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THE ROLE OF CHOLESTEROL 7α-HYDROXYLASE IN THE HYPOBETALIPOPROTEINAEMIC ACTIVITY OF SKF-525A IN RATS

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Abstract—Administration of SKF-525A to rats fed on a stock diet specifically decreased the serum concentration of low-density lipoprotein. SKF-525A and cholestryamine also reversed the rise in circulating concentration of both very-low-density and low-density lipoprotein that was observed in rats given a sucrose-based, cholesterol-supplemented diet. The enhancement of hepatic cholesterol 7α-hydroxylase by SKF-525A or by cholestyramine is accompanied by homeostatic responses by the liver which include induction of low-density lipoprotein clearance and increased cholesterogenesis to attempt to replenish sterol pools. These compensatory mechanisms are separately controlled.

SKF-525A, diethylaminoethyl-2,2-diphenyl valerate hydrochloride, originally reported as a potentiator of drug action [1], is hypocholesterolaemic in laboratory animals [2], although little is known of the effect on individual lipoproteins. Inhibition of cholesterol biosynthesis from mevalonate by SKF-525A can be demonstrated *in vitro* but not *in vivo* [3].

Our recent observation [4] that SKF-525A enhances the activity of cholesterol 7α-hydroxylase (EC 1.14.13.17), the rate-limiting enzyme for the pathway of cholesterol catabolism to bile acids [5], in association with the elevation of hepatic microsomal cytochrome P-450 concentration, suggests an alternative mechanism.

The present studies used SKF-525A to examine the role of cholesterol 7α-hydroxylase in the control of cholesterol metabolism. In particular, information was obtained by a comparison of diets to give a range of circulating concentrations of VLD and LD lipoproteins† and by comparing SKF-525A with cholestyramine, a hypolipidaemic agent known to increase bile acid turnover [6].

MATERIALS AND METHODS

Animals, diets and treatment. Male Sprague–Dawley rats (140–160 g) were obtained from A. Tuck & Son, Battlesbridge, U.K. and received food and water *ad lib.* The rats were fed on a stock pelleted diet (Oxoid Breeding Diet, Oxoid Ltd., Basingstoke, Hants., U.K.) or on a semi-synthetic diet containing (w/w): sucrose, 61%; casein, 22%; saturated fat (White cap fat, a blend of marine oils obtained from Van den Berghs, Burgess Hill, West Sussex, RH15 9AW, U.K.), 8%; methyl cellulose, 4%; cholesterol, 0.25%; cholic acid, 0.05%; and vitamins and mineral salts as described previously [7] for 7 days prior to commencement of drug treatment.

SKF-525A (50 mg/kg) in 0.9% NaCl (w/v) was administered by i.p. injection (2 ml/kg) once daily between 0830 and 0930 hr for 2 days. Control rats received 0.9% NaCl (w/v) alone. Cholestyramine was administered as a supplement to the diet (4% w/w) for 5 days. Rats were killed between 1000 and 1100 hr (daylight-dependent light cycle).

Lipogenesis in vivo. The synthesis of digitonin-precipitable sterols from ^3H_2O in liver was measured as previously reported [8]. Using these experimental conditions we have shown (R. Rush, unpublished results) by splitting the digitonide with pyridine [9] that the radiolabelled sterols are not contaminated by ^3H from ^3H_2O-binding, in contrast to the findings of others [10] who used precursor of much higher specific radioactivity and a different extraction procedure.

Measurement of serum lipids and lipoproteins, and tissue lipids. Total cholesterol and HDL cholesterol in serum were measured by previously documented procedures [8]. LD lipoprotein was measured following precipitation of VLD lipoprotein with sodium dodecyl sulphate [11]. Preliminary experiments showed that the concentration of LD lipoprotein cholesterol in rat serum measured by this method was similar to the concentration obtained following high-speed centrifugation (*d* 1.006–1.063).

Liver microsomal free and esterified fractions of cholesterol were extracted and separated as described previously [8] and the concentration of cholesterol was measured fluorimetrically [12].

Other measurements. Methods for the isolation of hepatic microsomes, determination of microsomal cytochrome P-450 and protein concentrations, and assay of cholesterol 7α-hydroxylase activity have been reported elsewhere [13].

