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Effects of Oxiracetam, Physostigmine, and Their Combination on Active and Passive Avoidance Learning in Mice

MARIO SANSONE,*[1] CLAUDIO CASTELLANO,* SERGIO PALAZZESI,†
MARIO BATTAGLIA* AND MARTINE AMMASSARI-TEULE*

**Istituto di Psicobiologia e Psicofarmacologia, CNR, via Reno 1, 00198 Roma, Italy*
†*Istituto Superiore di Sanitá, Roma, Italy*

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SANSONE, M., C. CASTELLANO, S. PALAZZESI, M. BATTAGLIA AND M. AMMASSARI-TEULE. *Effects of oxiracetam, physostigmine, and their combination on active and passive avoidance learning in mice.* PHARMACOL BIOCHEM BEHAV **44**(2) 451–455, 1993.—The nootropic drug oxiracetam (50 and 100 mg/kg) had no effect on one-trial passive avoidance acquisition in CD-1 mice, while the acetylcholinesterase inhibitor physostigmine improved passive avoidance performance at doses of 0.025 and 0.05 mg/kg given either pre- or posttraining. In a multitrial avoidance task (shuttle-box), a consistent tendency to better performance was displayed by mice receiving oxiracetam (50 and 100 mg/kg) or physostigmine (0.01 and 0.025 mg/kg, but not 0.05 mg/kg). Combinations of the two drugs never improved active or passive avoidance performance more than drugs given separately. This indicates no advantage in combining nootropics and anticholinesterase inhibitors to improve learning and memory.

Oxiracetam Physostigmine Avoidance learning Mice

PIRACETAM-like compounds, the so-called nootropics (4), are able to improve learning and memory and enhance resistance to learning impairment in various experimental situations (4,10). In studying the action of nootropic agents in combination with drugs facilitating active and passive avoidance learning, supraadditive effects have been often observed. Thus, combinations of oxiracetam and piracetam with methamphetamine (15) or nicotine (18) improved shuttle-box avoidance acquisition more than drugs given separately. Advantages of drug combination were especially evident in mice tested in a one-trial passive avoidance task, in which oxiracetam had no effect alone but enhanced the retention-improving effects exerted by cholinomimetic agents, such as nicotine (18) and secoverine (1). In view of these last findings, we assumed that, in avoidance tasks, a synergism may always be expected when nootropics are given in combination with drugs enhancing cholinergic activity. This hypothesis, however, is not supported by the present findings, which demonstrate that the nootropic drug oxiracetam (2) did not enhance the avoidance-improving effects of the acetylcholinesterase inhibitor physostigmine, a putative cholinomimetic cognition enhancer (19). The two drugs were tested, alone or in combination, in mice subjected to shuttle-box avoidance training or a one-trial passive avoidance test. Training was always preceded by a 5-day pretreatment with oxiracetam (a daily injection at the dose tested afterward during training) because it was previously demonstrated (16,23) that shuttle-box avoidance improvements by nootropics occur in pretreated animals only. Spontaneous locomotor activity was also tested to verify the specificity of the drug effects on learning.

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 (eight per cage) in standard transparent plastic cages (27 × 21 × 14 cm) 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 9:00 a.m. and 4:00 p.m. by using different animals for different behavioral tests.

Drugs

Saline solution (0.9% NaCl), oxiracetam (ISF, s.p.a., Milan, Italy; 50 or 100 mg/kg), and physostigmine sulfate (Merck, Darmstadt, Germany; 0.01, 0.025, or 0.05 mg/kg),

[1] To whom requests for reprints should be addressed.

dissolved in distilled water, were injected IP in a volume of 10 ml/kg.

Active Avoidance

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 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 five daily 100-trial avoidance sessions. Mice received a first injection with saline or oxiracetam (50 or 100 mg/kg) 30 min before each avoidance session and a second injection with saline or physostigmine (0.01, 0.025, or 0.05 mg/kg) 15 min later. Experimental groups consisted of 16 mice that received saline as a second injection; groups receiving physostigmine included eight animals.

