DEPLETION OF CYTIDINE TRIPHOSPHATE AS A CONSEQUENCE OF CELLULAR URIDINE TRIPHOSPHATE DEFICIENCY

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1. Introduction

The synthesis of CTP from UTP, catalyzed by CTP synthetase (EC 6.3.4.2), would imply that severe UTP deficiency causes a reduction of cellular CTP synthesis followed by a decrease in CTP pools. By contrast, UTP depletion induced by D-galactosamine in rat liver in vivo has been shown to be associated with a 2-fold increase in the hepatic content of CTP [1,2]. This result is confirmed in the present communication by means of high-pressure liquid chromatography of hepatic nucleotides. In vivo, the cytidine present in plasma [3] can contribute significantly to the synthesis of CTP on the salvage pathway. We have therefore studied hepatoma cell suspensions in vitro in order to evaluate the influence of the cellular level of UTP on CTP pools in the absence of extracellular cytidine. Under this condition a close correlation over a wide range was demonstrated between UTP and CTP contents. This correlation was independent from the agents used to induce UTP deficiency. These included inhibitors of de novo pyrimidine nucleotide synthesis and the uridylate-trapping amino sugars D-glucosamine (GlcN) and D-galactosamine (GalN). The analysis of pyrimidine nucleotides by high-pressure liquid chromatography has shown an excellent agreement with our earlier enzymatic measurements [4,5].

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2. Materials and methods

2.1. *Chemicals*

D-Galactosamine·HCl was purchased from C. Roth, Karlsruhe, D-glucosamine·HCl was from Sigma Chemical Co., St Louis and 6-azauridine from Calbiochem, San Diego. *N*-(Phosphonacetyl)-L-aspartate (PALA) was a kind gift from Dr G. Stark, Dept Biochemistry, School of Medicine, Stanford University. KH_2PO_4 and KCl were from E. Merck, Darmstadt, all other chemicals used in this study were analytical grade.

2.2. *Incubation of hepatoma cells and preparation of acid-soluble cell and tissue extracts*

AS-30D ascites hepatoma cells [6] were carried, incubated, collected, frozen and extracted with perchloric acid as in [7] except that phosphate and sodium bicarbonate concentrations in the suspension medium were changed to 2 and 25 mmol/l, respectively. Liver samples were obtained by freeze-clamping in situ under thiopental anesthesia, extracted with cold perchloric acid and neutralized with potassium bicarbonate [2,5].

2.3. *High-pressure liquid chromatographic analysis of nucleotides*

Cell or tissue extracts (usually 50 μl) were analyzed on a DuPont (Wilmington) chromatograph, model 848, with a single-beam ultraviolet detector operating at 254 nm. Nucleotides were separated on a Partisil PXS-1025 SAX column, 4.6 mm × 25 cm (Whatman,

Clifton) at ambient temperature using a linear gradient from low to high-concentration eluent within 30 min. The low-concentration eluent contained KH_2PO_4 (7 mmol/l) and was adjusted to pH 4.0, the high-concentration eluent was composed of KH_2PO_4 (250 mmol/l) and KCl (750 mmol/l) at pH 4.5. The flow rate was 1.5 ml/min. The area under each peak was measured by cutting and weighing on a microbalance; the nucleotides were quantitated by referring to standards of the highest purity available and run on the same column.

3. Results

3.1. *Changes in CTP and UTP contents of hepatoma cells suspended in vitro*

Combination of uridylate trapping by GalN with a blockade of de novo pyrimidine synthesis led to a depletion of UTP within 2 h (fig.1). UTP levels and time course were almost identical as in previous studies in which enzymatic nucleotide analyses were employed [8]. With a slight delay, UTP depletion was followed by a fall in CTP. The CTP level measured after 2 and 3 h corresponded to 28% of control (fig.1).

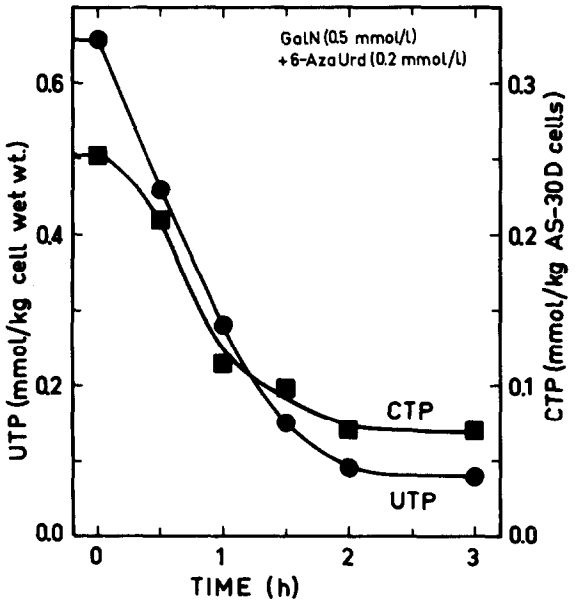

Fig.1. Decrease in CTP and UTP contents of AS-30D hepatoma cells incubated as in [7,8] in the presence of GalN (0.5 mmol/l) and 6-azauridine (6-AzaUrd; 0.2 mmol/l). CTP and UTP were analyzed as in section 2.3. Mean values from 3 different experiments; the mean variation coefficients were 14% and 22% for UTP and CTP, respectively.

