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Effect of a New Cognition Enhancer, Alpha-Glycerylphosphorylcholine, on Scopolamine-Induced Amnesia and Brain Acetylcholine

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LOPEZ, C. M., S. GOVONI, F. BATTAINI, S. BERGAMASCHI, A. LONGONI, C. GIARONI AND M. TRABUCCHI. *Effect of a new cognition enhancer, alpha-glycerylphosphorylcholine, on scopolamine-induced amnesia and brain acetylcholine.* PHARMACOL BIOCHEM BEHAV **39**(4) 835–840, 1991.—The present study investigates the effect of the administration of alpha-glycerylphosphorylcholine (alpha-GPC) on scopolamine-induced amnesia and on brain acetylcholine (ACh) levels and release in rats. The results indicate that alpha-GPC, when administered orally, reverses the amnesia caused by scopolamine in passive avoidance. The peak effect is observed using 600 mg/kg IG, 5 h before training. The effect of the drug is long lasting (up 30 h) in accordance with its pharmacokinetic characteristics. Since, alpha-GPC administered IG is cleaved within the gut mucosal cells to glycerophosphate and free choline, it is tempting to speculate that this drug acts by increasing the ACh precursor pool. This view is supported also by the observation that alpha-GPC partially counteracts the decrease of brain ACh levels elicited by scopolamine administration. The effect is observed in the hippocampus and cortex, but not in the striatum. Moreover, in ex vivo experiments, alpha-GPC is able to increase the amount of ACh released by rat hippocampus slices following potassium stimulation.

Rat Acetylcholine Release Choline precursors Scopolamine Passive avoidance

A variety of findings from both animals and humans suggests that the cholinergic system plays an important role in the process of learning and memory. Studies carried out on laboratory animals have shown that the lesion of the cholinergic forebrain nuclei projections produces an impairment of the cognitive functions (24). Thus, in rats, the destruction of the nucleus basalis magnocellularis with ibotenic acid can produce significant changes in the performance of several types of behavioral tasks such as radial maze (16), T maze (15) and active and passive avoidance (12). The administration of centrally active anticholinergic drugs also induces profound alterations of the behavior. Elrod et al. (10) demonstrated that the cholinolytic scopolamine alters the retention time of passive avoidance in rats when administered 30 min before the training, suggesting an action on the acquisition process. Scopolamine can indeed interfere with different stages of memory (acquisition, consolidation, storage and retrieval) (18).

The amnesia caused by the cholinolytic scopolamine and by chemical or electric destruction of forebrain cholinergic nuclei is widely used as a model of cognitive dysfunction (21, 24, 28, 31). Along this line, the effects of muscarinic agonists (14, 26, 28), selective M2 receptor antagonists (29), acetylcholinesterase inhibitors (14,26) and acetylcholine (ACh) precursors (2) have been characterized. The results of several studies indicate that these agents are able to antagonize the amnesic effects produced by scopolamine or by lesions, strengthening the concept of a causal link between cholinergic dysfunction and cognitive impairment (3,30). Clinical observations in Alzheimer's disease, which is characterized by severe cognitive dysfunctions, have demostrated that the degree of cognitive impairment is highly correlated with degeneration or atrophy of the basal forebrain cholinergic projection system (37) which provides major cholinergic afferent inputs to both hippocampus and neocortex (8). Precursors of ACh (11), cholinergic agonists (17), cholinesterase inhibitors (22,36) and the combination of the above (13) have been used in the attempt to ameliorate cognitive dysfunction in this pathology [for a review see (32)].

The purpose of the present study was to investigate the effects of the modulation of cholinergic transmission through the administration of a putative ACh precursor, alpha-glycerylphosphorylcholine (alpha-GPC), studying the reversal of scopolamine-induced amnesia in the passive avoidance conditioned response in rats. Alpha-GPC is a derivative of lecithin that contains in its structure one molecule of choline (Ch). After absorption, alpha-GPC is rapidly metabolized, and the free Ch is capable of crossing the blood brain barrier, reaching the brain

where it may be utilized for phospholipid or ACh synthesis (1). In addition, the correlation between the behavioral effects and neurochemical parameters linked to cholinergic transmission, i.e., ACh levels and release, was investigated.

