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Research Report

Oral uridine-5′-monophosphate (UMP) increases brain CDP-choline levels in gerbils

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Abstract

We examined the biochemical pathways whereby oral uridine-5′-monophosphate (UMP) increases membrane phosphatide synthesis in brains of gerbils. We previously showed that supplementing PC12 cells with uridine caused concentration-related increases in CDP-choline levels, and that this effect was mediated by elevations in intracellular uridine triphosphate (UTP) and cytidine triphosphate (CTP). In the present study, adult gerbils received UMP (1 mmol/kg), a constituent of human breast milk and infant formulas, by gavage, and plasma samples and brains were collected for assay between 5 min and 8 h thereafter. Thirty minutes after gavage, plasma uridine levels were increased from 6.6 ± 0.58 to 32.7 ± 1.85 μM ($P < 0.001$), and brain uridine from 22.6 ± 2.9 to 89.1 ± 8.82 pmol/mg tissue ($P < 0.001$). UMP also significantly increased plasma and brain cytidine levels; however, both basally and following UMP, these levels were much lower than those of uridine. Brain UTP, CTP, and CDP-choline were all elevated 15 min after UMP (from 254 ± 31.9 to 417 ± 50.2, [$P < 0.05$]; 56.8 ± 1.8 to 71.7 ± 1.8, [$P < 0.001$]; and 11.3 ± 0.5 to 16.4 ± 1, [$P < 0.001$] pmol/mg tissue, respectively), returning to basal levels after 20 and 30 min. The smallest UMP dose that significantly increased brain CDP-choline was 0.05 mmol/kg. These results show that oral UMP, a uridine source, enhances the synthesis of CDP-choline, the immediate precursor of PC, in gerbil brain.

Theme: Cellular and molecular biology
Topic: Membrane composition and cell-surface macromolecules

Keywords: UMP; Uridine; Cytidine; CDP-choline; CTP; PC

1. Introduction

Phosphatides are major constituents of cell membranes, forming the lipid bilayer and serving as reservoirs for such first and second messengers or their precursors as acetylcholine, eicosanoids, diacylglycerol (DAG), and inositol 1,4,5-trisphosphate (IP_3). In brain, the phosphatides phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are generated primarily via the CDP-choline and CDP-ethanolamine pathways ("Kennedy cycle") [15], whereas phosphatidylserine (PS), the third structural phosphatide, is formed from PC or PE via base-exchange [12,17]. PC, the most abundant phosphatide in neuronal membranes, is synthesized via a pathway that involves three enzymatic reactions, i.e., the phosphorylation of free choline to phosphocholine; the reaction of phosphocholine with cytidine triphosphate (CTP) to yield CDP-choline; and the reaction of CDP-

Abbreviations: UMP, uridine-5′-monophosphate; UTP, uridine triphosphate; CTP, cytidine triphosphate; CDP-choline, cytidine-5′-diphosphocholine; DAG, 1,2-diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; BBB, blood–brain barrier

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choline with DAG. The rate of PC synthesis can be regulated at each of these steps by processes that change the levels of the substrates choline [21], CTP [28], or DAG [3]; however, CTP levels are thought to be most important in overall PC synthesis under usual conditions. It is difficult to detect changes in PC levels after short treatments, because the pool size of cellular PC is large, and its mean turnover rate is relatively slow [4]. However, oral treatment of rats with CDP-choline, which in this species provides supplemental choline and cytidine, for a relatively long (6 weeks) period, did significantly elevate PC levels per brain cell by 19–22% [20]. Changes in PC synthesis can be more readily detected by measuring endogenous brain levels of CDP-choline, PC's immediate precursor, since these are orders of magnitude lower than those of PC. Hence, providing the brain with supplemental choline or with cytidine or uridine – the precursors of CTP – might be expected to increase levels of this marker of PC synthesis [27].

Uridine, a pyrimidine nucleoside, is a constituent of nucleotides, UDP-sugars, and nucleic acids. It is obtained both from dietary sources and, principally, de novo synthesis. UDP-sugars are intermediates in glycogen synthesis, while UTP is both a direct agonist for several P2Y purinergic receptors and an essential constituent of RNA. In humans, uridine is the principal circulating pyrimidine [33], but in rats, the principal circulating pyrimidine is cytidine, not uridine [33]. Pyrimidine metabolism in gerbils more closely resembles that in humans, and plasma uridine concentrations (6–7 μM) in this rodent species are, as in humans, substantially higher than those of cytidine (1.2–1.3 μM). For this reason, we used Mongolian gerbils and not rats in this study of plasma and brain uridine metabolism.

We used uridine-5′-monophosphate (UMP), as the uridine source in our study, because it is known to have few if any significant side effects; it is a constituent of human breast milk and infant formulas. In a recent study, we showed that adding cytidine or uridine to the medium in vitro caused dose-related increases in CDP-choline levels of PC12 cells [27]. These increases were shown to be mediated by elevations in the levels of intracellular uridine triphosphate (UTP) and ultimately, cytidine triphosphate (CTP). Hence, in the present study, we evaluated the ability of oral UMP to increase brain levels of CDP-choline, as well as those of its precursors UTP and CTP, in vivo.

2. Materials and methods

2.1. Animals

Male gerbils (*M. unguiculatus*) (Charles River Laboratories, Boston, MA, USA; 60–80 g) were housed two per cage and allowed free access to food (regular laboratory chow, not including any added nucleoside) and water. The experiments were conducted around 10 am. Two hours before an experiment, food and water were removed. All experiments were carried out in accordance with 1996 Guide for the Care and Use of Laboratory Animals (National Institute of Health) and Massachusetts Institute of Technology policies.

2.2. Experimental protocol

In the first set of experiments, gerbils were anesthetized with Telazol (80 mg/kg) after having received water (1 ml/kg) or UMP (1 mmol/kg) 5, 10, 15, 20, 30, 60, 120, 240, or 480 min earlier by gavage. Their heads were shaved and dipped [34] into liquid nitrogen and trunk blood and brain samples were then obtained. Time "zero" represents the control group in which gerbils were subjected to anesthesia only.

