Effects of Analogues of Ethanolamine and Choline on Phospholipid Metabolism in Rat Hepatocytes

By BJÖRN ÅKESSON

Department of Physiological Chemistry, University of Lund, Lund, Sweden

(Received 29 April 1977)

1. Analogues of ethanolamine and choline were incubated with different labelled precursors of phospholipids and isolated hepatocytes and the effects on phospholipid synthesis were studied. 2. 2-Aminopropan-1-ol and 2-aminobutan-1-ol were the most efficient inhibitors of [^{14}C]ethanolamine incorporation into phospholipids, whereas the incorporation of [^3H]choline was inhibited most extensively by *NN*-diethylethanolamine and *NN*-dimethylethanolamine. 3. When the analogues were incubated with [^3H]glycerol and hepatocytes, the appearance of ^3H in unnatural phospholipids indicated that they were incorporated, at least in part, via CDP-derivatives. The distribution of [^3H]glycerol among molecular species of phospholipids containing 2-aminopropan-1-ol and 1-aminopropan-2-ol was the same as in phosphatidylethanolamine. In other phospholipid analogues the distribution of ^3H was more similar to that in phosphatidylcholine. 4. *NN*-Diethylethanolamine stimulated both the conversion of phosphatidylethanolamine into phosphatidylcholine and the incorporation of [$\text{Me-}^{14}\text{C}$]methionine into phospholipids. Other *N*-alkyl- or *NN*-dialkyl-ethanolamines also stimulated [^{14}C]methionine incorporation, but inhibited the conversion of phosphatidylethanolamine into phosphatidylcholine. This indicates that phosphatidyl-*NN*-diethylethanolamine is a poor methyl acceptor, in contrast with other *N*-alkylated phosphatidylethanolamines. 5. These results on the regulation of phospholipid metabolism in intact cells are discussed with respect to the possible control points. They also provide guidelines for future experiments on the manipulation of phospholipid polar-headgroup composition in primary cultures of hepatocytes.

Analogues of ethanolamine and choline were first studied with respect to their ability to cure or aggravate choline deficiency (for references see Griffith & Nyc, 1971). Several analogues are incorporated into rat liver phospholipids in considerable amounts (Artom *et al.*, 1958; Longmore & Mulford, 1960; Katyal & Lombardi, 1974; Lee *et al.*, 1975). The incorporation occurs, at least in part, via CDP derivatives (Weiss *et al.*, 1958), although the analogues have been reported to be more poorly utilized than the corresponding derivatives of ethanolamine and choline, especially in the cytidyltransferase reactions (EC 2.7.7.14, 2.7.7.15) (Chojnacki, 1964; Ansell & Chojnacki, 1966; Chojnacki & Ansell, 1967). There has subsequently been renewed interest in these compounds in studies on membrane phospholipid modification (Glaser *et al.*, 1974; Katyal & Lombardi, 1974; Lee *et al.*, 1975; Blank *et al.*, 1975). For such investigations, it would be advantageous to know their relative capacity for incorporation into tissue phospholipids. Therefore the incorporation and effects on phospholipid metabolism of a wide range of analogues of ethanolamine and choline were studied in isolated hepatocytes. The results will be useful for studies on membrane phospholipid modification in primary culture of hepatocytes (Åkesson, 1977).

Experimental

Materials

Ethanolamine, choline chloride, *NN*-dimethylethanolamine (2-dimethylaminoethanol), 2-amino-2-methylpropan-1-ol and chlorocholine [(2-chloroethyl)trimethylammonium chloride] were obtained from BDH Chemicals, Poole, Dorset, U.K. DL-2-Aminopropanol and 3-aminopropan-1-ol were bought from Merck-Schuchardt, Darmstadt, Germany. 1-Aminopropan-2-ol, L-2-aminobutan-1-ol, 2-amino-methylpropan-2-ol hydrochloride, *N*-ethylethanolamine (2-ethylaminoethanol), *NN*-diethylethanolamine (2-diethylaminoethanol), 1-dimethylamino-propan-2-ol, 2-chloroethylamine hydrochloride, 2-aminoethanethiol hydrochloride, 2-dimethylaminoethylchloride hydrochloride (2-chloro-*NN*-dimethylethylamine hydrochloride) and bromocholine [(2-bromoethyl)trimethylammonium bromide] were obtained from Aldrich-Europe, Beerse, Belgium. *N*-Isopropylethanolamine (2-isopropylaminoethanol) and triethylcholine [triethyl(2-hydroxyethyl)-ammonium iodide] were obtained from Eastman Kodak Co., Rochester, NY, U.S.A., and *N*-methyl-ethanolamine was from Koch-Light, Colnbrook, Bucks., U.K. Methyl iodide, ethyl iodide and propyl

iodide were from Merck-Schuchardt. $[1(3)\text{-}^3\text{H}]$ -Glycerol, $[\text{Me-}^{14}\text{C}]$ methionine, $[2\text{-}^{14}\text{C}]$ ethanolamine and $[\text{Me-}^3\text{H}]$ choline were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Synthesis of phospholipids