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 waiting 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 (cut-off latency time 300 s) was recorded.

Drug treatment consisted of saline or oxiracetam (50 or 100 mg/kg) given 30 min before both the acquisition and retention trials. Physostigmine was given 15 min before training and testing at the doses of 0 (saline), 0.01, 0.025, or 0.05 mg/kg or immediately after training (0, 0.025, or 0.05 mg/kg). Each group included eight subjects.

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 30 min. Mice were subjected to the activity test after a 5-day pretreatment with saline solution or oxiracetam 100 mg/kg. On the sixth day, animals received saline or oxiracetam (as in the pretreatment) 30 min before the activity test. In addition, they received saline or physostigmine (0.01, 0.025, or 0.05 mg/kg) 15 min before testing. Each group consisted of eight subjects.

Statistics

The data concerning one-trial passive avoidance test and locomotor activity were evaluated by two-factor analysis of variance (ANOVA), the factors being oxiracetam (two or three levels) and physostigmine (three or four levels). Shuttle-box avoidance responses were evaluated by a three-factor ANOVA because in addition to the above two factors a third factor (repeated measures) was represented by daily sessions (five levels). Posthoc analysis was carried out, when appropriate, by Duncan's multiple-range test.

RESULTS

Active Avoidance

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

A three-factor ANOVA for avoidance responses showed no significant main effect of oxiracetam, $F(2, 108) = 0.28$, $p > 0.05$, or physostigmine, $F(3, 108) = 0.66$, $p > 0.05$, and no significant drug interaction, $F(6, 108) = 0.91$, $p > 0.05$, on the whole of the five training sessions. The analysis also showed a significant effect of training, $F(4, 432) = 221.54$, $p < 0.001$, but oxiracetam × sessions, $F(8, 432) = 0.59$, $p > 0.05$, physostigmine × sessions, $F(12, 432) = 0.60$, $p > 0.05$, and oxiracetam × physostigmine × sessions, $F(24, 432) = 1.19$, $p > 0.05$, interactions did not reach significance levels. The results of this complex three-factor ANOVA did not allow further analysis for simple individual drug effects. However, Fig. 1 shows that mice receiving oxiracetam or physostigmine alone clearly performed better than controls and combination of the two drugs did not produce any advantage. This observation was supported by simpler statistical analyses (a two-factor treatment × sessions ANOVA for each treatment).

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

Figure 2 shows mean step-through latencies exhibited on the retention trial by mice treated with saline or oxiracetam and receiving physostigmine before or after training. A two-factor ANOVA, for pretraining physostigmine showed a significant main effect of physostigmine, $F(3, 84) = 191.98$, $p < 0.001$, but not of oxiracetam, $F(2, 84) = 0.45$, $p > 0.05$, and no significant interaction, $F(6, 84) = 1.01$, $p > 0.05$. Similar results were obtained when physostigmine was given posttraining: a significant main effect of physostigmine, $F(2, 63) = 88.41$, $p < 0.001$, but not of oxiracetam, $F(2, 63) = 0.15$, $p > 0.05$, and no significant interaction, $F(4, 63) = 0.08$, $p > 0.05$. In both cases, a posthoc analysis indicated that physostigmine produced significant retention improvements at doses of 0.025 and 0.05 mg/kg.

Physostigmine had no effect in mice that did not receive foot-shock in the training session (data not shown).

