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Molecular Mechanisms Mediating the Effects of L-α-Glycerylphosphorylcholine, a New Cognition-Enhancing Drug, on Behavioral and Biochemical Parameters in Young and Aged Rats

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SCHETTINI, G., C. VENTRA, T. FLORIO, M. GRIMALDI, O. MEUCCI, A. SCORZIELLO, A. POSTIGLIONE AND A. MARINO. *Molecular mechanisms mediating the effects of L-α-glycerylphosphorylcholine, a new cognition-enhancing drug, on behavioral and biochemical parameters in young and aged rats.* PHARMACOL BIOCHEM BEHAV **43**(1) 139–151, 1992. – The behavioral effects of the acute and subchronic administration of L-α-glycerylphosphorylcholine (α-GPC) on passive and active avoidance behavioral tasks were investigated. When administered IP after training together with scopolamine 2 h before retest, α-GPC reverses the scopolamine-induced amnesia in the passive avoidance conditioning in young and old rats. Furthermore, the subchronic treatment with α-GPC positively and significantly influences the performance of both young and old animals in the active avoidance test. Moreover, in in vitro/ex vivo experiments α-GPC potentiates receptor-stimulated phosphatidylinositol hydrolysis in cortical synaptoneurosomes derived from young and old animals. In young but not old animals, α-GPC significantly potentiates potassium (40 mM)-stimulated intrasynaptosomal calcium oscillations in purified synaptosomes derived from the hippocampus. These results show that α-GPC improves the performance of animals in both active and passive conditioning tasks. Furthermore, subchronic treatment with the compound enhances in young and restores in aged animals the transduction of the signal, namely, the receptor-mediated production of inositol phosphate and the potassium-induced calcium mobilization. These modifications may represent at least part of the molecular mechanism of action of the compound.

Acetylcholine Choline precursors Scopolamine Passive avoidance Active avoidance Inositol phosphate Adenylate cyclase

DIFFERENT tissues and subcellular components possess a typical phospholipid composition, conferring to membrane-bound proteins the best physical state they require to function. For instance, many enzymatic reactions and transports are influenced by membrane fluidity (16,41), which, in turn, depends upon several factors, including fatty acids composition.

The peroxidative damage of biological membranes increases with age (31,32). Lipid peroxidation increases the rigidity of red blood cells and model membranes (13,35,39,41). Membrane receptors are particularly susceptible to the changes in membrane fluidity. Previous studies have shown that among different membrane receptor systems the B_{max} or functional efficacy is reduced in 70% of them, in 25% does not change and in 5% is increased with aging (1,36,37). This age-related loss of receptor binding and/or coupling efficacy could be ascribed to the impairment of membrane structural properties, such as transbilayer fluidity. Thus, alterations in membrane lipid content or composition as a function of age may modulate receptor number and affinity (10,42) and transduction of the signal via ionotropic and metabolotropic receptors in CNS cell membranes (7).

Within the basal forebrain of the rat, there is a core of cholinergic neurons that has been divided into several regions, which include the medial septum (MS), diagonal band of

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Broca, vertical (VDB) and horizontal limbs, respectively, and nucleus basalis magnocellularis (NBM) (43). MS and VDB send their afferents mainly to the hippocampal formation (27). This cholinergic system contributes to important behavioral functions since its structural and functional integrity has been related to learning and memory abilities (14,22). In Alzheimer's disease, which is characterized by a primary loss of memory function, structural alterations of MS and DB have been related to the reduction of choline acetyltransferase activity in the hippocampus. Changes in cholinergic markers, occurring also during physiological aging, suggest the involvement of cholinergic neurotransmission in the age-associated memory impairment (3).

These two lines of evidence suggest that pharmacological treatments capable of both reconstituting the physiological membrane fluidity and enhancing the basal forebrain cholinergic system might ameliorate cognitive functions. L-α-glycerylphosphorylcholine (α-GPC), the deacylated derivative of phosphatidylcholine, is a naturally occurring substance that is both a precursor of membrane phosphatidylcholine and a choline donor (33). For these reasons, it has been proposed for an integrated pharmacological approach (structural and functional) to senile mental decline.

The aim of our study has been to evaluate the effects of α-GPC on active and passive avoidance behavioral parameters and the possible effects of the drug on both basal and receptor-mediated second messenger systems in young and aged rats.

METHOD

Animals

Male Wistar rats, 4 (200–220 g) and 24–25 (350–400 g) months old, were used for the experiments. Animals were housed, four per cage, in a room with automatic light control (12 h of light, from 7:00 a.m.–7:00 p.m., and 12 h of dark) with constant temperature (22 ± 2°C) and 60% relative humidity at least 1 week before the experiments. Ad lib food and water was allowed. After several days' acclimatization, rats were weighed and randomly assigned to control or treatment groups.

Locomotor Activity

Animals were placed individually in Perspex activity cages (28 × 32 × 42 cm) (Basile Biological Research Apparatus, Comerio, Varese, Italy) connected with an electronic unit impact printer. The cage floor is made up of 30 evenly spaced stainless steel bars (3 mm diameter, 11 mm apart) insulated from each other. The odd bars are earthed. The bridges the animal makes or breaks with its paw link or disconnect one or more of the active bars with the earth, producing random configurations that change as the animal moves. These changes in configurations are converted into pulses by four solid-state resistance detectors. An electronic counter sums the pulses and prints the results at preset intervals. The printed figures are proportional to the spontaneous activity. The current running through the animal's body (1–1.5 μA) is below the threshold of feeling. Groups of five animals underwent 1-h trials 2 h after administration of saline and α-GPC (25, 50, 100, and 200 mg/kg, IP). Both the acute (2 h) and subchronic (one daily injection for 21 days) administration of α-GPC have been tested.

Active Avoidance

Drugs and injection procedure. Young animals were divided into five groups, each composed of 14 animals, and injected (IP) once daily (between 9 a.m. and 10 a.m.) for 21 days with saline or α-GPC 25, 50, 100, and 200 mg/kg, respectively.

Old rats were divided into four groups receiving saline and α-GPC 50, 100, and 200 mg/kg (IP), respectively. Soon after the last trial, animals were killed by decapitation, brains were removed, and the areas of interest dissected (29) for neurochemical evaluation.

Behavioral procedure. Active avoidance behavior was assessed using the automatic reflex conditioner apparatus (Shuttle Box, U. Basile Biological Research Apparatus, Comerio, Varese, Italy), which consists of a programmable recording unit and a rat cage divided into two sections by a partition, with an intercommunicating door at the floor level. The cage is provided with a visual stimulator, which supplies the conditioning stimulus. The reinforcement consists of an electrical stimulus applied to the floor bars of the cage by a static scrambler circuit. The 1-min conditioning program is scheduled as follows: pause 28 s; visual conditioning stimulus 12 s; conditioning stimulus + reinforcement (shock) 20 s. Each animal underwent daily 20-min sessions consisting of 20 1-min trials. A counter was started concurrently with the visual stimulus and stopped when animals went through the door, thus computing the sum of the response latencies (waiting times), expressed as s^{-2}. The number of shocks avoided by each animal during the 20-min sessions was also recorded, indicating the percentage of conditioned avoidance responses (CARs). These experiments were performed between 11 a.m. and 2 p.m. Animals received (IP) injections 1 h before the trial and were tested for 21 days.

