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Low-Level Microwave Irradiation and Central Cholinergic Systems

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LAI, H., M. A. CARINO, A. HORITA AND A. W. GUY. *Low-level microwave irradiation and central cholinergic systems.* PHARMACOL BIOCHEM BEHAV **33**(1) 131–138, 1989.—Our previous research showed that 45 min of exposure to low-level, pulsed microwaves (2450-MHz, 2-μsec pulses, 500 pps, whole-body average specific absorption rate 0.6 W/kg) decreased sodium-dependent high-affinity choline uptake in the frontal cortex and hippocampus of the rat. The effects of microwaves on central cholinergic systems were further investigated in this study. Increases in choline uptake activity in the frontal cortex, hippocampus, and hypothalamus were observed after 20 min of acute microwave exposure, and tolerance to the effect of microwaves developed in the hypothalamus, but not in the frontal cortex and hippocampus, of rats subjected to ten daily 20-min exposure sessions. Furthermore, the effects of acute microwave irradiation on central choline uptake could be blocked by pretreating the animals before exposure with the narcotic antagonist naltrexone. In another series of experiments, rats were exposed to microwaves in ten daily sessions of either 20 or 45 min, and muscarinic cholinergic receptors in different regions of the brain were studied by ^{3}H-QNB binding assay. Decreases in concentration of receptors occurred in the frontal cortex and hippocampus of rats subjected to ten 20-min microwave exposure sessions, whereas increase in receptor concentration occurred in the hippocampus of animals exposed to ten 45-min sessions. This study also investigated the effects of microwave exposure on learning in the radial-arm maze. Rats were trained in the maze to obtain food reinforcements immediately after 20 or 45 min of microwave exposure. Exposure to microwaves for 20 min prior to training had no significant main effect on maze learning but affected the shape of the learning curve, whereas 45-min exposure significantly retarded the performance of the rats.

Microwaves Cholinergic systems Choline uptake Naltrexone Muscarinic receptors Radial-arm maze

MICROWAVES used in both industrial and commercial environments raise concern about possible health hazards, especially in situations where repeated exposure to low-level microwave radiation is unavoidable (19,29). Numerous studies have reported on the biological effects of microwave irradiation, including endocrine and neurological activities, drug actions, and molecular and cellular functions (1, 17, 20).

We have been studying the effects of low-level microwave exposure on cholinergic activity in the central nervous system. In a previous paper (15) we reported that acute (45-min) exposure to low-level microwaves decreased cholinergic activity in the frontal cortex and hippocampus, as measured by sodium-dependent high-affinity choline uptake (28). Furthermore, the effects on hippocampal choline uptake can be blocked by pretreatment with the narcotic antagonists naloxone and naltrexone, suggesting activation of endogenous opioids by microwaves, which in turn causes the changes in cholinergic activity. In further experiments (16), we investigated the effects of repeated microwave exposure on central cholinergic activity. We found that tolerance developed to the effects of microwaves after repeated 45-min sessions of exposure, and that the effects of microwaves on central choline uptake can be classically conditioned to cues in the exposure environment.

This present paper reports experiments to further investigate this neurological effect of low-level microwave irradiation, and also the biochemical and behavioral consequences of repeated exposure. In the first series of experiments we examined the effects of acute exposure on choline uptake in the striatum, frontal cortex, hippocampus, and hypothalamus, four regions of the brain containing high amounts of cholinergic innervations (5). In previous research (15,16), we investigated the effects of microwaves on choline uptake in the brain after 45 min of exposure. The present study used a shorter period (20-min) of exposure and also investigated whether tolerance developed to the effects of microwaves on central choline uptake after repeated 20-min exposure sessions. Our studies and others have indicated a biphasic response characteristic of central cholinergic reaction to stressors such as restraint (6, 7, 12) and noise (10,11) depending on the duration or intensity of exposure, and also showed tolerance after ten daily 45-min sessions of microwave exposure (16).

In the second series of experiments we studied whether the effects of acute (20-min) microwave exposure on central cholinergic activity could be blocked by pretreatment with narcotic

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antagonist, since we have speculated that microwave irradiation is a stressor and some of their effects are mediated by endogenous opioids (17). In addition, we also investigated changes in muscarinic cholinergic receptors in different regions of the brain after repeated microwave exposure. Up- and down-regulations of cholinergic receptors are well documented after repeated perturbation of the cholinergic systems by various agents such as drugs and stressors (6, 25, 26).