Soya-bean phosphatidylethanolamine and soya-bean phosphatidylcholine were obtained from Dr. D. LeKim, Köln, Germany. They were purified by column chromatography on silicic acid (Lea *et al.*, 1955) and alumina (Singleton *et al.*, 1965) respectively, by using mixtures of chloroform/methanol. Phosphatidyl-*N*-methylethanolamine, phosphatidyl-*N*-ethylethanolamine, phosphatidylpropanolamine (containing 2-aminopropanol) and phosphatidyl-*NN*-diethylethanolamine were prepared by phospho-

lipase D hydrolysis of soya-bean phosphatidylcholine in the presence of the respective base (Yang *et al.*, 1967). The reactions were monitored by t.l.c. (see Figs. 1 and 2) and the products were separated from phosphatidate by silicic acid column chromatography using stepwise elution with methanol/chloroform (5–30%, v/v) and t.l.c. on silica gel [developing solvent, chloroform/methanol/conc. ammonia (12:6:1, by vol.)]. Phosphatidyl-*NN*-dimethylethanolamine was synthesized as described by LeKim & Betzing (1973). Quaternary-amine phospholipids were prepared from tertiary-amine phospholipids and alkyl iodides as described for phosphatidylcholine

Fig. 1. Thin-layer chromatography of phospholipids containing analogues of ethanolamine and choline

The applied substances are: phosphatidylethanolamine (1), phosphatidylpropanolamine (2), phosphatidyl-*N*-methylethanolamine (3), phosphatidyl-*N*-ethylethanolamine (4), phosphatidyl-*NN*-dimethylethanolamine (5), phosphatidyl-*NN*-diethylethanolamine (6), and rat liver phospholipids (7). Developing solvents: (a) chloroform/methanol/acetic acid/water (65:25:4:4, by vol.); (b) chloroform/methanol/conc. ammonia (12:6:1, by vol.). Spray: 0.6% (w/w) $\text{K}_2\text{Cr}_2\text{O}_7$ in 55% (w/w) H_2SO_4 . Horizontal lines indicate points of application.

Fig. 2. Thin-layer chromatography of phospholipids with a quaternary ammonium group

The applied substances are: phosphatidylcholine (1), phosphatidyl-*N*-ethyl-*NN*-dimethylethanolamine (2), phosphatidyl-*NN*-dimethyl-*N*-propylethanolamine (3), phosphatidyl-*NN*-diethyl-*N*-methylethanolamine (4), phosphatidyl-*NNN*-triethylethanolamine (5), and rat liver phospholipids (6). Solvents and spray were as in Fig. 1.

synthesis (LeKim & Betzing, 1973). Phosphatidylcholine was rapidly formed as described by LeKim & Betzing (1973), but when the alkyl chain in either the phospholipid or the alkyl iodide was elongated the rate of the reaction was considerably slower. For instance, only 50% of phosphatidyl-*NN*-diethylethanolamine was converted into phosphatidyl-*NNN*-triethyl-ethanolamine after 1 week in the presence of ethyl iodide. It was also observed that the reaction was more rapid when the reactants were dissolved in methanol than in chloroform. The products were extracted into chloroform, washed with methanol/water (1:1, v/v) and purified by t.l.c. (Figs. 1 and 2). Phosphatidylpropanolamine had an R_F value slightly higher than that of phosphatidylethanolamine. Phosphatidyl-*NN*-dimethylethanolamine and phosphatidyl-*NN*-diethylethanolamine had higher R_F values than the corresponding monoalkyl phospholipids. In an acidic solvent, ethyl derivatives had similar R_F values to methyl derivatives, whereas in an alkaline solvent they had significantly higher R_F values (Fig. 1). For quaternary-amine phospholipids the R_F value increased with increasing alkyl-chain length on the quaternary nitrogen, in both acidic and alkaline systems (Fig. 2). In the identification of labelled phospholipids formed by hepatocytes, the acidic solvent system was used for separation of quaternary-amine phospholipids, and the alkaline system for other phospholipids. All phospholipids could not be separated simultaneously in these systems. However, for the mixtures encountered in the present study they were quite adequate. Special separation steps were necessary for phospholipids containing *N*-methylethanolamine (Sundler & Åkesson, 1975), 2-aminopropanol and 2-aminobutanol. The last two phospholipids were separated from phosphatidylethanolamine in the solvent chloroform/methanol/conc. NH_3 (sp.gr. 0.880) (40:10:1, by vol.). The plates (12 cm in length) were run three to five times in this solvent before the separation was adequate.

