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A LIPOTROPE-DEPENDENT INCREASE OF HISTIDASE AND UROCANASE IN THE LIVERS OF CHOLINE-DEFICIENT RATS AND IN THE REUBER H-35 TRANSPLANTED HEPATOMA

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SUMMARY

The administration of choline to choline-deficient rats led to significant increases in the hepatic levels of histidase (L-histidine ammonia-lyase, EC 4.3.1.3) and urocanase (4-imidazolone-5-propionate hydro-lyase, EC 4.2.1.49). In contrast high dietary levels of methionine and choline had no significant effect on the levels of histidase and urocanase in the livers of chow-fed rats. Similarly, high dietary levels of methionine, choline and vitamin B_{12}, either alone or in combination, did not alter the levels of histidase and urocanase in the livers of rats bearing the Reuber H-35 transplanted hepatoma. However, a chow diet containing elevated levels of methionine, choline and vitamin B_{12} led to marked increases in the activities of both histidase and urocanase in the H-35 hepatoma. These results indicate that the altered control of histidase and urocanase seen in hepatomas may result from the abnormal metabolism of methyl donors generally seen in tumors.

INTRODUCTION

The lipotropes, methionine, choline, and vitamin B_{12}, have been shown both to play a determining role in hepatocarcinogenesis[1–4] and to be abnormally metabolized in tumors[2,5,6]. Histidase or histidine ammonia-lyase (EC 4.3.1.3) and urocanase or urocanate hydro-lyase (EC 4.2.1.49) are dependent upon lipotropes for their normal activity in liver[7]. Tumor-bearing rats are lipotropedeficient[8] and the histidase and urocanase levels of transplantable hepatomas are generally lower than those of host livers[6]. This research was undertaken to determine whether the abnormal tumor levels of histidase and urocanase could be linked with the abnormal lipotrope content or metabolism often seen in tumors.

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MATERIALS AND METHODS

Three experiments were done investigating the effects of dietary lipotropes on hepatic histidase and urocanase. In the first, male weanling Sprague–Dawley rats were placed on a choline-deficient diet[7] for 10 days; they were then given the same diet containing 2000 ppm choline chloride for 4 days. Groups of 6 rats were sacrified by decapitation immediately prior to and 4 days following the choline supplementation. Liver supernatants were obtained and stored as described below. In a second experiment the effects of high dietary levels of methionine and choline were investigated in the livers of rats fed a lipotrope-adequate diet. Groups of 5–6 young adult male Sprague–Dawley rats were fed Wayne Lab Blox Meal supplemented with 15 000 ppm of DL-methionine or 10 000 ppm of choline chloride; control rats were fed the same diet without supplement. Ten days later the animals were sacrificed; liver supernatants were obtained and stored as below. In a third experiment male Buffalo rats bearing the H-35 hepatoma were maintained on high lipotrope experimental diets for 5–6 weeks until the tumors reached approximately 2.0 cm in diameter. The tumor-bearing rats were housed 6 per cage in wire-screened cages, food (Wayne Lab Blox Meal) and water were available *ad libitum*. The following compounds and concentrations were added to the appropriate diets: choline chloride 10 000 ppm; methionine, 15 000 ppm; and vitamin B_{12}, 0.5 ppm. At the end of the experimental period the rats were decapitated. After exsanguination the livers and tumors were immediately excised and placed on ice. The viable neoplastic tissue of each tumor and the liver of each rat was weighed and individually homogenized with a Polytron homogenizer (Kinematica, Lucerne, Switzerland) with 4 vol. of 0.1 M K_2HPO_4 buffer at pH 8.1 containing $1 \cdot 10^{-3}$ M EDTA, $5 \cdot 10^{-5}$ M pyridoxal phosphate, and 15 mg/l dithiothreitol. The homogenates were centrifuged at 100 000 $\times$ *g* for 1 h in a refrigerated centrifuge, the supernatant then collected and frozen at -20 °C for subsequent enzyme analyses. Histidase and urocanase were measured in a Beckman Acta III Spectrophotometer by the respective rates of appearance and disappearance of urocanic acid at 277 nm[7,9].