Step-Down Passive Avoidance

We used the passive avoidance model described by Cumin et al. (12) with modifications. The test apparatus consists of a Skinner box (25 × 52 × 25 cm) with an electrifiable (Animal Test Cage Grid Floor Shocker, Coulbourn Instruments Inc., Lehigh Valley, PA) grid floor and an insulated platform (15 × 15 × 0.5 cm) in one corner.

Selection of animals. Before training, each rat was placed on the platform; only those animals that stepped down within 2 min (the grid was not electrified at that time) were trained.

Injections and behavioral procedures. During the training session, each rat was placed again on the platform and the latency time to step down was recorded (acquisition trial). As soon as the animal touched the grid floor, it received an unescapable electric foot-shock of 0.8 mA for 5 s. Then, the animal was returned to its home cage. After training, animals were divided into six groups: the first group received *saline IP*; the second received *α-GPC 100 mg/kg IP*; the third was injected with *α-GPC 200 mg/kg IP*; the fourth with *scopolamine hydrobromide (HBr) 1 mg/kg (SC) and saline (IP)*; the fifth with *scopolamine HBr 1 mg/kg SC + α-GPC 100 mg/kg IP*; and the sixth with *scopolamine HBr SC + α-GPC 200 mg/kg IP*.

Two hours later, animals were tested in the same manner as before (retention trial) and the latency time was registered.

The cutoff time adopted was 600 s.

Results are expressed as percent of the latency time.

Adenylate Cyclase Assay

The adenylate cyclase (AC) assay was performed according to the method described by Salomon et al. (38), with modifica-

tions (40). After dissection (29), the brain areas studied (frontal cortex and hippocampus) were weighed and manually homogenized with a glass homogenizer fitted with a Teflon pestle in ice-cold buffer (1 : 20 w/v) containing 10 mM HEPES (pH 7.4 at 4°C), 1 mM dithiotreitol, 1.2 mM EGTA, and 0.32 M sucrose. The homogenate was then centrifuged at 400 × *g* for 5 min. The pellet was discarded and the supernatant centrifuged at 13,000 rpm for 15 min in a Heraeus (Osterode, Germany) microfuge model Biofuge A. The supernatant was discarded and the pellet was resuspended in the same homogenizing buffer without EGTA. The reaction mixture contained 53 mM HEPES (pH 7.4 at 30°C), 0.3 mM EGTA, 1 mM dithiotreitol, 2 mM $MgCl_2$, 1 mM cAMP, 0.5 mM 3-isobutylmethylxantine, 10 μM GTP, 0.1 mM ATP, 5 mM creatine phosphate, 100 U/ml creatine phosphokinase, and 0.5 mM α-[^{32}P]ATP (25–50 cpm/pM) (Amersham Corp., Arlington Heights, IL). The final reaction volume was 100 μl, consisting of 50 μl reaction mixture containing α-[^{32}P]ATP, 25 μl test substances, and 25 μl membrane preparation. The reaction was started by the addition of 25 μl membrane preparation to 75 μl reaction mixture (50 μl) and test substances (25 μl) and was carried out for 10 min at 30°C. The reaction was stopped by the addition of 100 μl of a solution containing 2% sodium dodecyl sulphate, 20 mM ATP, and 6.25 mM cAMP (pH 7.5). [^{3}H]cAMP (15,000 cpm, ICN, San Diego, CA) was added to the stop reaction to monitor recovery from chromatography. Proteins were determined according to the method of Bradford (8) using the reagent purchased by BioRad (Milan, Italy).

Assessment of Inositol Monophosphate Production

Synaptoneurosomal preparation. The particulate preparations were obtained from the cerebral cortex in toto of rats using a modified Krebs-Henseleit buffer (KRB), pH 7.4, containing adenosine deaminase (10 μg/ml). The buffer consisted of 118.5 mM NaCl, 4,7 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.18 mM KH_2PO_4, 24.9 mM $NaHCO_3$, and 10 mM glucose, and was aerated with O_2:CO_2 (95 : 5) to adjust the pH to 7.4. Brains were removed immediately following decapitation and placed on an ice-cooled Petri dish; slices of gray matter were cut manually with a cooled razor blade from the cerebral cortex. The slices (1 g/brain) were then manually homogenized (five strokes) in 7 ml KRB using a glass homogenizer. The homogenate was then diluted with 35 ml KRB. For pressure filtration, the homogenate was passed through a prefilter consisting of three layers of a nylon material (100 mesh) using a Millipore filter holder and then filtered through a Millipore 10-μm filter (LCWP-047) or a 5-μm filter (SMWP-047). Filtered solutions were centrifuged at 100 × *g* for 10 min. The supernatant fractions were decanted and the pellets resuspended in 10 ml KRB (19). Proteins were determined according to the method of Bradford (8) using the reagent purchased by BioRad.

Inositol monophosphate assay. Synaptoneurosomes, prepared as described above, were labeled in 370 μl buffer containing 5 μCi [^{3}H]D-myo-inositol every 1.2 mg proteins. This suspension was gently stirred in a shaking bath for 60 min at 37°C in an atmosphere containing 5% CO_2. After incubation, the suspension was centrifuged at 1,000 × *g* for 10 min and the supernatant discarded; the labeled synaptoneurosomes were resuspended in a fresh buffer containing 10 mM LiCl such that aliquots of 370 μl would contain approximately 1.2 mg proteins. Aliquots were transferred to polypropylene tubes (Falcon, Milan, Italy, 2063) and, after 10 min at room temperature, agents in buffer or buffer alone were added. The tubes were then gassed briefly with O_2 : CO_2 (95 : 5), capped, and placed in a water bath at 37°C. Unless stated otherwise, incubations were carried out for 90 min.

The analysis of [^{3}H]inositol metabolites was performed as described previously (5,19). Briefly, the tubes were centrifuged for 5 min at 1,000 × *g* and the supernatant was discarded. The pellets were washed with 1 ml fresh buffer. To the washed pellet 0.94 ml methanol chloroform (2 : 1) was added. After 10 min at room temperature, 300 μl water, 300 μl buffer, and 300 μl chloroform were sequentially added to each tube. The extraction of water-soluble metabolites was performed by shaking the tubes manually for 30 s. The layers were separated by centrifugation, and 1 ml of the aqueous upper layer was placed on an AG (Richmond, CA) 1-X8 anion exchange column (formate-form, 0.5–0.8 ml). Free [^{3}H]inositol was eluted with 10 ml water. [^{3}H]Inositol phosphates were eluted in scintillation vials with 8 ml of a solution containing 1 M ammonium formate and 100 mM formic acid. Dynagel (7 ml, J. T. Baker, Deventez, Holland) was added to each vial for analysis of radioactivity in a scintillation counter.

