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Dietary cytidine (5′)-diphosphocholine supplementation protects against development of memory deficits in aging rats

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Abstract

The present study was designed to assess the effect of supplementation with dietary cytidine (5′)-diphosphocholine (CDP-choline), a source of cytidine and choline, on memory in young and older rats. Although the hippocampal-dependent memory deficits in aged rats are well documented, cognitive functioning in early aging has not been as thoroughly evaluated. Female Sprague–Dawley rats (3 or 15 months of age) consumed either a control diet or a diet supplemented with CDP-choline (approximately 500 mg/kg/day) for 8 weeks, after which they were trained to perform spatial and cued versions of the Morris water maze. Compared with young rats, aged rats exhibited a selective deficit in spatial memory tasks that required rats to retain information for 24 h or longer. CDP-choline supplementation protected against the development of this deficit, but had no memory-enhancing effect in normal young rats. These findings suggest that early-aged rats display a selective impairment in hippocampal-dependent long-term memory, and that dietary CDP-choline supplementation can protect against this deficit.

Keywords: Acetylcholine; Aging; CDP-choline; Hippocampus; Memory; Phospholipids

1. Introduction

Advanced age is associated with a decline in hippocampal-dependent memory capacities in humans (Craik, 1990; Albert, 1993), and animals (Gage et al., 1988; Gallagher et al., 1993). In rats, aging results in impairments of spatial learning and memory processes (Rapp et al., 1987; Gallagher and Pelleymounter, 1988) that are known to depend on hippocampal function (Morris et al., 1982; Packard and Teather, 1997, 1998). Interestingly, aging does not compromise procedural memory (Rapp et al., 1987; Gallagher and Pelleymounter, 1988), perhaps because procedural memory (also known as habit or stimulus-response memory) apparently is independent of hippocampal function (Packard et al., 1989; Packard and Teather, 1997, 1998).

Although many studies have addressed hippocampal-dependent memory dysfunction in aged rats, such changes have not been as thoroughly evaluated in early aging. Assessing memory function during early aging may provide us with a better understanding of subtle functional changes that may occur prior to the dramatic deficits characteristic of aged mammals. Also, assessing early changes in memory could assist in the identification of more specific, early biochemical or molecular changes that may underlie such changes.

Preliminary experiments showed that 17-month-old rats (which are used as a model of early aging) displayed poor spatial water maze task acquisition (relative to young rats aged 3–5 months) when given two to four training trials for several days, yet they readily acquired the task when given eight trials in a single training session (Teather and Wurtman, unpublished observations). This suggested that early aging might adversely affect the ability of rats to acquire a spatial task when acquisition requires that information be transferred from day to day. Such a deficit may indicate that early-aged rats have impaired consolidation and/or retrieval processes rather than impaired acquisition mechanisms. In this study, we investigated this hypothesis and assessed the performance of early-aged rats (relative to young adult rats) on a procedural task to ensure that any changes we might

Abbreviations: AA, arachidonic acid; CDP-choline, cytidine (5′)-diphosphocholine; PC, phosphatidylcholine; PAF, platelet-activating factor.

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find in spatial memory were not attributable to nonmnemonic processes common to aged rats, such as poor sensorimotor coordination, locomotion deficits, or changes in motivation or exploration behavior.

Cytidine (5′)-diphosphocholine (CDP-choline, also known as citicoline) is an endogenous intermediate in the biosynthesis of phosphatidylcholine (PC), a phosphatide (Kennedy and Weiss, 1956) that is the major lipid component of cell membranes. When orally administered to rats, CDP-choline rapidly increases the levels of cytidine and choline in plasma and brain (Lopez-Coviella et al., 1987). This enhances acetylcholine synthesis and release (Hirsch et al., 1978) and increases brain levels of PC and the other membrane phosphatides (Lopez-Coviella et al., 1987, 1992). Inasmuch as brain aging can be associated with cholinergic dysfunction (Hellweg et al., 1990; Rylett et al., 1993) and with alterations in cellular membranes (Ando et al., 2002; Salvador et al., 2002), CDP-choline could potentially act at several loci to prevent or ameliorate cognitive impairments associated with the aging process. In fact, CDP-choline has been shown to improve attentional and memory deficits in senile patients and the aged (Alvarez et al., 1997), and to prevent amnesia induced by the cholinergic antagonist, scopolamine, in rats (Mosharrof et al., 1987). Administration of CDP-choline also enhanced memory for an active avoidance task in aged rats (Mosharrof et al., 1987).

