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ORIGINAL INVESTIGATION

Effects of dimethylaminoethanol pyroglutamate (DMAE p-Glu) against memory deficits induced by scopolamine: evidence from preclinical and clinical studies

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Abstract
Rationale Dimethylaminoethanol pyroglutamate (DMAE p-Glu) is a compound resulting from the reaction between dimethylaminoethanol (an indirect precursor of acetylcholine) and pyroglutamic acid (a cyclic derivative of glutamic acid having procholinergic properties and promnesic effects in both animals and man).
Objectives The present study undertook preclinical and clinical evaluations to test a potential therapeutic utility for DMAE p-Glu in cognitive impairments related to central cholinergic deficit.
Materials and methods In preclinical study, DMAE p-Glu was studied in rats by intracerebral microdialysis in conscious freely moving animals, on performance of rats in the Morris water maze test of spatial memory, and on the deficit in passive avoidance behavior induced by scopolamine. The clinical study examined the effect of DMAE p-Glu on cognitive deficits induced by an intravenous injection of scopolamine in healthy young male subjects.
Results In rat experiments, DMAE p-Glu increased the extracellular levels of choline and acetylcholine in the medial prefrontal cortex, as assessed by intracerebral microdialysis, improved performance in a test of spatial memory, and reduced scopolamine-induced memory deficit in passive avoidance behavior. Clinical study results show that scopolamine induced a memory deficit and that DMAE p-Glu produced a significant positive effect on scores in the Buschke test, as well as a slight but significant difference on choice reaction time.
Conclusion These results indicate that DMAE p-Glu reduces the deleterious effect of scopolamine on long-term memory in healthy volunteers and suggest that DMAE p-Glu might be effective in reducing memory deficits in patients with cognitive impairment.

Keywords Dimethylaminoethanol pyroglutamate · Scopolamine · Cognitive tests · Human volunteers · Rat · Microdialysis

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Introduction

The cholinergic basis of memory dysfunction is well established. Acetylcholine plays an important role in learning and memory process (Drachman 1977; Coyle et al. 1983). In disorders in which a cognitive decline is seen,

such as Alzheimer's disease, the link between the reduction in cholinergic function and the cognitive decline has been established (Perry et al. 1978; Terry and Buccafusco 2003). Abnormalities in cognitive processes may be due to dysfunction in both nicotinic and muscarinic receptor systems (Erskine et al. 2004; Ellis et al. 2006). Recently, abnormalities in the cholinergic system have been documented in schizophrenia, another disease with cognitive disturbances, with cortical reductions in nicotinic receptors not related to tobacco use (Breese et al. 2000).

Scopolamine, an antagonist of muscarinic acetylcholine receptor subtypes (Huang et al. 2001), has been shown in pharmacological studies with normal subjects to produce impairments in learning and memory (Glick and Zimmerberg 1972; Rusted and Warburton 1988; Wesnes et al. 1988; Potter et al. 2000; Edginton and Rusted 2003) that mimic certain aspects of cognitive impairment in aging and dementia (Drachman and Leavitt 1974; Wesnes 2001). The scopolamine challenge has, thus, been widely used for identifying the potential of drug candidates to reverse the effects of cholinergic blockade (Hall et al. 1990; Molchan et al. 1990; Wesnes et al. 1991; Mauri et al. 1994; Riedel et al. 1995; Martinez et al. 1997; Gilles and Luthringer 2007).

Dimethylaminoethanol pyroglutamate (DMAE p-Glu) is a compound resulting from the reaction between DMAE and p-Glu. DMAE is a tertiary amine naturally present in the human brain and is considered to be a precursor of choline and, hence, acetylcholine. DMAE has been shown to have positive effects on memory in rodents that appear to result from its effects on cholinergic transmission (Pepeu et al. 1960; Flood et al. 1983). p-Glu is a cyclic derivative of glutamic acid that is present in free form in the mammalian brain and which has procholinergic properties (Antonelli et al. 1984) and promnesic effects in both animals and man (Drago et al. 1987). The combined pharmacological properties of DMAE and p-Glu would suggest a potential therapeutic utility of DMAE p-Glu in cognitive impairments related to central cholinergic deficit. The aim of the present study was to undertake both preclinical and clinical investigations to test this hypothesis.

DMAE p-Glu was first studied in preclinical experiments, to measure its effect on extracellular levels of acetylcholine and choline in the medial prefrontal cortex (mPFC) of rats, as assessed by intracerebral microdialysis in conscious freely moving animals. The mPFC was chosen as a region of interest since, in the rat, it is considered to be involved in arousal, vigilance and motivational responses, and interconnections with the hippocampus may underly a role in cognition and memory (Kolb and Tees 1990). Specific roles of neocortical acetylcholine itself have been attributed in attention, cognitive flexibility, and spatial memory (Sarter and Bruno 1997, 2000; Winkler et al. 1995) to modulate the general efficacy of the cortical processing of sensory or associational information, to mediate the ability to detect and select stimuli and associations for extended processing, and to allocate the appropriate processing resources to these functions (Sarter and Bruno 1997).

Subsequently, the effect of DMAE p-Glu was examined on performance of rats in the Morris water maze test of spatial memory (Morris 1981; Chopin et al. 2002) and on the deficit in passive avoidance behavior induced by scopolamine. These tests were considered to be valid for evaluating a promnesic potential of DMAE-pGlu, since they have been shown (e.g., Chopin et al. 2002) to be sensitive to systemic administration of cholinesterase inhibitors that are clinically effective in treating some of the memory deficits in Alzheimer's disease.

The promnesic potential of DMAE p-Glu was then examined in man. A double-blind, crossover, placebo-controlled clinical study assessed the effect of DMAE p-Glu, administered in repeated doses of 1,500 mg during 7 days, to reduce the deficits in attention and memory induced by intravenous administration of scopolamine (0.5 mg) in 24 healthy young male subjects.

Materials and methods

Preclinical studies

Animals Male Sprague–Dawley rats (Ico:OFA-SD (IOPS. Caw), IFFA CREDO, Domaine des Oncins, France; 200–250 g at the time of delivery) were housed and tested in an Association for the Assessment and Accreditation of Laboratory Animal Care-accredited facility, in strict compliance with all applicable regulations, and the protocol was carried out in compliance with French regulations and with local Ethical Committee guidelines for animal research.

