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Chronic Administration of UMP Ameliorates the Impairment of Hippocampal-Dependent Memory in Impoverished Rats[1]

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Abstract

We have previously shown that chronic, but not acute, dietary supplementation with CDP-choline prevents the hippocampal-dependent memory deficits manifested by aged rats and by rats reared under impoverished environmental conditions. In rats, dietary CDP-choline is rapidly metabolized into cytidine and choline; the cytidine is then readily converted to uridine, which enters the brain and, via conversion to UTP and CTP, increases brain levels of membrane phosphatides. Hence, we have assessed whether administering a uridine source (UMP) instead of CDP-choline can also ameliorate the memory deficits in rats reared under impoverished environmental conditions. At weaning, 32 male Sprague-Dawley rats were exposed to either enriched (EC) or impoverished (IC) conditions for 3 mo. Concurrently, IC and EC rats were given access to either a control diet or a diet supplemented with 0.1% UMP. Rats were then assessed for learning and memory skills using 2 versions of the Morris water maze, the hidden platform version that assesses hippocampal-dependent cognitive memory processing, and the visible platform version that assesses striatal-dependent habit memory. As expected, exposure to the impoverished environment impaired hippocampal-dependent, but not striatal-dependent learning and memory. Supplementation with UMP prevented this cognitive dysfunction, as had been observed with supplemental CDP-choline. These results suggest that IC rats do not use and/or remember their spatial strategies for task solving as well as EC rats, and that long-term dietary supplementation with UMP alleviates this dysfunction. J. Nutr. 136: 2834–2837, 2006.

Introduction

The findings of studies examining the organization of memory provide compelling evidence that memory is processed in multiple brain systems that differ in the type of memory they mediate (1). Dissociations between the roles of the hippocampal system and the dorsal striatum (i.e., caudate nucleus) on the acquisition and consolidation of various learning tasks have been observed following damage to these structures (2,3). These and other results suggest that the hippocampus and dorsal striatum may be part of distinct memory systems.

Advanced age is associated with a decline in hippocampal-dependent memory capacities in humans (4,5) and certain rats. For instance, aged rats show deficits in the performance of a variety of memory tasks, including the radial maze (6) and the spatial water maze (6,7), which require an intact hippocampus. Whereas the mnemonic functions served by the hippocampus are especially susceptible to age-related changes, striatal-based memory function appears to be little affected by the aging process (7), suggesting a dissociation in the effects of aging on distinct memory systems in the mammalian brain.

A similar pattern of cognitive dysfunction has been observed in rats raised under impoverished environmental conditions (8,9). Specifically, rats reared in impoverished conditions (IC)[4] display deficits in memory for a hippocampal-dependent version (i.e. hidden platform) of the water maze, but do not differ from rats reared in enriched conditions (EC) in a striatal-dependent (i.e. visible platform) water maze task. Thus, the IC/EC paradigm may constitute a useful model for investigating the behavioral and molecular changes that accompany the aging process and may be used to assess potential treatments for age-related memory impairment.

Research from our laboratory has concentrated on identifying possible nutritional or metabolic interventions that might reduce the age-related impairments in hippocampal-dependent memory processes. CDP-choline (citicoline) serves as a precursor for the biosynthesis of several important neurochemicals, including phosphatidylcholine (PC), acetylcholine, and platelet-activating factor. Chronic, but not acute, dietary supplementation with

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[4] Abbreviations used: EC, enriched conditions; EC-CONT, enriched control diet–treated rats; EC-UMP, enriched uridine monophosphate–treated rats; IC, impoverished conditions; IC-CONT, impoverished control diet–treated rats; IC-UMP, impoverished UMP–treated rats; PC, phosphatidylcholine.

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CDP-choline alleviated the hippocampal-dependent memory impairments that occur in aged rats (7) and also in rats raised under IC (9). The circulating breakdown products of CDP-choline in rats (i.e. cytidine and choline) are also precursors for many brain constituents that could underlie CDP-choline's beneficial mnemonic effects.

Whereas the importance of choline in learning and memory processes has been an active area of research for decades (for review, see 10), the roles of cytidine or a related pyramidine, uridine, have been far less studied. Young rats treated with uridine and cytidine exhibit facilitated acquisition of active avoidance behavior and increased retention of a passive avoidance task, compared with untreated control rats (11). Moreover, experimental evidence suggests that pyramidine nucleosides also improve learning and memory in aged rats (12). Specifically, subchronic treatment (i.e. 10 d) with either pyrimidine nucleoside alleviated the impairments in the acquisition of active avoidance behaviors and in the retention of passive avoidance responses in aged rats. Acute treatments (i.e. <10 d) had no influence on the age-related memory impairment in aged rats (12). We also found that long-term dietary supplementation with CDP-choline could prevent the hippocampal-dependent cognitive impairments in aged and impoverished rats (7,9). These results suggest that the beneficial cognitive effects of CDP-choline result at least in part from its liberation of cytidine.