METHOD

Animals

Adult male Wistar rats (Charles River, Calco, Italy) of 150–250 g of body weight were utilized for all the experiments. The animals were housed in plastic cages in groups of four for at least seven days before the behavioral task; food and water were provided ad lib, room temperature was 22°C, and a 7 a.m. to 8 p.m. light-dark cycle was maintained.

Step Down Passive Avoidance

The step-down apparatus was derived from that described by Kubanis et al. (20) and consisted of a box constructed with Plexiglas (23.5 cm long × 21 cm wide × 26 cm high). An aluminum platform (8 × 11 cm) was attached to one of the end walls approximately 7 cm above the floor of the appartatus. The floor is formed by a grid which is automatically electrified when the rat steps down. The apparatus is reset and activated by a foot pedal.

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

Procedure. During the training, 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 inescapable electric footshock of 0.8 mA, 5 seconds (Animal test cage grid floor shocker, Coulbourn Instruments). The animal was then returned to its home cage. Twenty-four hours after training, the animals were tested in the same manner as before (retention trial), and the latency time was registered. The cut-off time adopted was 600 seconds.

Treatment. The treated animals received alpha-GPC at different doses (100, 300, 600, 1000 mg/kg IG in 1 ml) 5 h before training. A second group received alpha-GPC (600 mg/kg IG) at different times (1, 3, 5, 20, 30, and 48 h) before training. When indicated, scopolamine (0.75 mg/kg SC) was given 30 min before training. Controls received 1 ml of tap water IG and 0.1 ml of saline solution SC.

ACh Levels

ACh levels were measured by HPLC with electrochemical detection according to Damsma et al. (9) with modifications. In brief, a 5 μm pore size cyanopropyl polar phase analytical column (30 mm large × 4.6 mm i.d., Brownlee Labs) was used to separate Ch and ACh. An enzymatic postcolumn reactor containing acetylcholinesterase and choline oxidase (Sigma Chemical, St. Louis, MO) attached to AX-300, 300 A pore size anion exchange column (30 mm large × 4.6 mm i.d.) converted the amines to hydrogen peroxide, which was electrochemically detected by an amperometric detector equipped with a platinum electrode. The electrode potential was set to +500 mV against an Ag/AgCl reference electrode.

The mobile phase consisted of 20 mM phosphate pH 7.2 containing 2 mmol/l of tetramethylammonium chloride (Merck), and was delivered by a pump Merck-Hitachi L-6000 at 0.5 ml/min.

In this condition, the retention time of ACh was 5 min, whereas the retention time of Ch was 3 min.

Preparation of the tissues for ACh levels assay. Immediately after the retest of the passive avoidance response, the rats were killed by fast focused microwave irradiation to the head (20 kW, 245 GHz, 75 W/cm^2, 5 seconds). The skull was opened and cortex, hippocampus and striatum were dissected.

The tissues were homogenized with acetonitrile (Carlo Erba) using a glass teflon homogenizer. The homogenates were centrifuged (4000 × g) at 4°C, 10 min and portions of the supernatant fluid were evaporated under nitrogen.

The dried extracts were dissolved in the mobile phase, filtered utilizing Millipore filters (0.45 μm) and injected for HPLC analysis.

Treatment. Alpha-GPC was administered IG at different doses (300, 600, 1000, 2000 mg/kg) 3.5 hours before sacrifice, and scopolamine (0.75 mg/kg SC) 1.5 hour before sacrifice. Controls received saline solution (0.1 ml) SC and tap water (1 ml) IG.

ACH Release Assays

Treatment and preparation of the slices. The release of endogenous ACh and the release of [^{3}H]-ACh following prelabelling with [^{3}H]-choline were determined. The treated rats received 300 mg/kg alpha-GPC IG and were sacrificed 3 hours after treatment. The control group received saline solution IG.

