

## Effect of Cytidine on Membrane Phospholipid Synthesis in Rat Striatal Slices

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**Abstract:** Using rat striatal slices, we examined the effect of cytidine on the conversion of [<sup>3</sup>H]choline to [<sup>3</sup>H]-phosphatidylcholine ([<sup>3</sup>H]PC), and on net syntheses of PC, phosphatidylethanolamine (PE), and phosphatidylserine, when media did or did not also contain choline, ethanolamine, or serine. Incubation of striatal slices with cytidine (50–500  $\mu$ M) caused dose-dependent increases in intracellular cytidine and cytidine triphosphate (CTP) levels and in the rate of incorporation of [<sup>3</sup>H]choline into membrane [<sup>3</sup>H]PC. In pulse–chase experiments, cytidine (200  $\mu$ M) also increased significantly the conversion of [<sup>3</sup>H]choline to [<sup>3</sup>H]PC during the chase period. When slices were incubated with this concentration of cytidine for 1 h, small (7%) but significant elevations were observed in the absolute contents (nmol/mg of protein) of membrane PC and PE ( $p < 0.05$ ), but not phosphatidylserine, the synthesis of which is independent of cytidine-containing CTP. Concurrent exposure to cytidine (200  $\mu$ M) and choline (10  $\mu$ M) caused an additional significant increase ( $p < 0.05$ ) in tissue PC levels beyond that produced by cytidine alone. Exposure to choline alone at a higher concentration (40  $\mu$ M) increased the levels of all three membrane phospholipids ( $p < 0.01$ ); the addition of cytidine, however, did not cause further increases. Concurrent exposure to cytidine (200  $\mu$ M) and ethanolamine (20  $\mu$ M) also caused significantly greater elevations ( $p < 0.05$ ) in tissue PE levels than those caused by cytidine alone. In contrast, the addition of serine (500  $\mu$ M) did not enhance cytidine's effects on any membrane phospholipid. Exposure to serine alone, however, like exposure to sufficient choline, increased levels of all three membrane phospholipids significantly ( $p < 0.01$ ). These data show that exogenous cytidine, probably acting via CTP and the Kennedy cycle, can increase the synthesis and levels of membrane PC and PE in brain cells. **Key Words:** Cytidine—Choline—Phosphatidylcholine—Phospholipid—Ethanolamine—Serine—Striatum. *J. Neurochem.* **64**, 378–384 (1995).

The nucleoside cytidine, once it is phosphorylated (Santos et al., 1968) to form the nucleotides cytidine triphosphate (CTP), cytidine diphosphate (CDP), and cytidine monophosphate (CMP), exerts important effects on a variety of brain functions (Geiger and Yamasaki, 1956; Roberts, 1973; Drago et al., 1990). In both

cell culture systems and in vivo experiments, exogenous cytidine has been shown to be taken up into cells and converted sequentially to CMP, CDP, and CTP (Plagemann, 1971a,b; Trovarelli et al., 1982; 1984; Lopez G.-Coviella and Wurtman, 1992). CTP is required to form key intermediates in the biosynthesis of both phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (quantitatively the most significant phospholipids of eukaryotic cells) via the Kennedy pathway (Kennedy and Weiss, 1956; Pelech and Vance, 1984; Vance, 1985) and also in the biosynthesis of phosphatidylinositol (Vance, 1985; Majerus, 1992; Pike, 1992). This pathway involves, sequentially, the phosphorylation of choline or ethanolamine, combination of the resulting phosphocholine or phosphoethanolamine with CTP to generate 5'-cytidinediphosphocholine (CDP-choline) or 5'-cytidinediphosphoethanolamine (CDP-ethanolamine), and, finally, their combination with diacylglycerol (DAG) to yield PC or PE (Kennedy and Weiss, 1956; Pelech and Vance, 1984). The rate of PC biosynthesis apparently depends upon the activity of CTP:phosphocholine cytidylyltransferase, which catalyzes the formation of CDP-choline from phosphocholine and CTP (Pelech and Vance, 1984). Brain CTP levels are low (Mandel and Edel-Harth, 1966; Abe et al., 1987) and below this enzyme's apparent  $K_m$  for the nucleotide (Pelech and Vance, 1984; Mages et al., 1988). Hence, we suspected that exogenous cytidine might, by increasing intracellular CTP levels, affect brain phospholipid synthesis. This hypothesis was previously confirmed in a stable cell line of PC12 cells (Lopez G.-Coviella and Wurtman, 1992) but had not yet been examined in animal tissue.