Finally, we examined the effects of microwave exposure on learning in the radial-arm maze. Central cholinergic systems especially the septo-hippocampal and basalis-cortical systems, are known to play a role in the performance in the maze (22,24). Since acute microwave exposure affects central cholinergic activity, a possible consequence is that learning in the radial-arm maze will be affected after exposure.

In our experiments, rats were exposed in waveguides to 2450-MHz pulsed microwaves at average power density of 1 mW/cm^2, which gives a whole-body specific absorption rate (SAR) of 0.6 W/kg. This dose rate is 1.5 times the limit recommended for human exposure by the American National Standards Institute (27).

METHOD

Animals

Male Sprague-Dawley rats (250–300 g) purchased from Tyler Laboratories, Bellevue, WA were used in our experiments. During the experiments they were housed in a room adjacent to the microwave exposure room and were maintained on a 12-hr light-dark cycle (light on from 0800 to 2000 hr). The ambient temperature was 23°C. The rats in the first two series of experiments were housed four to a cage and provided with food and water ad lib. Rats in the maze-learning experiments were caged individually and maintained on a food deprivation schedule at 80% of their ad lib weights.

Microwave Exposure System

The 2450-MHz cylindrical waveguide exposure system of Guy *et al.* (9) was used. Experimental and control rats were subjected simultaneously to either microwave or sham exposure, respectively. The microwave-exposed rats were irradiated with pulsed (2 μsec pulses, 500 pps), circularly-polarized microwaves at a spatially averaged power density of 1 mW/cm^2 within the waveguide. The specific absorption rate (SAR) was determined calorimetrically to be 0.6 W/kg for the size of animals used in our experiments (4). This dose rate does not significantly increase the colonic temperature of the rat after 45 min of microwave exposure. Apparently, the rat can effectively dissipate the heat load (14). Control animals were placed in similar waveguides for the same period of time as the experimental animals but received no irradiation (sham exposure). All experiments were performed blind, i.e., the experimenters doing the irradiation, biochemical assays, and learning experiments did not know whether a certain animal had received microwave or sham exposure. All exposures were done between 0800–1100 hr to control for possible circadian variation in response.

Effects of Acute and Repeated Microwave Exposure on Sodium-Dependent High-Affinity Choline Uptake in the Brain

Rats were exposed to microwaves for 20 min and sodium-dependent high-affinity choline uptake was determined in the striatum, frontal cortex, hippocampus, and hypothalamus immediately after exposure. To study whether tolerance developed, other animals were exposed to microwaves or sham exposed in 10 daily 20-min sessions. In an 11th session, they were exposed to microwaves or sham exposed, and then sacrificed immediately after exposure. High-affinity choline uptake was determined in different brain regions.

In a separate set of experiments, rats subjected to 20-min acute exposure (microwave or sham) were injected immediately before exposure with naltrexone hydrochloride (1 mg base/kg, DuPont Pharmaceuticals, Wilmington, DE; dissolved in sterile, pyrogen-free, physiological saline and injected intraperitoneally at a volume of 1 ml/kg) or 1 ml/kg of physiological saline. Sodium-dependent high-affinity choline uptake was determined in different regions of the brain immediately after the single 20-min exposure.

Rats were sacrificed by decapitation at a bench outside of the exposure room. Their brains were removed and dissected on ice for the striatum, hippocampus, and hypothalamus according to the method of Glowinski and Iversen (8). The frontal cortex also was assayed. It consisted of the cerebral cortex anterior to a coronal cut at the level of the optic chiasm, with the olfactory tubercles and the anterior portions of the striatum and septum removed.

Sodium-dependent high-affinity choline uptake was determined by the method of Zucker *et al.* (35). Tissue was homogenized in 2 ml of 0.32 M sucrose solution using a glass pestle homogenizer. The homogenate was centrifuged at 1,000 × g for 10 min. The supernatant was then recentrifuged at 12,000 × g for 15 min, and the 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 (containing 4% dextrose, 126 mM NaCl, 1.28 mM Na_2HPO_4, 4.75 mM KCl, 1.27 mM $CaCl_2$, and 1.42 mM $MgCl_2$; pH 7.2), 0.5 μM choline chloride, and 0.4 μCi of ^{3}H-choline (80 Ci/mmol, New England Nuclear, Boston, MA). Nonsodium-dependent choline uptake was determined by addition of 3.0 μM of hemicholinium-3 (Sigma Chemicals, St. Louis, MO) to a similar set of tubes. Each brain sample was assayed in triplicate.