RESULTS AND DISCUSSION

The results of these experiments are summarized in Tables I–III. Table I demonstrates that in accord with previous observations[7], in choline-deficient animals

TABLE I

A CHOLINE-INDUCED INCREASE IN THE HEPATIC LEVELS OF HISTIDASE AND UROCANASE IN CHOLINE-DEFICIENT RATS

Group No.	*Time of Choline Supplementation (days)*	*Enzyme activity* (μmoles/h per g liver)*	
		Histidase	*Urocanase*
1	0	4.5 ± 0.4	6.9 ± 0.4
2	4	9.3 ± 0.6	9.3 ± 1.0

* Mean of 5 animals ± S.E.

TABLE II

THE ABSENCE OF ANY EFFECT BY DIETARY METHIONINE AND CHOLINE ON THE HEPATIC LEVELS OF HISTIDASE AND UROCANASE IN RATS FED A LIPOTROPE-ADEQUATE DIET FOR 10 DAYS

Group	*Enzyme activtiy* (μmoles/h per g liver)*	
	Histidase	*Urocanase*
Control	12.0 ± 1.1	17.3 ± 0.4
+ 1.5% methionine	9.2 ± 2.4	16.0 ± 2.2
+ 1.0% choline chloride	14.0 ± 2.2	19.6 + 1.6

* Mean of 5–6 animals ± S.E.

the hepatic levels of histidase and urocanase respond to dietary supplementation with choline. Table II illustrates that in rats fed a diet containing adequate levels of lipotropes, no increase in the hepatic levels of histidase and urocanase can be observed following dietary supplementation with very high levels of methionine and choline. The results with the hepatoma-bearing rats were quite different and are summarized in Table III. When rats bearing the H-35 hepatoma were fed diets containing high levels of choline + methionine + vitamin B_{12} in combination, the tumor levels of histidase and urocanase were, respectively, found to be 70 and 104% above the corresponding enzyme levels noted in the animals fed the control diet; no significant change was observed in the histidase and urocanase levels of the host livers of the same animals fed the diet supplemented with all 3 lipotropes. When each of the 3 essential one-carbon compounds was added to the diet individually, no effect could be noted in the histidase levels of either the host or the neoplastic liver. Similarly, high dietary levels of methionine, choline and vitamin B_{12}, when administered singly, had no effect on the urocanase levels of host livers. On the other hand, dietary supplementation with vitamin B_{12} did lead to slightly increased levels of urocanase

TABLE III

THE ENZYMATIC ACTIVITY OF HISTIDASE AND UROCANASE IN THE H-35 HEPATOMA AND IN THE HOST LIVERS OF RATS FED DIETS SUPPLEMENTED WITH HIGH LEVELS OF CHOLINE, METHIONINE, AND VITAMIN B_{12}, EITHER SEPARATELY OR IN COMBINATIONS

Following tumor transplantaton, the animals were placed on diets containing metihonine, choline and vitamin B_{12} at levels of 15 000, 10 000 and 0.5 ppm, respectively, for 5–6 weeks. The animal sacrifices and enzyfme assays are described in the text.

Group	*Tumor transplant generation No.*	*Enzyme activity (μmoles/h per g liver)**			
		Histidase		*Urocanase*	
		Liver activity	*Tumor activity*	*Liver activity*	*Tumor activity*
Control	81	23.5 ± 3.6	39.2 ± 2.9	33.3 ± 1.6	5.7 ± 0.4
Methionine	81	21.2 ± 1.5	43.7 ± 1.7	33.5 ± 2.2	4.1 ± 0.8
Choline	81	20.0 ± 0.9	42.7 ± 3.3	35.2 ± 2.3	7.7 ± 1.3
Vitamin B_{12}	81	19.2 ± 1.7	42.4 ± 2.4	30.8 ± 1.5	7.5 ± 0.2
Choline + methionine + vitamin B_{12}	83	21.0 ± 2.1	66.7 ± 3.3	27.8 ± 1.4	11.6 ± 0.4

* Each value represents the mean of 5–6 animals ± S.E.

in the H-35 hepatoma (Table III); the apparent rise noted in the hepatoma levels of urocanase were not found to be statistically significant.

These results extend previous observations[7–9] that the hepatic levels of histidase and urocanase fall under the control of lipotropes. The results also indicate that the abnormal levels of histidase and urocanase generally found in hepatomas[6] may in part be the consequence of the abnormal lipotrope metabolism associated with such tumors.

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