STIMULATION OF HYDROXYMETHYLGLUTARYL-COENZYME A REDUCTASE IN MOUSE LIVER BY X-RAYS

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1. Introduction

From previous investigations it is known that cholesterol synthesis from acetate is elevated up to twenty-fold in the liver of X-irradiated mammals [1, 2], whereas cholesterol synthesis from mevalonic acid is only little affected by the X-rays [1, 3, 4]. Bucher et al. [5], Siperstein and Fagan [6, 7] and Hamprecht [8] have pointed out that reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonic acid (MVA) by HMG-CoA reductase (EC 1.1.1.34) is the regulating site in the biosynthesis of cholesterol. The radiation-induced stimulation of cholesterol synthesis from acetate was supposed to be due to a stimulation of HMG-CoA reductase [1, 3, 4]. This was, however, an assumption and has not been proved so far.

Using a simplified assay procedure for the microsomal HMG-CoA reductase, we were now able to demonstrate that the increase of cholesterol synthesis from acetate in the liver of X-irradiated mice depends in fact on an increased activity of HMG-CoA reductase. In our experiments, the activity of this enzyme was found to be 6–7 times higher in irradiated mice than in controls. As with acetate incorporation into cholesterol [1], the extent of stimulation of HMG-CoA reductase depends upon the radiation dose. It could further be demonstrated that stimulation of the enzyme after irradiation is inhibited by puromycin.

2. Experimental

Male white mice, 25–30 g, about 8 weeks old, bred in the institute, were fed with "ssniff" (Fa. Intermast, Bockum-Hövel) ad libitum. Whole-body X-irradiation (148 R/min) was performed as previously described [1]. Microsomes from mouse liver were prepared according to the method of Regen et al. [9], 3-^{14}C-HMG-CoA was prepared by the method of Hilz et al. [10]. Incorporation experiments with acetate are the same as previously described [1] and the corresponding data in tables 1–3 are taken from our previous investigation [1]. The details of the simplified HMG-CoA reductase assay procedure are given elsewhere [11, 12]. The HMG-CoA concentration in the experiments described here was 40–60 μM. All mice fasted for 24 hr before killing; the animals were killed between 8.00 and 8.30 a.m.

3. Results and discussion

After irradiation the food intake of the animals is very irregular. Since the activity of HMG-CoA reductase is highly sensitive to the nutritional state of the animal [8], in our experiments mice were without food for 24 hr before sacrifice. This has the advantage that all mice are in the same nutritional state at the time of acetate incorporation and enzyme measurements, respectively.

In table 1 the HMG-CoA reductase activity is compared with the incorporation rates of acetate into cholesterol at various times after whole-body irradiation

Table 1
Cholesterol synthesis from acetate in liver slices compared with HMG-CoA reductase activity in liver microsomes at various times after X-irradiation with 2000 R. Mice fasted for 24 hr before killing. Measurements were made at 37°.

Time after 2000 R (hr)	Ac → Chol (nmoles/g wet wt./hr incorporated)	n	HMG-CoA → MVA (nmoles/g wet wt./hr MVA formed)	HMG-CoA → MVA (nmoles/mg microsomal prot./hr MVA formed)	n
0	5.5 ± 0.5	20	1.34 ± 0.11	0.041 ± 0.003	16
18	8.2 ± 1.8	5	1.17 ± 0.18	0.045 ± 0.007	8
24	8.5 ± 1.2	10	1.48 ± 0.08	0.057 ± 0.003	8
48	18.0 ± 7.3	10	1.16 ± 0.12	0.040 ± 0.004	8
72	110 ± 16	10	7.98 ± 1.12	0.285 ± 0.040	8

with 2000 R. The activity of the enzyme is given as nmoles per mg microsomal protein (specific activity) and from these other activities (nmoles per g wet wt.) were calculated. Seventy two hr after the irradiation, the activity of the enzyme was found to be six times that of the control. For cholesterol synthesis from acetate in liver slices the stimulation also was highest 72 hr after irradiation.

Table 2 summarizes the experiment with various radiation doses. Mice were irradiated with 690–3000 R and 72 hr after exposure HMG-CoA reductase activity was determined in the microsomal fraction of the liver. The activity of the enzyme was compared with cholesterol synthesis from acetate in liver slices. Both acetate incorporation and activity of the enzyme increase with increasing radiation doses in a very similar manner. No significant differences were obtained between the effects of 2000 R and 3000 R.

