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Oxiracetam Prevents Mecamylamine-Induced Impairment of Active, But Not Passive, Avoidance Learning in Mice

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SANSONE, M., C. CASTELLANO, M. BATTAGLIA AND M. AMMASSARI-TEULE. *Oxiracetam prevents mecamylamine-induced impairment of active, but not passive, avoidance learning in mice.* PHARMACOL BIOCHEM BEHAV **36**(2) 389–392, 1990.—The nicotinic antagonist mecamylamine (2.5 and 5 mg/kg/IP) depressed both active (shuttle-box) and passive (step-through) avoidance learning in mice of the DBA/2 strain. The nootropic drug oxiracetam (50 and 100 mg/kg/IP) improved acquisition in the multitrial active avoidance test, but had no effect on one-trial passive avoidance learning. When the two drugs were combined, oxiracetam did not counteract mecamylamine-induced impairment of passive avoidance learning, even if it maintained a facilitating action on shuttle-box avoidance acquisition in mice receiving the nicotinic receptor blocker. Prevention of mecamylamine-induced shuttle-box avoidance depression by oxiracetam indicates that central nicotinic mechanisms are probably involved in the improving effects exerted by nootropic drugs on learning.

Mecamylamine Oxiracetam Avoidance learning Mice

NOOTROPIC drugs enhance resistance to learning and memory impairment induced by various agents (9). In particular, the amnesia induced by the muscarinic receptor blocking drug, scopolamine, is considered an useful model in the screening of nootropic agents (4, 22, 23). This behavioral model is supported by the findings demonstrating that cholinergic mechanisms play an important role in learning and memory processes as well as in the nootropic action of some drugs (2, 7, 21–23). However, the amnesic property of scopolamine, efficaciously utilized to uncover learning improving effects of nootropics in one-trial passive avoidance tasks (4, 20, 22, 23), is not always evident in active avoidance tasks, since antimuscarinic agents may increase the number of avoidance responses as a consequence of the enhanced general activity due to their disinhibitory action (8,19). On the other hand, it seems that nicotinic acetylcholine receptors may also contribute to the cholinergic involvement in cognitive functioning (11–13). As previously shown, the nicotonic receptor blocker, mecamylamine, impairs both active (6, 10, 17) and passive (3, 5, 10) avoidance acquisition in rodents. Thus, mecamylamine-induced impairment of avoidance learning could be a valid model to test nootropic agents, if nicotinic, besides muscarinic, receptors were involved in the action of these drugs.

The present study investigated the effects of the piracetam-like nootropic drug oxiracetam (1), given alone or in combination with mecamylamine, in mice tested for active (shuttle-box) or passive (step-through) avoidance learning. Locomotor activity and reactivity to electrical stimulation were also tested, in order to verify the specificity of drug effects on learning. Oxiracetam treatment was always preceded by a five-day pretreatment, since it was previously demonstrated (18,24) that shuttle-box avoidance improvements by nootropics occur in pretreated animals only.

METHOD

Animals

The subjects were naive male mice (25–28 g) of the inbred DBA/2 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment) the mice were housed in standard transparent plastic cages (8 per cage) under standard animal room conditions (free access to food and water, 12-hr light/dark 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. In the avoidance tasks and in the locomotor activity test, the experimental groups included 8 mice; the number of animals employed to test reactivity to the electric shock is reported in Table 3.

Drugs

Oxiracetam (ISF; 50 or 100 mg/kg) and mecamylamine hydrochloride (Sigma; 2.5 or 5 mg/kg), dissolved in distilled water, and saline solution (0.9% NaCl), were injected intraperitoneally in a volume of 10 ml/kg.

Active Avoidance

The apparatus consisted of 8 automated shuttle-boxes, each one 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 sec and overlapped it for 25 sec. The US was an electric shock (0.2 mA) applied continuously to the grid floor. The intertrial interval was 30 sec. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 5 sec after the onset of the CS. If animals failed to avoid the shock they could escape it by crossing during the US. Spontaneous crossings from the dark to the light compartment were punished and recorded as intertrial responses.