Chemicals. [4-^{14}C]Cholesterol, sp. radioact. 54–57 mCi/mmole, and ^3H_2O, sp. radioact. 18 Ci/mmole, were obtained from Amersham International (Amersham, U.K.). Cholestryamine, a quaternary ammonium anion exchange resin (Merck, Sharp & Dohme, Hoddesdon, U.K.), and SKF-525A (Smith, Kline & French, Welwyn Garden City,

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† HD lipoprotein, high-density lipoprotein; LD lipoprotein, low-density lipoprotein; VLD lipoprotein, very-low-density lipoprotein.

U.K.) were received as gifts. All other reagents and solvents were of analytical grade and obtained from Sigma Chemical Co. (Poole, U.K.), Boehringer–Mannheim Corp. (Lewes, U.K.), or British Drug Houses (Poole, U.K.).

RESULTS

The sucrose-based, cholesterol-supplemented semi-synthetic diet is well tolerated and supports a gain in body weight comparable to that obtained with a stock diet (results not shown). This diet has been devised to produce moderate elevations in the concentration of serum cholesterol carried on LD and VLD lipoproteins with the maintenance of HD lipoprotein concentration and without the appearance of lipoproteins of altered composition (β VLD lipoprotein, HDL_c) that accumulate when using other cholesterol-supplemented diets [8].

SKF-525A significantly decreased the serum cholesterol concentration of the hyperlipidaemic rats and this is attributed to the effects on VLD and LD lipoproteins (Table 1) although only the latter change achieved statistical significance. SKF-525A did not affect the serum concentration of total cholesterol of the rats receiving the stock diet but there was a specific decrease in LD lipoprotein concentration.

Table 1. Comparison of effects of SKF-525A on lipid metabolism in rats receiving stock and semi-synthetic diets

	Stock diet		Semi-synthetic diet	
	Control	SKF-525A (50 mg/kg body wt)	Control	SKF-525A (50 mg/kg body wt)
Serum lipid (mg/100 ml)				
Cholesterol	56 ± 4	54 ± 2	110 ± 6	87 ± 5*
HD lipoprotein cholesterol	41 ± 2	39 ± 1	44 ± 3	42 ± 2
LD lipoprotein cholesterol	17 ± 2	9 ± 1*	28 ± 3	18 ± 3*
VLD lipoprotein cholesterol	2 ± 1	5 ± 1	40 ± 4	27 ± 5
Hepatic sterol synthesis (nmole/min per g tissue)	2.04 ± 0.32	3.79 ± 0.94	0.46 ± 0.08	0.44 ± 0.05
Hepatic cholesterol 7α-hydroxylase (nmole/min per g tissue)	0.59 ± 0.05	1.16 ± 0.10*	0.12 ± 0.03	0.34 ± 0.07*
Hepatic microsomal cytochrome P-450 (nmole/mg protein)	1.16 ± 0.06	1.68 ± 0.08*	0.41 ± 0.02	0.72 ± 0.06*

Each rat was injected intraperitoneally with ^3H_2O (1.5 mCi/0.1 kg) 1 hr prior to killing. Results are the means ± S.E.M. for eight analyses; each analysis being of tissue from a single rat.
*Indicates a significant difference from a comparable control group ($P < 0.05$).

Table 2. Comparison of effects of SKF-525A and cholestyramine, alone and in combination, on lipid metabolism in rats receiving semi-synthetic diets

	Control	SKF-525A (50 mg/kg body wt)	Cholestyramine (4% w/w in diet)	Cholestyramine + SKF-525A (50 mg/kg body wt: 4% w/w in diet)
Serum lipid (mg/100ml)				
Cholesterol	116 ± 7	87 ± 8*	75 ± 5*	65 ± 5*
LD lipoprotein cholesterol	25 ± 3	18 ± 2	16 ± 1*	10 ± 1*
Sterol synthesis (nmole/min per g liver)	0.26 ± 0.04	0.26 ± 0.13	11.37 ± 0.82*	10.08 ± 0.65*
Cholesterol 7α-hydroxylase (nmole/min per g liver)	0.16 ± 0.03	0.46 ± 0.06*	1.53 ± 0.12*	1.57 ± 0.18*
Hepatic microsomal cholesterol (nmole/mg protein)				
Free	88 ± 3	79 ± 2*	88 ± 2	87 ± 3
Esterified	78 ± 5	50 ± 5*	18 ± 2*	21 ± 1*

Each rat received ^3H_2O as described in Table 1. Results are the means ± S.E.M. for six analyses; each analysis being of tissue from a single rat.
*Indicates a significant difference from the control value ($P < 0.05$).

