THE EFFECTS OF THIOLS ON CHOLESTEROL SYNTHESIS BY RAT LIVER IN VITRO

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Certain thiols such as β-mercaptoethylamine, β-mercaptoethanol, ethanethiol and dithiothreitol inhibit cholesterol synthesis from mevalonic acid (MVA) in rat liver homogenates and cause accumulation of a mixture of lanosta-8,24-dien-3β-ol (lanosterol) and lanost-8-en-3β-ol (Δ^8-lanostenol). Cysteine and glutathione inhibit cholesterol synthesis without accumulation of lanosterol and Δ^8-lanostenol. Since it has been proposed that the demethylation of lanosterol involves hydroxylation of the methyl group this process must differ from certain P450-dependent mixed function oxidases which are stimulated by similar concentrations of β-mercaptoethylamine.

In a further study of the effect of thiols[1] on mixed function oxidases[2] it has been found that cholesterol synthesis from mevalonic acid (MVA) by rat liver preparations is inhibited by certain thiols with, in some cases, accumulation of an intermediate. The present investigation was prompted by the possibility of using this system as a model for a study of factors controlling the natural accumulation of intermediates of cholesterol biosynthesis in skin [3]. The identity of the intermediate as a mixture of lanosta-8,24-dien-3β-ol (lanosterol) and lanost-8-en-3β-ol (Δ^8-lanostenol) has been established and the effect of various thiols on lanosterol demethylation[4] in rat liver preparations has been examined.

Methods and Materials

2-^{14}C-MVA lactone (Radiochemical Centre, Amersham) was

converted to the sodium salt before use by incubating for 1.5 hours at 37° with 1% sodium bicarbonate solution (pH 8.4). ^{14}C-Lanosterol/lanostenol was prepared by the method of Moller and Tchen[5] and was added to incubations in acetone solution.

20% Homogenates of rat liver in Bucher's medium[6] were centrifuged to give the microsomal + supernatant fraction (18,000 x g, 20 min) from which "floating lipid" was removed. Incubations (1 hr. at 37°) were carried out in air as follows: 18,000 g supernatant (2 ml equivalent to 0.5 g tissue), Bucher's medium (2 ml), $NADP^+$ (1 μmole, 0.1 ml) glucose-6-P (10 μmoles, 0.1 ml), glucose-6-P dehydrogenase (0.2 U/0.1 ml), NAD^+ (4 μmoles, 0.1 ml), GSH (20 μmoles/0.1 ml), fructose-1,6-P_2 (15 μmoles, 0.1 ml) and substrate (0.1 μC, 0.02 ml). Incubations were stopped with methanol (5 ml). The non-saponifiable lipid product was assayed by thin layer chromatography.

Results and Discussions

Addition of 9.5 mM β-mercaptoethylamine to the incubation medium resulted in the accumulation of radioactive material with the same R_f on TLC as lanosterol (62% of the non-saponifiable lipid compared with a 1% control). This was shown to be a mixture of lanosterol and Δ^8-lanostenol on the following evidence.

Incubation of the isolated material in the absence of β-mercaptoethylamine gave cholesterol, identified by silicic acid TLC and silver nitrate impregnated TLC. Incubation with an acetone powder of rat liver microsomes, in the presence of 105,000 x g supernatant and cofactors as above, omitting fructose-1,6-P_2 and NAD^+, gave acidic material the methyl ester of which had the same silicic acid TLC behaviour as the analagous compound formed from Δ^8-

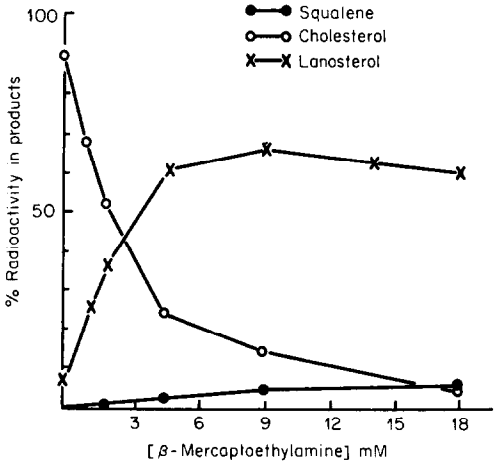

Figure 1 Effect of increasing β-mercaptoethylamine concentration on cholesterol synthesis from 2-^{14}C-MVA

