INFLUENCE OF FASTING AND CHOLESTEROL FEEDING ON THE CHOLESTEROL SYNTHESIS IN RATS IN VIVO

O. Wiss*)

Department of Vitamin and Nutritional Research of F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland

Received November 11,1975

SUMMARY

An inhibition of the cholesterol synthesis located between lanosterol and cholesterol could be shown in fasting rats in vivo with glucose, palmitate, acetate, and mevalonate as tracer substances. The same type of inhibition of the cholesterol synthesis was observed after cholesterol feeding. No indication was obtained for any other inhibiting effect located between acetyl-coenzyme A and lanosterol by these in vivo experiments.

INTRODUCTION

In a previous communication (1), the influence of a lipogenic diet on the cholesterol synthesis in rats was described. It was found that an inhibition between lanosterol and cholesterol leads to a substantial reduction of the incorporation of the label of glucose and of mevalonate into cholesterol in vivo in rat livers.

Results are reported now on the influence of fasting and cholesterol feeding on the cholesterol synthesis using the same technique. For that purpose, labelled glucose, palmitate, acetate and mevalonate were used for tracer experiments in rats.

MATERIALS AND METHODS

As described previously (1).

RESULTS AND DISCUSSION

The results of Table 1 show that the incorporation of radioactivity from labelled glucose, palmitate, and acetate into the lanosterol fraction is differently affected by fasting. Only with glucose, an

*) Present address: Institute of Biochemistry, University of Basle, Vesalianum, Basle, Switzerland

Table 1

Incorporation of radioactivity from D-[6-^{14}C] glucose (oral dose: 44 µCi/127 µg in 0.5 ml)	dpm per rat liver		
	16 h fasted mean of 8 rats	controls mean of 7 rats	fasted rats / controls
Into lanosterol	434	842	0.52
Into cholesterol	16 030	77 140	0.21
Incorporation of [1-^{14}C] palmitic acid (oral dose: 10 µCi/144 µg in 0.5 ml)	mean of 8 rats	mean of 7 rats	
Into lanosterol	334	341	1
Into cholesterol	3 088	6 243	0.49
Incorporation of [1-^{14}C]acetate (oral dose: 42 µCi/66 µg in 0.25 ml)	mean of 8 rats	mean of 8 rats	
Into lanosterol	12 760	7 992	1.6
Into cholesterol	204 260	257 000	0.79
Incorporation of [1-^{14}C]acetate (intraperitoneal dose: 36 µCi/75 µg in 0.25 ml)	mean of 10 rats	mean of 10 rats	
Into lanosterol	4 466	3 229	1.4
Into cholesterol	114 438	170 748	0.67

inhibition to about 50 % of the controls is observed, whereas, with palmitate, no effect and with acetate even a stimulation can be seen. It must be concluded, therefore, that the inhibition observed must occur before the formation of the acetyl-coenzyme A under the assumption that acetyl-coenzyme A deriving from these three precursors enters the same pool for the lanosterol synthesis.

With all three precursors, however, an inhibition of the incorporation of the label into cholesterol is evident. With glucose, a reduction to about 20 %, with palmitate to about 50 %, and with acetate to about 80 % of the controls occurs. It becomes thus clear that a regulation is effective, located between lanosterol and cholesterol resulting in an inhibition of the cholesterol synthesis for all three precursors to about half of the control group.

No significant effect of fasting on the incorporation of labelled mevalonate into cholesterol, but a stimulation of the lanosterol synthesis from mevalonate is observed (Table 2). If the cholesterol synthesis is related to the lanosterol formation, again an inhibition of the cholesterol synthesis to about half of the controls results from fasting.

Comparing the incorporation rates of mevalonate into squalene and lanosterol, no indication is obtained for an inhibition of the cholesterol synthesis between these two precursors by fasting.

