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TURNOVER OF CYTIDINE AND URIDINE COMPONENTS OF ACID-SOLUBLE POOL AND RNA OF CYTOPLASMIC RIBOSOMES AFTER REPEATED PHENOBARBITAL ADMINISTRATION

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Abstract—The administration of phenobarbital in drinking water (1 g/l) to rats that had received labeled orotic acid three days before does not affect the half-life of uridine components of the liver nucleotide pool ($t_{1/2}$ = 5·9 days). The half-life of the cytidine components in the control group is 6·2 days. In the experimental group the decrease of specific activity of cytidine components of the pool is biphasic. The decrease is faster during the first six days of phenobarbital administration ($t_{1/2}$ = 4·5 days) whereas a slower decrease ($t_{1/2}$ = 8·2 days) is observed during the subsequent administration of the drug. The decrease of specific activity of uridylic acid in RNA from cytoplasmic ribosomes is faster in the control group ($t_{1/2}$ = 4·9 days) than in the experimental group ($t_{1/2}$ = 5·9 days). Similarly, the decrease of the specific activity of cytidylic acid isolated from the ribosomal RNA is markedly slowed down ($t_{1/2}$ of controls = 5·9 days, $t_{1/2}$ of experimental group = 7·5 days).

In the mammalian organism the quantity of the main cell components (proteins, RNAs, phospholipids) and their turnover rates are controlled by simultaneous synthesis and degradation. The synthesis and the degradation of the cell components are usually investigated by determining the decrease of specific activity of isolated cellular macromolecules labeled with a low molecular weight precursor. The half-life values of the specific activities measured need not however represent the actual half-lives of the cell components investigated because of the reutilization of radioactive components.

The decrease of the specific activity of liver ribosomal RNA reflects not only the intracellular regeneration of the ribosomes but also the rate of cell division in the tissue. The decrease of specific activity caused by the division of liver cells is more significant in the growing animals than in the mature animals where the overall growth and also the growth of the organ proceed at a slower rate.

The changes in the decrease of the specific activity of RNA under various experimental conditions (starvation, regeneration, adrenalectomy, administration of extraneous drugs) can be brought about not only by a change in the synthesis and degradation of the macromolecules themselves but they can also reflect changes in the metabolism of the low molecular weight precursors and in the composition of the nucleotide pool. The determination of the biological half-life of rRNA labeled with orotic acid is complicated by differences in the utilization of the labeled precursor in the synthesis of uridine and cytidine nucleotides of rRNA [1, 2] and also by differences in the decrease of the specific activities of both pyrimidines of rRNA [3]. Since the administration of phenobarbital affects the utilization of labeled orotic acid in the synthesis of the cytidine components of the free nucleotide pool and of cytidylic acid of rRNA and tRNA [4], we investigated the effects of chronic administration of this drug on the metabolism of the pyrimidine components of the pool and of RNA in isolated plasmatic ribosomes.

METHODS

Male Wistar rats (Lysolaje), weighing 160 g at the beginning of the experiment, were used. Animals of the same weight were chosen for each time interval. Phenobarbital sodium salt (Merck) was administered to the animals in the drinking water (1 g/l). (6-^{14}C)Orotic acid (Institute for Research, Production and Uses of Radioisotopes, Prague; sp. act. 47 mCi/m-mole) was injected intraperitoneally (i.p.) in 0·9% NaCl (30 μCi/kg body wt) 3 days before the administration of phenobarbital. The rats were starved 12 hr before being sacrificed. The ribosomes were isolated by the method of Munro *et al.* [5]. The determination of the specific activities of the uridine and cytidine components of the pool and of the pyrimidine nucleotides of ribosomal RNA has been described elsewhere [4, 6].

The slopes of the curves characterizing the time profile of the logarithm of the specific activities of the components investigated were calculated by the method of

least squares. The half-life of the specific activity was calculated from the relation

$$t_{1/2} = \frac{\log 2}{-b}$$

where b is the slope of the line.

RESULTS

The decrease of the specific activities of uracil and cytosine in the hydrolyzed free nucleotide pool of control animals was essentially identical ($t_{1/2}$ of uracil = 5·9 days, $t_{1/2}$ of cytosine = 6·2 days). The administration of phenobarbital did not affect the rate of the decrease of the specific activity of uracil in the pool. The specific activity of the cytidine components of the nucleotide pool decreased faster ($t_{1/2}$ = 4·5 days) during the first 6 days of the drug administration; the decrease was significantly slower ($t_{1/2}$ = 8·2 days) at the later stage of phenobarbital administration (Fig. 1, Table 1).