Training consisted of 5 daily 50-trial (25 min) avoidance sessions. The mice were pretreated with 5 daily injections of saline or oxiracetam (50 or 100 mg/kg). During training, they received a first injection with saline or oxiracetam (as in the pretreatment) 30 min before each avoidance session, and a second injection with saline or mecamylamine 15 min later.

Passive Avoidance

Mice were subjected to a one-trial passive avoidance task in an apparatus consisting of two compartments, one light ($13.5 \times 6 \times 12$ cm high) and one dark ($27 \times 27 \times 27$ cm), connected via a sliding door. In the acquisition trial, each mouse was placed individually in the light 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 electrofootshock (0.7 mA for 1 sec) was delivered through the grid floor. The mouse was then returned to its own cage waiting for the retention trial, carried out 24 hr later. In the retention trial, the mouse was placed in the light compartment and the latency of the step-through response (cut-off latency time 180 sec) was recorded. Drug treatment consisted of saline or oxiracetam (as in the pretreatment), given 30 min before both the acquisition and the retention trial; mecamylamine (or saline) was given only before (15 min) the acquisition trial.

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 25 min. Thirty minutes before the activity test, the mice received saline or oxiracetam as in the pretreatment. In addition, they received saline or mecamylamine hydrochloride, 15 min before testing.

Pain Threshold to Electrical Stimulation

Sensitivity to the electric stimulation was evaluated by determining the pain threshold in mice placed in a plastic box ($27 \times 27 \times 27$ cm) having a grid floor, which was electrified by gradually increasing the current intensity to a maximum of 1000 μA. The minimal intensity eliciting vocalization in the mouse, indicated the pain threshold, expressed in μA; the maximum score (1000) was recorded for mice failing to squeak. Pretreated animals were tested 30 min after the administration of saline or oxiracetam and 15 min after mecamylamine (or saline).

RESULTS

Active Avoidance

Figure 1 reports the mean percent avoidance responses for each

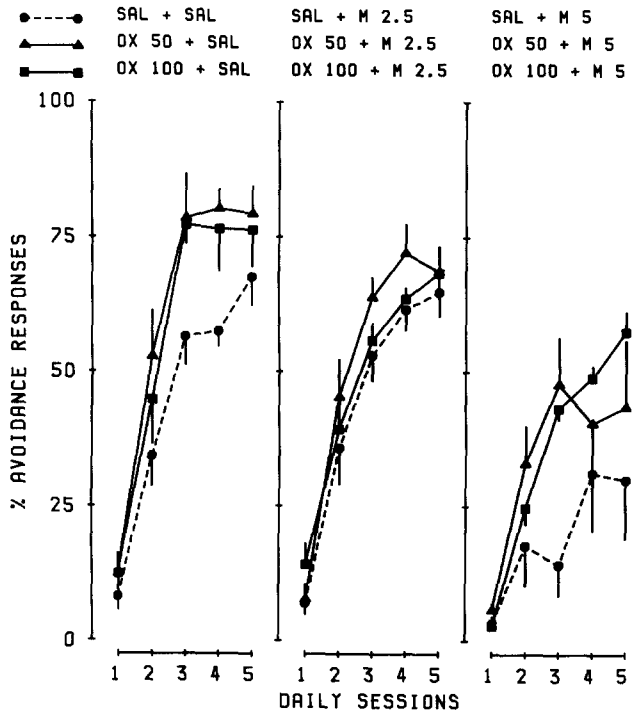

FIG. 1. Effect of mecamylamine and oxiracetam on shuttle-box avoidance acquisition. Mean percent avoidance responses (groups of 8 mice) in each of the five 50-trial sessions. Vertical bars indicate SEM. Mice pretreated with saline or oxiracetam (five daily injections) received, during training, a first injection with saline (SAL) or oxiracetam (OX; 50 or 100 mg/kg), 30 min before each session, and a second injection with saline (SAL) or mecamylamine hydrochloride (M; 2.5 or 5 mg/kg), 15 min later.

daily shuttle-box session and for each treatment group; escape responses are not reported, since escape failure seldom occurred.

