Phosphatidylcholine Synthesis in the Developing Small Intestine

Philip G. HOLTZAPPLE, Christopher M. STARR and Timothy MORCK
Department of Pediatrics and Medicine, State University of New York, Upstate Medical Center,
750 East Adams Street, Syracuse, NY 13210, U.S.A.

(Received 3 July 1979)

1. Phosphatidylcholine synthesis in the foetal, newborn and adult small intestine of rats was studied by determination of cytidine diphosphocholine–1,2-diacylglycerolcholine phosphotransferase (cholinephosphotransferase) and acyl-CoA–1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase (lysophosphatidylcholine acyltransferase) activities and the incorporation of [1-¹⁴C]oleic acid into phosphatidylcholine. 2. Cholinephosphotransferase activity was low in foetal jejunum and ileum, increased 3–4-fold in the ileum by 6 days of age and by 12 days in the jejunum. Jejunal activity remained constant throughout weaning; ileal activity gradually decreased to values 25% of that of the jejunum. 3. Lysophosphatidylcholine acyltransferase activity was high in foetal jejunum and ileum, decreased 70% immediately after birth in the jejunum and increased to adult values by 12 days of age. Ileal activity decreased by 20% after birth, but decreased more rapidly at weaning to 30% of the activity in jejunum. 4. Initial rates and steady-state incorporation of [1-¹⁴C]oleic acid into phosphatidylcholine by jejunal rings of 10 day-old rats exceeded that observed in jejunal rings from adult rats by 2–4-fold. 5. In the post-natal jejunum, neither cholinephosphotransferase and lysophosphatidylcholine acyltransferase activities nor oleic acid incorporation were stimulated by cortisone administration *in vivo*.

Phosphatidylcholine is the main active component of pulmonary surfactant, accounting for 70% of the phospholipid in the lung of the newborn. A decrease in the synthesis, secretion or function of lung surfactant is considered to be aetiologically related to the pathogenesis of the respiratory distress syndrome of the newborn (King, 1974). Antenatal induction of phosphatidylcholine synthesis in the lung of several animal species can be achieved by corticosteroid administration (Farrell & Zachman, 1973), and increased surfactant secretion into alveolar spaces in the foetal lung has been observed after thyrotropin-releasing hormone (Rooney *et al.*, 1979) or cholinergic stimulation (Corbet *et al.*, 1977). The acceleration of pulmonary maturation by corticosteroids, in part, is related to increased activity of cholinephosphotransferase (Farrell & Zachman, 1973), lysophosphatidylcholine acyltransferase (Rooney *et al.*, 1976), and glycerophosphate phosphatidyltransferase (Rooney *et al.*, 1975), and to increased incorporation of glucose into phosphatidylcholine (Gilden *et al.*, 1977).

Although considerable attention has been focused on phospholipid metabolism in the lung of the foetal or newborn animal, little information is available concerning phospholipid synthesis and metabolism

in the developing gastrointestinal tract, a rapidly proliferating organ. The present study was designed to determine the activity of CDP-choline–1,2-diacylglycerolcholine phosphotransferase (EC 2.7.8.2) and acyl-CoA–1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase (EC 2.3.1.23) in jejunum and ileum of foetal and newborn rats and the effect of cortisone administration on enzyme activity and incorporation of fatty acid into phosphatidylcholine by jejunal mucosa.

Materials and Methods

Animals

Timed pregnant Sprague–Dawley rats were housed and allowed to deliver in a controlled environment. Within 24 h of delivery the litter size was adjusted to eight pups per dam. Foetal rats were obtained by caesarian section 24 h before expected birth. Male rats (150 g) served as a source of adult material and were starved overnight before being killed.

Animals were killed in a cold room by cervical dislocation and the small intestine was immediately removed. After discarding the duodenum, the proximal third (jejunum) and distal third (ileum) of the

small intestine were flushed with cold 0.154 M-NaCl, opened, blotted gently, and the mucosal surface was scraped. Jejunum and ileum from the foetal, 1 and 6 day-old rats were used intact as source material.

The intestine or mucosal scrapings were weighed, homogenized in 0.28 M-mannitol/0.01 M- Na_2HPO_4 (pH 7.4) containing 2.5 mM-EDTA. The supernatant of two 20 min centrifugations (8700 g) was spun at 105 000 g for 60 min. The resultant microsomal pellet was resuspended in 0.154 M-KCl in 0.01 M- Na_2HPO_4 (pH 7.4). In the developmental studies, enzyme activities were determined in triplicate on microsomal fractions prepared daily.