The samples were transferred from an ice bath to a water bath at 37°C for incubation for 4 min. Uptake was terminated by returning the samples to the ice bath. Synaptosomes were collected by centrifugation at 8,000 × g for 20 min. The supernatant was discarded, and the pellet was surface washed with 1 ml of ice-cold 0.9% saline. The saline was removed, and the pellet was dissolved overnight with 0.7 ml of Protosol (New England Nuclear). Protosol was then neutralized with 30 μl of glacial acetic acid, and 8 ml of Econofluor (New England Nuclear) was added. Radioactivity was determined by liquid scintillation technique. High-affinity choline uptake was determined as the difference in uptake in the absence and presence of hemicholinium-3, and was found to comprise aproximately 40–50% of the total uptake. Protein concentration of the synaptosomal preparation was determined by the method of Lowry *et al.* (18) using bovine serum albumin as external standards. Choline uptake is expressed in pmol/mg protein/4 min.

Effects of Repeated Microwave Exposure on Muscarinic Cholinergic Receptors

Rats were exposed to microwaves in 10 daily 20- or 45-min sessions. They were sacrificed by decapitation 24 hr after the last exposure session, and their brains were removed and dissected on ice. Quinuclidinyl benzilate (^{3}H-QNB) binding sites were assayed in the striatum, frontal cortex, hippocampus, and hypothalamus using a method modified from that of Yamamura and Snyder (33). Tissue was homogenized in 20 vol. of 0.32 M sucrose using a glass pestle homogenizer. The homogenate was centrifuged at 1,000 × g for 10 min. The supernatant was then homogenized for

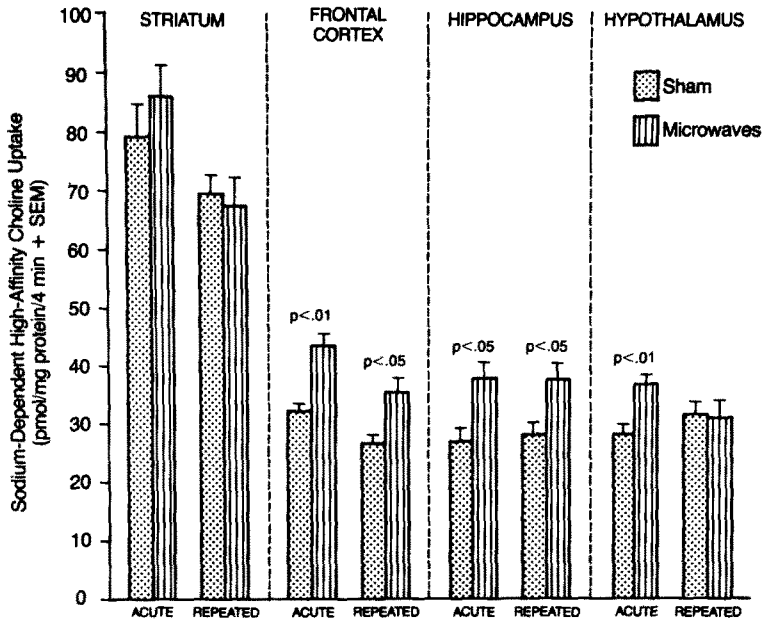

FIG. 1. Effects of acute (20-min) and repeated (ten 20-min sessions) microwave exposure on sodium-dependent high-affinity choline uptake in different regions of the rat brain. Each bar represents data from 7 animals.

20 sec with a Polytron (setting 5). Of this homogenate, 0.1 ml was added to each of a set of tubes containing 0.8 ml of 0.05 M Na-K phosphate buffer (pH 7.4) and 0.1 ml of ^{3}H-QNB (43.6 Ci/mmol, New England Nuclear) of different concentrations. Nonspecific binding was determined by addition of 1.0 μM of atropine sulfate to a similar set of tubes. The tubes and their contents were incubated for 60 min at 25°C in a water bath. Incubation was terminated by addition of 3 ml of cold buffer and filtration with suction through GF/B filter paper (Whatman). The filter paper was rinsed 3 times with 5 ml of the cold buffer and dried overnight. Radioactivity trapped was counted by liquid scintillation technique using a Liquifluor (New England Nuclear)-toluene scintillation cocktail. Nonspecific binding was 5–10% of the total binding. Protein concentration of the final tissue homogenate was determined by the method of Lowry *et al.* (18) using bovine serum albumin as external standards. Concentration (B_{max}) and affinity (K_d) of the binding sites were determined by the Scatchard analysis. B_{max} is expressed in fmol/mg protein and K_d in nM.