Oxiracetam alone. A 2-factor ANOVA (treatment × sessions) showed significant effects of training, $F(4,84) = 89.75$, $p<0.001$, and treatment, $F(2,21) = 5.27$, $p<0.05$: avoidance performance increased in all groups with practice and was higher in oxiracetam-injected mice.

Mecamylamine alone. A 2-factor ANOVA (treatment × sessions) gave significant training, $F(4,84) = 47.71$, $p<0.001$, and treatment, $F(2,21) = 12.92$, $p<0.001$, main effects and a significant treatment × sessions, $F(8,84) = 3.15$, $p<0.01$, interaction. A further analysis of this interaction (Duncan test) showed that mecamylamine had no effect at the dose of 2.5 mg/kg, but significantly reduced the number of avoidance responses, starting from the second session, at the dose of 5 mg/kg.

Drug combinations. When combined with the ineffective dose of mecamylamine (2.5 mg/kg), oxiracetam did not exert any significant effect. On the contrary, the nootropic drug almost completely reversed the avoidance depression induced by 5 mg/kg of the nicotinic antagonist. A 2-factor ANOVA (treatment × sessions), concerning the groups receiving 5 mg/kg mecamylamine, given alone or in combinations with the two doses of oxiracetam, showed a significant training, $F(4,84) = 28.48$, $p<0.001$, but not treatment main effect, $F(2,21) = 3.04$, $p>0.05$. However, a significant treatment × training interaction, $F(8,84) = 2.39$, $p<0.05$, indicated the occurrence of dose- and session-related effects. In fact, a further analysis (Duncan test) showed that a significant increase of avoidance responses was produced by 50 mg/kg oxiracetam in the third session and by 100

TABLE 1

EFFECT OF MECAMYLAMINE AND OXIRACETAM ON PASSIVE AVOIDANCE

Mecamylamine mg/kg	Oxiracetam 0	50	100
0	79.00 ±9.06	82.00 ±11.71	86.25 ±14.86
2.5	75.37 ±10.57	78.87 ±14.02	83.37 ±11.76
5	14.87 ±4.08	15.12 ±4.46	11.87 ±1.95

Mean (±SEM) step-through latencies (sec) on the retention trial (24 hr after the acquisition trial), in groups of 8 mice. The animals were pretreated (5 daily injections) with oxiracetam at the doses of 0 (saline), 50 or 100 mg/kg and received the same treatment 30 min before both the acquisition and the retention trial. In addition, 15 min before the acquisition trial, mice were injected with mecamylamine hydrochloride at the doses of 0 (saline), 2.5 or 5 mg/kg.

TABLE 2

EFFECT OF MECAMYLAMINE AND OXIRACETAM ON LOCOMOTOR ACTIVITY

Mecamylamine mg/kg	Oxiracetam 0	50	100
0	80.50 ±5.76	82.75 ±3.48	75.50 ±5.65
2.5	25.00 ±4.29	28.50 ±7.73	27.50 ±6.06
5	24.75 ±3.33	23.00 ±1.60	20.75 ±2.38

Mean (±SEM) activity crossings, during 25 min, in groups of 8 mice. The animals were pretreated (5 daily injections) with oxiracetam at the doses of 0 (saline), 50 or 100 mg/kg and received the same treatment 30 min before both the test. In addition, 15 min before the testing, mice were injected with mecamylamine hydrochloride at the doses of 0 (saline), 2.5 or 5 mg/kg.

mg/kg in third, fourth and fifth sessions.

