# Effect of ethanolamine on choline uptake and incorporation into phosphatidylcholine in human Y79 retinoblastoma cells

**Mark A. Yorek,[1] Joyce A. Dunlap, Arthur A. Spector, and Barry H. Ginsberg**

Veterans Administration Medical Center, Iowa City, IA 52240, and Departments of Internal Medicine, Biochemistry, and Diabetes-Endocrinology Research Center, University of Iowa, Iowa City, IA 52242

**Abstract** The effect of physiological concentrations of ethanolamine on choline uptake and incorporation into phosphatidylcholine was investigated in human Y79 retinoblastoma cells, a multipotential, undifferentiated retinal cell line that has retained many neural characteristics. These cells have a high-affinity uptake system for choline, and the majority of the choline taken up was incorporated into phosphatidylcholine via the CDP-choline pathway. The presence of extracellular ethanolamine significantly decreased high-affinity choline uptake and, subsequently, the amount of choline incorporated into phosphatidylcholine. When 100 μmol/L ethanolamine was added, there was a decrease of about 8% in the phosphatidylcholine content. Ethanolamine had no effect on choline incorporation into phosphatidylcholine, however, once choline was taken up by the cell. The $K'_M$ and $V'_{max}$ for high-affinity choline uptake was increased from 0.93 to 9.74 μM and 19.60 to 79.25 pmol/min per mg protein, respectively, by the presence of 25 μmol/L ethanolamine. In contrast, 25 μmol/L choline had no effect on the kinetic parameters of high-affinity ethanolamine uptake. Therefore, the reduction in high-affinity choline transport by ethanolamine apparently is not simply due to competitive inhibition. 2,2-Dimethylethanolamine and 2-methylethanolamine both reduced choline uptake to a greater extent than ethanolamine. However, because these compounds exist at much lower concentrations than ethanolamine, they probably have little physiological influence. These results suggest that changes in ethanolamine concentration within the physiologic range can regulate the synthesis and content of phosphatidylcholine in a neural cell by influencing the uptake of choline.—**Yorek, M. A., J. A. Dunlap, A. A. Spector, and B. H. Ginsberg.** Effect of ethanolamine on choline uptake and incorporation into phosphatidylcholine in human Y79 retinoblastoma cells. *J. Lipid Res.* 1986. **27:** 1205–1213.

**Supplementary key words** phospholipids • lipids

The human Y79 retinoblastoma is a multipotential neural cell line derived from a tumor of the inner layers of the human retina (1–3). This cell retains many neural characteristics (3–10) and, therefore, is a potentially useful cellular model for the human retina and central nervous system. Y79 cells have a high-affinity uptake system for choline (5), and we recently reported that they also have a separate high-affinity uptake system for ethanolamine (10). An extracellular source of ethanolamine apparently is required by the Y79 cell for phospholipid synthesis, especially for the synthesis of ethanolamine plasmalogen (10).

Choline and ethanolamine glycerophospholipids account for most of the phospholipid content of mammalian membranes (11). In most tissues, choline is primarily incorporated into phospholipid through a pathway involving the formation of CDP-choline (12). The major regulatory step in this pathway is catalyzed by phosphocholine cytidyltransferase (13, 14). Similarly, ethanolamine is incorporated into phosphatidylethanolamine primarily by the formation of CDP-ethanolamine (15, 16). Both choline and ethanolamine may also be incorporated into phospholipid by a $Ca^{2+}$-dependent base exchange reaction (16, 17). In addition, phosphatidylethanolamine may be converted to phosphatidylcholine in many tissues, including brain, by a series of methylation reactions utilizing S-adenosyl-methionine (18, 19).

Ethanolamine is a required nutrient for certain cultured cells (20–22); and, like Y79 cells, some tissues are reported to have a specific uptake mechanism for ethanolamine (23, 24). Furthermore, hamsters have high circulating levels of ethanolamine (24), and recently plasma levels of ethanolamine ranging from 10 to 75 μmol/L have been reported in rats and humans (25–27). Therefore, the availability of ethanolamine in the circulation may be an important factor in regulating and contributing to phospholipid synthesis in certain tissues. In this regard, Zelinski and Choy (28) have reported that high concentrations of ethanolamine reduce the uptake of choline in the perfused hamster heart. Furthermore, we have obtained evidence that ethanolamine may regulate phosphatidylserine decarboxylase activity in Y79 cells (10). To further elucidate

[1] To whom reprint requests should be addressed at: 3E-17, Veterans Administration Medical Center, Iowa City, IA 52240.

the role of ethanolamine availability on phospholipid synthesis in tissues of neural origin, we investigated the effect of physiological levels of ethanolamine on the uptake and incorporation of choline into the phospholipids of human Y79 retinoblastoma cells.