Assessment of Free Intracellular Calcium Concentrations

Synaptosomal preparation. Synaptosomes were prepared from rat hippocampus. The hippocampi were rigidly transferred into an ice-cold 0.32 M sucrose solution (pH 7) (see below) and mechanically homogenized by 7–10 strokes at 700 rpm in a Teflon-glass homogenizer (Wheaton, Millville, NJ). All purifications were carried out at 0–4°C. Large fragments were sedimented for 10 min in a Sorvall (Dupont, Wilmington, DE) SS-34 rotor. Synaptosomes were purified according to the protocol of Gray and Whittaker (17) with modifications (48). Synaptosomes were pelleted for 20 min at 9,500 × *g* (p2 fraction) and purified by layering on a gradient of 4 ml 1.2 and 0.8 M sucrose and centrifuged in a swing-out rotor (MSE, Dupont, Wilmington, DE) 43127-111) for 30 min at 10,000 × *g*. Two fractions were collected—the 0.8 M fraction (to be referred to as the "light fraction") and a band floating on the 1.2 M fraction (to be referred to as the "heavy fraction")—and diluted gradually to 10 volumes, over a 30-min period, with a low-calcium artificial cerebrospinal fluid (CSF) containing the following (in mM): NaCl, 132: KCl, 3; $MgSO_4$, 1; NaH_2PO_4, 1.2; D-glucose, 10; HEPES, 10; and variable $CaCl_2$ (0.02–1). Synaptosomes were pelleted for 30 min at 9,500 × *g* and resuspended in 3–4 ml CSF. Proteins were determined according to the method of Bradford (8) using the reagent purchased by BioRad.

Fura-2 measurements. Synaptosomes (2–5 mg/ml protein) were incubated, while gently shaking, with fura-2-AM (1–10 μM) for 20–40 min at 37 or 30°C. Frequently, a control suspension was incubated with only the solvent [dimethyl sulfoxide (DMSO)]. Incubations were stopped by 10-fold dilutions with ice-cold, low-calcium CSF and suspensions were immediately centrifuged for 10 min at 9,500 × *g*. Finally, synaptosomes were resuspended in 4 volumes of CF containing 1 mM Ca^{2+} and kept on ice in the dark until used. Fluorescence measurements were carried out with a Perkin-Elmer (Norwalk, CT) Luminescence Spectrometer (LS 5) in a thermostated cuvette with continuous stirring. Emission of Fura 2 was measured at 510 nm (slit 20 nm) and automatically averaged over 2-s intervals, with excitation at 340 (slit 2.5 nm). Before each experiment, an aliquot (50–100 μg synaptosomal proteins) was centrifuged at 15,000 × *g* for 30 s. The dry pellet was resuspended in 2.5 ml warm CSF containing 1 mM $CaCl_2$ and transferred to the cuvette. Calibration was per-

formed as described by Grynkiewicz et al. (18) for Fura 2 (K_D = 225 nM). R_{max} was taken after the addition of 0.1% (w/v) Triton-X-100. R_{min} was determined with 2–10 mM EGTA in a saturated Tris solution (final concentration, approximately 80 mM), raising pH in the cuvette above 8.2.

Synaptosomes were depolarized by the direct addition of 40 mM KCl in the cuvette.

Pharmacological Agents and Reagents

α-GPC was kindly provided by Sandoz Prodotti Farmaceutici SpA (Milan, Italy). Forskolin and Fura-2 AM were purchased from Calbiochem (La Jolla, CA). All other reagents, unless otherwise specified, were purchased from Sigma Chemical Corp. (St. Louis, MO).

Statistics

AC and inositol phosphate assays were carried out in triplicate and quadruplicate, respectively. Behavioral and biochemical experiments were repeated independently three or four times, respectively. Data are expressed as mean ± SE of each experimental group.

The Kruskal–Wallis test [nonparametric analysis of variance (ANOVA)] for the behavioral responses was used (23). Multiple comparisons were then performed according to Conver (11). Neurochemical data were analyzed by one-way ANOVA followed by the Newman–Kuels test to determine the statistical significance.

A p value less than 0.05 was considered statistically significant.

RESULTS

Locomotor Activity

In young as well as old rats, both acute (2 h) and subchronic (one injection per day for 21 days) administrations of 25, 50, 100, and 200 mg/kg α-GPC IP did not cause any significant change in locomotor activity vs. the control group (young: vehicle 1,236 ± 102, α-GPC 100 mg/kg 1,292 ± 87, α-GPC 200 mg/kg 1,177 ± 91; old: vehicle 969 ± 59, α-GPC 100 mg/kg 921 ± 54, α-GPC 200 mg/kg 934 ± 42; data are expressed as counts detected in 1-h trials).

Active Avoidance Conditioning

α-GPC 25 and 50 mg/kg did not significantly modify the percentage of CARs when compared to the control group (data not shown). Conversely, both young and aged animals treated with 100 mg/kg α-GPC showed a significant improvement of performance (CARs, Figs. 1a and 2a; waiting times, Figs. 1b and 2b) starting from the eighth day of trial (+25% of control value). The effect of 200 mg/kg α-GPC vs. controls was already detectable starting from the fifth day of trial (Figs. 1a and b). In old rats, the performance was worse both in controls and in α-GPC-treated animals (Figs. 2a and 2b) than in young ones. Nevertheless, α-GPC, 100 and 200 mg/kg, was able to induce a significant improvement of behavioral performance.

Passive Avoidance Conditioning

The efficacy of 100- and 200-mg/kg doses of α-GPC in reverting the amnestic effect of the anticholinergic drug scopolamine HBr (1 mg/kg in saline), both in young and aged rats, has been evaluated. Aged animals showed a worsened performance in passive avoidance behavior, as shown by the decrease in the latency times (Fig. 3b). However, the administration of α-GPC 2 h before the test significantly reversed, in a dose-dependent manner, scopolamine-induced memory failure (Figs. 3a and 3b) in aged as well as young rats.

AC Activity

We evaluated the effect of the in vivo treatment with 200 mg/kg α-GPC on AC activity in membranes derived from the frontal cortex of young and aged rats. After 21 days, α-GPC treatment did not show any significant effect on basal, forskolin (1 μM)- and norepinephrine (NE) (1, 10, and 100 mM)-stimulated AC activity (data not shown).

