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Mechanism of Action of 8-OH-DPAT on Learning and Memory

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MENESES, A. AND E. HONG. *Mechanism of action of 8-OH-DPAT on learning and memory.* PHARMACOL BIOCHEM BEHAV **49**(4) 1083–1086, 1994. – It has been previously demonstrated that pretraining injection of 8-hydroxy-2-(di-*n*-propilamino)tetralin (8-OH-DPAT, a 5-HT_{1A} agonist) impairs conditioned response (CR) in an autoshaping learning task. Therefore, in the present work we intended to determine whether such an effect could be prevented by pretraining, and whether pre- or postsynaptic 5-HT receptors are involved. Groups of rats received or did not receive food magazine training. On the next day, all groups in both conditions, pre- or posttraining, were treated with 8-OH-DPAT (0, 0.062, or 0.250 mg/kg). Posttraining groups were tested on a second session of autoshaping 24 h later. In a second experiment, naive rats received para-chlorophenylalanine (PCPA) (300 mg/kg × 3 days) before pre- or posttraining injection of 8-OH-DPAT. Results showed that in those groups trained to food magazine and treated 24 h later with 8-OH-DPAT, CR was not affected or enhanced. PCPA injection had no effect by itself, but blocked or attenuated the effect of a post- or pretraining injection of 8-OH-DPAT. The present data suggest that a) the pretraining effect of 8-OH-DPAT eliciting a decrease in CR can be eliminated by a food magazine training session; and b) presynaptic 5-HT_{1A} receptors are involved in the effect of 8-OH-DPAT on the acquisition and consolidation of learning.

Learning Autoshaping Serotonin Receptors 5-HT_{1A} Rats

MULTIPLE 5-hydroxytryptamine (5-HT) receptors have been characterized in mammalian species (13) and divided into the categories of 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, and 5-HT_5 based on differential affinity for diverse 5-HT agonists and antagonists, involvement of different second messenger systems, and gene structures (14). The 5-HT_1 receptor has been further divided into the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} subtypes (14,29), which exhibit distinct pharmacologic profiles (29) and second messenger systems (9). The 5-HT_{1A} agents elicit or modulate a variety of behaviors, such as anxiety, hyperphagia, and activity (20,36). Diverse evidence suggests that 5-HT_{1A} receptors are also involved in learning and memory processes (1,21–23,26). Thus, different authors have reported that 5-HT_{1A} agonists impair learning and memory (3,6,10,15,19,21,22, 25,31,34,37). However, it has been reported that the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propilamino)tetralin (8-OH-DPAT) improved the consolidation of a conditioned response when injected after training, but impaired it with a pretraining injection (23). This effect was observed under diverse food-deprivation conditions (23). The pretreatment impairment effect of 8-OH-DPAT may be due to a decrease in exploration of the food magazine. Because the administration of 8-OH-DPAT alters spontaneous activity and exploration behavior (12), we thought it interesting to investigate the influence of food magazine training, previous to any pharmacologic manipulation, on learning induced by the 5-HT_{1A} agonist. Furthermore, we intended to determine whether pre- or postsynaptic 5-HT_{1A} receptors are involved in the effect of 8-OH-DPAT on the CR. The behavioral task used was autoshaping, an associative learning model (22–24), which has been useful in detecting the facilitation effect of D-amphetamine (23,32) or impairment by atropine (22,32).

METHOD

Male Wistar rats 3 mo of age were used. They were collectively housed in a temperature- and light-controlled room under a 24-h light–dark cycle (light on at 0700 h). Water and food were provided ad lib for a week. After that period, the rats' body weights were gradually reduced to 85%.

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Apparatus

Operant chambers for rats with standard sound attenuation were used. The chambers were 25 cm wide, 29 cm long, and 25 cm high. A retractable lever was mounted 4 cm above the floor and 10 cm from the right and left walls. The lever required a force of 10 g for operation. A food magazine for rat pellets (Bio Serv, Frenchtown, NJ) was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner. Solid-state programming equipment was used for control and recording (Coulbourn Instruments, Lehigh Valley, PA.

Food Magazine Training

Each rat was placed into an experimental chamber and allowed to habituate to the experimental environment until the animal found and ate 30 food pellets (45 mg per pellet).