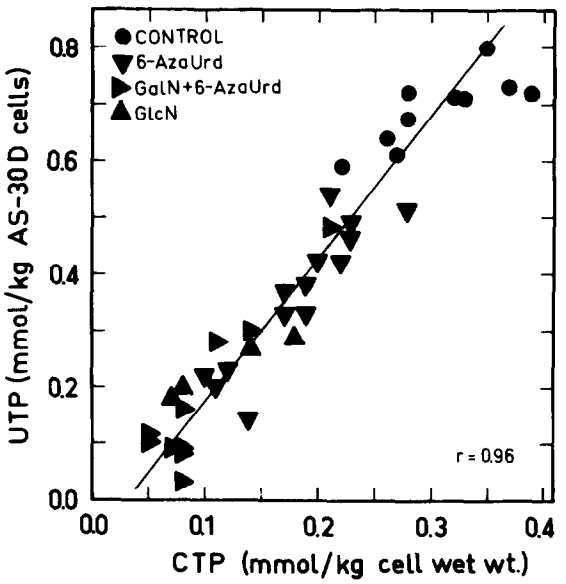

Fig.2. Correlation between CTP and UTP levels in hepatoma cells incubated for time periods up to 3 h in the presence or absence of different antipyrimidines. 6-azauridine (6-AzaUrd) was 0.2 mmol/l; GalN, 0.5 mmol/l; GlcN, 10 mmol/l. Freeze-pellets were obtained [7] and CTP and UTP determined as in section 2.3.

The close correlation between CTP and UTP contents of cells suspended in vitro was maintained too, when UTP levels were lowered by means of 6-azauridine or GlcN (fig.2), but also when PALA (0.8 mmol/l) or PALA + GalN [9] were used as antipyrimidines. The correlation coefficient was 0.96 and the regression line for the conditions described under fig.2 was calculated as CTP = 0.4 UTP + 0.03. These findings indicate that UTP deficiency in cell suspensions in vitro is not as selective as GalN-induced hepatic UTP depletion in vivo [2].

3.2. *Increase in CTP as a result of hepatic UTP deficiency in vivo*

In livers from fed female Wistar rats treated with GalN (1.85 mmol/kg body wt) UTP decreased after 3 h from 0.23 ± 0.02 (SD, n = 4) to 0.025 ± 0.003

(SD, $n = 4$) mmol/kg liver wet wt while CTP increased from 0.06 ± 0.005 (SD) to 0.15 ± 0.003 (SD) mmol/kg. ATP remained constant at 2.98 ± 0.31 (SD) mmol/kg. These nucleotide contents determined by high-pressure liquid chromatography are in close agreement with those measured in an earlier experiment under the same conditions [2] by means of enzymatic analysis and isotope dilution [4,5]. The 2.5-fold increase in the CTP content after 3 h of uridylate trapping and UTP deficiency suggests that CTP synthesis from cytidine plays a significant role under this condition.

4. Discussion

UTP and CTP are potent feedback inhibitors of uridine–cytidine kinase (EC 2.7.1.48) [10] which appears as the rate-determining enzyme in the conversion of cytidine to CTP [11]. UTP deficiency and relief of this feedback inhibition may thus lead to an increased utilization of cytidine for CTP synthesis. Cytidine in rat plasma at 10 μmol/l [3] is sufficient to provide a source for the paradoxical rise of CTP in GalN-treated liver described in section 3.2.

The fall of the CTP content in cell suspensions under conditions of UTP deficiency and in the absence of extracellular cytidine (fig.1,2) is in line with the reported K_m-value for UTP of CTP synthetase from rat or calf liver which is in the range of 0.1 mmol/l [12]. Evidence for a strongly reduced conversion of UTP to CTP was obtained furthermore in GlcN-induced UTP deficiency in chick fibroblasts using labeled uridine and its relative incorporation into the CMP and UMP moieties of RNA [13]. A strong parallel decrease of UTP and CTP was also described in murine lymphoma L5178Y cells cultured in vitro in the presence of 6-azauridine [14] or pyrazofurin [15,16]. However, when these cells were grown in vivo as ascites tumors, UTP deficiency was associated with normal CTP contents when determined at 1, 24 and 48 h after administration of pyrazofurin which is a powerful inhibitor of de novo uridylate synthesis [15,16].

A comparison of nucleotide concentrations determined by high-pressure liquid chromatography as described in section 2.3. with those measured by enzymatic analysis has shown a close agreement, and the former method seems to be more rapid and simpler. For UTP analyzed in the same sample by the two different methods a mean difference of 3% was found in 7 samples; the maximal difference detected in 1 sample was 10%.

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