In a second set, water (1 ml/kg) or various doses of UMP (0.02, 0.05, 0.1, or 1 mmol/kg) were administered by gavage and blood and brain samples were obtained 15 min thereafter as above. Dose "zero" represents the water-treated control group.

2.3. Preparation of samples

The frozen heads were removed using a guillotine and stored on dry ice; trunk blood was then collected into tubes containing EDTA (50 mg/ml blood). Brain samples were obtained using a dental trephine (7 mm diameter; George Tiemann and Company, Hauppauge, NY, USA) attached to a drill. The skull and dura were removed, and a sample of brain tissue, mostly derived from the cortex, was taken. The brain sample was then divided into two parts which were wrapped in foils and weighed immediately. One brain sample used for nucleotide assay was homogenized using a tissue degrader (Polytron PT 1200, Kinematica AG, Switzerland) in prechilled 0.4 N $HClO_4$ (1 ml/100 mg tissue) [5] which contained 10 μM bromoUTP as an internal standard. Aliquots were then centrifuged, and the pH of each supernatant fluid was adjusted to 6–6.5 using 6N KOH. Following removal of the $KClO_4$ by centrifugation [5], the extract was kept at −80 °C until components were measured by HPLC. The other brain sample that was used for nucleoside and CDP-choline assays was homogenized in 80% methanol. Aliquots were then centrifuged and supernatants were lyophilized; dried samples were reconstituted with 100–200 μl of deionized water, divided in two equal parts and kept at −80 °C until assayed for nucleosides and CDP-choline. Blood samples were centrifuged for 20 min at 3000 rpm and plasmas as well as the brain aliquots kept for nucleoside assay were subjected to a boronate affinity column procedure [27]. A known amount of 5-fluorouridine was used as an internal standard.

2.4. Analysis of nucleosides, nucleotides, and of CDP-choline

Brain and plasma uridine and cytidine were analyzed by a modification of the method of Savci and Wurtman [29]

as described by Richardson et al. [27]. Following fractionation on boronate affinity columns, samples were analyzed by HPLC on a reversed-phase column (Dynamax Microsorb C-18, 5 μm, 250 × 4.6 mm) using 4 mM potassium phosphate buffer containing 0.1% methanol at pH 5.8. Individual peaks were detected by UV absorption at 280 nm and were identified by comparison with the positions of authentic standards. Recoveries were determined by comparison with known amounts of 5-fluorouridine. The retention times were 6.3 and 8.6 min for cytidine and uridine, respectively.

Brain UTP, CTP, and CDP-choline were also analyzed by HPLC on an anion-exchange column (Alltech Hypersil APS-1, 3 μm, 150 × 4.6 mm) kept at 37 °C, with a flow rate of 1 ml/min. Individual peaks were detected by UV absorption at 280 nm, and were identified by comparisons with the positions of authentic standards. UTP and CTP were analyzed by a modification of the method of Richardson et al. [27], using an isocratic buffer containing 200 mM NaH_2PO_4 at pH 2.8. Retention times for CTP and UTP were 8.9 and 23.1 min, respectively. Recoveries for the nucleotides were determined by comparisons with known amounts of bromoUTP. In a separate run, CDP-choline was analyzed using an isocratic buffer containing 1.75 mM H_3PO_4 at pH 2.9 with a flow rate of 1 ml/min as described by Richardson et al. [27]. Retention time for CDP-choline was 6.5 min (CDP-ethanolamine was readily distinguished from CDP-choline; its retention time was 3.2 min).

2.5. Data analysis

Statistical analyses were carried out using Systat 10.0. Data were represented as means ± SEM. Unpaired Student's *t* test was used to compare the effect of 1 mmol/kg UMP with that of water controls at respective time points. Analysis of variance (ANOVA) followed by Tukey test was used to compare the effects of different doses of UMP with those of water controls at 15 min. The significance level was set at $P < 0.05$.

3. Results

3.1. Oral UMP increases plasma uridine and cytidine levels

UMP, administered orally, is rapidly degraded to uridine in the digestive system and this uridine enters the systemic circulation. Plasma uridine levels were indeed significantly elevated 10 min after the gerbils received UMP (1 mmol/kg), i.e., from 6.6 ± 0.58 μM to 32.7 ± 1.8 μM ($P < 0.001$) at 30 min (Fig. 1A), and remained so for at least 4 h. Plasma cytidine levels, which in this species were always much lower than those of uridine, also were increased 30 min after UMP, from 1.2 ± 0.2 μM to 1.9 ± 0.1 μM ($P < 0.001$); however, these increases persisted for only 1 h (Fig. 1B).

