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# Enhancement by Cytidine of Membrane Phospholipid Synthesis

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**Abstract:** Cytidine, as cytidine 5′-diphosphate choline, is a major precursor in the synthesis of phosphatidylcholine in cell membranes. In the present study, we examined the relationships between extracellular levels of cytidine, the conversion of [$^{14}$C]choline to [$^{14}$C]phosphatidylcholine, and the net syntheses of phosphatidylcholine and phosphatidylethanolamine by PC12 cells. The rate at which cytidine (as [$^{3}$H]cytidine) was incorporated into the PC12 cells followed normal Michaelis–Menten kinetics ($K_m$ = 5 $\mu M$; $V_{max}$ = 12 × $10^{-3}$ mmol/mg of protein/min) when the cytidine concentrations in the medium were below 50 $\mu M$; at higher concentrations, intracellular [$^{3}$H]cytidine nucleotide levels increased linearly. Once inside the cell, cytidine was converted mainly into cytidine triphosphate. In pulse–chase experiments, addition of cytidine to the medium caused a time- and dose-dependent increase (by up to 30%) in the incorporation of [$^{14}$C]choline into membrane [$^{14}$C]-phosphatidylcholine. When the PC12 cells were supplemented with both cytidine and choline for 14 h, small but significant elevations ($p < 0.05$) were observed in their absolute contents of membrane phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine, all increasing by 10–15% relative to their levels in cells incubated with choline alone. Exogenous cytidine, acting via cytidine triphosphate, can thus affect the synthesis and levels of cell membrane phospholipids. **Key Words:** Acetylcholine—Choline—Cytidine—PC12 cells—Phosphatidylcholine—Phospholipids. **López G.-Coviella I. and Wurtman R. J.** Enhancement by cytidine of membrane phospholipid synthesis. *J. Neurochem.* **59,** 338–343 (1992).

Most of the phosphatidylcholine (PtdCho) synthesized by cells is formed via a biosynthetic pathway that utilizes cytidine in the form of 5′-cytidine diphosphate choline (CDP-choline) as an intermediate (Kennedy and Weiss, 1956). This pathway involves, sequentially, the phosphorylation of choline, the combination of the resulting phosphorylcholine with CTP to generate CDP-choline, and the addition of diacylglycerol to this compound to yield PtdCho. We previously showed that administration of CDP-choline to animals increased plasma cytidine and choline levels (Lopez G.-Coviella et al., 1987). Moreover, measurements of the arteriovenous differences in plasma cytidine concentrations occurring during a constant intravenous cytidine infusion suggested that more than 30% of the circulating cytidine enters the brain with a single pass (unpublished observations). The fate of exogenous cytidine in neural tissues, however, was not explored. The present study examined the fate of exogenous cytidine in PC12 cells, a neuron-related line, and specifically its conversion to CTP and CDP-choline and its effects on intracellular PtdCho levels.

## MATERIALS AND METHODS

### Cell culture

PC12 cells were routinely grown in 250-ml flasks at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum and 10% horse serum in a humidified atmosphere of 10% $CO_2$/90% air. Cells were plated in 35-mm dishes at least 72 h prior to each experiment. When the medium was changed in the course of an experiment, the cells were washed three times with cold (4°C) phosphate-buffered saline (PBS) before addition of the new medium. To harvest the cells, their medium was aspirated and the cells washed and scraped off the dishes with cold methanol (1 ml). Cell counts and assays of protein (Lowry et al., 1951) and DNA (Labarca and Paigen, 1991) were done routinely in each experiment.

### Incorporation of radiolabeled cytidine into PC12 cells

PC12 cells were incubated in Hanks' balanced salt solution supplemented with 5, 10, 15, 20, 50, 100, or 200 $\mu M$ [$^{3}$H]cytidine (1 Ci/mol) at 37°C. After a 20-min incubation period, the medium was removed and the cells were washed with 5 ml of PBS and scraped from the dish in 1 *M* formic acid (pH 2). Duplicate samples were analyzed for the radio-

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*Abbreviations used:* CDP-choline, 5′-cytidine diphosphate choline; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdSer, phosphatidylserine.