## MATERIALS AND METHODS

### Cell culture

Human Y79 retinoblastoma cells (29) were cultured as a suspension in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 294 μg/ml glutamine (Gibco, Grand Island, NY). The cells were grown in Corning 150-$cm^2$ tissue culture flasks in an Imperial II $CO_2$ incubator (Lab-Line Instruments, Melrose Park, IL) maintained at 37°C, with 5% $CO_2$ in humidified air as the gas phase.

### Transport and incorporation studies

Actively growing Y79 cells were collected by centrifugation, washed with buffer solution, and resuspended either in a medium containing 10 mM HEPES, 128 mM NaCl, 5.2 mM KCl, 2.1 mM $CaCl_2$, 2.9 mM $MgSO_4$, and 5 mM glucose, pH 7.4, or in the RPMI growth medium. Tracer quantities of the following isotopes were added to the incubations: [methyl-$^3$H] or [methyl-$^{14}$C]choline, 78 Ci/mmol, and 50 mCi/mmol, respectively; [1-$^3$H]ethanolamine, 8.8 Ci/mmol, or $^{32}$Pi, carrier-free; purchased from Amersham/Searle, Arlington Heights, IL. Certain of the incubation media were also supplemented with unlabeled choline, ethanolamine, 2-methylethanolamine, or 2,2-dimethylethanolamine. For the kinetic and competition studies, the cells were incubated in a buffered salt solution and uptake was measured after 1 min incubation. After the incubation, two 200-μl aliquots of the suspension were transferred to ice-cold 1.5-ml Sarstedt microfuge tubes containing 0.8 ml of the HEPES buffer layered over 0.3 ml of an *n*-butyl phthalate–corn oil mixture (11:4, v/v) (30). The tubes were immediately centrifuged, the aqueous and oil layers were removed by aspiration, and the bottom of the tube containing the sedimented cells was cut off and added to a 7.0-ml scintillation vial containing 0.3 ml of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL). The cell pellet was then dissolved by incubating the contents of the scintillation vial for 1 hr at 45°C. After adding 5 ml of Neutralizer scintillation solution (Research Products International Corp., Mount Prospect, IL), the radioactivity was measured in a liquid scintillation spectrometer. Quenching was monitored with a $^{226}$Ra external standard. For other incubations, the cells were harvested by centrifugation, washed, and resuspended in buffer. Separate aliquots of the cells were taken to determine total isotope uptake and protein content as previously described (6, 8). The remainder of the cell sample was used for phospholipid analysis. Lipids were extracted with 20 ml of a 2:1:0.015 (v/v/v) mixture of chloroform–methanol–HCl, and the lipid fraction was isolated following phase separation produced by adding 4 ml of 154 mM NaCl containing 0.6 M HCl.

### Separation and analysis of the cellular phospholipids

The lipid extract was collected and dried under $N_2$. Following resuspension in 1 ml of chloroform–methanol–water 75:25:2 (v/v/v), a 100 μl aliquot was taken to determine the total amount of isotope in the lipid extract. Phospholipid classes were separated by a thin-layer chromatography method using LK5D silica gel plates (Whatman, Clifton, NJ) and a solvent system consisting of chloroform–methanol–40% methylamine–2.4 M HCl 60:36:5:5 (v/v/v/v) (31). This system routinely separated phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, inositol phospholipids, and the lysoglycerophospholipids derived from ethanolamine and choline plasmalogens (9). After development, the plates were sprayed with 0.05% 8-anilino-1-naphthalenesulfonic acid in water–methanol 1:1 (v/v), and the phospholipid bands were visualized using a fluorescent light. Each phospholipid was identified by comparison with standards, and the fractions then were collected and transferred into a 7.0-ml scintillation vial, containing 5 ml of Budget Solve scintillation solution (Research Products International Corp., Mount Prospect, IL). The amount of isotope present in each fraction was measured in a liquid scintillation spectrometer. Under these conditions, greater than 95% of the [methyl-$^3$H]choline incorporated into lipid was recovered in the phosphatidylcholine band and 3–4% of the remaining radioactivity in lysophosphatidylcholine. To determine the lipid phosphorus content, the phospholipid bands were extracted from the silica gel by adding the samples to separatory funnels containing 4 ml of acidic saline. After mixing, phase separation was induced by adding 10 ml of chloroform–methanol 1:1 (v/v). The chloroform phase was isolated, and the phosphorus content was determined by the method of Chalvardjian and Rudnicki (32). Total phosphorus phospholipid content was determined in an aliquot prior to phospholipid separation.

