Bioelectromagnetics 12:27–33 (1991)

Naltrexone Pretreatment Blocks Microwave-Induced Changes in Central Cholinergic Receptors

H. Lai, M.A. Carino, Y.F. Wen, A. Horita, and A.W. Guy

Departments of Pharmacology (H.L., M.A.C., A.H.) Psychiatry & Behavioral Sciences (Y.F.W., A.H.) and the Center for Bioengineering (H.L., A.W.G.), University of Washington School of Medicine, Seattle

Repeated exposure of rats to pulsed, circularly polarized microwaves (2,450-MHz, 2-μs pulses at 500 pps, power density 1 mW/cm^2, at an averaged, whole-body SAR of 0.6 W/kg) induced biphasic changes in the concentration of muscarinic cholinergic receptors in the central nervous system. An increase in receptor concentration occurred in the hippocampus of rats subjected to ten 45-min sessions of microwave exposure, whereas a decrease in concentration was observed in the frontal cortex and hippocampus of rats exposed to ten 20-min sessions. These findings, which confirm earlier work in the authors' laboratory, were extended to include pretreatment of rats with the narcotic antagonist naltrexone (1 mg/kg, IP) before each session of exposure. The drug treatment blocked the microwave-induced changes in cholinergic receptors in the brain. These data further support the authors' hypothesis that endogenous opioids play a role in the effects of microwaves on central cholinergic systems.

Key words: microwaves, muscarinic cholinergic receptors, frontal cortex, hippocampus, naltrexone, endogenous opioids

INTRODUCTION

Acute exposure to low-density, 2,450-MHz microwaves altered activity of cholinergic systems in the brains of rats [Lai et al., 1987a,b, 1988, 1989a,b]. A duration-dependent biphasic response was observed. After 20 min of acute exposure at 1 mW/cm^2, increased uptake in sodium-dependent, high-affinity choline occurred in the frontal cortex, hippocampus, and hypothalamus. In contrast, after 45 min of exposure, a decreased uptake of choline was observed in the frontal cortex and hippocampus. Furthermore, pretreatment of the animals with narcotic antagonists blocked both effects.

Received for review June 20, 1989; revision received July 27, 1990.

Address reprint requests to Henry Lai, Ph.D., Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195.

Y. F. Wen's permanent address is Hunan Medical University, 20, Bei Zhan Road, Changsha, Hunan, People's Republic of China.

In subsequent studies, we found that changes in the concentration of muscarinic cholinergic receptors occurred after repeated microwave exposure [Lai et al., 1989b]. We observed decreases in receptor concentration in the frontal cortex and hippocampus after ten, once-daily, 20-min exposures, and we found an increased concentration in the hippocampus after ten, once-daily, 45-min exposures. These changes may be compensatory responses to the acute effects of microwave radiation on cholinergic activity.

The investigation reported here is one of a series of experiments designed to clarify neural mechanisms involved in the effects of low-level microwave irradiation on the functions of the central cholinergic systems. In this study, we investigated the effect of pretreatment with the narcotic antagonist naltrexone on microwave-induced changes in muscarinic cholinergic receptors. Because the acute effect can be blocked by naltrexone, it is expected that the neurological consequences of repeated exposure, i.e., changes in neurotransmitter receptor concentrations, also can be blocked.

METHODS AND PROCEDURES

Animals

Male Sprague-Dawley rats (250–300 g at the start of the experiment) were used as subjects. They were purchased from Tyler Laboratories, Bellevue, Washington. The animals were housed four to a cage in a room adjacent to the microwave-exposure room, and they were maintained on a 12-h light-dark cycle (lights on 0800 h) at an ambient temperature of 23 °C. The rats were given food and water ad libitum. They were moved into the microwave-irradiation room immediately before each session of exposure and returned to the adjacent room afterward.

Microwave Exposure and Drug Treatment

The 2,450-MHz, cylindrical, waveguide-exposure system of Guy et al. [1979] was used in these studies. Experimental and control rats were treated simultaneously by microwave and sham irradiation. The microwave-exposed rats received pulsed (2-μs duration, 500 pps), circularly polarized waves at a spatially averaged power density of 1 mW/cm^2 within the waveguide. The averaged whole-body SAR was determined calorimetrically to be 0.6 W/kg for the animals used in our experiments [see Chou et al., 1984]. Control animals were placed in waveguides for durations that matched those of the experimental animals, but they were not irradiated by microwaves—they were sham exposed.

The animals were given ten, once-daily exposures for 20 or 45 min. Immediately before each exposure, each animal was injected with saline (1 ml/kg, IP) or with naltrexone hydrochloride (Research Biochemicals, Inc., 1 mg base/kg, IP, dissolved in pyrogen-free, sterile, physiological saline and injected at a volume of 1 ml/kg). For each treatment duration (20 or 45 min), there were four conditions: microwave-naltrexone, sham-exp.-naltrexone, microwave-saline, and sham-exp.-saline. All exposures occurred between 0800 and 1100 h to minimize effects of circadian variability. All experiments were performed in the blind, i.e., the investigator that performed biochemical assays did not know the treatments received by any given animal.