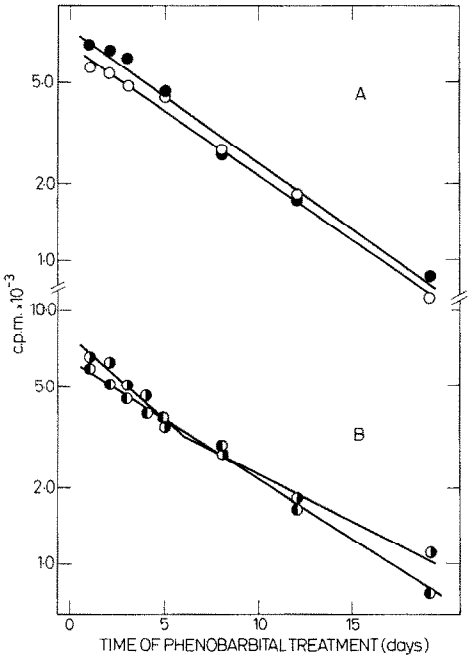

Fig. 1. Decrease of specific activities of the hydrolyzed liver acid-soluble pool during phenobarbital treatment.
(A) Specific activity of the uracil moiety of the nucleotide pool; ○——○ controls, ●——● phenobarbital treatment. (B) Specific activity of the cytosine moiety of the nucleotide pool; ◐——◐ controls, ◑——◑ phenobarbital treatment.

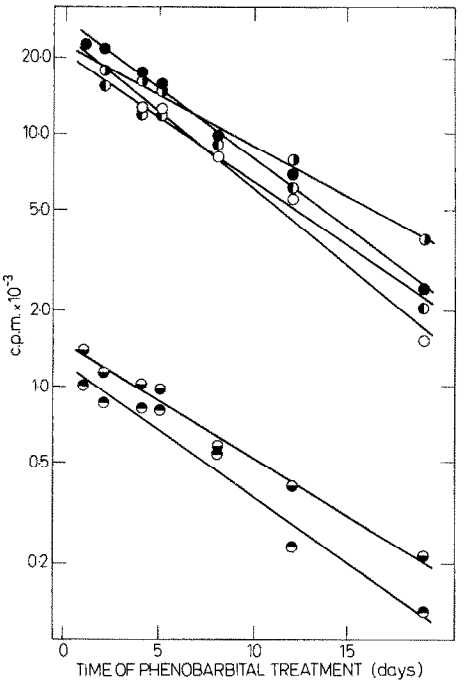

Fig. 2. Decrease of specific activities of uridylic and cytidylic acid of ribosomal RNA and whole ribosomal RNA hydrolysate during phenobarbital treatment.
○——○ 3′-UMP, controls; ●——● 3′-UMP, phenobarbital treated; ◐——◐ 3′-CMP, controls; ◑——◑ 3′-CMP, phenobarbital treated; ⊖——⊖ rRNA hydrolysate, controls; ⊖——⊖ rRNA hydrolysate, phenobarbital treated.

The rate of decrease of the specific activity of rRNA pyrimidine components of control animals was different. Radioactivity of UMP from rRNA ($t_{1/2}$ = 4·9 days) decreased faster than radioactivity of CMP iso-

Table 1. Turnover of uracil and cytosine moieties of the hydrolyzed liver acid-soluble pool

	Uracil		Cytosine		
	Control	Phb treated	Control	Phb treated 1–6 days	Phb treated 7–17 days
b	−0·051 ± 0·003	−0·053 ± 0·004	−0·048 ± 0·002	0·067 ± 0·010	−0·037 ± 0·003
log c	3·842 ± 0·021	3·908 ± 0·028	3·810 ± 0·012	3·905 ± 0·030	3·725 ± 0·040
$t_{1/2}$	5·902 ± 0·330	5·679 ± 0·398	6·206 ± 0·222	4·492 ± 0·583	8·224 ± 0·718

The decrease of the specific activity of the components examined can be characterized by the expression: $y = bx + \log c$, where y is the logarithm of the specific activity, b the regression coefficient, x the time (in days) after the injection of labeled orotic acid, and log c the intercept on the ordinate. The regression coefficient was computed by the method of least squares. Each value is the mean ± S.E. of seven or eight points on the line.