Cell incubations

Hepatocytes were isolated from male Sprague-Dawley rats, fed on a balanced diet, by the procedure described elsewhere (Nilsson *et al.*, 1973), except that CaCl_2 was added together with collagenase after the initial perfusion with Ca^{2+} -free buffer (Seglen, 1973). As described previously (Sundler *et al.*, 1974), incubations were performed in 25 ml Erlenmeyer flasks in a total volume of 0.5 ml of Hanks solution (Hanks & Wallace, 1949) buffered at pH 7.4 with 10 mM-sodium phosphate and 20 mM-Hepes.* After incubation at 37°C the reactions were terminated by

3 ml of chloroform/methanol (1:1, v/v) (Sundler *et al.*, 1974). Lipids were separated by t.l.c. as described above and assayed for radioactivity (Sundler *et al.*, 1974). For the separation of molecular species, the phospholipids were converted into acetyldiacylglycerol by treatment with phospholipase C from *Bacillus cereus* (Boehringer, Mannheim, Germany) (Van Golde & Van Deenen, 1966) and subsequent acetylation (Elovson, 1965). Acetyldiacylglycerol was purified by t.l.c. and separated by argentation t.l.c. (Åkesson *et al.*, 1970) with toluene/methanol (23:2, v/v) as developing solvent.

Results

Different analogues were tested for their ability to inhibit the incorporation of small amounts of [^{14}C]ethanolamine and [^3H]choline into hepatocyte phospholipids (Table 1). 2-Aminopropanol and 2-aminobutanol most efficiently inhibited the incorporation of ethanolamine. Other aminopropanols with a methyl group attached to the hydroxyl carbon or with three methylene groups between the hydroxy- and amino-groups were much less effective. Addition of another methyl group to the amino-carrying carbon (2-amino-2-methylpropanol) resulted in complete loss of inhibition. Ethanolamines containing *N*-alkyl groups also inhibited ethanolamine incorporation and the inhibition decreased with increasing number and chain length of the alkyl groups.

The pattern for inhibition of [^3H]choline incorporation was completely different (Table 1). *NN*-Diethylethanolamine and *NN*-dimethylethanolamine were most effective, but also *N*-alkylethanolamines inhibited significantly. *NNN*-Triethylethanolamine was clearly less effective. Some analogues containing a primary amino group inhibited [^3H]choline incorporation. Also in this context 2-aminobutanol and 2-aminopropanol were the most effective substances.

Analogues containing halogens or thiols instead of the hydroxy group did not inhibit very much. Aminoethanethiol and bromocholine inhibited [^3H]choline incorporation but not as effectively as the hydroxyl-containing analogues.

Some of the most inhibitory analogues were also tested at different larger concentrations of [^{14}C]ethanolamine (0.05–0.4 mM) and [^3H]choline (0.1–2 mM) (Figs. 3 and 4). The Lineweaver-Burk plots could be approximated to straight lines, in spite of the large number of steps involved in the incorporation of bases into phospholipids. The curves indicate competitive inhibition with respect to both ethanolamine and choline. For choline incorporation, the K_i was 1.04 mM for 2-aminopropanol, 0.82 mM for *N*-methylethanolamine (Fig. 4) and 1.10 mM for *NN*-diethylethanolamine. The standard error in the calculation of apparent K_m (Wilkinson, 1961) was 1–32%, the higher value being more typical. At larger

* Abbreviation: Hepes, 4-(2-hydroxyethyl)piperazine-1-ethanesulphonic acid.

Table 1. *Effect of different analogues on the incorporation of ethanolamine and choline into lipids in isolated hepatocytes* Isolated hepatocytes were incubated for 60 min in 0.5 ml of Hanks buffer containing 2% (w/v) albumin, [^{14}C]ethanolamine (0.5 nmol), [^3H]choline (0.1 nmol), and different bases at a concentration of 2 mM (ethanolamine, 0.4 mM). Data are expressed as percentage of the incorporation of isotope into lipid with no added base and are means $\pm$ S.E.M. ($n = 4-6$).

