INCREASED ACTIVITIES OF HEPATIC OROTIDINE 5'-PHOSPHATE PYROPHOSPHORYLASE AND OROTIDINE 5'-PHOSPHATE DECARBOXYLASE INDUCED BY OROTATE

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1. Introduction

Administration of orotate, an intermediate in pyrimidine nucleotide biosynthesis, has been shown to elevate the levels of acid soluble uracil [1–6] and cytosine nucleotides [5] in liver. Furthermore, activities of enzymes involved in the de novo synthesis of pyrimidine nucleotides increase following the administration of orotate; this has been demonstrated for aspartate carbamoyl transferase (EC 2.1.3.2) and dihydro-orotase (EC 3.5.2.3) in rat liver [7]. In yeast, a sequential induction of enzymes of pyrimidine biosynthesis by an intermediate of this pathway (dihydroorotate) was observed [8]. Elevated uracil nucleotide contents as well as an increased rate of uridylate biosynthesis in liver have been considered essential for the prevention of galactosamine-induced hepatitis by orotate [9, 10]. Data will now be presented indicating that orotate administration leads to increased activities of the hepatic enzymes catalyzing the conversion of orotate to UMP.

2. Experimental procedures

Orotate was administered intraperitoneally to female Wistar rats (150 g) as one single dose of Tris-orotate (3 mmole/kg body weight) or as 6 injections of choline orotate (0.57 mmole/kg body weight each) over a period of 2 days [9]. Untreated animals served as controls. Inhibitors of protein and RNA synthesis, respectively, were administered intraperitoneally 15 min before orotate treatment: cycloheximide (4 mg/kg body weight), actinomycin D (0.5 mg/kg body weight, the same dose repeated 4 hr later), and D-galactosamine-HCl (400 mg/kg body weight).

The specific radiochemical assays of the hepatic activities of orotidine 5'-phosphate pyrophosphorylase (EC 2.4.2.10) and orotidine 5'-phosphate decarboxylase (EC 4.1.1.23) were performed as described previously [11].

Alanine aminotransferase (EC 2.6.1.2) and L-iditol dehydrogenase (EC 1.1.1.14) activities in liver were measured according to Bergmeyer and Bernt [12] and Gerlach and Hiby [13], respectively. Specific activities of enzymes are given in international units at 25°.

3. Results

3.1. *Orotate effect*

The activity of orotidine 5'-phosphate (OMP)-pyrophosphorylase was elevated 8 hr after administration of Tris-orotate (table 1); it remained constant between 8 and 24 hr after orotate injection. Under the same conditions the activity of OMP-decarboxylase (in untreated animals, 0.60 ± 0.09 mU/mg protein (±S.D.)) was also enhanced significantly (fig. 1). Six doses of choline orotate increased the activities of OMP-pyrophosphorylase and of OMP-decarboxylase by 43 and 47% ($p < 0.001$), respectively.

To exclude unspecific effects of orotate on enzyme activities the hepatic activity of alanine aminotransferase and L-iditol dehydrogenase was monitored: in untreated rats their activities (± S.D.) were 718 ± 189 and 237 ± 35 mU/mg protein, respectively. Eight

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Table 1
Time-dependent changes of OMP-pyrophosphorylase activity after i.p. injection of 3 mmole Tris-orotate per kg body weight.

Time after orotate injection (hr)	OMP-pyrophosphorylase activity (mU/mg protein ± S.D.) (n)	Increase as compared to controls (%)	*p*
0 (controls)	0.53 ± 0.02 (5)	–	–
3	0.56 ± 0.05 (4)	6	–
8	0.71 ± 0.09 (11)	34	<0.001
12	0.69 ± 0.08 (4)	31	<0.002
24	0.72 ± 0.10 (4)	36	<0.002

For further details see Experimental procedures.

hr after application of Tris-orotate 801 ± 86 and 234 ± 5 mU/mg protein, respectively, were measured, the differences not being significant.

3.2. *Pretreatment with inhibitors of protein and RNA synthesis*

Cycloheximide completely suppressed the orotate-induced stimulation of OMP-pyrophosphorylase and OMP-decarboxylase (table 2, fig. 1). Furthermore, in animals pretreated with D-galactosamine or actinomycin D, OMP-pyrophosphorylase activity increased after orotate treatment by less than 9%. A similar and significant extent of suppression was also observed with OMP-decarboxylase. The inhibitors alone did not cause significant alterations of the basal enzyme activities. Accordingly, cycloheximide was shown not to affect the incorporation of orotate into UTP in short-term experiments *in vivo* [14].