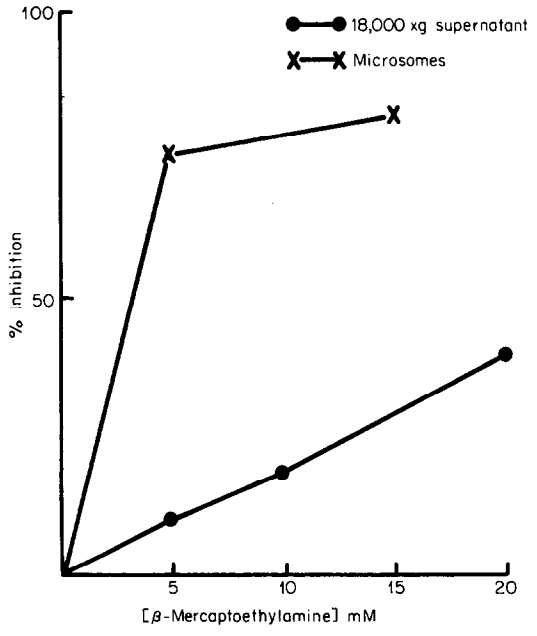

Figure 2 Inhibition of lanosterol demethylation by β-mercaptoethylamine

lanostenol[7]. Both radio-glc[8] (3% OV 17) and silver nitrate impregnated TLC showed two radioactive peaks corresponding to authentic lanosterol and Δ^8-lanostenol. The lanosterol from

the silver nitrate impregnated TLC was recovered and co-crystallised with non-radioactive lanosterol (1st crystn. 0.79 x 10^5 dpm/mmole, 2nd crystn. 0.94 x 10^5 dpm/mmole, 3rd crystn. 0.79 x 10^5 dpm/mmole ± 5%). A brominated sample of this material was also crystallised (0.90 x 10^5 dpm/mmole ± 5%).

The effect of varying the β-mercaptoethylamine concentration is shown in Fig. 1. The accumulation of lanosterol/lanostenol reaches a plateau at 5 mM β-mercaptoethylamine. That lanosterol demethylation was being inhibited was confirmed by using a lanosterol/lanostenol mixture as substrate (Fig. 2); using microsomes (105,000 x g/60 min) alone, a more rapid inhibition was observed (Fig. 2). In order to get information about the nature of this inhibition, a variety of thiols were tried using MVA as substrate (Table. Fig. 3). Glutathione and cysteine (Fig. 3) have a similar effect in stimulating

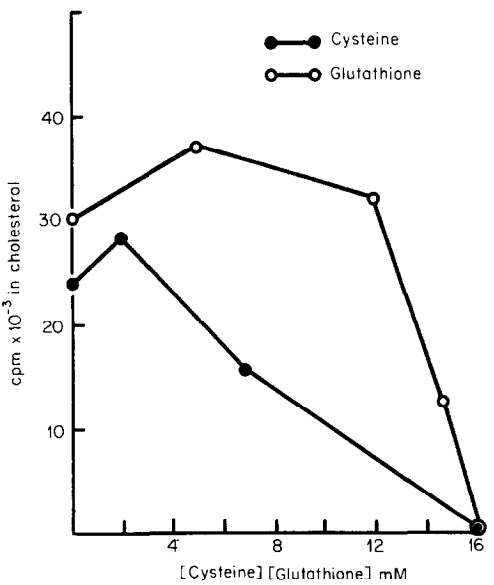

Figure 3 Inhibition of cholesterol synthesis from 2-^{14}C-MVA in 18,000g. supernatant by cysteine and glutathione. In no case did the total of lanosterol/lanostenol and squalene exceed 5 x 10^3 cpm.

TABLE

Effect of Thiols on Cholesterol Synthesis from 2-^{14}C-MVA

Thiol	Concentration mM	% of non-saponifiable		
		Squalene	Lanosterol/ lanostenol+	Cholesterol
Control	-	1.8	2.5	92.5
Ethanethiol*	15.4	1.8	71.8	24.1
β-Mercaptoethanol	7.0	-	95.2	4.8
Dithiothreitol	7.0	-	96.6	3.4
β-Mercaptoethylamine	7.0	4	62	18

* b.p. 34-7°; incubation carried out at 32°
+ characterised only for β-mercaptoethylamine

cholesterol synthesis at low concentrations and inhibiting at higher concentrations, but lanosterol/lanostenol is not accumulated. Ethylamine (0.6 to 12 mM) had no effect at any of the concentrations used, so it would seem that the thiol group of β-mercaptoethylamine is essential for the inhibition. In support of this view it was found that ethanethiol, dithiothreitol and β-mercaptoethanol gave accumulation of lanosterol/lanostenol. It may be significant that the two thiols which do not affect demethylation, cysteine and glutathione, differ from the 'active' thiols in possessing free carboxyl groups. Indeed glutathione has been routinely used in both this and previous work [9] to obtain maximum demethylase activity at concentrations between 3 and 5 mM.

The mechanism of lanosterol demethylation is still not clear, although it has been compared with that of fatty acyl desaturase[10] in that both require O_2 and NADPH, are cyanide sensitive, and CO insensitive. In neither case has a simple hydroxylated inter-

mediate been isolated. In demethylation the intermediates isolated appear to be carboxylic acids[7)11)12)]. The action of β-mercapto-ethylamine in preventing aberrant autoxidation reactions of cholesterol during 7α-hydroxylation[1)] is probably due to the radical scavenging activity of this thiol. That β-mercaptoethylamine and several other thiols have an inhibitory effect on demethylation may be due to a similar activity and suggests that the initial step in lanosterol demethylation is predominantly radical in nature.[13)]

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