Locomotor Activity

Table 1 reports the activity crossings exhibited by mice receiving oxiracetam (100 mg/kg) and physostigmine (0.01, 0.025, or 0.05 mg/kg) alone or in combination. A two-factor ANOVA showed a significant main effect of physostigmine,

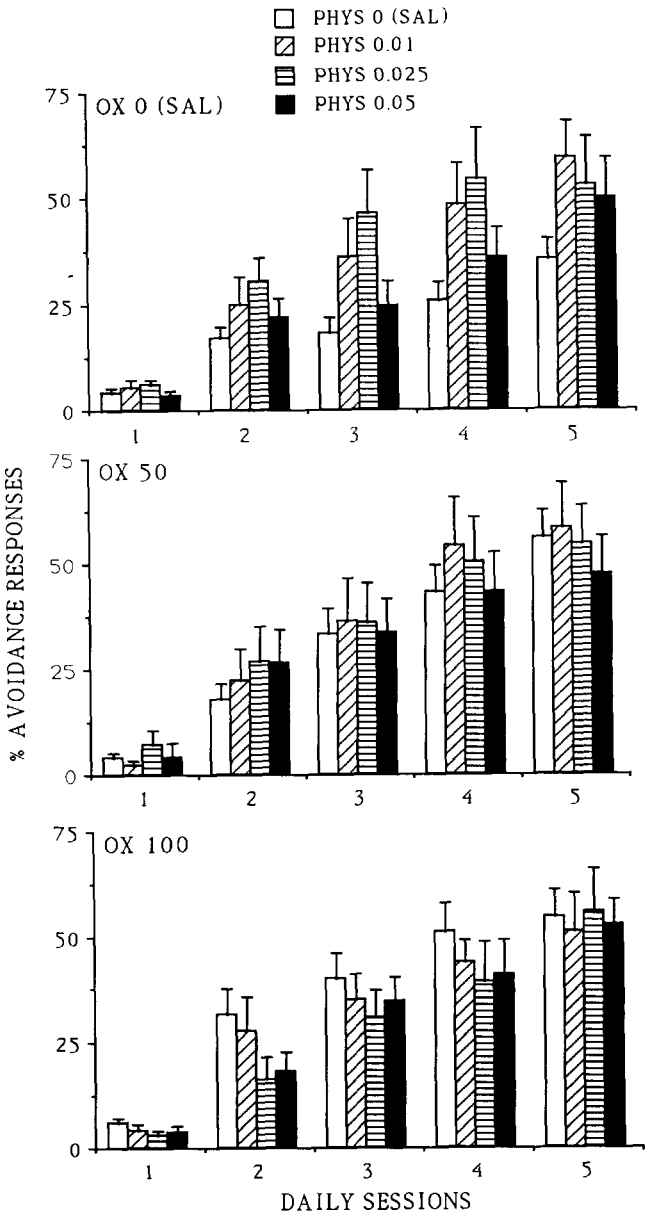

FIG. 1. Effect of oxiracetam (OX) and physosigmine (PHYS) on shuttle-box avoidance acquisition. Columns represent mean percent avoidance responses in each of the five 100-trial sessions. Vertical bars indicate SEM. Mice received a first injection with OX at doses of 0 (SAL), 50, or 100 mg/kg 30 min before each session and a second injection with PHYS sulfate at doses of 0 (SAL), 0.01, 0.025, or 0.05 mg/kg 15 min later.

$F(3, 56) = 2.97$, $p < 0.05$, but not of oxiracetam, $F(1, 56) = 0.45$, $p > 0.05$, and no significant interaction, $F(3, 56) = 0.25$, $p > 0.05$. A posthoc analysis indicated that physostigmine depressed locomotor activity at the dose of 0.05 mg/kg.