Effects of Microwave Exposure on Learning in the Radial-Arm Maze

Rats were housed in individual cages. They were given a weighed amount of rat chow daily to maintain their body weights at 80% of ad lib level. The 12-arm radial maze consisted of a center hub (86 cm in diameter, 20 cm high) surrounded by 12 equally-spaced arms (68 cm long, 10 cm wide) with a food well (2 cm diameter, 1 cm deep) situated at the end of each arm.

When the rats reached 80% of their ad lib weight, they were sham-exposed in the waveguides and then placed for 10 min each in the maze with pieces of rat chow (0.1 g each) scattered in the maze. This procedure was repeated for 4 more days and was designed to get the animals used to the experimental procedure, both exposure and maze running. In the next session (start of learning session), the rats were either exposed to microwaves (20 or 45 min) or sham-exposed and then placed in the center hub and allowed to explore the maze and obtain food baits placed at the wells only. Each rat remained in the maze until it had made 12 arm entries or 10 min had elapsed, whichever occurred first. An entry was recorded when an animal placed all four paws inside an arm. An experimenter in an adjacent room observed the performance using a closed circuit television system. The performance was recorded on videotape for detailed data analysis. The maze was cleaned with 2.5% cider vinegar after each session. This training procedure continued for 10 daily sessions. In data analysis, the first entry into an arm was scored as a correct choice, whereas a reentry into an arm was scored as an error.

Data Analysis

Data on the effects of acute and repeated microwave exposure and naltrexone pretreatment on choline uptake, and also the effects of repeated exposure on ^{3}H-QNB binding sites in each region of the brain were analyzed by the two-factor analysis of variance. Difference between two treatment groups was then compared by the Newman-Keuls test. Learning curve of the radial-arm maze (average error versus day of training) was evaluated by trend analysis. Significance of treatment effects (microwaves versus sham exposure effects) and 'treatment × training-session' interaction effect were assessed by repeated measurement analysis of variance. Difference or effect at $p<0.05$ was considered statistically significant.

RESULTS

Acute and Repeated Microwave Exposure and Choline Uptake

Figure 1 presents the data of acute and repeated microwave exposure on sodium-dependent high-affinity choline uptake in different regions of the brain. Two-way analysis of variance of the data gave the following F-values (degrees of freedom) for the irradiation (sham vs. microwave), duration of exposure (acute vs.

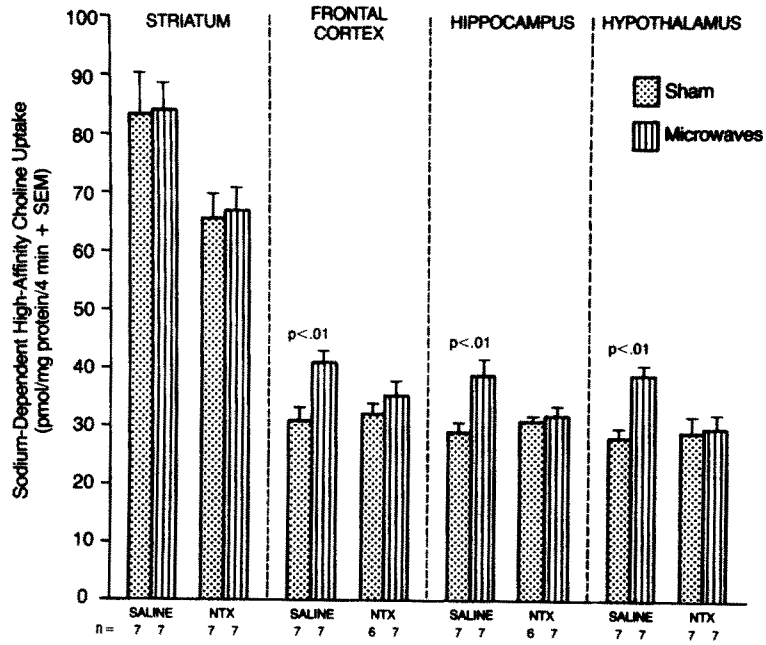

FIG. 2. Influence of pretreatment with naltrexone (NTX) on the effects of acute (20-min) microwave exposure on choline uptake in different regions of the rat brain. Data are compared with those of saline-injected animals.