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**Abbreviations used:** CDP, cytidine diphosphate; CDP-choline, 5'-cytidinediphosphocholine; CDP-ethanolamine, 5'-cytidinediphosphoethanolamine; CMP, cytidine monophosphate; CTP, cytidine triphosphate; DAG, diacylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

In this study, we investigated the effects of cytidine on the synthesis and levels of PC, PE, and phosphatidylserine (PS) in striatal slices, as well as its interactions with choline, ethanolamine, and serine in affecting phospholipid production.

## MATERIALS AND METHODS

### Slice preparation and experimental procedure

Male Sprague-Dawley rats (250–280 g) (Charles River, Cambridge, MA, U.S.A.) were killed by decapitation and brains were placed in ice-cold Krebs buffer (mM: NaCl 120, KCl 3.5, CaCl<sub>2</sub> 1.3, MgSO<sub>4</sub> 1.2, NaH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, glucose 10, eserine salicylate 0.02). The buffer was gassed with a mixture of 95% O<sub>2</sub> + 5% CO<sub>2</sub> for at least 30 min before use. Striata were rapidly excised, and slices (0.3 mm) were prepared using a McIlwain tissue chopper (Mickle Laboratory Engineering Co., Gomshall, U.K.). The slices were washed several times with Krebs buffer solution and transferred to incubation tubes (five to seven slices per tube); these were placed in a Dubnoff shaker and incubated at 37°C for the periods indicated. During incubation periods media were replaced every 10 min with fresh Krebs buffer continuously bubbled with 95% O<sub>2</sub> + 5% CO<sub>2</sub>. After a 60-min equilibration period, incubation media were discharged; slices were then incubated for 1 h with 1 ml of Krebs buffer containing 5  $\mu$ Ci of [<sup>3</sup>H]choline (86.7 Ci/mmol, Du Pont-New England Nuclear) (final concentration of choline per tube was 65 nM) and different concentrations of cytidine (50–500  $\mu$ M).

To determine the effect of incubation of slices with exogenous cytidine on tissue cytidine and nucleotides, slices were incubated with different concentrations of cytidine (50–200  $\mu$ M) for 1 h. For time-course experiments, incubations were of 10-, 30-, or 60-min duration. Two different sets of experiments were done for cytidine and nucleotide measurements.

In some experiments, unlabeled choline (10, 40  $\mu$ M), ethanolamine (20  $\mu$ M), or serine (500  $\mu$ M) was added to incubation media, alone or together with cytidine (200  $\mu$ M), before a 1-h incubation.

### Extraction and analysis of cytidine and nucleotides

Slices were homogenized in 1 ml of methanol (–30°C) and aliquots were taken for protein assay. The homogenates were mixed with 1 ml of formic acid (0.1 M) and then 2 ml of chloroform. After centrifugation (10 min, 1,500 g, +4°C), the aqueous phases (1,800  $\mu$ l) were taken and dried under vacuum. Methanol extraction was repeated one more time and dried samples were redissolved in 1 ml of ice-cold water and filtered (Millipore, 0.45  $\mu$ m filter). Filtered samples were again dried under vacuum and redissolved in water (70  $\mu$ l) before analysis by HPLC.

Cytidine was measured using a reverse-phase column (C18) with a UV detector (280 nm) and the following gradient system: solvent A, 10 mM KH<sub>2</sub>PO<sub>4</sub>, 1% MeOH, pH 6; solvent B, 10 mM KH<sub>2</sub>PO<sub>4</sub>, 50% MeOH, pH 6; 5-min linear gradient to 50% of solvent B followed by 5 min of 100% solvent B; at 0.7 ml/min, with a 15-min equilibration delay.

