INFLUENCE OF A LIPOGENIC DIET ON THE CHOLESTEROL SYNTHESIS IN RATS IN VIVO

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SUMMARY

An inhibition of the cholesterol synthesis by a lipogenic diet in rat liver is described. The inhibition could be shown with glucose or mevalonate as tracer substances. This inhibition is located between lanosterol and cholesterol and results in a reduction of the cholesterol synthesis to about one sixth of the control group. No indication for any other inhibiting effect was obtained by these in vivo experiments.

INTRODUCTION

The mechanism of the regulation of the cholesterol synthesis is the subject of a great number of publications. Special attention was given to the influence of fasting and of cholesterol feeding in rats. The results obtained are mainly based on incorporation experiments carried out in vitro or on comparing activities of isolated enzymes. According to a widely accepted theory, the β-hydroxy-β-methylglutaryl-coenzyme A reductase (E.C. No. 1.1.1.34) plays a key role in the regulation of the cholesterol synthesis in that sense that by inhibition of this enzyme a shunt to the synthesis of fatty acids and ketone bodies occurs. Other inhibiting influences for the regulation of the cholesterol synthesis, such as between acetyl-coenzyme A and hydroxy-methylglutaryl-coenzyme A or between this intermediate and squalene, are assumed to exist but to be of minor importance (1-3).

In this communication, results are reported on the influence of a lipogenic diet on the cholesterol synthesis by in vivo experiments, using labelled glucose and mevalonate as precursors and by measuring its incorporation into squalene, lanosterol and cholesterol.

MATERIALS AND METHODS

Rats weighing 180-200 g were kept on a stock diet consisting of 60 % cereals, 18 % plant proteins, 4 % minerals and 1 % vitamin mixture. The experimental group received a lipogenic diet during a period of

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Table 1

Incorporation of radioactivity from D-[6-^{14}C] glucose (oral dose: 10 µCi/30.5 µg in 0.5 ml)	dpm per rat liver		
	Lipogenic Diet 6 rats	Stock Diet 6 rats	Lipogenic Diet / Stock Diet
Into acetone extract	268 400	63 800	4.2
Into squalene	682	425	1.6
Into cholesterol	6 140	22 163	0.28

Table 2

Incorporation of DL-[2-^{3}H]mevalonate (oral dose: 5 µCi/1.7 µg, together with a load dose of 1 g glucose*))	dpm per rat liver		
	Lipogenic Diet 7 rats	Stock Diet 6 rats	Lipogenic Diet / Stock Diet
Into unsaponifiable	704 x 10^3	396 x 10^3	1.8
Into squalene	308 x 10^3	66 x 10^3	4.7
Into lanosterol	154 x 10^3	35 x 10^3	4.4
Into cholesterol	220 x 10^3	286 x 10^3	0.76

*) Before the administration of the load dose, the rats were fasted for 16 hours.

five days of the following composition: 70 % glucose, 24 % vitamin-free casein, 5 % minerals, and 1 % vitamin mixture. D-[6-^{14}C]glucose with a specific activity of 324 µCi/mg and DL-[2-^{3}H] mevalonic acid lactone with a specific activity of 3.85 mCi/mg were administered by stomach tube in the morning. Two hours later, the animals were sacrificed by taking care that the intervals between the application of the tracer substances and the removal of the liver were the same for each animal.

Liver homogenate was used for extraction. The saponification was carried out with equal quantities of homogenate and 30 % methanolic KOH under reflux at 100°C during 30 min. The unsaponifiable was chromatographed on alumina I inactivated with 7 % water. The various fractions were eluted with petroleum ether/ether mixture 100/0 for squalene, 95/5

for lanosterol and 80/20 for cholesterol. The substances were identified by determination of the molecular weight with mass spectroscopy. It turned out that the lanosterol fraction contained some dihydrolanosterol.

RESULTS AND DISCUSSION

It was to be expected that the synthesis of the total lipids in rat livers is clearly enhanced by the administration of a lipogenic, glucose-rich diet. Surprisingly, however, a strong reduction of the incorporation of the ^{14}C-label from glucose into cholesterol is observed to about one fourth of that of the control group. As the incorporation of radioactivity from glucose into squalene is not inhibited but even increased by the lipogenic diet, it must be concluded that the inhibition is located between squalene and cholesterol (Table 1). For further evaluation of the inhibition mechanism mevalonate was used as tracer and, in addition, its incorporation into lanosterol was measured (Table 2). It turned out that the cholesterol synthesis from mevalonate is only slightly reduced by the lipogenic diet, whereas the incorporation of mevalonate into squalene and lanosterol is strongly enhanced. It became thus clear that the section of the pathway of steroid synthesis between mevalonate and lanosterol is considerably activated by the lipogenic diet and that the inhibition is actual ly located between lanosterol and cholesterol.

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