Kinetic Modeling of Phosphatidylcholine and Phosphatidylethanolamine Biosynthesis Using 13C-NMR Spectroscopy

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Introduction

Phosphatidylcholine (PtdC) and phosphatidylethanolamine (PtdE) are the two major components of biological membranes. Biosynthesis and metabolism of these phospholipids (PL) affects processes of signal transduction, cell proliferation, differentiation and apoptosis, including carcinogenesis. Yet our understanding of this metabolism and its regulation is far from complete.

De novo synthesis of PtdE from ethanolamine occurs through the CDP-ethanolamine pathway. PtdC can be synthesized from choline via the CDP-choline pathway (de novo) or by methylation of PtdE catalyzed by PtdE-N-methyltransferase (PEMT). Recent studies suggest that PtdC synthesized by these two pathways may be functionally different and that the fluxes through these pathways are coordinately regulated (1). Such processes may play a role in cell proliferation and carcinogenesis (2). Radioisotope methods have previously been used to measure rates of PtdC and PtdE synthesis in liver, but these methods lack the specificity to analyze the metabolic pathways. We investigated the biosynthesis of PtdC and PtdE in rat liver in vivo during a 60 min. infusion of [1-13C]choline and [2-13C]ethanolamine, and conducted a kinetic model analysis of the data (Figure 1). PEMT and de novo pathways were monitored simultaneously by analysis of metabolite concentrations and 13C enrichments in liver extracts using 13C and 31P NMR.

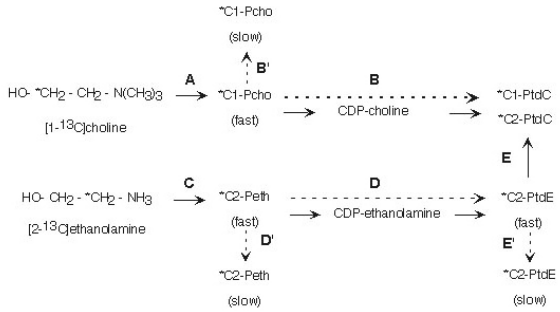

Figure-1

Methods

Rats were infused with an equimolar solution of 13C-labeled choline and ethanolamine for 0, 15, 30, 45 and 60 min (n = 4 to 5 at each timepoint). After infusion, livers were surgically exposed and freeze-clamped. Chemical extracts of the tissues were prepared using a dual-phase extraction procedure (3). High-resolution proton-decoupled 31P and 13C spectra on the extract samples were acquired at 8.5T, respectively. NMR spectral intensities from aqueous and lipid extracts yielded metabolite concentrations, and an assessment of the 13C-enrichment of the metabolite pools. All data were considered statistically significant at the 95% confidence level. Kinetic models were coded using a numerical integration package, Advanced Computing Simulation Language. As a first approximation, each reaction was assumed to progress at a constant rate expressed in umol/h/g of liver. Alternative models were also evaluated.

Results

Data reveal that 71% of newly synthesized PtdC is derived via the de novo pathway, while 29% is by action of PEMT. The kinetic analyses required "fast" and "slow" pools for some metabolites derived from exogenous choline and ethanolamine indicating that they remain discrete from intracellular pools, but are preferentially used in the pathways for PL synthesis. Thus the CDP-choline, CDP-ethanolamine, and PEMT pathways are "channeled" metabolic processes. The kinetics of the de novo pathways for PtdC and PtdE biosynthesis are uniquely different and the data do not fit a simple model assuming constant fluxes (Model-cf). An alternative model that provided a better fit to the data allowed some fluxes to be unregulated (Model-uf). The model results are given in the Table 1 (letters refer to pathways in the figure). Fluxes are in umol/h/g of liver. The flux from ethanolamine to phosphoethanolamine (Peth) exceeded the flux from choline to phosphocholine (Pcho), and the rate of PtdE synthesis was 2-to-3-times greater than the rate for de novo synthesis of PtdC.

Discussion

These NMR methods are superior to earlier radioisotope studies since they provide simultaneously in a single experiment information about the specific metabolic pathways of phospholipid biosynthesis. The results of the current study indicated that metabolites derived from exogenous choline and ethanolamine are preferentially utilized in the pathways for PL synthesis. Biosynthesis of PtdC and PtdE through the CDP pathways are channeled processes. We also show for the first time that channeling is involved in the PEMT pathway for interconversion of PtdE into PtdC.

References

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TABLE 1: Kinetic Model Fluxes

	Model-cf	Model-uf
FLUX A	0.31	0-1.05
FLUX B	0.21	0-0.7
FLUX B'	0.1	0-0.35
FLUX C	0.94	1.04
FLUX D	0.92	1.04
FLUX D'	0.02	0-0.06
FLUX E	0.12	0.14
FLUX E'	0.8	0.9