In some mammalian species, including humans, cytidine apparently is minimally transported across the blood-brain barrier brain capillaries that lack the high-affinity CNT1 transport protein known to mediate pyrimidine transport (13). In contrast, uridine, which is readily formed from cytidine, can enter the brain via an unsaturated transport system. Moreover, in both humans and gerbils, unlike rats, oral CDP-choline provides the blood with uridine rather than cytidine. Hence, this study examined the effect of long-term dietary supplementation with a uridine source (UMP) on hippocampal-dependent and striatal-dependent forms of memory processing among rats exposed to EC or IC (EC-CONT, EC-UMP, IC-CONT, IC-UMP) for 3 mo starting at weaning.

Materials and Methods

The following experiment was carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Rats and diet. All rats were maintained under standard environmental conditions (room temperature, 20–25°C; relative humidity, 55–60%; light/dark schedule, 12/12 h). Seven pregnant Sprague Dawley rats (Charles River Laboratories) were obtained 1 wk before giving birth. At postnatal d 23, male pups were removed and separated into small groups and allowed to acclimatize for 1 wk. At this time, 32 rats were matched according to body wt and assigned to either the IC or EC group. One subgroup of IC rats ($n = 8$) and 1 subgroup of EC rats ($n = 8$) were given access to a control laboratory diet (IC-CONT, EC-CONT) (Teklad Global 16% protein rodent diet, TD.00217, Harlan Teklad) with a proximate composition of: crude protein (16.7%), crude fat (3.5%), and carbohydrate (60.89%). The remaining subgroups ($n = 8$/group) received this diet supplemented with UMP disodium (IC-UMP, EC-UMP) (0.1% UMP-$2Na^+$; TD.03273). This diet provides ~200 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} of UMP-$2Na^+$ or ~132 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} or uridine based on the mean daily intake of adolescent rats (data not shown). We weighed rats weekly to ensure that UMP-treated and untreated rats were eating equivalent amounts of food. We have previously shown that body wt between the EC and IC groups does not differ with the use of this EC/IC paradigm (8,9).

Rearing conditions. Rearing conditions have been previously described in detail (8,9). Briefly, rats were housed in the same rack in plastic cages (52 × 32 × 20 cm high) with wire lids. Bedding and water were regularly changed, and rats were weighed each wk, at which time general health assessments were made. Rats had ad libitum access to food and water. EC rats were housed in groups of 2–3. Plastic toys (blocks, balls, PVC tubing, etc.) placed in the EC cages were rotated between groups weekly; new toys were introduced monthly. EC rats were taken to a "playroom" (3.7 × 2 m; containing cabinets, desks, chairs, boxes, and toys) every other d for 45 min. The IC rats were housed individually, without toys, and handled 3 times/wk to acclimatize the rats to handling by the experimenter and to alleviate fear and anxiety in subsequent behavioral training procedures. To avoid the typical wt gain caused by impoverished conditions, relative to enriched rats, IC rats were allowed to exercise 3 times/wk for 15 min in an empty 1.3- × 2-m room with only the experimenter present.

Water maze apparatus. The water maze was a galvanized circular tank, 185 cm in diameter and 55 cm in height. The tank was filled with water (25°C ± 2°C) to a depth of 20 cm and located in a dimly lit room containing several extramaze cues. Four starting positions (north, south, east, and west) were spaced around the perimeter of the tank to divide the pool into 4 equal quadrants. For the visible platform 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. We mounted a video camera directly above the water maze; this camera was linked to a computer with video tracking software to automatically record the escape latency (time to reach the platform), distance traveled (length of swim path taken to find the platform), and swim speed of all rats (HVS Image).

Behavioral procedures. Memory processing was assessed in EC and IC rats given a control diet (EC-CONT, IC-CONT) or a diet enriched in UMP (EC-UMP, IC-UMP) as previously described (9). All behavioral training was carried out between 1000 and 1400 h, by an experimenter who did not know rats' treatment groups. Briefly, rats were given 4 trials/d (i.e., block) for 4 d to locate the hidden platform (1.5 cm below the water surface), which remained in the same position for all trials for individual rats (within 1 of 4 quadrants). If a rat 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, rats were removed from the maze and placed in a holding cage for a 30-s intertrial interval.

