EFFECT OF ACTINOMYCIN D AND PUROMYCIN ON THE CONVERSION OF CHOLESTEROL INTO BILE ACIDS IN BILE FISTULA RATS

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1. Introduction

In a bile fistula rat the daily production of bile acids is ten to fifteen times greater than in an intact rat [1]. In a previous communication [2] the effect of biliary drainage on some reactions in the conversion of cholesterol into taurocholic acid was studied. It was found that the increase in bile acid formation in the bile fistula rat was parallelled by an increase in the 7α-hydroxylation of cholesterol. The other reactions studied, the conversion of cholest-5-ene-3β,7α-diol into 7α-hydroxycholest-4-en-3-one, the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one, and the 7α-hydroxylation of taurodeoxycholic acid, were influenced much less by biliary drainage. It was suggested that the formation of cholest-5-ene-3β,7α-diol from cholesterol might be a rate-determining step in the conversion of cholesterol into bile acids. However, no conclusions could be drawn concerning the nature of the increase in activity of the 7α-hydroxylating enzyme system. It was shown that the increase in enzyme activity was not due to any general increase in liver protein. An increase in enzyme activity might depend on (1) an activation of pre-existing enzyme, (2) an induced increase in the synthesis of the enzyme and/or (3) a decrease in the breakdown of the enzyme. According to current concepts [3] the rate of synthesis may be the preferential way of the mammalian organism to control its levels of enzyme activity. During recent years specific inhibitors of protein synthesis have been used in studies of the mechanisms involved in increased enzyme activity. Thus, actinomycin D, which inhibits the DNA-dependent synthesis of RNA [4], and puromycin, which interferes with the transfer of amino acids to ribosomes [5], have been used in studies of for instance tryptophan pyrrolase [6, 7] and δ-aminolevulinic acid synthetase [8]. To obtain some information on the mechanisms of the increased formation of cholest-5-ene-3β,7α-diol from cholesterol, induced by biliary drainage, actinomycin D and puromycin were given to bile fistula rats, and the effect on the 7α-hydroxylation of cholesterol, and for comparison, on the conversion of cholest-5-ene-3β,7α-diol into 7α-hydroxycholest-4-en-3-one and on the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one was studied.

2. Materials and methods

4-^{14}C-cholesterol (specific radioactivity, 145 μC/mg) was obtained from Radiochemical Centre, Amersham, England. Prior to use, the labeled cholesterol was purified by chromatography on a column of aluminum oxide, grade I (Woelm, Eschwege, Germany). 7β-^{3}H-Cholest-5-ene-3β,7α-diol (specific radioactivity, 10 μC/mg) and tritium-labeled 7α-hydroxycholest-4-en-3-one (specific radioactivity, 10 μC/mg) were prepared as described previously [9, 10]. NAD, NADPH and puromycin (dihydrochloride, grade II) were obtained from Sigma Chemical Co., St. Louis, Mo. Actinomycin D was a gift from Merck, Sharp & Dohme Res. Lab., Rahway, N. J.

White male rats of the Sprague-Dawley strain weighing 150-200 g were used. The bile fistula opera-

tion was performed in the usual manner and the bile fistula rats were kept in restraining cages. They were given 0.9% solution of sodium chloride to drink and had free access to a chow diet. Actinomycin D and puromycin, dissolved in 1 ml of saline, were administered by intraperitoneal injection. The control rats were given the same amount of saline. Liver homogenates, 20%, were prepared in a modified Bucher medium [11] with a Potter-Elvehjem homogenizer using a loosely fitting teflon pestle. The 20000 × *g* supernatant fluid and the microsomal fraction were prepared as described in a previous communication [2]. The labeled compounds were added to the incubation mixtures dissolved in 50 μl of acetone. 4-^{14}C-Cholesterol, 10 μg, was incubated with 3 ml of 20000 × *g* supernatant fluid, diluted with 2 ml of Bucher medium, for 1 hour at 37°. 7β-^{3}H-Cholest-5-ene-3β,7α-diol, 40 μg, was added to a mixture of 1 ml of microsomal fraction, 2 ml of Bucher medium and 1 μmole of NAD, and incubation was carried out for 20 min at 37°. Tritium-labeled 7α-hydroxycholest-4-en-3-one, 80 μg, was incubated with 3 ml of microsomal fraction fortified with 3 μmoles of NADPH for 10 min at 37°. Termination of the incubations and further analysis were carried out as described in a previous communication [2].