DMAE p-Glu drug solutions A stock solution of DMAE p-Glu (40.0 g per 100 ml of 24.4% ethanol/water *v:v*) was obtained from PFM Production-PROGIPHARM (Gien, France). The stock solution was diluted in distilled water to obtain a working solution of 12.8 g DMAE p-Glu per 100 ml. At the injection, volume of 10 ml/kg body weight used in all of the animal experiments, and this solution provided a dose of 1,280 mg/kg. Lower doses of DMAE p-Glu were obtained by serial dilution in 7.8% ethanol/water, which subsequently served as the vehicle treatment and which corresponded to a dose of 0.62 g ethanol per kilogram body weight.

Intracerebral microdialysis in conscious rats Intracerebral microdialysis in conscious freely moving rats was

performed using methods previously described (Tellez et al. 1999). Commercially available microdialysis probes were surgically implanted into the mPFC at the following stereotaxic coordinates: 3.0 mm anterior to bregma, 1.5 mm lateral to the midline suture, and 4.5 mm ventral from the dura, at a medially directed vertical angle of 14°. Twenty-four hours after surgery, the probes were perfused with Ringer's solution (mM: NaCl, 147; KCl, 4.0; $CaCl_2$, 1.3) containing 0.5 μM neostigmine (cholinesterase inhibitor). Three 20-min dialysate samples were collected for measurement of baseline levels of acetylcholine and choline. Immediately thereafter, DMAE p-Glu (10–1,280 mg/kg) or vehicle was administered by oral gavage, and 20-min dialysate samples were collected continuously for the next 4 h. At the end of the experiment, brains were fixed in 4% paraformaldehyde, sectioned and examined to verify the location of the probe placements. Acetylcholine and choline levels were quantified in dialysate samples by high-performance liquid chromatography (HPLC) with electrochemical detection (Damsma et al. 1987). Levels were expressed as a percentage of "baseline," defined as the average amounts of acetylcholine and choline measured in the three dialysate samples preceding the oral administration of DMAE p-Glu or vehicle. Levels were also expressed as the percentage baseline values averaged over the entire 4-h posttreatment sampling period, which is analogous to and directly proportional to an "area under the curve" measurement. For the comparison of drug and vehicle treatment effects, analyses of variance (ANOVAs) followed by Newman–Keuls test or Mann–Whitney *U* test were used (Friedman 1991). Differences were considered significant when values of *P* were less than 0.05.

Scopolamine-induced deficit in passive avoidance behavior of rats This procedure has been described previously (Chopin and Briley 1992; Chopin et al. 2002). The apparatus consisted of a white brightly lit (750 lux) compartment connected by an opening to a black unlit compartment with a metal grid floor. Initially, each rat was placed in the bright compartment and allowed 30 s to freely explore that side of the apparatus. The entrance to the dark compartment was then opened; as soon as the rat had entered with all four paws on the grid, the door was closed, and an inescapable scrambled footshock (0.8 mA for 2 s) was delivered through the grid floor by a Coulbourn shock generator. Immediately afterwards, the rat was returned to its home cage. Forty-eight hours later, the rat was again placed in the brightly lit compartment; after 30 s, the door was opened, and the delay in entering the dark compartment (step-through latency) was recorded to a maximum of 180 s. DMAE-pGlu was given 35 min before the initial trial to study its effect on the deficit induced by an intraperitoneal administration of 2.5 mg/kg of scopolamine hydrobromide (Fluka) given 30 min before the initial trial. Results are expressed as the mean ± standard error of the mean (SEM) and were compared using a Kruskal–Wallis nonparametric one-way ANOVA corrected for ties followed by a two-tailed Mann–Whitney *U* test (Friedman 1991).

Morris water maze test of spatial memory in rats The water maze test was adapted from Morris (1981), and the procedure has been described previously (Chopin et al. 2002). Rats are placed into a circular pool containing water and are required to find an escape platform just beneath the surface of the water and thus, invisible to the animal. This test appears to measure spatial memory, which is highly conditional on the presence of extramaze cues (Morris 1981). Performance is dependent on the number of training days. In a probe trial without platform, 24 h after three or four training days, rats spend significantly more than 25% of their time (chance performance) in the quadrant that contained the platform during the training sessions, indicating that they have learned the location of the platform in that quadrant. However, after only one or two training days, equal time is spent in all the four quadrants of the pool, suggesting that rats have not learned the platform location. Since, after two training days, the spatial memory performance of control animals is not significant, and a potential promnesic effect of a compound can be detected. The test apparatus consisted of a circular fiberglass pool (130 cm in diameter, 50 cm deep) filled to a height of 30 cm with water at room temperature and divided into four virtual quadrants (Q1, Q2, Q3, and Q4) of equal surface area. A transparent escape platform made of Plexiglas was placed in a fixed location in the tank, 1 cm below the water surface, and not visible to the animals at water level (Gage et al. 1984). Many extramaze cues were placed around the pool for the rats to use in locating the escape platform. On the training trials, the platform remained in a constant location in the center of one quadrant (Q4). Each rat received three training trials per day for 2 days, which involved placing the rat into the pool facing the wall at one of the three quadrants Q1, Q2, and Q3. A different starting point was randomly used on each trial. The rats were allowed to swim freely until they found the escape platform. The latency to find the hidden platform was recorded and used as a measure of acquisition of the task. If a rat failed to locate the platform within 100 s, it was then manually guided to the escape platform by the experimenter. The intertrial interval was 20 s during which the rat remained on the platform. Twenty hours after the last training trial, the platform was removed from the pool, the rats were allowed to swim for 60 s in the pool, and the time spent in the target quadrant Q4 (the quadrant in which the platform was during training) was recorded. The percent time spent in the previous training quadrant Q4 was used as an index of memory. Rats were injected intraperitoneally (i.p.) once daily for 14 days with vehicle or DMAE p-Glu (160, 320 or

640 mg/kg). On the 13th and the 14th days, animals were treated immediately after the last of the three training sessions. Rats were tested in a probe trial, without platform, on the 15th day (without drug treatment). Results were expressed as the mean ± SEM of performance (latency to find the platform and percentage time spent in target quadrant) and were analyzed by one-way ANOVA, with drug treatment as the factor, followed by a two-tailed Students *t* test (Friedman 1991). In the probe trial, without platform, results were also compared to chance performance (25%) by a one-sample Student's *t* test (Friedman 1991).