activity in total cell material and in acid-soluble and -insoluble materials. To measure total radioactivity, an aliquot of the cell suspension was dried under vacuum, redissolved in 200 μl of 0.5 *M* trichloroacetic acid, heated at 70°C for 30 min, mixed with scintillator-solvent mixture, and counted in a liquid scintillation spectrophotometer (Beckman Instruments, Berkeley, CA, U.S.A.). To measure the radioactivity associated with the acid-insoluble material, an aliquot was centrifuged at 2,000 *g* for 10 min; the resulting pellet was then washed twice with cold 0.5 *M* perchloric acid and 0.5 *M* trichloroacetic acid, and its radioactivity was counted after heating at 70°C for 30 min in 0.5 *M* trichloroacetic acid. To measure acid-insoluble radioactivity, the supernatant fluid was centrifuged again at 5,000 *g* for 15 min (4°C), and an aliquot was taken for counting.

### Ribonucleotide extraction and analysis

Cells were harvested with 1 *M* formic acid, as above. The formic acid was collected and centrifuged at 4°C to remove cells and debris, and the supernatant fluid was lyophilized. Nucleotides were purified by boronate-affinity chromatography (Affi-gel 601, Bio-Rad Laboratories, Richmond, CA, U.S.A.) prior to analysis by HPLC. To separate the ribonucleotide fractions, dried residues of the samples were redissolved in 1 *M* ammonium acetate (pH 8.8) and applied at 4°C to gel columns (1.0 × 1.5 cm) which had been equilibrated with 1 *M* 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 ribonucleotides were dried under vacuum and redissolved in water (100 μl) before analysis by HPLC.

Nucleotides were resolved by ion-pair chromatography, using a reverse-phase column (Dynamax, 250 × 4.6 mm, ODS 3 μm) and the following gradient system: solvent A, 5 m*M* tetrabutylammonium phosphate, 30 m*M* $KH_2PO_4$, pH 6; solvent B, 50% acetonitrile in solvent A; 35-min concave gradient (no. 5) to 55% of solvent B; at 1.4 ml/min, with a 23-min equilibration delay.

### Choline and acetylcholine assays

Choline was assayed by a modification of the method of Goldberg and McCaman (1973). Aliquots of cell extracts were diluted with an equal volume of sodium phosphate buffer (10 m*M*, pH 6.7), and the quaternary amines were extracted into a solution of tetraphenylboron dissolved in heptanone (5 mg/ml), reextracted into 0.4 *M* HCl, and dried. The residues were then incubated for 15 min at 38°C with 30 ml of a buffer solution [50 m*M* sodium phosphate, 5 m*M* $MgCl_2$, 1 m*M* ATP, 0.5 unit/ml choline kinase (EC 2.7.1.32) from *Saccharomyces cerevisiae*, pH 8] containing [$^{32}$P]ATP (sp. act. 30 Ci/mmol). The resulting [$^{32}$P]-phosphocholine was isolated using an ion-exchange resin (Dowex AG-1 × 8; Bio-Rad Corp.), eluted with ammonium acetate buffer (75 m*M*, pH 10), and quantitated by liquid scintillation spectrophotometry. Acetylcholine levels in aliquots of sample extracts previously incubated with acetylcholinesterase were determined using the same method.

### Extraction and separation of cell phospholipids

Cell phospholipids were extracted according to the method of Folch et al. (1957). Tissues were sonicated in methanol, brought to 20% (vol/vol) with chloroform/methanol (2:1), and mixed with two volumes of a 50% solution of methanol/water. After centrifugation, both the organic phase (locus of the phospholipids) and the aqueous phase (locus of the water-soluble choline-containing metabolites) were dried under vacuum. The residue from the organic phase was reconstituted in chloroform/methanol (1:1).

An aliquot (50 ml) of the phospholipid extract was purified subsequently by TLC on silica gel G plates (LK-6D or LK-5D plates, Whatman) using a system consisting of chloroform/ethanol/triethylamine/water (30:34:30:8, by volume) 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 radioactivities associated with the different phospholipid bands were counted by liquid scintillation spectrometry, and the total amounts of each of the phospholipids were determined by phosphate assay (Svanborg and Svennerholm, 1961).