Table 2. Turnover of uridylic and cytidylic acid in ribosomal RNA and whole ribosomal RNA hydrolysate

	Uridylic acid		Cytidylic acid		rRNA hydrolysate	
	Control	Phb treated	Control	Pbh treated	Control	Phb treated
b	$-0{\cdot}061 \pm 0{\cdot}004$	$-0{\cdot}055 \pm 0{\cdot}002$	$-0{\cdot}051 \pm 0{\cdot}004$	$-0{\cdot}040 \pm 0{\cdot}002$	$-0{\cdot}052 \pm 0{\cdot}005$	$-0{\cdot}045 \pm 0{\cdot}002$
$\log c$	$4{\cdot}386 \pm 0{\cdot}034$	$4{\cdot}451 \pm 0{\cdot}014$	$4{\cdot}321 \pm 0{\cdot}033$	$4{\cdot}348 \pm 0{\cdot}017$	$3{\cdot}085 \pm 0{\cdot}036$	$3{\cdot}165 \pm 0{\cdot}014$
$t_{1/2}$	$4{\cdot}934 \pm 0{\cdot}303$	$5{\cdot}473 \pm 0{\cdot}200$	$5{\cdot}902 \pm 0{\cdot}429$	$7{\cdot}525 \pm 0{\cdot}358$	$5{\cdot}788 \pm 0{\cdot}507$	$6{\cdot}689 \pm 0{\cdot}285$

For details see legend to Table 1.

lated from the same rRNA ($t_{1/2}$ = 5·9 days) (Table 2). The radioactivity half-life of cytidylic acid of ribosomal RNA in control animals was essentially the same as the radioactivity half-life of the pyrimidine components of the hydrolyzed free nucleotide pool. The rate of radioactivity decrease of the total hydrolysate of ribosomal RNA was between the values obtained for ribosomal uridylic and cytidylic acids ($t_{1/2}$ = 5·8 days).

Chronic administration of phenobarbital caused a significant increase of the half-life of both pyrimidine components of ribosomal RNA ($t_{1/2}$ = 5·5 days for UMP and 7·5 days for CMP). The specific activity decrease of cytidylic acid of RNA remained markedly slower during phenobarbital administration than the decrease of the corresponding values characterizing the remaining pyrimidines investigated (Fig. 2, Table 2). Similarly, the half-life ($t_{1/2}$ = 6·7 days) of the specific activity of the whole hydrolysate of ribosomal RNA was prolonged with respect to the control value during the phenobarbital administration.

DISCUSSION

The radioactivity of the pyrimidine components in the hydrolyzed whole free nucleotide pool, determined several days after the administration of labeled orotic acid is predominantly in degraded nucleic acids and in pyrimidine coenzymes. The specific activity decrease with time represents a measure of the rate of synthesis and degradation of all these components; at the same time, the possibility cannot be excluded that the half-lives of these individual components are different. Chronic administration of phenobarbital does not affect the radioactive half-life of uracil in the whole hydrolyzed pool of free nucleotides. This correlates with the previous observation that the utilization of orotic acid in the synthesis of uridine nucleotides in the liver is not affected by chronic administration of the drug. During the first 6 days of phenobarbital administration, when a hypertrophy of both the cells and of the organ can be observed, the decrease of specific activity of cytosine in the whole hydrolyzed nucleotide pool is significantly faster than the decrease observed with the control group. By contrast, the decrease of specific activity of cytosine is slower during the subsequent drug administration after a steady state has been achieved. The effect of phenobarbital administration on the synthesis of cytidine nucleotides has been observed before [4].

Chronic drug administration increases the activity of cytidine triphosphate synthetase in the cytoplasm of liver cells; it decreases, however, the utilization of orotic acid in the synthesis of cytidine nucleotides [6] and also of cytoplasmic RNA [4].

Studies of the biosynthesis of free pyrimidine nucleotides and on their incorporation into ribonucleic acids have provided evidence of the existence of various nucleotide compartments in the cell. Two separate compartments of the free nucleotide pool have been reported to exist in the Novikoff hepatoma cells; the larger, in volume, cytoplasmic compartment and the smaller nuclear compartment which is used for RNA synthesis [7]. By contrast, no separate compartments of nuclear and cytoplasmic nucleotides have been found in HeLa cells; however, indirect evidence for the existence of a mitochondrial compartment of the free nucleotide pool has been obtained [8]. If similar compartments of the free nucleotide pool also exist in the normal liver cells, then the turnover rate of uracil and cytosine in the hydrolyzed acid-soluble pool of the whole liver tissue need not necessarily be directly related to the turnover rate of RNA; likewise, the half-life of uracil and cytosine in the pool need not be in relation to the half-lives of uridylic and cytidylic acids of ribosomal RNA.