Clinical study

Subjects Twenty-four healthy young male volunteers, aged 20–28 years (mean ± standard deviation (SD) : 22.7±2.0), 64.0–88.6 kg weight (mean ± SD : 73.7±6.1), 168–186 cm height (mean ± SD : 179.8±4.9), and with a body mass index between 20.1 and 25.9 (mean ± SD : 22.8±1.6) participated in the study. All subjects were screened with a complete history, medical examination (weight, height, measurement of intraocular pressure, vital signs, and electrocardiogram) and biology screening (hematology, biochemistry, urinalysis, screening tests for drugs of abuse, and hepatitis B, hepatitis C, and HIV serologies). Individuals with personal history of psychiatric illness, substance abuse, medical illness, in particular ophthalmic disorder, and abnormal biology were excluded. Women were excluded from the study due to the influence of gender on C_{max} of scopolamine after intravenous infusion (Ebert et al. 2000). Subjects were not matched on predrug performance measures, but all the subjects carried out a training for the battery of tests at the selection visit in order to familiarize them with the battery of psychometric tests and to obtain a performance plateau. Subjects were medication free at the beginning of the protocol. The protocol was approved by the local Ethical Committee, declared to the French Health Care Authorities, performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, and all subjects signed an informed consent form prior their inclusion in the study.

Drugs DMAE p-Glu (1,500 mg) and placebo were administered orally with solution obtained by extemporaneous dilution of powder in 150 ml of fresh water. The dose of 1,500 mg of DMAE p-Glu was extrapolated from the data obtained in preclinical studies in rats. Moreover, the pharmacokinetic profile of DMAE p-Glu at this dose was investigated in a previous study, showing a mean C_{max} of 1.44 μg/mL, T_{max} of 36 min, and $T_{1/2}$ of 73 min (unpublished internal reports, Pierre Fabre Medicament). Scopolamine (0.5 mg) was administered intravenously (infusion during 15 min, scopolamine vials for intravenous infusion Scopolamine Cooper 0.5 mg/2 ml). A previous study has shown that intravenous infusion is the only appropriate route of administration to achieve a high enough serum concentration in all volunteers (Ebert et al. 2001).

Study design This was a monocentric, double-blind, randomized, placebo-controlled, crossover study, divided in two periods of 7 days. From day 1 to day 6 of each period, subjects received oral repeated doses of 1,500 mg of DMAE p-Glu or placebo, once a day in the morning 1 h before the breakfast, according to the randomization. Included subjects were instructed to come to the clinical unit at day 1 in the morning for the first administration. They were kept under medical supervision for 2 h; after which time, the subjects left the clinical unit with the investigator's agreement and were instructed to take the study drug at home once a day in the morning for 5 days (from day 2 to day 6). On the evening of day 6, subjects were hospitalized in the clinical unit. On the morning of day 7, after completion of the baseline battery of tests, scopolamine was administered intravenously. One hour after the beginning of the scopolamine infusion, subjects were administered either DMAE p-Glu (1,500 mg) or placebo according to the crossover design. Neuropsychological tests were performed at T1.5 h, T3 h, and T6 h after the beginning of the infusion of scopolamine (T0). The two periods were separated by a washout period of at least 2 weeks.

Cognitive and psychomotor tests Cognitive and psychomotor tests included the Buschke Selective Reminding Test, the digit symbol substitution test (DSST), the visual analog scale "Bond & Lader," and the choice reaction time (CRT). Tests were performed on day 7 of each treatment period, at T0 and at T1.5 h, T3 h, and T6 h after the beginning of the infusion. A training session was included in the selection visit in order to familiarize the subjects with the battery of psychometric tests and to obtain a performance plateau.

Buschke Selective Reminding Test (Buschke 1973) This test has demonstrated the effect of scopolamine on verbal memory and learning in many psychopharmacological studies (Molchan et al. 1990; Patat et al. 1991; Martinez et al. 1997). This task analyzes several components of memory and learning in verbal free recall. Subjects were listening a list of 12 words without logical connection. They had to remember all the words. During eight trials, subjects had to recall all of the words and after each recall, they were listening only words not recalled on the immediately preceding trial. That procedure permits an analysis of long-term storage (LTS), retrieval from long-term storage (LTR), and recall from short-term storage.

Nine different lists of 12 words were used. The volunteers had never the same list to learn. The LTS represents the number of words learned in the long-term memory by a cumulative sum of words that have been at least reminded without recall. The LTR score is the number of words that have been recovered from the LTS at a trial (one to eight). The consistent long-term retrieval (CLTR) is the sum of words definitively learned in the long-term memory, i.e., words having been recovered from the LTS for a trial and having ever been recovered at the following trials. NALL corresponds to the number of the trial (one to eight) when all the words were recovered for the first time (the first of two following trials with all words). This test was chosen as the primary assessment because of its known sensitivity to the effects of scopolamine (Caine et al. 1981; Molchan et al. 1990).

Digit symbol substitution test This test is a faster assessment that permits the evaluation of attention, working memory, and skilled coordination that are impaired by scopolamine (Mintzer and Griffiths 2003; Mauri et al. 1994). Volunteers had a set time (90 s) in which to copy as many different nonsense symbols as possible into boxes (substitution) according to a code on the top of the sheet (pencil and paper tasks). The main parameter is the number of correct signs copied over 90 s.

Visual analog scale "Bond & Lader" (Bond and Lader 1974) This is a self-subjective scale used in pharmacodynamic assessment to evaluate changes in vigilance and mood (McCann et al. 1999; Vernikos-Danellis et al. 1977). Cognitive functioning and more specifically, memory, can be modified by attention and vigilance. Sixteen lines representing 16 aspects of mood were defined by terms at the two extremities. Three mood factors were used as dependant variables: alertness, contentedness, and calmness. These three subscales ranged from zero to 100, with high values indicating a deterioration of each mood component (alertness, contentedness, and calmness). Each subject was to draw a vertical line on the point of the horizontal axis that best reflected his feelings at the time of testing. The use of behavior scale is reported in several studies with scopolamine (Patat et al. 1991; Mintzer and Griffiths 2003).

Choice reaction time The CRT is a test sensitive to the effects of psychotropic drugs and particularly, for detecting a deficit in vigilance. The CRT task is used as an indicator of sensorimotor performance, assessing the ability to attend and respond to a critical stimulus. Subjects were required to extinguish one of six equidistant red lights, illuminated at random, by pressing the associated response button as quickly as possible. The mean reaction time of 48 trials was recorded in milliseconds for three components: recognition, motor, and total reaction time. Recognition reaction time (RRT) is the time it takes for the subject to notice the light, i.e., the time between stimulus onset and the subject lifting his finger from the start button. Motor reaction time (MRT) indexes the movement component of this task and is the time between the subjects lifting his finger from the start button and touching the response button. Total reaction time (TRT) is the sum of RRT and MRT.