Nucleotides were separated by ion-pair chromatography, using a reverse-phase column (Dynamax, 250  $\times$  4.6 mm, 5  $\mu$ m C18) (Waters Associates, Milford, MA, U.S.A.) and UV detector (280 nm) and the following gradient system: solvent A, 5 mM tetrabutylammonium phosphate, 30 mM

KH<sub>2</sub>PO<sub>4</sub>, 4% acetonitrile, pH 6; solvent B, 50% acetonitrile in solvent A; 35-min concave gradient (no. 3) to 50% of solvent B; at 1.5 ml/min, with a 25-min equilibration delay.

### Extraction and separation of phospholipids and water-soluble choline-containing metabolites

After incubation, slices were rinsed several times with ice-cold Krebs solution and homogenized in 1 ml of distilled water containing eserine (20  $\mu$ M). Three milliliters of methanol/chloroform (1:2; vol/vol) was then added. After centrifugation, both the organic phase (1,800  $\mu$ l) (locus of phospholipids) and the aqueous phase (1,800  $\mu$ l) (locus of water-soluble choline-containing metabolites) were dried under vacuum.

Aliquots of the lower phase were used for determination of phosphorus and for separation of individual classes of phospholipids. These classes were purified by TLC on silica gel G plates (LK-6D plates, Whatman), using a system consisting of chloroform/ethanol/triethylamine/water (30:34:30:8, by volume) as a mobile phase (Touchstone et al., 1980). Bands containing the individual phospholipids were scraped off the plates and their radioactivities counted by liquid scintillation spectrophotometry; the total amount of each phospholipid was determined by phosphate assay (Svanborg and Svennerholm, 1961).

Aliquots of the aqueous phase (containing water-soluble, choline-containing metabolites) were separated by TLC on silica gel G plates (LK-5D plates, Whatman). The following composition was used as a mobile phase: acetonitrile, ethanol, 30% NH<sub>4</sub>OH, 8.3% NaCl (40:8:3:24, by volume) (Slack et al., 1991). Corresponding bands containing choline, acetylcholine, and phosphocholine were scraped and their radioactivities counted.

### Measurement of choline and acetylcholine

During the extraction of lipids, the upper aqueous phase (1,800  $\mu$ l) was separated for determination of choline and acetylcholine and dried under vacuum. Aliquots of dried aqueous phases were resuspended in water and subjected to HPLC analysis using polymeric reverse-phase column (BAS, West Lafayette, IN, U.S.A.) with a mobile phase of 50 mM phosphate (pH 8.5) containing 0.005% Kathon CG (Rohm and Hass, Philadelphia, PA, U.S.A.) as a bactericide. Once separated, acetylcholine and choline were converted to hydrogen peroxide by a postcolumn enzymatic reactor (BAS) containing acetylcholinesterase and choline oxidase. The hydrogen peroxide was detected electrochemically using a platinum electrode (500 mV vs. Ag/AgCl) (model 200a, BAS). Fifty-microliter aliquots of tissue homogenates were used for protein measurement (Lowry et al., 1951).

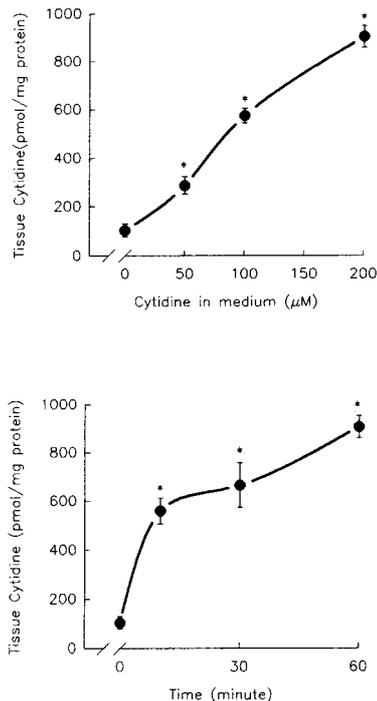
### Data analysis

Data are expressed as mean  $\pm$  SEM. Student's *t* test was used to compare the effect of cytidine on tissue choline and acetylcholine levels. Statistical analysis of all other data was performed using ANOVA with post hoc Newman-Keuls test. The effect of serine (500  $\mu$ M) and choline (40  $\mu$ M) on phospholipid levels was evaluated by using both Student's *t* test and ANOVA with post hoc Newman-Keuls test.