Added compound	Incorporation of [^{14}C]ethanolamine	Incorporation of [^3H]choline
Ethanolamine	4 ± 1	68 ± 4
Choline	89 ± 1	4 ± 1
1-Aminopropan-2-ol	69 ± 4	79 ± 3
2-Aminopropan-1-ol	25 ± 2	43 ± 2
3-Aminopropan-1-ol	95 ± 2	86 ± 5
2-Aminobutan-1-ol	34 ± 1	23 ± 2
2-Amino-2-methylpropan-1-ol	96 ± 3	64 ± 4
2-(Aminomethyl)propan-1-ol	100 ± 2	99 ± 1
<i>N</i> -Methylethanolamine	52 ± 4	31 ± 2
<i>NN</i> -Dimethylethanolamine	75 ± 4	7 ± 1
<i>N</i> -Ethylethanolamine	59 ± 1	21 ± 1
<i>NN</i> -Diethylethanolamine	92 ± 1	6 ± 0
<i>NNN</i> -Triethylethanolamine	104 ± 4	65 ± 6
1-Dimethylaminopropan-2-ol	100 ± 4	27 ± 1
<i>N</i> -Isopropylethanolamine	—	16 ± 1
2-Chloroethylamine	95 ± 4	113 ± 3
2-Aminoethanethiol	102 ± 2	58 ± 14
2-Dimethylaminoethyl chloride	91 ± 5	84 ± 7
Bromocholine	99 ± 5	59 ± 3
Chlorocholine	98 ± 3	80 ± 5

Fig. 3. *Inhibition of the incorporation of [^{14}C]ethanolamine into hepatocyte phospholipids by analogues of ethanolamine and choline*

Isolated hepatocytes were incubated with 2 mM base as described in Table 1, except that the concentration of [^{14}C]ethanolamine was varied. Symbols: $\circ$, no inhibitor; Δ , 2-aminopropan-1-ol; $\square$, *N*-methylethanolamine. The straight lines were fitted according to Wilkinson (1961).

concentrations of [^{14}C]ethanolamine, 2-aminopropanol ($K_i = 1.11$ mM, Fig. 3) and 2-aminobutanol ($K_i = 1.88$ mM) were the only efficient inhibitors,

Fig. 4. *Inhibition of the incorporation of [^3H]choline into hepatocyte phospholipids by analogues to ethanolamine and choline*

Isolated hepatocytes were incubated with 2 mM base as described in Table 1 except that the concentration of [^3H]choline was varied. Symbols are as in Fig. 3.

whereas *N*-methylethanolamine (Fig. 3), *N*-ethylethanolamine and *NN*-diethylethanolamine had negligible effects.

Although the extent of incorporation of analogues into phospholipids via different pathways probably

is related to the inhibitory potency described above, it was desirable to show their incorporation directly. To study their incorporation via CDP-esters, they were incubated with [^3H]glycerol and isolated hepatocytes, and the appearance of ^3H in different phospholipid fractions was followed (Table 2). In the absence of added base, phosphatidylethanolamine and phosphatidylcholine were the major phospholipids. Addition of ethanolamine markedly increased ^3H incorporation into phosphatidylethanolamine. All aminopropanols were incorporated into phospholipids, although to a lesser degree than ethanolamine, and 2-aminobutan-1-ol was even less efficient. The phospholipids formed from these compounds (except that from 3-aminopropan-1-ol) had a slightly higher R_F value than phosphatidylethanolamine, as described in the Experimental section. The addition of 3-amino-1-propan-1-ol gave an increase of incorporation of ^3H into the phosphatidylethanolamine fraction. This phosphatidylpropanolamine therefore probably does not separate from phosphatidylethanolamine, which agrees with the fact that the free bases ethanolamine and 3-aminopropan-1-ol had the same R_F values on silica-gel t.l.c. *N*-Alkyl- and *NN*-dialkyl-ethanolamines were incorporated into phospholipids approximately as effectively as ethanolamine, with *NN*-dimethylethanolamine as the most effective compound. *NNN*-Triethylethanolamine, on the other hand, was only slowly incorporated.