Statistical analysis The main variables in the test of Buschke were the retrieval from LTR score, CLTR, and NALL. The primary analysis was the comparison of changes of LTR, CLTR, and NALL scores from T0 to T1.5 h between the two treatment groups using ANOVA with treatment group, sequence and period as fixed effects. The secondary analysis was the comparison of changes from T0 of scores obtained at T3 h and at T6 h using ANOVA with treatment, sequence and period as fixed effects. All efficacy criteria were analyzed using repeated measures analysis of variance (T0, T1.5 h, T3 h, and T6 h) with treatment group, sequence and period, and the interactions, followed, if necessary, by tests of effect sizes.

Results

Preclinical studies

Intracerebral microdialysis in conscious rats In microdialysis experiments performed in conscious freely moving rats, the oral administration of DMAE p-Glu (10–1,280 mg/kg) resulted in dose-dependent changes in dialyzed levels of acetylcholine and choline in the mPFC in comparison to vehicle treatment (Fig. 1a, b). The 4-h posttreatment average level of acetylcholine was significantly reduced by 20% at the dose of 10 mg/kg, was unchanged by the doses of 40 and 160 mg/kg, and was increased significantly (+68% and +91%, respectively) by the doses of 640 and 1,280 mg/kg. Levels of choline in the same animals were increased by all tested doses of drug, in an orderly dose-related manner. Significant increases in choline levels first occurred within 40 min after drug administration and persisted for at least 220 min at doses greater than or equal to 40 mg/kg. Average increases in choline were maximal and comparable at the doses of 640 and 1,280 mg/kg (+412% and +459%, respectively, in comparison to the vehicle treatment).

Scopolamine-induced deficit in passive avoidance behavior of rats Previous studies in our laboratory (see Chopin and Briley 1992; Chopin et al. 2002) have shown that scopolamine (2.5 mg/kg i.p.), administered 30 min before

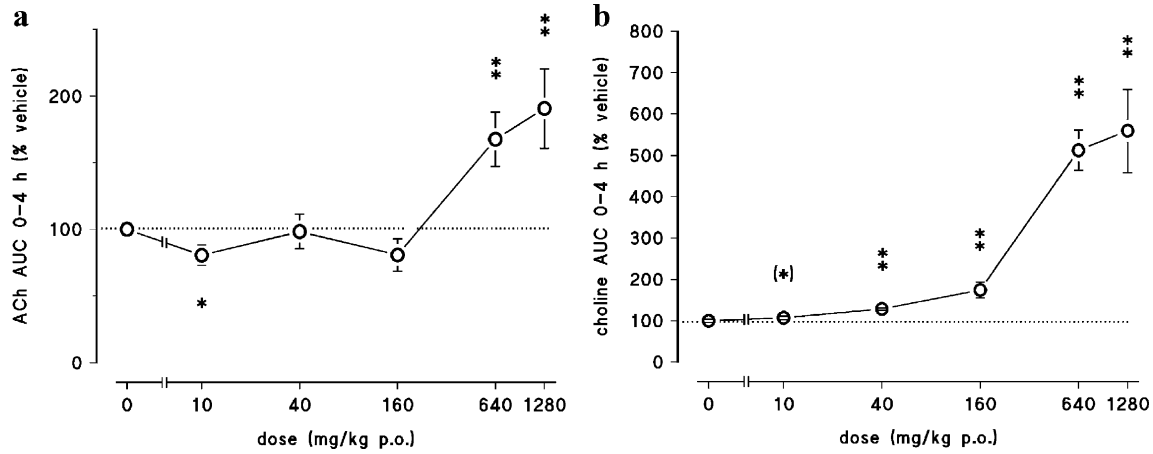

Fig. 1 Dose-response effect of DMAE p-Glu (10–1,280 mg/kg orally) on extracellular levels of acetylcholine (*ACh*; **a**) and choline (**b**) in the medial prefrontal cortex of conscious freely moving rats, as measured by intracerebral microdialysis. Data points represent the average change in levels (± SEM) over the 4- posttreatment sampling period (*area under the curve measurement*) expressed as a percentage of vehicle controls. Mann–Whitney *U* test, n=5–6 rats per treatment group. *P<0.1; *P<0.05; **P<0.01 compared to the vehicle group

training, routinely produces a robust, reproducible, and significant reduction in step-through latencies (latencies in vehicle-treated control animals, 160–180 s; latencies in scopolamine-treated animals, 30–40 s) under the experimental conditions used in the present study (Fig. 2). At the doses of 320 and 640 mg/kg i.p., DMAE p-Glu, given 35 min before training, significantly attenuated the effect of scopolamine, whereas its vehicle was without significant effect (Fig. 2). The dose–response curve was bell-shaped with a maximal effect at the dose of 320 mg/kg. This antiscopolamine effect of DMAE p-Glu occurred at doses that had otherwise no observable effects on behavior and that were without apparent effect on spontaneous activity. Moreover, the effect occurred in animals that were tested at retention trial (i.e., 24 h after administration of DMAE p-Glu), thereby arguing against possible state-dependent effects or an effect of the drug on performance at the time of testing.

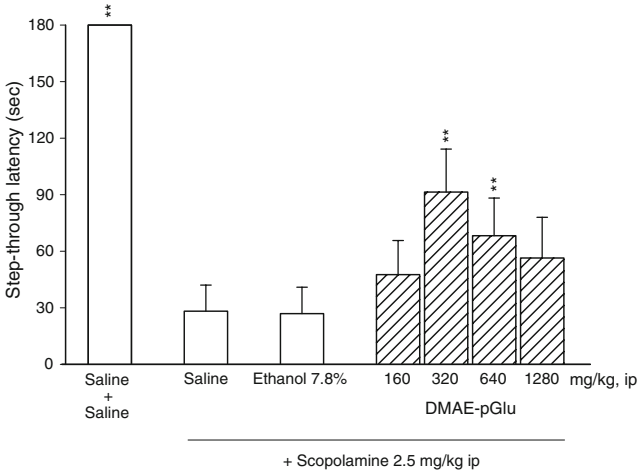

Fig. 2 Effects of dimethylaminoethanol pyroglutamate (DMAE p-Glu) or vehicle on acquisition and/or retention of a passive avoidance behavior after deficit induced by scopolamine in rats. DMAE p-Glu was administered i.p. 35 min before the initial trial (training). Scopolamine was administered i.p., at a dose of 2.5 mg/kg, 30 min before training. Results are means ± SEM of 12 rats per treatment group. **P<0.01 compared to (scopolamine+7.8% ethanol vehicle) group (Mann–Whitney *U* test)

Morris water maze test of spatial memory in rats A repeated daily intraperitoneal administration of DMAE p-Glu (320 and 640 mg/kg) for 14 days, but not for 2 days (data not shown), improved performance of rats in this test (Fig. 3), as indicated by the significantly higher percentage of time (32.9±1.6% and 36.7±3.3%, respectively) spent by the animals in the target quadrant during the probe trial (without platform), in comparison to both the vehicle group (27.8±2.1%) and to chance performance (25%). The lowest dose tested (160 mg/kg i.p.) was without significant effect in the same test (29.2±2.2%). DMAE p-Glu produced no significant effects on the latency (second) to find the hidden platform during the training phase in this test (three trials per day in the 2 days immediately preceding the proble trial; Table 1).

