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A POSSIBLE TRANSLATIONAL CONTROL OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE INDUCTION BY ML-236B(COMPACTIN) IN ISOLATED RAT HEPATOCYTES

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ML-236B ("Compactin"), a competitive inhibitor of 3-hydroxy-3-methylglutaryl(HMG)-CoA reductase, increased the cholesterol synthesis and the HMG-CoA reductase activity in isolated rat hepatocytes. These increases were prevented by 0.2 mM puromycin, but not by 10 μg/ml actinomycin D and 40 μg/ml α-amanitin. These results indicated that the increases in cholesterol synthesis and HMG-CoA reductase activity by ML-236B required the enzyme synthesis but not newly synthesized mRNA. The regulatory site of feed-back inhibition by cholesterol for the HMG-CoA reductase synthesis in liver may be at the translational level.

Liver is a major organ for cholesterol synthesis. The conversion of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) to a mevalonic acid catalyzed by HMG-CoA reductase [EC 1.1.1.34] is a rate-limiting step for cholesterol biosynthesis. The reductase has been shown to be changed by diet as well as various hormones and to have a remarkably rapid turnover. Edwards and Gould estimated the half-life of degradation of the enzyme to be 4 hours (1). In rat liver, the HMG-CoA reductase activity shows a circadian rhythm. The nocturnal increase in the microsomal reductase activity was completely prevented by cycloheximide (2) and puromycin (3) but not however by actinomycin D (4,5). Kirsten and Watson (6) and Krone *et al*. (7) suggested a post-transcriptional control of lipoprotein-induced HMG-CoA reductase in hepatoma tissue culture cells and human lymphocytes.

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Incubation of hepatocytes (8) and human fibroblasts (9) in the presence of ML-236B (Compactin), a specific competitive inhibitor of HMG-CoA reductase, results in a significant increase in the HMG-CoA reductase activity because of a blockage of endogenous cholesterol synthesis and an elimination of feed-back inhibition by cholesterol in the cells. The majority of the increase in the enzyme activity by ML-236B was proved to be due to an increase in the number of enzyme molecules in rat hepatocytes by immunotitration (8). We report here the effects of protein synthesis inhibitors on the ML-236B-induced increase in cholesterol synthesis and HMG-CoA reductase activity. Available evidence indicates that the HMG-CoA reductase induction may be regulated at the translational level.

MATERIALS and METHODS

Preparation of isolated rat hepatocytes: Male Wistar rats weighing 300∿400 g which had been fed ad libitum, were used. Rats were killed at noon and hepatocytes were prepared by the method described previously (10). Hepatocytes were 80∿95 % viable as tested by trypan blue dye exclusion.

Measurements of cholesterol and fatty acid synthesis: Hepatocytes were suspended in Eagle's minimal essential medium (pH 7.4) containing 1.5 % bovine fatty acid-free albumin (Sigma Chemical Co.), 5 % lipoprotein-poor serum (less then 3 mg cholesterol/ml) prepared by ultracentrifugation, and 1 μCi/ml [2-^{14}C]acetate sodium salt (55 mCi/mmol, The Radiochemical Centre, Amersham). Incubations were done in stoppered plastic vials at 90 osc/min in a metabolic shaker at 37°C in an atmosphere of 5 % CO_2 - 95 % O_2. Radioactive cholesterol and fatty acids were determined as described by Endo et al. (11).

Assay of HMG-CoA reductase activity: Microsomes of isolated rat hepatocytes were prepared as described by Nicolau et al. (12). The sedimented microsomes were washed three times by incubating at 37°C for 10 min in 100 mM potassium phosphate (pH 7.4) containing 10 mM EDTA, 10 mM mercaptoethanol and 5 % brij 96, followed by centrifugation at 100,000 g for 60 min (13). Assay of HMG-CoA reductase activity was carried out by the method of Brown et al.(14). [^{14}C]-Mevalonolactone formed was isolated by silica thin-layer chromatography in acetone-benzene (1:1 vol/vol) and then counted (15).

