has been shown (14, 15) that even a two fold increase in enzyme activity can lead to a several fold increase in poly ADP ribose levels. Poly ADP-ribose is of particular relevance and interest in carcinogenesis and tumor promotion, as it involves chromosomal proteins and provides the cell with a mechanism for modulating gene expression by epigenetic mechanism. The induction of poly ADPR polymerase seen in liver and testis is accompanied by drop in NAD^+ , which acts as a substrate for the enzyme. NAD^+ pools decrease, in association with the polymer formation when rate of consumption exceeds cells capacity for resynthesis. However, in the brain there is an increased NAD^+ pool because of low enzyme activity. Since this enzyme has been reported to be involved in DNA repair (16) the decrease in its activity on MW exposure may probably account for increased rates of malignant brain tumors reported (2) in MW workers. One explanation of low enzyme activity levels in brain could be that the enzyme poly ADPR polymerase might itself act as a self acceptor of the polymer in the brain. We have shown earlier (17) that this is the case with other tumor promoters, such as phorbol esters. Extensive poly ADP-riboseylation of the enzyme leads to its inactivation and results in a drop in its activity. This has been widely reported in the literature (18).

The coomassie blue staining did not show any differential induction in the protein band pattern of the different tissues examined. Our results indicate that MW can affect pathways of signal transduction especially post translational modification of proteins such as by poly ADP-riboseylation, at least in some organs like liver, testis and brain. The alterations of poly ADP-riboseylation may be at the cross road of a series of branching signalling networks.

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