To determine the amount of phosphatidylethanolamine that was converted into phosphatidylcholine, the uptake and incorporation into phospholipid of L-[methyl-$^3$H]methionine was examined (10).

### Separation and analysis of phosphocholine and CDP-choline

Phosphocholine and CDP-choline were collected from the aqueous fraction of the lipid extract, separated, and analyzed for radioactivity (33). Following the incubation of the cells with [methyl-$^{14}$C]choline or $^{32}$Pi, the cells were extracted and the aqueous layer was collected, washed, and evaporated to dryness under $N_2$. The sample was redissolved in 100 $\mu$l of 50% ethanol and an aliquot was taken to determine total radioactivity. The remaining extract was then applied to LK5D silica gel plates and chromatographed with a solvent system containing methanol–0.5% NaCl–ammonia 100:100:2 (v/v/v). After drying, the plates were exposed for 96 hr to X-ray film (XAR-5, Kodak) to determine the location of the radioactive bands which were identified by comparison with [methyl-$^{14}$C]choline, phosphoryl[methyl-$^{14}$C]choline, and cytidine 5′-diphospho[methyl-$^{14}$C]choline standards. The radioactive bands were scraped and radioactivity was measured in a liquid scintillation spectrometer. CDP-ethanolamine and phosphoethanolamine did not co-elute with the corresponding choline derivatives in this system.

### Data analysis

Values were calculated per mg of cell protein. The protein content of the cells was determined by a modification of the Lowry method (34). Kinetic analysis of the choline and ethanolamine uptake data was done with an IBM personal computer using a weighted least-squares fit of the experimental points to a double reciprocal plot (35). All comparisons for significance were made by Student's t-test.

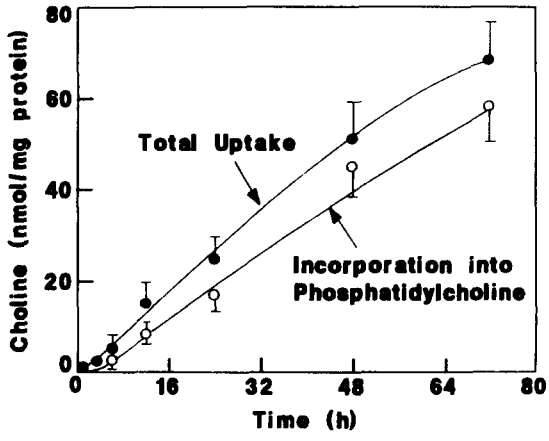


**Fig. 1.** Choline uptake and incorporation into phosphatidylcholine. Y79 cells were incubated for up to 72 hr in RPMI medium containing 21.5 $\mu$mol/L choline together with tracer amounts of [methyl-$^{3}$H]choline. Samples were taken at the indicated times, and the amount of choline taken up and incorporated into cellular lipid was measured. Each value is the average of four separate determinations, and the standard error of the mean is indicated by the vertical line.

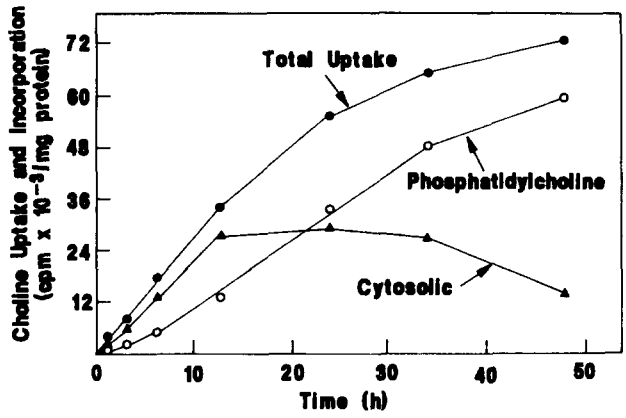


**Fig. 2.** Choline incorporation into phosphocholine, CDP-choline, and phosphatidylcholine. Y79 cells were incubated for up to 48 hr in RPMI medium containing 21.5 $\mu$mol/L choline together with tracer amounts of [methyl-$^{14}$C]choline. Samples were taken at the indicated times, and the amount of choline taken up and incorporated into phosphocholine, CDP-choline, and phosphatidylcholine was determined. The cytosolic fraction consists of free intracellular choline, phosphocholine, and CDP-choline. Each value is the average of three separate determinations. The standard error of the mean is less than 15% for each point.

## RESULTS

### Choline uptake and incorporation into phosphatidylcholine

The uptake of [methyl-$^{3}$H]choline and its incorporation into phosphatidylcholine by human Y79 retinoblastoma cells was followed during a 72-hr incubation period (**Fig. 1**). Choline was rapidly taken up by the cells and, following a short lag period, was incorporated into phosphatidylcholine. After 48 hr and 72 hr of incubation, more than 90% of the choline taken up by the cells was recovered in phosphatidylcholine.