This study characterizes the changes in learning and memory function associated with early aging and the ability of dietary CDP-choline supplementation to protect against these changes.

2. Methods

2.1. Animals

Three- ($n=16$) and 15-month-old ($n=16$) female Sprague–Dawley rats (Charles River Laboratories, Boston, MA) were housed in polycarbonate cages in groups of two to three per cage. One subgroup of aged rats ($n=8$) and one subgroup of young rats ($n=8$) were fed a diet of laboratory chow (Teklad Global 16% protein rodent diet; Harlen Teklad, Madison, WI) supplemented with CDP-choline (kindly provided by Ferrer Internacional, Barcelona, Spain) for 8 weeks. The remaining subgroups (n's=8) received the Teklad control diet. Four diets were used with different amounts of CDP-choline added. Mean weekly intakes were monitored for all rats and the diets were adjusted so that rats received approximately 500 mg/kg/day. It should be noted that rats were housed in groups; therefore, a precise determination of amount of CDP-choline ingested per rat was not available.

Animals were maintained under standard environmental conditions (room temperature: 20±1 °C; relative humidity: 55–60%; light/dark schedule: 12/12 h) with free access to tap water and food. The health of all subjects was assessed by routine examination during the experiment.

2.2. Water maze apparatus

The water maze was a black circular tank 6 ft (1.83 cm) in diameter and 1.5 ft (0.55 cm) in height. The tank was filled with water (25±2 °C) to a depth of 20 cm and was in a well-lit room containing several extramaze cues. Four starting positions (north, south, east and west) were spaced around the perimeter of the tank, dividing the pool into four quadrants. The rectangular Plexiglas escape platform used for the spatial task (11 × 14 × 19 cm) was submerged at a depth of 1 cm. For the cued version of the water maze, a white rubber ball (8 cm in diameter) was attached to the top of the submerged platform and protruded above the water surface. The platform could be used as a step to mount the ball to escape the water. All behavioral procedures were videotaped using a CCD camera positioned on the ceiling above the center of the maze. This camera was connected to a monitor and video recorder kept in an observation room adjacent to the training room.

2.3. Behavioral procedures

The following experiments were carried out in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Behavioral testing was carried out in a blind manner.

2.4. Spatial memory: 4-day/four training trials per day paradigm

Rats received a 4-day training session consisting of four trials (i.e., swims) per day in the spatial water maze task. On each trial the animal was placed into the tank facing the wall at one of four designated start points (N, S, E and W) and allowed to escape onto the hidden platform. The submerged platform was in the same quadrant (quadrant N) on every trial for all 4 days. A different starting point was used on each trial such that each starting point was used once each day. If an animal did not escape within 90 s, it was manually guided to the escape platform by the experimenter. After mounting the platform, rats remained on the platform for 20 s. Following each trial, animals were removed from the maze and placed in a holding cage for a 30-s intertrial interval (ITI). The latency to mount the escape platform was recorded and used as a measure of task acquisition.

Twenty-four hours after the final training day (i.e., Day 5), rats were given a 60-s probe test (where the platform was removed from the pool). During the probe trials, the time spent in each of the four quadrants was recorded and analyzed.

2.5. Cued memory

One week after completion of the 4-day/four trials per day spatial training task, rats received three training ses-

