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Effects of Oxiracetam-Nicotine Combinations on Active and Passive Avoidance Learning in Mice

MARIO SANSONE, CLAUDIO CASTELLANO, MARIO BATTAGLIA AND MARTINE AMMASSARI-TEULE

Istituto di Psicobiologia e Psicofarmacologia, CNR, via Reno 1, 00198 Roma, Italy

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SANSONE, M., C. CASTELLANO, M. BATTAGLIA AND M. AMMASSARI-TEULE. *Effects of oxiracetam-nicotine combinations on active and passive avoidance learning in mice.* PHARMACOL BIOCHEM BEHAV **39**(1) 197–200, 1991.—Tested alone, in CD-1 mice, the nootropic drug oxiracetam (50 mg/kg) improved learning in a multitrial active avoidance task (shuttle-box), but did not affect one-trial passive avoidance acquisition. Nicotine, which was ineffective at the dose of 0.25 mg/kg, improved both active and passive avoidance at the dose of 0.5 mg/kg; 1 mg/kg nicotine still exerted facilitating effects on passive avoidance, but slightly depressed shuttle-box performance. Combinations of oxiracetam and nicotine improved passive avoidance more than either drug given separately. In the active avoidance task, a combination of oxiracetam with the lower dose of nicotine exerted improving effects never observed with nicotine alone, even at higher doses. The nootropic drug also prevented the slight depressant action exerted by 1 mg/kg nicotine. Thus, contrary to what was previously supposed, at least in mice subjected to shuttle-box avoidance training, nicotinic activation does not appear as the main neurochemical mechanism involved in the action of oxiracetam. Perhaps, oxiracetam and nicotine activate different types of cholinergic mechanisms, but it cannot be excluded that other neurotransmitters, particularly catecholamines, may be involved in the avoidance facilitating effects produced by nicotine and by combinations of the two drugs.

Nicotine Oxiracetam Avoidance learning Mice

WE have recently observed (15) that oxiracetam, a piracetam-like nootropic drug (1), administered to mice tested in a passive avoidance task (step-through), did not improve acquisition nor facilitate performance when learning was impaired by the nicotinic antagonist mecamylamine. Instead, in an active avoidance task (shuttle-box), oxiracetam improved learning and maintained its facilitating action in mice receiving the nicotinic receptor blocker.

It is well known that cholinergic mechanisms play an important role in learning and memory processes as well as in the nootropic action of some drugs (3, 5, 16, 17, 19). Since nicotinic mechanisms may contribute to the cholinergic involvement in cognitive functioning (8,9), prevention of mecamylamine-induced shuttle-box avoidance depression by oxiracetam suggested that nicotinic acetylcholine receptors could be involved in the improving effects exerted by nootropic drugs on learning (15). However, this hypothesis should be verified through other experimental findings, because depression of locomotor activity and reduction of sensitivity to electric shock, produced by mecamylamine, could have aspecifically contributed to the impairing effects exerted by the nicotinic antagonist on active and passive avoidance performance. A further evaluation of the role played by nicotinic mechanisms, in the action of oxiracetam, might be obtained by studying the effects of the nootropic drug in comparison and in combination with those of nicotine, the specific agonist of the nicotinic acetylcholine receptors.

In the present study, oxiracetam and nicotine, given alone or in combination, were tested on active (shuttle-box) and passive (step-through) avoidance learning in mice. Spontaneous locomotor activity was also tested, in order to verify the specificity of the drug effects on learning. Treatment with oxiracetam was always preceded by a five-day pretreatment, since it was previously demonstrated (14,18) that facilitation of shuttle-box avoidance learning by nootropics occurs in pretreated animals only.

METHOD

Animals

The subjects were naive male mice (age, 7–8 weeks; weight, 28–32 g) of the randomly bred CD-1 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-h light/dark cycle, ambient temperature of 23°C). The experiments were carried out between 9 a.m. and 4 p.m., by using different animals for different behavioral tests.

Drugs

Saline solution (0.9% NaCl), oxiracetam (50 mg/kg) and nicotine bitartrate (0.25, 0.5 or 1 mg/kg, as base), dissolved in dis-

tilled water, were injected intraperitoneally in a volume of 10 ml/kg. The pH of nicotine solutions was adjusted to 7 with NaOH.

