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Metabolism of 3β-hydroxycholest-5-en-26-oic acid in hamsters

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Summary. Metabolism of 26-hydroxycholesterol to 3β-hydroxychol-5-en-24-oic acid and other C24-bile acids has been expected to occur by way of 3β-hydroxycholest-5-en-26-oic acid in studies in vitro. 3β-Hydroxycholest-5-en-26-oic acid was infused intravenously into bile fistula hamsters and the following C24-bile acids were identified: 3β-hydroxychol-5-en-24-oic acid, lithocholic acid, chenodeoxycholic acid, and a small amount of cholic acid.
Key words. 3β-Hydroxycholest-5-en-26-oic acid; bile acids; hamsters; metabolism.

Previous studies in hamsters reported that 26-hydroxycholesterol is metabolized to 3β-hydroxychol-5-en-24-oic acid, lithocholic acid, chenodeoxycholic acid, and cholic acid[1] and that 3β-hydroxychol-5-en-24-oic acid is also metabolized to lithocholic acid and chenodeoxycholic acid[2,3]. In vitro studies of rat liver by Mitropoulos and Myant indicated that 3β-hydroxycholest-5-en-26-oic acid was the intermediate in the conversion of the C24 bile acids from cholesterol[4]. We synthesized [16,22-^{3}H]-3β-hydroxycholest-5-en-26-oic acid from [16,22-^{3}H]-26-hydroxycholesterol[5] and determined its metabolism in the hamster in vivo.

Materials and methods

[16,22-^{3}H]-3β-hydroxycholest-5-en-26-oic acid (0.28 μCi/μmol) was previously prepared from [16,22-^{3}H]-26-hydroxycholesterol which was synthesized from diosgenin[1,5]. 24-^{14}C-labeled lithocholic, chenodeoxycholic, and cholic acids were purchased from Amersham/Searle (Arlington Heights, IL) and/or New England Nuclear (Boston, MA). [24-^{14}C]-3β-hydroxychol-5-en-24-oic acid was the compound described previously[3].

The steps used to identify metabolites were (a) solvolysis, (b) hydrolysis, (c) excretion, (d) methylation, (e) column chromatography using glycophase G on controlled-pore glass 80–100 mesh (Pierce Chemical Co., Rockford, IL), (f) HPLC using μPorasil (10 μM silica, Waters Associates, Milford, MA) or thinlayer chromatography, and (g) reverse isotope dilution. All these steps have previously been described in detail, including the quantification of radioactivity by liquid scintillation spectrometry using a Beckmann CPM200 instrument[2,3,6].

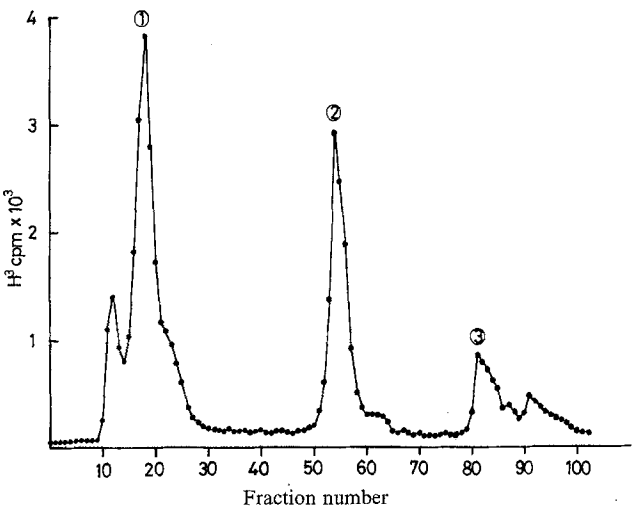
Male Syrian hamsters weighing about 100 g each were prepared during pentobarbital anesthesia as described previously[6]. The animal's bile acid pool was depleted by overnight drainage. Then an infusion, made by dissolving the sodium salt of [16,22-^{3}H]-3β-hydroxycholest-5-en-26-oic acid in 0.2 ml of propylene glycol and adding it, with vortexing, to a sterile solution of 5% dextrose in 0.45% NaCl containing 25% of human serum albumin, was administered intravenously over a 10-min period.