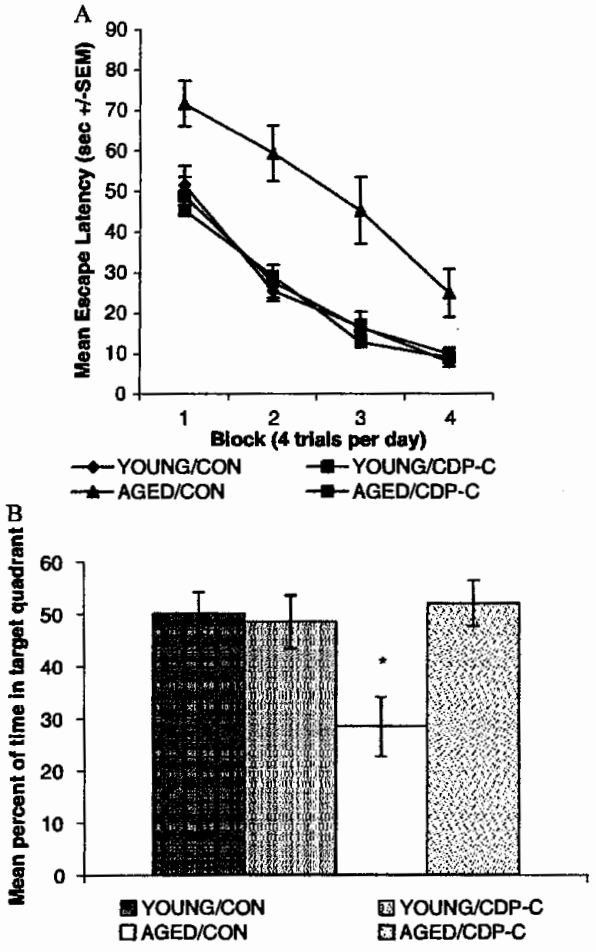

Fig. 1. (A) Mean escape latencies ± S.E.M.s for 4 days of acquisition of a spatial water maze task. Young and aged rats were fed either a control diet or a diet supplemented with CDP-choline (approximately 500 mg/kg/day) for 2 months. $n = 8$ for all four experimental groups. Rats were given four training trials (i.e., block) for four consecutive days. The submerged platform was always in the same quadrant. * Different from other groups, $P < .05$ (Scheffe's post hoc test). (B) Bar graph represents the percentage of total swim time spent by rats in the target quadrant (i.e., that previously contained the platform) during the 60-s probe test. * Different from other groups, $P < .05$ (Scheffe's post hoc test).

sions consisting of four trials (i.e., swims) per day in the cued water maze task. On each trial the animal was placed into the tank facing the wall at one of four designated start points (N, S, E and W) and allowed to escape onto the visibly cued platform. A different starting point was used on each trial such that each starting point was used each day. If an animal did not escape within 90 s, it was manually guided to the escape platform by the experimenter. After mounting the platform, rats remained on the platform for 20 s. Following each trial, animals were removed from the maze and placed in a holding cage for a 30-s ITI. The latency to mount the escape platform was recorded and used as a measure of task acquisition. The visible escape platform was placed in a different quadrant on each trial such that each of the four quadrants contained the escape platform once each day.

2.6. Spatial memory: 2-day/eight training trials per day paradigm

One week after completion of the cued task, rats were given 2 days of spatial training with eight trials administered each day. For all eight trials of Day 1, the submerged platform was located in the same quadrant (quadrant S). Sixty minutes after completion of the final trial, rats were given a 60-s probe test (as described above). The following day, rats were given eight training trials with the submerged platform in a new location (quadrant W). Twenty-four hours after the completion of training, the rats were given a final, 60-s probe test.

2.7. Data analysis

Data are expressed as means ± S.E.M.s. Experimental groups were compared using two-way analysis of variance (ANOVA) (Diet × Age) with repeated measures, followed by Scheffe's post hoc test to compare groups if overall significance was revealed by ANOVA. P values of $< .05$ were considered statistically significant.

3. Results

3.1. Body weight

Animals were weighed weekly to ensure that treated and untreated rats were eating approximately equivalent amounts