repeated exposure), and radiation × duration interaction effects in each brain area: striatum, 0.256(1,24), n.s, 8.08(1,24), $p<0.01$, 0.689(1,24), n.s.; frontal cortex, 21.24(1,24), $p<0.005$, 10.78(1,24), $p<0.005$, 0.38(1,24), n.s.; hippocampus, 12.13(1,24), $p<0.005$, 0.007(1,24), n.s., 0.07(1,24), n.s.; and hypothalamus, 1.4(1,24), n.s., 0.019(1,24), n.s., 2.62(1,24), n.s. Further analysis of the data with the Newman-Keuls test showed that after 20 min of acute microwave exposure, significant increases in choline uptake were observed in the frontal cortex, hippocampus, and hypothalamus, whereas no significant effect was seen in the striatum, when compared with the uptake activity of sham-irradiated animals.

After repeated exposure, tolerance developed to the effects of microwaves on the hypothalamus (i.e., no significant difference in hypothalamic choline uptake between the repeated microwave- and sham-exposed animals was observed after the last exposure). However, no tolerance developed to the responses of the frontal cortex and hippocampus (i.e., these brain regions of the repeated microwave-exposed rats still showed significant increases in

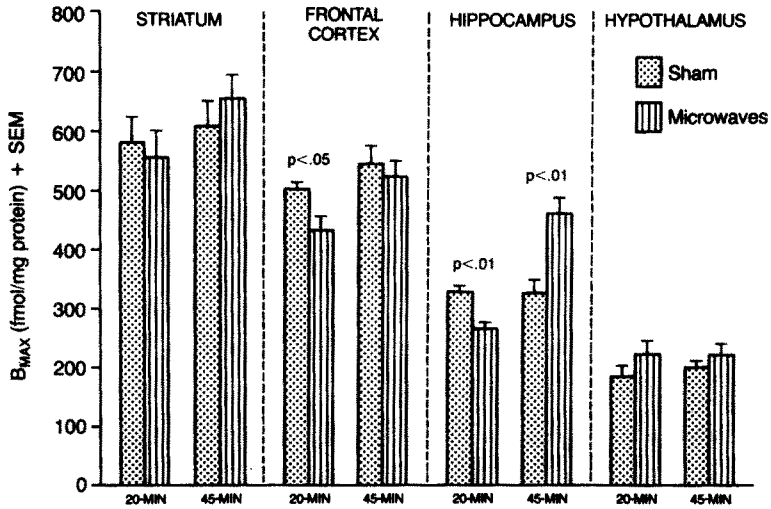

FIG. 3. Concentrations (B_{max}) of ^{3}H-QNB binding sites in different regions of the brains of rats exposed to microwaves in 10 daily 20- or 45-min sessions. Assays were done 24 hr after the last exposure session. Each bar represents data from 8 animals.

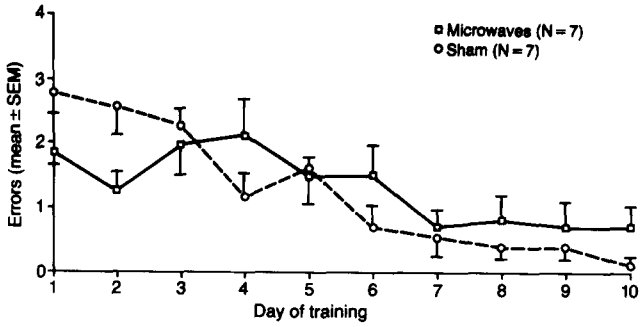

FIG. 4. Effects of exposure to 20-min session of microwaves on learning in the radial-arm maze.

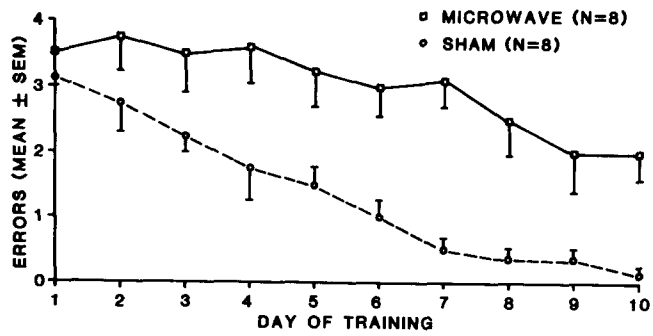

FIG. 5. Effects of exposure to 45-min sessions of microwaves on learning in the radial-arm maze.

choline uptake after the last session of microwave exposure compared to data of repeated sham-exposed animals). Furthermore, repeated-exposure procedure decreased the choline uptake activity in the striatum of both microwave- and sham-exposed animals [$F(1,24) = 8.08$, $p<0.01$, comparing data of acute versus repeated exposure]. The reason for this decrease is not clear.