Radioactivity was determined with a methane gas flow counter. Under the conditions of the assay 1 μC of ^{14}C corresponds to 1 × 10^6 cpm and 1 μC of ^{3}H to 6 × 10^5 cpm.

3. Results and discussion

Four bile fistula rats were given three injections of actinomycin D (100 μg/kg body weight) at 12, 24 and 36 hours after operation. The animals were killed after 48 hours. Three control rats were injected in the same way with saline. The conversion of cholesterol into 7α-hydroxylated products (cholest-5-ene-3β,7α-diol, 7α-hydroxycholest-4-en-3-one, and 7α,12α-dihydroxycholest-4-en-3-one) was on an average 4.4% (range, 3.2 - 5.6%) in the control rats and 0.9% (range 0.4 - 1.5%) in the actinomycin D-treated rats. No marked differences were observed with respect to the pattern of products formed between control rats and actinomycin D-treated rats. Actinomycin D thus prevented an increase of the 7α-hydroxylating activity in bile fistula rats.

Bile fistula rats were given a single injection of actinomycin D (500 μg/kg body weight) or puromycin (100 mg/kg body weight) 72 hours after operation, when the activity of the 7α-hydroxylase, catalyzing the conversion of cholesterol into cholest-5-ene-3β, 7α-diol, has reached a plateau. After 1 - 7.5 hours the rats were killed and the extent of conversion of cholesterol into 7α-hydroxylated products was determined. For comparison, the extent of conversion of cholest-5-ene-3β,7α-diol into 7α-hydroxycholest-4-en-3-one and the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one were determined. No marked differences were observed between the rats with respect to the pattern of

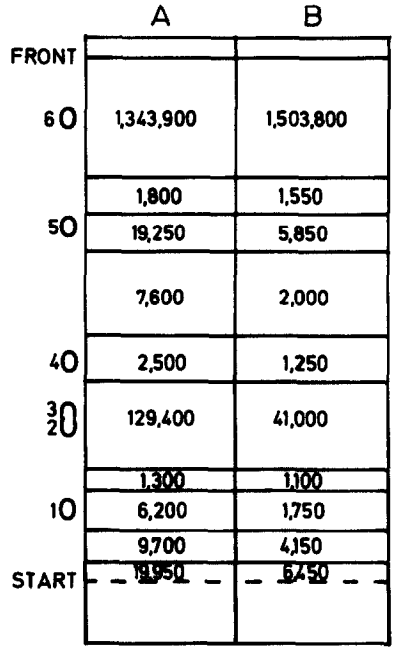

Fig. 1. A, thin layer chromatogram of chloroform extract of incubation of 4-^{14}C-cholesterol, 10 μg, with the 20000×*g* supernatant fluid of homogenate of liver from bile fistula rat. The numbers on the chromatogram represent cpm. Reference compounds were: 1, 5β-cholestane-3α,7α,12α-triol; 2, 7α,12α-dihydroxycholest-4-en-3-one; 3, cholest-5-ene-3β, 7α-diol; 4, cholest-5-ene-3β,7β-diol; 5, 7α-hydroxycholest-4-en-3-one; 6, cholesterol. Solvent, benzene-ethyl acetate (2 : 3). B, thin layer chromatogram of chloroform extract of incubation of 4-^{14}C-cholesterol, 10 μg, with the 20000×*g* supernatant fluid of homogenate of liver from bile fistula rat injected with puromycin 4 hours before sacrifice. Reference compounds and chromatographic conditions as in A.

