Pharmacology Biochemistry and Behavior, Vol. 42, pp. 797–801, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Oxiracetam Prevents Haloperidol-Induced Passive Avoidance Impairment in Mice

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Received 2 January 1992

CASTELLANO, C., M. BATTAGLIA AND M. SANSONE. *Oxiracetam prevents haloperidol-induced passive avoidance impairment in mice.* PHARMACOL BIOCHEM BEHAV 42(4) 797–801, 1992.—The nootropic drug oxiracetam (50 mg/kg) prevented passive avoidance impairment induced by posttraining administration of haloperidol (0.25 and 0.5 mg/kg). Conversely, oxiracetam did not antagonize either locomotor depression or suppression of active avoidance responses induced by the dopamine receptor blocking agent. The results indicate that prevention of haloperidol-induced retention impairment, by oxiracetam, may be due to a not yet defined protective action, common to other nootropic agents, on different types of experimental amnesias, rather than to a specific interaction with dopaminergic mechanisms.

Oxiracetam Haloperidol Passive avoidance Mice

EXPERIMENTAL amnesias represent a useful tool to evidentiate memory-improving effects of nootropic drugs. In particular, these drugs exert a protective action against the disrupting effects produced by various treatments on retention performance of rodents subjected to a one-trial passive avoidance test. These retention impairments, antagonized by nootropics, were induced by drugs interfering with specific neurotransmitter systems, such as anticholinergic agents (5,6,9,20, 22,23) and NMDA receptor antagonists (14), but also by other less specific drug treatments (5,9,12) and by electroconvulsive shock (5,9,19,21).

As dopamine is believed to have an important role in aversive learning (16), in the present study we wanted to investigate whether the nootropic agent oxiracetam (1) might interfere with the effects of the dopamine receptor blocking agent haloperidol on passive avoidance learning. Haloperidol was given after training since posttrial administration of this drug impaired memory consolidation of two-way avoidance (8). In addition, experiments were carried out to verify if the nootropic agent influences other behavioral effects of haloperidol, such as the reduction of locomotor activity, a common effect of dopamine receptor blocking drugs (3), and the selective inhibition of active avoidance responses, a typical action of neuroleptic drugs (4).

METHOD

Animals

Subjects were naive, male mice (age 7–8 weeks; weight 28–33 g) of the randomly bred CD-1 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment) mice were housed in standard transparent plastic cages (eight per cage) under standard animal room conditions (free access to food and water, 12 L : 12 D cycle, ambient temperature of 23°C). The experiments were carried out between 9 a.m. and 2 p.m. by using different animals for different behavioral tests.

Drugs

Saline solution (0.9% NaCl), oxiracetam, and haloperidol (dissolved in distilled water) were injected intraperitoneally in a volume of 10 ml/kg.

Passive Avoidance

Mice were subjected to a one-trial passive avoidance task in an apparatus consisting of two compartments, one lighted (13.5 × 6 × 12 cm) and one dark (27 × 27 × 27 cm), connected via a sliding door. In the acquisition trial, each mouse was placed individually in the lighted compartment and the time taken to enter the dark compartment was measured. As soon as the mouse entered the dark compartment, the sliding door was closed and a strong foot-shock (0.7 mA for 1 s) was delivered through the grid floor. The mouse was then returned to its own cage to wait for the retention trial, carried out 24 h later. In the retention trial, the mouse was placed in the lighted compartment and the latency of the step-through response was recorded. Oxiracetam, at doses of 0 (saline), 10, 25, or 50 mg/kg, was given 30 min before both the acquisition and retention trials after a 3-day pretreatment (a single daily injection); haloperidol, at the doses of 0 (saline), 0.25, or 0.5 mg/