The significantly shorter radioactive half-life of UMP of ribosomal RNA reflects probably the half-life of the ribosomes themselves. It appears that uridine is not reutilized (or if it is reutilized then to a considerably lower degree than cytidine) in the subsequent synthesis of RNA. By contrast, CMP of ribosomal RNA seems to be reutilized considerably more. This is evidenced both by the slowest decrease of specific activity of cytidylic acid of ribosomal RNA and also by the relatively long period necessary for an equal distribution of specific activity between ribosomal UMP and CMP after the single-dose application of labeled orotic acid. The ratio of the specific activities of UMP and CMP in ribosomal RNA reaches 1·0 approx. 9 days after the administration of one dose of ^{14}C orotic acid [4].

The quantity of the 45S ribosomal precursors synthetized in the hepatocyte nuclei is approximately 80 per cent higher than the quantity necessary for the replacement of the degraded cytoplasmic ribosomes [9]. This "surplus" RNA is degraded in the nucleus. An exoribonuclease degrading the RNA molecule from the 3'OH terminus and releasing 5'-monophosphates was reported to exist in the nuclear fraction of various tissues [10]. These nucleotides could be phosphorylated by a kinase in the nucleus to triphosphates and again utilized in RNA synthesis. The ribosomal RNA contains 70 per cent more cytidine than uridine [11]. If the pyrimidine nucleotides from the RNA ribosomal precursors still in the nucleus are reutilized, a higher reutilization of cytidine nucleotides can be assumed because their quantity formed by the degradation of rRNA is higher. On the contrary, the quantity of cytidine nucleotides in the total acid-soluble pool, which represents the predominant part of the cytoplasm, is approximately six times lower than the quantity of uridine nucleotides [12]. This fact also indicates the necessity of a more effective utilization of cytidine nucleotides in RNA synthesis. This utilization could be made possible not only by a faster transport of CTP from the cytoplasm to the nucleus but also by its synthesis from UTP directly in the nucleus [7].

The administration of phenobarbital affects the synthesis and degradation of RNA in the liver. During the initial stage after the first dose of phenobarbital a

higher incorporation of labeled orotic acid into cytoplasmic RNA has been observed [13], especially into the RNA ribosomes bound to membranes [14]. The increase of specific activity of cytoplasmic RNA shortly after the administration of phenobarbital could be related to the changes in the transport mechanism rather than to the increase of RNA synthesis in the nucleus. Phenobarbital does not increase the incorporation of the radioactive precursors into nuclear RNA at any of the time intervals examined [15]. However, in liver of immature rats, an increase of the degree of methylation and of the quantity of cytidine-guanosine rich RNA after the administration of the drug has been observed [16]. A study of the specific activities of RNAs of isolated cytoplasmic ribosomes during phenobarbital administration has shown that this drug decreases the utilization of labeled orotic acid for synthesis of cytidylic acid of rRNA [4]. The specific activities of uridylic acid of rRNA are altered only a little. The incorporation of labeled orotic acid into the pyrimidine nucleotides of rRNA can be affected after the phenobarbital administration also by changes in the metabolism of pyrimidine nucleoside triphosphates. It has been observed that chronic administration of the drug increases the activity of cytidine triphosphate synthetase, CTPase and UTPase in the particle-free fraction of the liver. On the contrary, the nucleoside triphosphatase activities of pyrimidines in the microsomal fraction are decreased after chronic phenobarbital administration [6]. These findings indicate that the results of the determination of RNA synthesis *in vivo* in terms of incorporation of labeled orotic acid can be distorted by changes in the specific activities of pyrimidine nucleoside triphosphates.

The half-life of the specific activity of RNA of the cytoplasmic ribosomes is prolonged during phenobarbital treatment [17]. The turnover rates of uridine and cytidine in this RNA, however, are affected differently. The half-life of UMP of ribosomal RNA is approximately 10 per cent longer in the experimental group. This prolongation can be explained by decreased degradation of both the cytoplasmic [18–21] and the nuclear [15] RNA. The specific activity of CMP of ribosomal RNA decreases during phenobarbital treatment with a half-life approx. 30 per cent longer than in the control group. An additional increase of the reutilization of the cytidine RNA precursors after phenobarbital administration most likely participates on this prolongation. A slower decrease of the specific activity of CMP of ribosomal RNA is related also to changes in the pyrimidine metabolism [6] and to the increase of the metabolic stability of 45S RNA in the nuclei of liver cells after phenobarbital administration [15].

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