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Habituation of Exploratory Activity in Mice: Effects of Combinations of Piracetam and Choline on Memory Processes

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PLATEL, A., M. JALFRE, C. PAWELEC, S. ROUX AND R. D. PORSOLT. *Habituation of exploratory activity in mice: Effects of combinations of piracetam and choline on memory processes.* PHARMACOL BIOCHEM BEHAV 21(2) 209–212, 1984.—The effects of various piracetam + choline combinations on an experimental model of memory were investigated. Mice were given two sessions in a simple photo-cell activity cage and the decrease in activity at the second session (habituation) served as an index of retention. Retention was facilitated by post-session administration of 2000 mg/kg piracetam IP and 50 mg/kg piracetam + 50 mg/kg choline IP. Similar injections of choline alone (10 to 200 mg/kg IP), piracetam alone (10 to 1000 mg/kg IP) or other combinations of piracetam and choline were without effect. These results, consistent with those reported elsewhere, suggest that piracetam can interact with choline to facilitate memory processes in mice.

Habituation Memory Mice Nootropics Choline Piracetam

THE nootropic drug piracetam has been reported to improve learning and memory in several animal models [5] and even in some cases in man [9]. In recent years, numerous studies have indicated that age-related cholinergic deficits may account, at least partially, for the memory loss observed in senile demented patients [2] and in aging rats [7]. Unfortunately, most attempts to improve memory performance in aged humans with cholinergic agonists or precursors have not succeeded [3]. Recently, two reports have been published which suggest that a combination of piracetam and choline is more effective than the constituent drugs alone in restoring memory impairment in aged Fischer rats in a one-trial passive avoidance task [1] and in enhancing retention in a conditioned avoidance response in young adult rats [6]. There has even been one report suggesting modest improvements in memory performance in some senile demented patients tested with a piracetam + choline combination [4].

The purpose of the present study was to examine the effects of acute post-session administration of various doses of piracetam, choline and piracetam + choline combinations on the habituation of exploratory activity in mice. Previous experiments [8] have shown that this model of memory is sensitive to post-session administration of memory enhancing or impairing drugs, is subject to forgetting with time, possesses a consolidation period of limited duration and is not affected by post-session administration of presumably noxious or rewarding stimuli. The experiments described below show that: (1) in a dose range from 10 to 200 mg/kg, post-session administration of choline did not affect memory. (2) In a dose range from 10 to 2000 mg/kg, piracetam enhanced retention only at 2000 mg/kg. (3) The combination of piracetam + choline enhanced retention at 50 mg/kg each drug, doses which were ineffective when either piracetam or choline were injected alone. (4) Combinations of 50 mg/kg choline with other doses of piracetam were without effect. (5) The facilitation observed with 2000 mg/kg piracetam alone disappeared when this dose was combined with 50 mg/kg choline.

METHOD

Animals

Adult male CD1 mice (Charles River France) were used. The animals were housed in groups of 10 in standard plastic cages, in a temperature (20–22°) and light (non reversed 12 hour light/dark cycle) controlled room. The mice were kept at least 4 days after their arrival before being used and weighed 20 to 35 g at the time of the experiment. Food and water were given ad lib.

Apparatus

A standard photo-cell activity meter was used ("Apelab"). Briefly, it consisted of six 26×20.5 cm Plexiglas cages, 10 cm high and covered with a lid. Each cage was fitted with two photo-cell assemblies with visible light beams and connected to electromechanical counters which totalized