Clinical study

Cognitive and psychomotor tests The statistical analysis of the Buschke Selective Reminding Test at baseline (T0) showed a significant periods effect with means values of LTR and CLTR in the second period higher than in the first period, whatever the treatment group (LTR: period 1: DMAE p-Glu, 78.33±9.25; placebo, 79.58±8.31; period 2: DMAE p-Glu, 84.83±5.02; placebo, 85.00±12.83, P=0.0091. CLTR: period 1: DMAE p-Glu, 68.00±14.24 versus

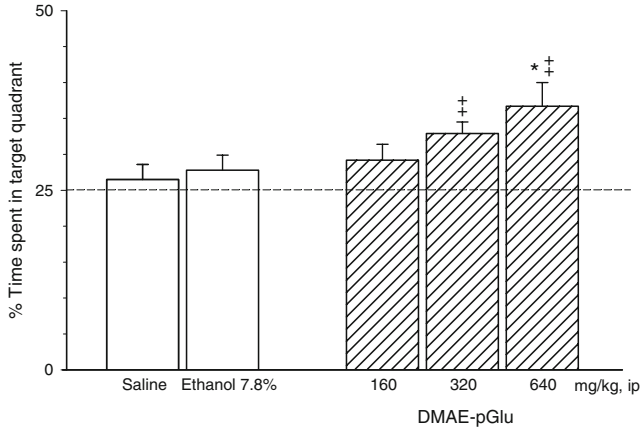

Fig. 3 Effects of repeated administration of dimethylaminoethanol pyroglutamate (DMAE p-Glu) on the percentage of the 60-s swim spent in the target quadrant (without platform) during the probe trial (24 h after the last training trial) in the Morris water maze test in rats. DMAE p-Glu was administered i.p. once daily for 14 days. On the 13th and the 14th days, animals were treated with DMAE p-Glu immediately after the last of three training sessions. On day 15, 24 h after the last injection of DMAE p-Glu, animals were tested in the probe trial. Results are means ± SEM of ten rats per treatment group. *$P<0.05$ compared to the 7.8% ethanol vehicle group. $^{++}P<0.01$ compared to chance performance (25%; Student's t test)

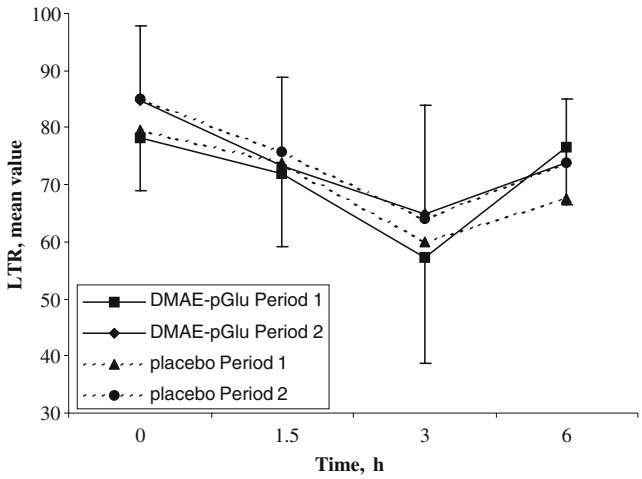

Fig. 4 Evolution of LTR (long-term retrieval) scores over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

placebo, 68.08±14.99; period 2: DMAE p-Glu, 80.33±9.74 versus placebo, 82.25±15.56, $P=0.0003$). This result might be linked to a learning effect. After administration of scopolamine, in both treatment groups, the LTR and CLTR scores showed impairment at T1.5 h, T3 h, and T6 h versus T0 with a decrease of the score to T3 h, followed at T6 h by a tendency to return to baseline. The most pronounced impairment was found at T3 h as shown in Fig. 4 and 5. The comparison of changes (from T0) of LTR and CLTR scores between treatment groups indicated a significant effect at T6 h. For LTR, the statistical analysis revealed a significant treatment effect at T6 h ($P=0.040$), with a decrease of the score in the DMAE p-Glu group that was significantly lower than in the placebo group, as shown in Fig. 6. The complementary analysis performed only on the data collected at the period 1 ($n=24$, 12 subjects in DMAE p-Glu group and 12 subjects in placebo group) confirmed this result at T6 h ($P=0.012$). For CLTR, the statistical analysis revealed a significant period effect ($P=0.021$) and a significant treatment effect ($P=0.049$) on changes at T6 h, the decrease of CLTR score in DMAE p-Glu group being significantly lower than in placebo group, as shown in Fig. 7. However, in spite of the lack of sequence effect ($P=0.131$), this significant treatment effect was probably due to the scores obtained by the subjects with DMAE p-Glu in the first period. The complementary analysis performed only on data collected at the period 1 ($n=24$, 12 subjects in DMAE p-Glu group and 12 subjects in placebo group) confirmed this result at T6 h ($P=0.035$) with a variation of CLTR score from baseline significantly less important in the DMAE p-Glu group versus the placebo group. Scopolamine impaired the NALL score mainly at T3 h in both groups (Fig. 8). At T6 h, the comparison of treatment groups on NALL changes between T6 h and T0 showed a significant treatment effect on changes at T6 h

Table 1 Effects of dimethylaminoethanol pyroglutamate (DMAE-pGlu) on the latency (second) to find the hidden platform during the three trials per day for 2 days in the water maze task in rats