Method of Assay of Muscarinic Cholinergic Receptors

Twenty-four hours after their last exposure, rats were killed by decapitation. From their brains, the frontal cortex and hippocampus were dissected and placed on

ice. The frontal cortex consisted of the cerebral cortex anterior to a coronal section at the level of the optic chiasm; the olfactory tubercles and the anterior portions of the septum and striatum were excluded from analysis.

Muscarinic receptors in the tissue samples were assayed by a method modified from that of Yamamura and Snyder [1974] with ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) as ligand. Tissue was homogenized in 20 vol of 0.32 M sucrose by a glass-pestle homogenizer (with five up-and-down strokes). The homogenate was centrifuged at 1,000*g* for 10 min. The supernatant was then homogenized for 20 s with a Polytron (at setting 5). A 0.1-ml quantity of the homogenate 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 the addition of 3 ml of cold buffer and filtration with suction through GF/B filter paper (Whatman). The filter paper was rinsed three times with 5 ml of the cold buffer and dried overnight.

Trapped radioactivity was counted by liquid-scintillation technique in a Liquifluor (New England Nuclear)-toluene scintillation cocktail. Nonspecific binding was 5% to 10% of the total binding. Protein concentration of the final tissue homogenate was determined by the method of Lowry et al. [1951] with bovine serum albumin as external standard. Concentration (B_{max}) and apparent affinity (K_d) of the binding sites were determined by the Scatchard analysis. B_{max} was expressed in fmol/mg protein, and K_d in nM.

Data Analysis

The data from the binding assay were analyzed by a two-way analysis of variance. The statistical significance of the microwave and naltrexone treatment effects, and that of the microwave-by-naltrexone interaction, were determined for each brain region studied. Differences between paired treatment groups were determined by the Newman-Keuls test. An alpha limit of .05 was used in making statistical decisions.

RESULTS

Effect of naltrexone pretreatment on the concentrations of cholinergic receptors in the frontal cortex and hippocampus of rats exposed to ten 20-min sessions of microwave irradiation are shown in Figures 1 and 2. Data analysis of the concentration (B_{max}) of the cholinergic receptors in the frontal cortex showed that microwave irradiation ($F[1,28] = 4.205$, $P < .05$) and the microwave-by-naltrexone interaction ($F[1,28] = 9.47$, $P < .005$) were significant sources of variation. Naltrexone as such was not a significant source ($F[1,28] = 1.25$, $P > .05$). The test revealed that the B_{max} of the cholinergic receptors in the frontal cortex of the microwave-saline animals was significantly lower than the B_{max} of the sham-exp.-saline rats ($P < .01$). This result confirmed our previously reported finding that ten 20-min sessions of microwave irradiation increased the concentration of cholinergic receptors in the frontal cortex of rats that had not received a saline injection [Lai et al., 1989b].

The B_{max} values of the microwave-naltrexone and sham-exp.-naltrexone groups did not differ significantly from each other, and they were similar to those of the

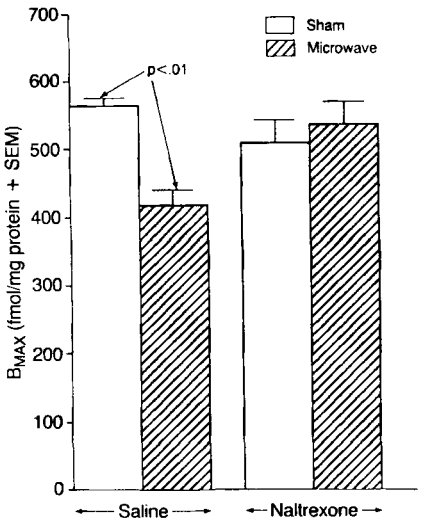

Fig. 1. Effects of naltrexone pretreatment on the concentrations of cholinergic receptors in the frontal cortex of rats exposed to ten 20-min sessions of microwave irradiation. N = 8 in each group.