### Determination of water-soluble choline-containing metabolites from cultured cells

The water-soluble choline-containing metabolites were purified by HPLC, using a normal phase silica column (3-μm spherical particles, 10 × 4.6 mm) and a gradient elution system based on increasing polarity and ionic strength (Liscovitch et al., 1985). The mobile phase consisted of two solvents: solvent A, acetonitrile/water/ethanol/acetic acid/0.83 *M* ammonium acetate (800:127:68:2:3, by volume); and solvent B, same components as solvent A (400:400:68:53:79, by volume; pH 3.6). A linear gradient from 0 to 100% of solvent B was started 7 min after the injection (100 μl). The flow rate was 1.5 ml/min and the temperature 55°C.

Phosphocholine in the aqueous extracts was quantitated by collecting the corresponding HPLC fraction, hydrolyzing it with alkaline phosphatase, and measuring the choline derived from it. The purified phosphocholine fraction was dried under vacuum and reconstituted in 200 μl of a mixture (pH 10.7) of 0.1 *M* glycylglycine, 1 m*M* $MgCl_2$, 2 m*M* zinc sulfate, and 2 units of alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] (Sigma Type III, St. Louis, MO, U.S.A.). This mixture was incubated at 37°C and the reaction stopped after 4 h by adding 250 ml of chilled tetraphenylboron dissolved in heptanone (5 mg/ml). Subsequently, choline was determined by radioenzymatic assay.

### Data analysis

Statistical analysis of the data was performed using *t* tests for comparisons between two groups, and by one-way or two-way analysis of variance, followed by multiple comparison tests, when more than two groups were compared. Transport kinetics and regression analyses of the data were done following linear and nonlinear models.

## RESULTS

### Kinetics of cytidine incorporation into PC12 cells

To measure the rate of incorporation of cytidine, PC12 cells were incubated with various concentrations of [$^3$H]cytidine (1 Ci/mol), and the radioactivities associated with total cell extract and with acid-soluble and -insoluble materials were measured. The rate of incorporation of cytidine into PC12 cells was found to follow normal Michaelis–Menten kinetics ($K_m$ = 5 μ*M*) when the cytidine concentrations in the medium were below 50 μ*M* (Fig. 1). However, when

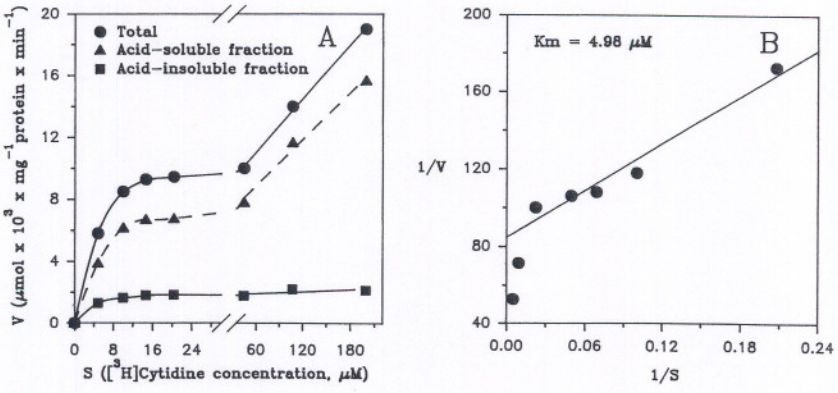


**FIG. 1.** Incorporation of cytidine into PC12 cells as a function of the concentration of cytidine in the medium. PC12 cells were incubated in Hanks' balanced salt solution supplemented with 5, 10, 15, 20, 50, 100, and 200 $\mu M$ [$^3$H]cytidine (1 mCi/mmol) at 37°C. After a 20-min incubation period, the medium was removed and the cells were washed with PBS and scraped from the dish into 1 *M* formic acid. Duplicate samples were analyzed for the radioactivities in total cell material and in the acid-soluble and -insoluble fractions.

cytidine concentrations in the medium were increased above this level, the amounts of intracellular [$^3$H]cytidine nucleotides increased linearly, suggesting a simple diffusion mechanism or a low-affinity transport system. At all concentrations of cytidine tested, its rate of incorporation into the acid-soluble pool was more than four times that of its incorporation into acid-insoluble material. Theoretical $V_{max}$ values for cytidine incorporation into acid-soluble and -insoluble material were estimated as $9 \times 10^{-3}$ and $2 \times 10^{-3}$ mmol/mg of protein/min.

