Pharmacology Biochemistry and Behavior, Vol. 41, pp. 445–448, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Behavioral Effects of L-α-Glycerylphosphorylcholine: Influence on Cognitive Mechanisms in the Rat

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Received 8 April 1991

DRAGO, F., F. MAUCERI, L. NARDO, C. VALERIO, N. LAURIA, L. RAMPELLO AND G. GUIDI. *Behavioral effects of L-α-glycerylphosphorylcholine: Influence on cognitive mechanisms in the rat.* PHARMACOL BIOCHEM BEHAV **41**(2) 445–448, 1992.—The phosphorylcholine precursor, L-α-glycerylphosphorylcholine (α-GPC), was injected at the dose of 100 mg/kg/day for 20 days to aged male rats of the Sprague-Dawley strain, 24 months old, showing a deficit of learning and memory capacity. The drug was also administered to rats with amnesia induced pharmacologically with bilateral injections of kainic acid into the nucleus basalis magnocellularis (NBM). Learning and memory capacity of the animals, studied with tests of active and passive avoidance behavior, was improved after treatment with α-GPC in all experimental groups. These results indicate that this drug affects cognitive mechanisms in the rat through an involvement of central neurotransmission.

L-α-Glycerylphosphorylcholine Learning and memory Active avoidance Passive avoidance Central neurotransmission Nucleus basalis magnocellularis

EVIDENCE has been presented that central neurotransmission, particularly acetylcholine (5a,21) and dopamine neurotransmission (3,20), plays an important role in learning and memory processes. These processes appear to be altered in aging (9,14,18) and in other conditions of central neurotransmission disruption (4,15,21). In particular, the loss of memory following specific pharmacological manipulations has been considered as an experimental model of cognitive disturbances in human pathology (16,17).

The use of drugs affecting cognitive processes (cognition enhancers) is now well established for the therapy of memory disturbances, and many of these drugs possess a mechanism of action involving central cholinergic and dopaminergic neurotransmission (8). Among these substances are the choline precursors, which provide the biochemical substrate for the synthesis of acetylcholine (11).

L-α-glycerylphosphorylcholine (α-GPC) is a natural substance that may be present in low amounts in food (23) and serves as a precursor of phosphorylcholine, the active form of choline (13). This drug has been demonstrated to possess cholinomimetic properties and increase the striatal content of the dopamine metabolite, dihydroxyphenylacetic acid (DOPAC), suggesting an interaction with both cholinergic and dopaminergic neurotransmission (19). This prompted us to study the effects of α-GPC on cognitive deficits occurring in various experimental models of central neurotransmission disruption, mainly involving the cholinergic and dopaminergic neuronal systems.

METHOD

Animals

Male rats of Sprague-Dawley strain (purchased from Charles River, Italy), weighing 250 ± 20 g, were used throughout all experiments. A group of 10 male rats of the same strain, weighing 550 ± 20 g, 24 months old, was also used in some experiments. The animals were housed two to three per cage under a constant light-dark cycle (lights on

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between 0800 and 2000) at 21°C. Food and water were available ad lib.

All animals were used only once in the behavioral experiments.

Surgical Manipulations

A group of animals, fixed in a Kopf stereotaxic apparatus after being anesthesized with pentobarbital, received the bilateral implantation of stainless steel cannulas into the nucleus basalis magnocellularis (NBS, 0.8 mm anterior and 3.0 mm lateral of the bregma, 7.2 mm deep).

At the end of behavioral procedures, all animals were killed by decapitation. Localization of cannulas was checked by injecting Evans blue and microscopical inspection of the coloring of brain tissue in formalin-fixed preparations.

Drugs

L-α-glycerylphosphorylcholine (LPB Research Laboratories, Italy) was dissolved in saline and injected IP at doses of 100 mg/kg/day for 20 days, the last injection being made 1 h prior to behavioral testing. Control animals received an injection of placebo with the same procedure.