On d 5, the rats were given a probe test. For this, the platform was removed and the swim path and time spent searching in the quadrant of the pool that previously contained the platform were measured over 60 s. This provides a measurement for the retention of spatial memory and indicates whether a spatial strategy was used during hidden platform training.

Stimulus-response memory processing was assessed in UMP-treated and -untreated EC and IC rats in the visible platform water maze, as previously described (9). Briefly, rats were given 4 trials/d for 4 d to locate the visible platform; the visible escape platform was placed in a different quadrant on each of the 4 trials. If a rat 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, rats were removed from the maze and placed in a holding cage for a 30-s each trial, rats were removed from the maze and placed in a holding cage for a 30-s intertrial.

Data analysis. Results are expressed as means ± SEM. Experimental groups were compared using a 2 × 2 ANOVA (Diet and Environment) with repeated measures (block; mean of 4 trials/d) when appropriate. If overall significance was revealed by ANOVA, we conducted a Fisher's PLSD for post hoc comparison. Differences with a value of $P < 0.05$ were considered significant.

Results

Body wt. Body wt did not differ between UMP-supplemented and control groups (data not shown), indicating that rats were eating equivalent amounts of diet with or without UMP supplementation.

Effect of UMP and environmental conditions on hippocampal-dependent memory. All groups were able to learn the spatial water maze task to some degree, showing a decrease in mean escape latency (**Fig. 1***A*) and indicated by a significant main effect of day (block of 4 training trials/d) ($P < 0.001$). A main effect of environment ($P < 0.01$) and an environment × diet interaction ($P < 0.05$) were also observed. IC-UMP and EC rats treated with either diet acquired the task at a faster rate than did IC-CONT rats ($P < 0.05$). Moreover, EC rats treated with UMP tended to acquire the task at a faster rate than EC-CONT rats ($P < 0.09$). These results indicate that long-term dietary treatment with UMP prevents the spatial memory impairments caused by impoverished environmental conditions.

The results of the 60-s probe test indicated that most rats spent more time in the quadrant that originally contained the platform, suggesting that all experimental groups used spatial skills to some degree to acquire the hidden platform task (Fig. 1*B*). The percentage of swim time in the 4 quadrants during the probe test was effected by environment ($P < 0.01$), quadrant ($P < 0.001$), and diet × environment interaction ($P < 0.01$). IC-UMP and treated or untreated EC rats spent more time in the correct quadrant than did the IC-CONT rats ($P < 0.01$) during the 60-s probe test.

Effect of UMP and environmental conditions on striatal-dependent memory. All groups were able to learn the cued water maze task to some degree, showing a decrease in mean escape latency (**Fig. 2**) and indicated by a main effect of day (block of 4 training trials/d) ($P < 0.001$). No other significant main effects were determined, suggesting that environment and a UMP-supplemented diet have little or no effect on striatal-based stimulus-response forms of learning and memory.

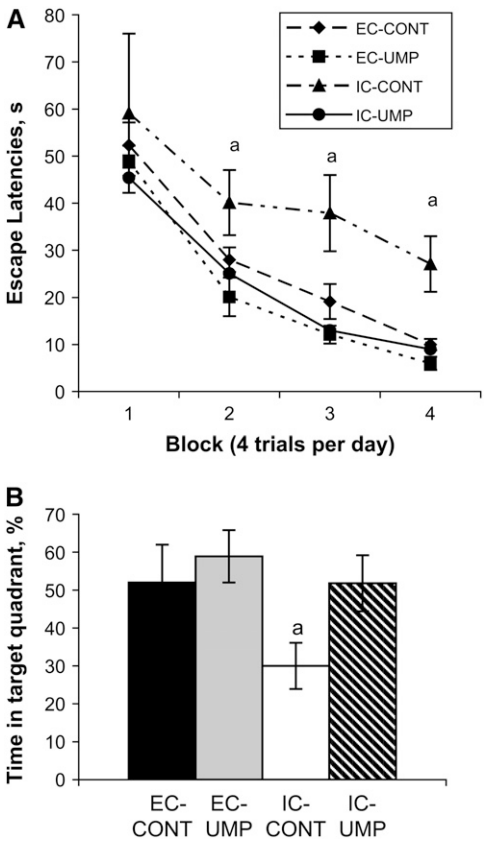

Figure 1 The effects of environment and of a UMP-supplemented diet on memory for a hippocampal-dependent hidden platform water maze task in rats reared under IC or EC procedures for 3 mo immediately postweaning. Values are means ± SEM, $n = 8$. Acquisition of the task (*A*) was affected blocks of trials ($P < 0.001$), environment ($P < 0.01$), and environment × diet interaction ($P < 0.05$). The 60-s probe task data (*B*) were affected by environment ($P < 0.01$), quadrant ($P < 0.001$), and the diet × environment interaction ($P < 0.05$). [a]Different from the other groups, $P < 0.5$.