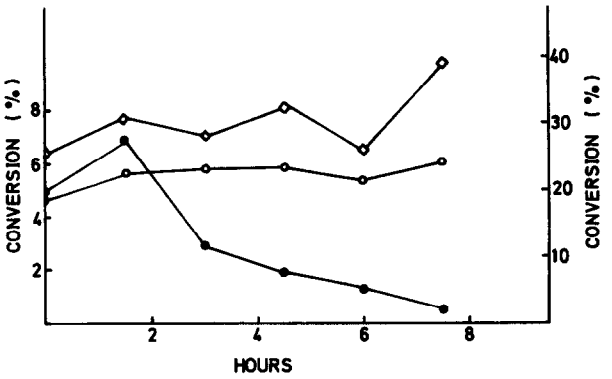

Fig. 2. Effect of actinomycin D on some reactions in the conversion of cholesterol into cholic acid in homogenates of liver from bile fistula rats. ●—●, conversion of 4-^{14}C-cholesterol into 7α-hydroxylated products (cholest-5-ene-3β,7α-diol, 7α-hydroxycholest-4-en-3-one, and 7α,12α-dihydroxycholest-4-en-3-one) in the presence of 20000 X g supernatant fluid. ○—○, conversion of 7β-^{3}H-cholest-5-ene-3β,7α-diol into 7α-hydroxycholest-4-en-3-one in the presence of microsomal fraction fortified with NAD. ◇—◇, conversion of tritium-labeled 7α-hydroxycholest-4-en-3-one into 7α,12α-dihydroxycholest-4-en-3-one in the presence of microsomal fraction fortified with NADPH.

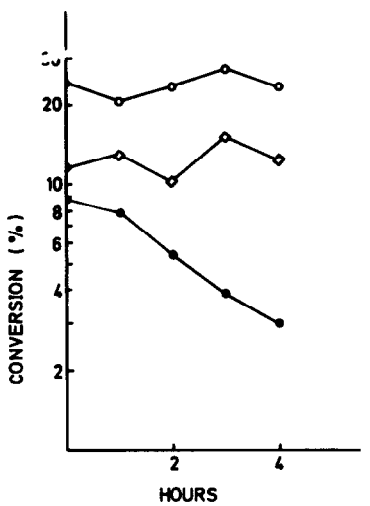

Fig. 3. Effect of puromycin on some reactions in the conversion of cholesterol into cholic acid in homogenates of liver from bile fistula rats. Symbols as in fig. 2.

products formed (cf. fig. 1). After treatment with actinomycin D, the activity of the 7α-hydroxylase decreased rapidly (fig. 2), indicating that the 7α-hydroxylase and its messenger-RNA have short half-life times in the bile fistula rat [12]. The half-life time for the breakdown of the 7α-hydroxylase was calculated to be 2-3 hours from the experiments with puromycin (fig. 3). No significant decrease in the conversion of cholest-5-ene-3β,7α-diol into 7α-hydroxycholest-4-en-3-one or in the 12α-hydroxylation of 7α-hydroxycholest-4-en-3-one could be seen after treatment with actinomycin D (fig. 2) or with puromycin (fig. 3), indicating a longer half-life time for these enzymes than for the 7α-hydroxylase.

The increase in the activity of the 7α-hydroxylase in the bile fistula rat may depend on an increased enzyme synthesis or on a diminished rate of breakdown of the enzyme. The data presented do not exclude the possibility of a decrease in breakdown of the enzyme but this would mean that the half-life time of the enzyme should be even shorter in the intact rat, which seems improbable. It should also be pointed out that the preferential way of the mammalian organism to increase its levels of enzyme activity is increased enzyme synthesis [3]. The half-life time for the breakdown of the 7α-hydroxylase in the bile fistula rat was calculated to be 2-3 hours, whereas the half-life time for total liver protein is 2-3 days [13]. It has been suggested that inducible liver enzymes will have half-life times of the order of a few hours, whereas noninducible enzymes have much longer half-life times [14]. The present results are also consistent with the view that the conversion of cholesterol into cholest-5-ene-3β,7α-diol is a rate-determining step in the biosynthesis of bile acids from cholesterol.

Acknowledgements

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