The rats were killed by decapitation and the hippocampus quickly removed and sliced into 0.4 mm sections using a McIlwain tissue chopper.

Endogenous release. Slices were equilibrated at 37°C for 30 min in 3 ml oxygenated (95% O_2/5% CO_2) Krebs-Ringer Bicarbonate Buffer (KRB) pH 7.4 having the following composition (mM): 120 NaCl, 4.6 KCl, 2.4 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 9.9 glucose, 25 $NaHCO_3$. Following the 30 min equilibration period, slices were resuspended in KRB containing 2 mmol/l neostigmine and incubated for an additional 20 min at 37°C.

After this incubation period, samples were resuspended in KRB containing 30 mM KCl (NaCl was 94 mM to maintain the isotonic solution) and incubated at 37°C for 20 min.

The concentration of ACh in the superfusate was determined by HPLC after filtration using 0.45 μm Millipore filters. Chromatographic conditions were different from those used to measure ACh levels because a greater sensitivity was required. In particular, the HPLC-column used was Nucleosil 5SA (60 mm large × 4.6 mm i.d.), the mobile phase was 100 mM phosphate containing 5 mM TMA pH 8.0, at a flow rate of 0.7 ml/min. In this condition, the retention time of ACh was 8.0 min and the minimum amount of ACh detectable was 500 fmoles.

[^{3}H]-Acetylcholine release. Slices from rat hippocampus were incubated for 30 min at 37°C in 2.0 ml of calcium free Earle Balanced Saline Solution (EBSS) having the following composition (mM): 117 NaCl, 5.3 KCl, 0.4 $MgSO_4$, 0.9 NaH_2PO_4, 26 $NaHCO_3$, 5.5 glucose, containing 0.1 μM [^{3}H]-choline (88.0 Ci/mmol, NEN). Following a washout period of 20 min, the slices were incubated with EBSS containing 10 μM hemicholinium-3 (Sigma Chemical) and 1.8 mM $CaCl_2$ for 10 min at 37°C, and the efflux of radioactivity measured (basal release). The samples were then resuspended in EBSS medium containing 20 mM KCl, 10 min at 37°C and the evoked release was estimated.

At the end of the experiment, the slices were solubilized in 2 N NaOH, and the tritium was determined by liquid scintillation spectrometry. The protein content of the tissue was determined as described by Lowry et al. (25).

Statistical Analysis

Kruskal-Wallis test (nonparametric analysis of variance) for the behavioral responses was used (19). Multiple comparisons

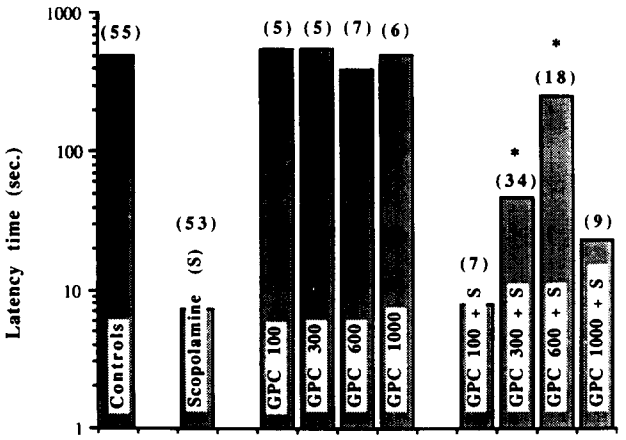

FIG. 1. Effect of pretraining alpha-GPC administration on passive avoidance latency times. Data are expressed as medians. The number of the animals is indicated in parentheses. *$p<0.01$ vs. scopolamine-treated rats; Kruskal-Wallis test statistic (112.62, $p<0.0001$) followed by multiple comparisons.

