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BRIEF COMMUNICATION

Low-Level Microwave Irradiation Attenuates Naloxone-Induced Withdrawal Syndrome in Morphine-Dependent Rats

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LAI, H., A. HORITA, C. K. CHOU AND A. W. GUY. *Low-level microwave irradiation attenuates naloxone-induced withdrawal syndrome in morphine-dependent rats.* PHARMACOL BIOCHEM BEHAV **24**(1) 151–153, 1986.—The effect of microwave irradiation on naloxone-induced withdrawal syndrome was studied in rats made morphine dependent by subcutaneous implantation of morphine pellets. Morphine-dependent rats were either exposed to pulsed low-level microwaves (2450 MHz, 1 mW/cm^2, 500 pps, 2 μsec pulses) or sham-irradiated for 45 min before the naloxone injection. We found that microwave-exposed rats showed significantly less weg-dog-shakes and had higher body temperature than the sham-exposed animals during withdrawal. There was no significant difference in the incident of diarrhea between the two groups of animals. These data further support the results of our previous research suggesting that pulsed low-level microwave irradiation activates endogenous opioids in the rat.

Microwaves Morphine-withdrawal Wet-dog-shake Naloxone

DATA from our previous research suggest that acute low-level microwave irradiation activates endogenous opioids in the brain of the rat. Experimental data supporting this hypothesis can be summarized as follows: (1) acute microwave exposure elicited a hyperthermic response at 15 min postexposure and the response was blocked by the narcotic antagonists, naloxone and naltrexone [7]; (2) the effects of microwaves on the actions of certain psychoactive drugs, including amphetamine and ethanol, could be blocked by pretreatment with naloxone [8,10]; (3) the cataleptic effect of morphine was potentiated by prior acute exposure to microwaves [6]; and (4) acute microwave exposure reduced choline uptake in the hippocampus and the effect could be blocked by naloxone and naltrexone [9].

In this present study, we investigated the effects of microwave exposure on naloxone-induced withdrawal syndrome in morphine-dependent rats. Since activation of endogenous opioids in the brain has been shown to attenuate morphine withdrawal syndrome in animals [2, 13, 15], it would be interesting to investigate whether microwave irradiation could also attenuate morphine withdrawal.

METHOD

Animals

Male Sprague-Dawley rats (weighing 250–300 g at the start of an experiment) from Tyler Laboratory, Bellevue, WA were used. They were housed in a temperature-controlled vivarium maintained on a 12-hr light-dark cycle (lights on at 8:00 hr) and provided with food and water ad lib. All experiments were run between 9:00 and 11:00 hr at an average ambient temperature of 22.0°C (range: 21–24°C). Each animal was used once in the experiment.

Method of Microwave Exposure

The 2450-MHz cylindrical waveguide exposure system of Guy *et al.* [5] was used. The waveguide exposure system consists of a number of individual cylindrical exposure tubes connected through a power divider network to a single power source. Each cell consists of a section of circular waveguide constructed of galvanized wire screen in which a circularly polarized TE_{11} mode field configuration is excited. It contains a plastic chamber to house the rat. The floor of the chamber consists of glass rods, allowing waste to fall through plastic funnels into collection containers outside of the waveguide. Rats were irradiated by pulsed, circularly polarized microwaves (2 μsec pulses, 500 pps) at a spatially averaged power density of 1 mW/cm^2 within the guide. Control animals were run simultaneously in similar waveguides but received no irradiation (sham exposure). In our system, eight rats could be subjected to either microwave or sham

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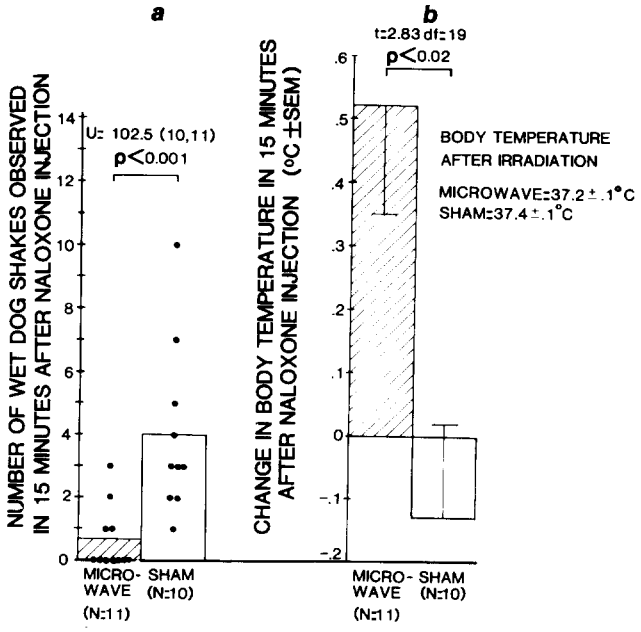