SKF-525A significantly elevated hepatic cholesterol 7α-hydroxylase activity in rats fed on either diet; the basal level was lower on the semi-synthetic diet because of feedback repression by dietary cholic acid. Significant increases in the hepatic microsomal cytochrome P-450 concentration were also elicited by SKF-525A although the increase observed in rats on the semi-synthetic diet was relatively greater (75% compared to 45%) and corresponded with the greater stimulation of cholesterol 7α-hydroxylase. Administration of SKF-525A to the rats receiving the stock diet elevated the hepatic rate of cholesterogenesis *in vivo* in contrast with the effect reported *in vitro*. These elevated rates of cholesterol 7α-hydroxylase and sterol synthesis were significantly correlated ($r = 0.73$, $P < 0.01$). In the hyperlipidaemic rats, basal cholesterogenesis was low due to feedback repression by dietary cholesterol and was not affected by SKF-525A.

On retesting, the activity of SKF-525A was similar to that observed previously. Cholestyramine, included in the diet, was also hypocholesterolaemic and decreased the concentration of LD lipoprotein (Table 2) and VLD lipoprotein (result not shown). However, cholesterol 7α-hydroxylase was much more markedly stimulated (1300% compared with 300% for SKF-525A) and was associated with a large increase in hepatic cholesterogenesis (2500%). The co-administration of SKF-525A with cholestyramine gave a further, small reduction in the serum concentrations of total cholesterol and LD lipoprotein although the increases in both cholesterol 7α-hydroxylase and cholesterogenesis were of the same order to those found for cholestryamine alone. SKF-525A, alone, slightly decreased the microsomal concentration of free cholesterol (believed to constitute the substrate pool for cholesterol 7α-hydroxylase) and, more markedly, decreased the esterified fraction. The effect of cholestyramine on the concentration of esterified cholesterol was much more pronounced; the combination of both agents produced no extra effect.

DISCUSSION

The content of cholesterol in the body is controlled by the balance between absorption, synthesis, catabolism and excretion. In experiments using rats, input may be principally dependent on synthesis, for example when using a stock diet, or on absorption, for example when using a cholesterol-supplemented diet.

In the present studies, administration of SKF-525A was observed to lower the concentration of circulating LD lipoprotein cholesterol using both types of diet, but in contrast to results *in vitro*, hepatic cholesterol synthesis is not inhibited. We consider that the explanation for the hypobetalipoproteinaemic action of SKF-525A is similar to the explanation proposed for cholestyramine [14] which also acts to increase cholesterol 7α-hydroxylase activity. Cholestyramine promotes the high affinity receptor-mediated clearance of LD lipoprotein by inducing receptor activity in the liver, probably by increasing receptor synthesis. The present results suggest that when hepatic cholesterol pools are depleted as a result of increased bile acid synthesis then two compensatory mechanisms can be brought into operation to attempt to maintain pool sizes: first, induction of receptor synthesis; secondly, induction of hepatic β-hydroxy-β-methylglutaryl-CoA reductase (EC 1.1.1.34) synthesis leading to enhanced cholesterogenesis. The observation that SKF-525A induces increased LD lipoprotein clearance but not cholesterogenesis (semi-synthetic diet) suggests that the two homeostatic responses are separately controlled, either by discrete regulatory sterol pools or by the extent of depletion of a common pool. The compensatory response to the consequences of SKF-525A administration is less in rats receiving the semi-synthetic diet because hepatic esterified cholesterol stores are now larger than in rats receiving the stock diet (approximately 10 nmole/mg protein).

Thus, under at least some circumstances, the response by the liver differs from other tissues where an increased requirement for cholesterol leads to concomitant increases in LD lipoprotein receptor synthesis and cholesterogenesis [15]. This greater complexity of control in the liver may be related to the discrete cholesterol pools required for independent functions, for example, secretion of VLD lipoprotein, nascent HD lipoprotein and bile acids. Depletion of liver cholesterol may lead to impaired secretion of VLD lipoprotein, but perhaps not HD lipoprotein (Table 1) in addition to enhanced LD lipoprotein uptake.

Due to the multiplicity of effects on intermediary metabolism and the possibility of toxicity on long-term administration [2], SKF-525A is of no direct interest as a hypolipidaemic agent. However, the relationship between the rate of bile acid synthesis and LD lipoprotein turnover observed for SKF-525A and cholestyramine, and oestrogens [16, 17] supports the importance of cholesterol 7α-hydroxylase as a regulatory step in the control of VLD and LD lipoprotein levels.

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