Compounds (mg/kg i.p.)	Day 1			Day 2		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Saline	68.1±13.0	44.0±12.7	37.0±11.4	52.4±12.5	30.4±10.6	32.5±11.0
Ethanol 7.8%	68.8±13.0	54.6±10.5	33.0±8.2	63.3±9.7	36.9±11.3	29.4±9.0
DMAE-pGlu						
160	62.6±10.9	43.4±10.1	17.7±4.0	46.4±12.2	31.8±6.5	19.7±5.1
320	54.5±10.9	43.8±10.3	21.9±5.5	43.4±9.1	24.3±4.8	17.3±2.4
640	85.0±5.7	28.7±2.8	26.5±8.8	43.4±10.3	32.9±11.8	17.7±3.8

There were no statistically significant differences between experimental groups within trials (Student's t test)

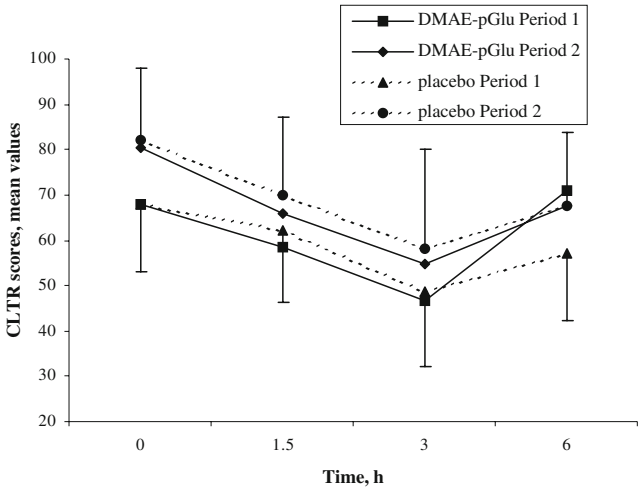

Fig. 5 Evolution of CLTR (consistent long-term retrieval) scores over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

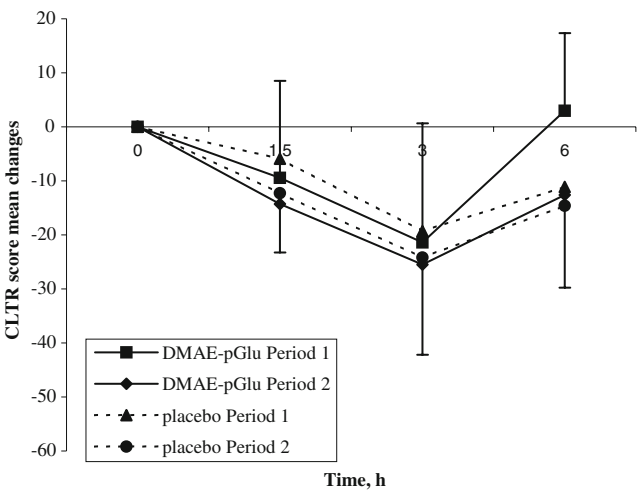

Fig. 7 Mean CLTR (consistent long-term retrieval) score changes (± SD) over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

(P=0.034), as shown in Fig. 9. The increase of NALL in the DMAE p-Glu group was significantly lower than in placebo group, i.e., the number of trials necessary to retrieve the whole list from the LTS was less increased in the DMAE p-Glu group. However, the complementary analysis performed only on data collected at the period 1 did not reveal a significant difference between the two treatment groups. Scopolamine induced an impairment of the performance in the two treatment groups with DSST, with a decrease, from baseline, of the total number of responses at T1.5 h and T3 h, followed at T6 h by a reversal to baseline scores (Table 2). However, no significant difference was found between the two treatment groups. On the visual analog scale "Bond & Lader", scopolamine induced an impairment of alertness and contentedness scores in the two groups, as shown by an increase of the score from baseline at T1.5 h and T3 h, with a trend to return to baseline at T6 h (Table 2). However, no significant difference was found between the two treatment groups. Scopolamine did not significantly affect the calmness mood factor (Table 2), and there was no difference between the two groups whatever the time of assessment. With the CRT, the comparison between treatment groups of RRT changes pointed out a significant sequence effect on changes at T1.5 h (P=0.002) and at T3 h (P=0.011), as shown in Fig. 10. The DMAE p-Glu group was faster than placebo group for recognition at T1.5 h and T3 h only for period 1. No effect was found on changes at T6 h, as shown in Fig. 11. The complementary analysis performed on data collected only during the period 1 confirmed this result, with a significant difference at T3 h

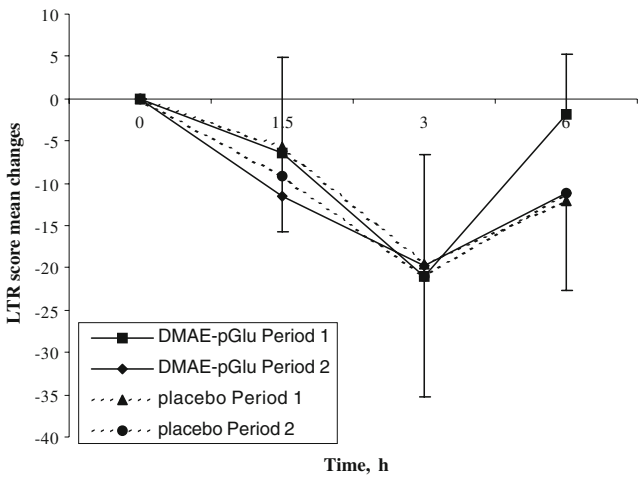

Fig. 6 Mean LTR (long-term retrieval) score changes (± SD) over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

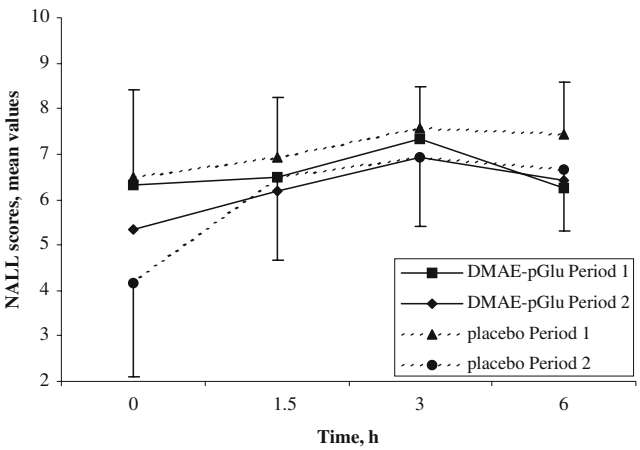

Fig. 8 Evolution of NALL scores over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