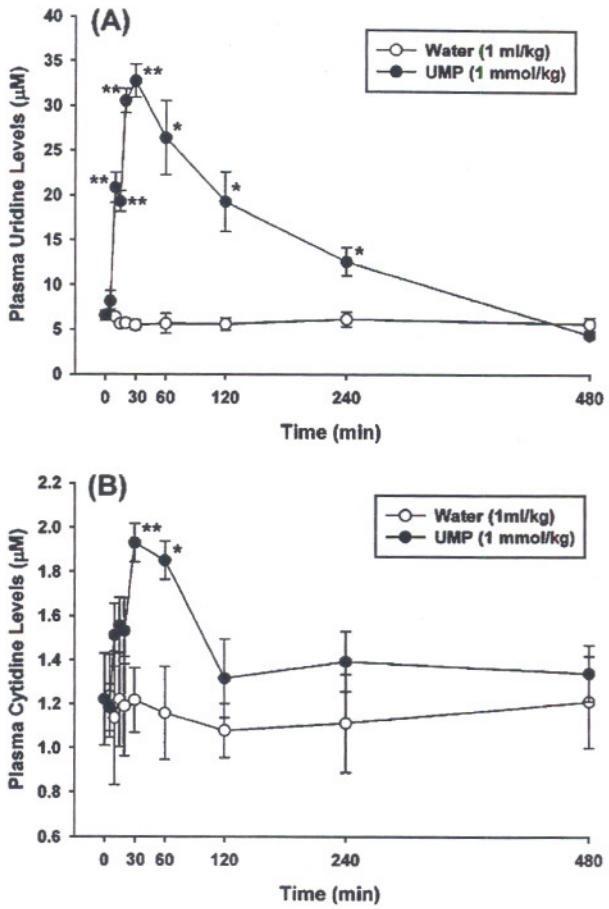

Fig. 1. Effect of UMP on plasma uridine and cytidine levels. Plasma (A) uridine and (B) cytidine levels in gerbils given oral UMP (1 mmol/kg). Basal plasma uridine at zero time was 6.6 ± 0.58 μM ($n = 8$). These levels increased significantly by 10 min after UMP administration, and remained significantly elevated for up to 4 h. Highest elevations ($P < 0.001$) were observed 30 min after UMP (32.7 ± 1.8 μM, $n = 20$). *$P < 0.05$ and **$P < 0.001$ compared to corresponding water-treated controls. Basal plasma cytidine at zero time was 1.2 ± 0.2 μM ($n = 8$). These levels increased significantly ($P < 0.001$), yielding the highest levels, 30 min after UMP administration (1.9 ± 0.1 μM, $n = 20$). No further increases were observed after 1 h. *$P < 0.05$ and **$P < 0.001$ compared to corresponding water-treated controls. (UMP: Uridine-5′-monophosphate, μM: micromolar).

3.2. Brain uridine and cytidine are elevated following oral UMP

UMP (1 mmol/kg), also significantly elevated brain uridine levels 10 min after its oral administration, i.e., from 22.6 ± 2.9 to 89.1 ± 8.8 pmol/mg tissue ($P < 0.001$) at 30 min (Fig. 2A), and these increases also persisted for at least 4 h. Brain cytidine levels, which also were always much lower in gerbils than those of uridine, increased after 30 min, and remained significantly elevated for 2, but not 4 h. Highest brain cytidine levels were observed 60 min after UMP administration, rising from 5.9 ± 0.4 to 12.1 ± 1.1 pmol/mg tissue ($P < 0.05$) (Fig. 2B).

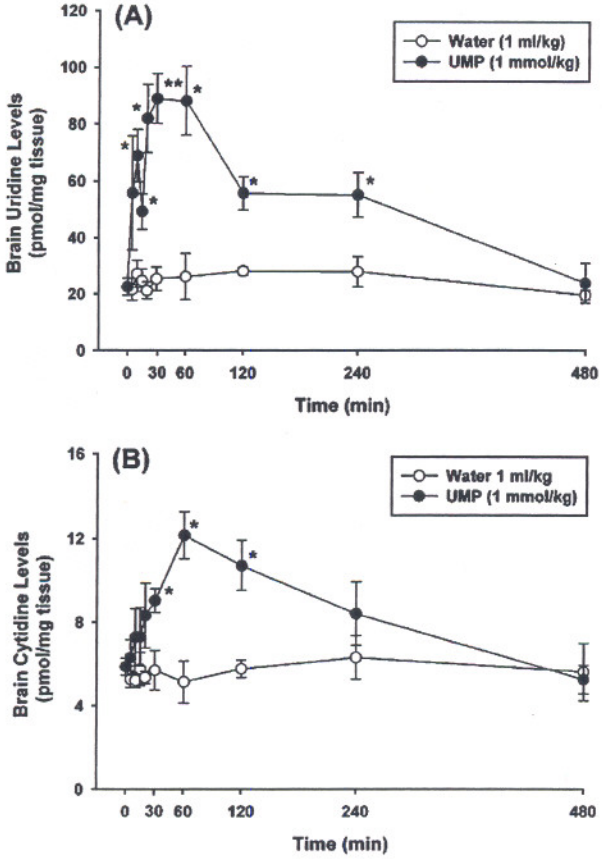

Fig. 2. Effect of UMP on brain uridine and cytidine levels. Brain (A) uridine and (B) cytidine levels in gerbils given oral UMP (1 mmol/kg). Basal brain uridine at zero time was found to be 22.5 ± 2.9 μM (n = 6). These levels increased significantly 10 min after UMP administration, and remained significantly elevated for up to 4 h. Highest elevations (P < 0.001) were observed 30 min after UMP (89.1 ± 8.8 μM, n = 18). *P < 0.05 and **P < 0.001 compared to corresponding water-treated controls. Basal brain cytidine at zero time was 5.8 ± 0.4 μM (n = 6). Brain cytidine levels increased significantly 30 min after UMP administration, and remained significantly elevated for up to 2 h. Highest elevations (P < 0.001) were observed 60 min after UMP (12.1 ± 1.1 μM, n = 18). *P < 0.05 and **P < 0.001 compared to corresponding water-treated controls. (UMP: Uridine-5′-monophosphate).

3.3. Oral UMP rapidly elevates brain UTP and CTP

Brain UTP levels increased significantly 15 min after UMP (1 mmol/kg) administration rising from 254 ± 31.9 to 417 ± 50.2 pmol/mg tissue (P < 0.05) (Fig. 3A). Brain CTP levels similarly rose from 56.8 ± 1.8 to 71.7 ± 1.8 pmol/mg tissue (P < 0.001) after 15 min (Fig. 3B). Both brain UTP and CTP levels returned to baseline by 1 h after UMP administration.

3.4. Oral UMP increases brain CDP-choline levels

In the rate-limiting step of the Kennedy cycle for phosphatide biosynthesis, CTP combines with phosphocholine to yield CDP-choline [15]. Consistent with this role for CTP, brain CDP-choline levels were significantly elevated 10–20 min after UMP administration. Highest levels were observed 15 min after UMP, CDP-choline increasing from 11.3 ± 0.5 to 16.4 ± 1 pmol/mg tissue (P < 0.001) (Fig. 4).