One would expect that compounds similar to ethanolamine would be incorporated via ethanolaminophosphotransferase (EC 2.7.8.1), and compounds similar to choline via choline phosphotransferase (EC 2.7.8.2). Some compounds did, however, inhibit the incorporation of both [^3H]choline and [^{14}C]ethanolamine (Table 1). Therefore, another method was sought to obtain information on which phosphotransferase was responsible for the incorporation of a specific base. Ethanolamine phosphotransferase primarily selects hexaenoic diacylglycerol, whereas choline phosphotransferase is less selective, as studied after the administration of [^3H]glycerol (Åkesson *et al.*, 1970; Sundler *et al.*, 1974). If the phosphotransferases retain this specificity when incorporating unnatural analogues, it would be possible to distinguish between the two enzymes by analysing the distribution of ^3H among molecular species from the unnatural phospholipid (Table 3). In both phosphatidylpropanolamines the hexaenoic fraction dominated, as in phosphatidylethanolamine. In all other phospholipids tested, the distribution of ^3H among molecular species was similar to that in phosphatidylcholine. This is somewhat surprising only for *N*-methylethanolamine, since previous results indicated that it was incorporated similarly to ethanolamine (Radomińska-Pyrek *et al.*, 1969; Sundler & Åkesson, 1975). There is evidence that ethanolamine phosphotransferase and choline phosphotransferase are separate enzymes (Kano & Ohno, 1976), but the possibility that their selectivity towards different diacylglycerols is influenced by the structure of the CDP ester cannot be excluded.

Unnatural phospholipids may be methylated in the same way as the stepwise methylation of phosphatidylethanolamine to phosphatidylcholine (Bremer *et al.*, 1960). We found previously that addition of the naturally occurring bases *N*-methylethanolamine or *NN*-dimethylethanolamine decreased the conversion of phosphatidylethanolamine into phosphatidylcholine, but increased the incorporation of [^{14}C]methionine into phospholipids (Sundler & Åkesson, 1975), and therefore the same parameters were investigated for the unnatural analogues (Table 4).

The primary amines slightly inhibited the conversion of phosphatidylethanolamine into phosphatidylcholine, but did not change the incorporation of [^{14}C]methionine. *N*-Methylethanolamine, *NN*-dimethylethanolamine and *N*-ethylethanolamine also inhibited phosphatidylethanolamine conversion, but instead caused increased [^{14}C]methionine incorporation. *NN*-Diethylethanolamine was the only compound tested that stimulated both phosphatidylethanolamine conversion and [^{14}C]methionine incorporation into phospholipids. In all cases most of the lipid ^{14}C was located in phosphatidylcholine. When *N*-methylethanolamine was added, 1.0–2.8% was located in phosphatidyl-*NN*-dimethylethanolamine,

Table 2. *Effect of added bases on the synthesis of special phospholipids*

Isolated hepatocytes were preincubated for 60 min at 37°C in Hanks buffer containing 2% (w/v) albumin and different bases. Then albumin-bound oleic acid (0.5 mM) and 2 mM- ^3H glycerol was added and incubation was continued for 60 min. The final concentration of bases was 2 mM (except ethanolamine, 0.4 mM). The values show the percentage of the total lipid ^3H that was isolated in the lipid containing the added base. In parentheses is the ^3H found in phosphatidylethanolamine and phosphatidylcholine in incubations without added bases. The other phospholipids contained insignificant radioactivity in this case. Neutral lipids contained 70–84% of lipid ^3H . Results from two experiments are shown.

Added base	Expt. 1	Expt. 2
Ethanolamine	7.2 (2.3)	9.6 (3.3)
Choline	1.6 (14.8)	2.9 (12.5)
1-Amino-propan-2-ol	2.0	2.8
2-Aminopropan-1-ol	3.1	4.0
3-Aminopropan-1-ol	2.8	4.0
2-Aminobutan-1-ol	1.3	1.0
<i>N</i> -Methylethanolamine	6.1	4.3
<i>NN</i> -Dimethylethanolamine	11.3	12.0
<i>N</i> -Ethylethanolamine	7.3	5.6
<i>NN</i> -Diethylethanolamine	5.8	4.3
<i>NNN</i> -Triethylethanolamine	1.0	1.0

Table 3. *Biosynthesis of molecular species of different phospholipids*

Isolated hepatocytes were preincubated for 60 min with 2% (w/v) albumin and different bases at a concentration of 2 mM (ethanolamine, 0.4 mM). Then a trace amount of [^3H]glycerol was added and the incubation was continued for another 60 min. Results are the percentage distribution of ^3H among molecular species in the phospholipid containing the added base.