Inositol Phosphate Assay

Both in young and old rats, subchronic treatment for 21 days with α-GPC 100 and 200 mg/kg/day significantly increased the production of inositol phosphate, from cerebral cortex synaptoneurosomes, induced by 100 μM and 1 mM carbamilcholine (Cch) (Fig. 4a and 4b). The dose of 50 mg/kg/day was ineffective in modulating phosphatidylinositol (PI) turnover in young as well as old animals (Figs. 4a and 4b). NE-stimulated production of inositol phosphate was significantly potentiated both in young and old rats after subchronic treatment with α-GPC 100 and 200 mg/kg/day (Figs. 5a, 5b). A significant difference was observed in basal values between young and old rats (Figs. 6a and 6b), basal values in old rats being significantly reduced. Although α-GPC treatment was not able to modify the difference existing between basal values, it caused a significant potentiation of CCh- and NE-stimulated PI hydrolysis both in young and aged animals (Figs. 6a and 6b).

Intrasynaptosomal Calcium Concentration

Subchronic (21 days) treatment with α-GPC 200 mg/kg did not significantly affect basal intrasynaptosomal calcium concentration in young rats when compared with vehicle controls, while determining a statistically significant potentiation of potassium (K+ 40 mM)-induced increase of intrasynaptosomal Ca^{2+} concentration (Fig. 7a). It is noteworthy that intrasynaptosomal calcium concentration in aged animals was almost doubled when compared with young ones (Fig. 7a and 7b) and that α-GPC did not modify intrasynaptosomal Ca^{2+} rise evoked by the potassium-induced depolarization (Fig. 7b).

DISCUSSION

In young rats, α-GPC induced a dose-dependent improvement in active and passive avoidance conditioning, with the highest doses (100 and 200 mg/kg/day, IP) being significantly effective.

It is interesting to observe that, both in young and old rats, α-GPC 200 mg/kg/day significantly reduced the latency of effect of the drug, improving the performance already after the fifth day of trial, compared to the 12 days required to obtain a significant improvement of the active avoidance conditioning in rats treated with 100 mg/kg α-GPC.

Data from passive avoidance experiments match those obtained in the active avoidance conditioning. In agreement with previous reports (26), α-GPC significantly reverts the amnestic effect of scopolamine both in young and old rats. Although the reversal of the scopolamine effect is not direct proof of a cholinergic mechanism of action, since drugs acting on a variety of neurotransmitters (4,22) have been shown to exert the

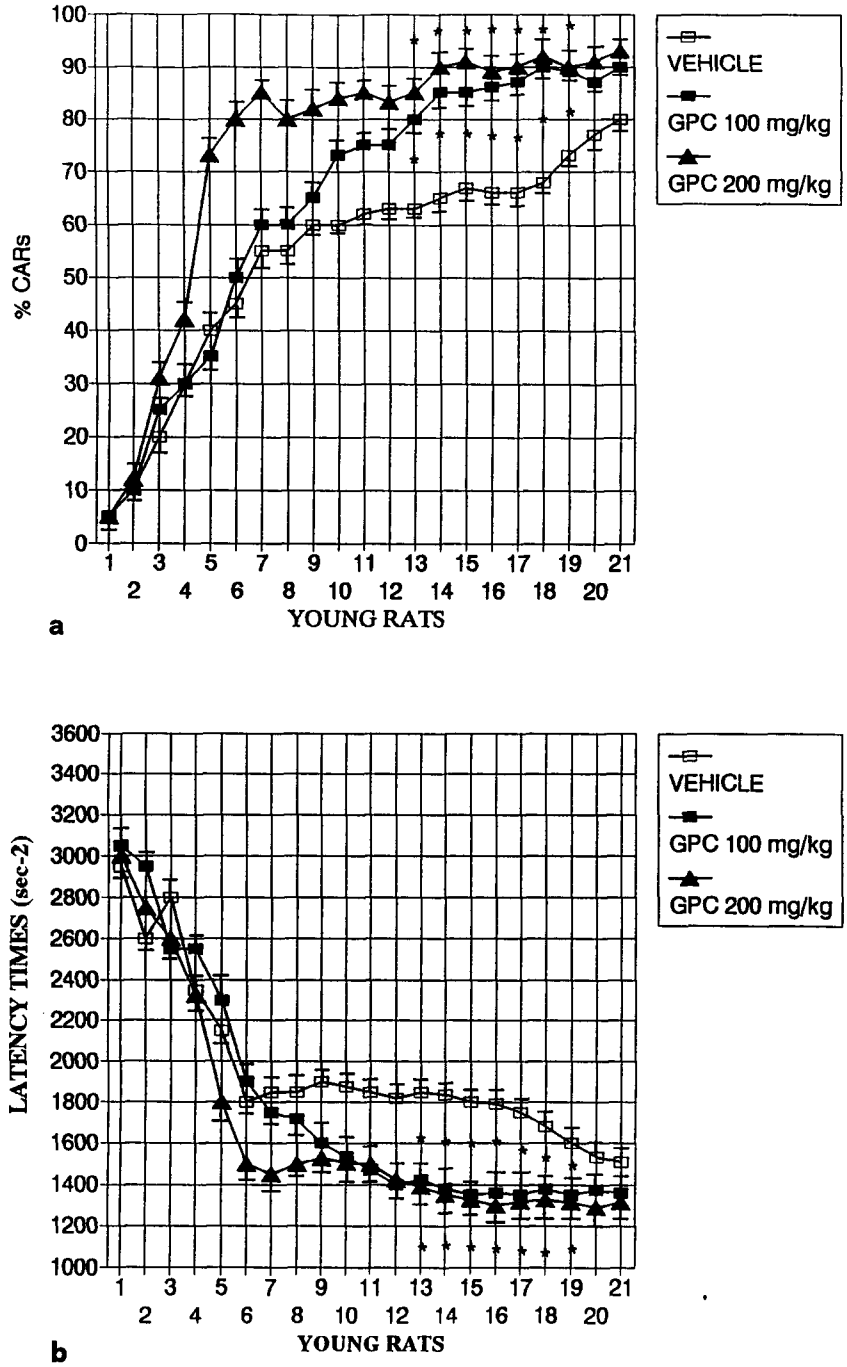


FIG. 1. Effect of subchronic administration (once a day for 21 days) treatment with α-GPC 100 and 200 mg/kg IP in young (3 months) rats at the active avoidance behavioral task. Results are expressed as percent of conditioned avoidance responses (a) and as latency time (s_{-2}) (b). * $p < 0.05$ vs. respective control (vehicle) values; $K(3) < 7.93$.

same effect, the recent observation that α-GPC administration strongly enhances acetylcholine release, both in the hippocampus and in the striatum, as measured by means of in vivo microdialysis (20), along with the reversal by α-GPC of the effect of scopolamine, suggest a cholinergic mechanism of action of the drug in the behavioral tests.