3.5. Effects of various doses of UMP on plasma and brain uridine levels

We examined the effects of various oral UMP doses (0.02, 0.05, 0.1, or 1 mmol/kg) on plasma and brain uridine levels in gerbils sacrificed 15 min after receiving the UMP. Plasma uridine rose from 5.6 ± 0.6 to 9.5 ± 0.6 (P < 0.001); 13.9 ± 2.4 (P < 0.05); 14.2 ± 1.5 (P <

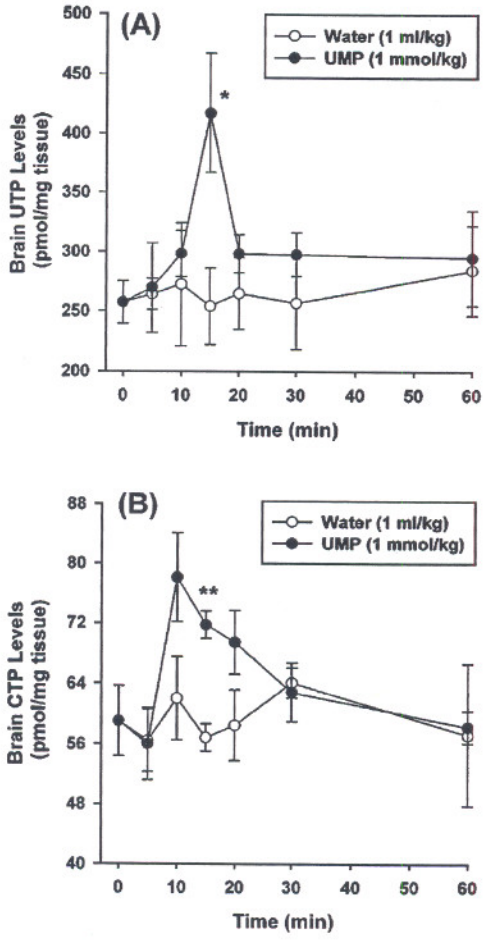

Fig. 3. Effect of UMP on brain UTP and CTP levels. Brain (A) UTP and (B) CTP levels in gerbils given oral UMP (1 mmol/kg). Basal brain UTP at zero time was 253.9 ± 31.9 pmol/mg tissue (n = 6). These levels increased significantly (P < 0.05) 15 min after UMP administration (416.9 ± 50.2 pmol/mg tissue, n = 8). No further increases were observed. *P < 0.05 compared to corresponding water-treated controls. Basal brain CTP at zero time was 56.8 ± 1.8 pmol/mg tissue (n = 6). These levels increased significantly (P < 0.001) 15 min after UMP administration (71.7 ± 1.8 pmol/mg tissue, n = 8). No further increases were observed. **P < 0.001 compared to corresponding water-treated controls. (UMP: Uridine-5′-monophosphate, UTP: Uridine triphosphate, CTP: Cytidine triphosphate).

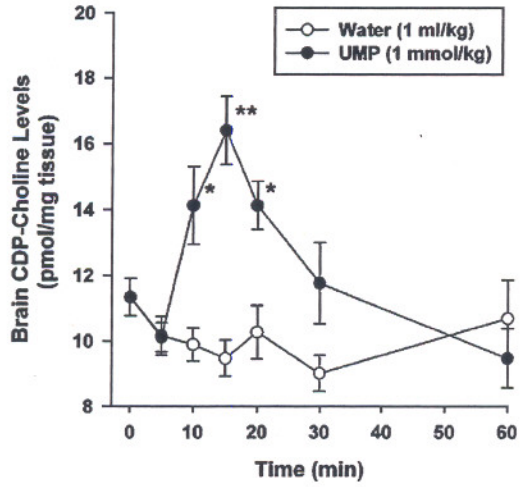

Fig. 4. Effect of UMP on brain CDP-choline levels. Brain CDP-choline levels in gerbils given oral UMP (1 mmol/kg). Basal brain CDP-choline at zero time was 11.3 ± 0.5 pmol/mg tissue (n = 8). These levels increased significantly 10 min after UMP administration, and returned to control values by 30 min. Highest elevations ($P < 0.001$) were observed 15 min after CDP-choline (16.4 ± 1 pmol/mg tissue, n = 8). *$P < 0.05$ and **$P < 0.001$ compared to corresponding water-treated controls. (UMP: Uridine-5′-monophosphate, CDP-choline: Cytidine-5′-diphosphocholine).

0.001); and 20.1 ± 1.7 μM ($P < 0.001$) after 0.02, 0.05, 0.1, or 1 mmol/kg doses, respectively (Fig. 5A). Brain uridine levels also increased from 22.6 ± 2.9 to 47.7 ± 2.8 ($P < 0.001$) after the 0.02 mmol/kg dose. Higher doses of UMP did not further elevate brain uridine levels; these levels were 42.6 ± 6 ($P < 0.05$); 40 ± 2.6 ($P < 0.05$); and 49 ± 2.6 pmol/mg tissue ($P < 0.001$) after the 0.05, 0.1, and 1 mmol/kg doses (Fig. 5B).

Plasma and brain cytidine levels failed to change significantly 15 min after the UMP doses tested (data not shown).

3.6. Effects of various doses of UMP on brain UTP and CTP levels

Brain UTP and CTP levels failed to increase 15 min after the 0.02 mmol/kg dose; however, higher doses caused significant increases. Brain UTP levels rose from 254 ± 31.9 to 388 ± 39.6 ($P < 0.05$); 390 ± 26.6 ($P < 0.05$); and 407 ± 21.4 pmol/mg tissue ($P < 0.05$), respectively, after the 0.05, 0.1, and 1 mmol/kg doses (Fig. 6A), and brain CTP levels rose from 56.8 ±1.8 to 71.6 ± 5.9 ($P < 0.05$); 75.9 ± 5.7 ($P < 0.05$); and to 73.4 ± 2.9 pmol/mg tissue ($P < 0.001$) (Fig. 6B).

