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Intraseptal Microinjection of β-Funaltrexamine Blocked a Microwave-Induced Decrease of Hippocampal Cholinergic Activity in the Rat

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LAI, H., M. A. CARINO, A. HORITA AND A. W. GUY. *Intraseptal microinjection of β-funaltrexamine blocked a microwave-induced decrease of hippocampal cholinergic activity in the rat.* PHARMACOL BIOCHEM BEHAV 53(3) 613–616, 1996.—Acute (45 min) exposure to pulsed (2 μs pulse width, 500 pulses per second) 2450-MHz microwaves at a power density of 1 mW/cm^2 (whole body specific absorption rate 0.6 W/kg) microwaves caused a decrease in cholinergic activity in the hippocampus of the rat as measured by the sodium-dependent high-affinity choline uptake. Microinjection of β-funaltrexamine (1 μg) into the septum before microwave exposure blocked this effect. These data indicate that μ-opioid receptors in the septum mediate a microwave-induced decrease in cholinergic activity in the hippocampus and support our hypothesis that microwaves at a whole body SAR of 0.6 W/kg can activate endogenous opioids in the brain.

Microwaves Hippocampus Cholinergic activity Endogenous opioids

HIGH-POWER, continuous-wave, as well as modulated microwaves are a source of heating for industrial processing of materials and food, in microwave ovens, and in medical therapy. Low- to high-power continuous-wave, modulated, and pulsed microwaves are extensively used for air-traffic control systems, police and military radars, earth-to-satellite television broadcast systems, and long-distance telephone communication. The possibility that accidental and work-related exposure to microwaves is a current societal concern.

Our laboratory has been involved in research on the effects of microwave radiation on neurological functions and behavior (8). A goal of our research is to understand neural mechanisms affected by microwave exposure. In previous research (10–12), we have found that acute exposure to pulsed 2450-MHz microwaves induces a decrease in cholinergic activity in the hippocampus of the rat. The effect is mediated by endogenous opioids in the brain, because it can be blocked by pretreatments by either systemic administration of naloxone or naltrexone or by intracerebroventricular injection of opioid antagonists. An increase in the concentration of muscarinic cholinergic receptors was observed in the hippocampus of rats subjected to 10 45-min sessions of microwave exposure, and this change could also be blocked by pretreating the rats with naltrexone before each session of exposure (13). A microwave-induced decrease in central cholinergic activity has also been shown by us to be responsible for deficits in spatial learning and memory in animals exposed to microwaves at an average whole-body specific absorption rate (SAR) of 0.6 W/kg. These behavioral deficits could also be blocked by treatment with opioid antagonists (9). We have hypothesized that microwaves activate endogenous opioids in the brain, which in turn, causes a decrease in cholinergic activity in the hippocampus (8).

In this research, we investigated the brain site where endogenous opioids act to cause a decrease in cholinergic activity in the hippocampus by microinjecting the μ-opioid antagonist β-funaltrexamine into the brain in an attempt to block the inhibitory effect of microwaves. Because cholinergic cell bodies innervating the hippocampus are located in the medial septum, and because endogenous opioids are known to play a modulatory role on septal cholinergic neurons (18), we microinjected β-funaltrexamine into the septum to investigate whether opioid receptors in the septum mediate the micro-

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wave-induced decrease in hippocampal cholinergic activity. Sodium-dependent high-affinity choline uptake in the hippocampus was used as a measure of cholinergic activity (2,7).

METHOD AND PROCEDURES

Animals

Male Sprague-Dawley rats (250–300 g) purchased from Tyler Laboratory, Bellevue, WA, were used in this research. They were housed three to a cage in a temperature-controlled (23°C) room adjacent to the microwave-exposure room. The rooms were maintained on a 12 L : 12 D cycle (lights on 0700–1900 h) and at a humidity of 65%. Animals were provided with food and water ad lib during the experiment.

Implantation of Cannula for Intraseptal Drug Injection

At least 5 days before an experiment, rats were implanted with guide cannulae for injection of drug into the septum. Because a metallic cannula can perturb absorption pattern of microwaves in the head of the rat, fine Teflon tubing (Small Parts Inc., Miami, FL; STT-22 tubing, i.d. 0.711 mm, wall thickness 0.154 mm) was used as guide cannula. During implantation, rats were anesthetized with pentobarbital sodium (50 mg base/kg, IP) and injected with atropine methyl bromide (2 mg base/kg, IP) to prevent respiratory congestion. They were then positioned on a stereotaxic instrument (Kopf Instruments, Tujunga, CA) by a pair of blunt-tip earbars. The guide cannula was anchored on the skull by miniature nylon screws (Small Parts Inc., D-MN-080-2) and cold-cure dental cement. The tip of the guide cannula was positioned 1.5 mm above the site of injection, which, according to the rat brain stereotaxic map of Paxinos and Watson (19), corresponds to the coordinates of AP 9.2 mm, L 0 mm, and DV 6.6 mm from the surface of the cortex. Rats were returned to their home cages after they recovered from anesthesia.