RESULTS

As shown in Fig. 1, ML-236B caused a significant inhibition of [2-^{14}C]acetate incorporation into cholesterol, but caused no inhibition of [2-^{14}C]acetate incorporation into fatty acids in isolated rat hepatocytes.

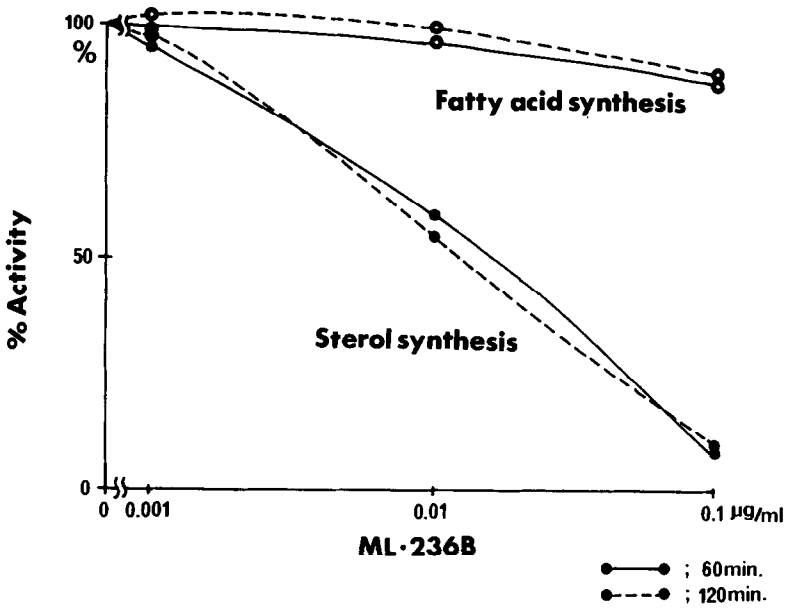

Fig. 1 Effects of ML-236B on sterol and fatty acid synthesis in isolated rat hepatocytes.

Isolated hepatocytes were incubated in the presence of ML-236B at 37°C for 60 min or 120 min as described in MATERIALS AND METHODS. Each point represents the average of duplicate determinations.

Table I shows the increases in cholesterol synthesis/cell dry weight and cholesterol synthesis/fatty acid synthesis in isolated hepatocytes incubated in the medium containing various

Table I. The increases in the cholesterol synthesis/cell dry weight and the cholesterol synthesis/fatty acid synthesis by ML-236B in isolated rat hepatocytes.

concentration of ML-236B	cholesterol synthesis /cell dry weight	cholesterol synthesis /fatty acid synthesis
μg/ml	% of control	% of control
0.05	115.0 ± 7.2	113.3 ± 3.8
0.1	113.0 ± 0.0	127.7 ± 5.4
0.5	182.7 ± 14.7	171.3 ± 14.3

Isolated hepatocytes were incubated in the presence of various concentrations of ML-236B as described in MATERIALS AND METHODS. After incubation for 90 min, the medium was removed and cells were washed three times with the medium without ML-236B. Incubations were continued with [2-^{14}C]acetate for 30 min and [^{14}C]incorporations into cholesterol and fatty acids were determined. Results obtained in cells incubated with ML-236B for 90 min were compared with the controls which were incubated with the same concentration of ML-236B for 30 min. Each value is mean ± SEM of triplicate determinations.

Table II. Effects of protein inhibitors on the increases in cholesterol synthesis/cell dry weight and cholesterol synthesis/fatty acid synthesis.

inhibitors	cholesterol synthesis /cell dry weight	cholesterol synthesis /fatty acid synthesis
	%	%
puromycin (0.2 mM)	34.0 ± 6.8	31.9 ± 8.0
actinomycin D (10 μg/ml)	279.8 ± 4.6	166.4 ± 13.3