3.7. Effects of various doses of UMP on brain CDP-choline levels

Brain CDP-choline levels increased from 10.1 ± 0.5 to 15.4 ± 0.9 ($P < 0.05$) after the 0.05 mmol/kg dose. Higher doses did not cause significantly greater increases, brain CDP-choline levels rising to 15.6 ± 1.1 ($P < 0.05$); and 17.1 ± 0.9 pmol/mg tissue ($P < 0.001$), 15 min after 0.1 and 1 mmol/kg of oral UMP, respectively (Fig. 7).

4. Discussion

These data show that oral uridine-5′-monophosphate (UMP) increased plasma and brain uridine levels as well as brain UTP, CTP, and CDP-choline in male Mongolian gerbils. All of these increases were time-dependent, and all except the increases in brain uridine levels were dose-dependent. Other investigators [16,25,36], studying other species, showed similar increases in plasma uridine after administration of uridine itself; ours is apparently the first study to show that administering a uridine source accelerates the usual rate-limiting step in phosphatidylcholine synthesis,

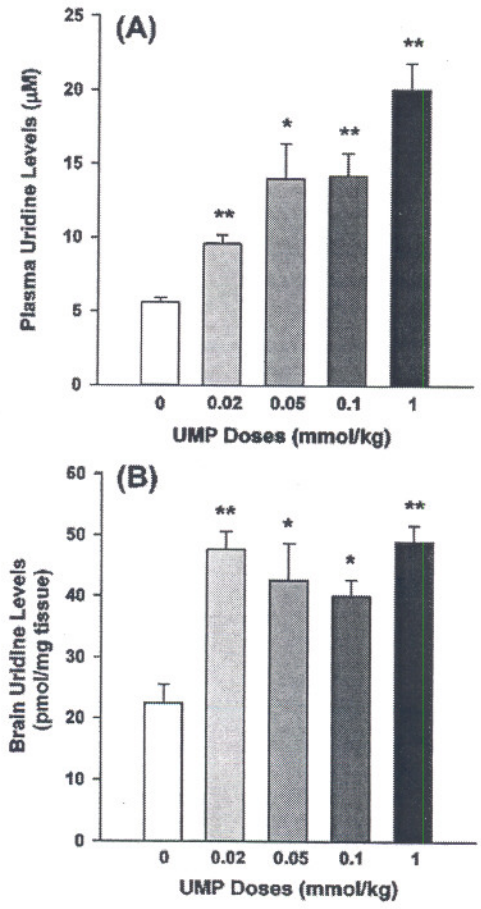

Fig. 5. Effect of various UMP doses on plasma and brain uridine levels. Plasma (A) and brain (B) uridine levels in gerbils 15 min after various UMP doses. Dose "zero" represents water (1 ml/kg) treatment. UMP (0.02, 0.05, 0.1, or 1 mmol/kg) increased plasma uridine levels significantly after 15 min. *$P < 0.05$ and **$P < 0.001$ compared to water-treated controls (5.6 ± 0.3 μM, n = 6) at 15 min. UMP (0.02, 0.05, 0.1, or 1 mmol/kg) caused significant increases in brain uridine levels after 15 min. *$P < 0.05$ and **$P < 0.001$ compared to water-treated controls (22.5 ± 2.9 pmol/mg tissue, n = 6) at 15 min. (UMP: Uridine-5′-monophosphate, μM: micromolar).

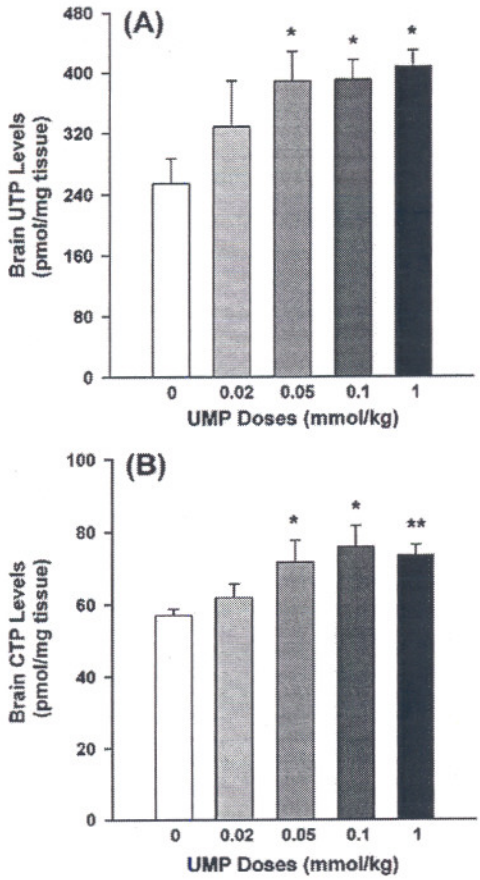

Fig. 6. Effect of various UMP doses on brain UTP and CTP levels. Brain (A) UTP (B) CTP levels in gerbils 15 min after various UMP doses. Dose "zero" represents water (1 ml/kg) treatment. UMP (0.05, 0.1, or 1 mmol/kg) increased brain UTP levels significantly after 15 min. $^{*}P < 0.05$ compared to water-treated controls (253.9 ± 31.9 pmol/mg tissue, $n = 6$) at 15 min. UMP (0.05, 0.1, or 1 mmol/kg) caused significant increases in brain CTP levels after 15 min. $^{*}P < 0.05$ and $^{**}P < 0.001$ compared to water treated controls (56.8 ± 1.8 pmol/mg tissue) at 15 min. (UMP: Uridine-5′-monophosphate, UTP: Uridine triphosphate, CTP: Cytidine triphosphate).

the formation of CDP-choline. Highest plasma uridine levels, in animals receiving our highest UMP dose (1 mmol/kg), were observed 30 min after its oral administration, and these levels remained significantly elevated for up to 4 h (Fig. 1A). Lower doses of UMP (0.02, 0.05, or 0.1 mmol/kg), also caused dose-related increases in plasma uridine (Fig. 5A). The large increases in plasma uridine levels indicate that plasma uridine is poorly regulated, if at all, and – like the amino acids after eating [8] – are allowed to vary over a broad dynamic range when uridine-containing compounds are administered. Similar conclusions have been drawn by others [14,16,36].