Kainic acid (Sigma, USA) was dissolved in saline and injected bilaterally into the NBM at the dose of 1 μg/1 μl. Placebo-treated animals served as controls.

Behavioral Tests

Shuttle-box acquisition was studied in a single-session test as described elsewhere (2). Briefly, rats were trained to avoid the unconditioned stimulus (US) of a scrambled electrical foot shock (0.20 mA) delivered through the grid floor. The conditioned stimulus (CS) was a buzzer presented for 3 s prior to the US. If no escape occurred within 20 s of CS/US presentation, the shock was terminated. A maximum of 30 conditioning trials was given with a variable intertrial interval averaging 60 s. The learning criterion was five consecutive conditioned avoidance responses (CAR's). For those animals that reached the criterion in less than 30 trials, the remaining trials until 30 were considered as CAR's. Indexes of avoidance behavior were the total number of CAR's and the number of learners per group.

Passive avoidance behavior was studied in a step-through type of passive avoidance situation (1). Briefly, rats were adapted to the apparatus consisting of a large, dark compartment equipped with a grid floor and a mesh-covered elevated runway attached to the front center of the dark chamber. Adaption training was followed by a single trial in which rats were placed on the elevated platform and allowed to enter the dark box. Three such trials were given on the next day with an intertrial interval of 5 min. After the third trial, rats received a single 2-s unavoidable scrambled foot-shock (0.20 mA) immediately after entering the dark compartment. Retention of the response was tested 24 h after the learning trial. Rats were placed on the elevated runway and the latency to reenter the shock compartment was recorded up to a maximum of 300 s.

Animals were killed by decapitation at the end of behavioral procedures. Data were used only from those animals appearing physically healthy during the tests and that showed no gross abnormalities on postmortem examination. No animals were found unhealthy and were discarded.

All experiments were performed blind to treatment between 900 and 1400.

Experimental design. In the first experiment, the effect of α-GPC on the acquisition of the shuttle-box active avoidance behavior and on the retention of passive avoidance response was studied in aged rats. The same behavioral parameters were studied in animals with bilateral lesions of NBM.

Statistical Analysis

The Dunnett's test for multiple comparisons was used for statistical analysis of data from multiple active groups compared to a control group. Paired comparisons have been made using the Student's *t*-test. The Fisher exact probability test was used for the frequency analysis of data on the percentage of learners. Data of nonparametric systems were analyzed with the Mann–Whitney *U*-test. A level of 0.05 or less was accepted as indicative of significant difference.

RESULTS

Table 1 shows the effects of α-GPC administration on the acquisition of shuttle-box active avoidance behavior of aged rats. The number of CAR's and the percentage of learners were significantly lower in saline-treated rats as compared to those of young animals ($p < 0.01$, Student's *t*-test). Repeated injection in aged rats of 100 mg/kg of α-GPC was followed by an increase in the number of CAR's and in the percentage of learners. Also, the retention of passive avoidance behavior was lower in saline-treated aged rats than in young rats ($p < 0.01$, Mann–Whitney *U*-test). However, retention of this behavior in aged rats was improved relative to control animals when α-GPC was administered at the dose of 100 mg/kg.

Animals bearing a bilateral lesion of NBM also showed a decrease in learning capacity, as indicated by the lower number of CAR's and percentage of learners at the shuttle-box test than those of controls (Table 2). A decrease in the retention of passive avoidance response was also present. However, the treatment with α-GPC was followed by a significant increase in the behavioral parameters concerning the acquisition of active avoidance behavior (CAR's and percentage of learners) and the retention of passive avoidance (latency to reenter the dark box).

TABLE 1

EFFECTS OF α-GPC ADMINISTRATION ON THE ACQUISITION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR AND RETENTION OF PASSIVE AVOIDANCE RESPONSE OF AGED MALE RATS

Experimental groups	CAR's (mean ± SEM)	Learners (%)	Latency in s (median)
Young animals			
Vehicle (8)	16.0 ± 1.2	75.0	74
Old animals			
Vehicle (5)	7.4 ± 0.6	0.0	25
α-GPC 100 mg/kg/day (6)	14.7 ± 0.5*	50.0†	57‡

Treatment with α-GPC was made for 20 days, the last administration being made 1 h prior to behavioral testing. In parentheses is shown the number of animals per each group.