Intertrial responses (spontaneous crossings from the dark to the light compartment), which were punished by electric shock, were always at rather low levels.

Passive Avoidance

In the acquisition trial, all mice entered the dark compartment within 30 sec, but animals treated with mecamylamine required a significantly longer time: 12 sec on average, against the 6 sec of the controls. In the retention trial, mice that had received 5 mg/kg mecamylamine before training, exhibited much shorter latencies for the step-through responses (Table 1). Oxiracetam had no effect either alone or in combination with mecamylamine. A 2-factor analysis of variance, concerning retention latencies, showed a significant mecamylamine, $F(2,63)=43.41$, $p<0.001$, but not oxiracetam, $F(2,63)=0.12$, $p>0.05$, main effect and no significant interaction, $F(4,63)=0.9$, $p>0.05$.

Locomotor Activity

A 2-factor analysis of variance, concerning the number of activity crossings exhibited by the experimental groups during the 25-min test, showed a significant mecamylamine, $F(2,63)=127.52$, $p<0.001$, but not oxiracetam, $F(2,63)=0.39$, $p>0.05$, main effect and no significant interaction, $F(4,63)=0.24$, $p>0.05$. In fact, mecamylamine (2.5 and 5 mg/kg) depressed locomotor activity, while oxiracetam (50 and 100 mg/kg) did not change the number of activity crossings (Table 2).

Pain Threshold to Electrical Stimulation

A 2-factor analysis of variance, concerning pain threshold values, showed significant main effects of oxiracetam, $F(2,77)=3.86$, $p<0.05$, and mecamylamine, $F(2,77)=271.03$, $p<0.001$, but no significant interaction, $F(4,77)=0.32$, $p>0.05$. Oxiracetam slightly reduced sensitivity of mice to electrical stimulation, only at the dose of 100 mg/kg; conversely, both doses of mecamylamine, 2.5 and 5 mg/kg, strongly raised squeak thresholds (Table 3), indicating a strong reduction by the drug of the reactivity to the electric shock.

DISCUSSION

Oxiracetam, in the present study, produced task-dependent effects on learning. The nootropic drug improved shuttle-box avoidance acquisition as in previous studies (18,19), but had no effect on passive avoidance learning. Such a discrepancy may be due to the substantial differences characterizing learning in the two avoidance tasks, in which the type of response (active or passive) is quite different. Shuttle-box avoidance acquisition develops gradually, during several multitrial daily sessions, and it is possible that, in these conditions, nootropics may well exert their facilitative effects on learning. On the contrary, the acquisition of a passive avoidance response by normal animals occurs in a single trial, so that nootropic effects can be better observed in amnesic animals (16). However, a few cases of passive avoidance facilitation by nootropics in normal animals have been reported (24,25) and it seems that the intensity of the footshock, applied in the training trial, plays a determinant role in this respect (15).

A well-known property of nootropics is represented by their

TABLE 3

EFFECT OF MECAMYLAMINE AND OXIRACETAM ON SENSITIVITY TO ELECTRICAL STIMULATION

Mecamylamine mg/kg	Oxiracetam 0	50	100
0	367.00 (20) ±13.70	327.33 (15) ±21.63	411.33 (15) ±16.87
2.5	640.00 (6) ±65.31	660.00 (6) ±41.86	700.00 (6) ±42.50
5	943.33 (6) ±25.51	900.00 (6) ±34.25	983.33 (6) ±16.66

Mean (±SEM) pain thresholds (μA); number of mice in parentheses. The animals were pretreated (5 daily injections) with oxiracetam at the doses of 0 (saline), 50 or 100 mg/kg and received the same treatment 30 min before the test. In addition, 15 min before testing, mice received mecamylamine hydrochloride at the doses of 0 (saline), 2.5 or 5 mg/kg.

