Loss of Feedback Control of Hydroxymethylglutaryl Coenzyme A Reductase in Hepatomas

MARVIN D. SIPERSTEIN, ANN M. GYDE, AND HAROLD P. MORRIS

Department of Internal Medicine, The University of Texas (Southwestern) Medical School at Dallas, Dallas, Texas 75235, and Howard University College of Medicine, Washington, D. C.

Communicated by Bernard B. Brodie, December 4, 1970

ABSTRACT The feedback response of mevalonate: NADP oxidoreductase (acylating CoA; EC 1.1.1.34) in two varieties of hepatoma has been examined. In marked contrast to the feedback inhibition of this enzyme that is regularly observed in normal liver, the feedback control is completely lost in minimal-deviation hepatomas 9121 and 3924A. This finding provides the first specific biochemical localization of the defect in cholesterol feedback control, which has been shown previously to be a characteristic of all hepatomas.

Since the original observations of Gould (1), abundant evidence has been accumulated to demonstrate that cholesterol synthesis in the livers of all higher animals is markedly suppressed by exogenous cholesterol (2). Indirect evidence that this cholesterol feedback reaction involves reactions prior to the synthesis of mevalonate was first provided by the studies of Gould and Popjak (3), of Bucher *et al.*, (4), and of Siperstein and Guest (5). It was subsequently demonstrated in this laboratory by directly measuring the synthesis of mevalonic acid and of 3-hydroxy-3-methyl glutarate that the feedback control of cholesterol synthesis is specifically localized to the enzymatic step responsible for the conversion of 3-hydroxy-3-methylglutarate to mevalonate (6). The finding of Linn (7, 8), recently confirmed in several laboratories (9-11), that the conversion of labeled 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate is inhibited by cholesterol feeding, supports the conclusion that the primary site of cholesterol feedback control is at the reaction responsible for the synthesis of mevalonate.

We have previously reported that in striking contrast to the uniform presence of the cholesterol feedback reaction in normal liver cells of all species, this feedback control is consistently lost when the hepatocyte becomes malignant (12-14). In fact, deletion of the cholesterol feedback system has to date been demonstrated in all hepatomas regardless of animal species, degree of tumor differentiation, or method of induction of the hepatoma (13, 15). This finding, coupled with the fact that loss of the feedback control of cholesterol synthesis represents the only example of a consistent deletion of a feedback system in a tumor tissue, has led us to suggest (14, 16) that a unique disturbance in the enzyme site responsible for the synthesis of mevalonate might represent a characteristic of cancer. The purpose of the present communication is to provide direct and specific evidence that the cholesterol feedback lesion of cancer is in fact localized to the enzyme mevalonate:

NADP oxidoreductase (acylating CoA) (hydroxymethylglutaryl CoA reductase EC 1.1.1.34).

MATERIALS AND METHODS

The rats used throughout these studies were of the inbred ACI/f Mai strain (Microbiological Associates, Bethesda, Md.) The two minimal-deviation hepatomas studied, the Morris hepatomas 9121 and 3924A, were grown intramuscularly in the same strain of rat. The 5% cholesterol diet in B & D Master-Rat Cubes (B and D Mills, Grapevine, Texas) was prepared as described (5) and fed ad libitum for 6 days to those rats on the high-cholesterol diet. The rats fed the low-cholesterol diet received 720 μ g of cholesterol per gram of chow.

Microsomes from both livers and tumors were prepared exactly as described by White and Rudney (17). [3- 14 C]HMG-CoA was synthesized from [14 C]HMG anhydride as described by Knappe *et al.* (18) and further purified by chromatography on Munktells' S-302 paper with 0.1 M sodium acetate (pH 4.5)-ethanol 1:1 as the developing solvent.

HMG-CoA reductase was determined by measuring the incorporation of tracer amounts of [14 C]HMG-CoA into mevalonate employing the incubation conditions of White and Rudney (17). The method of isolating mevalonate by gas-liquid chromatography involved a slight modification of the procedure previously described from this laboratory (19). We noted that contaminating radioactivity and extraneous chromatographic peaks could largely be removed without formation of the dibenzylethylenediamine salt of mevalonate by means of the following simplified procedure.