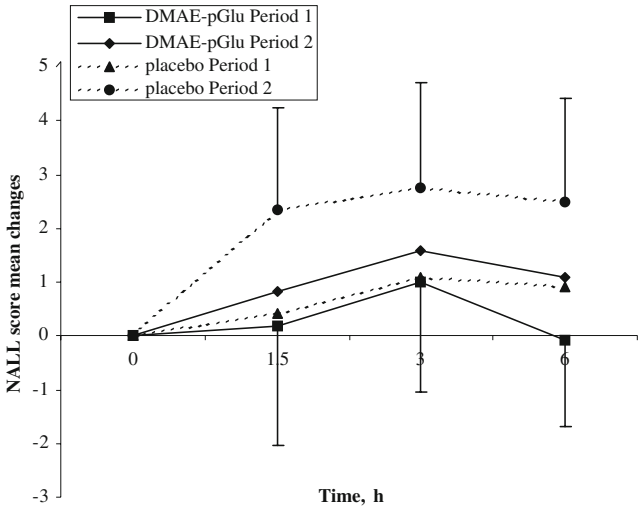

Fig. 9 Mean NALL score changes (± SD) over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

between the two treatment groups (P=0.009; DMAE p-Glu period 1 change from T0: 38±31.76 ms; placebo period 1 change from T0: 39.55±26.48 ms). The evolution of MRT and TRT scores was comparable between the two treatment groups, and no significant difference was found on changes at each time versus baseline in both groups (Table 2). The complementary analysis on data of the period 1 also showed no significant difference between the two groups.

Adverse effects In this study, the DMAE p-Glu was found to be very safe. There was no premature withdrawal and no serious adverse event.

Discussion

The aim of this combined preclinical and clinical study was to assess the cholinomimetic activity of DMAE p-Glu and to test its ability to reverse scopolamine-induced memory impairment. In microdialysis experiments, DMAE p-Glu

Table 2 Mean values of DSST (digit symbol substitution test), visual analog scale Bond & Lader (alertness, contentedness, and calmness), MRT (motor reaction time), and TRT (total reaction time) scores in each period for DMAE p-Glu and placebo groups

		DMAE p-Glu		Placebo	
		Period 1	Period 2	Period 1	Period 2
DSST (number of total response)	T0	51.67±8.81	49.75±6.12	46.50±5.47	56.67±9.73
	T1.5 h	48.17±9.87	43.33±6.34	43.92±5.05	48.83±9.38
	T3 h	46.67±8.99	45.58±5.95	40.42±4.56	52.17±11.07
	T6 h	53.92±10.20	51.33±5.77	49.50±5.82	59.25±11.40
Alertness	T0	29.06±12.72	27.20±18.03	24.38±15.31	22.77±12.35
	T1.5 h	63.35±10.79	55.93±15.49	56.67±16.96	57.48±15.81
	T3 h	53.72±18.02	51.00±19.18	53.02±20.01	50.03±18.77
	T6 h	30.02±14.38	35.58±15.85	34.14±14.21	29.93±15.21
Contentedness	T0	19.67±13.65	19.00±13.17	15.88±12.42	16.82±9.74
	T1.5 h	29.05±16.24	26.18±13.58	25.97±11.80	27.22±15.53
	T3 h	26.48±13.62	24.65±12.64	23.83±13.21	25.03±14.19
	T6 h	20.75±12.17	21.32±9.91	16.20±10.36	21.50±12.87
Calmness	T0	19.00±14.10	20.50±11.12	20.50±16.55	17.88±10.16
	T1.5 h	20.29±14.80	20.96±10.35	18.42±9.93	20.83±12.26
	T3 h	21.13±11.87	23.42±10.87	20.50±13.72	21.67±12.91
	T6 h	21.08±11.68	19.42±12.16	15.77±9.32	22.46±13.82
MRT (ms)	T0	183.2±28.15	222.8±25.28	229.1±27.16	189.7±26.53
	T1.5 h	228.9±47.61	258.1±43.17	262.0±37.90	219.0±38.32
	T3 h	218.2±47.76	259.9±37.06	248.54±41.87	219.0±37.65
	T6 h	214.2±31.09	247.6±24.54	236.2±32.83	214.8±34.64
TRT (ms)	T0	553.6±43.27	575.2±63.29	592.0±46.33	541.7±40.03
	T1.5 h	626.2±66.63	657.3±82.48	675.4±71.64	603.7±59.19
	T3 h	598.0±65.43	649.8±87.09	650.9±68.64	599.6±54.81
	T6 h	656.5±32.51	607.3±53.77	591.8±38.56	558.2±60.09

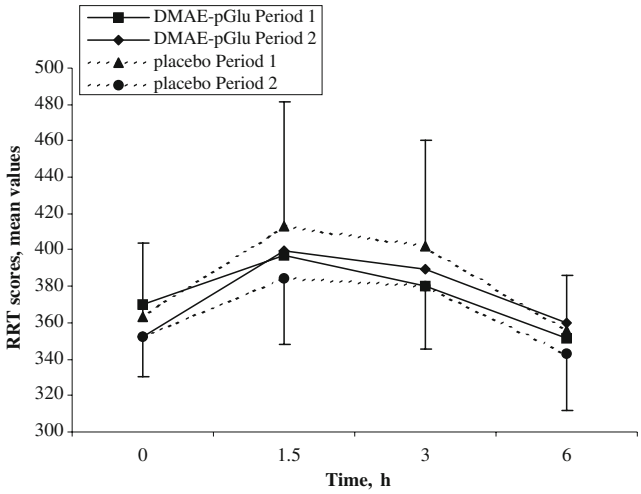

Fig. 10 Evolution of RRT (recognition reaction time) scores over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

was shown in rats to dose dependently increase extracellular levels of choline, and acetylcholine in the increase in extracellular levels of choline observed in the present study was not totally unexpected, given that an increased content of brain choline following acute systemic administration of DMAE has been reported in the past (e.g., Jope and Jenden 1979). On the other hand, the increase in acetylcholine release was a novel and somewhat unexpected result. The rate of acetylcholine formation is limited by the intracellular concentration of choline, which is determined by the active uptake of choline into the nerve ending. However, increased availability of extracellular levels of choline per se is not