Exposure System and Experimental Procedures

At 24 h before exposure, rats were given intraseptal injection of β-funaltrexamine (Research Biochemicals Inc., Natick, MA; 1 μg in 0.5 μl, dissolved in methanol and then diluted with 5 vol of double distilled water immediately before injection). Drug treatment controls were injected with 0.5 μl of the vehicle (methanol/water). This drug treatment schedule has been previously shown to block the mu-opioid receptors in the rat brain [e.g., (1)].

During injection, rats were lightly restrained. A stainless steel injection cannula (30 gauge) was inserted into the guide cannula and injection was started 30 s after insertion and lasted for 30 s. The injection cannula was withdrawn 30 s after injection. Microwave exposure was started 24 h after drug treatment.

The circular waveguide system of Guy et al. (5) was used to expose rats to 2450-MHz microwaves. The waveguide system consists of eight individual cylindrical exposure tubes connected though a power divider network to a single microwave source. However, each waveguide could be turned on individually. Each tube consists of a section of circular waveguide constructed of galvanized wire screen in which a circularly polarized TE11 mode field configuration is excited. The tube also contains a plastic chamber to house a rat. The floor of the chamber is formed of glass rods, allowing waste to fall through plastic funnels into a collection container outside of the waveguide.

Experimental and control animals were subjected simultaneously to microwave or sham exposure, respectively. The microwave-exposed rats were irradiated with pulsed (2 μs pulses, 500 pps), circularly polarized 2450-MHz microwaves at a spatially averaged power density of 1 mW/cm^2. Such a power density gives an average whole body specific absorption rate of 0.6 W/kg for the rats (250–300 g) used in our experiment (3). This dose rate exceeds by 1.5 times the exposure limit recommended for human exposure by the IEEE Standard Co-ordinating Committee (6). During sham exposures, animals were placed in similar waveguides for the same period of time as the microwave-exposed animals, but they did not receive irradiation. Thus, the experiment consisted of four treatment groups: funaltrexamine/microwave, funaltrexamine/sham, vehicle/microwave, and vehicle/sham.

Exposure was done between 0800–1000 h to control for possible circadian variation in response. In this experiment, each animal was subjected to a 45-min session of microwave or sham exposure. At the end of the exposure, animals were decapitated in a small animal guillotine, and the brain was immediately removed from the skull. The hippocampus was dissected on ice according to the method of Glowinski and Iversen (4). The site of injection was examined during brain dissection. Brain dissection and tissue sample removal took approximately 1 min. The variation in brain weight was ± 10 mg among samples. The experiments were set up such that the researchers doing the brain dissection and choline uptake assay did not know the exposure condition (microwave or sham) or the drug treatment (funaltrexamine or vehicle injection) of the animals.

Method of Sodium-Dependent High-Affinity Choline Uptake Assay

Sodium-dependent, high-affinity choline uptake by brain synaptosomes was assayed by the method we described previously (11). Brain tissue was homogenized in 2 ml of 0.32 M sucrose solution with a glass pestle homogenizer. The homogenate was centrifuged at 1,000 × *g* for 10 min. The supernatant was then centrifuged at 12,000 × *g* for 15 min, and the resulting pellet was reconstituted in 2 ml of 0.27 M sucrose. Of this synaptosomal preparation, 0.1 ml was added to each of a set of tubes containing 0.9 ml of a buffer (4% dextrose, 126 mM NaCl, 1.28 mM Na_2HPO_4, 4.75 mM KCl, 1.27 mM $CaCl_2$, and 1.42 mM $MgCl_2$, at pH 7.2), 0.5 mM choline chloride, and 0.4 mCi of [^{3}H]-choline (80 Ci/mmol, New England Nuclear, Boston, MA). Nonsodium-dependent choline uptake was determined by addition of 3.0 mM of hemicholinium-3 (Sigma Chemical Co., St. Louis, MO) to a similar set of tubes. Each brain sample was assayed in triplicates. The samples were then transferred from an ice bath to a water bath at 38°C to incubate for 4 min. Uptake was terminated by return of the samples to the ice bath. Synaptosomes were then collected by centrifugation at 8,000 × *g* for 20 min. The supernatant was discarded and the pellet washed with 1 ml of ice-cold 0.9% saline. The saline was removed and the pellet dissolved in 0.7 ml of hyamine hydroxide (ICN Biochemicals, Inc., Irvine, CA). Ten milliliters of Cytoscint (ICN Biomedicals, Inc.) were then added. Radioactivity was determined by liquid scintillation technique. High-affinity choline uptake was the differnce in uptake in the absence and presence of hemicholinium-3. Protein concentration of the synaptosomal fraction was determined by the method of Lowry et al. (15), with bovine serum albumin as an external standard. High-affinity choline uptake was expressed as pmol/mg protein/4 min.