In additional studies the simultaneous incorporation of [methyl-$^{3}$H]choline and $^{32}$Pi into phosphatidylcholine was examined over a 48-hr incubation period in RPMI media. Throughout the 48-hr period, about the same amount of [methyl-$^{3}$H]choline and $^{32}$Pi were incorporated into phosphatidylcholine. After 48 hr 40.5 ± 2.3 and 38.6 ± 1.2 nmol/mg of protein of [methyl-$^{3}$H]choline and $^{32}$Pi, respectively, were incorporated into phosphatidylcholine, suggesting that most of the choline incorporation occurs by de novo synthesis through the CDP-choline pathway and not by base exchange. This conclusion is supported by the results of studies presented in **Fig. 2,** which shows the amount of radioactivity (cpm/mg protein) from [methyl-$^{14}$C]choline incorporated into the choline-containing phospholipid precursors during an incubation of up to 48 hr. The amount of [methyl-$^{14}$C]choline (**Fig. 2**) appearing in the nonlipid fractions (cytosolic) reached saturation after 12 hr and remained constant for 24 hr

before declining. In contrast, [methyl-$^{14}$C]choline incorporation into phosphatidylcholine approached linearity after 6 hr of incubation and remained linear until 36 hr, after which the rate of incorporation also declined slightly. Further analysis of the nonlipid fraction showed that phosphocholine accounted for about 85% of the [methyl-$^{14}$C]choline radioactivity. The remaining 15% of the radioactivity in this fraction was divided between choline (5%) and CDP-choline (10%). Both the phosphocholine and CDP-choline fractions reached a constant radioactivity content after 12 hr of incubation. Similar studies with $^{32}$Pi showed that the amount of $^{32}$Pi-derived radioactivity appearing in the nonlipid fraction, consisting of phosphocholine and CDP-choline also approached a constant level after 12 hr of incubation. This level was maintained for the ensuing 36 hr. The incorporation of $^{32}$Pi into total phospholipid approached linearity after 24 hr. Taken together, these data suggest that the amounts of phosphatidylcholine synthesis calculated from the [methyl-$^{3}$H]choline and $^{32}$Pi incorporation results obtained after 24 hr are probably accurate estimates because after 12 hr of incubation the intracellular pools of phosphatidylcholine precursors have attained a constant radioactivity content, based on cell protein.

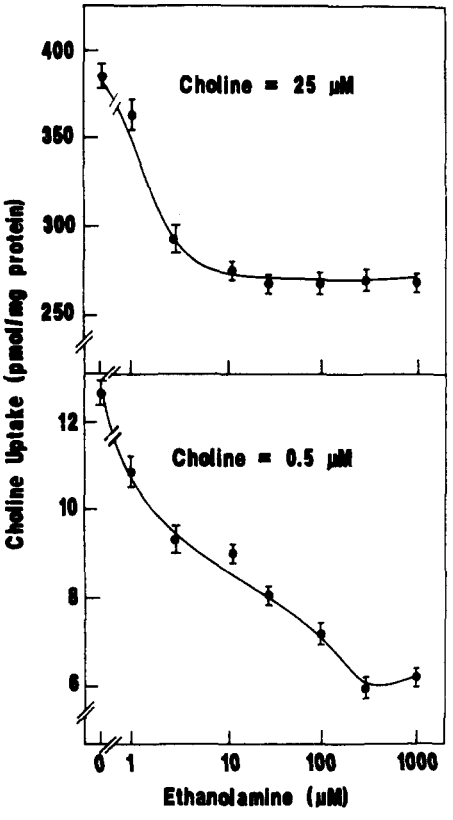


**Fig. 3.** Effect of ethanolamine on high-affinity choline uptake. The Y79 cells were incubated in HEPES buffer containing 0.5 or 25 μmol/L choline together with tracer amounts of [methyl-$^{3}$H]choline, in the presence of 0–1,000 μmol/L ethanolamine. The cells were preincubated for 10 min in buffer alone or with ethanolamine, and the reaction was then started by adding the [methyl-$^{3}$H]choline solution. After 1 min of incubation, aliquots were taken and the total amount of choline uptake was measured. Each value is the mean of four separate determinations, and the standard error of the mean is indicated by the vertical line.