A possible effect of the drug on the locomotor activity could somehow affect the behavioral performance. Thus, we evaluated the effect of both acute and 21 days treatment with α-GPC (50, 100, and 200 mg/kg/day, IP) on the locomotor activity of these animals, being unable to detect any effect of the drug on this parameter both in young and old rats; thus, the behavioral effects of α-GPC reported here are not due to a nonspecific locomotor activation.

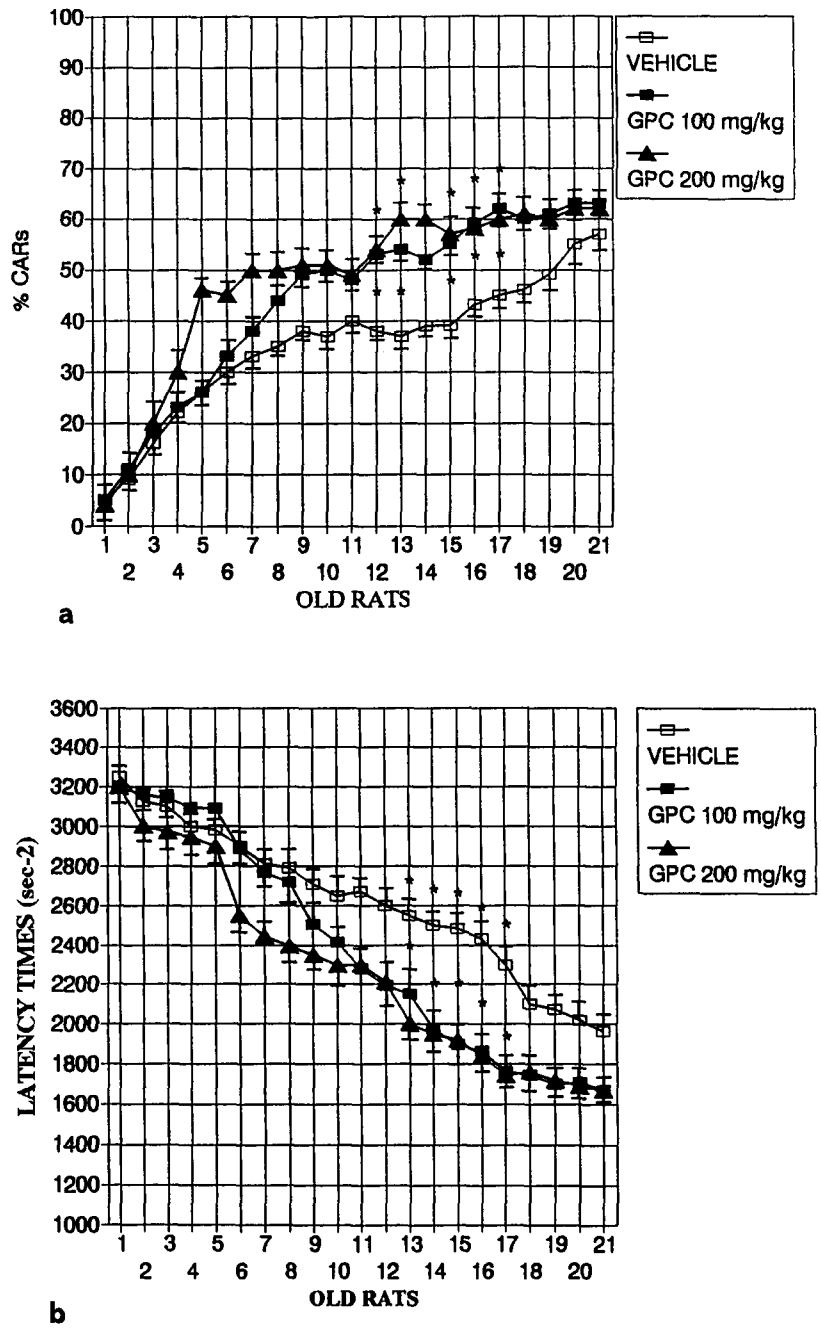
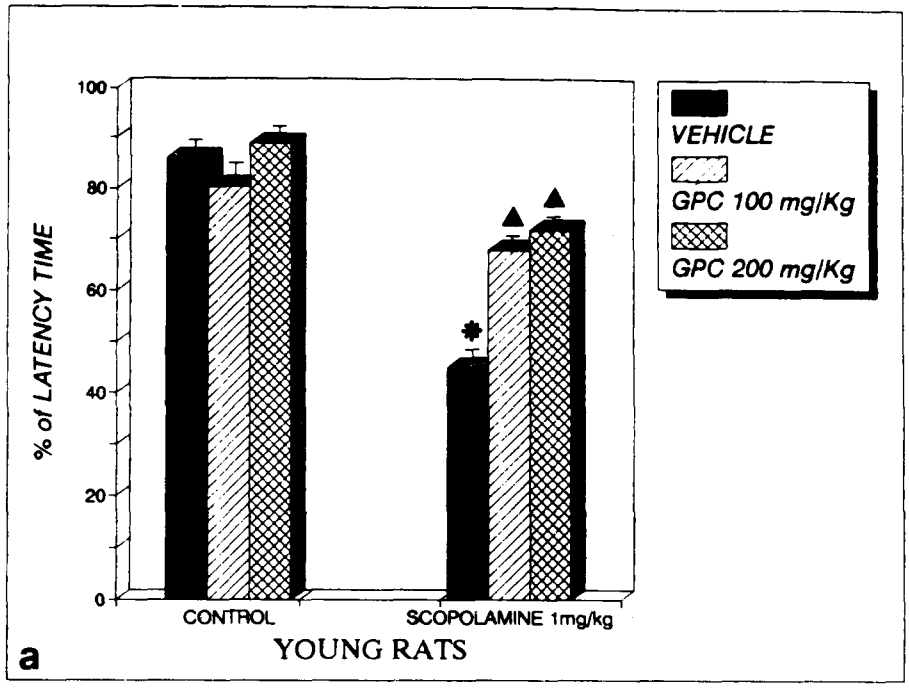


FIG. 2. Effect of subchronic administration (once a day for 21 days) treatment with α-GPC 100 and 200 mg/kg IP on the behavioral performance of old (24 months) rats at the active avoidance task. Results are expressed as percent of conditioned avoidance responses (a) and as latency times (s_{-2}) (b). * $p < 0.05$ vs. respective control (vehicle) values; $K(3) < 7.87$.

Biochemically, we evaluated the modulation, by the subchronic administration of the compound, of the second messenger systems mediating the effects of neurotransmitter-activated receptors. In basal conditions, we observed significant differences between young and aged animals, namely, lowered inositol phosphate production and increased intrasynaptosomal calcium levels with aging, while no change was detectable in AC activity. The effects of aging on PI metabolism have been repeatedly examined (15,46,47), showing a significant decrease in basal, receptor-, and G-protein-stimulated PI turnover.