DISCUSSION

The present results show significant improving effects of physostigmine on passive avoidance acquisition in CD-1 mice. Active avoidance was also apparently facilitated by the drug, even if this facilitating action was not supported by a statistical significance. Improvement of passive avoidance learning by physostigmine is in agreement with previous findings, demonstrating that this acetylcholinesterase inhibitor, administered either pre- or posttraining, enhances retention performance in both rats and mice tested in one-trial inhibitory avoidance tasks (8). Conversely, previous works indicated that physostigmine exerted impairing rather than improving effects on active avoidance acquisition. In particular, learning impairment was observed in rats subjected to shuttle-box training after administration of the drug at doses higher (5,13) or even near (14) those employed in the present study. Thus, it seems that mice are less sensitive than rats to the depressant action of physostigmine so low doses of the drug, devoid of aspecific impairing effects, show a tendency to facilitate shut-

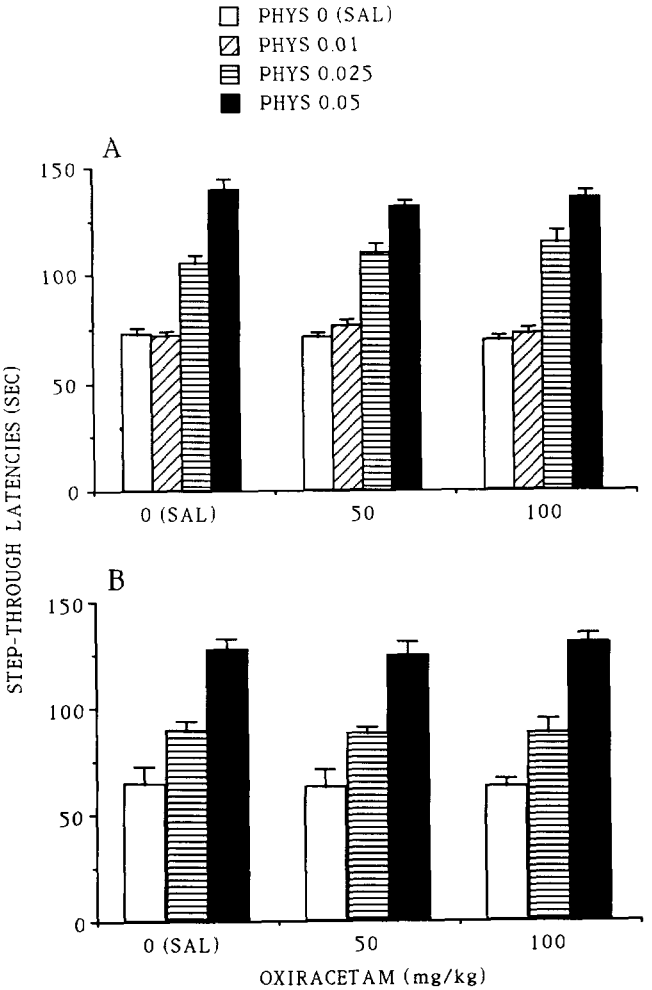

FIG. 2. Effect of oxiracetam and physositgmine (PHYS) on passive avoidance acquisition. Columns represent mean step-through latencies (seconds) on the retention trial (24 h after the acquisition trial). Vertical bars indicate SEM. Mice received oxiracetam at doses of 0 (SAL), 50, or 100 mg/kg 30 min before both the acquisition and retention trials. PHYS sulfate was injected (A) 15 min before both trials at doses of 0 (SAL), 0.01, 0.025, or 0.05 mg/kg or (B) immediately after the acquisition trial at doses of 0 (SAL), 0.025, or 0.05 mg/kg.

TABLE 1

EFFECT OF OXIRACETAM AND PHYSOSTIGMINE ON LOCOMOTOR ACTIVITY

Physostigmine (mg/kg)	Oxiracetam (mg/kg)	
	0	100
0	80.75 ± 6.17	75.00 ± 6.48
0.01	72.00 ± 4.70	70.50 ± 3.99
0.025	80.00 ± 8.40	72.25 ± 6.40
0.05	59.00 ± 9.71	61.75 ± 2.40

Mean (±SEM) activity crossings during 30 min. Mice received oxiracetam at doses of 0 (saline) or 100 mg/kg 30 min before testing and physostigmine sulfate at doses of 0 (saline), 0.01, 0.025, or 0.05 mg/kg 15 min later.

tle-box avoidance acquisition. On the other hand, failure of the highest dose (0.05 mg/kg) of physostigmine to increase shuttle-box avoidance responses in mice might be due, at least in part, to aspecific depressant effects, as suggested by a slight reduction in locomotor activity and sensitivity to the electric shock (squeak response; data not shown) induced by this dose of the drug.