Added base	Molecular species				
	Monoenoic	Dienoic	Trienoic	Tetraenoic	Hexaenoic
Ethanolamine	11.1	20.9	7.7	15.8	44.6
Choline	23.6	31.4	9.0	17.4	18.6
2-Aminopropan-1-ol	7.6	21.0	12.8	13.3	45.3
1-Aminopropan-2-ol	10.1	22.9	8.8	14.0	44.3
<i>N</i> -Methylethanolamine	22.9	37.4	10.2	9.5	20.0
<i>NN</i> -Dimethylethanolamine	20.6	31.8	9.2	13.0	25.4
<i>N</i> -Ethylethanolamine		78.6		8.1	13.3
<i>NN</i> -Diethylethanolamine	19.2	35.6	11.8	13.1	20.3
<i>N</i> -Isopropylethanolamine	74.7		7.0	9.7	8.7

Table 4. *Effect of different analogues on the synthesis of phospholipids by methylation*

Isolated hepatocytes were incubated for 120 min in 0.5 ml of Hanks buffer containing 2% (w/v) albumin, different bases at concentration of 2 mM (ethanolamine, 0.4 mM) and either 0.8 mM- $[\text{Me-}^{14}\text{C}]$ methionine or 0.8 mM-methionine and $[\text{C}^{14}]$ ethanolamine (1.1 nmol). In the latter case, the ratio of ^{14}C in phosphatidylcholine/(phosphatidylethanolamine + phosphatidylcholine) was calculated. All data are expressed as % of control (with no added base) and are means $\pm$ S.E.M. ($n = 4-6$).

Added base	Phosphatidylcholine	Incorporation of $[\text{C}^{14}]$ methionine into lipids (% of control)
	Phosphatidylcholine + Phosphatidylethanolamine (% of control)	
Ethanolamine	74 $\pm$ 8	102 $\pm$ 5
Choline	119 $\pm$ 9	97 $\pm$ 4
2-Aminopropan-1-ol	86 $\pm$ 5	104 $\pm$ 6
2-Aminobutan-1-ol	72 $\pm$ 4	92 $\pm$ 5
<i>N</i> -Methylethanolamine	50 $\pm$ 4	119 $\pm$ 3
<i>NN</i> -Dimethylethanolamine	81 $\pm$ 6	162 $\pm$ 12
<i>N</i> -Ethylethanolamine	86 $\pm$ 6	118 $\pm$ 7
<i>NN</i> -Diethylethanolamine	115 $\pm$ 4	135 $\pm$ 8
<i>NNN</i> -Triethylethanolamine	93 $\pm$ 1	99 $\pm$ 3

in agreement with previous results (Sundler & Åkesson, 1975). When *N*-ethylethanolamine was added, approx. 8% of the phospholipid ^{14}C was located in a substance with chromatographic mobility expected for phosphatidyl-*N*-ethyl-*N*-methylethanolamine and approx. 4% migrated with phosphatidyl-*N*-ethyl-*NN*-dimethylethanolamine. The latter percentage may be underestimated, since this substance had an R_F value similar to that of phosphatidylcholine. After the addition of *NN*-diethylethanolamine to hepatocytes only 0.5% of lipid ^{14}C migrated a sphosphatidyl-*NN*-diethyl-*N*-methyl-ethanolamine. Also in incubations with 2-aminopropan-1-ol and 2-aminobutan-1-ol approx. 1% of lipid ^{14}C was in substances with higher chromatographic mobility than phosphatidylethanolamine, and this proportion probably represents partially methylated phospholipids containing these bases.

Discussion

A variety of ethanolamine and choline analogues can be incorporated into tissue phospholipids, but only in a few studies has the pathway of incorporation been investigated. In rat liver, the phosphotransferases (EC 2.7.8.1, 2.7.8.2) are not very specific towards different CDP esters, but the cytidylyltransferases utilizing phosphate esters are more specific for phosphoethanolamine and phosphocholine (Chojnacki, 1964; Ansell & Chojnacki, 1966; Chojnacki & Ansell, 1967). There was, however, no good correlation between the cytidylyltransferase activity with different phosphoaminoalcohols found by these workers and the extent of incorporation of the corresponding bases found here. For instance, *NN*-diethylethanolamine and 1-aminopropan-2-ol were significantly incorporated into hepatocytes

(Table 2), but their phosphate esters were very slowly converted into CDP esters (Chojnacki & Ansell, 1967). Maybe the optimum conditions for activity were not attained in the referred studies. The fact that 1-aminopropan-2-ol is efficiently phosphorylated in rat liver mitochondria (Willets, 1974) does not seem to increase its incorporation into phospholipids (Table 2), which agrees with the suggestion that the phosphorylation of bases is not a rate-limiting process in phospholipid biosynthesis (Sundler & Åkesson, 1975).