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TABLE 1

PROTECTIVE EFFECT OF OXIRACETAM ON HALOPERIDOL-INDUCED PASSIVE AVOIDANCE IMPAIRMENT

Treatment 30 min Before Testing	Posttrial			
	SAL	HAL 0.25	HAL 0.5	H (2)
SAL	93.5 (72-101)	34.5* (8-56)	15.0* (6-33)	16.16†
OX 10	76.5 (70-82)	45.5* (37-50)	19.0* (17-23)	20.76†
OX 25	81.5 (77-87)	60.5*‡ (55-67)	40.5*‡ (35-46)	18.89†
OX 50	86.0 (80-96)	82.5‡ (66-95)	64.5* (52-75)	6.41§
H (3)	2.80	18.52†	23.56†	

Median step-through latencies (seconds) with interquartile ranges (Q1-Q3: in parentheses) on the retention trial (24 h after the acquisition trial) in groups of eight mice. Animals were pretreated (three daily injections) with saline solution (SAL) or oxiracetam (OX: 10, 25, or 50 mg/kg) and received the same treatment 30 min before both the acquisition and retention trials. In addition, immediately after the acquisition trial mice were injected with SAL or haloperidol (HAL: 0.25 or 0.5 mg/kg).

*‡Significances ($p < 0.05$) in the Mann-Whitney U-test: haloperidol alone (SAL-HAL) vs. saline (SAL-SAL) and drug combinations vs. oxiracetam alone (OX-HAL) at the corresponding doses, and oxiracetam alone (OX-SAL) vs. saline (SAL-SAL) and drug combinations vs. haloperidol alone (SAL-HAL) at the corresponding doses, respectively.

†§Significance ($p < 0.05$ and $p < 0.001$, respectively) in the Kruskal-Wallis one-way ANOVA (values of H: df in parentheses) for each dose of OX or HAL.

kg, was injected immediately after the acquisition trial. Each experimental group consisted of eight subjects.

Additional experiments were carried out to test the effect of haloperidol injected 120 min after training and drug effects in mice that did not receive foot-shock in the training session.

Active Avoidance—Trained Mice

The apparatus consisted of eight automated shuttle-boxes, each divided into two 20 × 10 cm compartments, connected by a 3 × 3 cm opening. A light (10 W) was switched on alternately in the two compartments and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 and overlapped it for 25 s. The US was an electric shock (0.2 mA) applied continuously to the grid floor. The intertrial interval was 30 s. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 5 s after onset of the CS. If animals failed to avoid the shock, they could escape it by crossing during the US. Mice were subjected to 13 daily 100-trial training sessions. The last three training sessions were preceded (45 min) by injection of saline solution or oxiracetam 50 mg/kg. Only animals reaching a criterion of 70% avoidance responses were used to test the effect of haloperidol 24 h after the last training session.

Training consisted of five daily 100-trial avoidance sessions. In the test session, mice received a first injection with saline or oxiracetam 45 min before the avoidance task and a second injection with saline or haloperidol (0.1 or 0.15 mg/kg) 30 min later.

Locomotor Activity

Spontaneous locomotor activity was measured by using the same apparatus employed to measure active avoidance. For this purpose, the lamps of the shuttle-boxes were switched off and no electric shock was applied to the floor. For each mouse, the number of crossings from one compartment to the other was recorded for 60 min. Mice pretreated (three daily injections) with saline or oxiracetam 50 mg/kg received the same treatment 45 min before the activity test. In addition, they received saline or haloperidol (0.05 or 0.1 mg/kg) 15 min before testing.

RESULTS

Passive Avoidance

In the acquisition trial, all mice entered the dark compartment within 15 s.