Naltrexone Pretreatment and Effects of Microwaves on Choline Uptake

Results of the naltrexone pretreatment experiment are presented in Fig. 2. Data of naltrexone-pretreated animals are compared with those of the saline-pretreated rats. Analysis of variance gave the following F-values (degrees of freedom) for the irradiation (sham vs. microwave), drug pretreatment (naltrexone vs. saline), and irradiation × drug-pretreatment interaction effects of each brain area: striatum, 0.017(1,24), n.s., 10.55(1,24), $p<0.005$, 0.0002(1,24), n.s.; frontal cortex, 8.35(1,23), $p<0.025$, 1.01(1,23), n.s., 2.42(1,23), n.s.; hippocampus, 7.36(1,23), $p<0.025$, 1.76(1,23), n.s., 4.74(1,23), $p<0.05$; and hypothalamus, 6.68(1,24), $p<0.025$, 3.61(1,24), n.s., 5.33(1,24), $p<0.05$.

In the saline-injected rats, increases in choline uptake were observed in the frontal cortex, hippocampus, and hypothalamus after 20 min of microwave exposure. These effects of microwaves were blocked by pretreating the rats with naltrexone. Furthermore, naltrexone pretreatment did not significantly affect the choline uptake in the sham-irradiated rats, except in the striatum in which a reduction of activity was observed in both the microwave- and sham-exposed rats after the naltrexone treatment. This effect of narcotic antagonist on striatal choline uptake has been previously reported by us (10,15).

Microwave Exposure and ^{3}H-QNB Binding Sites

Data of repeated 20- and 45-min exposure sessions on ^{3}H-QNB binding sites in different regions of the brain are presented in Fig. 3. Analysis of variance of the data gave the following F-values (degrees of freedom) for the irradiation (sham vs. microwave), duration of exposure (20 vs. 45 min), and irradiation × exposure-duration interaction effects for the different areas of the brain: striatum, 0.049(1,28), n.s., 2.254(1,28), n.s., 0.75(1,28), n.s.; frontal cortex, 3.441(1,28), n.s., 7.025(1,28), $p<0.025$, 0.74(1,28), n.s.; hippocampus, 3.244(1,28), n.s., 26.35(1,28), $p<0.005$, 27.23(1,28), $p<0.005$; and hypothalamus, 2.432(1,28), n.s., 0.092(1,28), n.s., 0.304(1,28), n.s.

In the 20-min session exposure group, decrease in concentration of binding sites (B_{max}) was observed in the frontal cortex and hippocampus. No significant effect was observed in the striatum and hypothalamus. Furthermore, there was no significant effect of repeated microwave exposure on receptor binding affinity (K_d) in any of the brain areas studied. K_ds (nM ± SEM) of binding sites in the brains of the microwave- and sham-exposed animals were: striatum 0.207 ± 0.032, 0.240 ± 0.017; frontal cortex 0.194 ± 0.026, 0.212 ± 0.017; hippocampus 0.176 ± 0.007, 0.191 ± 0.018; and hypothalamus 0.130 ± 0.031, 0.136 ± 0.016 (N = 8 in each group).

In rats exposed to repeated 45-min sessions of microwaves, increase in B_{max} was observed in the hippocampus, whereas no significant effect was observed in the striatum, frontal cortex, and hypothalamus. Again, there was no significant difference in K_d (nM ± SEM) of binding sites in the brains of the microwave- and sham-exposed groups: striatum 0.295 ± 0.035, 0.248 ± 0.058; frontal cortex 0.240 ± 0.024, 0.228 ± 0.038; hippocampus 0.229 ± 0.042, 0.181 ± 0.021; and hypothalamus 0.162 ± 0.034, 0.137 ± 0.020 (N = 8 in each group).

Microwaves and Radial-Arm Maze Learning

Data of effects of microwave exposure on learning in the radial-arm maze are presented in Figs. 4 and 5. The average errors made were plotted against the training sessions. Exposure to 20 min of microwaves before maze running had no significant effect on learning (Fig. 4). Trend analysis showed improvement in maze running with training in both the microwave- $F(9,54) = 4.08$, $p<0.005$, and sham- $F(9,54) = 12.73$, $p<0.005$, exposed groups. Repeated measurement analysis of variance of the data showed no significant treatment main effect (microwave vs. sham), $F(1,12) = 0.047$. However, a significant treatment × training-session interaction effect was found, $F(9,108) = 3.499$, $p<0.005$. This suggests that the learning curves of the two treatment groups are not the same form. The performance of the microwave-exposed rats is significantly better than the sham-exposed animals at the first 2 training sessions ($p<0.01$, Newman-Keuls test).

