Short Communication

Effects of orally administered cytidine 5′-diphosphate choline on brain phospholipid content

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Cytidine, as cytidine 5′-diphosphate choline (CDP-choline), is important for the synthesis of phosphatidylcholine in cell membranes. To investigate whether exogenous CDP-choline could affect brain phospholipid composition, we supplemented the diet of mice with this drug (500 mg/kg/day) for 27 months in 3-month-old mice and for 90, 42, and 3 days in 12-month-old mice, and measured their levels of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and the content of phosphatidylinositol plus phosphatidic acid in the cerebral cortex. After 27 months of treatment, PC and PE increased significantly by 19% ($P < 0.05$) and by 20% ($P < 0.01$), respectively. PS levels increased by 18% (not statistically significant). Similar elevations in PC and PE levels were obtained when older mice were treated for only 3 months ($P < 0.05$). No changes were observed with shorter treatment periods. These results suggest that chronic administration of CDP-choline can have effects on brain phospholipid composition that may underlie its reported utility in various neurologic disorders.

Keywords: CDP-choline; choline; cytidine; dopamine; membranes; phospholipids

Introduction

Functional integrity of neuronal cell membranes is required not only for cell survival and growth, but also for neurotransmitter release and interneuronal communication. Although choline and ethanolamine glycerophospholipids comprise more than half of the total phospholipid content in cell membranes,[1] their levels and metabolism are not constant throughout aging.[2] In old animals, the rates of synthesis of these phospholipids are decreased.[3]

We have shown that cytidine, as cytidine 5′-diphosphate choline (CDP-choline) ingestion by humans or rats increases blood levels of two compounds needed for phosphatidylcholine (PC) synthesis, cytidine and choline,[4] and that cytidine supplementation can increase PC and phatidylethanolamine (PE) synthesis in cultured cells (unpublished results). We, and others, have described effects of exogenous CDP-choline on levels of dopamine and norepinephrine metabolites in the central nervous system and periphery.[5–7] It is not known whether these later effects are related to those on cell membranes. In the present study, we investigated whether chronic administration of CDP-choline could affect phospholipid synthesis of mouse frontal cortex in vivo.

Materials and methods

Eight groups of female CD-1 mice were housed in conventional cages and given free access to food (A04, U.A.R., France) and water; their food intake was monitored. The diet consisted of 17.2% protein, 2.7% fat, and 59.7% carbohydrate (the remaining 20.4% being minerals, fiber, and water), and contained adequate amounts of vitamins and 1.58 g/kg of choline (no CDP-choline was detected). Starting at 3 months of age, one group of mice received 500 mg/kg of body weight/day of CDP-choline incorporated into the diet for 27 months. Three groups, initially 12 months old, received the same dose of CDP-choline for 3, 42, and 90 days. The remaining four groups of animals, used as controls

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for each of the treatment groups, were pair-fed and received only placebo (the initial diet). At the end of each treatment period (when animals were 12 months and 3 days, 13 months and 12 days, 15 months, and 30 months old), the mice were sacrificed at noon by decapitation under light anesthesia (penthotal, 25 mg/kg, i.p.); their heads were dropped into liquid nitrogen and then stored at −70° C for subsequent analyses.

Brain phospholipids were extracted according to the method of Folch et al.[8] Tissues were sonicated in methanol, brought to 20 vol/vol with chloroform and methanol (2:1), and mixed with two volumes of a 50% solution of methanol and water. After centrifugation the organic phase (locus of the phospholipids) was dried under vacuum. The residue was reconstituted in chloroform-methanol (1:1), and an aliquot (50 μL) of the phospholipid extract was subsequently purified by thin layer chromatography on silica gel G plates (LK-6D or LK-5D plates, Whatman Inc., Clifton, NJ, USA), using a system consisting of chloroform, ethanol, triethylamine, and water (30:34:30:8, vol/vol) as the mobile phase. Phospholipid standards were used to identify the corresponding bands under UV light after spraying the plates with 0.1% diphenylhexatriene in petroleum ether. The total amounts of each of the phospholipids were determined by phosphate assay.[9]

Plasma choline was assayed by a modification of the method of Goldberg and McCaman.[10] Aliquots of plasma were diluted with an equal volume of sodium phosphate buffer (10 mmol/L; pH 6.7), and the quaternary amines were extracted into a solution of tetraphenylboron dissolved in heptanone (5 mg/mL), reextracted into 0.4 N HCl, and dried. The residues were then incubated for 15 minutes at 38° C with 30 mL of a buffer solution (50 mmol/L sodium phosphate, 5 mmol/L $MgCl_2$, 1 mmol/L ATP, 0.5 U/mL choline kinase [EC 2.7.1.32] from Saccharomyces cerevisiae; pH 8) containing [^{32}P]-ATP (specific activity 30 Ci/mmol). The resulting [^{32}P]-phosphocholine was isolated using an ion exchange resin (Dowex AG-1×8; Bio-Rad Laboratories, Richmond, CA, USA), eluted with ammonium acetate buffer (75 mmol/L; pH 10), and quantitated by liquid scintillation spectrophotometry.