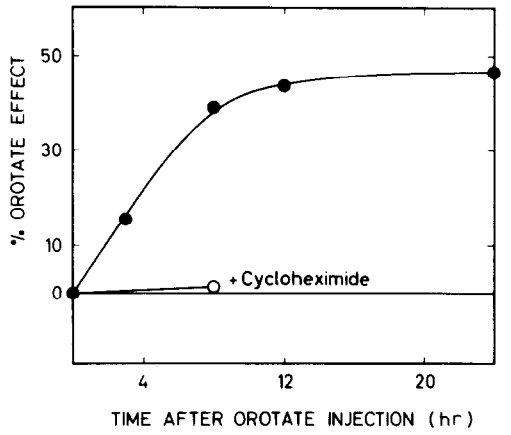

Fig. 1. Time-dependent increase in OMP-decarboxylase activity by Tris-orotate. The increase in activity is expressed as % over untreated controls. The experimental conditions are the same as in table 2.

4. Discussion

Changes of *in vitro* activities of the enzyme catalyzing uridylate biosynthesis from orotate were found to parallel alterations of the rate of stimulated uridylate synthesis *in vivo*. The latter was measured as the increase of the sum of uracil nucleotides with time under conditions of depleted hepatic pools of uridine phosphates and UDP-hexoses and calculated to be 0.35 μmoles/g liver/hr in adult rats [15]. Orotate administration increased this value significantly [10]. OMP-pyrophosphorylase and OMP-decarboxylase activities are elevated about 2-fold in livers of young rats (10 days after birth) and of rats 5 days after partial hepatectomy [11]; here, the rates of stimulated uridylate biosynthesis exceed those of adult animals by a factor of 2.3 and 1.7, respectively [11]. The prerequisites for the prevention of galactosamine-induced hepatitis by orotate are a higher capacity of uridylate biosynthesis and increased tissue contents of uracil nucleotides [10]. Both result in part from the orotate-induced elevation of hepatic OMP-pyrophosphorylase and OMP-decarboxylase activities.

Table 2
Suppression of the orotate effect on OMP-pyrophosphorylase and OMP-decarboxylase by cycloheximide.

Treatment	OMP-pyrophosphorylase (mU/mg protein ± S.D.)	(n)	OMP-decarboxylase (mU/mg protein ± S.D.)	(n)
None (controls)	0.53 ± 0.02	(5)	0.60 ± 0.09	(5)
Orotate	0.71 ± 0.09	(11)	0.94 ± 0.08	(11)
Orotate + cycloheximide	0.56 ± 0.03	(5)	0.68 ± 0.03	(5)

The enzyme activities were determined 8 hr after i.p. injection of 3 mmole Tris-orotate per kg body weight. 4 mg cycloheximide per kg body weight had been injected intraperitoneally 15 min before orotate administration. Differences between controls and orotate treated animals and the suppression of the orotate effect by cycloheximide are significant for both enzymes ($p < 0.002$).

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References

[1] L.H. von Euler, R.J. Rubin and R.E. Handschumacher, J. Biol. Chem. 238 (1963) 2464.
[2] M. Marchetti, P. Puddu and C.M. Caldarera, Biochem. J. 92 (1964) 46.
[3] E.A. Valli, D.S.R. Sarma and P.S. Sarma, Indian J. Biochem. 5 (1968) 120.
[4] D. Keppler, J. Rudigier and K. Decker, Anal. Biochem. 38 (1970) 105.
[5] W. Domschke, D. Keppler, E. Bischoff and K. Decker, Hoppe-Seyler's Z. Physiol. Chem. 352 (1971) 275.
[6] H.G. Windmueller and L.H. von Euler, Proc. Soc. Exp. Biol. Med. 136 (1971) 98.
[7] E. Bresnick, E.D. Mayfield, Jr., and H. Mossé, Mol. Pharmacol. 4 (1968) 173.
[8] F. Lacroute, J. Bacteriol. 95 (1968) 824.
[9] D. Keppler, J. Rudigier, W. Reutter, R. Lesch and K. Decker, Hoppe-Seyler's Z. Physiol. Chem. 351 (1970) 102.
[10] K. Decker, D. Keppler, J. Rudigier and W. Domschke, Hoppe-Seyler's Z. Physiol. Chem. 352 (1971) 412.
[11] J. Pausch, D. Keppler and K. Decker, Biochim. Biophys. Acta, in press.
[12] H.U. Bergmeyer and E. Bernt, in: Methoden der enzymatischen Analyse, ed. H.U. Bergmeyer (Verlag Chemie, Weinheim/Bergstrasse, second edition 1970) p. 717.
[13] U. Gerlach and W. Hiby, in: Methoden der enzymatischen Analyse, ed. H.U. Bergmeyer (Verlag Chemie, Weinheim/Bergstrasse, second edition 1970)p. 527.
[14] R.F. Brown, T. Umeda, Sh.-J. Takai and I. Lieberman, Biochim. Biophys. Acta 209 (1970) 49.
[15] D.O.R. Keppler, J.F.M. Rudigier, E. Bischoff and K.F.A. Decker, European J. Biochem. 17 (1970) 246.