Since HMG-CoA reductase is the rate limiting enzyme of cholesterol synthesis from acetate [5–8], acetate incorporation and HMG-CoA reductase activity should be of the same order of magnitude. The enzyme activities in table 1 are, however, much lower than the cholesterol synthesis from acetate and the control values in table 1 and 2 differ by a factor of 4–5. Very high differences in the activity of HMG-CoA reductase in untreated rats were obtained also by Hamprecht [13], Linn [14] and White and Rudney

Table 2
Cholesterol synthesis from acetate in liver slices compared with HMG-CoA reductase activity in liver microsomes 72 hr after various radiation doses. Mice fasted for 24 hr before killing. Measurements were made at 37°.

72 hr after radiation	Ac → Chol (nmoles/g wet wt./hr incorporated)	n	HMG-CoA → MVA (nmoles/g wet. wt./hr MVA formed)	HMG-CoA → MVA (nmoles/mg microsomal prot./hr MVA formed)	n
0	5.7 ± 0.5	20	5.74 ± 0.62	0.221 ± 0.024	8
690 R	4.9 ± 0.8	10	5.23 ± 1.19	0.194 ± 0.041	8
1000 R	15.0 ± 3.9	5	14.19 ± 2.74	0.546 ± 0.105	8
1500 R	–	–	21.21 ± 3.62	0.816 ± 0.140	8
2000 R	110 ± 16	10	31.62 ± 5.18	1.072 ± 0.175	8
3000 R	90 ± 13	10	29.51 ± 6.90	1.093 ± 0.255	8

Table 3
Influence of puromycin on the radiation induced stimulation of cholesterol synthesis from acetate and on HMG-CoA reductase activity 72 hr after 2000 R. Mice fasted for 24 hr before killing. Measurements were made at 37°.

Treatment	Ac → Chol (moles/g wet wt./hr incorporated)	*n*	% inhibition	HMG-CoA → MVA (nmoles/g wet wt./hr MVA formed)	HMG-CoA → MVA (nmoles/mg microsomal prot. /hr MVA formed)	*n*	% inhibition
24 hr fasted (control)	5.7 ± 0.5	20	–	5.74 ± 0.62	0.221 ± 0.024	8	–
Puromycin alone[a]	7.6 ± 0.5	10	–	3.21 ± 0.26	0.146 ± 0.012	8	–
72 hr after 2000 R	110 ± 16	10	0	31.62 ± 5.18	1.072 ± 0.175	8	0
72 hr after 2000 R + 3 X 2 mg puromycin[b]	19.1 ± 5.6	5	83	6.55 ± 0.81	0.281 ± 0.035	8	74

[a] Puromycin, 2 mg each, was given 72 hr, 48 hr, and 24 hr before killing
[b] Puromycin, 2 mg each, was given 30 min, 24 hr and 48 hr after 2000 R.

[15] and factors up to 40 in two different experiments were observed [15]. The preparation of the microsomal fraction, the time of the day [16, 17] as well as light and dark periods and the age of the animals [13] obviously influence the activity of HMG-CoA reductase. Further, it must be considered, in this regard, that preparation of liver contain, besides HMG-CoA reductase, at least one additional HMG-CoA consuming enzyme: HMG-CoA acetoacetate lyase (EC 4.1.3.4) [18]. In incorporation experiments HMG-CoA is formed from high acetate levels in situ [1] and substrate deficiency for reductase reaction is, therefore, very improbable. On the other hand, HMG-CoA reductase was determined in microsomal preparations with (precious) labeled HMG-CoA as substrate. As Hamprecht showed recently [18], microsomal preparations contain considerable amounts of HMG-CoA lyase and measurements of HMG-CoA reductase activity in microsomes have probably not been carried out under optimal conditions with respect to substrate concentrations, particularly not after high radiation doses (see table 2). For these reasons one can expect that the activity of this enzyme shows high variabilities and may be below the incorporation rates of acetate.

In table 3 inhibition experiments with puromycin are shown. Mice were irradiated with 2000 R and the antibiotic was given three times (30 min, 24 hr and 48 hr) after the irradiation. The radiation-induced elevation of acetate incorporation is inhibited by about 83 percent under these conditions, the stimulation of the enzyme is inhibited by about 74 percent (by about 80 percent on the nmoles/g wet wt basis). As mentioned before in these experiments, mice were fasted for 24 hr before killing.

Our results lead to the conclusion that radiation-induced increase of cholesterol synthesis in mammals is caused by a stimulation of HMG-CoA reductase, the rate limiting enzyme of the whole reaction chain. Nothing is known about this mechanism; probably a de novo synthesis of this enzyme occurs after irradiation. This idea is supported by the inhibition experiments with puromycin (table 3). Another antibiotic, however, actinomycin D, is unsuitable for inhibition experiments in this case, because it stimulates cholesterol synthesis from acetate itself [1, 19, 20].

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