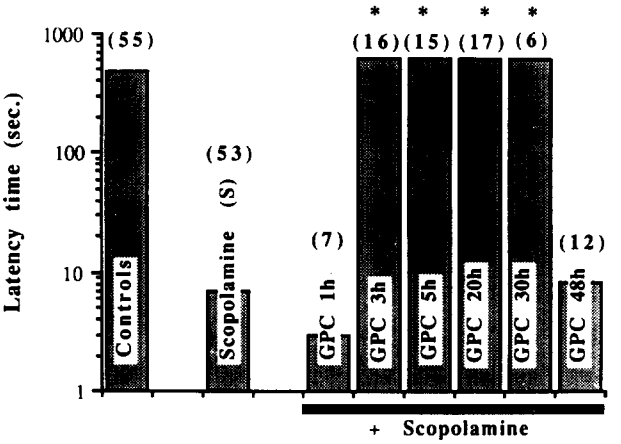

FIG. 2. Time course of the effect of a single dose (600 mg/kg IG) alpha-GPC on passive avoidance latency times. Data are expressed as medians. The number of animals is given in parentheses. *$p<0.01$ vs. scopolamine-treated rats; Kruskal-Wallis test statistic (54.00, $p<0.0001$) followed by multiple comparisons.

where then performed according to Conver (7). Results are reported as medians. Dunnet's t-test was applied to the neurochemical studies and the results are reported as means ± standard deviation.

RESULTS

Passive Avoidance

The results indicate that alpha-GPC is capable of effectively antagonizing the disruption of the passive avoidance conditioned response exerted by scopolamine administered 30 min before acquisition trial.

Rats receiving 300 and 600 mg/kg IG alpha-GPC, 5 hours before training (4.5 hours before scopolamine), performed significantly better in comparison with those treated with scopolamine. The peak effect was obtained with 600 mg/kg IG alpha-GPC. Alpha-GPC at the dose of 300 mg/kg IG also gave significant protection, although latency times were shorter than those of controls. Rats receiving 100 and 1000 mg/kg IG did not differ from those of the group treated only with scopolamine. Alpha-GPC administered alone (at the same doses and times that were used in the combined treatment with scopolamine) did not modify the latency time of the retention trial (Fig. 1). The time course studies (Fig. 2) indicated that the effect of 600 mg/kg IG alpha-GPC was long lasting. A significant reversal of scopolamine-induced amnesia was observed following pretreatment with alpha-GPC as long as 30 hours before training. No effect was observed 1 and 48 hours before training. The latency time of the acquisition trial was similar in all experimental groups.

ACh Levels

As shown in Table 1, scopolamine 0.75 mg/kg administered subcutaneously 1.5 h before sacrifice reduced ACh content by 32%, 26% and 45% respectively in the cerebral cortex, hippocampus, and striatum. Alpha-GPC by itself did not modify brain

TABLE 1

EFFECT OF IN VIVO TREATMENT WITH SCOPOLAMINE AND DIFFERENT DOSES OF ALPHA-GPC ON ACh LEVELS IN RAT CORTEX, HIPPOCAMPUS AND STRIATUM

	ACh Level (pmoles/mg ww)		
Treatment	Cortex	Hippocampus	Striatum
Saline	13.3 ± 2.8 (21)	18.9 ± 2.4 (17)	99.5 ± 11.2 (6)
Scopolamine	9.1 ± 1.7†(29)	14.0 ± 2.1†(15)	55.0 ± 9.9†(5)
Alpha-GPC 300 + Scopolamine	11.9 ± 3.0* (8)	16.9 ± 2.9†(11)	52.3 ± 9.6†(6)
Alpha-GPC 600 + Scopolamine	10.1 ± 2.3†(29)	17.9 ± 2.2*(21)	54.0 ± 11.1†(5)
Alpha-GPC 1000 + Scopolamine	10.7 ± 2.7†(12)	16.8 ± 2.1†(12)	ND
Alpha-GPC 2000 + Scopolamine	7.4 ± 0.2† (5)	11.2 ± 2.2† (5)	ND

Alpha-GPC was given orally 3.5 hours before sacrifice and scopolamine at dose of 0.75 mg/kg SC 1.5 hour before sacrifice.