## RESULTS

### Cytidine and nucleotide levels in brain striatal slices incubated with cytidine

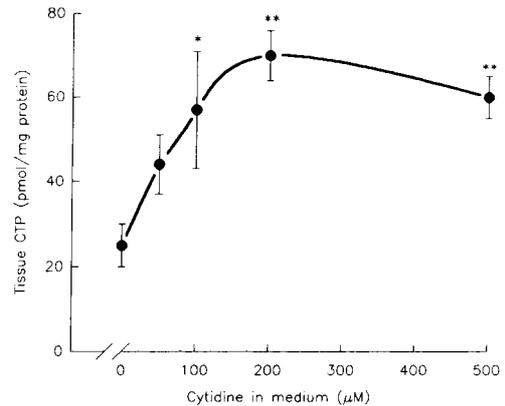
Incubation of striatal slices with various concentrations of cytidine (50–500  $\mu$ M) for 1 h caused dose-



**FIG. 1.** Concentration- and time-dependent increases in striatal cytidine levels after incubation of tissue slices with cytidine. **Top:** Slices were incubated with various concentrations of cytidine (0–200  $\mu\text{M}$ ) for 1 h. **Bottom:** Slices were incubated for various times in the presence of 200  $\mu\text{M}$  cytidine. After incubation, the slices were rinsed several times with fresh Krebs buffer and homogenized in 1 ml of methanol ( $-30^{\circ}\text{C}$ ). Then 1 ml of formic acid (0.1 M) and 2 ml of chloroform were added subsequently. Aqueous phases containing cytidine were separated and dried under vacuum. This extraction procedure was repeated one more time and dried samples were redissolved with ice-cold water and filtered. Filtered samples were dried again and cytidine was measured by reverse-phase HPLC with UV detection (280 nm). Data represent mean  $\pm$  SEM of five to ten measurements. Statistics were performed using an ANOVA with a post hoc Newman–Keuls test ( $*p < 0.01$ , compared with basal levels). The difference between the means was significant at  $p < 0.01$ .

dependent increases in tissue cytidine levels (Fig. 1, top). These were time dependent during the 60 min examined (Fig. 1, bottom).

Levels of triphosphate nucleotides in striatal slices were  $2,190 \pm 128$ ,  $397 \pm 79$ ,  $99 \pm 12$ , and  $25 \pm 5$  pmol/mg of protein ( $n = 5$ ) for ATP, GTP, UTP, and CTP, respectively. These levels are lower than those described in brain samples obtained from rats killed differently (i.e., by freezing the brain before decapitation and, thereafter, not subjecting the tissues to incubation) (Mandel and Edel-Harth, 1966; Abe et al., 1987). Dose-dependent increases in tissue CTP levels were observed after striatal slices were incubated with cytidine for 1 h (Fig. 2). Maximum increases occurred with 200  $\mu\text{M}$  cytidine, which caused a threefold increase in CTP levels (Fig. 2). Further elevations in the cytidine concentration of the medium did not cause further elevations in CTP. Cytidine, at concentrations

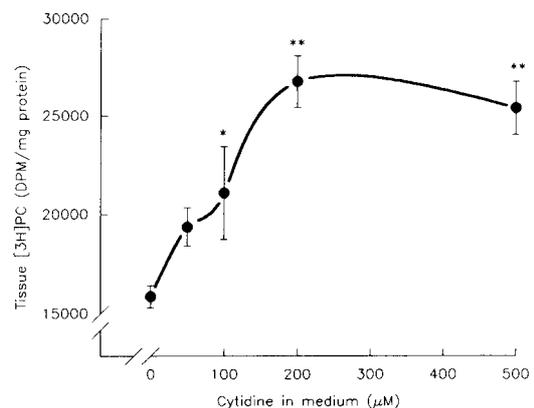


**FIG. 2.** Effect of cytidine on tissue CTP concentrations: dose-response relationships. Slices were incubated with various cytidine concentrations for 1 h. After the incubation period, they were immediately homogenized with cold methanol ( $-30^{\circ}\text{C}$ ) and prepared for HPLC separation as described previously in the legend to Fig. 1. Nucleotides were separated by ion-pair HPLC with UV detection (280 nm). Data represent mean  $\pm$  SEM of five to six measurements. Statistics were performed using an ANOVA with a post hoc Newman–Keuls test ( $*p < 0.05$ ;  $**p < 0.01$ ). The difference between means was significant at  $p < 0.01$ .

of 500  $\mu\text{M}$  and 50  $\mu\text{M}$ , also increased tissue CMP and CDP levels, respectively, but had no effects on those of uridine, adenine, or guanidine nucleotides (data not shown).