Oxiracetam, as physostigmine, exerted facilitating effects, even if not statistically significant, on shuttle-box avoidance acquisition but, contrary to physostigmine, had no effect on passive avoidance learning. These findings agree with the results obtained in previous experiments, carried out under similar experimental conditions (17,18), showing learning facilitation by oxiracetam in the multitrial active but not in the one-trial passive avoidance task. Conversely, oxiracetam did not enhance either active or passive avoidance improvement induced by physostigmine, contrary to what was expected in view of previous effects obtained with drug combinations including this nootropic agent (1).

The mechanism of action of nootropics is still unknown, as it is unknown whether the same neurochemical mechanisms are responsible for both the cognition-enhancing and protective action upon brain injuries exerted by these drugs (10). It has been suggested that the central action of nootropics might initiate at the peripheral level through a stimulation of the adrenal glands. Activation of the adrenal medulla, increasing epinephrine release and glucose blood levels, might facilitate learning and memory through an enhanced availability and utilization of glucose in the brain (22). Alternatively, a stimulation of the adrenal cortex by nootropics could release adrenocortical steroids, which might exert a modulatory action on the central biochemical effects of the same agents (9). Other experimental findings indicate a direct action of oxiracetam on brain structures (12). Anyway, nootropic agents may interfere with various neurotransmitter systems (19) and an activation of brain cholinergic function by oxiracetam has been reported (20,21). In view of the role that cholinergic mechanisms might play in the action of nootropics, we recently hypothesized (1) that retention improvements produced by combinations of oxiracetam and secoverine, a presynaptic muscarinic antagonist (7), as previous similar effects produced by combinations of physostigmine and secoverine (6), might be ascribed to a simultaneous activation of different cholinergic mechanisms. However, it should not be disregarded that catecholaminergic mechanisms were probably involved in the strong avoidance facilitation induced by a combination of oxiracetam with methamphetamine (15), or even with nicotine (18), and that the nootropic drug counteracted the passive avoidance impairment induced by the dopamine receptor blocker haloperidol (3). On the other hand, passive avoidance facilitation by combinations of oxiracetam and secoverine could also be explained by considering that secoverine, which antagonizes acetylcholine at the muscarinic autoreceptors, was less effective at the muscarinic receptors mediating potentiation of dopamine release (7). These last considerations, together with the present results, showing failure of oxiracetam to enhance physostigmine-induced avoidance facilitation, suggest that the role of cathecholaminergic mechanisms should always be considered in evaluation of the effects on avoidance learning exerted by combinations of nootropics with other drugs. In this respect, it is important to note that central catecholamines are in particular involved in acquisition and maintenance of aversive learning (11). Although an involvement of catecholaminergic mechanisms in the action of nootropics has been suggested (10), it is at present difficult to hypothesize a role of cathecholamines in the effects of oxiracetam on avoidance behavior. However, it is possible that nootropics, whatever their mechanism of action may be, exert additive or supraadditive facilitating effects on avoidance learning when combined with arousing catecholaminergic agents more than with cholinomimetic cognition enhancers. Further researches, carried out by combining nootropics with other catecholaminergic and cholinergic agents, could clarify this point.