Discussion

These data show that rats raised under IC display hippocampal-dependent cognitive memory impairments and that a long-term supplemental uridine source (0.1% UMP) supplied via the diet can protect against development of these impairments.

These results confirm and extend our previous findings (8,9) that IC rats are impaired in acquiring and/or consolidating spatial information pertaining to the location of a hidden platform, a task thought to be dependent upon an intact hippocampus. Moreover, we found that IC rats were able to learn and/or remember the location of a visible platform as well as their EC counterparts, confirming previous work that indicated environment has a limited impact on the striatal-based memory system (8,9). Thus, the IC/EC paradigm used in this and previous investigations appears to reliably elicit differences in hippocampal-dependent cognitive behavior while having no mnemonic effect on striatal-based memory processing. As the cognitive impairments seen in rats resemble those that occur during normal aging, this IC/EC paradigm may be an effective way to study the molecular changes that accompany the hippocampal dysfunction in aged mammals.

Chronic supplementation with UMP completely ameliorated the learning and memory deficit in IC rats, and mildly enhanced the performance of EC rats, in the hippocampal-dependent water maze task. The memory-enhancing effect does not appear to involve alterations in sensorimotor performance or motivation, because UMP treatment had no effect on striatal-based mnemonic memory processes in either IC or EC rats. We have previously shown that, whereas long-term treatment with CDP-choline prevented the memory impairments in aged and IC rats, supplementation with this compound had no beneficial cognitive effects in young or enriched rats (7,9). These findings indicate

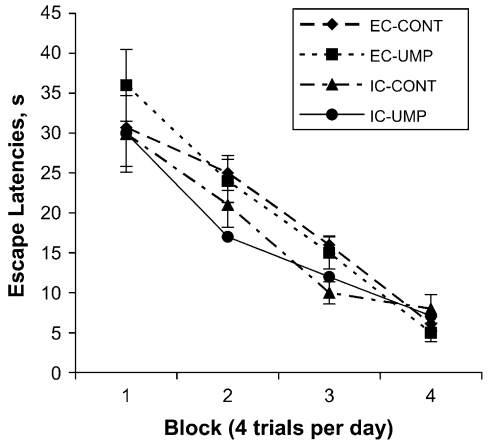

Figure 2 The effects of environment and of a UMP-supplemented diet on memory for a striatal-dependent visible platform water maze task in rats reared under IC or EC procedures for 3 mo immediately postweaning. The significance of differences among the 4 experimental groups with respect to acquisition of the task was determined by a 2 × 2 (diet and environment) ANOVA with repeated measures (block); a main effect of block was determined ($P < 0.001$).

that supplementation with a diet that increases uridine levels (UMP) not only prevents the hippocampal-dependent memory deficits that occur in rats raised under impoverished environmental conditions, but that this dietary treatment may have beneficial mnemonic properties in enriched rats as well.

Although the mechanisms of UMP's (and presumably cytidine's) beneficial effects on hippocampal-dependent forms of cognition remain to be elucidated, several interesting possibilities exist. Oral administration of UMP elevates plasma uridine and then sequentially elevates brain levels of uridine, UTP, CTP, and CDP-choline (14), key intermediates in the biosynthesis of phosphatides. In fact, the synthesis of the phosphatide PC (the most abundant constituent of cellular membranes) is increased by consumption of a dietary uridine source (14). PC and other phosphatides (phosphatidylinositol, spingomyelin) act not only as structural constituents of cellular membranes but also as reservoirs for the production of many first and second messengers, including eicosanoids, diacylglcerol, inositol triphosphate, and platelet-activating factor). All of these molecules are required for memory consolidation (7,15,16); thus, it is possible that supplementation with UMP prevents the memory impairment that occurs in impoverished rats by increasing membrane phosphatide levels and their related signal transduction cascades. Moreover, the enhancement of membrane phosphatide synthesis can increase the release of neurotransmitters (striatal dopamine) (17), possibly by increasing synaptic membranes (18).

In conclusion, the present data suggest that chronic dietary supplementation with UMP might prove a useful therapeutic strategy to delay or diminish the cognitive deficits associated with poor environmental conditions or aging, as well as the changes in membrane lipid composition associated with the aging process. Because the membrane lipid composition can be significantly affected by the aging process, it is feasible that the protective function may arise from UMP's ability to enhance the production of brain membrane phosphatides.

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