#### Effect of cytidine on [ $^3\text{H}$ ]PC synthesis in striatal slices

Striatal slices incorporated [ $^3\text{H}$ ]choline into both glycerolipids and water-soluble compounds. The effects of various cytidine concentrations on the incorporation of [ $^3\text{H}$ ]choline into PC are shown in Fig. 3.



**FIG. 3.** Effect of cytidine on the incorporation of [ $^3\text{H}$ ]choline into PC. Slices were incubated for 1 h in Krebs buffer containing various cytidine concentrations plus [ $^3\text{H}$ ]choline. Slices were then washed and homogenized. PC was purified by TLC and its radioactivity counted. Data represent mean  $\pm$  SEM of five to eight measurements. Statistics were performed using an ANOVA with a post hoc Newman–Keuls test ( $*p < 0.05$ ;  $**p < 0.01$ ). The difference between means was significant at  $p < 0.01$ .

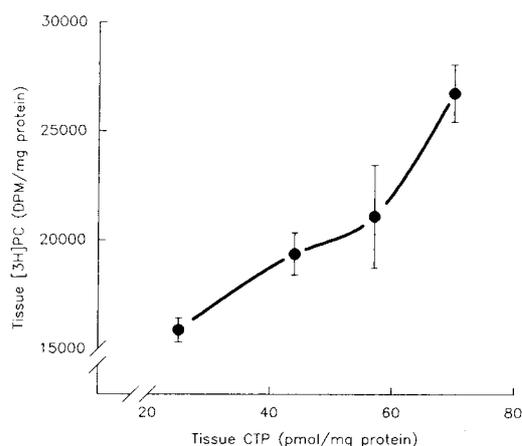


FIG. 4. Relationship between the incorporation of [ $^3\text{H}$ ]choline into [ $^3\text{H}$ ]PC and tissue CTP levels.

Cytidine's effect was concentration dependent in the range of 50–200  $\mu\text{M}$ ; higher concentrations (500 and 1,000  $\mu\text{M}$ ) did not cause further changes. The increase in tissue [ $^3\text{H}$ ]PC bore a linear relationship to tissue CTP levels (Fig. 4). We did not observe significant changes in the levels of [ $^3\text{H}$ ]phosphocholine and [ $^3\text{H}$ ]choline when slices were incubated with cytidine. However, because the increased labeling of PC under this condition might have been due to an increase in the specific radioactivity of any of PC's water-soluble precursors, and not to an actual change in PC's rate of synthesis, we examined the ability of cytidine to enhance [ $^3\text{H}$ ]choline's incorporation into [ $^3\text{H}$ ]PC under chase conditions (i.e., in which the initial specific radioactivity of the precursor [ $^3\text{H}$ ]choline pool would be the same both in control and cytidine-treated slices). As shown in Fig. 5, PC labeling during the chase period was also stimulated in the presence of cytidine, indicating that the nucleoside does affect PC biosynthesis. Moreover, incubation of slices with 200  $\mu\text{M}$  cytidine did not change significantly the radioactivity of lysophosphatidylcholine, the breakdown product of PC [ $457 \pm 35$  vs.  $572 \pm 83$  (dpm/mg of protein); control vs. cytidine;  $n = 5$ ].

#### Levels of acetylcholine, choline, and phospholipids in striatal slices incubated with cytidine: effects of concurrent exposure to cytidine and to choline, ethanolamine, or serine

To determine whether the accelerated synthesis of PC in cytidine-supplemented slices produced a net increase in absolute tissue PC levels, we measured tissue phospholipid contents in slices incubated with cytidine 200  $\mu\text{M}$  for 1 h. In three separate experiments, cytidine caused 7–10% increases in both PC and PE levels (Table 1), it failed to affect tissue PS (Table 1) or choline levels (Table 2), and it reduced tissue acetylcholine by  $\sim 18\%$  (Table 2). Cytidine did not influence the release of acetylcholine or choline into the medium (data not shown).