Isolated hepatocytes were preincubated for 30 min in the medium with or without the indicated inhibitors and 0.5 μg/ml ML-236B was added to the medium. Incubations were continued for 90 min and cholesterol and fatty acid synthesis were measured in MATERIALS AND METHODS. Results are given as the percentage of the increase :

$$\frac{\text{cholesterol synthesis after the incubation for 90 min with ML-236B}}{\text{chelesterol synthesis after the incubation for 30 min with ML-236B}} - 1$$

obtained in the cells without the indicated inhibitors. Each value is mean ± SEM of triplicate determinations.

concentrations of ML-236B. Treatment with 0.5 μg/ml ML-236B caused about a 170 % increase of cholesterol synthesis. As shown in Table II, the increase in cholesterol synthesis was prevented by 0.2 mM puromycin, which is known to prevent protein synthesis at the step of translational level, but not by 10 μg/ml actinomycin D, which is known to be sufficient to prevent the formation of mRNA (16, 17).

Table III. Effects of ML-236B on HMG-CoA reductase activity in isolated rat hepatocytes.

concentration of ML-236B	HMG-CoA reductase activity
μg/ml	% of control
0.1	155.9
0.5	277.1

Isolated hepatocytes were incubated in the medium containing ML-236B for 90 min and microsomes were prepared. Microsomal HMG-CoA reductase activity was measured as described in MATERIALS AND METHODS. Results represent the percentage of the relative activity (% of initial activity) obtained in the cells treated without ML-236B for 90 min. Each value is the average of duplicate determinations.

Table IV. Effects of protein inhibitors on the increase in HMG-CoA reductase activity in isolated rat hepatocytes.

inhibitors	HMG-CoA reductase activity
	%
puromycin (0.2 mM)	8.1
actinomycin D (10 μg/ml)	126.7
α-amanitin (40 μg/ml)	292.2

Isolated hepatocytes were preincubated for 30 min in the medium with or without the indicated inhibitors and 0.5 μg/ml ML-236B was added to the medium. Incubations were continued for 90 min and microsomal HMG-CoA reductase activity was measured as described in MATERIALS AND METHODS. Results are given as the percentage of the increase :

$$\frac{\text{HMG-CoA reductase activity after the incubation with ML-236B}}{\text{HMG-CoA redictase actovotu after the incubation without ML-236B}} - 1$$

obtained in the cells without the indicated inhibitors. Each value is the average of duplicate determinations.

Table III and IV show results of measurements of HMG-CoA reductase activity in isolated hepatocytes 90 min after the administration of ML-236B. The microsomal HMG-CoA reductase activity in the cells treated with ML-236B increased compared with the control cells which were not treated with ML-236B. The increase in HMG-CoA reductase activity by ML-236B was prevented by 0.2 mM puromycin but not by 10 μg/ml actinomycin D nor 40 μg/ml α-amanitin, another specific inhibitor of mRNA synthesis (18).

DISCUSSION

Recently, the rates of protein synthesis in eukaryotic cells are shown to be closely correlated with the amount of the corresponding mRNA, and the protein synthesis is believed to be regulated at the transcriptional level. Itoh and Okamoto, however, demonstrated the translational control by glucose of proinsulin synthesis in pancreatic islets (19,20).

In the present study the increases in cholesterol synthesis and HMG-CoA reductase activity by ML-236B were prevented by puromycin but not by actinomycin D nor α-amanitin. The data obtained with puromycin suggested that the enzyme synthesis was required for the increase in HMG-CoA reductase activity by ML-236B in hepatocytes, and the data obtained with actinomycin D and α-amanitin suggested that mRNA synthesis was not required for the increase in the enzyme synthesis. Therefore, the ML-236B-induced induction of HMG-CoA reductase may be regulated at the translational level. Krone et al. (7) and Kirsten et al. (6) also suggested the post-transcriptional control in regulation of sterol synthesis by lipoproteins. On the other hand, in human fibroblasts the increase in HMG-CoA reductase activity after removal of low density lipoprotein from the incubation medium was prevented by actinomycin D (9) and the induction of HMG-CoA reductase by epinephrine was prevented by actinomycin D (5). These different results may be due to the differences of the organs or due to the agents by which the induction of HMG-CoA reductase was produced.

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