In tissue-ring experiments, jejunum was obtained from 10 day-old rats, flushed with cold 0.154 M-NaCl, everted over polyethylene tubing and cut into rings weighing approx. 10–20 mg each. Rings from one litter were pooled. Three rings were weighed and incubated in 2 ml of Ca^{2+} - and Mg^{2+} -free Krebs–Ringer bicarbonate buffer (pH 7.4) containing 10 mM-sodium taurocholate and 0.5 mM-choline chloride. At the end of the incubation the rings were removed, rinsed twice in non-radioactive buffer, blotted and placed in 0.5 ml of acid 0.15 M-NaCl (pH 2). Uptake and incorporation of 1 mM-[1- ^{14}C]-oleic acid (sp. radioactivity 270 000 d.p.m./ μmol) into phospholipid in triplicate flasks was followed.

In the cortisone experiments, four pups in each litter received intraperitoneal cortisone acetate (0.5 mg/10 g body wt.) on days 3 and 5. The remaining four pups in each litter received an equal volume of 0.154 M-NaCl intraperitoneally. On day 6 the pups were killed and microsomal fractions were prepared from the jejunum. For tissue-ring experiments, four pups per litter received cortisone acetate intraperitoneally (0.5 mg/10 g body wt.) on days 6–9. On day 10 the pups were killed and jejunal rings were prepared.

Enzyme activity

CDP-choline–1,2-diacylglycerolcholine phosphotransferase (cholinephosphotransferase) activity was determined by the method of Weiss *et al.* (1958), as modified by Mansbach (1973). The complete reaction mixture contained (in 1 ml): Tris/HCl (pH 7.6), 80 μM ; cysteine/HCl, 10 μM ; MgCl_2 , 30 μM ; EDTA, 10 μM ; *sn*-1,2-dipalmitoylglycerol, 1 μM (dissolved in 0.2 ml of 0.1% Tween 20); [Me^{-14}C]CDP-choline, 300 μM (sp. radioactivity 300 000 d.p.m./ μmol); 200–400 μg of microsomal protein. Incubation was started with the addition of the microsomal fraction and carried out at 37°C for 10 min and terminated with the addition of 0.1 ml of 2 M- H_2SO_4 .

Acyl-CoA–1-acyl-*sn*-glycerol-3-phosphocholine acyltransferase (lysophosphatidylcholine acyltransferase) was determined by the method of Lands (1960). The complete reaction mixture (0.5 ml) contained: Tris/HCl (pH 7.4), 100 μM ; EDTA, 10 μM ;

[1- ^{14}C]oleoyl-CoA, 0.4 μM (sp. radioactivity 800 000 d.p.m./ μmol); palmitoyl α -lysophosphatidylcholine; 200–400 μg of microsomal protein. Incubation was started with the microsomal addition, carried out at 28°C for 1 min and terminated with the addition of 3 ml of methanol.

Lipid analysis

Lipids from both the tissue-ring experiments and enzyme studies were extracted by the method of Folch *et al.* (1957). Phospholipids were separated by t.l.c. on silica gel G plates (Analtech, Newark, DE, U.S.A.) developed with chloroform/methanol/acetic acid/water (25:15:4:2, by vol.). The areas containing phosphatidylcholine and phosphatidylethanolamine were detected by I_2 vapour, scraped into scintillation vials and counted for radioactivity in a Beckman LS-355 spectrometer by using the external-standard ratio technique for quench correction. Protein was determined by the method of Lowry *et al.* (1951).

Reagents

All reagents were of analytical grade obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., or Fisher Scientific Co., Rochester, NY, U.S.A. Solvents for lipid extraction and chromatography were doubly distilled in glass (Burdick & Jackson Laboratories, Muskegon, MI, U.S.A.). Radioisotopes were obtained from New England Nuclear, Boston, MA, U.S.A.

Results

The activity of cholinephosphotransferase in jejunum and ileum during the perinatal period is shown in Fig. 1. Jejunal activity is low in the foetal rat and increases during the first 12 days to a value of 2.4 nmol/mg per min and remains essentially unchanged during weaning and into maturity. Ileal activity increases during the first 6 days to 2.5 nmol/mg per min and then gradually decreases to a lower activity of 0.3 nmol/mg per min in the weanling and mature animal. Combining jejunal and ileal microsomal fractions from mature rats produced activity of intermediate value between either those of the jejunum or ileum alone. The activity of cholinephosphotransferase mixture of microsomal fractions from the jejunum of adult and foetal rats was additive, indicating that an inhibitor did not account for the low activity in foetal intestine.