Incubation was terminated by acidifying to pH 1 with 6 N HCl. When ready for analysis, the samples were neutralized to pH 7 with KOH and extracted three times with 3 vol of ethyl ether. They were then reacidified to pH 1 and the resulting mevalonolactone was recovered with three further extractions of ethyl ether. The combined ether extracts were dried under nitrogen, the [14 C]mevalonolactone was taken up in 40 μ l of chloroform, and an aliquot was injected onto a 6 ft (183 cm), 15% diethylene glycol succinate, gas-liquid chromatographic column, at a temperature of 185°C and flow rate of 200 ml/min. The retention time of mevalonolactone under these conditions is 10 min. The [14 C]mevalonolactone was collected on toluene-soaked cotton in glass tubes with a Packard automatic sampling device, and counted in a liquid scintillation counter with *p*-bis-[2-(5-phenyloxazolyl)]-benzene ("POPOP")-2,5-diphenyloxazole ("PPO")-toluene, as the scintillation solution.

Abbreviation: HMG, hydroxymethylglutaryl.

TABLE 1. *Feedback control of HMG-CoA reductase in normal liver and hepatoma 9121*

Experiment	Tissue	Treatment	Cholesterol synthesis*	HMG-CoA reductase†
A	Liver	—	90	0.77 ± 0.26
	Liver	5% Cholesterol	1.6	0.007 ± 0.01
	Hepatoma 9121	—	91	1.34 ± 0.32
	Hepatoma 9121	5% Cholesterol	192	1.69 ± 0.38
	Liver	—	—	2.21 ± 0.29
B	Liver	5% Cholesterol	—	0.18 ± 0.05
	Hepatoma 9121	—	—	2.00 ± 0.25
	Hepatoma 9121	5% Cholesterol	—	1.59 ± 0.26

* [2-¹⁴C]Acetate incorporation into cholesterol by 200-mg liver slices incubated in 2 ml of Krebs-Ringer phosphate buffer with 6 μ mol of acetate (0.3 Ci/mol) for 2 hr, in nmol/gm.

† Conversion of [3-¹⁴C]HMG-CoA to [¹⁴C]mevalonate by microsomes isolated from 800 mg of liver incubated for 1 hr with 10 mM dithiothreitol, 1.4 mM EDTA, 3.6 mM glucose-6-phosphate, 2.5 units of glucose-6-phosphate dehydrogenase, 1.4 mM NADP, 33 nmol (3 Ci/mol) [3-¹⁴C]HMG-CoA, 1.0 mM potassium mevalonate, and 43 mM potassium phosphate in a total volume of 7 ml. Figures represent mean ± standard deviation of quintuplicate incubations (Exp. A) or triplicate incubations (Exp. B) in nmol/sample per hr.

RESULTS

The data in Table 1 clearly demonstrate that the feeding of cholesterol for 3 days leads to a consistent 90% reduction in the activity of the HMG-CoA reductase of normal liver. This result fully confirms the previous findings of Linn (8).^{*} The results of two comparable experiments, in which the well-differentiated minimal-deviation hepatoma 9121 was examined, are in striking contrast to those obtained in normal liver. While the activity of HMG-CoA reductase in the tumor microsomes is at least equal to that of the normal liver, the tumor enzyme consistently failed to show a significant decrease in activity after cholesterol feeding. It is apparent therefore that the normal feedback control of HMG-CoA reductase has been lost in this minimal deviation hepatoma.

In Table 2 are presented the data of a similar experiment in which feedback inhibition of a second minimal-deviation hepatoma, 3924A, was examined. Normal liver was again shown to respond to cholesterol feeding with a drastic—in this experiment 99%—inhibition of HMG-CoA reductase activity. By contrast, the HMG-CoA reductase in 3924A Morris hepatoma proved to be completely insensitive to cholesterol feeding.