Table 2

Incorporation of [2-^{14}C]mevalonate (oral dose: 9.4 µCi/248 µg in 0.5 ml)	dpm per rat liver		
	16 h fasted mean of 8 rats	controls mean of 8 rats	fasted rats / controls
Into squalene fraction	293 300	106 682	2.7
Into lanosterol fraction	194 161	76 433	2.5
Into cholesterol fraction	916 362	782 877	1.2

Table 3

Incorporation of radioactivity from D-[6-^{14}C] glucose (oral dose: 10.5 µCi/30.5 µg in 0.5 ml	dpm per rat liver		
	stock diet + 5 % cholesterol for 24h (St + Ch) mean of 11 rats	stock diet (St) mean of 12 rats	St + Ch / St
Into squalene	1 417	994	1.4
Into cholesterol	9 556	26 064	0.36

Table 3 shows results of cholesterol feeding on the cholesterol synthesis. It is evident that no inhibition of the squalene synthesis occurs, on the contrary, a slight stimulation of the incorporation of radioactivity from labelled glucose into squalene can be seen. This effect contrasts with a strong reduction of the incorporation of the label of glucose into cholesterol, indicating that the inhibition is located between squalene and cholesterol. By comparing the mevalonate incorporation into squalene and lanosterol, it turned out again that the inhibiting effect takes place between lanosterol and cholesterol (Table 4).

By reviewing the results obtained with the lipogenic diet (1) with fasting and with cholesterol feeding, it is evident that, under all three conditions, the same type of regulation of the cholesterol synthesis seems to occur located between lanosterol and cholesterol. At the present stage of the investigations, no definite answer is possible whether lanosterol itself is involved in the inhibiting reaction or possibly an isomer of it with a very similar structure, which could not be separated by the methods used.

Surprisingly, no evidence was obtained for all three conditions, namely fasting, cholesterol feeding and administration of a lipogenic diet, that any enzyme located between acetyl-coenzyme A and lanosterol is involved in the regulation of the cholesterol synthesis. Thus, these results are in clear disagreement to those obtained by in vitro experiments, especially to those pointing to a key function of the β-hydroxy-β-methylglutaryl-coenzyme A reductase (E.C. No. 1.1.1.34) for the regulation of the cholesterol synthesis. Further experiments will be necessary to solve the various questions involved.

Table 4

Incorporation of [2-^{14}C]mevalonate (oral dose: 10 µCi/248 µg in 0.25 ml)	dpm per rat liver		
	stock diet + 5 % cholesterol for 24h (St + Ch) mean of 10 rats	stock diet (St) mean of 10 rats	$\frac{St + Ch}{St}$
Into squalene	233 814	113 873	2.1
Into lanosterol	209 746	101 477	2.1
Into cholesterol	690 838	714 339	0.95

An important aspect has not been evaluated up to now, namely the question to which substance the portion of lanosterol is metabolized which is not transformed to cholesterol by the inhibiting influence. As a working hypothesis, it may be assumed that a bile acid is formed from it by bypassing the cholesterol as intermediate. No direct evidence in favour of or against such an assumption is available at present. Certain indications for it, however, result from recent studies demonstrating that in rats, cholic acid is mainly formed from glucose, whereas chenodeoxycholic acid is formed from exogenous cholesterol (2). Two possibilities can be considered. Either that the rat organism can distinguish between endogenous and exogenous cholesterol for bile acid synthesis, as it is assumed by Uchida et al. (2), or that cholic acid can be formed by bypassing cholesterol as intermediate. These hypotheses will be checked by incorporation experiments.

A regulation of the cholesterol synthesis between lanosterol and cholesterol would enable the organism to inhibit the cholesterol synthesis selectively without affecting other pathways, such as ubiquinone, dolichol and branched chain fatty acid synthesis, as it would be the case if the β-hydroxy-β-methylglutaryl-coenzyme A reductase has the decisive function in the regulation of the cholesterol synthesis.

REFERENCES

1. Wiss, O., Biochem.Biophys.Res.Commun., this issue, paper before.
2. Uchida, K., Takeuchi, N., and Yamamura, Y. (1975) Lipids 10: 473-477.