*$p<0.05$ in comparison with scopolamine treated.

†$p<0.005$ in comparison with saline treated.

Two-tailed Dunnett's t-test. Number of cases is indicated in parentheses.

TABLE 2

EFFECT OF ALPHA-GPC IN VIVO TREATMENT ON THE KCl-STIMULATED RELEASE OF ACh FROM RAT HIPPOCAMPUS SLICES

(A) Endogenous Release

	Evoked Release (pmoles/mg prot/h)	%
Saline	241 ± 71	—
Alpha-GPC*	597 ± 136†	+147

(B) Efflux of [^{3}H]-ACh

	Evoked Release (% of stimulus)
Saline	105 ± 56
Alpha-GPC*	159 ± 28†

*Alpha-GPC was administered at the dose of 300 mg/kg 3 h before sacrifice.

Values are means ± SD of (A) 2 experiments with 5 to 6 replicates for each condition and (B) 3 experiments with triplicate samples. KCl was 30 mM (A) and 20 mM (B).

In (B) the % of stimulus was calculated in respect with the basal release (5 mM KCl) which was equivalent to 3.93 ± 0.94% of the radioactivity present in the slice.

†$p<0.05$.

acetylcholine concentrations at any of the doses tested (300, 600, 1000, 2000 mg/kg) (data not shown).

The effect of the combined treatment of alpha-GPC + scopolamine was complex and different according to the dose and area investigated. Alpha-GPC at the dose of 300 mg/kg IG partially, but significantly, prevented the decrease of ACh levels induced by scopolamine in the cortex, but not in the hippocampus nor in the striatum. At 600 mg/kg, the effect of alpha-GPC against scopolamine was significant in the hippocampus, but not in the cortex nor in the striatum. At 1000 and 2000 mg/kg, alpha-GPC did not affect the scopolamine-induced decrease of ACh concentration neither in the cortex nor in the hippocampus.

ACh Release

Table 2A reports the KCl (30 mM) stimulated release of endogenous ACh from hippocampal slices prepared from animals treated in vivo with alpha-GPC (300 mg/kg IG, 3 h).

In the presence of neostigmine, the K^+ evoked endogenous ACh release from the slices prepared from alpha-GPC-treated rats was 2.5 times that of the control group.

The effect ot alpha-GPC (300 mg/kg IG, 3 h) on the evoked [^{3}H]-ACh release is shown in Table 2B. Alpha-GPC increased the KCl (20 mM) evoked [^{3}H]-ACh release by 51% above control values. Preliminary data suggest that the effect is observed also with 600 mg/kg, and that the release is still increased 5 h following alpha-GPC administration, while is back to control values after 8 h (data not shown).

DISCUSSION

The results indicate that alpha-GPC, a putative precursor of ACh, when administered orally, reverses the amnesic effect caused by scopolamine in passive avoidance, supporting the concept that the effect of this muscarinic receptor blocker can be antagonized by agents suggested to boost cholinergic transmission. In fact, alpha-GPC administered IG is cleaved within the gut mucosal cell by action of glycerylphosphoryl-choline diesterase (L-3-glyceryl-phosphorylcholine glycero-phosphohydrolase, EC 3.1.4.2) (38) to glycerylphosphate and free choline which enters the portal circulation and can reach brain tissues, increasing ACh precursor pool.

Time course studies indicate that alpha-GPC has a long lasting effect, since the reversal of scopolamine-induced amnesia still persists 30 hours after administration. These results are in agreement with pharmacokinetic data. Indeed, the concentration of alpha-GPC in the brain increases slowly and attains maximum levels 8 hours after administration and remains constant over 30 hours (1).

