EFFECT OF MICROWAVE RADIATION ON LEARNING AND MEMORY

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According to some reports microwave radiation has an action on learning and memory, but these reports are contradictory and not systematic in character. The possible neurochemical mechanisms of these effects has virtually not been examined.

The aim of this investigation was to study the effect of low-energy microwave radiation on learning and memory and also to look for neurochemical correlates of these effects.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 150-200 g. In the experiments of series I the effect of microwave radiation on learning was studied by the conditioned passive avoidance reaction (CPAR). The technique was described in detail by the writers previously [2]. Immediately after training the animals were exposed for 1 h to microwave irradiation with a power flux density of 1 mW/cm^2. A field of this intensity was formed by a "Luch-58-1" physiotherapy apparatus, working at a frequency of 2375 MHz (wavelength 12.6 cm). During irradiation the rats were kept in individual cages made from radio-opaque material, and were arranged on a horizontal surface in regions with uniform power flux density. Immediately after irradiation preservation of the learned reaction was tested for 60 sec. Two groups of rats served as the control: undergoing mock irradiation (the animals of this group were fixed for 1 h in individual cages made from radio-opaque material, which were placed in the experimental apparatus but with the microwave generator switched off) and intact. The animals of this group were returned to their ordinary cage after training to be kept in a group and to have free access to food and water. In the experiments of series II the effect of microwave radiation on parameters of the rats' behavior in an open field was studied. In the experiments of series III the effect of microwave radiation on nociceptive sensitivity of the rats to electrodermal stimulation of the limbs was determined. The threshold of nociceptive sensitivity was recorded as the voltage of an ac current evoking a limb withdrawal reaction from the electrode floor of the CPAR forming apparatus. In the experiments of series IV the ligand-binding capacity of the muscarinic acetylcholine receptors in different parts of the brain was determined and their functional activity assessed in the control and after exposure to microwave radiation. To obtain the synaptic membrane fraction the animals were decapitated and the brain removed quickly in the cold. Regions of the brain corresponding to the cortex, brain stem, and cerebellum were isolated by the method in [8].

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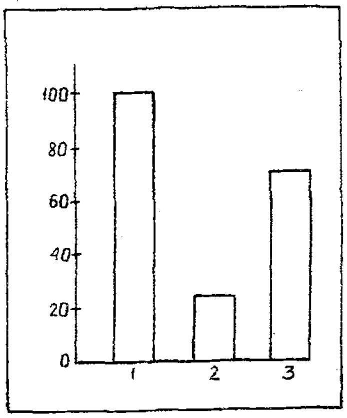

Fig. 1. Effect of microwave radiation on training of rats for the CPAR test. Ordinate, number of animals jumping off plastic platform on to electrode floor (in %); 1) untrained animals, 2) training + mock irradiation; 3) training + microwave irradiation.

As ligand of the muscarinic acetylcholine receptors we used ^{3}H-quinuclidinyl benzoate (^{3}H-QB), produced by the firm of "Amersham International" (UK) with specific activity of 91 Ci/mmole. To investigate the functional activity of the muscarinic acetylcholine receptors we studied the effect of acetylcholine (ACh) on the concentration of free calcium ions and camp in cerebral cortical synaptosomes, which were isolated by the method in [6]. The Ca^{2+}-sensitive fluorescent probe Fura-2 was used [1]. The cAMP concentration in the synaptosomes was determined by radioimmunoassay, using commercial kits (^{125}I-RIA kit, Czechoslovakia) in accordance with recommendations of the manufacturer.

EXPERIMENTAL RESULTS

The results of the study of the effect of microwave radiation on learning and memory are shown in Fig. 1. By means of the method used, no fewer than 75% of the rats were trained (i.e., in the control group after a training session and the subsequent period of mock irradiation lasting 1 h, only 25% of the rats left the plastic platform). It must be emphasized that the normal response of intact untrained animals was to jump off the platform on to the electrode floor, and to inspect the experimental chamber. Exposure to microwaves for 1 h led to the development of retrograde amnesia in 70% of the rats.

To judge the specificity of the effect of a factor on memory processes, the probability of its action on certain behavioral parameters, which could simulate an amnesic or, on the contrary, an antiamnesic effect, must be ruled out [3]. Considering the technical details of the previous experiment, it was considered advisable to study the effect of microwave radiation on parameters of the rats' open field behavior, and in particular, their motor activity and also their nociceptive sensitivity. The experiments showed that microwave radiation caused no significant changes in the rats' behavioral parameters in the open field or in their nociceptive sensitivity.

Central muscarinic acetylcholine receptors are involved in the realization of cognitive functions [5]. In the next part of the work, we therefore studied the effect of microwave radiation on parameters of specific binding of muscarinic acetylcholine receptors in different parts of the brain (Table 1). The results showed that during exposure to microwaves the number of specific binding sites of ^{3}H-QB located in the cerebral cortex was significantly

TABLE 1. Parameters of Specific Binding of ^{3}H-Quinuclidinyl Benzoate by Synaptic Membranes from Different Parts of the Rat Brain (M ± m)

Experimental conditions	Cerebral cortex		Brain stem		Cerebellum	
	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}
Control	4,0±0,3	0,91±0,06	6,2±0,4	0,26±0,005	7,0±1,0	0,12±0,01
Microwave irradiation	4,2±0,3	1,32±0,05*	5,3±0,5	0,29±0,04	8,0±2,0	0,11±0,01

Legend. K_d) Equilibrium dissociation constant of ligand-receptor complex (nM); B_{max}) maximal concentration of binding sites (pmoles/mg protein); asterisk indicates significant difference from control ($p < 0.05$).

increased by 31%, but the affinity of the muscarinic acetylcholine receptors for the labeled ligand was unchanged ($p > 0.05$). Activation of muscarinic acetylcholine receptors is known to lead to a change in the content of secondary intracellular messengers, namely calcium ions and cAMP [4]. To investigate the functional activity of the muscarinic acetylcholine receptors we studied the effect of ACh on the concentration of free calcium ions (FCC) and of cAMP in cerebral cortical synaptosomes of control and microwave-irradiated rats. No significant difference was found between values of basal FCC in the two groups of animals. Meanwhile, comparison of the paired results revealed a significant decrease ($p < 0.01$), on average by 60%, in the ability of ACh to cause an increase in intrasynaptosomal FCC under the influence of microwave radiation. Incubation of a suspension of synaptosomes from the rat cerebral cortex with 10^{-5} M ACh for 2 min led to an increase in FCC up to 189 ± 13 nM, whereas in the control group the increase in FCC reached values of 315 ± 17 nM. No significant change was found in the cAMP concentration under the influence of ACh in the control and after microwave irradiation.

These results are evidence that rats exposed to microwave irradiation develop retrograde amnesia, and that the cholinergic system of the brain is evidently involved in its realization.

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