The data of these two studies therefore clearly demonstrate that in contrast to normal liver both varieties of minimal-deviation hepatomas examined in this study have lost the

^{*} While previous attempts in this laboratory to demonstrate the direct conversion of [¹⁴C]HMG-CoA to mevalonate have been unsuccessful (6) with the incubation media and conditions described by White and Rudney (17), this reaction could be readily demonstrated in rat liver microsomes.

normal cholesterol-mediated feedback control of HMG-CoA reductase.

DISCUSSION

This laboratory has previously reported that the cholesterol feedback system that is a characteristic of all normal livers is consistently lost when the liver cell undergoes malignant change. To date this deletion of the cholesterol feedback system has been demonstrated in every variety of hepatoma studied including a spontaneous mouse hepatoma (13), a series of 14 minimal-deviation rat hepatomas (14, 15), two spontaneous hepatomas of man (13), and the aflatoxin-induced hepatomas of fish (15). Moreover, it has recently been shown that this lesion is not confined solely to liver tumors in that loss of the cholesterol feedback system is also observed in leukemic cells (20).

Finally, the finding that this feedback system is absent in the livers of rats and fish treated for only short periods of time with the carcinogen, aflatoxin (20, 21), suggests that deletion of the cholesterol feedback system may actually occur at an early stage of carcinogenesis many months before the liver cell becomes malignant.

This consistent relationship between the development of malignancy and the loss of the cholesterol feedback system, coupled with the fact that this lesion remains the only consistent example of the deletion of a normal feedback system in cancer tissue, has emphasized the importance of elucidating the biochemical alterations responsible for the loss of the cholesterol feedback control mechanism in the malignant cell. As a first step to this end, it was apparent that the enzymatic site of this feedback defect in malignant tissue should be specifically identified. While previous studies from this laboratory have demonstrated that in normal liver the cholesterol feedback system operates specifically at the site of conversion of 3-hydroxy-3-methylglutarate to mevalonate (6), for technical reasons it has to date only been possible to localize the cholesterol feedback lesion of cancer tissue to the reactions between acetate and mevalonate (13).

The finding in this study that, in contrast to the situation in normal liver, hepatomas continue to convert HMG-CoA to mevalonate at normal rates despite the feeding of cholesterol therefore provides the first direct evidence that HMG-CoA reductase represents the specific enzymatic site of the characteristic feedback lesion of cancer tissues.

It should be emphasized that the present studies, while localizing the biochemical site of this feedback defect of malignancy, provide no direct information as to whether this lesion represents an alteration in HMG-CoA reductase itself, a derangement in either transcriptional or translational control

TABLE 2. *Feedback control of HMG-CoA reductase in normal liver and hepatoma 3924A*

Tissue	Treatment	HMG-CoA reductase*
Liver	—	1.14 ± 0.10
Liver	5% Cholesterol	0.11 ± 0.01
Hepatoma 3924A	—	4.7 ± 0.34
Hepatoma 3924A	5% Cholesterol	4.5 ± 0.25

* Conditions and units as in Table 1. The figures represent the means ± standard deviations for triplicate incubations, in nmol/sample per hr.

of the synthesis of this enzyme, or an alteration of an inhibitor of this reaction. Obviously, defects at any of these sites could lead to the deletion of the feedback response of HMG-CoA reductase to exogenous cholesterol observed in these studies; the exact mechanism by which the malignant cell loses the normal feedback control of 3-hydroxy-3-methylglutaryl coenzyme A reductase remains to be established.

We acknowledge the invaluable assistance of Mrs. Lynne J. Luby, Mrs. Alice E. Clark, and Mrs. Judy K. Johns.

This investigation was supported by U.S. Public Health Service Grant 5 RO1 CA 08501 and Damon Runyon Memorial Fund for Cancer Research, Inc. no. 747. Dr. Siperstein is the recipient of a Research Career Award 5 KO6 HE 01958, U.S. Public Health Service.

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