ability to prevent impairment of cognitive functions produced by various brain injuries (9, 16, 20), including drug-induced deficits in learning and memory, such as the amnesia induced by the anticholinergic agent scopolamine (4, 22, 23). Since the blockade of nicotinic cholinergic receptors by mecamylamine may also produce learning impairments in various tasks (see Introduction), a facilitative effect of nootropics on learning performances depressed by the nicotinic antagonist might also be expected. In the present study mecamylamine, at the dose of 5 mg/kg, depressed both active and passive avoidance responses. Oxiracetam failed to counteract mecamylamine-induced impairment of one-trial passive avoidance acquisition, but maintained its facilitating action on active avoidance learning in mice receiving the nicotinic antagonist. In fact, the depressant action exerted by mecamylamine on shuttle-box avoidance acquisition was almost completely reversed by the nootropic drug.

Findings showing learning and memory impairment by mecamylamine have previously proved that nicotinic cholinergic receptors are involved in cognitive functions (12,13). However, depression of locomotor activity and reduction of sensitivity to electric shock, exerted by mecamylamine in the DBA/2 mice in the present study, suggest that a specific factors may contribute to active and passive avoidance impairments produced by the nicotinic antagonist. This hypothesis is supported by the observation that a rather high dose of mecamylamine (10 mg/kg) had no effect on passive avoidance learning (unpublished data), when given soon after training. In this respect, it must be noted that posttrial administration, in one-trial passive avoidance task, is considered a valid tool to test effects of drugs on memory processes (14). Moreover, it must be considered that mecamylamine blocks central but also peripheral nicotinic acetylcholine receptors (11) and that blockade of peripheral receptors, when the drug is given before training, may produce unwanted effects aspecifically interfering with avoidance performance.

An involvement of a specific factors may limit the validity of mecamylamine-induced learning deficit as a tool for testing nootropic agents and for investigating possible interactions of these drugs with central nicotinic mechanisms. In this respect, the existence of structurally distinct subtypes of neuronal nicotinic acetylcholine receptors (11) could allow, in the future, a selective central nicotinic blockade and, consequently, the availability of more adequate behavioral models.

However, even if oxiracetam does not affect at all mecamylamine-induced passive avoidance impairment, the present results, showing prevention by oxiracetam of mecamylamine-induced shuttle-box avoidance depression, demonstrate that central nicotinic mechanisms may be involved in the facilitating effects exerted by nootropics in some learning tasks.