### Nucleotide levels in PC12 cells supplemented with cytidine

When PC12 cells were incubated for 2 h in the presence of various concentrations of cytidine, they showed a dose-dependent increase in the intracellular levels of cytidine nucleotides (Fig. 2A). Intracellular CDP and CTP levels increased by 200 and 287%, respectively, when cells were incubated with 25 $\mu M$ cytidine. Further elevations in the cytidine concentration of the medium led to comparatively smaller increases. CMP levels apparently did not change with any of the concentrations of cytidine used in this study, and CDP-choline was found only in trace amounts in both control and supplemented cells, making its quantification difficult. No significant changes in any of the other ribonucleotides were observed as a consequence of supplementing PC12 cells with cytidine.

PC12 cells incubated with 100 $\mu M$ cytidine for periods of 0.5–8 h (Fig. 2B) showed maximal increases in CTP and CDP levels (by more than five and three times, respectively) at the earliest time tested; these persisted for at least 8 h. The uridine nucleotide pools were also increased, cellular UTP rising gradually from 3.66 to a maximum of 5.86 nmol/mg of protein after 4 h of incubation, and UDP and UMP peaking at 8 h (from 3.20 to 5.38 and from 1.09 to 1.84 nmol/mg of protein, respectively).

### Effect of cytidine on [$^{14}$C]PtdCho synthesis in PC12 cells

To determine if the cytidine-induced increase in intracellular CTP levels could affect PtdCho biosynthesis, PC12 cells, incubated in serum-containing DMEM with and without cytidine (100 $\mu M$), were given a 1-h pulse with radiolabeled choline (34 $\mu M$, 7.4 mCi/mmol) and "chased" every 30 min thereafter (for a total of 2.5 h). The rate of [$^{14}$C]choline incorporation into PtdCho was found to be significantly higher in cytidine-supplemented cells (by 30%; $p < 0.01$), and was maintained for a longer period of time (Fig. 3). The observed change in radioactive PtdCho was not due to an isotope dilution effect, because the amounts of radioactive choline and the specific activity of the phosphocholine, measured at the end of the pulse and at 30, 60, and 150 min thereafter, did not differ between cytidine-supplemented and unsupplemented cells. We were not able to quantify [$^{14}$C]CDP-choline, because only trace amounts of this compound could be detected (see Discussion).

In other experiments, cells incubated for 15 min with 0, 10, 25, or 100 $\mu M$ cytidine showed dose-de-

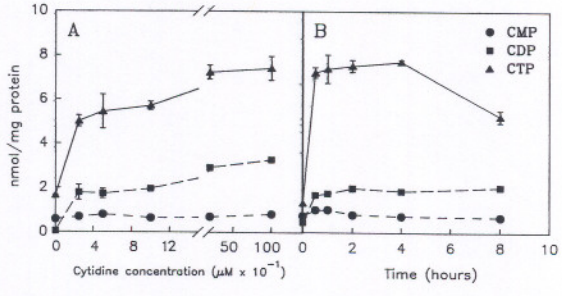


**FIG. 2.** Dose- and time-dependent increase in cytidine nucleotides in PC12 cells. **A:** Nucleotide content as a function of cytidine concentration in the medium. PC12 cells were incubated in N2 medium supplemented with 0, 25, 50, 100, 200, and 1,000 $\mu M$ cytidine, and harvested 2 h thereafter. **B:** Time course of the effect of cytidine supplementation on nucleotide levels in PC12 cells. PC12 cells were incubated in N2 medium supplemented with 100 $\mu M$ cytidine, and their nucleotide contents measured at 30 min, and at 1, 2, 4, and 8 h. At appropriate times, media were removed and the cells washed with PBS and scraped from the dish with 3 ml of ice-cold 1 *M* formic acid. Cell extracts were centrifuged and the nucleotides in the supernatant (acid-soluble) fraction measured by boronate-affinity chromatography and reverse-phase ion-pair HPLC with UV detection (254 nm). Data represent the means ± SEM of three experiments.