A bell-shaped dose effect relationship was obtained in passive avoidance experiments, as it has been observed in the case of other cognition-enhancing agents (29, 33, 35). The reason for the loss of response when increasing the dose still eludes explanation. It is possible that the higher levels of the drug lead to excess acetylcholine synthesis, triggering homeostatic mechanisms such as receptor desensitization or activation of presynaptic inhibitory receptors. On the other hand, it should be stressed that the bell-shaped dose response curve to cognition-enhancing agents is described with drugs of different chemical structure and mechanism of action.

Based on these results, we attempted to investigate the biochemical correlation between behavioral effects and ACh levels after treatment with scopolamine. Scopolamine significantly reduced ACh levels in different brain areas as found by others (34,35). This effect may be due to excess acetylcholine release caused by the removal of an inhibitory presinaptic muscarinic control on ACh release, or to a long loop feed-back mechanism directed to overcome receptor blockade.

The maximum decrease of ACh levels induced by scopolamine is between 45 and 90 min after administration. Therefore, ACh content measurements were carried 90 min after scopolamine administration. This time schedule is somewhat different from that used in the behavioral experiments so far described, and was purposely adopted to better detect changes in ACh levels. On the other hand, alpha-GPC also in this treatment protocol was able to improve the performance of the rats in passive avoidance (retention time test 30 min after acquisition trial, data not shown).

At the neurochemical level, alpha-GPC partially prevented the scopolamine-induced decrease in ACh levels in the cortex and hippocampus, but not in striatum. Interestingly, the effect in the hippocampus was observed with 600 mg/kg, which is the dose giving the best protection against scopolamine amnesia in passive avoidance. In addition, higher doses which are behaviorally inactive were also ineffective on ACh content. The lack of effect in the striatum is in line with the observations made by Spignoli and Pepeu (35) on the effect of the combined treatment of scopolamine and oxiracetam.

When a dynamic parameter of cholinergic function (namely the release of ACh from slices of hippocampus of rats treated with alpha-GPC) was studied, a significant increase in the release of this neurotransmitter was observed with two independent methods evaluating the release of [^{3}H]-ACh following preloading with [^{3}H]-choline and the release of endogenous ACh.

Although the correlation between ACh levels and the behavioral response is tempting, it should be stressed that the effect of alpha-GPC in the cortex and hippocampus was indeed weak, and did not occur simultaneously in both areas for all the behaviorally active doses. It should be stressed that the action on ACh levels and release is observed in a narrow time window, in contrast to the long lasting effect on behavior. It is tempting to speculate that the effect on acetylcholine may be related to cortical activation (23), but not sufficient to explain the antagonism of scopolamine-induced amnesia. Accordingly, other mechanisms

may contribute to mediate the effect of alpha-GPC on passive avoidance, possibly involving the metabolism of phospholipids (of which alpha-GPC can be considered a precursor) and of phospholipid-regulated events.

Along this line, it would be interesting to investigate the effect of cognition enhancers on general behavior and on brain neurotransmitters in other conditions associated with decreased memory performance, such as aging or ethanol exposure. In fact, these conditions are characterized by severe derangement of several neurochemical parameters, including those such as the calcium channels (6,27) and the calcium phospholipid dependent protein kinase (4,5), which are linked to memory trace formation.