Results

Bile samples were collected separately at 30-min intervals over a period of 4 h and were then pooled. Eighty percent of the radioactivity from the infused material was recovered in the bile. An aliquot of bile was solvolyzed, hydrolyzed, extracted with ethyl acetate, and methylated. The methyl esters of the radioactive metabolites obtained from hamster bile were fractionated on glycophase G (fig.).

After addition of a [24-^{14}C] labeled standard (3β-hydroxychol-5-en-24-oic acid or lithocholic acid) to the radioactive samples obtained from the monohydroxy region, HPLC analysis as both the methyl ester and the methyl ester acetate detected both 3β-hydroxychol-5-en-24-oic acid and lithocholic acid. A small amount (4–10%) of the infused 3β-hydroxycholest-5-en-26-oic acid was still present in this fraction.

On HPLC analysis, the radioactive material obtained from the dihydroxy region of the glycophase column had the retention time of chenodeoxycholic acid methyl ester. Addition of authentic [^{14}C]-chenodeoxycholic acid methyl ester and HPLC analysis as both the methyl ester



Chromatographic separation of metabolites of radioactive 3β-hydroxycholest-5-en-26-oic acid on glycophase G using a solvent system of 25% ethyl acetate/hexane that was increased to 60% ethyl acetate after fraction No. 40, and 90% ethyl acetate after fraction No. 80. Flow rate = 1.5 ml/min, volume of each fraction = 2.0 ml. Peak No. 1 was found by HPLC to be a mixture of 3β-hydroxycholest-5-en-26-oic acid, 3β-hydroxychol-5-en-24-oic acid, and lithocholic acid. Peaks No. 2 and 3 were found to be chenodeoxycholic acid and cholic acid, respectively, by HPLC or TLC and reverse isotope dilution.

and the methyl ester diacetate also indicated that the [^{3}H]-dihydroxy bile acid was chenodeoxycholic acid.

The radioactive material obtained from the trihydroxy region of the glycophase column was subjected to thin-layer chromatography on silica gel and was developed with a mixture of isooctane, ethyl acetate, and acetic acid (20:40:3, v/v). The radioactive material corresponding to the methyl cholate zone was scraped off and was eluted with methanol. After addition of authentic methyl [^{14}C]-cholate, the combined eluate was rechromatographed in the same solvent system and was then extracted with the solvent.

After HPLC or TLC analysis, the identity of the bile acids derived from 3β-hydroxycholest-5-en-26-oic acid was further established by reverse isotope dilution (table).

Discussion

Cholesterol is transformed into 26-hydroxycholesterol by oxidation in the hepatic mitochondria in rats[4, 7] and mice[8]. In humans 26-hydroxycholesterol is found to be normally present in plasma[9]. Wachtel et al.[1] reported that 26-hydroxycholesterol is metabolized in hamsters to 3β-hydroxychol-5-en-24-oic acid, lithocholic acid, chenodeoxycholic acid, and cholic acid. The presence of 3β-hydroxychol-5-en-24-oic acid in the bile indicates that

Isotope dilution experiments on metabolites of 3β-hydroxycholest-5-en-26-oic acid