Incubation of striatal slices with 10  $\mu\text{M}$  choline did not change membrane PC levels. However, the combination of cytidine (200  $\mu\text{M}$ ) and choline (10  $\mu\text{M}$ ) caused an additional increase in membrane PC over that produced by cytidine alone. Exposure to 40  $\mu\text{M}$  choline increased the levels of PC, PE, and PS; inclusion of cytidine in the medium failed to amplify these responses (Table 1).

Incubation of striatal slices with 20  $\mu\text{M}$  ethanolamine did not change membrane phospholipid levels; however, combining the ethanolamine with cytidine (200  $\mu\text{M}$ ) caused an additional significant increase in membrane PE over that produced by cytidine alone (Table 1).

Incubation of striatal slices with 500  $\mu\text{M}$  serine increased tissue levels of all three phospholipids (Table 1). Although cytidine (200  $\mu\text{M}$ ) alone increased membrane PC and PE, it failed to enhance the phospholipid changes produced by serine alone (Table 1).

## DISCUSSION

These data show that exogenous cytidine, acting via CTP and the Kennedy cycle, can increase the synthesis and levels of cell membrane phospholipids in striatal slices.

It has previously been shown that exogenous cytidine can be taken up into cells and converted to cytidine nucleotides (Kennedy and Weiss, 1956; Plagemann, 1971*a,b*). In cultured cells, cytidine and uridine are, at concentrations  $< 50 \mu\text{M}$ , taken up primarily via a transport system; at higher concentrations (i.e.,  $> 100 \mu\text{M}$ ), simple diffusion becomes the predominant mode of entry (Plagemann, 1971*b*). In rat cerebral cortical

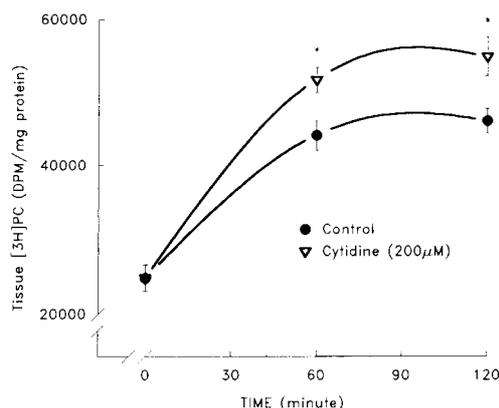


FIG. 5. Effect of cytidine on pulse-chase labeling of PC. Slices were pulse-labeled by incubation with [ $^3\text{H}$ ]choline in 1 ml of Krebs buffer for 45 min. Media were then replaced with unlabeled Krebs buffer, with or without added cytidine (200  $\mu\text{M}$ ). After incubation, tissue [ $^3\text{H}$ ]PC was extracted and assayed as described in Materials and Methods. Data represent mean  $\pm$  SEM of six to eight measurements. Statistics were performed using an ANOVA with post hoc Newman-Keuls test. \* $p < 0.05$  (values significantly different from corresponding controls at the same time point).