REFERENCES

1. Ammassari-Teule, M.; Castellano, C.; Sansone, M. Enhancement by oxiracetam of passive avoidance improvement induced by the presynaptic muscarinic antagonist secoverine in mice. Behav. Brain Res. 47:93-95; 1992.
2. Banfi, S.; Fonio, W.; Allievi, E.; Pinza, M.; Dorigotti, L. Cyclic GABA-GABOB analogues. IV—Activity on learning and memory. Farmaco Ed. Sci. 39:16-22; 1984.
3. Castellano, C.; Battaglia, M.; Sansone, M. Oxiracetam prevents haloperidol-induced passive avoidance impairment in mice. Pharmacol. Biochem. Behav. 42:797-801; 1992.
4. Giurgea, C.; Salama, M. Nootropic drugs. Prog. Neuropsychopharmacol. 1:235-247; 1977.
5. Gonzales, L. P.; Altshuler, H. L. Time-dependent changes in the effects of cholinesterase inhibitors on shuttle-box avoidance. Pharmacol. Biochem. Behav. 12:847-850; 1980.
6. Gower, A. J. Enhancement by secoverine and physostigmine of retention of passive avoidance response in mice. Psychopharmacology (Berl.) 91:326-329; 1987.
7. Marchi, M.; Raiteri, M. Differential antagonism by dicyclomine, pirenzepine and secoverine at muscarinic receptor subtypes in the rat frontal cortex. Eur. J. Pharmacol. 107:287-288; 1985.
8. McGaugh, J. L. Drug facilitation of learning and memory. Annu. Rev. Pharmacol. 13:229-241; 1973.
9. Mondadori, C.; Ducret, T.; Petschke, F. Blockade of the nootropic action of piracetam-like nootropics by adrenalectomy: An effect of dosage? Behav. Brain Res. 34:155-158; 1989.
10. Nicholson, C. D. Pharmacology of nootropics and metabolically active compounds in relation to their use in dementia. Psychopharmacology (Berl.) 101:147-159; 1990.

11. Oei, T. P. S.; King, M. G. Catecholamines and aversive learning: A review. Neurosci. Biobehav. Rev. 4:161–173; 1980.
12. Ponzio, F.; Pozzi, O.; Banfi, S.; Dorigotti, L. Brain entry and direct central pharmacological effects of the nootropic drug oxiracetam. Pharmacopsychiatry 22:111–115; 1989.
13. Rech, R. H. Effects of cholinergic drugs on poor performance of rats in a shuttle-box. Psychopharmacologia 12:371–383; 1968.
14. Rosic, N.; Bignami, G. Depression of two-way avoidance learning and enhancement of passive avoidance learning by small doses of physostigmine. Neuropharmacology 9:311–316; 1970.
15. Sansone, M.; Ammassari-Teule, M.; Oliverio, A. Interaction between nootropic drugs and methamphetamine on avoidance acquisition but not on locomotor activity in mice. Arch. Int. Pharmacodyn. Ther. 278:229–235; 1985.
16. 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.
17. Sansone, M.; Castellano, C.; Battaglia, M.; Ammassari-Teule, M. Oxiracetam prevents mecamylamine-induced impairment of active, but not passive, avoidance learning in mice. Pharmacol. Biochem. Behav. 36:389–392; 1990.
18. Sansone, M.; Castellano, C.; Battaglia, M.; Ammassari-Teule, M. Effects of oxiracetam–nicotine combinations on active and passive avoidance learning in mice. Pharmacol. Biochem. Behav. 39:197–200; 1991.
19. Sarter, M. Taking stock of cognition enhancers. Trends Pharmacol. Sci. 12:456–461; 1991.
20. Spignoli, G.; Pepeu, G. Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia. Eur. J. Pharmacol. 126:253–257; 1986.
21. Spignoli, G.; Pepeu, G. Interactions between oxiracetam, aniracetam and scopolamine on behavior and brain acetylcholine. Pharmacol. Biochem. Behav. 27:491–495; 1987.
22. Wenk, G. L. An hypothesis on the role of glucose in the mechanism of action of cognitive enhancers. Psychopharmacology (Berl.) 99:431–438; 1989.
23. Wolthuis, O. L. Behavioural effects of etiracetam in rats. Pharmacol. Biochem. Behav. 15:247–255; 1981.