According to previous reports testing the effect of phosphatidylcholine (24), α-GPC treatment did not modify basal and stimulated adenylate cyclase activity, thus excluding the involvement of second messenger pathways in the mechanism of action of the drug.



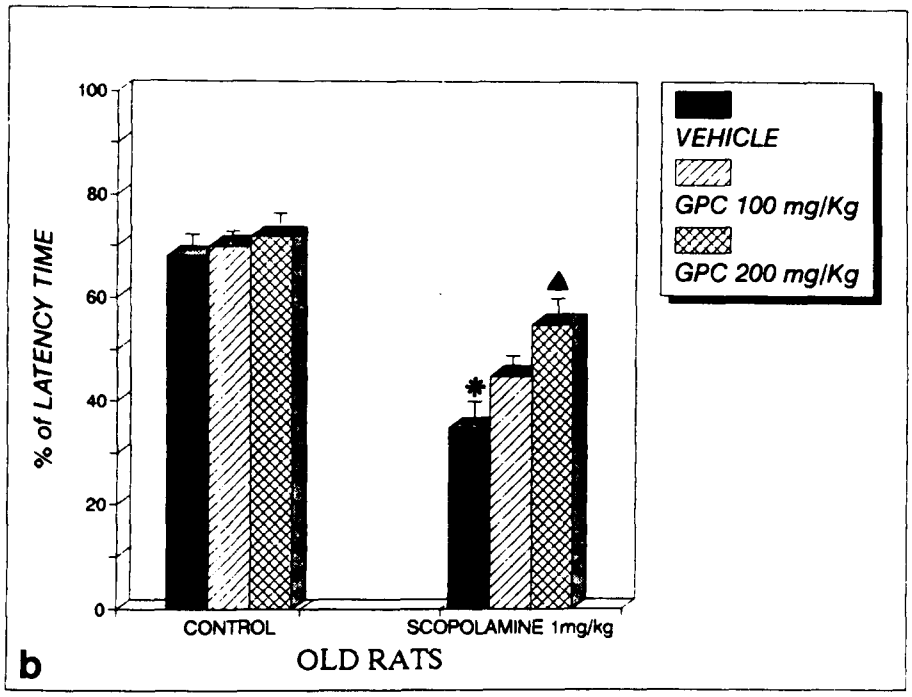


FIG. 3. Effect of acute treatment with α-GPC 100 and 200 mg/kg/day IP, administered soon after the test, on the memorizing effect of scopolamine HBr (SC) administered at the same time, as evaluated by means of the passive avoidance behavioral task, both in young (a) and old (b) rats (3 and 24 months, respectively). Results are expressed as percent of latency times (measured in s). * $p < 0.01$ vs. control (vehicle) values; $K(3) < 5.99$. ▲ $p < 0.05$ vs. scopolamine values; $K(3) < 5.91$.

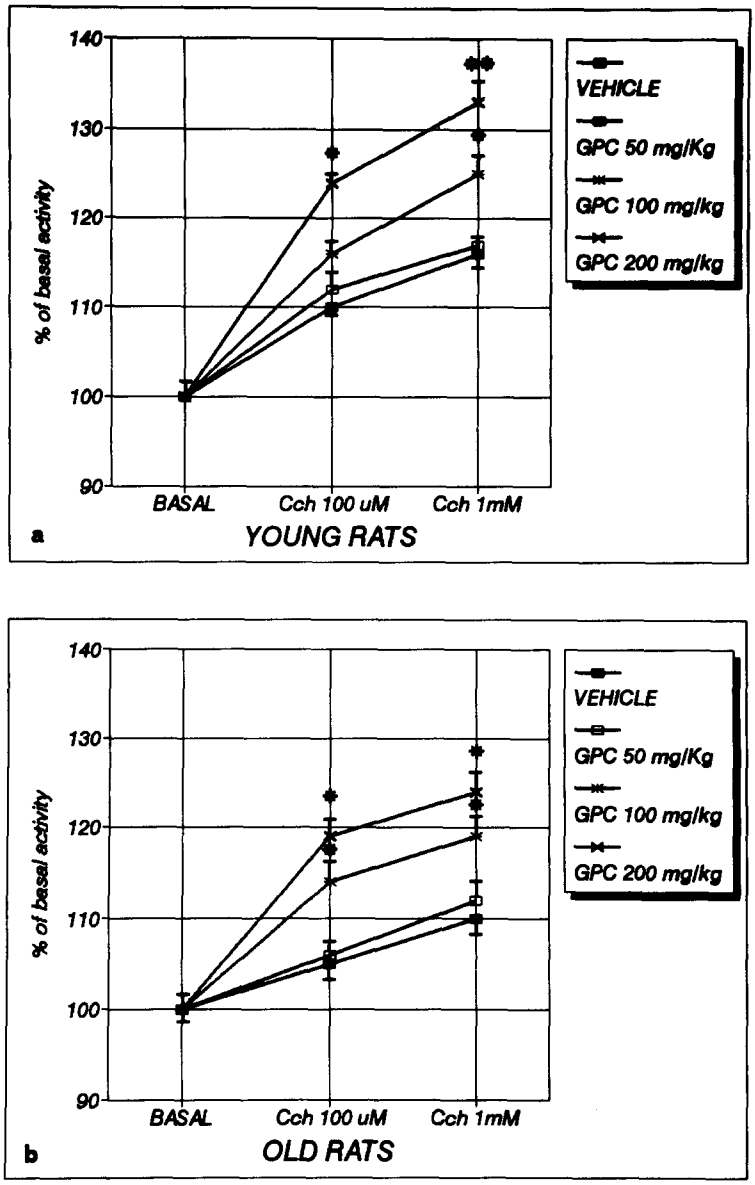
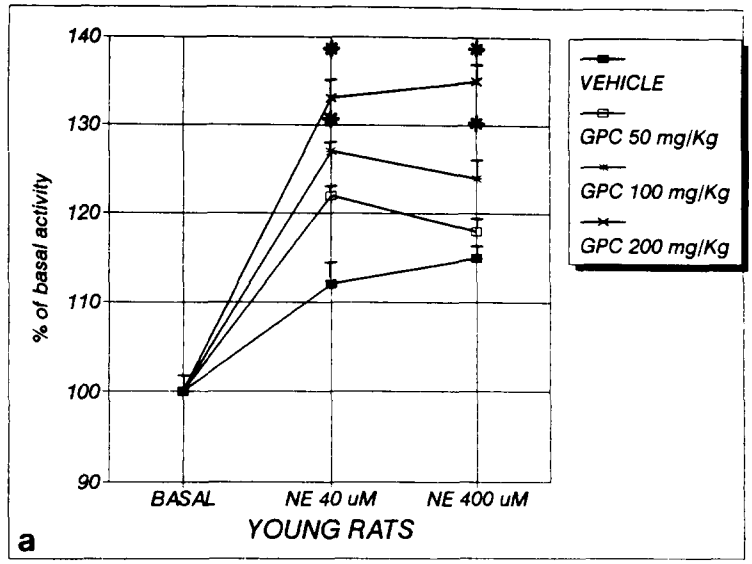


FIG. 4. Effect of the subchronic (once a day for 21 days) administration of α-GPC 50, 100, and 200 mg/kg IP on carbachol (100 μM and 1 mM))-stimulated inositol monophosphate production in young (a) and old (b) rats (3 and 24 months, respectively). Results are expressed as percent of basal activity. $*p < 0.05$ vs. respective control (vehicle) values; F (11, 24) < 2.25. $**p < 0.01$ vs. respective control (vehicle) values; F(11, 24) < 3.17.