Exposure to microwaves for 45 min before maze running significantly affected the learning of the animals (Fig. 5). Trend analysis showed a significant learning effect in both the microwave- $F(9,63) = 3.77$, $p<0.005$, and sham- $F(9,63) = 15.97$, $p<0.005$, exposed rats. Analysis of variance of the data showed significant treatment main effect [microwave vs. sham, $F(1,14) = 12.36$, $p<0.005$], and treatment × training-session interaction effects, $F(9,126) = 2.22$, $p<0.025$. Thus, the data indicate that learning of the microwave-exposed rats in the radial-arm maze was significantly retarded compared to the sham-exposed animals.

DISCUSSION

Our data show that low-level microwave irradiation affects

cholinergic activity in the central nervous system of the rat. Acute exposure changes the activity of the cholinergic innervations and repeated exposure leads to alterations in the concentrations of the muscarinic cholinergic receptors in various regions of the brain. These effects predict deficit in radial-arm maze learning, a learning task that requires normal central cholinergic functions.

Effects of Microwaves on Central Choline Uptake

Data presented in this paper, together with data reported in our previous papers (15,16), show that the effects of microwaves on central choline uptake is duration-dependent. A biphasic response function was observed in the frontal cortex and hippocampus, whereas effect on hypothalamic choline uptake was observed only after 20 min of exposure, but not after 45 min of exposure. A brain site specificity also was suggested since no significant effect was observed in the striatum after both durations of exposure even though the striatum has the highest choline uptake activity among the brain regions studied.

Previous reports have described the biphasic response characteristics of cholinergic systems. Restraint stress causes an increase in choline uptake in the hippocampus after short duration, but longer duration of restraint leads to a decrease (6, 7, 12). Furthermore, exposure to white noise also shows an intensity-dependent biphasic effect (10): increase in choline uptake in the frontal cortex and hippocampus after 45 min of exposure to 70-dB noise, and decrease in uptake after exposure to 100-dB noise. Also, increase in choline uptake was observed in the hypothalamus after exposure to the 70-dB noise and no significant effect was seen after exposure to the 100-dB noise. However, it may be relevant that, even though the cholinergic systems show a duration-dependent biphasic response to microwaves, no intensity-dependency was observed. Recently, we investigated the dose-response relationship of microwaves on central choline uptake by varying the power density of 45-min exposure session (13). We observed decreases in choline uptake in the hippocampus and frontal cortex at power intensities greater than 0.75 mW/cm^2 (whole-body average SAR 0.45 W/kg), but no increase in uptake at lower power densities, as would be expected if the responses are biphasic depending on the power density of exposure.

The finding that pretreatment with the narcotic antagonist naltrexone blocked the effects of microwaves on choline uptake suggests that endogenous opioids are involved. These data further support our hypothesis that low-level microwaves activate endogenous opioids (17). It also is interesting that in the frontal cortex, increase in choline uptake after 20 min of microwave exposure was blocked by naltrexone but the decrease after 45 min of exposure was not, whereas in the hippocampus narcotic antagonist blocked both increase and decrease in choline uptake after 20 and 45 min of exposure, respectively [data presented in this paper and in (15)].

It is well known that endogenous opioids modify cholinergic activity in the hippocampus. Activation of an endogenous opioid system in the septum inhibits activity in the septohippocampal cholinergic pathway (22). Relevant to the biphasic activation and depression effects of microwaves on hippocampal choline uptake is that morphine has been reported to both increase and decrease hippocampal cholinergic activity, probably depending on the dosage administered (30). It is possible that either the septohippocampal cholinergic pathway is controlled by two opposing endogenous opioid inputs (one activating and the other inhibitory) or the end-effect on cholinergic activity depends on the duration or extent of activation of the endogenous opioid systems.