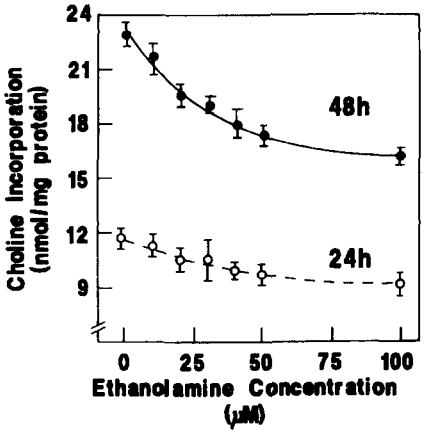


**Fig. 4.** Effect of ethanolamine on choline incorporation into phosphatidylcholine. The Y79 cells were incubated for up to 48 hr in RPMI medium containing 21.5 μmol/L choline together with tracer amounts of [methyl-$^{3}$H]choline. Cells were also incubated with various concentrations of ethanolamine from 0–100 μmol/L. After 24 hr (open circles) and 48 hr (closed circles) of incubation, aliquots were taken and total amount of choline incorporated into phosphatidylcholine was measured. Each value is the mean of four separate determinations, and the standard error of the mean is indicated by the vertical line.

### Effect of ethanolamine on choline uptake

To determine the effect of ethanolamine on choline uptake, cells were incubated with increasing amounts of ethanolamine (0–1,000 μmol/L) and the uptake of physiological concentrations of choline was determined after a 1-min incubation. **Fig. 3** shows the effect of ethanolamine concentration on the uptake of 0.5 (bottom) or 25 (top) μmol/L choline by Y79 retinoblastoma cells. The presence of 5 μmol/L ethanolamine significantly decreased choline uptake at both concentrations by about 25% ($P < 0.02$ and 0.01, respectively). Increasing ethanolamine caused an even greater reduction of choline uptake, and maximal inhibition occurred within the approximate range of physiological ethanolamine concentrations, 10–100 μmol/L.

### Effect of ethanolamine on choline incorporation into phosphatidylcholine

**Fig. 4** shows the effect of physiological concentrations of ethanolamine on the incorporation of choline (21.5 μmol/L) into phosphatidylcholine. These studies were

conducted to determine the longer term effects of ethanolamine on choline metabolism. Ethanolamine caused a concentration-dependent reduction in choline incorporation into phosphatidylcholine after either a 24- or 48-hr incubation, the reduction being about 25% at 100 $\mu$M ethanolamine.

In contrast, ethanolamine had no effect on the incorporation of [methyl-$^3$H]choline into phosphatidylcholine once choline had been taken up by the Y79 cells. Data in **Fig. 5** show that the amount of [methyl-$^3$H]choline incorporated into phosphatidylcholine following a pulse labeling of the cells increased linearly for 6 hr then remained constant. During this period, the incorporation was the same for control cells or those exposed to 10–1,000 $\mu$mol/L ethanolamine.

### Phosphatidylcholine and ethanolamine phospholipid content of Y79 cells exposed to ethanolamine

Data in **Table 1** show that the total phospholipid content of Y79 cells was not altered by the presence of 100 $\mu$mol/L ethanolamine. However, ethanolamine caused an 8% decrease in the phosphatidylcholine content of the cells. This was accompanied by a consistent increase in the amount of ethanolamine phospholipids within the cell, although this change did not reach statistical significance.

The methylation of phosphatidylethanolamine occurs slowly in Y79 cells (10), and we find that the addition of 100 $\mu$mol/L ethanolamine causes only a 25% increase in the activity of this pathway. This contributes 0.43 nmol to the synthesis of phosphatidylcholine in a 24-hr incubation period (data not shown).

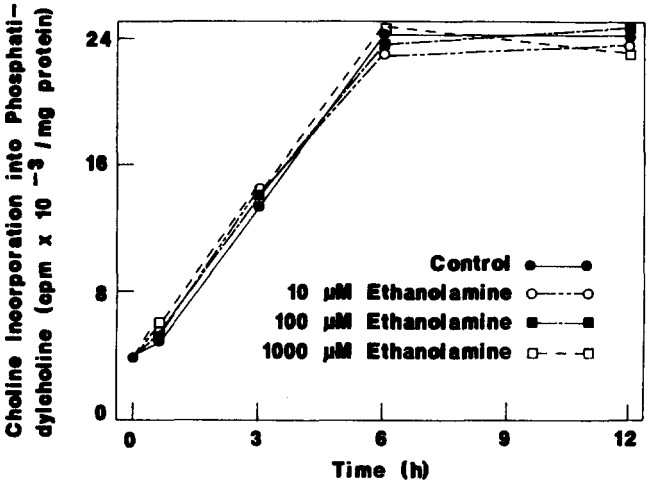


**Fig. 5.** Effect of ethanolamine on choline incorporation into phosphatidylcholine after labeling the cells with choline. The Y79 cells were pulse-labeled with [methyl-$^3$H]choline for 30 min. This medium was removed and the cells were washed, resuspended, and incubated in RPMI 1640 medium containing 0–1000 $\mu$mol/L ethanolamine. At the indicated times, samples were taken and the amount of [methyl-$^3$H]choline incorporated into phosphatidylcholine was measured. Each value is the mean of three separate determinations. The standard error of the mean is less than 10% for each point.