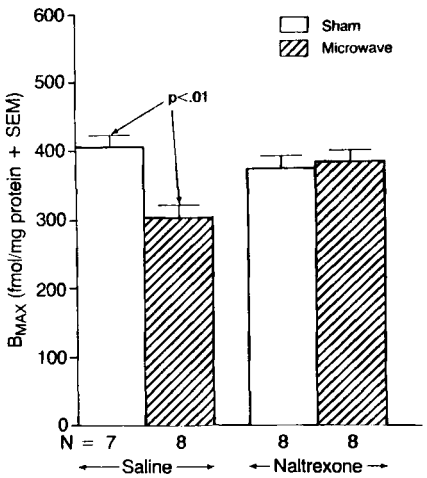

Fig. 2. Effects of naltrexone pretreatment on the concentration of cholinergic receptors in the hippocampus of rats exposed to ten 20-min sessions of microwave irradiation.

sham-exp.-saline group, which indicates that the drug treatment may have blocked the effect of microwaves. Receptor affinities (K_d, nM ±SEM) of the microwave-saline, sham-exp.-saline, microwave-naltrexone, and sham-exp.-naltrexone groups were, respectively, 0.146 ±0.016, 0.167 ±0.017, 0.177 ±0.019, and 0.195 ±0.020 (for N = 8 in each group). No significant treatment effects on K_d were found among the four treatment conditions: microwave: F [1,28] = 1.545; naltrexone: F [1,28] = 3.181; microwave-by-naltrexone interaction: F [1,28] = 0.009; all *Ps* >.05.

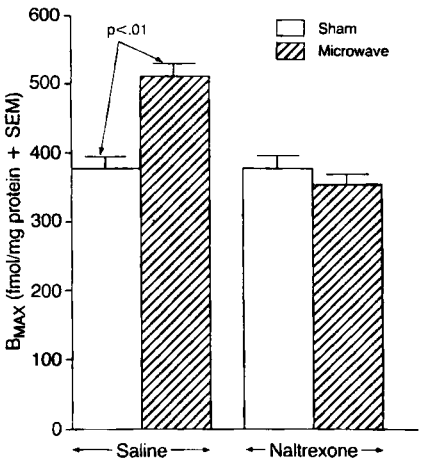

Fig. 3. Effects of naltrexone pretreatment on the concentrations of cholinergic receptors in the hippocampi of rats subjected to ten 45-min sessions of exposure. N = 8 in each group.

Analysis of the B_{max} data on hippocampal receptors (Fig. 2) showed that microwave irradiation ($F[1,27] = 9.493$, $P < .005$) and the microwave-by-naltrexone interaction ($F[1,27] = 13.81$, $P < .005$) were highly significant sources of variation. Naltrexone was not a significant source ($F[1,27] = 2.23$, $P > .05$). A significant difference was found between the B_{max} of the microwave-saline and sham-exp.-saline animals (Newman-Keuls' $P < .01$). However, no significant difference was observed between the B_{max} values of the microwave-naltrexone, sham-exp.-naltrexone, and sham-exp.-saline rats. K_ds (nM ±SEM) of the receptors of the microwave-saline, sham-exp.-saline, microwave-naltrexone, and sham-exp.-naltrexone rats were, respectively, 0.147 ±0.018 (N = 8), 0.161 ±0.012 (N = 7), 0.144 ±0.011 (N = 8), and 0.170 ±0.012 (N = 8). No significant treatment effects on K_d were observed among the different treatment groups: microwave: $F[1,27] = 2.11$, naltrexone: $F[1,27] = 0.158$, and microwave-by-naltrexone interaction: $F[1,27] = 0.368$; all Ps $> .05$.

Effects of naltrexone pretreatment on B_{max} of cholinergic receptors in the hippocampi of rats subjected to ten 45-min sessions of exposure are shown in Figure 3. A two-way analysis of variance of the data revealed that microwave irradiation ($F[1,28] = 11.05$, $P < .005$), naltrexone ($F[1,28] = 23.88$, $P < .005$), and the microwave-by-naltrexone interaction ($F[1,28] = 23.06$, $P < .005$) were significant sources of variation. A significant increase in receptor concentration was observed in the microwave-saline animals compared with that of the sham-exp.-saline rats (Newman-Keuls' $P < .01$). No significant differences in B_{max} were found in paired comparisons between the microwave-naltrexone, sham-exp.-naltrexone, and sham-exp.-saline conditions. K_ds (nM ±SEM) of the receptors of the microwave-saline, sham-exp.-saline, microwave-naltrexone, and sham-exp.-naltrexone groups were, respectively, 0.145 ±0.016, 0.157 ±0.020, 0.133 ±0.019, and 0.159 ±0.016 (N = 8 in all four groups). No significant treatment effects on receptor affinity were found: microwave: $F[1,28] = 1.17$, naltrexone: $F[1,28] = 0.083$, and the microwave-by-naltrexone interaction: $F[1,28] = 0.166$; all Ps $> .05$.