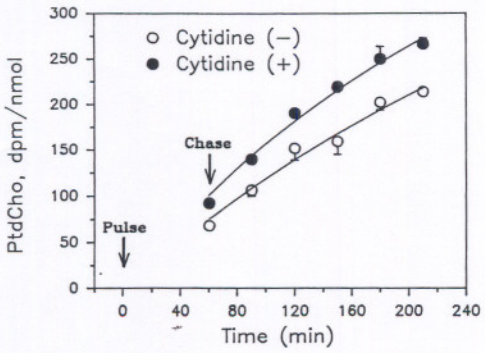


**FIG. 3.** Effects of cytidine on [$^{14}$C]PtdCho synthesis in PC12 cells. Cells were incubated in serum-containing DMEM in the presence of [$^{14}$C]choline (34 $\mu M$, 7.4 mCi/mmol), with and without cytidine (100 $\mu M$). After 60 min, the medium was removed, and the cells were washed twice with PBS and further incubated with unlabeled medium. At 30-min intervals (for 2.5 h), the media were discarded, and the cells were washed with PBS, scraped from the dish, and collected in 1 ml of ice-cold methanol. Two volumes of chloroform were added to the cell suspension, and the mixture was washed with 1 ml of water. Phospholipids in the organic extract were purified by TLC and the radioactivity corresponding to PtdCho counted. The aqueous phase was analyzed for choline-containing metabolites by HPLC at the end of the pulse and at 30, 60, and 150 min thereafter. Data represent means ± SEM of three experiments.

pendent increases in the incorporation of [$^{14}$C]choline into PtdCho (Table 1). Increasing the concentration of cytidine over 100 $\mu M$ did not result in further increases in radiolabeled PtdCho.

### Levels of phospholipid and choline metabolites in cytidine-supplemented PC12 cells

To determine if the accelerated rate of PtdCho synthesis in cytidine-supplemented cells produced a net increase of total PtdCho content, PC12 cells were incubated with or without choline (100 $\mu M$) for 48 h and then supplemented with choline (50 $\mu M$), cytidine (50 $\mu M$), or both. Cellular levels of the phospholipids PtdCho, phosphatidylethanolamine (PtdEtn), and phosphatidylserine (PtdSer), and of choline and acetylcholine, were measured 14 h thereafter (Table 2). Cells incubated in the absence of choline for 48 and 14 h had lower choline and acetylcholine levels than those supplemented with choline in all groups studied. Incubation with cytidine for 14 h did not affect the levels of either compound, whereas incubation with choline significantly increased both ($p < 0.01$).

Total PtdCho concentration was increased slightly, but significantly, in cells incubated with both choline and cytidine, compared with its levels in control cells or in cells supplemented with choline alone ($p < 0.05$; Table 2). Choline supplementation for 14 h significantly elevated PtdCho levels only in cells which had been incubated previously without choline for 48 h. Cytidine-supplemented cells also exhibited increased levels of PtdEtn and PtdSer, whether or not the medium also contained choline ($p < 0.05$; Table 2). Cytidine supplementation failed to affect cellular sphingomyelin content (data not shown).

## DISCUSSION

These data show that cytidine is taken up into PC12 cells and converted to CTP, and that the exogenous pyrimidine increases both the rate of synthesis and the total contents of PtdCho, PtdEtn, and PtdSer in the cells.

When cytidine concentrations in the medium are low, cytidine incorporation follows normal Michaelis–Menten kinetics, and its incorporation into the acid-soluble pool is greater than that into nucleic acids. Similar observations have been made for the incorporation of uridine and other nucleosides in a wide variety of cells (Jacquez, 1962; Scholtissek, 1968; Plagemann, 1971*c*); this suggests that at concentrations below 50 $\mu M$, cytidine is taken up by PC12 cells mainly by a transport system, perhaps involving facilitated diffusion, whereas at concentrations above this level, simple diffusion or a low-affinity uptake system becomes the predominant mode of entry.

Chromatographic analysis of acid extracts from PC12 cells showed that the total content of pyrimidine nucleotides in these cells is about one-fourth that of purine nucleotides (3.85 versus 11.32 nmol/$10^6$ cells). The estimated values in the present study are very close to those reported by Plagemann (1972) for Novikoff rat hepatoma cells, lower than those found by Khym et al. (1978) for some cell types, and in the same range as those reported by Brenton et al. (1977) for lymphoid cells and by Weber and Edlin (1971) for 3T3 cells. Considering that the radius of PC12 cells is approximately 5 $\mu$m (Watanabe et al., 1983), and on the basis of a total volume of 2.5 $\mu$l/$10^6$ cells (Ward and Plagemann, 1969), the average overall intracellular concentration of cytidine nucleotides is 0.17 m$M$, and at least 55% of this may be present as CTP.