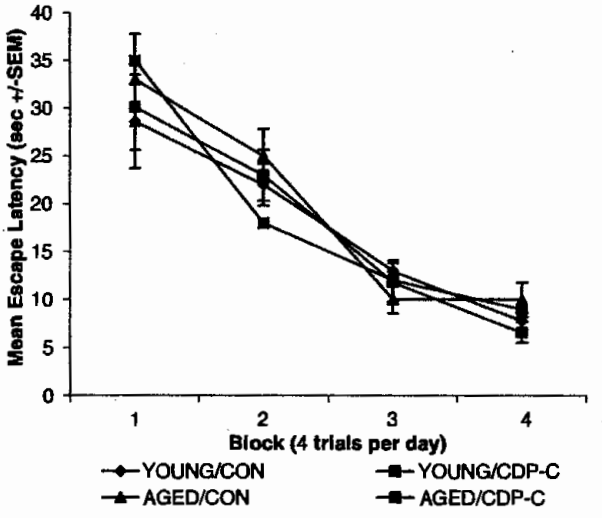

Fig. 2. Mean escape latencies ± S.E.M.s for 3 days of acquisition of a cued water maze task. Young and aged rats were fed either a control diet or a diet supplemented with CDP-choline (approximately 500 mg/kg/day) for 2 months. $n = 8$ for all four experimental groups. Rats were given four training trials (i.e., block) for three consecutive days. The visible platform was in a different quadrant for all four training trials.

of food. As expected, aged rats weighed significantly more than young rats ($P<.01$). No significant differences in mean body weight were found between the CDP-choline-supplemented and the respective control groups (data not shown).

3.2. Spatial memory: 4-day/four training trials per day paradigm

All groups were able to learn the spatial water maze task to some degree, showing a decrease in mean escape latency across the 4 days of training (Fig. 1); as indicated by the main effect of day [$F(3,84)=145.1$, $P<.001$]. ANOVA analysis also revealed significant main effects of age [$F(1,28)=14.535$, $P<.005$], diet [$F(1,28)=17.5$, $P<.001$], as well as a Diet × Age interaction [$F(1,1)=20.12$, $P<.001$). Post hoc analysis indicated that untreated (i.e., no CDP-choline supplementation) aged rats had significantly higher escape latencies on all 4 days than did the other groups (P's $<.05$). It should be noted that untreated aged rats showed large individual differences in performance (as shown in the standard error bars in Fig. 1). Specifically, 5 of the 8 untreated aged rats showed considerably less learning across days than did the young rats, while the 3 remaining rats were almost (or) as proficient as the young rats. Only 1 aged rat supplemented with CDP-choline failed to perform as well as the young rats.

The escape latencies of the initial trial on each day are considered the most sensitive measure of memory for the previous day's learning (Packard and Teather, 1997). When the mean escape latencies for the initial trials on the 4 days of training were analyzed, untreated aged rats