TABLE 1. Phospholipid contents of rat brain striatal slices

	Total phospholipids	PC	PE	PS
Control (n = 15)	426 ± 9	177 ± 3	177 ± 5	60 ± 1
Cytidine (n = 19)	478 ± 12 <sup>a</sup>	190 ± 3 <sup>a</sup>	190 ± 4 <sup>a</sup>	62 ± 2
Choline (10 μM) (n = 10)	459 ± 24	183 ± 5	180 ± 7	61 ± 3
Choline (10 μM) + cytidine (200 μM) (n = 13)	494 ± 19 <sup>a</sup>	202 ± 5 <sup>a</sup>	194 ± 4 <sup>a</sup>	61 ± 3
Choline (40 μM) (n = 10)	492 ± 14 <sup>b</sup>	209 ± 8 <sup>a</sup>	209 ± 7 <sup>b</sup>	66 ± 3 <sup>d</sup>
Choline (40 μM) + cytidine (200 μM) (n = 10)	517 ± 18 <sup>b</sup>	206 ± 8 <sup>a</sup>	199 ± 5 <sup>a</sup>	67 ± 3 <sup>d</sup>
Control (n = 12)	419 ± 17	168 ± 3	183 ± 4	59 ± 2
Cytidine (n = 13)	484 ± 14 <sup>a</sup>	180 ± 3 <sup>a</sup>	195 ± 3 <sup>a</sup>	64 ± 3
Ethanolamine (20 μM) (n = 10)	439 ± 12	161 ± 4	178 ± 4	55 ± 3
Ethanolamine (20 μM) + cytidine (200 μM) (n = 13)	501 ± 23 <sup>a</sup>	188 ± 4 <sup>b</sup>	208 ± 4 <sup>c</sup>	66 ± 2
Control (n = 8)	404 ± 11	161 ± 4	168 ± 6	60 ± 3
Cytidine (n = 8)	446 ± 10 <sup>a</sup>	177 ± 3 <sup>a</sup>	194 ± 6 <sup>a</sup>	67 ± 4
Serine (500 μM) (n = 7)	453 ± 12 <sup>a</sup>	191 ± 7 <sup>b</sup>	211 ± 11 <sup>b</sup>	74 ± 6 <sup>d</sup>
Serine (500 μM) + cytidine (200 μM) (n = 7)	443 ± 13 <sup>a</sup>	183 ± 3 <sup>b</sup>	201 ± 10 <sup>a</sup>	67 ± 6

Levels of membrane phospholipids (expressed as nanomoles per milligram of protein) in striatal slices incubated with cytidine alone or in combination with choline, ethanolamine, or serine. Slices were incubated for 1 h in the presence of cytidine (200 μM); choline, ethanolamine, or serine; or cytidine plus choline, ethanolamine, or serine. Phospholipid components were purified by TLC and the total amounts of each determined by phosphate assay. Statistics were performed using an ANOVA with post hoc Newman-Keuls test. n, number of measurements in each group.

<sup>a</sup>  $p < 0.05$ ; <sup>b</sup>  $p < 0.01$ , compared with respective control levels.

<sup>c</sup>  $p < 0.01$  vs. control and  $p < 0.05$  vs. cytidine alone.

<sup>d</sup>  $p < 0.05$ , compared with respective control levels (unpaired Student's *t* test).

slices, cytidine and other nucleosides may simply diffuse into cells (Santos et al., 1968). The present study confirms that exogenous cytidine can be taken up into brain cells and can thereby increase tissue cytidine and CTP levels. Incubating striatal slices with cytidine caused increases in CTP that were dose dependent to 200 μM; at this dose, CTP rose from 25 ± 5 to 70 ± 6 pmol/mg of protein, and CDP rose from 103 ± 30 to 196 ± 19; other nucleotides did not change.

The de novo synthesis of PC from choline is mediated by the Kennedy, or CDP-choline, cycle. The synthesis of CDP-choline from phosphocholine and CTP, catalyzed by the enzyme CTP:phosphocholine cytidyltransferase, appears to be rate limiting in this pathway (Pelech and Vance, 1984). In cell culture systems, it has been shown that CTP levels can be a critical regulator in

PC synthesis (Whitehead et al., 1981). In rat brain, the apparent  $K_m$  of the cytidyltransferase for CTP is 10 mM (Mages et al., 1988). CTP concentrations in our samples were lower than those previously reported in tissue from rats killed by in situ brain freezing, and were not subjected to an incubation period. However, those CTP levels (~70 μM) (Abe et al., 1987) are also well below the CTP's  $K_m$  for CTP:phosphocholine cytidyltransferase. The relative amounts of nucleotides found in our slices were the same as those previously reported. Thus, the cytidine-induced increase in tissue CTP levels would be expected to enhance PC synthesis and to increase the incorporation of radiolabeled choline into PC (Figs. 3 and 5) and the absolute level of membrane PC (Table 1).

It has been demonstrated that the activity of choline kinase, the enzyme that catalyzes the phosphorylation of choline in the first step of PC biosynthesis via the CDP-choline pathway, varies with extracellular choline concentrations (Ando et al., 1987). Ganglionic choline kinase activity is low at low extracellular choline concentrations (1–5 μM) but rises as the choline concentration is raised to 10–50 μM (Ando et al., 1987). Moreover, administration of choline to rats has been shown to increase brain phosphocholine levels (Millington and Wurtman, 1982). In the present study, incubating striatal slices with both cytidine and choline caused a greater increase in membrane PC levels than incubation with cytidine alone. This confirms observations made on cultured cells that both cytidine and choline availability can affect the net rate of PC synthesis (Lopez G.-Coviella and Wurtman, 1992).