Plasma uridine is known to cross the blood–brain barrier (BBB) [7], and, in sufficient doses, can thereby increase brain uridine levels (Fig. 2A). We found that an effective dose to elevate brain uridine could be as low as 0.02 mmol/kg (Fig. 5B), and higher doses did not produce greater increases. The 0.02 mmol/kg dose increased plasma uridine from 5.6 ± 0.6 to 9.5 ± 0.6 μM (Fig. 5A), suggesting that the BBB transporter protein for uridine uptake may become saturated at plasma uridine concentrations of about 10 μM. Indeed, the Km for uridine transport by a protein, N1, which was initially studied in vitro in kidney, liver, and other tissues not including BBB, was estimated to range between 9 and 40 μM [11] (this protein is now recognized as CNT2 [18,24], a member of the concentrative nucleoside transporter family SLC28 [10], as discussed below). We estimated brain uridine concentrations by measuring them in pmol/mg tissue and assuming that 1 g of tissue corresponded to 1 ml of brain. Using this approach, we found that brain uridine concentrations were substantially greater than those in plasma; moreover, they continued to be greater than those in plasma after all of the UMP doses tested (Figs. 1A and 2A).

Plasma cytidine levels were also significantly elevated 30 min after oral UMP (1 mmol/kg), returning to control levels after 1 h (Fig. 1B). In correlation with previous findings in humans [40], we found that basal plasma cytidine levels in gerbils (1.2–1.3 μM) were substantially lower than those of uridine (6–7 μM); moreover, the increases in cytidine after UMP treatment were much smaller in magnitude than those of uridine (1.6-fold vs. 5-fold 30 min after oral UMP, for cytidine and uridine, respectively) (Fig. 1). The increase in plasma cytidine after UMP could reflect amination of uridine to cytidine within liver or other peripheral organs, in the presence of high uridine levels, or amination of a uridine nucleotide followed by hydrolysis [13].

Unlike uridine, cytidine may not be transported across the BBB to a significant extent; brain capillaries apparently lack the high-affinity CNT1 transport protein which is known to mediate pyrimidine transport [18]. Instead, rat

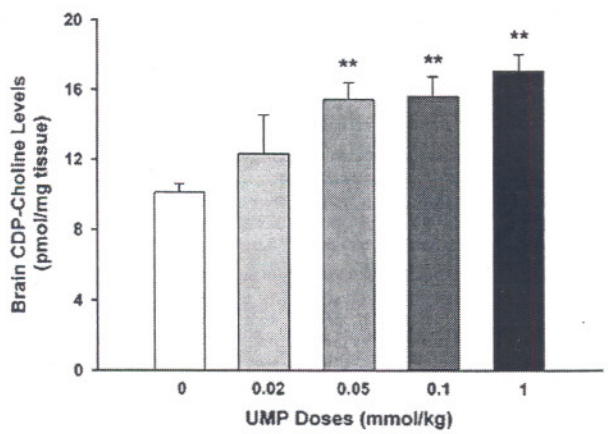

Fig. 7. Effect of various UMP doses on brain CDP-choline levels. Brain CDP-choline levels in gerbils 15 min after various UMP doses. Dose "zero" represents water (1 ml/kg) treatment. UMP (0.05, 0.1 or 1 mmol/kg) caused significant increases in brain CDP-choline levels after 15 min. $^{**}P < 0.001$ compared to water-treated controls (10.1 ± 0.5 pmol/mg tissue) at 15 min. (UMP: Uridine-5′-monophosphate, CDP-choline: Cytidine-5′-diphosphocholine).

BBB contains high-affinity CNT2 proteins which mediate the transport of purines as well as the pyrimidine uridine [18]. Mouse BBB reportedly contains a low-affinity ENT1 system [22] which can transport a variety of both purines and pyrimidines [11]. The transport protein mediating the passage of uridine across the gerbil or human BBB has not yet been identified, but our in vivo data also suggest a rapid and efficient transport of uridine into gerbil brain (Fig. 2A). The increase in brain cytidine levels (Fig. 2B) that we observed 30 min after UMP administration could reflect transport of plasma cytidine into cerebrospinal fluid by the choroid plexus [1,2,38,39], and then into brain. However, such transport is much less efficient than BBB transport since the surface area of BBB is about 1000 times that of choroid plexus [23]. As discussed below, the rise in brain cytidine could also reflect conversion of brain uridine to UTP and then to CTP, some of which could be dephosphorylated to cytidine (Figs. 2 and 3). Since the maximal increase in brain cytidine levels after UMP administration was actually preceded by significant increases in CTP and CDP-choline, it seems unlikely that an elevation in brain cytidine is responsible for those increases. The failure of the elevations in brain cytidine to increase brain CTP or its product CDP-choline could reflect feedback inhibition of uridine–cytidine kinase by UTP or CTP [6].

Brain UTP levels were increased 15 min after UMP administration, returning to control levels soon thereafter (Fig. 3A). This rapid return may again reflect feedback inhibition of uridine–cytidine kinase by UTP [6]. This mechanism could also explain the failure of higher UMP doses to provide greater increases in brain UTP than that seen after the 0.05 mmol/kg dose (Fig. 6A).

