BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **229,** 158–162 (1996)
ARTICLE NO. 1773

Phosphatidylethanolamine N-Methyltransferase 2 and CTP-Phosphocholine Cytidylyltransferase Expressions Are Related with Protein Kinase C Isozymes in Developmental Liver Growth

Eliana Sesca,* Gian Paolo Perletti,† Valentina Binasco,*
Marica Chiara,* and Luciana Tessitore*,[1]

**Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi di Torino, Turin, Italy; and †Istituto di Farmacologia, Università degli Studi di Milano, Milan, Italy*

Received October 17, 1996

PEMT and CT activities were reciprocally regulated during the perinatal period. Consistently, PEMT2 expression was undetectable before birth when CT was highly expressed. Surprisingly, PEMT2 was relatively highly expressed at birth when the cell division and CT expression were still high. During development liver cell growth was associated with enhanced levels in the activity of β, zeta and, particularly, α PKC. The activity of δPKC was lower in foetal, higher in the newborn and again slightly lower than adult liver 10 days after birth. These data show that CT expression and α, β and zeta PKC activities are positively, whereas PEMT2 expression and δ PKC activity are negatively associated with the liver cell division during development. © 1996 Academic Press, Inc.

All eucariotic cells synthetize PC via the direct incorporation of preformed choline, derived mostly from the diet. This pathway is essential for both cell survival and cell division (1). However, hepatocytes also have the ability to synthetize PC from step methylations of PE, catalyzed by at least two phosphatidylethanolamine N-methyitransferases. PEMT1 is mainly found on the endoplasmic reticulum, while PEMT2 is localized on mitocondria-associated membranes exclusively and it has recently been cloned (2). PEMT2 is inactivated in human and rat hepatoma cell lines (2) as well as in the hepato-cellular carcinoma induced by the Solt and Farber model (3). Overexpression of PEMT2 in hepatoma cells markedly slowed down the rate of cell division (4), suggesting a role of PEMT2 in the negative regulation of liver cell proliferation. PC derived from the methylation pathway failed to rescue the growth phenotype of mutant CHO cells, implying that this PC could not substitute PC via the CDP-choline pathways (5).

The degradation of PC is known to play the major role in sustaining PKC activation pattern by generating diacylglycerol, free fatty acids and lysophosphatidylcholine. The activation of different PKC isozymes is involved in the signal transduction of cell proliferation, differentiation and carcinogenesis (6). In this respect, the turnover of the two molecular subspecies of PC could lead to activation of different PKC isozymes. The α, β and zeta isoforms of PKC are positively, while δ PKC are negatively associated with the liver regeneration (7). Moreover, α and β PKC were down regulated during the lead-induced liver hyperplasia (8).

Aim of this work was to investigate whether: 1. PEMT2 was modulated during the developmental growth in relation to CT expression. 2. changes in PEMT2 expression correlated with the activation of PKC isoforms.

[1] Correspondence. Fax number: 39-11-9038639.

Abbreviations: Phosphatidylcholine (PC), protein kinase C (PKC), phosphatidylethanolamine N-metiltransferase (PEMT), CTP-phosphocholine cytidylyltransferase (CT), glyceraldheide phosphate dehidrogenase (GAPDH).

0006-291X/96 $18.00

TABLE 1
Liver Growth

		Liver weight	
Day	Body weight g	g	%BW
−3	1.95 ± 0.30	0.18 ± 0.01	9.12 ± 0.61
0	6.03 ± 0.58	0.26 ± 0.01	4.25 ± 0.23*
10	19.80 ± 2.30	0.58 ± 0.07	3.24 ± 0.41*
56	204.00 ± 6.00	7.86 ± 0.45	3.50 ± 0.25*

* $P < 0.05$ versus −3 days; $n = 5$.

MATERIALS AND METHODS

Chemicals. Oligo(dT)cellulose were from Sigma Chemical Co., St Louis, MO; [32P] dCTP, Methyl-^{3}H-choline chloride (15 Ci/mmol) S-adenosyl-L-methyl-^{3}H metionine (15 Ci/mmol), the ECL™ kit were from Amersham, Italy, all chromatographic materials were from Pharmacia, Italy.

Development. Wistar virgin female rats of about 200 g b.w. (Charles River) were left with adult males of 350 g b.w. until impregnation. Groups of pregnant rats were randomly selected. The first group was killed on day 19 after impregnation. Their foetuses were removed after caesarian section and were beheaded with scissors. The second group of pregnant rats was allowed to give birth. The last group of pregnant nurse their offspring until 10 days after birth. All animals were killed and livers (foetus, newborn and 10 day-weaning rats) were collected and immediately frozen at −80°C.