The significant changes, induced by α-GPC, of the receptor-mediated PI hydrolysis, both in young and old rats, could be explained, at least partially, by an amelioration of the efficacy of NE and CCh receptor binding (21,25) and of the transmembrane mechanisms coupling these neurotransmitters to phospholipase C. The improvement of basal and receptor-mediated inositol phosphate production could be ascribed to a possible trophic effect of the drug on membrane phospholipids, particularly on phosphatidylcholine, α-GPC being a direct precursor (deacylated) of this phospholipid, or to the possible involvement of α-GPC in the synthesis rate of other primary membrane phosholipids (9).

The striking differences we observed in resting synaptosomal calcium levels between young and aged rats can be due to a change in Ca^{2+} permeability of the cell membrane or to mobilization from intracellular stores. Ca^{2+} buffering can be achieved by calcium binding proteins, calcium entry into mitochondria or into Ca^{2+} storing organelles, or CA^{2+} extrusion from the cell using Ca^{2+} pumps or $Na+/Ca^{2+}$ exchange.

Two main membrane-associated calcium pumps contribute to the homeostasis of cytosolic calcium levels: a) the sodium-calcium exchanger and b) the calcium-magnesium ATPase. The sodium-dependent calcium exchanger requires external sodium since it couples calcium efflux with sodium influx



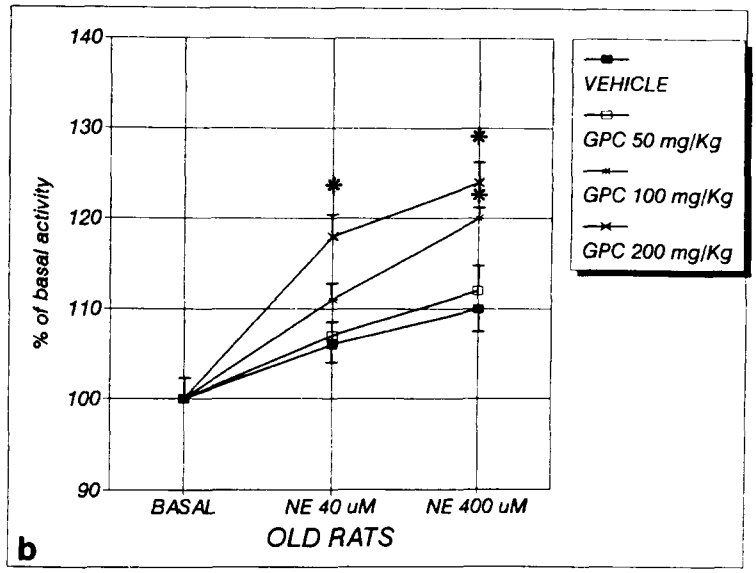


FIG. 5. Effect of the subchronic (once a day for 21 days) administration of α-GPC 50, 100, and 200 mg/kg IP on norepinephrine (40 and 400 μM)-stimulated inositol monophosphate production in young (a) and old (b) rats (3 and 24 months, respectively). Results are expressed as percent of basal inositol monophosphate production. $*p < 0.05$ vs. respective control (vehicle) values; $F(11, 24) < 2.30$.

down the sodium electrochemical gradient (6,34,44). Efflux by the Ca++, Mg++-ATPase requires energy, and Ca++ uptake increases in ATP-depleted synaptosomes (2,16). Acidic phospholipids are necessary for the activation of Ca++-ATPase (28) and polyunsaturated fatty acids decrease with age in mitochondria of old rats, indicative of lipid peroxidation. Concomitantly, ATP pools decrease by 25%, thereby lowering the amount of ATP available for transport activities by energy-dependent ion pumps (2); thus, the higher Ca^{2+} concentration observed in aged rats may be ascribed to a reduced Ca^{2+} extruding ability of the senescent cell. α-GPC potentiates potassium-evoked synaptosomal calcium increases in young rats without showing any effect in synaptosomes derived from old rats. A possible explanation of our results could reside in the ameliorating effects of the drug on membrane fluidity and, likely, on the functioning of voltage-