The UMP-induced increase in intracellular UTP levels might give rise to significant UTP release from brain cells, causing activation of membrane P2Y receptors and thereby affecting cell metabolism. Pooler et al. [26] found that the ability of uridine to enhance NGF-induced neurite outgrowth in vitro could be suppressed by drugs that block pyrimidine-sensitive P2Y receptors. Some of the newly formed UTP apparently is converted to CTP, levels of which also rose significantly 15 min after UMP in the present study (Fig. 3B); these increases in CTP exhibited the same dose–response relationship to UMP dose as did the increases in UTP (Fig. 6B), peaking after the low 0.05 mmol/kg dose. Enzymatic amination of UTP to CTP [19] has been shown to occur in brain [9]. This amination is potentially very important, inasmuch as the product, CTP, is, as discussed above, rate-limiting in the Kennedy cycle [15] of membrane PC synthesis.

The present data also show that oral UMP increases CDP-choline levels in gerbil brain; these were elevated 10–20 min after UMP (1 mmol/kg) administration, peaking after 15 min at levels about 50% greater than those present basally (Fig. 4). Significant increases in CDP-choline levels were also observed after lower UMP doses (0.05 and 0.1 mmol/kg) (Fig. 7). A similar UTP-mediated effect of uridine on cellular CDP-choline levels was previously observed in PC12 cells [27].

The significance of the observed increase in endogenous CDP-choline production is that this compound is beyond the usual rate-limiting step in PC synthesis [15]. We did not expect cellular PC levels to exhibit measurable increases minutes after the single dose of UMP, and thus did not measure phosphatides. However, we previously observed that when rats received another pyrimidine (and choline) source, i.e., CDP-choline, for 6 weeks, brain PC levels per cell increased significantly by 19–22% [20]. In that circumstance and after various other treatments that principally affected the levels of a single phosphatide [30,35], the stoichiometric relationships between PC, PE, and PS in the cell membrane tended to be maintained, suggesting that functionally-normal, and not PC-enriched, membrane was being formed. The UMP-induced increase in brain phosphatide synthesis is likely associated both with enhanced release of certain neurotransmitters (e.g., K^+-evoked striatal dopamine release in rats [37]) and with increases in the number of neurites produced by PC12 cells in response to nerve growth factor (NGF) [26]. Moreover, administration of oral CDP-choline for 6 weeks can restore certain cognitive functions in aged rats [31] or in rats maintained in a socially-restricted environment [32]. Thus, the increase in phosphatide intermediate synthesis observed here after a single dose of UMP suggests a process by which uridine availability might affect membrane synthesis, neuronal function, and perhaps behavior.

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References

[1] C.M. Anderson, W. Xiong, J.D. Geiger, J.D. Young, C.E. Cass, S.A. Baldwin, F.E. Parkinson, Distribution of equilibrative, nitrobenzylthioinosine-insensitive nucleoside transporters (ENT1) in rat brain, J. Neurochem. 73 (1999) 867–873.

[2] C.M. Anderson, S.A. Baldwin, J.D. Young, C.E. Cass, F.E. Parkinson, Distribution of mRNA encoding a nitrobenzylthioinosine-insensitive nucleoside transporter (ENT2) in rat brain, Brain Res. Mol. Brain Res. 70 (1999) 293–297.

[3] W. Araki, R.J. Wurtman, Control of membrane phosphatidylcholine biosynthesis by diacylglycerol levels in neuronal cells undergoing neurite outgrowth, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11946–11950.