Enzyme assays. PKC was partially purified and the different isoform assayed as described (9). CT activity was determined as described by Yao et al (10) and PEMT activity was assayed as reported by Ridgway and Vance (11).

Immunoblot analysis. Western Blot analysis of rat liver tissue was performed as described (12). The PEMT2 specific antibody was provided by Prof. Vance.

Northern blot analysis. Poly(A^+)-RNAs were prepared by oligo(dT)cellulose chromatography (13). Northern Blot analysis was performed with 10 μg samples of poly(A^+)-RNA by electrophoresis in 1% agarose-formaldeide gel and transfer to nitrocellulose filters. The 1.3 Kb CT cDNA and 0.9 Kb PEMT2 cDNA were provided by Prof. Vance.

RESULTS AND DISCUSSION

Developmental Growth

The whole embryo weight has been taken as a phenotypic criterion of the exponential growth of embryo. Such growth then slows down in the foetus and in the postnatal period. It has also been assumed that the liver follows a similar growth pattern. The relative liver weight is high in the foetus, it decreases at birth and in the postnatal period, but remaining still higher than in adults (Table 1). Accordingly, the mitotic index is 15-20 fold higher in the liver of suckling (10 day-old) than in young adults (14).

A reciprocal regulation was found for PEMT and CT activities in the liver during developmental growth: the PEMT activity was lower while the total CT activity was higher than in the adult liver, the maximal fluctuations being before birth (Fig. 1). In order to clarify whether the drop in PEMT activity during liver development was associated with a modulation in PEMT2 expression, an immunoblot analysis with isoform-specific antibodies was performed. Fig. 2 shows that PEMT2 protein was undetectable in the foetal liver, it became detectable at birth and remained at similar level 10 days later. Comassie blue staining for total protein was performed as control. To determine whether the changes in PEMT2 protein mass were due to a modulation of PEMT2 mRNA, Northern Blot analysis was performed. The PEMT2 gene, highly expressed in the adult liver, is quite undetectable in the foetus, while at birth was already expressed at about 50% of the adult liver level and it remained unchanged 10 days later. In contrast, CT expression was very high before birth, then slightly decreasing in newborn liver and 10 days later. The same membrane was also probed with GAPDH cDNA as internal

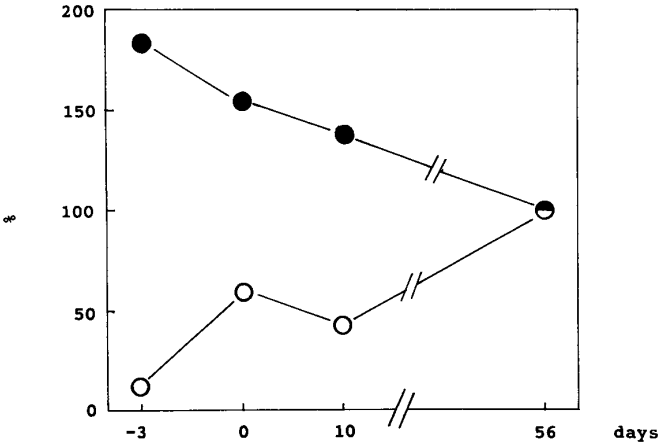

FIG. 1. PEMT and CT activities during liver growth. CT (●), PEMT (○).

control (Fig. 3). The lack of PEMT2 gene expression before birth associated with high expression of CT gene was completely in agreement with previous reports on the reciprocal regulation of the two enzymes for PC biosynthesis in non neoplastic liver growth such as regeneration and lead-induced hyperplasia (Tessitore, Houweling, Cui, Vance, unpublished results). Consistently, PEMT2 was inactivated in the chemical carcinogen-induced rat hepatocellular carcinoma when CT expression was extremely high, suggesting that PEMT2 plays a role in the negative regulation of hepatocyte cell division (3). The high expression of PEMT2 at birth was completely unexpected because newborn liver was known to be characterized by high cell division rates. Anyway, development involved not only cell division but also cell differentiation (15).

PC catabolism is known to provide various second messangers (diacylglycerol, free fatty acids, lysoPC) able to activate different isoforms of PKC (6). The activity of PKC β, zeta

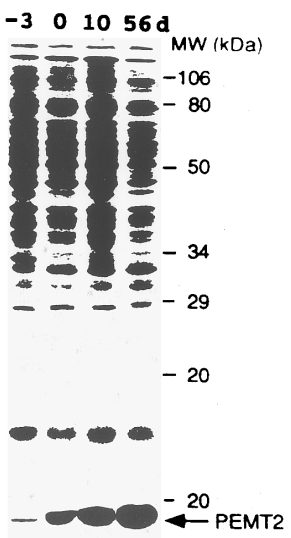

FIG. 2. Immunoblot analysis of PEMT2 during liver growth. The proteins were probed with PEMT2 specific antibody. A representative immunoblot is shown which was repeated with similar results.