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 s 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 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 100-trial avoidance sessions. The mice were pretreated with 5 daily injections of saline or oxiracetam (50 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 nicotine, 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 × 6 × 12 cm high) and one dark (27 × 27 × 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 s) was delivered through the grid floor. The mouse was then returned to its own cage

TABLE 1

EFFECT OF OXIRACETAM AND NICOTINE ON PASSIVE AVOIDANCE

Nicotine mg/kg	Oxiracetam 0	Oxiracetam 50
0	88.75 ± 8.46	92.25 ± 14.33
0.25	103.87 ± 17.26	180.75 ± 27.86*†
0.5	157.62 ± 19.07*	222.00 ± 36.70*
1	207.00 ± 21.05*	278.75 ± 13.00*†

Mean (±SEM) step-trough latencies (s) on the retention trial (24 h after the acquisition trial), in groups of 8 mice. The animals were pretreated (5 daily injections) with oxiracetam at the doses of 0 (saline) or 50 mg/kg and received the same treatment 30 min before both the acquisition and the retention trial. In addition, 15 min later, mice were injected with nicotine at the doses of 0 (saline), 0.25, 0.5 or 1 mg/kg. Significances ($p<0.05$) in the Duncan multiple-range test:

*Nicotine alone vs. saline (dose 0 of the drug) and drug combinations vs. oxiracetam alone (dose 0 of nicotine);

†Drug combinations vs. nicotine alone (dose 0 of oxiracetam).

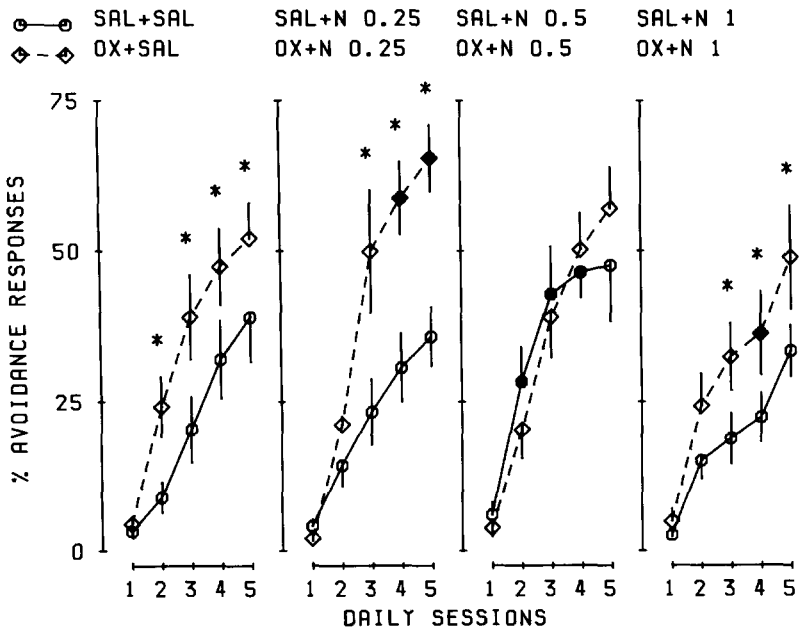

FIG. 1. Effect of oxiracetam and nicotine on shuttle-box avoidance acquisition. Mean percent avoidance responses (groups of 16 mice) in each of the five 100-trial sessions. Vertical bars indicate SEM. Mice pretreated with saline or oxiracetam (five daily injections) received, during training, a first injection with saline (SAL) or oxiracetam (OX; 50 mg/kg), 30 min before each session, and a second injection with saline (SAL) or nicotine (N; 0.25, 0.5 or 1 mg/kg), 15 min later. Asterisks denote a significant difference ($p<0.05$) of oxiracetam alone (OX + SAL) vs. controls (SAL + SAL) and of drug combinations (OX + N) vs. nicotine alone (SAL + N), at corresponding doses. Full symbols indicate a significant difference ($p<0.05$) of nicotine alone (SAL + N) vs. controls (SAL + SAL) and drug combinations (OX + N) vs. oxiracetam alone (OX + SAL).