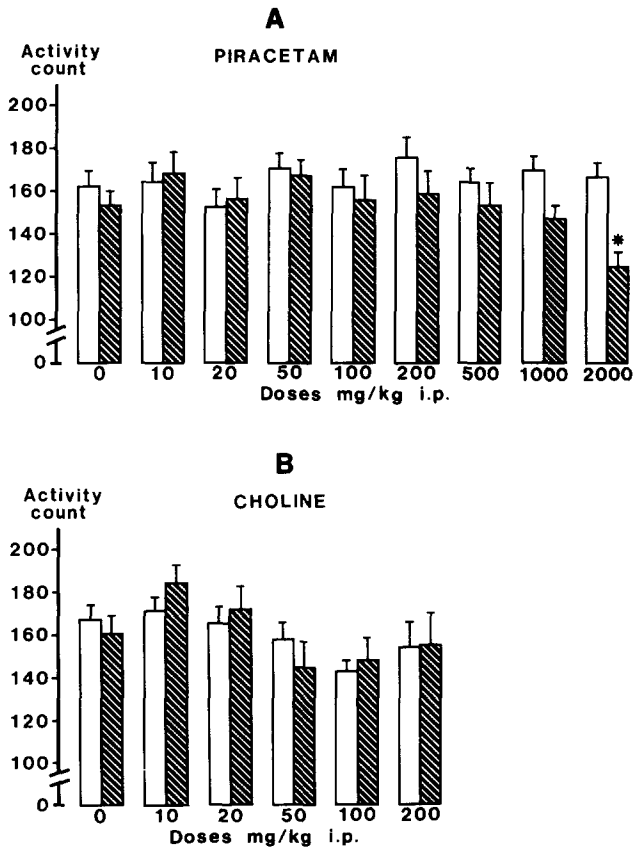

FIG. 1. Effects of post-session administration of piracetam (A) and choline (B) on the habituation of exploratory activity between Session 1 (white columns) and Session 2 (hatched columns). Means and Standard Errors. *: $p<0.05$ (Dunnett test). N=20 per group.

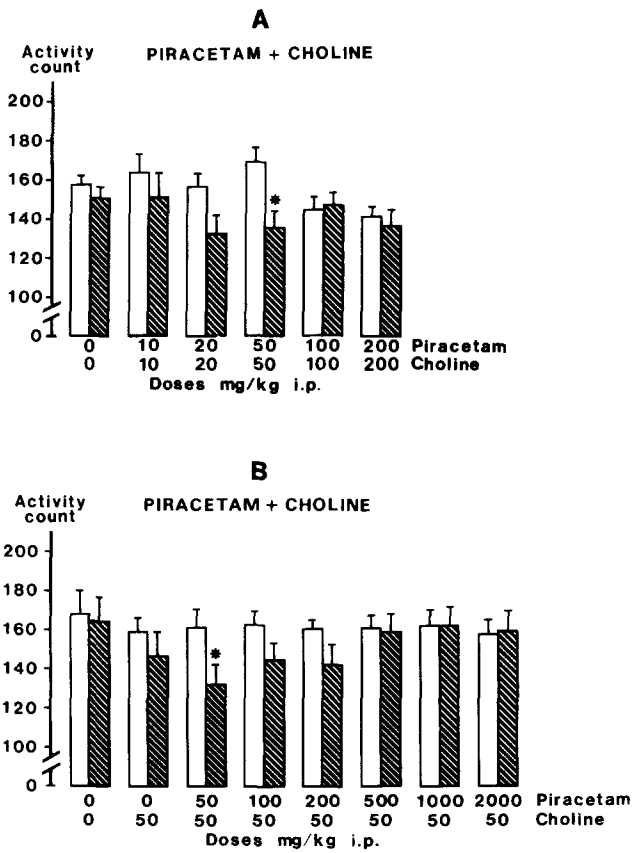

FIG. 2. Effects of post-session administration of various combinations of piracetam + choline on the habituation of exploratory activity between Session 1 (white columns) and Session 2 (hatched columns). Means and Standard Errors. *: $p<0.05$ (Dunnett test). N=20 per group.

the activity scores for each cage. The six cages were enclosed in a metal cabinet with no illumination except the light provided by the photo-cell beams.

General Procedure

All animals within a cage received the same treatment which was assigned at random. For testing, each group was divided into two half-groups containing five animals. All animals within each half-group were tested at the same time with one animal in each activity cage. To avoid order-of-testing effects, all different half-groups were tested in a counterbalanced order, always between 0900 hr and 1230 hr.

An acquisition session consisted of placing the animals into the activity cages, one to a cage, and scoring the photocell beams interruptions over a 5 min period. At the end of this session, the animals were removed, immediately injected with the test compound or its vehicle, and then replaced in their home cages. Between tests with different animals, the activity cages were cleaned using dry Kleenex® tissues. The retention session, given 7 days after the acquisition session, was the same except that no injections were given; animals were retested in the same cages and in the same order as during the acquisition session. All experiments were performed with two replications with 10 animals in each group at each replication. A decrease in activity between the two sessions (habituation) served as an index of retention.

Drugs

Piracetam was prepared using injectable ampoules of Gabacet® (Carrion, France) diluted where appropriate with physiological saline (0.9% w/v NaCl). Choline chloride was dissolved in physiological saline. For the piracetam + choline combinations both compounds were dissolved in the same solution. All drugs were injected IP in a volume of 10 ml/kg immediately after the acquisition session in order to act on the so-called "memory consolidation process." Doses are expressed in terms of base or salt where appropriate.