TABLE 1. Effect of ethanolamine availability on phosphatidylcholine and ethanolamine phospholipid content

| Addition | Phospholipid | | |
|---|---|---|---|
| | Total | Phosphatidylcholine | Ethanolamine Phospholipids |
| | *nmol P / mg protein* | % | |
| None | 144.4 ± 10.1 | 55.2 ± 1.0 | 35.5 ± 2.5 |
| Ethanolamine | 147.1 ± 15.0 | 50.6 ± 0.8[a] | 39.3 ± 2.0 |

Y79 cells were incubated for 48 hr in RPMI media ± 100 $\mu$M ethanolamine. Afterwards, cells were collected, washed, and resuspended in buffer. Aliquots were taken to determine protein and phospholipid content. The phospholipid classes were separated by thin-layer chromatography and the lipid phosphorus content was determined. Each value is the mean of nine separate determinations ± SEM.

[a] $P < 0.005$, as compared to control.

### Kinetic parameters of high-affinity choline and ethanolamine uptake

The data in **Fig. 6** show the Lineweaver-Burk plots of the effect of ethanolamine (25 $\mu$mol/L) on high-affinity choline uptake (left), and the effect of choline (25 $\mu$mol/L) on high-affinity ethanolamine uptake (right). When ethanolamine was present, choline uptake was inhibited. In contrast, choline had no appreciable effect on ethanolamine uptake. **Table 2** contains the kinetic parameters for the uptake processes. When 25 $\mu$mol/L ethanolamine was added, the $K'_M$ and $V'_{max}$ for high-affinity [methyl-$^3$H]choline uptake was increased. The presence of 25 $\mu$mol/L choline in the medium, however, had no significant effect on the high-affinity uptake parameters for [1-$^3$H]ethanolamine.

### Effect of ethanolamine derivatives on choline uptake

To determine the effect on choline uptake of the two methylated forms of ethanolamine that occur physiologically, Y79 cells were preincubated for 10 min with ethanolamine or the methylated ethanolamine derivatives and then assayed for [methyl-$^3$H]choline uptake (**Table 3**). 2,2-Dimethylethanolamine and 2-methylethanolamine were both more effective inhibitors than ethanolamine at all concentrations examined. 2,2-Dimethylethanolamine (5 $\mu$mol/L) reduced [methyl-$^3$H]choline uptake by about 65%, while 2-methylethanolamine (5 $\mu$mol/L) caused a 50% reduction. Ethanolamine (5 $\mu$mol/L) reduced [methyl-$^3$H]choline uptake by 30% in these studies.

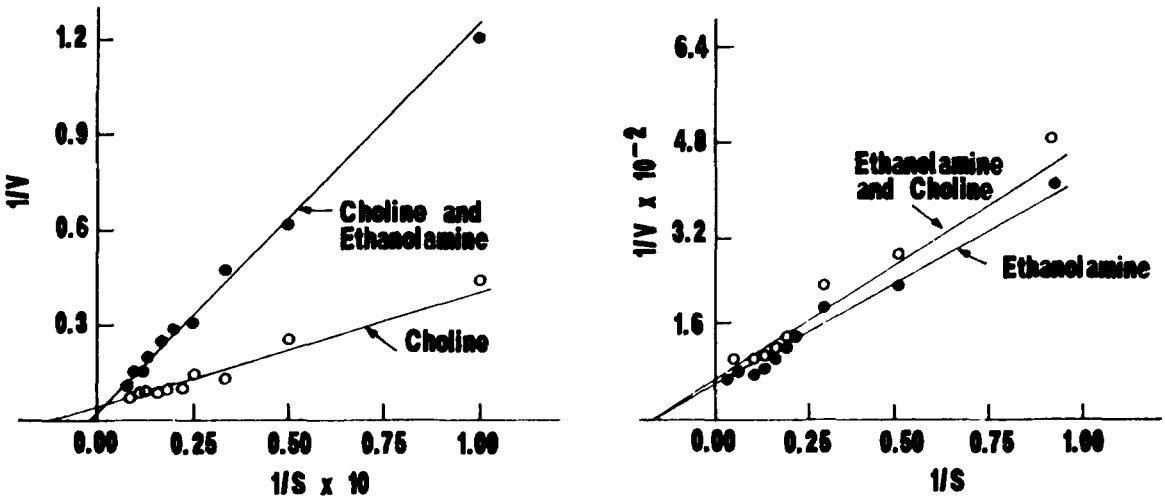


**Fig. 6.** Lineweaver-Burk plots of choline or ethanolamine high-affinity uptake. The effects of 25 μmol/L ethanolamine on high-affinity choline uptake (left) and 25 μmol/L choline on high-affinity ethanolamine uptake (right) are shown. The Y79 cells were incubated as described in Table 2. Each value is a mean of four separate determinations. The standard error of the mean is less than 10% for each point.