The activity of intestinal lysophosphatidylcholine acyltransferase is shown in Fig. 2. Activity in microsomal fractions from both the jejunum and ileum is high in the foetal animal (12.4 and 13.8 nmol/mg per min respectively), but differs considerably thereafter. Jejunal activity decreases rapidly during the first several postnatal days (1.9 nmol/mg per min at

Fig. 1. Developmental pattern of cholinephosphotransferase in the small intestine of the rat

Microsomal fraction from jejunum (○) and ileum (●) was prepared from the intestine of the eight to nine pups in each litter. The assay, as described in the Materials and Methods section, was performed in triplicate. The values for the activity at each age represents the mean $\pm$ S.E.M. for at least four litters.

Fig. 2. Developmental pattern of lysophosphatidylcholine acyltransferase in the small intestine of the rat

Assay conditions are the same as described in the legend to Fig. 1 and the Materials and Methods section. Symbols: ○, jejunum; ●, ileum.

6 days), but increases by the twelfth day of life with little change thereafter into maturity (9.4 nmol/mg per min). The decrement in ileal activity is less than that of the jejunal activity in the early postnatal period, but declines rapidly in the weaning and post-weaning period (2.8 nmol/mg per min).

Incorporation of oleic acid into phosphatidylcholine by jejunal rings from 10 day-old rats was contrasted with that observed in jejunal rings of mature rats (Fig. 3). At all time intervals the incorporation of oleic acid into phosphatidylcholine by jejunal rings from 10 day-old rats exceeded that observed by the jejunum of adult rats. In the presence of 5 mM-glucose the initial rate of incorporation of oleic acid did not differ; however, at longer periods of incubation from 15 to 30 min increased incorporation was observed in the jejunum of the suckling animal. The addition of 5 mM-glucose increased the incorporation of oleic acid into phosphatidyl-

Fig. 3. Incorporation of oleic acid into phosphatidylcholine by rat jejunum

The incorporation of oleic acid into phosphatidylcholine by jejunal rings of 10 day-old (○) and adult rats (△) is compared. The closed symbols (● and ▲) represent oleic acid incorporated in the presence of 5 mM-glucose in jejunal rings of 10 day-old and adult rats respectively. Conditions of incubation are described in the Materials and Methods section.

choline by jejunal rings of adult rats; however, incorporation always remained less than that observed with rings prepared from 10 day-old rats incubated in the absence of glucose.

Cortisone effect

The effect of cortisone on phospholipid synthesis was evaluated both by determination of enzyme activity of cholinephosphotransferase and lysophosphatidylcholine acyltransferase (6 day-old pups) and by oleic acid incorporation into phosphatidylcholine by jejunal rings (10 day-old pups) (Table 1). In these experiments cortisone administration decreased both cholinephosphotransferase and lysophosphatidylcholine acyltransferase activity. Oleic acid incorporation into phosphatidylcholine was decreased in jejunum of cortisone-treated pups compared with jejunum of control pups.

Discussion

Phosphatidylcholine, an important component of cellular and chylomicron membranes, is synthesized via two pathways in the intestinal epithelial cell. The cholinephosphotransferase pathway is more active in the rough endoplasmic reticulum than in the smooth endoplasmic reticulum of epithelial cells (Mansbach, 1976). The distribution of this enzyme activity appears to be fairly equal in cells derived either from

Table 1. *Effect of cortisone on phospholipid synthesis in jejunum of suckling rat*

In Expt. A, microsomal fraction, prepared from the jejunum of 6-day-old rats, was assayed for enzyme activity as described in the Materials and Methods sections. In Expt. B, jejunal rings were prepared from 10 day-old rats and incubated as described in the Materials and Methods section. Half of each litter received 0.5 mg of cortisone acetate intraperitoneally on days 3 and 5 in Expt. A and on days 6–9 in Expt. B. The number of litters studied are given in parentheses. The results are expressed as means $\pm$ S.E.M. for the control and cortisone-injected littermates.