REFERENCES

1. Abbiati, G.; Fossati, T.; Arrigoni, M.; Rolle, P.; Dognini, G. L.; Castiglioni, C. High-performance liquid chromatographic assay of L-alpha-glycerophosphorylcholine using a two-step enzymatic conversion. J. Chromatogr. Biomed. Applic., in press; 1991.
2. Bartus, R. T.; Dean, R. L.; Goas, J. A.; Lippa, A. S. Age-related changes in passive avoidance retention: modulation with dietary choline. Science 209:301–303; 1980.
3. Bartus, R. T.; Dean, R. L., III; Beer, B.; Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. Science 217:408–417; 1982.
4. Battaini, F.; Del Vesco, R.; Govoni, S.; Trabucchi, M. Chronic alcohol intake modifies phorbol ester binding in selected rat brain areas. Alcohol 6:169– 172; 1989.
5. Battaini, F.; Del Vesco, R.; Govoni, S.; Trabucchi, M. Regulation of phorbol ester binding and protein kinase C activity in aged rat brain. Neurobiol. Aging 11:563–566; 1990.
6. Bergamaschi, S.; Govoni, S.; Rius, R. A.; Trabucchi, M. Acute ethanol and acetaldehyde administration produce similar effects on L-type calcium channels in rat brain. Alcohol 5:337–340; 1988.
7. Conver, W. J. Practical non parametric statistics. New York: John Wiley; 1980:229–233.
8. Coyle, J. T.; Price, D. L.; DeLong, M. R. Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
9. Damsma, G.; Westerink, B. H. C.; Horn, A. S. A simple, sensitive and economic assay for choline and acetylcholine using HPLC, an enzyme reactor, and an electrochemical detector. J. Neurochem. 45:1649–1652; 1985.
10. Elrod, K.; Buccafusco, J. J. An evaluation of the mechanism of scopolamine-induced impairment in two passive avoidance protocols. Pharmacol. Biochem. Behav. 29:15–21;1988.
11. Etienne, P.; Gauthier, S.; Johnson, G.; Collier, B.; Mendis, T.; Dastoor, D.; Cole, M.; Muller, H. F. Clinical effects of choline in Alzheimer's disease. Lancet 1:508–509; 1978.
12. Flicker, C.; Dean, R. L.; Watkins, D. L.; Fisher, S. K.; Bartus, R. T. Behavioral and neurochemical effects following neurotoxic lesions of a major cholinergic input to the cerebral cortex in the rat. Pharmacol. Biochem. Behav. 18:973–981; 1983.
13. Gauthier, S.; Bouchard, R.; Lamontagne, A.; Bailey, P.; Bergman, H.; Ratner, J.; Tesfaye, Y.; Saint-Martin, M.; Bacher, Y; Carrier, L.; Charbonneau, R.; Clarfield, M.; Collier, B.; Dastoor, D.; Gauthier, L.; Germain, M.; Kissel, C.; Krieger, M.; Kushnir, S.; Masson, H.; Morin, J.; Vasavan, N.; Neirinck, L.; Suissa, S. Tetrahydroaminoacridine-lecithin combination treatment in patients with intermediate-stage Alzheimer's disease. N. Engl. J. Med. 322: 1272–1276; 1990.
14. Haroutunian, V.; Barnes, E.; Davis, K. L. Cholinergic modulation of memory in rats. Psychopharmacology (Berlin) 87:266–271; 1985.
15. Hepler, D.; Olton, D.; Wenk, G.; Coyle, J. Lesions in nucleus basalis magnocellularis and medial septal area of rats produce qualitatively similar memory impairments. J. Neurosci. 5:866–873; 1984.
16. Hodges, H.; Ribeiro, A. M.; Gray, J. A.; Marchbanks, R. M. Low dose tetrahydroaminoacridine (THA) improves cognitive function but does not affect brain acetylcholine in rats. Pharmacol. Biochem. Behav. 36:291–298; 1990.
17. Hollander, E.; Davidson, M; Mohs, R. C.; Horvath, T. B.; Davis, B. M.; Zemishlany, Z; Davis, K. L. RS 86 in the treatment of Alzheimer's disease: cognitive and biological effects. Biol. Psychiatry 22:1067–1078; 1987.
18. Izquierdo, J. Mechanisms of action of scopolamine as an amnestic. Trends Pharmacol. Sci. 10:175–177; 1989.