## DISCUSSION

Phosphatidylcholine, the most abundant phospholipid in mammalian cells, can be synthesized by three separate pathways (13). In the central nervous system, the majority of phosphatidylcholine is synthesized de novo from choline via the CDP-choline pathway (19). The major rate-limiting step in this pathway is catalyzed by phosphocholine cytidyltransferase (13, 14). The present results indicate that the majority of the choline incorporated into phosphatidylcholine by the Y79 retinoblastoma, a human retinal cell line that retains many neural characteristics (3–10), also occurs primarily by the de novo CDP-choline pathway. This is demonstrated by the data showing that when the cells are incubated with [methyl-$^3$H]choline and $^{32}$Pi long enough for the internal pools to reach constant specific activity, the incorporation of each isotope into phosphatidylcholine is about the same. This is also suggested by the lag of about 30 min that occurs before the appearance of [methyl-$^3$H]choline in phosphatidylcholine. The lag period is likely due to the time required for enough [methyl-$^3$H]choline to accumulate in the CDP-choline pool before radioctivity can apear in phosphatidylcholine. Overall, these data suggest that the contribution of the base exchange reaction to the synthesis of phosphatidylcholine in human cells of neural origin probably is minor (14).

Ethanolamine in physiological concentrations significantly reduced [methyl-$^3$H]choline uptake by the Y79 cells. This resulted in a decrease in the amount of [methyl-$^3$H]choline incorporated into phosphatidylcholine. These results are consistent with the findings of Zelinski and Choy (36) who showed that ethanolamine inhibits choline

TABLE 2. Kinetic parameters of choline and ethanolamine high-affinity uptake: effect of extracellular ethanolamine and choline

| Substrate | Addition | Kinetic Parameters | |
|---|---|---|---|
| | | $K'_m$ | $V'_{max}$ |
| | | *μM* | *pmol/min per mg protein* |
| [$^3$H]Choline | None | 0.93 ± 0.01 | 19.60 ± 1.67 |
| [$^3$H]Choline | 25 μM ethanolamine | 9.74 ± 0.57[a] | 79.25 ± 8.29[a] |
| [$^3$H]Ethanolamine | None | 9.8 ± 0.6 | 318.0 ± 15.0 |
| [$^3$H]Ethanolamine | 25 μM choline | 10.2 ± 0.4 | 242.4 ± 15.4 |

Y79 cells were incubated for 1 min with 0.1 to 1.0 μmol/L [methyl-$^3$H]choline ± 25 μM ethanolamine, or for 1 min with 1 to 10 μmol/L [1-$^3$H]ethanolamine ± 25 μM choline. Each value is the average of four separate determinations ± SEM.

[a] $P < 0.001$, as compared to choline alone.

TABLE 3. Effect of ethanolamine, methylethanolamine, or dimethylethanolamine on choline uptake

| Additions | Concentration | Uptake | P |
|---|---|---|---|
| | μM | pmol/mg protein | |
| None | | 28.4 ± 0.8 | |
| Ethanolamine | 1 | 23.7 ± 1.4 | 0.05 |
| Ethanolamine | 5 | 20.7 ± 1.9 | 0.02 |
| Ethanolamine | 25 | 13.8 ± 1.4 | 0.001 |
| 2-Methylethanolamine | 1 | 14.9 ± 1.0 | 0.001 |
| 2-Methylethanolamine | 5 | 13.4 ± 0.7 | 0.001 |
| 2-Methylethanolamine | 25 | 8.8 ± 0.8 | 0.001 |
| 2,2-Dimethylethanolamine | 1 | 14.6 ± 0.7 | 0.001 |
| 2,2-Dimethylethanolamine | 5 | 9.4 ± 0.4 | 0.001 |
| 2,2-Dimethylethanolamine | 25 | 5.3 ± 0.2 | 0.001 |

Y79 cells were preincubated for 10 min in either buffer alone or with the compounds listed at the indicated concentrations. The incubations were started by the addition of 1.0 μmol/L [methyl-$^3$H]choline. After 1 min, samples were taken to measure choline uptake. Each value is the average of three separate determinations ± SEM.