Although the specificities of cytidyltransferases may be major determinants in the incorporation of different bases (Table 2) and also in the competition between different bases (Table 1), additional competition may occur at other steps, such as tissue uptake, phosphorylation of bases (Weinhold & Rethy, 1974), incorporation of CDP esters into phospholipids (Kano & Ohno, 1976), and incorporation via base exchange (Bjerve, 1973). *NNN*-Triethylethanolamine and *NN*-diethylethanolamine inhibited choline uptake in synaptosomes (Simon *et al.*, 1975; Bosmann & Hemsworth, 1974). In rats given 2-amino-2-methylpropanol, incorporation of ethanolamine and *NN*-dimethylethanolamine, but not that of choline, was decreased (Longmore & Mulford, 1962). In our system 2-amino-2-methylpropanol only inhibited choline incorporation (Table 1). Chlorocholine inhibited choline incorporation into mammary tissue (Infante & Kinsella, 1973) and 2-chloroethylamine inhibited the incorporation of both ethanolamine and choline into cultured brain cells (Yavin & Kanfer, 1975). No effects on the corresponding incorporation into hepatocyte phospholipids were observed (Table 1). This may indicate that the mechanism of uptake of these compounds differs in different cells, since the above authors suggested that the chlorine-containing compounds exerted their effect mainly on cell uptake.

The administration of different *N*-methylethanolamines will influence phospholipid methylation in yeast (Waechter & Lester, 1973), intact rat liver (Kobayashi, 1970) and isolated hepatocytes (Sundler & Åkesson, 1975). As observed earlier (Sundler & Åkesson, 1975) *N*-methylethanolamine and *NN*-dimethylethanolamine increase the incorporation of [¹⁴C]methionine into phospholipids and decrease the conversion of [³H]phosphatidylethanolamine into phosphatidylcholine. This indicates that the formed phosphatidyl-*N*-methylethanolamine and phosphatidyl-*NN*-dimethylethanolamine are utilized for methylation, preferably to phosphatidylethanolamine, and that their presence increases the total number of methyl groups incorporated into phospholipids. *N*-Ethylethanolamine had similar effects to *N*-methylethanolamines (Table 4). Chromatographic analysis also indicated that the phosphatidyl-*N*-ethylethanolamine formed could act as a methyl-acceptor. Phosphatidyl-*NN*-diethylethanolamine was

evidently much less effective as methyl acceptor, and this is probably why its parent base caused stimulation rather than inhibition of the conversion of [³H]-phosphatidylethanolamine into phosphatidylcholine. This indicates that there is a short-term regulation of phospholipid methylation dependent on the presence of different bases and their derivatives. In addition, *N*-methylethanolamines may cause fluctuations in the activity of *N*-methyltransferase(s) as studied mainly in yeast (Waechter & Lester, 1973).

Several authors have suggested that *N*-alkylated ethanolamines or their derivatives can also undergo dealkylation (Chojnacki & Ansell, 1967; Lee *et al.*, 1975; Åkesson, 1977). In the present short-term experiments, no influence from such reactions on the distribution of radioactivity among phospholipids was detected.

Although there are indications that phospholipid analogues of phosphatidylcholine and phosphatidylethanolamine differ in chromatographic mobility (Chojnacki, 1964; Chojnacki & Ansell, 1967; Bell & Strength, 1968; Mehendale *et al.*, 1970; Lee *et al.*, 1975), this has not been systematically investigated. Figs. 1 and 2 show that an increase in the number and chain length of *N*-alkyl groups increases *R_F* values on t.l.c. The mobilities are dependent on the pH of the developing solvent. This effect is useful in resolving the very complex phospholipid mixtures created by adding different base analogues, especially when methylated phospholipid analogues are also formed. Some of the relative chromatographic mobilities observed for diacylphospholipids are analogous to those observed for monoacylphospholipids (Jezyk & Hughes, 1973).

Excellent technical assistance was given by Ms. Birgitta Mårtensson and Ms. Elizabeth Nilsson. This work was supported by The Swedish Medical Research Council (Project nos. 3968 and 4931), A. Pålsson's Foundation and H. Jeansson's Foundation.