Plasma nucleosides were assayed by mixing samples with 1 N formic acid. The formic acid was collected and centrifuged at 4° C to remove debris, and the supernatant fluid was lyophilized. Nucleosides were purified by boronate-affinity chromatography (Affi-gel 601, Bio-Rad Laboratories) prior to analysis by high performance liquid chromatography. To separate the ribonucleoside fractions, dried residues of the samples were redissolved in 0.25 N ammonium acetate (pH 8,8) and applied at 4° C to gel columns (1.0 × 1.5 cm) that had been equilibrated with 0.25 N ammonium acetate (pH 8.8). The columns were washed twice with 5 mL of the ammonium acetate solution and the ribonucleotides eluted with 5 mL of 0.1 M formic acid. Fractions containing ribonucleosides were dried under vacuum and redissolved in water (100 μL) before analysis by high performance liquid chromatography.

Nucleosides were resolved by using a reverse phase column (Dynamax$_R$, 250 × 4.6 mm, ODS 3 μm) and the following gradient system: solvent A, 60 mmol/L KH_2PO_4, pH 5.6; solvent B, 60% methanol in solvent A; 25-minute concave-gradient (No. 5; WISP controller, Waters Assoc. Milford, MA, USA) to 30% of solvent B; at 1.2 mL/minute, with a 10-minute equilibration delay.

Data were analyzed by two-way analysis of variance, and then by multiple comparison tests among means, or Student *t* test when appropriate. Statistical significance was $P < 0.05$.

Results

Phospholipid levels (as the sum of PC + PE + phosphatidylserine [PS] + phosphatidylinositol plus phosphatidic acid [PI/PA]) per mg protein (or per mg of DNA) in the frontal cortex of CDP-choline–treated animals were significantly higher than those of animals not receiving the drug ($P < 0.05$). In mice treated with the CDP-choline for 27 months *(Table 1)*, PC levels increased by 18% ($P < 0.01$); PE levels by 20% ($P < 0.05$); and PS levels by 18% (not statistically significant). In these two groups, the number of animals in the placebo and CDP-choline groups are different because only 4 of 10 placebo-treated animals survived the 27-month treatment period, while 8 of 10 CDP-choline–treated animals survived.

Administration of CDP-choline also significantly increased brain phospholipid levels in 12-month-old mice after 90 days of treatment ($P < 0.05$; *Table 2*). PC levels increased by 9% ($P < 0.05$), while PE levels increased by 18% ($P < 0.01$). PS levels again did not increase significantly. The content of PI/PA, taken together (i.e., because the two compounds had identical Rf chromatographic values), was also significantly elevated in the animals that received the CDP-choline (18.1 versus 15.7 ng/mg protein in control animals; $P < 0.01$). However, brain phospholipid levels after 3 and 42 days of CDP-choline treatment were not significantly different from those found in mice that received the placebo *(Table 2)*.

Plasma choline and cytidine (or uridine) levels did not differ among the groups at the time of sacrifice (noon).

Discussion

These data show that long-term administration of CDP-choline to young mice for 27 months can increase the concentrations of the principal phospholipids in the cerebral cortex. Moreover, similar, if smaller, elevations are produced when adult animals are treated for 3 months.

CDP-choline, by providing choline, could increase

Table 1 Effects of chronic CDP-choline administration on brain phospholipid levels in 30-month-old mice

nmol/mg protein	Placebo (4)	CDP-choline (8)
PC	190 ± 6.9	225 ± 4.9[a]
PE	109 ± 3.2	131 ± 2.4[b]
PS	78 ± 3.4	92 ± 4.9
PI/PA	9 ± 0.3	9 ± 0.9

Groups of female CD-1 mice received CDP-choline (500 mg/kg/day, incorporated into the diet) or placebo for 27 months and were sacrificed at 30 months. Brain phospholipids were measured as described in the Materials and methods section. Numbers within parentheses represent the number of animals used in each group. Values are means ± SEM.

[a] $P < 0.05$.

[b] $P < 0.01$.