Data Analysis

Data were analyzed by the two-way analysis of variance (ANOVA) and the difference between two treatments was compared by the Newman-Keuls test. A difference at $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results of intraseptal injection of β-funaltrexamine on microwave-induced decrease in hippocampal cholinergic activity are shown in Fig. 1. The two-way ANOVA showed a significant drug-treatment effect, $F(1, 23) = 8.922$, $p < 0.025$, but nonsignificant microwave, $F(1, 23) = 0.139$, effect and microwave × drug interaction, $F(1, 23) = 3.441$. The Newman-Keuls test indicated that microwave exposure significantly decreased the high-affinity choline uptake activity in the hippocampus (vehicle/microwave vs. vehicle/sham, $p < 0.01$). No significant difference was found between the responses of the funaltrexamine/sham and funaltrexamine/microwave treatment groups.

In addition, we have also assayed high-affinity choline uptake from the hippocampus of unhandled rats. These rats were housed in the laboratory for 48 h and then hippocampal choline uptake was assayed without any experimental manipulation. High-affinity choline uptake activity in the hippocampus of these animals was 23.1 ± 1.7 pmol/mg protein/4 min (mean ± SEM from 12 animals), which is not significantly different from that of the vehicle/sham-treated rats. Thus, the experimental procedures of the present study did not significantly affect cholinergic activity in the hippocampus.

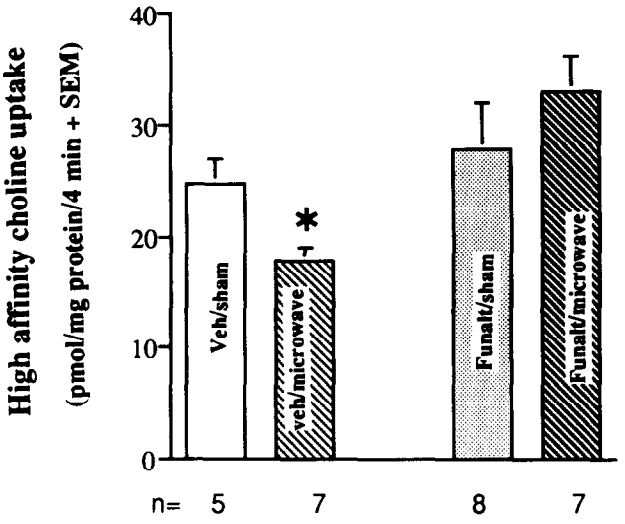

FIG. 1. Effect of intraseptal injection of β-funaltrexamine on microwave-induced decrease in sodium-dependent high-affinity choline uptake in the hippocampus of the rat. (Funalt = β-funaltrexamine; veh = vehicle) *Significantly different from veh/sham at $p < 0.01$.

The results of this experiment further support our hypothesis that acute exposure to microwaves can affect the septohippocampal cholinergic system via activation of endogenous opioid mechanism. Our previous experiment (11), based on intracerebroventricular injection of opioid antagonist, suggested involvement of μ-opioid receptors. The present experiment confirmed this finding and further indicates that the interaction between endogenous opioids and acetylcholine activity occurs in the septum, probably on the cell bodies of the septo-hippocampal cholinergic neurons. This is possible because it has been shown that endogenous opioid neurons in the lateral septum synapse on cholinergic cell bodies in the medial septum and exert an inhibitory effect (18). The majority of opioid receptors in the septal area are μ-receptors. An earlier study (22), using the radioligand binding technique, reported that the septum of the rat contains high concentration of μ-opioid receptors but no detectable amount of δ and κ opioid receptors. More recent studies (16,17) with in situ hybridization assay also showed that the septum and diagonal band of the rat have high concentrations of μ-opioid receptors relative to δ and κ receptors.

The possible involvement of δ and κ opioid receptors in the effect of microwaves on hippocampal cholinergic activity cannot be discounted. Both μ and δ opioid agonists have been shown to depress acetylcholine turnover in the hippocampus of the rat (21). Opioids can also affect hippocampal cholinergic activity via other pathways. For example, it is reported that, though μ-, δ-, and κ-agonists have no significant effect on spontaneous release of acetylcholine from hippocampal slices from the rat, μ-agonist, but not δ nor κ-agonists, could decrease potassium-evoked release (14). However, our data indicate that opioid action in the septum plays a major role in the effect of microwaves on hippocampal cholinergic activity, because the effect of the radiation was completely blocked by intraseptal injection of funaltrexamine. Thus, our data imply that acute exposure to microwaves leads to activation of endogenous opioids in the septum, which then inhibits the activity of the septohippocampal cholinergic pathway. Related to this conclusion is that Stein et al. (20) showed that both electric foot shock and water-deprivation could affect μ-, but not δ-, opioid receptors in the septum of the rat, suggesting a role of septal opioid-mechanism mediating the effect of experimental stressors. This is in agreement with our speculation that microwave radiation is a stressor (8).

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