[4] W. Araki, R.J. Wurtman, How is membrane phospholipid biosynthesis controlled in neural tissues? J. Neurosci. Res. 51 (1998) 667–674.
[5] P. Bernocchi, C. Ceconi, A. Cargnoni, P. Pedersini, S. Curello, R. Ferrari, Extraction and assay of creatine phosphate, purine and pyridine nucleotides in cardiac tissue by reversed-phase high-performance liquid chromatography, Anal. Biochem. 222 (1994) 374–379.
[6] N. Cheng, R.C. Payne, T.W. Traut, Regulation of uridine kinase. Evidence for a regulatory site, J. Biol. Chem. 261 (1986) 13006–13012.
[7] E.M. Cornford, W.H. Oldendorf, Independent blood–brain barrier transport systems for nucleic acid precursors, Biochim. Biophys. Acta 394 (1975) 211–219.
[8] J.D. Fernstrom, R.J. Wurtman, B. Hammarstrom-Wiklund, W.M. Rand, H.N. Munro, C.S. Davidson, Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino-acids: effect of dietary protein intake, Am. J. Clin. Nutr. 32 (1979) 1912–1922.
[9] D.D. Genchev, P. Mandel, CTP synthetase activity in neonatal and adult rat brain, J. Neurochem. 22 (1974) 1027–1030.
[10] J.H. Gray, R.P. Owen, K.M. Giacomini, The concentrative nucleoside transporter family, SLC28, Pflugers Arch. Eur. J. Physiol. 447 (2004) 728–734.
[11] D.A. Griffith, S.M. Jarvis, Nucleoside and nucleobase transport systems of mammalian cells, Biochem. Biophys. Acta 1286 (1996) 153–181.
[12] G. Hubscher, R.R. Dils, W.F. Pover, Studies on the biosynthesis of phosphatidyl serine, Biochim. Biophys. Acta 36 (1959) 518–528.
[13] R.B. Hurlbert, H.O. Kammen, Formation of cytidine nucleotides from uridine nucleotides by soluble mammalian enzymes: requirements for glutamine and guanosine nucleotides, J. Biol. Chem. 235 (1960) 443–449.
[14] J.M. Karle, L.W. Anderson, D.D. Dietrick, R.L. Cysyk, Determination of serum and plasma uridine levels in mice, rats, and humans by high-pressure liquid chromatography, Anal. Biochem. 109 (1980) 41–46.
[15] E.M. Kennedy, S.B. Weiss, The function of cytidine coenzymes in the biosynthesis of phospholipids, J. Biol. Chem. 222 (1956) 193–214.
[16] P. Klubes, D.B. Geffen, R.L. Cysyk, Comparison of the bioavailability of uridine in mice after either oral or parenteral administration, Cancer Chemother. Pharmacol. 17 (1986) 236–240.
[17] O. Kuge, M. Nishijima, Phosphatidylserine synthase I and II of mammalian cells, Biochim. Biophys. Acta 1348 (1997) 151–156.
[18] J.Y. Li, R.J. Boado, W.M. Pardridge, Cloned blood–brain barrier adenosine transporter is identical to the rat concentrative Na+ nucleoside cotransporter CNT2, J. Cereb. Blood Flow Metab. 21 (2001) 929–936.
[19] I. Lieberman, Enzymatic amination of uridine triphosphate to cytidine triphosphate, J. Biol. Chem. 222 (1956) 765–775.
[20] I. Lopez-Coviella, J. Agut, V. Savci, J.A. Ortiz, R.J. Wurtman, Evidence that 5′-Cytidinediphosphocholine can affect brain phospholipid composition by increasing choline and cytidine plasma levels, J. Neurochem. 65 (1995) 889–894.
[21] W.R. Millington, R.J. Wurtman, Choline administration elevates brain phosphorylcholine concentrations, J. Neurochem. 38 (1982) 1748–1752.
[22] H. Murakami, A. Ohkura, H. Takanaga, H. Matsuo, N. Koyabu, M. Naito, T. Tsuruo, H. Ohtani, Y. Sawada, Functional characterization of adenosine transport across the BBB in mice, Int. J. Pharm. 290 (2005) 37–44.
[23] Invasive brain drug delivery, in: W.M. Pardridge (Ed.), Brain Drug Targeting: The Future of Brain Drug Development, Cambridge Univ. Press, Cambridge, 2001, pp. 13–35.
[24] D.H. Patel, C.R. Crawford, C.W. Naeve, J.A. Belt, Cloning, genomic organization and chromosomal localization of the gene encoding the murine sodium-dependent, purine-selective, concentrative nucleoside transporter (CNT2), Gene 242 (2000) 51–58.
[25] G.J. Peters, C.J. van Groeningen, E.J. Laurensse, J. Lankelma, A. Leyva, H.M. Pinedo, Uridine-induced hypothermia in mice and rats in relation to plasma and tissue levels of uridine and its metabolites, Cancer Chemother. Pharmacol. 20 (1987) 101–108.
[26] A.M. Pooler, D.H. Guez, R. Benedictus, R.J. Wurtman, Uridine enhances neurite outgrowth in nerve growth factor-differentiated pheochromocytoma cells, Neuroscience 134 (2005) 207–214.
[27] U.I. Richardson, C.J. Watkins, C. Pierre, I.H. Ulus, R.J. Wurtman, Stimulation of CDP-choline synthesis by uridine or cytidine in PC12 rat pheochromocytoma cells, Brain Res. 971 (2003) 161–167.
[28] B.M. Ross, A. Moszczynska, J.K. Blusztajn, A. Sherwin, A. Lozano, S.J. Kisch, Phospholipid biosynthetic enzymes in human brain, Lipids 32 (4) (1997) 351–358.
[29] V. Savci, R.J. Wurtman, Effect of cytidine on membrane phospholipids synthesis in rat striatal slices, J. Neurochem. 64 (1995) 378–384.
[30] B.E. Slack, M. Liscovitch, J.K. Blusztajn, R.J. Wurtman, Uptake of exogenous phosphatidylserine by human neuroblastoma cells stimulates the incorporation of [methyl-14C]choline into phosphatidylcholine, J. Neurochem. 53 (1989) 472–481.
[31] L.A. Teather, R.J. Wurtman, Dietary cytidine (5′)-diphosphocholine supplementation protects against development of memory deficits in aging rats, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 27 (2003) 711–717.
[32] L.A. Teather, R.J. Wurtman, Dietary CDP-choline supplementation prevents memory impairment caused by impoverished environmental conditions in rats, Learn. Mem. 12 (2005) 39–43.
[33] T.W. Traut, Physiological concentrations of purines and pyrimidines, Mol. Cell. Biochem. 140 (1994) 1–22.
[34] H. Ueda, K. Tagawa, E. Furuya, M. Matsumoto, T. Yanagihara, A combined analysis of regional energy metabolism and immunohistochemical ischemic damage in the gerbil brain, J. Neurochem. 72 (1999) 1232–1242.
[35] I.H. Ulus, R.J. Wurtman, C. Mauron, J.K. Blusztajn, Choline increases acetylcholine release and protects against the stimulation-induced decrease in phosphatide levels within membranes of rat corpus striatum, Brain Res. 484 (1989) 217–227.
[36] C.J. van Groeningen, G.J. Peters, J.C. Nadal, E. Laurensse, H.M. Pinedo, Clinical and pharmacologic study of orally administered uridine, J. Natl. Cancer Inst. 83 (1991) 437–441.
[37] L. Wang, A.M. Pooler, M.A. Albrecht, R.J. Wurtman, Dietary uridine-5′-monophosphate supplementation increases potassium-evoked dopamine release and promotes neurite outgrowth in aged rats, J. Mol. Neurosci. 27 (2005) 137–146.
[38] X. Wu, M.M. Gutierrez, K.M. Giacomini, Further characterization of the sodium-dependent nucleoside transporter (N3) in choroid plexus from rabbit, Biochim. Biophys. Acta 1191 (1994) 190–196.
[39] X. Wu, G. Yuan, C.M. Brett, A.C. Hui, K.M. Giacomini, Sodium-dependent nucleoside transport in choroid plexus from rabbit. Evidence for a single transporter for purine and pyrimidine nucleosides, J. Biol. Chem. 267 (1992) 8813–8818.
[40] R.J. Wurtman, M. Regan, I. Ulus, L. Yu, Effect of oral CDP-choline on plasma choline and uridine levels in humans, Biochem. Pharmacol. 60 (2000) 989–992.