TABLE 2. Effect of cytidine on tissue ACh and Ch contents

	Control	Cytidine
ACh	3,037 ± 214	2,477 ± 82 <sup>a</sup>
Ch	1,785 ± 70	1,852 ± 53

Tissue acetylcholine (ACh) and choline (Ch) levels (expressed as picomoles per milligram of protein) of striatal slices. Slices were incubated with 200 μM cytidine for 1 h and homogenized. ACh and Ch were measured by HPLC with electrochemical detection as described in Materials and Methods. Data represent mean ± SEM of 13–16 measurements. Statistics were performed using unpaired Student's test.

<sup>a</sup>  $p < 0.01$ , vs. control levels.

In this study, cytidine also increased the absolute levels of membrane PE. This phospholipid can be synthesized by the decarboxylation of PS (Hubscher et al., 1959), the exchange of ethanolamine with the head groups of other phospholipids (Bjerve, 1973), and, predominantly, by the Kennedy cycle (i.e., the transfer of phosphoethanolamine from CDP-ethanolamine to DAG) (Sundler, 1973; Dykes et al., 1976). CTP:phosphoethanolamine cytidyltransferase, the enzyme that catalyzes the formation of CDP-ethanolamine, has received little attention in the literature but may be an important factor regulating PE synthesis (Vance, 1985), just as CTP:phosphocholine cytidyltransferase activity can regulate PC synthesis. Because coinubation of cytidine with ethanolamine caused a greater increase in PE levels than incubation with ethanolamine alone (Table 1), this enzyme may be unsaturated with phosphoethanolamine at usual tissue levels. Hence, exogenous ethanolamine could enhance PE synthesis by increasing tissue phosphoethanolamine levels.

The failure of exogenous cytidine to increase tissue PS levels is consistent with the fact that PS, unlike PC or PE, is not synthesized by the CDP-choline (or CDP-ethanolamine) pathway but by the exchange of serine with the head groups of other phospholipids (Porcellati et al., 1971; Holbrook and Wurtman, 1988).

In these experiments, we found significant increases in levels of all three membrane phospholipids after slices were incubated with 40  $\mu\text{M}$  choline or 500  $\mu\text{M}$  serine. It has previously been reported that giving supplemental choline to laboratory animals can increase brain levels of PC and of total phospholipids (Wecker, 1985a,b) and that superfusing brain striatal slices with choline can similarly increase PC, PE, and PS levels (Ulus et al., 1989). The increments of all three membrane phospholipid levels after incubation with serine could support the hypothesis that serine is an ultimate precursor of phospholipids by entering the PC cycle (Blusztajn and Wurtman, 1983). Moreover, incubation of cultured LA-N-2 cells with PS liposomes increased the incorporation of choline into PC and of ethanolamine into PE (Slack et al., 1989), ultimately increasing cellular levels of all three of the phospholipids. Our findings, taken with these observations, suggest that the proportions of PC, PE, and PS in cell membranes may be regulated such that a selective increase in the production or breakdown of one of the phospholipids ultimately affects the fates of the others.

Phospholipids, especially PC, have major structural functions in biological membranes. They can also influence cell growth and serve as reservoirs for fatty acids, and for other substituents that, with or without further transformation, are important mediators and modulators of transmembrane signaling (Exton, 1990; Zeisel, 1993). Various brain diseases, for example, hypoxia, the ischemia related to strokes or injury, and aging per se affect brain levels of these phospholipids (Alberghina et al., 1981a,b; Goracci et al., 1981; Wie-

loch et al., 1984; Abe et al., 1987; Abe and Kogure, 1989). Evidence has been presented that exogenous CDP-choline or CDP-ethanolamine, which are completely degraded to cytidine plus choline or ethanolamine when administered orally, can reverse these membrane changes (Trovarelli et al., 1981; Dorman et al., 1983) and thereby perhaps ameliorate the clinical findings associated with the disease (De la Morena, 1991). The present report provides a neurochemical basis for such effects.

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