DISCUSSION

The data show that pretreatment with the narcotic antagonist naltrexone blocked the effect of repeated microwave exposure on the concentration of muscarinic cholinergic receptors in the brains of rats. This result confirms and extends our previous finding that naltrexone blocked the acute effect of microwaves on central cholinergic activity [Lai et al., 1987a,b]. Changes in muscarinic receptors are compensatory responses to changes in cholinergic activity [Overstreet, 1984]. A decrease in cholinergic activity after 45 min of acute exposure leads to up-regulation of cholinergic receptors after repeated exposure, whereas an increase in activity after 20 min of exposure leads to a decrease in concentration of the receptors. Because the acute effect can be blocked by naltrexone, this finding is evidence that the response after repeated exposure also can be blocked. These data add further support to our hypothesis that endogenous opioids play a role in some of the neurological effects of low-density, low-SAR (circa 1 mW/cm^2, 0.6 W/kg) microwave irradiation [Lai et al., 1987c].

In similar research, we found that an acute (45-min) exposure to white noise at 100 dB decreased the uptake of sodium-dependent, high-affinity choline in the hippocampus of the rat [Lai, 1987]. Repeated exposure led to an increase in cholinergic receptors in the hippocampus. Furthermore, similar to the results of the present experiment, we found that pretreatment with naltrexone blocked the effect of repeated exposure to noise on hippocampal cholinergic receptors [Lai et al., 1989c].

Changes in muscarinic cholinergic receptors after repeated microwave exposure may have important implications for the behavior of the animal. These changes may affect an animal's adaptation to further perturbation by microwaves on brain cholinergic functions [Dilsaver, 1988]. On the other hand, functional changes in cholinergic receptors may lead to detrimental behavioral consequences. For example, changes in sensitivity of central cholinergic systems after repeated exposure to stress have been implicated in the development of depression in human beings [Janowsky et al., 1973]. Changes in cholinergic functions in the brain, especially in the hippocampus, are also known to affect the learning and memory of animals [Deutsch, 1983]. Our data indicate that treatment with a narcotic antagonist may prevent the development of these adverse consequences. Thus, it is important to understand the neurochemical effects of acute and repeated microwave exposure on central cholinergic systems. In this respect, the finding that endogenous opioids play a mediating role is significant. Further investigations are needed to determine how the endogenous opioid systems in the brain are activated by low-level microwave irradiation, and to identify the neural pathways involved in the activation.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Institute of Environmental Health Sciences (ES-03712). We thank Dr. C.-K. Chou of the City of Hope National Medical Center for valuable comments on the manuscript.

REFERENCES

Chou CK, Guy AW, Johnson RB (1984): SAR in rats exposed in a 2450-MHz circularly polarized waveguide. Bioelectromagnetics 5:389–398.

Deutsch JA (1983): The cholinergic synapse and the site of memory. In Deutsch JA (ed): "The Physiological Basis of Memory." New York: Academic Press, pp 367–386.

Dilsaver SC (1988): Effects of stress on muscarinic mechanisms. Neurosci Biobehav Rev 12:23–28.

Guy AW, Wallace J, McDougall JA (1979): Circularly polarized 2450-MHz waveguide system for chronic exposure of small animals to microwaves. Radio Sci 14:63–74.

Janowsky DS, Davis JM, El-Yousef MK, Sekerke HJ (1973): Acetylcholine and depression. Psychosom Med 35:568.

Lai H (1987): Acute exposure to noise affects sodium-dependent high-affinity choline uptake in the central nervous system of the rat. Pharmacol Biochem Behav 28:147–151.

Lai H, Horita A, Chou CK, Guy AW (1987a): Low-level microwave irradiation affects central cholinergic activity in the rat. J Neurochem 48:40–45.

Lai H, Horita A, Chou CK, Guy AW (1987b): Effects of low-level microwave irradiation on hippocampal and frontal cortical choline uptake are classically conditionable. Pharmacol Biochem Behav 27: 635–639.

Lai H, Horita A, Chou CK, Guy AW (1987c): A review of microwave irradiation and actions of psychoactive drugs. IEEE Engin Med Biol 6:31–36.

Lai H, Horita A, Guy AW (1988): Acute low-level microwave exposure and central cholinergic activity: studies on irradiation parameters. Bioelectromagnetics 9:355–362.

Lai H, Carino MA, Horita A, Guy AW (1989a): Low-level microwave irradiation and central cholinergic activity: a dose-response study. Bioelectromagnetics 10:203–208.

Lai H, Carino MA, Horita A, Guy AW (1989b): Low-level microwave irradiation and central cholinergic systems. Pharmacol Biochem Behav 33:131–138.

Lai H, Carino MA, Wen YF (1989c): Repeated noise exposure affects muscarinic cholinergic receptors in the rat brain. Brain Res 488:361–364.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951): Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275.

Overstreet DH (1984): Behavioral plasticity and the cholinergic system. Prog Neuropsychopharmacol Biol Psychiatry 8:133–151.

Yamamura H, Snyder SH (1974): Muscarinic cholinergic binding in rat brain. Proc Natl Acad Sci USA 71:1725–1728.