FIG. 1. (a) Microwave exposure significantly attenuated the wet-dog-shake induced by naloxone in morphine-dependent rats (each dot represents the response from one animal). Data were compared by the two-tailed Mann-Whitney U-test. (b) Change in body temperature in the microwave- and sham-exposed rats at 15 min after naloxone injection. Data were compared by the two-tailed Student's *t*-test. There was no significant difference in body temperature between the microwave- and sham-exposed rats immediately after exposure.

exposure simultaneously. The specific absorption rate (SAR) was determined calorimetrically to be 0.6 W/kg of body weight for the size of animals used in our experiments. This level of energy absorption is 1.5 times that of the human exposure safety standard recommended by the American National Standards Institute. This dose-rate did not cause a significant change in body temperature in the rat after 45 min of exposure, i.e., there was no significant difference in body temperature between the microwave- and sham-exposed rats immediately after exposure.

All experiments were performed blind, i.e., the experimenter doing the study did not know whether a certain rat had received microwave- or sham-exposure.

Measurement of Body Temperature

Core body temperature was monitored by a thermister probe (YSI-402, Yellow Springs Instrument) lubricated with glycerol and inserted 8 cm into the rectum and recorded by a telethermometer (YSI-43T, Yellow Springs Instrument). Animals were restrained by holding on the bases of their tails and the probe was inserted only during the time of temperature measurement and withdrawn afterwards. Each temperature measurement took approximately 20 sec.

Studies on the Effect of Microwaves on Naloxone-Induced Morphine-Withdrawal

Rats (n=21) were made morphine-dependent by subcutaneous implantation of morphine pellets. They were lightly anesthetized with ether, and two morphine pellets (each contained 75 mg of morphine) were inserted subcutaneously at both sides of the flank. After 72 hr, the pellets were removed and the sites of implantation were rinsed thoroughly with physiological saline (cf. [4,14]). Thirty-six hours after the removal of the morphine pellets, the animals were randomly divided into two groups and subjected to either microwave or sham exposure for 45 min. Immediately after the exposure, their body temperature was taken and they were then injected with 0.5 mg/kg of naloxone (dissolved in distilled water and injected subcutaneously at a volume of 1 ml/kg). They were then placed individually in a 35×30×17 cm clear plastic cage with a metal-grid cover. From each animal, the number of wet-dog-shake in the next 15 min was scored (cf. [3]) and body temperature was taken again at the end of the 15-min observation period. The number of animals in each group that developed diarrhea during this period was also noted.

Data Analysis

Frequencies of wet-dog-shake of microwave- and sham-exposed rats were compared by the 2-tailed Mann-Whitney U-test, and changes in body temperature (i.e., difference in body temperature at 15 min after and immediately before naloxone injection) were compared by the 2-tailed Student's *t*-test.

RESULTS AND DISCUSSION

Data of the withdrawal study are presented in Fig. 1 a and b. The frequency of wet-dog-shake was found to be significantly attenuated by microwave exposure (Fig. 1a) (frequenty of shakes of microwave-exposed rats versus that of sham-exposed rats, $p<0.001$). No wet-dog-shake was observed in seven out of the eleven microwave-exposed rats studied, whereas wet-dog-shake was observed in all of the sham-exposed animals. There was no significant difference in body temperature between the microwave- and sham-exposed rats immediately after exposure (37.2±0.1 and 37.4±0.1°C, respectively). However, a significant increase in body temperature was observed in the microwave-exposed animals at 15 min after the injection of naloxone in comparison with the sham-exposed animals (Fig. 1b) (change in body temperature of microwave-exposed rats versus that of shame-exposed rats, $p<0.02$). Microwave irradiation did not significantly affect morphine-withdrawal diarrhea. Five out of the eleven microwave-exposed rats and six out of the ten sham-exposed rats developed diarrhea within 15 min after naloxone injection.

Results from this experiment are consistent with our hypothesis that microwave irradiation activates endogenous opioids (see the introduction). Increase in endogenous opioid activity counteracts the effect of naloxone at opioid receptors and thus attenuates the morphine-withdrawal syndrome, i.e., wet-dog-shake. Similar to our results on microwaves, intracerebroventricular injection of morphine sulfate or met-enkephalin has also been shown to attenuate naloxone-induced withdrawal in mice made dependent by morphine pellet implantation, but not to affect withdrawal defecation [2].

It is not known how microwave irradiation activates endogenous opioids. A possible hypothesis is that it acts like a "stressor," since changes in activity of the hypothalamo-hypophyseal-adrenocortical axis, similar to those observed after exposure to stress, have been reported in microwave-

irradiated animals [11]. Endogenous opioids have been implicated to play a role in an animal's reaction to stress [1]. In a recent study [12], naltrexone-induced withdrawal syndrome was shown to be attenuated in morphine-dependent rats subjected to noise-light stress immediately before the naltrexone challenge.

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