Expt.	Treatment ...	Control	Cortisone	Cortisone (+5 mM-glucose)
A	Cholinephosphotransferase (nmol/mg per min)	0.56 $\pm$ 0.11 (3)	0.05 $\pm$ 0.02	—
	Lysophosphatidylcholine acyltransferase (nmol/mg per min)	34.96 $\pm$ 3.96 (3)	23.34 $\pm$ 3.18	—
B	Oleic acid incorporation (nmol/g of tissue per 15 min)	42.99 $\pm$ 7.2 (4)	15.72 $\pm$ 3.89	21.45 $\pm$ 5.91

the villous tip or from the crypt area of the mucosa (Mansbach, 1973). Lysophosphatidylcholine acyltransferase activity is only slightly higher in the rough endoplasmic reticulum of mucosal epithelial cells, but it is 2–3-fold more active in microsomal fractions prepared from villous tip cells than in those from the cells of the crypt area. The presence of both enzymes in the villous tip area indicates that phosphatidylcholine synthesis may be derived by either synthesis *de novo* or by reacylation of absorbed lysophosphatidylcholine derived from either biliary or dietary sources (Mansbach, 1977). Under physiological conditions the contribution of each pathway to the phosphatidylcholine membrane of chylomicrons depends on the intraluminal phosphatidylcholine concentrations. As the amount of phosphatidylcholine is increased in perfused jejunal segments, the proportion of chylomicron phospholipid derived by the reacylation of lysophosphatidylcholine increases as well.

Our findings of low cholinephosphotransferase activity in the jejunum of foetal rats is consistent with the observation of phospholipid synthesis in the foetal rat by other investigators (Weinhold & Vilee, 1965; Weinhold, 1969; Weinhold *et al.*, 1973b). In the foetal liver radioactive phosphate and choline incorporation into acylglycerophosphocholines has been reported to be low, with the total quantity of liver acylglycerophosphates approximately half that of the adult (Weinhold & Vilee, 1965; Weinhold, 1969). Cholinephosphotransferase activity is low in the foetal rat liver, whereas choline kinase and phosphocholine cytidyltransferase activities are equivalent to that found in microsomal fractions of adult liver (Weinhold *et al.*, 1973b). In studies of rat embryos and placentas, cholinephosphotransferase activity in homogenates of rat embryos was approximately one-third of the activity found in homogenates of corresponding placentas (Chepenik *et al.*, 1977). In the foetal rat lung Maniscalco *et al.* (1978) found cholinephosphotransferase activity to be lower than in the lung of the adult rat, confirming similar observations of Weinhold *et al.* (1973a). In the rat

lung there is little change in cholinephosphotransferase activity during foetal and early postnatal development.

In contrast with the low cholinephosphotransferase activity in the jejunum of the foetal rat lysophosphatidylcholine acyltransferase activity is high. A marked diminution in jejunal activity is found during the first several days of suckling. Other studies demonstrated low activity in the foetal rat (Dobiasova & Hahn, 1968) and rabbit lung (Tsao & Zachman, 1977), but increased activity in the early postnatal period. The lysophosphatidylcholine pathway for phosphatidylcholine synthesis is functionally active as shown by the incorporation of double-labelled lysophosphatidylcholine into phosphatidylcholine in foetal rabbit lung slices (Hallman & Raivo, 1975).

In the suckling rat, chylomicrons appear in the serum for the first time 24–72 h after the initiation of feeding (Koldovsky *et al.*, 1963). Our findings of low lysophosphatidylcholine acyltransferase activity at day 1 and 6 may account for the observation made by Koldovsky and his co-workers (1963). Other mechanisms such as altered apolipoprotein synthesis, chylomicron assembly or secretion by the intestinal epithelial cell could also be an important determinant for this observation.

The incorporation of oleic acid into phosphatidylcholine by the jejunum of 10 day-old rats exceeded that in the jejunum of adult rats. In previous studies, fatty acid uptake by the jejunum of 10 day-old rats exceeded that observed in the jejunum of adult rats by 4–5-fold, resulting in increased oleic acid incorporation into triacylglycerol (Holtzapple *et al.*, 1975). Consequently the observed increased incorporation of oleic acid may occur as a result of an enlarged intracellular fatty acid pool. Additional studies are required to evaluate this possibility.