The expansion of the cytidine nucleotide pool in the present study, when PC12 cells were supple-

**TABLE 1.** *Dose-dependent increase in [$^{14}$C]PtdCho in PC12 cells supplemented with cytidine*

| Cytidine ($\mu M$) | [$^{14}$C]PtdCho (dpm × $10^{-3}$/mg) | % increase |
|---|---|---|
| 0 | 2.20 ± 0.04 | — |
| 10 | 2.35 ± 0.04[a] | 7 |
| 25 | 2.81 ± 0.03[a] | 22 |
| 100 | 3.19 ± 0.20[a] | 32 |

PC12 cells were incubated in serum-containing DMEM in the presence of [$^{14}$C]choline (34 $\mu M$, 7.4 mCi/mmol), with 0, 10, 25, or 100 $\mu M$ cytidine. After 15 min, the medium was removed and the cells were washed with PBS, scraped from the dish, and collected in 1 ml of ice-cold methanol. Phospholipids were extracted, separated by TLC, and their radioactivity counted as described in Materials and Methods. Values represent means ± SEM of four experiments.
[a] $p < 0.01$ versus preceding value.

**TABLE 2.** *Choline, acetylcholine, and phospholipid contents (nmol/mg of protein) in PC12 cells*

| | Preincubation in the absence of Ch | | | | Preincubation in the presence of Ch | | | |
|---|---|---|---|---|---|---|---|---|
| | — | Ch | Cyd | Ch + Cyd | — | Ch | Cyd | Ch + Cyd |
| Ch | 0.09 ± 0.01 | 0.53 ± 0.04[a] | 0.07 ± 0.01 | 0.58 ± 0.02[a] | 0.20 ± 0.02 | 0.71 ± 0.07[a] | 0.02 ± 0.01 | 0.65 ± 0.02[a] |
| ACh | 0.02 ± 0.01 | 0.27 ± 0.03[a] | 0.04 ± 0.01 | 0.27 ± 0.03[a] | 0.06 ± 0.01 | 0.29 ± 0.03[a] | 0.07 ± 0.01 | 0.23 ± 0.01[a] |
| PtdCho | 39.2 ± 1.7 | 66.3 ± 2.7 | 44.0 ± 1.4 | 73.1 ± 0.9[a,b] | 73.6 ± 1.2 | 75.7 ± 1.9 | 76.1 ± 2.8 | 82.2 ± 1.0[b,c] |
| PtdEtn | 27.4 ± 1.0 | 28.8 ± 1.5 | 33.0 ± 1.1[b,c] | 31.9 ± 0.7[c] | 29.9 ± 0.7 | 33.6 ± 1.7 | 35.4 ± 1.8[c] | 34.5 ± 0.4[c] |
| PtdSer | 8.1 ± 0.2 | 9.6 ± 0.1[c] | 9.4 ± 0.3[c] | 10.7 ± 0.5[a] | 12.3 ± 0.7 | 11.3 ± 0.7 | 13.0 ± 0.6 | 13.2 ± 0.7 |

Intracellular choline (Ch) and acetylcholine (ACh) levels and membrane phospholipid levels of PC12 cells supplemented with cytidine (Cyd). PC12 cells were incubated with and without choline (100 $\mu M$) for 48 h, and then supplemented with choline (50 $\mu M$), cytidine (50 $\mu M$), or both during the following 14 h. Values represent means ± SEM of three experiments.

[a] $p < 0.01$ versus control.

[b] $p < 0.05$ versus choline alone.