Autoshaping Training

The autoshaping program consisted in the presentation of an illuminated lever for 8 s (conditioned stimulus; CS), followed by the delivery of a food pellet (unconditioned stimulus; US) each 60 s. When the animal pressed the CS, it was considered to be a conditioned response (CR). The trial was then shortened, the lever was retracted, the light turned off, and US was delivered. The increase in number of CR was considered to be an enhancement in learning. The first session consisted of 10 trials, and the second session 20 trials.

Statistical Analysis

The CR were transformed to the percentage of the total trials for each session. Multiple group comparisons were made using analysis of variance (ANOVA) with one or two factors (i.e., pre- vs. posttraining and vs. treated groups), followed by Dunnett's t-test. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance. There were eight animals per group, and they were used only once.

Drug Treatment

The drugs used were para-chlorophenylalanine (PCPA) and 8-OH-DPAT (Research Biochemical, Wayland, MA). These were dissolved in distilled water or saline and were administered intraperitoneally (IP). In Experiment 1, animals received a pre- or posttraining injection of 8-OH-DPAT or saline (0.9%) in a volume of 1 ml/kg. In Experiment 2, PCPA (300 mg/kg; 5.0 ml/kg) was injected daily for 3 days. Behavioral testing was conducted 24 h after the last dose.

Experiment 1: Effects of 8-OH-DPAT on Animals With or Without Food Magazine Training Before Autoshaping Task

The aim of this experiment was to prevent the impairment of CR induced by a pretraining injection of 8-OH-DPAT (23); therefore, six groups of animals were used. Three groups did not receive food magazine training, and they were directly trained on the autohaping program. The other three groups received food magazine training; 24 h later they had the first training session on the autoshaping task. All groups were treated with 8-OH-DPAT (pre- or posttraining). Posttraining groups were tested 24 hours later. Therefore, each behavioral or pharmacologic manipulation was separated by 24 h.

Experiment 2: Modification of the 8-OH-DPAT Effect on Autoshaping by Subchronic Pretreatment With PCPA

The aim of this experiment was to determine the effect of subchronic PCPA treatment on the effects induced by the acute administration of 8-OH-DPAT. Animals treated with 8-OH-DPAT (0.062 mg/kg) before and after training were compared with groups of rats pre- or posttraining treated with 8-OH-DPAT and PCPA (300 mg/kg × 3 days). These were injected with 8-OH-DPAT 24 h after the last dose of PCPA. Two control groups were included: one received only saline and another was treated with PCPA. Unlike in Experiment 1, all animals received food magazine training immediately before the autoshaping session.

RESULTS

Experiment 1

The aim of this experiment was to prevent the impairment of CR induced by pretraining injection of 8-OH-DPAT (23). ANOVA revealed a significant difference between trained and untrained groups to the food magazine $F(1, 84) = 123.4, p < 0.05$, between pre- and posttraining treated groups $F(5, 84) = 6.5, p < 0.05$; and there was a significant interaction between groups $F(5, 84) = 6.5, p < 0.05$. Preexposure to the food magazine eliminated the impairment of CR induced by pretraining injection of 8-OH-DPAT, but did not modify the increase of such a response elicited by the posttraining administration of 8-OH-DPAT (Table 1).

Experiment 2

The purpose of this experiment was to determine the effect of subchronic PCPA treatment on the effects induced by the acute administration of 8-OH-DPAT. The ANOVA with two factors (i.e., pre- vs. posttraining vs. treated groups) revealed that there was a significant difference between control and treated groups $F(2, 42) = 10.5, p < 0.05$. There was no difference between pre- and posttraining treated groups $F(1, 42) = 2.4, p > 0.05$. Pretraining injection of 8-OH-DPAT impaired the CR, but when injection took place after training, the response was enhanced. PCPA treatment itself did not alter the acquisition of CR, but blocked both the decrement in pretraining animals and the increase in posttraining of CR induced by 8-OH-DPAT, (Table 2). The interaction between factors was significant $F(2, 42) = 23.8, p < 0.05$.

DISCUSSION

The experimental evidence regarding the effects of 5-HT_{1A} agonists on learning and memory tasks is controversial (22,23). Thus, there are studies reporting that 5-HT_{1A} agonists impair (3,6,10,15,19,21,22,25,31,34,37), improve (18,23,37), or have no effect (6,20,24,25,26) on learning. In several of

TABLE 1

EFFECTS OF FOOD MAGAZINE TRAINING BEFORE AUTOSHAPING LEARNING TASK IN PRE- OR POSTTRAINING TREATED ANIMALS WITH 8-OH-DPAT (mg/kg).