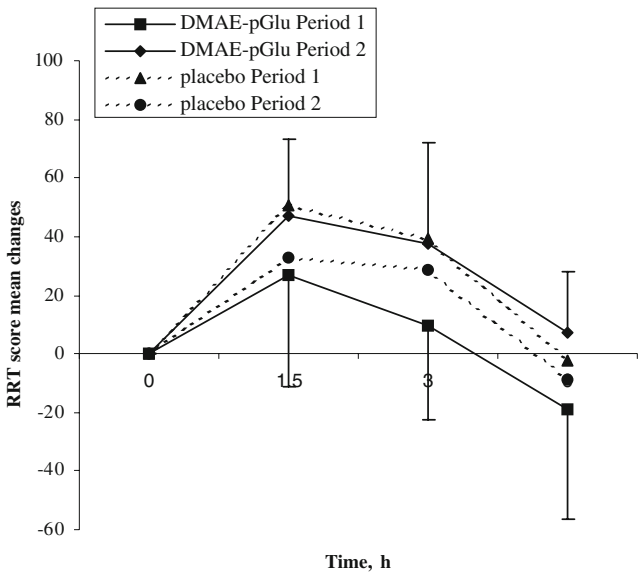

Fig. 11 Mean RRT (recognition reaction time) score changes (± SD) over 6 h after the scopolamine infusion, by treatment (DMAE p-Glu or placebo) and period (period 1 and period 2)

considered to be a determining or sufficient factor in cholinergic augmentation therapies. Thus, the mechanism(s) by which DMAE-pGlu increases acetylcholine release and produces promnesic effects in memory tests, if unrelated to the increase in brain-free choline availability, remains unclear at present.

In the rat behavioral studies, DMAE-pGlu was found to improve the spatial memory performance in the Morris water maze test following a 14-day repeated i.p. administration and to partially reduce following its acute i.p. administration the performance deficit induced by scopolamine in a passive avoidance paradigm. In this later test, the magnitude of effect of DMAE p-Glu against the scopolamine-induced deficit was similar to that observed under the same experimental conditions with acetylcholinesterase inhibitors including tacrine (see Chopin et al. 2002), galanthamine, physostigmine, and rivastigmine (unpublished in-house data). Together, these findings suggested that DMAE p-Glu might have a potential benefit in the treatment of cognitive disorders related to central cholinergic deficit. This hypothesis was further tested in the subsequent clinical study, wherein a potential effect of DMAE p-Glu to antagonize the deficits of attention and memory functions induced in healthy young male volunteers by the anticholinergic agent, scopolamine (0.5 mg intravenously), was examined.

It is well established that scopolamine induces an impairment of cognitive processes (Rusted and Warburton 1988; Wesnes et al. 1988; Potter et al. 2000; Wesnes 2001; Edginton and Rusted 2003). The peak effect in man is observed at 2 to 3 h after subcutaneous drug administration and persists beyond 6 h after dosing (Patat et al. 1991). In the present study, the effects of scopolamine peaked 3 h after intravenous administration. Results show that scopolamine induced a memory deficit characterized by a deterioration of long-term memory (reflected by a decrease, from baseline to T3 h, of LTR and CLTR scores) and in parallel, an increased intervention of short-term memory, a deficit in vigilance and attention reflected by the elevation of the scores of alertness and contentedness factors, and a deficit in attention, working memory, and skilled coordination, reflected by a decrease of the DSST score.

Under these experimental conditions, DMAE p-Glu, given at a daily dose of 1,500 mg for six consecutive days and 1 h after the infusion of scopolamine on day 7, showed a significant effect on LTR and CLTR scores, these two scores being less altered than during the placebo session. The interpretation of statistical results was complicated by a period effect, with better performance in period 2 whatever the treatment group, probably link to a learning effect. This also implies that the practice effects across the battery were not removed by "practicing" the volunteers at the selection phase. However, a complementary analysis performed only

on the data obtained in period 1 confirmed this tendency, with a reversal of the scopolamine-induced impairment on the long-term memory scores at T6 h with DMAE p-Glu. At least two reasons could explain the absence of treatment effect in period 2: it could be due to a lack of task sensitivity above a certain learning threshold, or it could also be explained in terms of a DMAE p-Glu effect. Indeed, the treatment could protect against scopolamine-induced deficits, but when tasks were underlearned, it could exist limited capacity to improve performance of well-learned tasks. The results of the other tests were similar in the two treatment groups, except for the CRT that showed a significant but slight difference at T3 h in favor of DMAE p-Glu. These results suggest that DMAE p-Glu at 1,500 mg could act on scopolamine-induced memory impairment, but this requires to be confirmed in another study.

The anatomical localization where DMAE p-Glu reverses scopolamine-induced memory impairment is uncertain. The rat microdialysis study shows an increase in acetylcholine release in the mPFC, but did not examine other cholinergic afferent target areas that can also participate in cognitive function (e.g., hippocampus). Thus, an exclusive implication of the prefrontal cortex in the clinical effects of DMAE p-Glu remains speculative at this point. It is perhaps noteworthy that an functional Magnetic Resonance Imaging (fMRI) study in humans has shown scopolamine to modulate prefrontal cortical activity during recollection (Bozzali et al. 2006), suggesting an involvement of this brain structure in scopolamine-induced memory deficit, but certainly cannot exclude extracortical loci of actions.

The mechanism of action of DMAE p-Glu suggests that this compound, by increasing synaptic levels of acetylcholine, could have a dual cholinomimetic action, mediated by both muscarinic and nicotinic receptor systems. DMAE p-Glu was demonstrated here, in both preclinical and clinical settings, to reverse cognitive deficits induced by scopolamine, a muscarinic receptor antagonist. The scopolamine challenge model does not permit an evaluation of the effect of DMAE p-Glu on nicotinic neurotransmission, since scopolamine is preferential for muscarinic receptors. Thus, it might be hypothesized that the cholinomimetic activity of DMAE p-Glu has been underestimated in the present study, and that the overall clinical effect might be more important than that observed here. It could, thus, be of interest to assess the effect of DMAE p-Glu on memory and cognitive processes of patients with dementia of the Alzheimer type, which involves a dysfunction in both nicotinic and muscarinic receptor systems.

In conclusion, DMAE p-Glu, administered at a daily oral dose of 1,500 mg for six consecutive days and 1 h after the infusion of scopolamine on day 7, was able to shorten the deleterious effect of scopolamine on long-term memory in healthy volunteers, with a faster return to the baseline level of performance, without diminishing the peak sedative and amnesic effects of scopolamine. The results of the clinical study also reinforce the predictive validity of the animal models used in the preclinical testing. Together, both the preclinical and clinical investigations, under the experimental conditions described, support the notion that DMAE p-Glu might be an effective drug in reducing memory deficits observed in patients suffering from cognitive impairment, but this requires to be confirmed in another study.

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