Presentation and Statistical Treatment of the Results

Mean activity scores and standard errors for the acquisition sessions (white columns) and the retention sessions (hatched columns) are presented in the figures for each control and treated group. The mean difference scores between Session 1 and Session 2 for each treated group was compared with the same measure for the control group using a Dunnett test (two tailed).

RESULTS

The effects of post-session administration of piracetam alone are shown in Fig. 1A and indicate a statistically significant difference with 2000 mg/kg piracetam. No significant effects were observed with the other doses. This result suggests that habituation was improved by post-session IP administration of 2000 mg/kg piracetam.

The effects of post-session administration of choline chloride alone are presented in Fig. 1B. No statistically significant differences between control and treated groups were observed at any dose investigated (10 to 200 mg/kg). Doses above 200 mg/kg were toxic. This result suggests that post-session administration of choline alone did not affect habituation.

The effects of post-session administration of the piracetam + choline combinations are presented in Fig. 2. Figure 2A shows a statistically significant difference between control and the treated groups which received 50 mg/kg piracetam + 50 mg/kg choline with a similar but non statistically significant tendency ($p=0.07$) at 20 mg/kg of both compounds. Figure 2B shows a statistically significant difference between control and the treated group which received 50 mg/kg piracetam + 50 mg/kg choline replicating results shown in Fig. 2A but no significant effects with other combinations. In particular the statistically significant effect observed with 2000 mg/kg piracetam alone (Fig. 1A) was no longer present when 2000 mg/kg piracetam was combined with 50 mg/kg choline. These results suggest that habituation was enhanced by post-session administration of 50 mg/kg piracetam + 50 mg/kg choline chloride, doses of each drug which were ineffective when injected alone, but by no other combination of these two drugs.

GENERAL DISCUSSION AND CONCLUSIONS

Habituation of exploratory activity in mice has been suggested to be a simple but valid model of memory [8]. Thus the statistically significant increases in habituation observed after a single post-session administration of piracetam alone at 2000 mg/kg or the combination of 50 mg/kg piracetam + 50 mg/kg choline can be interpreted as a facilitation of memory processes. The results obtained with 2000 mg/kg piracetam alone confirm those reported in our previous publication [8]. Bartus and coworkers [1] have also shown a facilitation of memory in aged Fischer rats with treatments (100 mg/kg piracetam + 100 mg/kg choline) administered chronically for 1 week and 30 min prior to the acquisition and retention of a passive avoidance task. Similarly Giurgea *et al.* [6] administered 100 mg/kg piracetam + 100 mg/kg choline for one week before the acquisition of a conditioned paw retraction in young adult rats. In contrast to these two studies, treatments in the present study were administered only once immediately after the acquisition session. The present results suggest therefore that in our conditions the piracetam + choline combination acts on so-called "memory consolidation."

While choline alone was ineffective and piracetam alone improved memory only at the highest dose (2000 mg/kg), the two drugs together induced an improvement of retention at 50 mg/kg, a dose 40 times lower than the effective dose of piracetam alone. Choline at 50 mg/kg did not potentiate the effects of any other dose of piracetam and in particular caused the significant effect observed with 2000 mg/kg piracetam alone to disappear. These results differ somewhat from those reported by Bartus *et al.* [1] and by Giurgea *et al.* [6] who found that rats receiving 100 mg/kg piracetam alone performed slightly better than control, but that rats receiving 100 mg/kg piracetam + 100 mg/kg choline exhibited retention scores several times better than those given piracetam alone.

Bartus *et al.* [1] suggested that piracetam reverses a deficit in hippocampal cholinergic transmission in aging rats. Such a hypothesis would not however account for the present results nor those reported by Giurgea *et al.* [6] because in these two studies young adult animals were used. Our results and those of Giurgea *et al.* [6] together with those of Bartus *et al.* [1] suggest nonetheless that piracetam may act at least in part via a cholinergic process which might depend on the synaptic availability of acetylcholine or its precursor choline. For example Wurtman *et al.* [10] reported that piracetam accelerated acetylcholine release in the hippocampus of young rats. It would seem furthermore that the facilitation we observed depends on a delicate equilibrium because the facilitatory effect only appeared with the combination of 50 mg/kg of each compound, doses which are nevertheless considerably lower than the 2000 mg/kg needed when piracetam was given alone. Regardless of the particular mechanisms responsible for the interaction observed, the fact that piracetam + choline can show synergistic facilitatory effects on learning and memory in animals suggests that the use of such combinations in the clinical treatment of memory disorders merits further detailed investigation.

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