No. of crystallizations	Solvent	^{3}H/^{14}C ratio	Specific activity ^{3}H cpm/mg
3β-Hydroxychol-5-en-24-oic acid			
Acetyl methyl ester, 55.4 mg was added			
0	–	1.10	1730
2	MeOH*	1.10	
3	Acetone	1.08	1713
Free acid			
2	MeOH	1.09	1708
3	DMF**-H_2O	1.08	1710
Lithocholic acid			
Acetyl methyl ester, 16.8 mg was added			
0	–	0.63	
1	MeOH	0.84	
2	MeOH	0.88	
Free acid			
1	MeOH	1.08	
2	MeOH	0.89	
Chenodeoxycholic acid			
Acetyl methyl ester, 45.5 mg was added			
0	–	1.07	379
2	MeOH-H_2O	1.01	329
3	MeOH-H_2O	1.01	
Free acid			
2	Ethyl acetate-Hexane	1.01	331
3	Ethyl acetate-Hexane	1.01	332
Cholic acid			
Methyl ester, 11.57 mg was added			
0	–	1.64	
1	MeOH	1.34	
2	MeOH	1.28	
Free acid			
1	MeOH	1.32	

* MeOH: methanol; ** DMF: dimethylformamide.

oxidation of the side chain of cholesterol could occur before any modification of the ring system, as already suggested by Mitropoulos and Myant[4]. The present in vivo experiment shows that the hamster liver can cleave the side chain of 3β-hydroxycholest-5-en-26-oic acid, which could logically be expected to be derived from 26-hydroxycholesterol, giving 3β-hydroxychol-5-en-24-oic acid, together with lithocholic and chenodeoxycholic acids. As previously reported[2,3,10], 3β-hydroxychol-5-en-24-oic acid is converted to lithocholic acid and chenodeoxycholic acid. In addition, in hamsters[3,6] but not in rabbits[3], lithocholic acid is metabolized into chenodeoxycholic acid.

It has been shown that 3β,7α-dihydroxychol-5-en-24-oic acid can be metabolized to chenodeoxycholic acid[11,12]. The present studies also permit the conclusion that 7 alpha hydroxylation of the monohydroxy C-27 bile acid can also provide a pathway for the production of chenodeoxycholic acid.

Although 26-hydroxycholesterol is not an important precursor of cholic acid in the rat[1,13], it can provide sufficient amounts of the acid in the hamster[1], rabbit[5], and human[14] to be considered a possible precursor along with 7α-hydroxycholesterol. However, the current findings in hamsters strongly suggest that 7 alpha hydroxylation of 26-hydroxycholesterol rather than further oxidation of the side chain must occur to account for significant amounts of cholic acid.

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Biosynthesis of a monoene and a conjugated diene sex pheromone component of the lightbrown apple moth by Δ11 desaturation

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Summary. Fatty acyl moieties present in the female sex pheromone gland of the lightbrown apple moth, *Epiphyas postvittana*, include the analogues of the two sex pheromone components, (*E*)-11-tetradecenyl acetate and (*E,E*)-9,11-tetradecadienyl acetate. Application of deuterium-labelled fatty acids followed by analysis by gas chromatography-mass spectrometry showed that biosynthesis of the two pheromone components involved initial Δ11-desaturation of myristic and palmitic acids respectively.
Key words. Sex pheromone; biosynthesis; Lepidoptera; *Epiphyas postvittana*; deuterium-labelling; (*E*)-11-tetradecenyl acetate; (*E,E*)-9,11-tetradecadienyl acetate.

Over the last 10 years, the biosynthetic pathways of sex pheromone components have been studied in a number of species of Lepidoptera[1]. These sex pheromone components are biosynthesized by a modified fatty acid synthesis occuring in the sex pheromone gland of females. Two important processes involved in these biosyntheses are desaturation and 2 carbon chain-shortening through limited β-oxidation[1]. The different desaturases and the action of these two processes at different stages of pheromone biosynthesis are largely responsible for the structural diversity of common pheromone components found in families such as the Tortricidae and Noctuidae[2].

In the biosynthesis of lepidopteran sex pheromone components the most common type of desaturation is apparently Δ11[1], although Δ9[3] and Δ10[4] are also known. For the biosynthesis of monoenic components a single desaturation step occurs[1]. However, somewhat surprisingly, a single desaturation step has also been found to be involved in the biosynthesis of conjugated dienic compo-