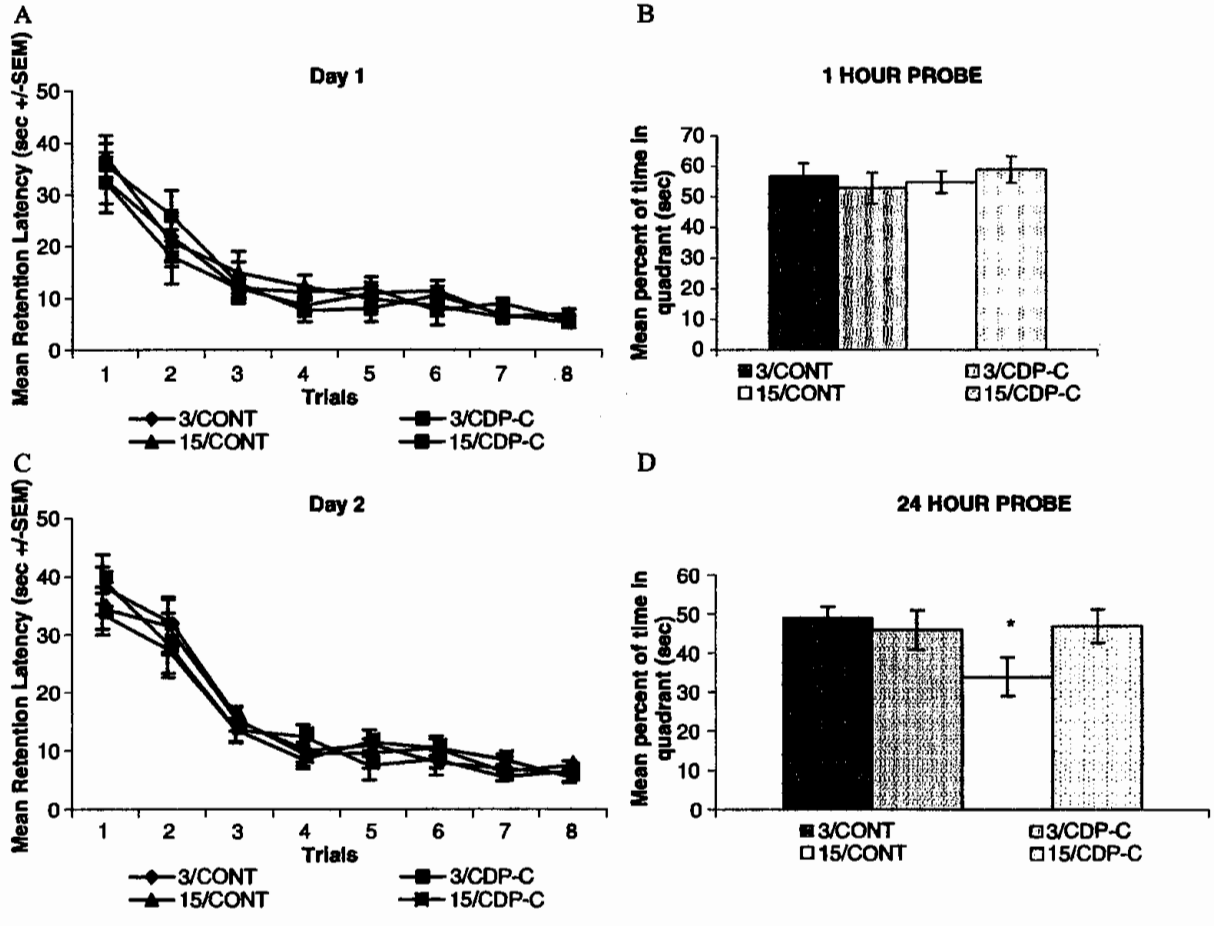

Fig. 3. (A) Mean escape latencies ± S.E.M.s for the initial day of training in the spatial water maze task with eight training trials in 1 day. Young and aged rats were fed either a control diet or a diet supplemented with CDP-choline (approximately 500 mg/kg/day) for 2 months. $n=8$ for all four experimental groups. Rats were given eight training trials with the submerged platform always in the same quadrant. * Different from other groups, $P<.05$ (Scheffe's post hoc test). (B) Bar graph represents the percentage of total swim time spent by rats in the target quadrant (i.e., that previously contained the platform) during the 60-s probe test administered 1 h after initial eight training trials. * Different from other groups, $P<.05$ (Scheffe's post hoc test). (C) Mean escape latencies ± S.E.M.s for the second day of training in the spatial water maze task with eight training trials in 1 day. Rats were given eight training trials with the submerged platform always in the same quadrant (different from the initial day of eight training trial training). * Different from other groups, $P<.05$ (Scheffe's post hoc test). (D) Bar graph represents the percentage of total swim time spent by rats in the target quadrant (i.e., that previously contained the platform) during the 60-s probe test administered 24 h after completion of the eight training trials. * Different from other groups, $P<.05$ (Scheffe's post hoc test).

had significantly higher escape latencies than those of the other groups (P's $<.05$), indicating that untreated rats displayed less memory for the previous day's learning (data not shown).

Improvement across trials (and days) in the spatial water maze task is not necessarily an indication of spatial learning because these measures do not provide information about the search strategy, as nonspatial strategies can often be successfully used to locate the hidden platform (Gallagher et al., 1993). Thus, we carried out a probe test to assess search strategy and memory for the platform's location. ANOVA analysis of the mean percentage of swim time in the four quadrants during the 60-s probe test revealed significant main effects of age [$F(3,28)=7.03$, $P<.005$], and quadrant [$F(3,28)=8.28$, $P<.001$], as well as an Age × Diet interaction [$F(3,3)=4.1$, $P<.01$]. Post hoc analysis indicated that untreated aged rats swam for a significantly shorter time in the target quadrant than did the other groups (P's $<.05$).