uptake and the subsequent incorporation of choline into phosphatidylcholine in hamster heart. 2,2-Dimethylethanolamine and 2-methylethanolamine both were more effective than ethanolamine in reducing choline uptake. However, because dimethylethanolamine and methylethanolamine exist in the circulation at much lower concentrations than ethanolamine, they probably have comparatively little physiological effect (26). An 8% decrease in the cellular content of phosphatidylcholine resulted after a 48-hr incubation in the presence of 100 μmol/L ethanolamine, indicating that the reduction in choline uptake is sufficient to produce some perturbation in the composition of the cell phospholipids. Since ethanolamine had no effect on choline incorporation into phosphatidylcholine following choline uptake (Fig. 5), the effect of ethanolamine on choline metabolism must be due to the decrease that it produces in the uptake. Although this is a small change, it could have physiological implications considering the unequal distribution of phosphatidylcholine across the lipid bilayer, the distinctive fatty acid content of cellular phospholipids, and the possibility that the effect could be localized to certain membrane lipid domains. The increase in ethanolamine availability did not result in an appreciable increase in phosphatidylethanolamine methylation, probably because the activity of the methylation pathway in these cells is low (10). The content of the ethanolamine phospholipids also was not significantly increased. Furthermore, as opposed to what occurs in mammary carcinoma cells (21), ethanolamine supplementation did not change the growth rate of the Y79 cells.

Zelinski and Choy (36) have also reported that choline regulates phosphatidylethanolamine synthesis by limiting ethanolamine phosphorylation and conversion to CDP-ethanolamine. Unlike these findings in heart, however, we found that choline had no effect on ethanolamine uptake or incorporation into phospholipid in Y79 cells (10).

Previous studies with Y79 cells have shown that they contain a high- and low-affinity uptake system for choline similar to retina (37), cultured brain cells (33, 38), and neuroblastoma x glioma hybrid cells NG108-15 (39). Y79 cells also have a high-affinity uptake system for ethanolamine which is distinct from the choline transporter (10). In the present studies, we observed that the presence of 25 μmol/L ethanolamine significantly increases both the $K'_M$ and $V'_{max}$ for high-affinity choline uptake by Y79 cells. This suggests a more complex mechanism of inhibition than competitive. Assuming that transport processes behave similarly to enzymatic reactions and that the analysis used in enzymatic studies is valid for carrier-mediated systems, it appears that ethanolamine may reduced choline uptake by a complex-mixed type of inhibition. This interpretation is different from what Zelinski and Choy (28) observed with the perfused hamster heart, where ethanolamine reduced high-affinity choline uptake by competitive inhibition. Our data would seem to eliminate the possibility of competitive inhibition in the Y79 cell because the $K'_M$ and $V'_{max}$ for high-affinity choline uptake are both significantly altered, the inhibition of choline transport by ethanolamine is not totally dependent on the choline concentration, and choline has no effect on high-affinity ethanolamine uptake. The latter observation also rules out the possibility that choline and ethanolamine uptake are mediated by a common transporter. Therefore, ethanolamine probably either binds irreversibly to the choline binding site of the transporter or it binds to an adjacent region which prevents the choline transporter from operating effectively. Although the presence of ethanolamine causes an increase in the $V'_{max}$ for high-affinity choline transport and presumably an increase in the number of transporters, the overall effect is a decrease in choline uptake. Therefore, the increase in the $K'_M$ corresponding to a decrease in choline's affinity for its transporter appears to be the critical factor that mediates ethanolamine's influence on choline uptake. Zelinski and Choy (28) did not examine the effect of choline on ethanolamine uptake, and they also used a much higher concentration of ethanolamine in their studies. This could explain the differences between their findings and the present results. Alternatively, species or tissue differences may account for the apparent discrepancy. The difficulty in analyzing metabolic effects in perfused organs also may have contributed to the difference between the results in the two systems.

In conclusion, these studies suggest that ethanolamine availability in the extracellular fluid may affect choline

uptake in human retina and neural cells. These findings suggest another mechanism whereby phosphatidylcholine synthesis can be regulated in the nervous system, by limitation of the intracellular supply of choline. Since little is known about the fluctuations in ethanolamine concentration in vivo, it is possible that the influence of ethanolamine on choline transport may vary in different physiologic states. In this regard, Baba et al. (26) have reported that circulating ethanolamine levels are increased in patients with kidney disorders. The present results, together with those of Zelinski and Choy (28), suggest that, by changing the relative amounts of choline and ethanolamine that are available to the cells, the membrane levels of phosphatidylcholine might be altered, perhaps leading to altered membrane function in certain excitable tissues.

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