EFFECT OF URACIL ANALOGUES ON THE OXIDATIVE AND PHOSPHORYLATING FUNCTION OF ALBINO RAT LIVER MITOCHONDRIA

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After intraperitoneal injection of 4-methyluracil (4MU) and 4-methyl-5-hydroxy-uracil (4M5HU) for four days in doses of 100 mg/kg daily partial inhibition of malate- and succinate-dehydrogenase activity was found in the liver mitochondria. The utilization of inorganic phosphate for ATP resynthesis was inhibited, and this was evidently one cause of the decrease in the ATP content in the liver. Administration of 4M5HU in a dose of 30 mg/kg was accompanied by similar changes but they were less marked. Under the influence of 4 MU in a dose of 30 mg/kg only the rate of phosphorylation in the mitochondria and the ATP content in the liver were significantly changed (increased).

KEY WORDS: 4-methyluracil; 4-methyl-5-hydroxyuracil; liver mitochondria; oxidative phosphorylation.

Analogues of the natural pyrimidines, with a clearly defined protein-anabolic action [2, 3] can activate the glycolytic breakdown of carbohydrates in the rat liver [7]. This paper describes an investigation of the effect of 4-methyluracil (4MU) and 4-methyl-5-hydroxyuracil (4M5HU) on the activity of mitochondrial malate dehydrogenase (MDH; EC 1.1.1.37) and succinate dehydrogenase (SDH; EC 1.3.99.1), on oxidative phosphorylation, on the ATP content in the rat liver, and on the incorporation of P^{32} into ATP.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar rats weighing 230-280 g. For four days the animal of the control group received 2.5 ml distilled water by intraperitoneal injection, whereas the experimental animals received a 0.6% solution of 4MU or 4M5HU in a dose of 30 or 100 mg/kg live weight. The rats were decapitated on the 5th day. Protein [10] and activity of MDH [9] and FDH [5] were determined in the liver mitochondria [4]. Respiration was determined in a Warburg's apparatus (15 min, 26°, atmosphere air). The incubation mixture [8] contained 10 μmoles potassium glutamate or succinate as the oxidation substrates. Phosphorylation was estimated from the decrease in the content of inorganic phosphate (P_{in}) in the small containers [6]. The quantity of O_2 assimilated and the decrease in P_{in} were expressed in μA/mg protein/h of incubation. ATP extracted from the liver was isolated by high-voltage electrophoresis [1]. The incorporation of P^{32}, injected intraperitoneally into the rats as a solution of $Na_2HP^{32}O_4$ 1 h before decapitation in a dose of 1 μCi/g live weight, was investigated by means of the B-2 apparatus.

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that administration of 4MU and, in particular, of 4M5HU by intraperitoneal injection in doses of 100 mg/kg daily for four days led to partial inhibition of the oxidative and phosphorylating functions of the liver mitochondria; this was evidently one cause of the decrease in the ATP level in the liver tissue compared with the control. As Table 1 shows, after injection of 4MU and 4M5HU in doses of 100 mg/kg the MDH activity fell by 18.4 and 40.5% and the FDH activity by 17.6 and 26.9% respectively com-

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TABLE 1. Change in SDH and MDH Activity in Oxidative Phosphorylation in the Mitochondria, ATP Content, and Incorporation of P^{32} into ATP in the Liver of Rats Receiving 4MU and 4M5HU (M ± m)

Compounds injected and their doses	SDH activity (in mmoles/g mitochondrial protein/min)	MDH activity (in mmoles NAD·H_2/g mitochondrial protein/min)	P/O		Specific radioactivity of ATP (in counts/min/μmole ATP/g tissue)	ATP content (in μmoles/g tissue)
			oxidation substrate succinate	oxidation substrate glutamate		
Control	20,20±0,78	19,18±0,80	1,72±0,055	2,63±0,050	79060,6±494,2	3,022±0,057
4-MU						
30 mg/kg	21,23±0,77	20,00±0,75	1,78±0,043	2,79±0,041	86288,5±474,4	3,365±0,062
P	>0,2	>0,5	>0,2	<0,05	<0,001	<0,001
100 mg/kg	16,64±0,78	15,66±0,71	1,35±0,045	2,29±0,076	67989,6±377,3	2,703±0,057
P	<0,01	<0,01	<0,001	<0,01	<0,001	<0,001
4-M-5-HU						
30 mg/kg	18,57±0,78	16,38±0,80	1,43±0,062	2,42±0,043	76647,6±504,8	3,184±0,057
P	>0,1	<0,05	<0,01	<0,01	<0,001	>0,05
100 mg/kg	14,77±0,74	11,42±0,65	1,28±0,053	2,22±0,043	63216,9±397,5	2,573±0,044
P	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001

Note. Each experiment was carried out on 8 animals.

pared with the control. The P/O ratio in these experiments also was lowered. One result of this decrease was a lower utilization of P_{in} for ATP resynthesis by comparison with the decrease in O_2 assimilation by the mitochondria in the presence of the oxidation substrates, both glutamate and succinate. Investigation of the specific radioactivity of ATP in the liver of rats receiving the test preparations in doses of 100 mg/kg also showed that the intensity of incorporation of P^{32} into ATP under these circumstances was lower than in the control.

After injection of 4M5HU into the rats in a dose of 30 mg/kg no significant differences in the ATP content in the liver were found in the experimental and control series. However, the action of this compound in this dose on the activity of enzymes of the Krebs' cycle and on phosphorylation processes in the mitochondria was identical in direction with the action of the same compound in a dose of 100 mg/kg and it differed only in the smaller magnitude of the changes produced.

The ATP content in the liver during the action of 30 μg/kg 4MU was 14.1% higher than in the control. Investigation of the specific radioactivity of ATP showed that the incorporation of P^{32} into the liver ATP of the experimental animals took place more rapidly than in the controls. The P/O ratio in samples with potassium glutamate showed a small increase under these circumstances ($P < 0.05$).

The decrease in SDH and MDH activity in the liver mitochondria observed in most of these experiments, especially after injection of the compounds into the rats in doses of 100 mg/kg, does not agree with the hypothesis that uracil analogues can stimulate oxidative processes in the Krebs' cycle [7]. The energy supply of the liver during exposure to these analogues is evidently provided mainly by the glycolytic type of ATP resynthesis [8], since according to the results of the present experiments oxidative phosphorylation was partly inhibited, especially after injection of 4MU and 4M5HU into the rats in doses of 100 mg/kg.

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