[c] $p < 0.05$ versus control.

mented with cytidine, was fast and dose-dependent (Fig. 2), and due primarily to increased levels of CTP. The presence of 25 $\mu M$ cytidine in the medium was sufficient to cause a two- to threefold increase in CTP levels after 2 h of incubation. In spite of the marked increase in cytidine nucleotide levels, the total concentration of nucleotides in the cells was affected only little (74.6 versus 60.7 nmol/mg of protein in unsupplemented cells). A slight increase in uridine nucleotides was apparent, with time, in cells supplemented with 100 $\mu M$ cytidine, but cytidine had practically no effect on the levels of other nucleotides. This observation agrees with previous reports by Plagemann (1971*a,b,* 1972) on Novikoff cells: uridine and cytidine nucleotide levels fell when these cells were incubated with high concentrations of guanosine, inosine, or adenosine, but levels of other nucleotides failed to change when cells were incubated with cytidine or uridine.

It is generally assumed that the formation of CDP-choline is a rate-limiting step in the synthesis of PtdCho (Vance et al., 1980). Thus, it would not be surprising for intracellular levels of this intermediate to be low. Despite the increase in CTP levels in cytidine-supplemented cells, we could not measure CDP-choline levels in these cells or in unsupplemented cells. However, we have found that PC12 cells incubated with [$^{14}$C]choline (8.6 $\mu M$, 0.5 $\mu$Ci, 58.5 mCi/mmol, 20 min) and subsequently "chased" in the presence of depolarizing concentrations of potassium (50 m$M$) exhibited higher radiolabeled CDP-choline levels than cells incubated without potassium. In these circumstances, addition of cytidine to the medium (100 $\mu M$) produces a two- to threefold increase in [$^{14}$C]CDP-choline (data not shown). The effect of potassium may be explained by activation of choline kinase by this ion (Ando et al., 1987), which may increase the availability of radiolabeled phosphocholine that reacts with CTP to form CDP-choline.

The enhancement by cytidine of the incorporation of radiolabeled choline into PtdCho in PC12 cells (Table 1) may reflect increased synthesis of this phospholipid, secondary to elevations in intracellular CTP levels. These findings resemble previous observations in poliovirus-infected HeLa cells, in which a two- to threefold increase in the concentration of CTP in the cytosolic compartment stimulated the incorporation of [*methyl*-$^{3}$H]choline into PtdCho and produced a twofold increase in cytidylyltransferase activity (presumably the rate-limiting step in PtdCho synthesis) (Vance et al., 1980). That CTP could be a critical regulator in PtdCho synthesis is also suggested in experiments in BHK-21 cells incubated with Semliki Forest virus: a 70% depletion in this nucleotide was associated with inhibition of PtdCho formation (Whitehead et al., 1981). However, the activity of microsomal cytidylyltransferase in PC12 cells is not known. In HeLa cells, the apparent $K_m$ for CTP of the cytidylyltransferase is 0.2 m$M$ (Choy et al., 1980), and in rat hepatocytes 0.54 m$M$ or more (Pelech and Vance, 1984). If one assumes that the $K_m$ for CTP of the enzyme is within this range in PC12 cells, then the concentration of CTP in these cells (~90 $\mu M$) would be well below this value. Thus, by providing cytidine, we could increase intracellular CTP levels and the rate of synthesis of PtdCho, and explain the observed increase in the incorporation of radiolabeled choline into PtdCho.

If this hypothesis is true, one might expect to find an absolute increase in the total PtdCho levels in cytidine-supplemented cells. A slight, but significant, increase of this phospholipid was indeed found in cells supplemented with cytidine when choline was also present in the medium. Interestingly, this increase was also accompanied by increased levels of two other major phospholipid constituents of cell membranes, PtdEtn and PtdSer. These data suggest that the relative composition of cell membrane phospholipids is maintained within certain limits, and that an increase in the synthesis of one phospholipid constituent can lead to increased synthesis (or decreased breakdown) of some of the others. Similar conclusions have been drawn from studies on LA-N-2 cells incubated with PtdSer liposomes (Slack et al., 1989); the increase in

membrane PtdSer was followed by activation of the incorporation of choline into PtdCho and of ethanolamine into PtdEtn. Thus, cytidine, by increasing intracellular CTP, and as a consequence membrane PtdCho levels, may stimulate the overall formation of cell membranes or slow the degradation of other phospholipids. Because, in humans cytidine plasma levels range between 1 and 2 $\mu M$ (Lopez G.-Coviella et al., 1987), it would not be surprising if, in some circumstances (i.e., cell membrane deterioration and neuronal cell loss), this substrate may limit the synthesis of membrane phospholipids.

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