Step-through latencies exhibited by mice in the retention trial are reported in Table 1. Kruskal-Wallis one-way analysis of variance (ANOVA) showed significant impairing effects of posttraining haloperidol (0.25 and 0.5 mg/kg) either in saline- or oxiracetam-pretreated mice, but the retention-impairing action of the neuroleptic was attenuated by 25 and especially 50 mg/kg oxiracetam. In particular, the highest dose of oxiracetam completely abolished the effect of 0.25 mg/kg haloperidol (see the results of the Mann-Whitney U-test in Table 1). The protective action of oxiracetam toward haloperidol-induced impairment of avoidance acquisition was confirmed

by a Kruskal-Wallis one-way ANOVA for each dose of haloperidol (or saline), which showed no statistical difference between groups receiving oxiracetam and posttraining injection of saline but significant differences between groups receiving oxiracetam and haloperidol. Mice receiving either dose of haloperidol after 25 or 50 mg/kg oxiracetam performed better than mice pretreated with saline (Mann-Whitney *U*-test).

Additional experiments demonstrated that haloperidol (0.5 mg/kg) when given 120 min after training did not affect passive avoidance acquisition. Median step-through latencies (seconds) and interquartile ranges (Q1-Q3; in parentheses) in the retention trial were 79.5 (74-87.5) for the saline group and 83.0 (73-92.5) for haloperidol-injected mice. Posttrial administration of haloperidol (0.5 mg/kg) also failed to affect retention performance when given either to saline- or oxiracetam (50 mg/kg)-pretreated mice, which did not receive foot-shock in the training trial. Median step-through latencies and interquartile ranges were: 5.5 (5-7) for the saline-saline, 5.0 (5-6) for oxiracetam-saline, 6.0 (5-6.5) for saline-haloperidol, and 5.0 (4-6.5) for oxiracetam-haloperidol treatment.

Active Avoidance – Trained Mice

In shuttle-box trained mice pretreated with saline or oxiracetam (50 mg/kg), haloperidol (0.1 and 0.15 mg/kg) significantly depressed avoidance performance in comparison with the previous control session (*t*-test for related samples; Table 2). The number of avoidance responses exhibited by mice receiving oxiracetam was not significantly different from that of mice receiving saline either in the control or in the haloperidol test sessions (*t*-test for independent samples).

Failure of escape responses was never observed after haloperidol administration.

Locomotor Activity

Table 3 shows that haloperidol (0.05 and 0.1 mg/kg) significantly decreased activity crossings both in saline- and oxiracetam-pretreated mice (Kruskal-Wallis one-way ANOVA). No significant difference was found between oxiracetam and the corresponding saline groups (Mann-Whitney *U*-test).

DISCUSSION

In the present study, posttraining administration of haloperidol induced a dose- and time-dependent impairing effect on one-trial passive avoidance acquisition in mice. Failure of haloperidol to affect step-through latencies of unshocked mice indicates that retention impairment was not due to nonspecific effects on response latencies. The nootropic drug oxiracetam, as in previous experiments (17,18), had no effect on passive avoidance behavior when given alone but prevented retention impairment induced by posttraining administration of the dopamine receptor blocking agent. Conversely, oxiracetam did not antagonize locomotor depression and suppression of stabilized active avoidance responses induced by haloperidol given before testing, even at doses lower than those inducing amnesia. These findings are in agreement with the results of Lenegre et al. (9), showing that piracetam antagonized amnesia but not other behavioral effects of scopolamine, diazepam, and electroconvulsive shock. According to these authors, the antagonism of various kinds of experimental amnesias by nootropics indicates that these drugs possess specific antiamnesic properties unrelated to the specific nature of the amnesia-inducing agents. The present findings support such a hypothesis since protection by oxiracetam of haloperidol-induced amnesia does not seem consequent to a general antagonistic action of the nootropic agent against the behavioral disrupting effects induced by the dopamine receptor blockade.