*Significantly different as compared to vehicle-injected old animals ($p < 0.05$, Dunnett's test for multiple comparisons).

†Significantly different as compared to vehicle-injected old animals ($p < 0.05$, Fischer exact probability test).

‡Significantly different as compared to vehicle-injected old animals ($p < 0.05$, Mann–Whitney *U*-test).

TABLE 2

EFFECTS OF α-GPC ADMINISTRATION ON THE ACQUISITION OF SHUTTLE-BOX ACTIVE AVOIDANCE BEHAVIOR AND RETENTION OF PASSIVE AVOIDANCE RESPONSE OF RATS WITH KAINATE-INDUCED BILATERAL LESIONS OF NBM

Experimental groups	CAR's (mean ± SEM)	Learners (%)	Latency in s (median)
Intact animals			
Vehicle (6)	17.5 ± 1.2	75.0	68
Lesioned animals			
Vehicle (6)	8.6 ± 0.5	16.6	12
α-GPC 100 mg/kg/day (6)	12.6 ± 0.5*	50.0†	26‡

Treatment with α-GPC was made for 20 days, the last administration being made 1 h prior to behavioral testing. In parentheses is shown the number of animals per each group.

*Significantly different as compared to vehicle-injected lesioned animals ($p < 0.05$, Dunnett's test for multiple comprisons).

†Significantly different as compared to vehicle-injected lesioned animals ($p < 0.05$, Fischer exact probability test).

‡Significantly different as compared to vehicle-injected lesioned animals ($p < 0.05$, Mann-Whitney U-test).

DISCUSSION

Evidence for an interference of α-GPC with central neurotransmission has been presented with a biochemical approach (19). Here, it has been shown that this drug improves learning and memory disturbances in aged rats and in pharmacologically induced cognitive alterations, and that this effect seems to involve the central neurotransmission.

The primary loss in learning and memory capacity occurring in aged animals and humans has been related to age-related alterations in central neurotransmission, including dopamine neurotransmission (9). Evidence has been presented that central neurotransmission, particularly acetylcholine (5a,21) and dopamine neurotransmission (3,20), plays an important role in learning and memory processes and appears to be altered in aging brain (7,10,12,22). The present data provide an experimental evidence that α-GPC can improve the learning and memory deficits of aged rats. Since α-GPC has been found to serve as choline precursor and to increase acetylcholine synthesis (13), it is possible that the behavioral effect of this drug in aged animals may depend on a facilitation of central cholinergic neurotransmission. In fact, amnesia induced by bilateral lesions of NBM [site of origin of the major cholinergic projections to frontal and temporal cortex, (6)] was corrected in the present experiments by α-GPC. Interestingly, in other experiments, after repeated injection of this drug aged rats showed a reduction in catalepsy induced by the dopamine receptor antagonist, haloperidol (Drago, unpublished observation). These data suggest that α-GPC interferes with dopamine neurotransmission in the brain of aged rats. This concept is supported by the finding that α-GPC increases the striatal content of the dopamine metabolite, DOPAC (19), and it has been shown that memory loss caused by 6-OHDA-induced central dopamine neurotransmission depletion may be corrected by the repeated administration of α-GPC (5b).

Another possible explanation of the present results is that α-GPC may interfere with neuronal metabolic processes. In fact, choline produced by the breakdown of α-GPC may serve as substrate for the synthesis of betaine, phosphorylcholine, and phospholipids (23). These substances are important in maintaining neuronal membrane structure and in the biosynthesis of substances acting as second messengers or neurotransmitters.

The present findings suggest that α-GPC may be useful in further studies on memory deficits because of its strong effect on several aspects of animal performance in operant tasks associated with memory and cognition.

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