References

- Åkesson, B. (1977) *Biochem. Biophys. Res. Commun.* **76**, 93–99
- Åkesson, B., Elovson, J. & Arvidson, G. (1970) *Biochim. Biophys. Acta* **210**, 15–27
- Ansell, G. B. & Chojnacki, T. (1966) *Biochem. J.* **98**, 303–310
- Artom, C., Lofland, H. B. & Oates, J. A., Jr. (1958) *J. Biol. Chem.* **233**, 833–837
- Bell, O. E., Jr. & Strength, D. R. (1968) *Arch. Biochem. Biophys.* **123**, 462–467
- Bjerve, K. S. (1973) *Biochim. Biophys. Acta* **296**, 549–562
- Blank, M. L., Piantadosi, C., Ishaq, K. S. & Snyder, F. (1975) *Biochem. Biophys. Res. Commun.* **62**, 983–988
- Bosmann, H. B. & Hemsworth, B. A. (1974) *Biochem. Pharmacol.* **23**, 523–532
- Bremer, J., Figard, P. A. & Greenberg, D. M. (1960) *Biochim. Biophys. Acta* **43**, 477–488
- Chojnacki, T. (1964) *Acta Biochim. Pol.* **11**, 11–23

- Chojnacki, T. & Ansell, G. B. (1967) *J. Neurochem.* **14**, 413–420
- Elovson, J. (1965) *Biochim. Biophys. Acta* **106**, 480–494
- Glaser, M., Ferguson, K. A. & Vagelos, P. R. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4072–4076
- Griffith, W. H. & Nyc, J. F. (1971) in *The Vitamins* (Sebrell, W. H., Jr. & Harris, R. S., eds.), pp. 63–69, Academic Press, New York
- Hanks, J. H. & Wallace, R. E. (1949) *Proc. Soc. Exp. Biol. Med.* **71**, 196–200
- Infante, J. P. & Kinsella, J. E. (1973) *Biochem. J.* **134**, 825–827
- Jezyk, P. F. & Hughes, H. N. (1973) *Biochim. Biophys. Acta* **296**, 24–33
- Kanoh, H. & Ohno, K. (1976) *Eur. J. Biochem.* **66**, 201–210
- Katyal, S. L. & Lombardi, B. (1974) *Lipids* **9**, 81–85
- Kobayashi, M. (1970) *J. Vitaminol.* **16**, 119–128
- Lea, C. H., Rhodes, D. N. & Stoll, R. D. (1955) *Biochem. J.* **60**, 353–363
- Lee, T.-C., Blank, M. L., Piantadosi, C., Ishaq, K. S. & Snyder, F. (1975) *Biochim. Biophys. Acta* **409**, 218–224
- LeKim, D. & Betzing, H. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1490–1492
- Longmore, W. J. & Mulford, D. J. (1960) *Biochem. Biophys. Res. Commun.* **3**, 566–569
- Longmore, W. J. & Mulford, D. J. (1962) *J. Biol. Chem.* **237**, 694–698
- Mehendale, H. M., Dauterman, W. C. & Hodgson, E. (1970) *Int. J. Biochem.* **1**, 429–437
- Nilsson, Å., Sundler, R. & Åkesson, B. (1973) *Eur. J. Biochem.* **39**, 613–620
- Radomińska-Pyrek, A., Matysiak, Z. & Chojnacki, T. (1969) *Acta Biochim. Pol.* **16**, 357–363
- Seglen, P. O. (1973) *Exp. Cell Res.* **82**, 391–398
- Simon, J. R., Mittag, T. W. & Kuhar, M. J. (1975) *Biochem. Pharmacol.* **24**, 1139–1142
- Singleton, W. S., Gray, M. S., Brown, M. L. & White, J. L. (1965) *J. Am. Oil Chem. Soc.* **42**, 53–56
- Sundler, R. & Åkesson, B. (1975) *J. Biol. Chem.* **250**, 3359–3367
- Sundler, R., Åkesson, B. & Nilsson, Å. (1974) *J. Biol. Chem.* **249**, 5102–5107
- Van Golde, L. M. G. & Van Deenen, L. L. M. (1966) *Biochim. Biophys. Acta* **125**, 496–509
- Waechter, C. J. & Lester, R. L. (1973) *Arch. Biochem. Biophys.* **158**, 401–410
- Weinhold, P. A. & Rethy, V. B. (1974) *Biochemistry* **13**, 5135–5141
- Weiss, S. B., Smith, S. W. & Kennedy, E. P. (1958) *J. Biol. Chem.* **231**, 53–64
- Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332
- Willetts, A. (1974) *Biochim. Biophys. Acta* **362**, 448–456
- Yang, S. F., Freer, S. & Benson, A. A. (1967) *J. Biol. Chem.* **242**, 477–484
- Yavin, E. & Kanfer, J. N. (1975) *J. Biol. Chem.* **250**, 2885–2890