The lack of specificity for a particular group of amnesic agents indicates that the antiamnesic activity of nootropics may be due to a general, nonspecific action. Indeed, the mechanism of action of nootropics is still unknown and it is not even clear whether the neurochemical mechanisms involved in the cognition-improving effects of these drugs are the same responsible for their protective action upon brain injuries (13). Some reports suggest that specific, in particular cholinergic, mechanisms (2,6,21,22,25) may play a role in the action of nootropics, but these drugs are also reported to affect cerebral metabolism and modulate brain neurotransmitter activity (3,7,20). It may be that more than one biochemical mechanism is involved in the effects of the nootropic agents. It has also been suggested that some central effects of nootropics initiate at the peripheral level. Thus, an initial stimulation of adrenal

TABLE 2

LACK OF EFFECT OF OXIRACETAM ON HALOPERIDOL-INDUCED ACTIVE AVOIDANCE DEPRESSION IN TRAINED MICE

Treatment 15 min Before Test Session	45 min Before Both Control and Test Sessions			
	HAL 0.1		HAL 0.15	
	Control Session	Test Session	Control Session	Test Session
SAL	91.3 ± 1.5	50.6* ± 7.7	82.5 ± 2.6	20.6* ± 6.6
OX 50	82.3 ± 2.4	34.4* ± 5.7	80.5 ± 2.9	20.3* ± 7.8

Effect of haloperidol (HAL: 0.1 and 0.15 mg/kg) on shuttle-box avoidance performance of trained mice pretreated with saline solution (SAL) or oxiracetam (OX: 50 mg/kg). Mean (±SE) percent avoidance responses in the last training session (control session) and in the test session following haloperidol administration in groups of 10 mice. No statistical difference was found between saline- and oxiracetam-pretreated groups receiving the same dose of haloperidol (*t*-test for independent samples).

*$p < 0.001$ vs. control session (*t*-test for related samples).

TABLE 3

LACK OF EFFECT OF OXIRACETAM ON HALOPERIDOL-INDUCED LOCOMOTOR DEPRESSION

Treatment 45 min Before Testing	15 min Before Testing			
	SAL	HAL 0.05	HAL 0.1	H (2)
SAL	85 (73-90)	55* (46-62)	32* (27-49)	14.25†
OX 50	78 (69-85)	57* (46-66)	48* (42-66)	9.78‡

Median activity crossings with interquartile ranges (Q1-Q3: in parentheses) during 30 min in groups of eight mice. Animals were pretreated (three daily injections) with saline solution (SAL) or oxiracetam (OX: 50 mg/kg) and received the same treatment 45 min before the test. In addition, 15 min before testing mice were injected with SAL or haloperidol (HAL: 0.05 or 0.1 mg/kg). Comparisons of oxiracetam alone (OX-SAL) with saline (SAL-SAL) and of drug combinations (OX-HAL) with haloperidol alone (SAL-HAL) at the corresponding doses did not show any significant differences between groups.

*Significant ($p < 0.05$; Mann-Whitney U-test) of haloperidol alone (SAL-HAL) vs. saline (SAL-SAL) and of drug combinations (OX-HAL) vs. oxiracetam alone (OX-SAL) at the corresponding doses.

†Significant difference ($p < 0.01$) effect of haloperidol either in saline- or oxiracetam-pretreated mice (Kruskal-Wallis one-way ANOVA; values of H; df in parentheses).

medulla, increasing epinephrine release and glucose blood levels, might enhance availability and utilization of glucose in the brain, with consequent increase of cerebral biochemical activity and synthesis of various neurotransmitters (24). Alternatively, a stimulation of adrenal cortex by nootropics could release adrenocortical steroids, which modulate centrally the biochemical effects of the same agents (10,11). Regardless, at least for what concerns oxiracetam it has been reported that the drug crosses the blood-brain barrier and accumulates in some brain structures, such as hippocampus, where it exerts a direct pharmacological action (15).

In conclusion, prevention by oxiracetam of passive avoidance impairment, induced by the dopamine receptor blocking agent haloperidol, could be ascribed to a specific interaction of the nootropic with dopaminergic methanisms. However, this hypothesis is weakened by the lack of protective action of oxiracetam upon other behavioral disrupting effects of haloperidol. Prevention by nootropics of various kinds of experimental amnesias makes it more likely that the protective action exerted by oxiracetam upon haloperidol-induced retention impairment may be due to a nonspecific, not yet defined antiamnesic action of the nootropics.

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