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Acute exposure to high-peak-power pulsed microwaves affecting the histamine H_3 receptor expression in rat hippocampus

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Abstract In the Morris Water Maze test, high-peak-power pulsed microwave (MW)-exposed rats displayed some learning and memory behavior dysfunctions, and their escape time and swimming distance to the submerged platform were longer than those of the sham-exposed rats. To understand the molecular mechanism involved, the reverse transcription-polymerase chain reaction (RT-PCR) and the Western-blotting technique were used for investigating the mRNA and protein expression patterns of the histamine H_3 receptor (H_3R) in rat hippocampus. High-peak-power pulsed microwave-exposure did not remarkably lead to the change in expression of H_3R mRNA in rat hippocampi; however, it promoted the up-regulatory expression of the H_3R protein, which was possibly triggered through the mitogen-activated protein kinase (MAPK) pathways. Therefore, further investigation of the molecular mechanism of the MW effects on the learning and memory behaviors is required.

Key words Histamine H_3-receptor, Rat hippocampus, Microwave, Expression

CLC numbers R122, R122.4

1 Introduction

Numerous *in-vivo* and *in-vitro* studies have been performed to investigate the effects on the biological system resulting from exposure to microwaves (MW), including the use of animal models, the cultured cell model, as well as human volunteers [1]. Some of these were focused on the central nerve system (CNS), especially on the learning and memory behaviors of MW-exposed rats. Similarly, MW at different doses reported by many laboratories using the diverse methods and experimental designs [2] may affect the learning and memory behaviors in rats and mice [3]. These will require considerably more researches for a complete understanding of the molecular mechanisms involved in the learning and memory effects of the MW exposure.

The hippocampus is directly involved in synaptic plasticity and cognitive functions[4]. The N-methyl D-aspartate (NMDA) receptor is a crucial receptor in the production of long-term potential (LTD) in post-synapse, but the formation of the learning and memory behaviors requires more complex interactions among histaminergic, cholinergic, dopaminergic, GABAergic, and other systems [5,6]. In the recent years, many studies have reported that as one of the four histamine receptors (H_1R, H_2R, H_3R, and H_4R), in rat hippocampi, H_3R plays an important role in the learning and memory behaviors[7,8]. The development of H_3R knockout mice has identified the importance of H_3R in the memory processes [9].

Since H_3R predominantly located presynaptically as an autoreceptor was originally discovered in 1983[10], it has been shown to be involved in the regulation of the release of various neurotransmitters (his-

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tamine, γ-aminobutyric acid, noradrenaline, 5-HT, and acetylcholine). As a member of the G-Protein-coupled receptor family of proteins [11], when H_3R was coupled to Gαi, it inhibited the production of cAMP[12]. Rat H_3R cDNA was successfully cloned by Lovenberg *et al* and Drutel *et al*[13,14] in 2000, and therefore, it is now feasible to investigate the mRNA and protein expressions of the H_3R gene in rat hippocampi.

In the present study, the expression pattern of the H_3R gene in rat hippocampi at different times after acute exposure to pulsed MW was investigated at the molecular level. The H_3R mRNA and its proteins were analyzed at different times by the reverse transcription-PCR (RT-PCR) and Western-blotting analysis, and their expression patterns were compared after acute exposure to pulsed MW.

2 Methods and materials

2.1 Animals

Male Sprague–Dawley rats (2 months old, 200—220 g) were purchased from the Experimental Animal Center of Third Military Medicine University, Chongqing, China. The rats were housed in a room with a 12-h light–dark cycle (light on from 7:00 to 19:00 h) at an ambient temperature of 22°C. The animals were provided with food and water during the experiment.

2.2 MW exposures

Thirty rats were simultaneously exposed to pulsed MW, and 10 rats were exposed to sham for 20 min. The MW-exposed rats were exposed to 2.45 GHz pulsed microwaves with a pulse duration of 50 ns and a peak power density of 3.5–6.0 $kW \cdot cm^{-2}$. Owing to the extremely short duration of the pulses, the specific absorption rate (SAR) of the local and whole body could not be measured [15]. The rats were decapitated at different times of 0.08 h, 3 h, 6 h, 12 h, and 24 h post-exposure. During the sham exposures, the animals were placed in similar waveguides for the same periods of time as the microwave-exposed animals, except that the waveguides were not activated.

2.3 Morris Water Maze

The water maze was a steel circular pool filled with water (22°C) to a depth of 28 cm. A steel platform was placed at the center of the S-E quadrant of the maze and was submerged 5 cm below the surface of the water. In each training session, the animals were exposed to MW ($n=10$ rats) or were sham-exposed ($n=10$ rats) for 20 min in the waveguides. The sessions and modes of the training set of rats were as described by Wang *et al* [3]. The traces were recorded using a video recorder, and the escape time was measured using a stop watch.

2.4 RT-PCR

At different time phases of 0.08 h, 3 h, 6 h, 12 h, and 24 h after exposure to pulsed MW, all rats were decapitated and their hippocampi were isolated; the intact rat hippocampi were then homogenized in a glass homogenizer on ice. RNA was extracted with Tripure (Roche Co., Germany), and the concentration of RNA was evaluated by the ratio of the A260/A280 OD values on the spectrophotometer (DU640, Beckman Co., USA). For each sample, the expression of H_3R mRNA was analyzed using PCR with the primers: 5'-ACAGGTATGGGGTGGGTGAG-3' (forward) and 5'-TGTAGTGGCACAGTGGGTAG-3' (reverse). The length of the H_3R PCR products was 435 bp. From the control β-actin mRNA, a 250-bp region was amplified with the primers: 5'-TAAAGACCTCTATGCCAACACAGT-3' (forward) and 5'-CACGATGGAGGGGCCGGACTCATC-3' (reverse). The reaction mixtures were all used at a 25 μL reaction volume (each sample contained 4 μg mRNA). The mixtures contained 2.5 μL PCR buffer, 5 μL $MgCl_2$ (25 mmol/L), 2.5 μL dNTPs (10 mmol/L), 0.5 μL RNAase inhibitor (40 U/μL), 0.5 μL AMV Rtase XL (5 U/μL), 0.5 μL AMV- optimized Taq polymerase (5 U/μL), 25 pmole H_3R primers (or 10 pmole β-actin primers), and 20 μL of RNase-free dH_2O (TaKaRa RNA PCR Kit, TaKaRa Biotechnology Co., Japan). The RT-PCR conditions were: reverse transcription at 50°C for 30 min, denaturation at 94°C for 3 min, 35 cycles at 94°C for 45 s/58°C for 45 s/72°C for 45 s, and the final elongation at 72°C for 10 min.

The electrophoresis of the PCR products was performed on a 1.5% agarose gel stained with EB. The ratio of the H_3R products to the β-actin products was