19. Kruskal, W. H.; Wallis, W. A. Use of ranks in one criterion variance analysis. J. Am. Stat. Assoc. 47:583–621; 1952.
20. Kubanis, P.; Zornetzer, S. F.; Freund, G. Memory and postsynaptic cholinergic receptors in aging mice. Pharmacol. Biochem. Behav. 17:313–322; 1982.
21. Kudo, Y.; Shiosaka, S.; Matsuda, M.; Tohyama, M. An attempt to cause the selective loss of the cholinergic neurons in the basal forebrain of the rat: A new animal model of Alzheimer's disease. Neurosci. Lett. 102:125–130; 1989.
22. Kumazr, V. Efficacy and side effects of THA in Alzheimer's disease patients. In: Giacobini, E.; Becker, R., eds. Current research in Alzheimer therapy. New York: Taylor and Francis; 1988:225–229.
23. Lacomba, C.; Cagiano, R.; Cortese, I.; Renna, G.; Tortorella, C.; Valerio, J.; Cuomo, V. Effects of alpha-glycerylphosphorylcholine (alpha-GPC) and phosphatidyl-choline (PC) on the electrocorticogram (ECoG) in the rat. Pharmacol. Res. 22(Suppl. 2):256; 1990.
24. LoConte, G.; Bartolini, L.; Casamenti, F.; Marconcini-Pepeu, I.; Pepeu, G. Lesions of cholinergic forebrain nuclei: Changes in avoidance behavior and scopolamine actions. Pharmacol. Biochem. Behav. 17:933–937; 1982.
25. Lowry, O. H.; Rosebrough, N. J.; Farr, L.; Randall, R. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
26. Miyamoto, M.; Narumi, S.; Nagaoka, A.; Coyle, J. T. Effects of continuous infusions of cholinergic drugs on memory impairment in rats with basal forebrain lesions. J. Pharmacol. Exp. Ther. 248:825–835; 1989.
27. Moresco, R. M.; Govoni, S.; Battaini, F.; Trivulzio, S.; Trabucchi, M. Omega-conotoxin binding decreases in aged rat brain. Neurobiol. Aging 11:433–436; 1990.
28. Nakahara, N.; Fujise, N.; Kawanishi, G.; Mizobe, F. Central muscarinic activities of an M1-selective agonist: preferential effect on reversal of amnesia. Brain Res. 507:172–175; 1990.
29. Packard, M. G.; Regenold, W.; Quirion, R.; White, N. M. Post-training injection of the acetylcholine M2 receptor antagonist AF-DX 116 improves memory. Brain Res. 524:72–76; 1990.
30. Perry, E. K. The cholinergic hypothesis—ten years on. Br. Med. Bull. 42:63–69; 1986.
31. Piercey, M. F.; Vogelsang, G. D.; Franklin, S. R.; Tang, A. H. Reversal of scopolamine-induced amnesia and alterations in energy metabolism by the nootropic piracetam: Implications regarding identification of brain structures involved in consolidation of memory traces. Brain Res. 424:1–9; 1987.
32. Pomponi, M.; Giacobini, E.; Brufani, M. Present state and future development of the therapy of Alzheimer disease. Ageing 2:125–153; 1990.
33. Shnindler, U.; Rush, D. K.; Fielding, S. Nootropic drugs: Animal models for studying effects on cognition. Drug Dev. Res. 4:567–576; 1984.
34. Spignoli, G.; Pepeu, G. Oxiracetam prevents electroshock-induced decrease in brain acetylcholine and amnesia. Eur. J. Pharmacol. 126:253–257; 1986.
35. Spignoli, G.; Pepeu, G. Interactions between oxiracetam, aniracetam and scopolamine on behavior and brain acetylcholine. Pharmacol. Biochem. Behav. 27:491–495; 1987.
36. Summers, W. K.; Majovski. L. V.; Marsh, G. M.; Tachiki, K.; Kling, A. Oral tetrahydroaminoacridine in long term treatment of senile dementia, Alzheimer type. N. Engl. J. Med. 315:1241–1245; 1986.

37. Whitehouse, P. J.; Price, D. L.; Struble, R. G.; Clark, A. W., Coyle, J. T., DeLong, M. R. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. Science 215:1237–1239; 1982.
38. Zeisel, S. H. Dietary choline: Biochemistry, physiology and pharmacology. Annu. Rev. Nutr. 95–121; 1981.