3.3. Cued memory

Analysis of the mean escape latencies across the 3 days of training in the visible platform task showed a significant main effect of day [$F(3,28)=64.05$, $P<.001$]. No main effects of diet or age (or interactions) were revealed, indicating that all groups (regardless of age or diet) acquired the procedural water maze task at equivalent rates (Fig. 2).

3.4. Spatial memory: 2-day/eight training trials per day paradigm

When rats were given eight training trials to acquire the location of the hidden platform, all groups successfully mastered the spatial memory task, as evidenced by the significant main effect of trial on both Day 1 (i.e., platform in the S quadrant) [$F(7,28)=22.38$, $P<.001$] and Day 2 (i.e., platform in the W quadrant) [$F(7,28)=20.3$, $P<.001$] of training. No significant main effects of diet or age were revealed for either day (Fig. 3A and C).

Sixty minutes after the last training trial of Day 1, a probe test, in which the platform had been removed, was given. The results in Fig. 3B demonstrate that all animals spent more time in the quadrant where the platform had been (significant main effect of quadrant [$F(3,28)=6.89$, $P<.005$]), indicating preserved short-term spatial memory function. However, when the probe test was given 24 h after Day 2 of training, not only was there a significant main effect of quadrant [$F(3,28)=8.01$, $P<.005$], but significant main effects of age [$F(1,28)=5.68$, $P<.01$] and diet [$F(1,28)=4.2$, $P<.05$] as well as a significant Age × Diet interaction [$F(1,1)=10.4$, $P<.005$]. Post hoc analysis indicated that untreated aged rats spent less time in the quadrant where the platform had been, compared with other groups (P's $<.05$). Thus, although the untreated rats acquired Day 2 of the working memory task as readily as other groups, they displayed a deficit in long-term memory function (i.e., location of the platform 24 h later) (Fig. 3D).

4. Discussion

4.1. Age-associated memory impairment: effect of CDP-choline supplementation

These data show that the persistence of spatial memory decreases in early aging, possibly due to defective consolidation or impaired retrieval mechanisms. Moreover, providing supplemental CDP-choline via the diet can protect against this decline.

The ability of 17-month-old rats to retain long-term spatial memory was less than that of 5-month-old rats (Fig. 1A). Moreover, as shown in the probe test results, the untreated aged rats spent less time than did the CDP-choline-treated, the treated or untreated young rats in the quadrant that had previously contained the platform (Fig. 1B). It should be noted that not all of the untreated aged rats displayed such impairments. Rather, a subset (5 of 8) of aged rats exhibited poor spatial memory, while a few displayed spatial learning skills equivalent to those of young rats. Such individual differences are common in aged rodents (Gage et al., 1984, Gallagher et al., 1993); our data indicate that the same is true for early aging. These results suggest that early aging, unlike more advanced aging, does not impair spatial acquisition processes, but rather selectively impairs consolidation and/or retrieval mechanisms.

Aged rats appear to have the ability to acquire spatial tasks, suggesting that their deficit results from an inability to transfer or consolidate information acquired during previous learning. Support for this hypothesis is provided by the finding that aged rats perform as well as young rats in the spatial task when they are subjected to the eight-training-trial protocol (Fig. 3A and C). Acquisition of this task occurs in a single training session, and does not require that animals remember the previous day's training. Familiarity with the environment (i.e., training conditions) could also be a factor producing these observed differences between four-trial and eight-trial training due to sequence of task training. In this study, rats were initially trained in the 4-day/four-trial spatial task first, followed by training in the cued task, and finally training in the 2-day/eight-training-trial spatial task. In preliminary work, we investigated the effect of varying the order of task acquisition, and found that the order did not affect the results (Teather and Wurtman, unpublished observations). Furthermore, data from the probe tests that followed the eight training trial sessions showed that aged rats had diminished memory for the platform location when the probe test was administered after a long-term delay (24 h; following Day 2 of training; Fig. 3D), but not after a short-term delay (60 min; following Day 1 of training;

Fig. 3B). If memory for the eight trial per day task was improved by experience in the water maze, we would expect that rats would show the most improvement on the probe test that followed the final day of eight-trial-per-day training; this was not the case.