The administration of glucocorticoids and thyroxine accelerates the development of many systems by changing enzyme activities in lung, liver, brain and gastrointestinal tract (Greengard, 1976). In the gastrointestinal tract, precocious development of

sucrose α -glucohydrolase (EC 3.2.1.48), an epithelial cell brush-border enzyme, is the most notable example of this effect (Herbst & Koldovsky, 1972). In the present studies, cortisone administration did not result in increased enzyme activity in the 6 day-old rat nor in phosphatidylcholine synthesis in the 10 day-old rat. The timing of the cortisone stimulus is critical. Although cholinephosphotransferase activity is reported to be increased with enhanced phosphatidylcholine synthesis in lung slices, the effect is best observed in the foetal animal (Farrell & Zachman, 1973; Possmayer *et al.*, 1977). Further studies are required to evaluate the susceptibility of phosphatidylcholine synthesis in foetal intestine to hormonal manipulation. This system may not be influenced by corticosteroid, but by other more selective trophic hormones for the intestine, such as gastrin.

This work was supported by Grant-In-Aid no. 76-950 from the American Heart Association. We thank Dr. O. Koldovsky for his helpful discussions.

References

- Chepenik, K. P., Borst, G. E. & Waite, B. M. (1977) *Dev. Biol.* **60**, 463–472
- Corbet, A. J. S., Flax, P. & Rudolph, A. J. (1977) *J. Appl. Physiol.* **43**, 1039–1045
- Dobiasova, M. & Hahn, P. (1968) *Physiol. Bohemoslov.* **17**, 26–35
- Farrell, P. M. & Zachman, R. D. (1973) *Science* **179**, 297–298
- Folch, J., Lees, M. & Sloane Stanley, G. H. (1957) *J. Biol. Chem.* **266**, 497–509
- Gilden, C., Sevanian, A., Tierney, D. F., Kaplan, S. A. & Barrett, C. T. (1977) *Pediatr. Res.* **11**, 845–848
- Greengard, O. (1976) *J. Steroid Biochem.* **6**, 639–643
- Hallman, M. & Raivo, K. I. (1975) *Biol. Neonate* **27**, 329–338
- Herbst, J. J. & Koldovsky, O. (1972) *Biochem. J.* **126**, 471–476
- Holtzapple, P. G., Smith, G. & Koldovsky, O. (1975) *Pediatr. Res.* **9**, 786–791
- King, R. J. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 2238–2247
- Koldovsky, O., Hahn, P., Melichar, V., Novak, M., Prochazka, P., Rokos, J. & Vacek, Z. (1963) *Biochem. Probl. Lipids, Proc. Int. Conf. 7th* **1**, 161–169
- Lands, W. E. M. (1960) *J. Biol. Chem.* **235**, 2233–2237
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Maniscalco, W. M., Wilson, C. M., Gross, I., Gobran, L., Rooney, S. A. & Warshaw, J. B. (1978) *Biochim. Biophys. Acta* **530**, 333–346
- Mansbach, C. M. (1973) *Biochim. Biophys. Acta* **296**, 386–400
- Mansbach, C. M. (1976) *Enzyme* **21**, 137–141
- Mansbach, C. M. (1977) *J. Clin. Invest.* **60**, 411–420
- Possmeyer, F., Duwe, G., Metcalb, R., Stewart-DeHaan, P. J., Wong, C., Las Heras, J. & Harding, P. G. R. (1977) *Biochem. J.* **166**, 485–494
- Rooney, S. A., Gross, I., Gassenheimer, L. N. & Motoyama, E. K. (1975) *Biochim. Biophys. Acta* **398**, 433–441
- Rooney, S. A., Gobran, L., Gross, I., Wai-Lee, T. S., Nardone, L. L. & Motoyama, E. K. (1976) *Biochim. Biophys. Acta* **450**, 121–130
- Rooney, S. A., Marino, P. A., Gobran, L. I., Gross, I. & Warshaw, J. B. (1979) *Pediatr. Res.* **13**, 623–625
- Tsao, F. H. C. & Zachman, R. D. (1977) *Pediatr. Res.* **11**, 858–861
- Weinhold, P. A. (1969) *Biochim. Biophys. Acta* **187**, 85–93
- Weinhold, P. A. & Vilee, C. A. (1965) *Biochim. Biophys. Acta* **106**, 540–550
- Weinhold, P. A., Sanders, R. & Stern, W. (1973a) in *Respiratory Distress Syndrome* (Vilee, C. A., Vilee, D. B. & Van Dolle, L. M. G., eds.), pp. 29–45, Academic Press, New York
- Weinhold, P. A., Skinner, R. S. & Sanders, R. D. (1973b) *Biochim. Biophys. Acta* **326**, 43–51
- Weiss, S. B., Smith, S. W. & Kennedy, E. P. (1958) *J. Biol. Chem.* **231**, 53–64