TABLE 2

EFFECT OF OXIRACETAM AND NICOTINE ON LOCOMOTOR ACTIVITY

Nicotine mg/kg	Oxiracetam 0	Oxiracetam 50
0	115.25 ± 16.24	109.25 ± 5.38
0.25	111.50 ± 14.25	126.75 ± 13.09
0.5	130.00 ± 9.86	141.25 ± 8.45
1	106.00 ± 11.47	132.50 ± 16.25

Mean (±SEM) activity crossings, during 60 min, in groups of 8 mice. The animals were pretreated (5 daily injections) with oxiracetam at the doses of 0 (saline) or 50 mg/kg and received the same treatment 30 min before the test. In addition, 15 min before testing, mice were injected with nicotine at the doses of 0 (saline), 0.25, 0.5 or 1 mg/kg.

waiting for the retention trial, carried out 24 h 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 300 s) was recorded.

Drug treatment consisted of saline or oxiracetam (as in the pretreatment), given 30 min before both the acquisition and the retention trial; nicotine (or saline) was given 15 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. Thirty minutes before the activity test, the mice received saline or oxiracetam as in the pretreatment. In addition, they received saline or nicotine, 15 min before testing.

RESULTS

Active Avoidance

Figure 1 reports the mean percent avoidance responses for each daily shuttle-box session and for each treatment group; escape responses are not reported, since escape failure seldom occurred.

A 2-factor ANOVA (treatment × sessions) for avoidance responses showed significant main effects of treatment, $F(7,120)=3.10$, $p<0.05$, and training (repeated measures), $F(4,480)=194.86$, $p<0.001$, and a significant treatment × sessions interaction, $F(28,480)=2.85$, $p<0.001$. A further analysis for between-treatment comparisons, in single sessions, was carried out by employing the Duncan multiple-range test.

Given alone, oxiracetam significantly improved avoidance performance, starting from the second session; nicotine had no effect at the dose of 0.25 mg/kg, facilitated avoidance responses at 0.5 mg/kg (second, third and fourth session) and exerted some slight, not significant, depressant effects, in the last training sessions, at the dose of 1 mg/kg. Thus, both drugs were able to improve avoidance acquisition, but the stronger improving effect was observed when oxiracetam was combined with the lower dose (0.25 mg/kg) of nicotine. Mice receiving such a combination performed better than mice treated with drugs given separately at the corresponding doses and exhibited, in the two last sessions, avoidance levels significantly higher ($p<0.05$) than those observed in mice treated with the effective dose (0.5 mg/kg) of nicotine alone. Oxiracetam also prevented the slight depressant effect produced by 1 mg/kg nicotine.

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

Drug treatments did not affect step-through latencies in the training trial: all mice entered the dark compartment within 20 s. Conversely, significant effects occurred in the retention trial, as demonstrated by an overall one-way ANOVA: $F(7,56)=10.49$, $p<0.001$. Individual between-treatment comparisons (Duncan test) indicated that performance of mice injected with nicotine alone (0.5 and 1, but not 0.25 mg/kg) was significantly higher than that of controls. Oxiracetam (50 mg/kg), which had no effect alone, significantly enhanced the facilitating action of nicotine on retention performance: even a combination of oxiracetam with the ineffective dose of nicotine (0.25 mg/kg) improved retention of the avoidance response (Table 1).

Locomotor Activity

Oxiracetam (50 mg/kg) and nicotine (0.25, 0.5 or 1 mg/kg), either alone or in combination, did not affect locomotor activity, measured during the whole 60-min session (Table 2). An overall one-way ANOVA, concerning the activity crossings, showed no significant effect of drug treatments, $F(7,56)=1.06$, $p>0.05$. However, a significant short-lasting depressant action was produced by nicotine 1 mg/kg during the first 10 min of the activity test (mean number of crossings: saline 44.25 ± 5.01; nicotine 1 mg/kg: 28.25 ± 5.09).