REFERENCES

1. Banfi, S.; Fonio, W.; Allievi, E.; Pinza, M.; Dorigotti, L. Cyclic GABA-GABOB analogues. IV—Activity on learning and memory. Il Farmaco, Ed. Sci. 39:16–22; 1984.
2. Bartus, R. T.; Dean, R. L., III; Sherman, K. A.; Friedman, E.; Beer, B. Profound effects of combining choline and piracetam on memory enhancement and cholinergic function in aged rats. Neurobiol. Aging 2:105–111; 1981.
3. Chiappetta, L.; Jarvik, M. E. Comparison of learning impairment and activity depression produced by two classes of cholinergic agents. Arch. Int. Pharmacodyn. Ther. 179:161–166; 1969.
4. Cumin, R.; Bandle, E. F.; Gamzu, E.; Haefely, W. E. Effects of the novel compound aniracetam (Ro 13-5057) upon impaired learning and memory in rodents. Psychopharmacology (Berlin) 78:104–111; 1982.
5. Dilts, S. L.; Berry, C. A. Effect of cholinergic drugs on passive avoidance in the mouse. J. Pharmacol. Exp. Ther. 158:279–285; 1967.
6. Driscoll, P.; Battig, K. The effect of small doses of mecamylamine on shuttlebox behavior in guinea-pig. Experientia 29:991–993; 1973.
7. Franklin, S. R.; Sethy, V. H.; Tang, A. H. Amnesia produced by intracerebroventricular injections of hemicholinium-3 in mice was prevented by pretreatment with piracetam-like compounds. Pharmacol. Biochem. Behav. 25:925–927; 1986.
8. Frontali, M.; Amorico, L.; De Acetis, L.; Bignami, G. A pharmacological analysis of processes underlying differential responding: A review and further experiments with scopolamine, amphetamine, lysergic acid diethylamide (LSD-25), chlordiazepoxide, physostigmine, and chlorpromazine. Behav. Biol. 18:1–74; 1976.
9. Giurgea, C.; Salama, M. Nootropic drugs. Prog. Neuropsychopharmacol. 1:235–247; 1977.
10. Goldberg, M. E.; Sledge, K.; Hefner, M.; Robichaud, R. C. Learning impairment after three classes of agents which modify cholinergic function. Arch. Int. Pharmacodyn. Ther. 193:226–235; 1971.
11. Gotti, C.; Sher, E.; Chini, B.; Fornasari, D.; Esparis Ogando, A.; Clementi, F. The nicotinic receptors in the nervous system. Pharmacol. Res. Commun. 20:637–662; 1988.
12. Levin, E. D.; Castonguay, M.; Ellison, G. D. Effects of the nicotinic receptor blocker mecamylamine on radial-arm maze performance in rats. Behav. Neural Biol. 48:206–212; 1987.
13. Levin, E. D.; McGurk, S. R.; South, D.; Butcher, L. L. Effects of combined muscarinic and nicotinic blockade on choice accuracy in the radial-arm maze. Behav. Neural Biol. 51:270–277; 1989.
14. McGaugh, J. L.; Herz, M. J. Memory consolidation. San Francisco: Albion Publishing Company; 1972.
15. Mondadori, C.; Ducret, T.; Borkowski, J. The memory-enhancing effects of the piracetam-like nootropics are dependent on experimental parameters. Behav. Brain Res. 33:79–82; 1989.
16. Nicolaus, B. J. R. Chemistry and pharmacology of nootropics. Drug Dev. Res. 2:463–474; 1982.
17. Oliverio, A. Effects of mecamylamine on avoidance conditioning and maze learning of mice. J. Pharmacol. Exp. Ther. 154:350–356; 1966.
18. Sansone, M.; Castellano, C.; Ammassari-Teule, M. Improvement of avoidance acquisition by the nootropic drug oxiracetam in mice. Arch. Int. Pharmacodyn. Ther. 275:86–92; 1985.
19. Sansone, M.; Libri, V.; Pavone, F.; Ammassari-Teule, M. Effects of oxiracetam-scopolamine combinations on shuttle-box avoidance acquisition in mice. Arch. Int. Pharmacodyn. Ther. 291:208–216; 1988.
20. Schindler, U.; Rush, D. K.; Fielding, S. Nootropic drugs: Animal models for studying effects on cognition. Drug Dev. Res. 4:567–576; 1984.
21. Spignoli, G.; Pepeu, G. Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia. Eur. J. Pharmacol. 126:253–257; 1986.
22. Spignoli, G.; Pepeu, G. Interactions between oxiracetam, aniracetam and scopolamine on behavior and brain acetylcholine. Pharmacol. Biochem. Behav. 27:491–495; 1987.
23. Verloes, R.; Scotto, A. M.; Gobert, J.; Wulfert, E. Effects of nootropic drugs in a scopolamine-induced amnesia model in mice. Psychopharmacology (Berlin) 95:226–230; 1988.
24. Wolthuis, O. L. Behavioural effects of etiracetam in rats. Pharmacol. Biochem. Behav. 15:247–255; 1981.
25. Wurtman, R. J.; Magil, S. G.; Reinstein, D. K. Piracetam diminishes hippocampal acetylcholine levels in rats. Life Sci. 28:1091–1093; 1981.
26. Yamada, K.; Inoue, T.; Tanaka, M.; Furukawa, T. Prolongation of latencies for passive avoidance responses in rats treated with aniracetam or piracetam. Pharmacol. Biochem. Behav. 22:645–648; 1985.