The role of endogenous opioids on frontal cortical cholinergic activity is not clear. Injections of narcotic agonists and partial agonists cause no significant effect on acetylcholine turnover in the frontal cortex of the rat (32). On the other hand, morphine elicits a naltrexone-reversible decrease in turnover rate of acetylcholine in the rat cerebral cortex (34). More recently, Wenk (31) reported a decrease in choline uptake in the frontolateral cortex of rats after injection of enkephalin into the substantia innominata, a major source of cholinergic innervation to the cerebral cortex. Furthermore, the effects of white noise on choline uptake in the frontal cortex of the rat can be blocked by pretreatment with naltrexone, suggesting a modulatory role of endogenous opioids on frontal cortical cholinergic activity (10).

Contrary to the responses after repeated 45-min exposure sessions, no tolerance developed to the choline uptake responses in the frontal cortex and hippocampus after repeated 20-min exposure sessions. It is possible that longer cumulative duration of exposure is required for tolerance to develop. However, it is interesting that hypothalamic responses developed tolerance after the same repeated exposure sessions.

Microwaves and Muscarinic Cholinergic Receptors

Numerous studies (25,26) have reported changes in muscarinic cholinergic receptors in the brain after repeated perturbation of the central cholinergic systems. In general, an up-regulation of receptor occurs after treatments that decrease cholinergic functions, e.g., after administration of cholinergic antagonists, and vice versa. Our study partially confirmed such a relationship. Twenty min of microwave exposure increases cholinergic activity in the brain. Thus, decreases in concentration of muscarinic receptors were observed in the frontal cortex and hippocampus after repeated exposure. On the other hand, increase in receptor concentration in the hippocampus occurs after ten 45-min sessions of exposure, which decrease cholinergic activity in the hippocampus. Similar to our data, increase in B_{max} of ^{3}H-QNB binding sites in the hippocampus has been reported in rats subjected to repeated sessions of restraint stress, a treatment that also decreased hippocampal choline uptake (6,7).

However, receptors in the frontal cortex did not respond after repeated 45-min exposure to microwaves, and similarly for the hypothalamus after repeated 20-min exposures. Research shows that muscarinic receptors in the cortex do not up-regulate. In one study (3), lesion of the nucleus basalis magnocellularis led to significant decreases in frontal cortical choline acetyltransferase and choline uptake activities. However, ^{3}H-QNB binding sites in the cortex were not significantly altered. In another study (2), lesion of the nucleus basalis with ibotenic acid caused decreases in levels of choline acetyltransferase and acetylcholinesterase in the cortex, but no significant change in ^{3}H-QNB binding sites. Lack of response of muscarinic receptors in the hypothalamus after repeated 20-min exposure may be due to rapid development of tolerance (Fig. 1). However, this is probably not the case for the frontal cortex after repeated 45-min exposure, since no tolerance developed after 10 sessions (15). The frontal cortical and hypothalamic muscarinic receptors may need more prolonged or repeated decreases in activity to up-regulate.

Microwaves and Radial-Arm Maze Performance

Exposure to microwaves for 20 min before maze training had no significant main effect on learning, but a significant ($p<0.005$) treatment $\times$ training-session interaction effect was observed, suggesting that the learning curves of the microwave- and sham-exposed animals are different. It seems that the learning curve of the microwave-exposed rats is flatter since the animals showed better performance on the first two days of training. It is not known whether enhancement of central cholinergic activity can improve learning in the radial-arm maze. Thus, the significance

and underlying neuromechanism of this effect is not clear.

Retardation in learning was observed after 45 min of microwave exposure, which decreases choline uptake in the frontal cortex and hippocampus. Both cholinergic systems are known to participate in learning and performance in the maze involving "working memory." Deficits in learning and memory have been reported in rats given anticholinergic drugs (23), and with lesions of the septohippocampal and basalis-cortical cholinergic systems (22,24). Thus, it is not surprising that rats after 45 min of exposure to microwaves showed learning deficit in the maze. In our case, the differential contributing effects of the hippocampal and frontal cortical cholinergic systems cannot be separated. Most likely, decrease in activity in both systems contributes to the learning deficit observed. On the other hand, possible effects of microwaves on other transmitter systems in the brain that affects learning (e.g., GABA) cannot be discounted.

Our research shows the effects of acute and repeated low-level microwave exposure on the central nervous system and also the behavior consequence of the neurological effects. These results may have important implications for work-related or accidental exposure to microwaves in both industrial and occupational situations. Such exposure may lead to detrimental health effects and alteration in work performance. Further studies are required to understand the underlying mechanisms by which microwaves affect neural functions. A coordinated approach of investigation at systemic, cellular, and molecular levels may provide fruitful answers.

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