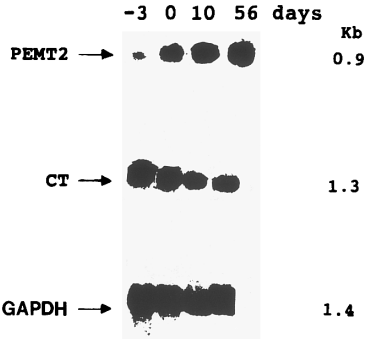

FIG. 3. Expression of PEMT and CT genes during liver growth. GAPDH was used as a control. Representative northern blot are shown which were reproduced twice with similar results.

and, particularly, α were higher (approximatively two-fold) in foetal and newborn liver and remained higher 10 days after birth, when compared with adult liver (Fig. 4). Of interest, α, β and zeta isoforms were positively correlated to cell proliferation in several model systems (15-17), included the compensatory (7) and adaptive (8) liver growth. The activity of δ PKC was lower in the foetal than in the adult liver, but surprisely it increased dramatically in the newborn liver (Fig. 4). This could be related to the high differentiation rate which is usually observed in the perinatal period, and not to the proliferation rate, which is similar before birth, at birth and during the perinatal period. In fact, δ PKC activity dropped to the levels of foetus and adults 10 days after birth, when the differentiation rates slowed down but the proliferative rates were still high. Consistently, δ PKC appeared to play a negative control on cell division since δ PKC overexpression slowed down the NIH 3T3 (18) and CHO (19) cell growth rates.

A possible interpretation of these data is that during liver development, when growth rates

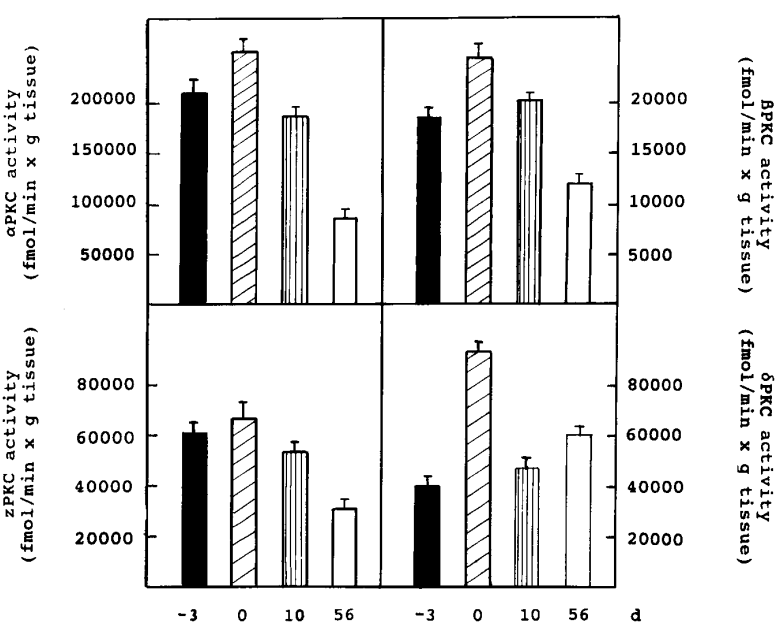

FIG. 4. α, β, zeta and δ PKC activities during liver growth. −3 day (■); 0 day (▨); 10 day (▩); 56 day (□).

are high the breakdown of PC, produced by the CDP-choline pathway, might activate the α, β and zeta PKC isoforms; while at birth, when the liver need not only to grow but also to differentiate, PC from the methylation pathway must also be produced to activate δ PKC isoform.

Our results indicate that, the high liver growth rates during the development are associated with enhancement of the CT expression and the activity of α, β, zeta PKC isoforms. At birth, when also the differentiation rates are high, PEMT2 is well expressed at about 50% of adult liver level and δ PKC activity is increased.

ACKNOWLEDGMENTS

This work was supported by grants from the Associazione Italiana per la Ricerca sul Cancro, Milano, the Consiglio Nazionale delle Ricerche (Grant 95.02456.CT04) and MURST, Roma. We thank Prof. Dennis E. Vance (Department of Biochemistry, Lipid and Lipoprotein Research Group, University of Alberta, Edmonton, Canada) who provided the PEMT2 antibody, and cDNA of PEMT2 and CT.

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