	Drug Administration			
	Pretraining		Posttraining	
	Yes	No	Yes	No
Control	2 ± 1	1 ± 1	11 ± 1	0
0.062	13 ± 2	1 ± 1	34 ± 5*	0
0.250	16 ± 3	1 ± 1	33 ± 6*	1 ± 1

*Values are significantly different from those of the control group ($p < 0.05$ by Dunnett's t-test).

TABLE 2

EFFECT OF 8-OH-DPAT ON AUTOSHAPING LEARNING TASK (CR%) IN PRE- OR POSTTRAINING UNTREATED OR PCPA TREATED RATS

	Drug Administration (mg/kg)	
	Pretraining	Posttraining
Control	10 ± 1	14 ± 3
8-OH-DPAT (0.062)	2 ± 1*	33 ± 5*
PCPA (300 mg/kg × 3 days)	8 ± 2	9 ± 3
PCPA (300 mg/kg × 3 days) + 8-OH-DPAT (0.062)	16 ± 4*	8 ± 2

*Values are significantly different from the control group ($p < 0.05$ by Dunnett's t-test).

these works, drugs were injected before the trial; thus, unspecific effects cannot be excluded. For instance, we have previously reported that pretraining systemic administration of 8-OH-DPAT decreased CR (23). In that experiment, animals received food magazine training immediately before being subjected to the autoshaping test (as groups described in this work in Table 2). These findings were the result of an alteration in exploratory behavior, because such a decrement was prevented when animals had learned to eat from the food magazine, where food pellets were delivered 24 h previous to the autoshaping test (Table 1). Moreover, the present findings indicate that the impairment effect of pretraining injection of 8-OH-DPAT on CR previously described by us (23) was eliminated by allowing animals to eat from food magazine 24 h before the autoshaping task. A possible explanation for this difference could be that when a 24-h period was allowed between the food magazine training and the autoshaping test (Table 1), consolidation of the learning process could be more complete, and therefore, animals became resistant to the impairment effects of the pretraining injection of 8-OH-DPAT (Table 2). Impairment of learning after pretraining administration of 8-OH-DPAT has also been described by other authors (10,15,19,21,37). In contrast to the results obtained with 8-OH-DPAT, pre- (unpublished results) or posttraining (22) injection of buspirone (another 5-HT_{1A} agonist) impaired CR. We cannot explain the difference between 5-HT_{1A} agonists; however, it could be attributed to their different affinity binding, intrinsic activity, regional differences, or different subtypes of receptors not yet defined (8,38). With regard to the mechanism of action of 8-OH-DPAT, the present results show that PCPA treatment prevented both the impairment effect produced by pretraining administration of 8-OH-DPAT and the improvement of CR induced by posttraining injection of 8-OH-DPAT. This suggests that 5-HT_{1A} presynaptic receptors are involved in the two effects. However, other authors disagree; thus, PCPA treatment did not alter the impairment induced by 5-HT_{1A} agonists in a passive avoidance task (21); subchronic administration (systemic or intrahippocampal) of 8-OH-DPAT impaired learning (6,7), and this effect was mediated by 5-HT_{1A} receptors. Such discrepancies could be attributed to differences in the behavioral tasks, doses, and pharmacologic treatments and environmental manipulations used. Furthermore, there is evidence that both pre- and postsynaptic 5-HT_{1A} receptors are involved in the discriminative stimulus effects of 8-OH-DPAT (33). In fact, the distribution of 5-HT receptors in brain structures (17,27-30) implicated in learning and memory processes (5,11,16,39) suggests that serotonergic projections have a role in learning and memory (1,35). It has been demonstrated that the presynaptic 5-HT autoreceptors controlling the nerve impulse flow within serotonergic neurons in the dorsal raphe nucleus belong to the 5-HT_{1A} subtype (30). Outside the raphe nucleus, 5-HT_{1A} receptors are mainly postsynaptic and are highly concentrated in the limbic areas (17,27,28,30). Several findings suggest that 5-HT_{1A} postsynaptic receptors acting in different subregions of hippocampus produce distinct physiologic responses (2, 4,8,27), indicating the great complexity of the serotonergic transduction. In conclusion, previous (23) and present findings suggest that pretraining injection of 8-OH-DPAT alters food intake and/or exploration behavior, but not learning. On the other hand, 5-HT_{1A} presynaptic receptors are involved in the acquisition and consolidation of learning.

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