These results suggest that early aging may selectively impair long-term retention or consolidation of spatial memory without adversely affecting short-term spatial memory and acquisition of spatial information. Moreover, early aged rats apparently acquire and remember procedural memory (i.e., cued version of the water maze task) at a rate comparable to that of young rats (Fig. 2), as shown in previous studies using aged rats (Zyzak et al., 1995; Gallagher et al., 1993; Gallagher and Pelleymounter, 1988; Gage et al., 1984). Hence, the spatial memory deficit observed in early-aged rats does not appear to result from nonmnemonic effects such as impairments in motivation or sensorimotor skills due to aging, but rather to specific changes in the ability to consolidate or retrieve information.

4.2. Potential mechanisms of CDP-choline action

Although the mechanism of CDP-choline action was not explored in this study, previous findings allow us to speculate on the compound's potential sites of action. It has been suggested that age-associated impairments of spatial memory result from central cholinergic dysfunction (Gibson et al., 1981). As the choline derived from CDP-choline does increase acetylcholine synthesis and release (Hirsch et al., 1978), the improvement in memory function in rats consuming this intermediate could reflect increased synthesis and release of brain acetylcholine. However, we do not believe this to be the sole, or even major, factor for several reasons. First, while hippocampal acetylcholine release is indeed critical for spatial learning (Day and Schallert, 1996), acetylcholine antagonists are more apt to block task acquisition than retention (Carlton, 1963; Harley, 1979; Whishaw, 1985); however, the present results suggest that the memory deficit is due to impaired retention and/or consolidation, rather than to impaired acquisition.

Second, hippocampal acetylcholine is required for both short- and long-term memory (Hironaka et al., 2001; Levin et al., 2002). In fact, short-term memory impairments are more pronounced than long-term impairments in animals receiving moderate doses of cholinergic antagonists, while lower doses impair only short-term memory (Ohno et al., 1996). This suggests that acetylcholine release would be required for both the long-term (4-day paradigm) and the short-term (eight-trial acquisition) learning paradigms. However, we found no evidence of impairment in short-term spatial memory (as evidenced by the results of the probe test administered 60 min after training).

Third, we did not observe enhanced spatial learning and memory capacity in young rats that consumed CDP-choline (relative to rats that received the control diet). Agents that stimulate acetylcholine release (particularly in the hippocampus) often enhance the acquisition and retention of spatial memory (Everitt and Robbins, 1997). Thus, if CDP-choline were acting to enhance acetylcholine synthesis and/or release we would expect to find superior learning and memory skills in the supplemented young rats; however, this was not the case.

Finally, 8 weeks of dietary supplementation of CDP-choline (approximately 500 mg/kg/day) improved the spatial memory deficits in early-aged rats. Preliminary studies in our laboratory suggest that 8 weeks may be the minimum time required for CDP-choline supplementation to alleviate memory dysfunction in early-aged rats; treatment for 4 weeks or less had no memory-improving effect (Teather and Wurtman, unpublished observations). Similarly acute doses of CDP-choline can increase acetylcholine synthesis and release, but treatment of about 6 weeks is needed to cause significant increases in brain phosphatide levels (Lopez-Coviella et al., 1987, 1992).

Membrane phosphatides like PC serve as reservoirs for such second and third messenger signaling systems as inositol triphosphate (IP_3) and diacylglycerol (DAG), and for such lipid mediators as platelet-activating factor (PAF) and arachidonic acid (AA), the precursor for prostaglandins. Most of these molecules have been implicated in hippocampal-dependent spatial memory processing in rats (Holscher et al., 1995; Moriyama et al., 1996; Teather et al., 1998, 2002). CDP-choline-induced increase in membrane phosphatides might also affect the size or density of synapses (Araki and Wurtman, 1988). Any such effects might protect against the age-associated decline in hippocampal-related memory functions.

5. Conclusions

Orally administered CDP-choline improved hippocampal-based memory function in early-aged rats, but had no effects on young rats. This protective function may arise from CDP-choline's ability to enhance the production of brain neuronal membrane phosphatides.

In conclusion, the present data suggest that chronic dietary supplementation of CDP-choline might prove to be a valuable therapeutic strategy for delaying or diminishing the cognitive deficits associated with aging.

Acknowledgements

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