DISCUSSION

In the present study the nootropic drug oxiracetam (50 mg/kg) improved active, but not passive, avoidance learning in mice of the CD-1 strain. A similar discrepancy was previously observed in DBA/2 mice and was ascribed to the opposite conditions made by the two avoidance tasks, requiring the acquisition of an active response during several multitrial sessions, in one case, and of a passive response in a single trial, in the other case (15). Nicotine improved performance in both tasks, in agreement with previous findings showing facilitation by the drug of both active (2,4) and passive (6,11) avoidance learning. In particular, nicotine, which had no effect at the dose of 0.25 mg/kg, improved both active and passive avoidance performance at the dose of 0.5 mg/kg, but facilitated only passive avoidance at the dose of 1 mg/kg. Since nicotine, at the dose of 1 mg/kg, produced a short-lasting locomotor depression, it might be supposed that a reduced motility could have contributed to the facilitating action exerted by the drug in the passive avoidance task, consisting of one trial only. However, this hypothesis can be ruled out, since no difference was found between step-through latencies of saline- and nicotine-treated mice in the training session. Moreover, similar facilitating effects on passive avoidance acquisition were observed when 1 mg/kg nicotine was injected 30 min, instead of 15 min, before testing (data not shown).

The main findings of the present study concern, however, the effects produced by combinations of oxiracetam and nicotine. In the active avoidance task, a strong performance improvement was observed when oxiracetam was combined with 0.25 mg/kg nicotine, a dose ineffective by itself. In the passive avoidance task, doses of the two drugs (50 mg/kg oxiracetam

and 0.25 mg/kg nicotine), ineffective when given separately, improved retention performance when combined. The nootropic drug also enhanced the facilitating effects of the higher doses of nicotine on passive avoidance acquisition. It seems unlikely that the avoidance facilitating effects produced by oxiracetam and nicotine, given alone or in combination, may be ascribed to an action of the two drugs on shock sensitivity. In fact, the evaluation of pain threshold (squeak response) to electrical stimulation showed that the two drugs, at the doses tested in avoidance studies, had no effect or slightly reduced sensitivity of mice to the electric shock (data not shown).

Previous findings (15), showing prevention by oxiracetam of shuttle-box avoidance depression induced by the nicotinic antagonist mecamylamine, suggested an involvement of central nicotinic mechanisms in the facilitating effects exerted by the nootropic drug on avoidance learning. However, the actual role of the nicotinic receptors was not clearly ascertained, because aspecific factors could have been involved in the learning impairment produced by mecamylamine (15). On the basis of the present results, it seems now unlikely that an activation of central nicotinic receptors may represent the neurochemical mechanism mainly involved in the improving effects of oxiracetam on learning. In fact, mice trained in the shuttle-box after receiving oxiracetam, combined with a low dose of nicotine, exhibited avoidance levels never reached by mice treated with nicotine alone, even at higher doses. Moreover, the slight depressant action of 1 mg/kg nicotine was prevented by the nootropic drug. On the other hand, it seems also important to note that oxiracetam, which in the present study enhanced the facilitating effects of nicotine on passive avoidance acquisition, was unable to counteract mecamylamine-induced impairment in the same learning task (15). Of course, even if central nicotinic receptors do not exert a determinant role in the avoidance facilitating effects of oxiracetam, other cholinergic systems may be activated by the drug. Thus combinations of oxiracetam and nicotine could improve both active and passive avoidance performances by simultaneously activating different types of cholinergic mechanisms. However, a possible interference with other neurotransmitter systems cannot be excluded, since nicotine does not act exclusively on cholinergic synapses (20). Nicotine stimulates the release of norepinephrine and dopamine from brain tissues (7) and its effects on learning can be, at least in part, based on adrenergic mechanisms (10). Moreover, central catecholamines are strongly implicated in the acquisition and maintenance of aversive learning (12) and a shuttle-box avoidance facilitation, stronger than that produced by combinations of oxiracetam and nicotine, was previously (13) observed when the nootropic agent was combined with methamphetamine, a sympathomimetic drug. In view of these last observations, it may be supposed that brain catecholamines are involved in the avoidance facilitation produced by combinations of oxiracetam and nicotine, without questioning the role of cholinergic mechanisms in the action of nootropic drugs.

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