Table 2 Effects of administering CDP-choline for 3, 42, or 90 days on brain phospholipid levels in 12–15-month-old mice

	3 days of treatment		42 days of treatment		90 days of treatment	
nmol/mg protein	Placebo (10)	CDP-choline (9)	Placebo (9)	CDP-choline (10)	Placebo (10)	CDP-choline (9)
PC	178 ± 5.6	165 ± 5.6	206 ± 3.9	218 ± 4.9	203 ± 4.9	222 ± 5.4[a]
PE	106 ± 4.4	103 ± 4.9	114 ± 2.9	117 ± 6.1	128 ± 1.5	152 ± 5.5[b]
PS	116 ± 2.3	109 ± 4.0	83 ± 6.1	94 ± 4.8	70 ± 2.4	73 ± 3.6
PI/PA	19 ± 0.8	17 ± 0.9	22 ± 0.5	23 ± 6.1	15 ± 0.5	18 ± 0.5[a]

Groups of 12-month-old female CD-1 mice received CDP-choline (500 mg/kg/day, incorporated into the diet) or placebo for 3, 40, or 90 days and were then sacrificed. Brain phospholipids were measured as described in the Materials and methods section. Numbers within parentheses represent the number of animals used in each group. Values are means ± SEM.
[a] $P < 0.01$.
[b] $P < 0.05$.

brain acetylcholine[11] and enhance brain levels of phosphocholine,[12] which is a precursor in the synthesis of PC. Exogenous CDP-choline is also metabolized in the blood to yield cytidine,[4] increasing brain levels of cytidine (in preparation); this in turn, could generate CTP, a rate-limiting substrate in the syntheses of CDP-choline and CDP-ethanolamine, precursors of PC and PE.[13] In this study we did not find elevations in basal plasma choline and cytidine levels. This is not surprising because the CDP-choline was administered with the diet, mostly consumed at night, and measurements of these compounds were done on blood samples obtained during the day, at the time of sacrifice (noon). We have previously shown that plasma choline and cytidine levels return to normal 6 hours after the oral administration of CDP-choline.[4] Because the minimum amount of time necessary to produce the increase in brain phospholipids was 3 months, these results suggest that this is not an acute effect of CDP-choline administration.

References

1 Svennerholm, L. (1964). The distribution of lipids in the human nervous system-I. Analytical procedure. Lipids of foetal and newborn brain. *J. Neurochem.* **11,** 839–853

2 Sun G.Y. and Foudin L.L. (1985). Phospholipid composition and metabolism in the developing and aging nervous system. In *Phospholipids in Nervous Tissue,* (J. Eichberg, ed), p. 79–134, J. Wiley & Sons Ltd., New York, NY, USA

3 Gaiti A., Brunetti M., Piccinin G.L., Woelk H., and Porcellati G. (1982). The synthesis in vivo of choline and ethanolamine phosphoglycerides in different brain areas during aging. *Lipids* **17,** 291–296

4 Lopez G.-Coviella I., Agut J., and Wurtman R.J. (1987). Metabolism of cytidine (5')-diphosphocholine (CDP-choline) following oral and intravenous administration to the human and the rat. *Neurochem. Internat.* **11,** 293–297

5 Agut J., Lopez G.-Coviella I., and Wurtman R.J. (1984). Cytidine (5') diphosphocholine enhances the ability of haloperidol to increase dopamine metabolites in the striatum of the rat and to diminish stereotyped behavior induced by apomorphine. *Neuropharmacol.* **23,** 1403–1406

6 Lopez G.-Coviella I., Agut J., and Wurtman R.J. (1986). Effect of cytidine (5')-diphosphocholine (CDP-choline) on the total urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG) by rats and humans. *J. Neural Trans.* **66,** 129–134

7 Saligaut C., Daoust M., Moore N., and Boismare F. (1987). Circling behavior in rats with unilateral lesions of the nigrostriatum induced by 6-hydroxydopamine: changes induced by oral administration of cytidine-5'-diphosphocholine. *Neuropharmacol.* **26,** 1315–1319

8 Folch J., Lees M., and Sloan-Stanley G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497–509

9 Svanborg, A. and Svennerholm, L. (1961). Plasma total lipids, cholesterol, triglycerides, phospholipids, and free fatty acids in a healthy scandinavian population. *A. Med. Scan.* **169,** 43–49

10 Goldberg A.M. and McCaman R.E. (1973). The determination of picomole amounts of acetylcholine in mammalian brain. *J. Neurochem.* **20,** 1–8

11 Wurtman, R.J. and Zeisel S.H. (1982). Brain choline: its sources and effects on the synthesis and release of acetylcholine. In *Alzheimer's Disease: a Report of Progress,* (S. Corkin, J.H. Growdon, and R.J. Wurtman, eds.), p. 303–313, Raven Press, New York, NY, USA

12 Millington, W.R. and Wurtman, R.J. (1982). Choline and physostigmine enhance haloperidol-induced HVA and DOPAC accumulation. *Eur. J. Pharmacol.* **80,** 431–434

13 Sundler R., Arvidson G., and Akesson B. (1972). Pathways for the incorporation of choline into rat liver phosphatidylcholines in vivo. *Biochim. Biophys. Acta* **280,** 559–568