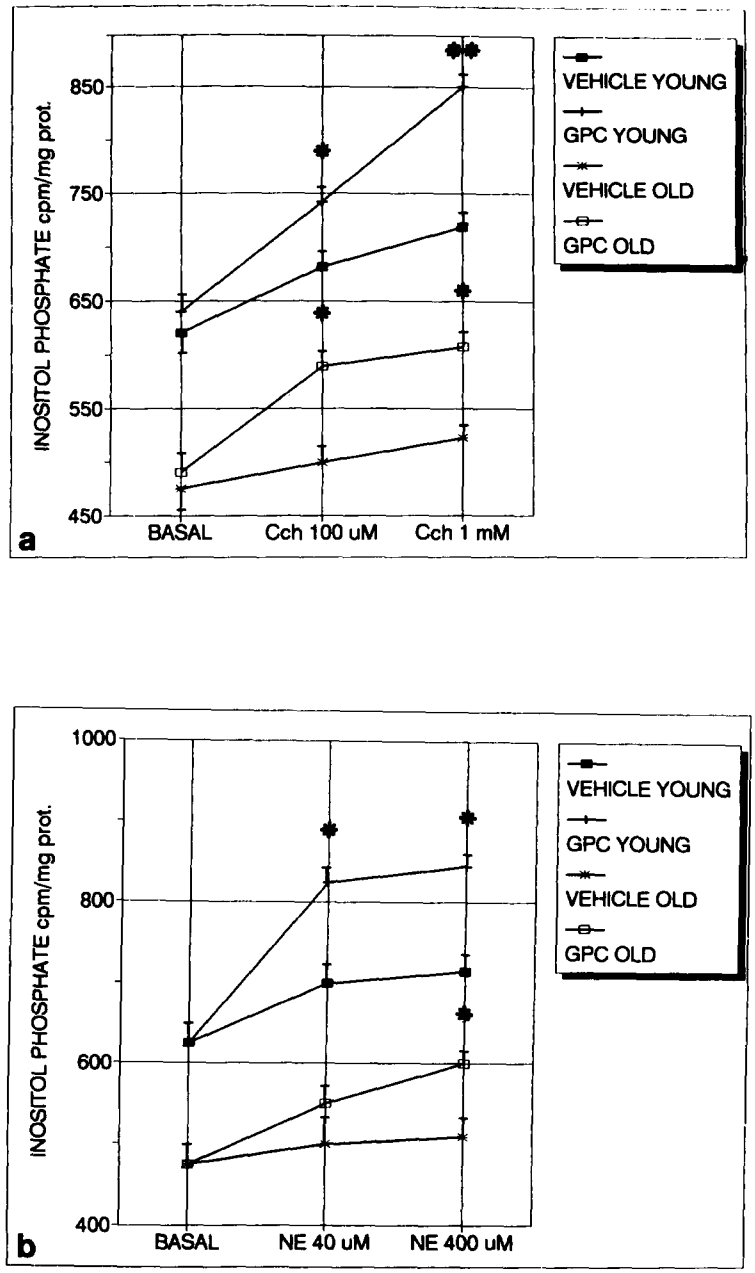


FIG. 6. Effect of the subchronic (once a day for 21 days) administration of α-GPC 200 mg/kg IP on carbachol (100 μM and 1 mM)- (a) and norepinephrine (40 and 400 μM)- (b) stimulated inositol monophosphate production in young and old rats (3 and 24 months, respectively). Results are expressed as cpm of tritiated inositol monophosphate produced per milligram synaptoneurosomal protein added. * $p < 0.05$ vs. respective control (vehicle) values; $F(11, 24) < 2.36$. ** $p < 0.01$ vs. respective control (vehicle) values; $F(11, 24) < 3.23$.

dependent calcium channels (30,45) in young but not old rats; however, this issue needs further clarification.

In conclusion, our results confirm previous reports (26) about the ameliorating effects of α-GPC on the behavioral performance, both in young and aged rats, and suggest that the behavioral effects of this compound, besides an enhancement of cholinergic neurotransmission (20), may be assumed also due to an improvement of the transduction of the signal occurring through the activation of PI hydrolysis and calcium mobilization at nerve terminals.

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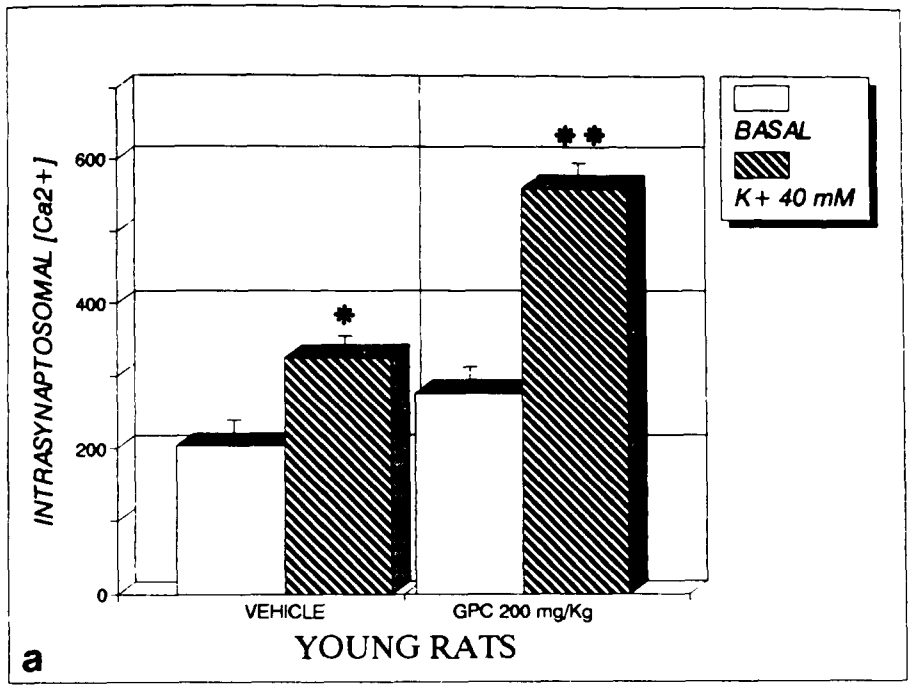

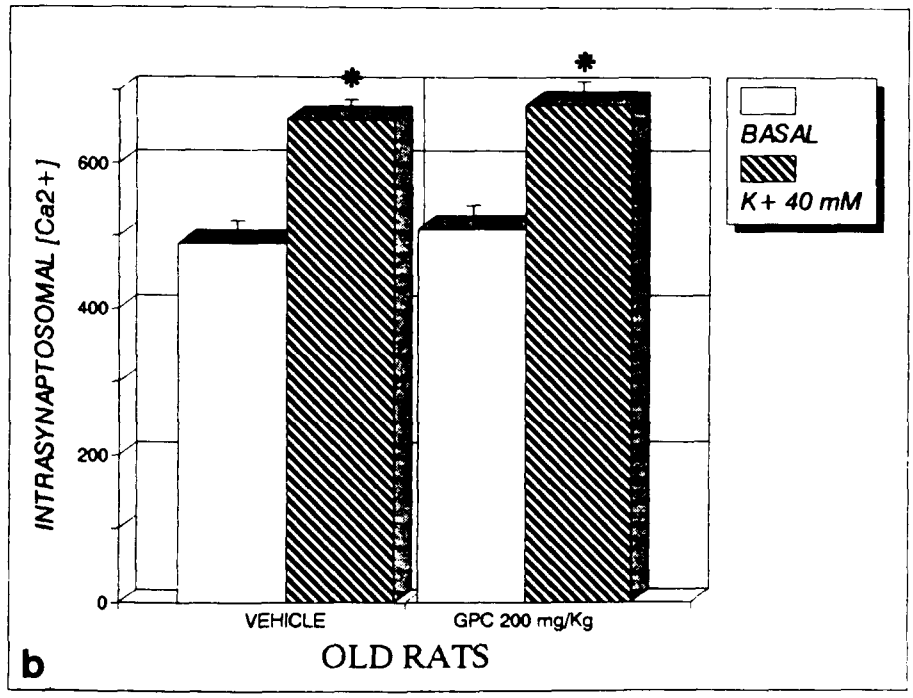

FIG. 7. Effect of the subchronic (once a day for 21 days) administration of α-GPC 50, 100, and 200 mg/kg IP on potassium (40 mM)-stimulated intrasynaptosomal calcium oscillations in young (a) and old (b) rats (3 and 24 months, respectively). Results are expressed as intrasynaptosomal calcium concentrations. $*p < 0.05$ vs. respective control (vehicle) values; $F(11, 24) < 2.31$. $**p < 0.01$ vs. respective control (vehicle) values; $F(11, 24) < 3.24$.

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