Bile Acid Synthesis in Humans

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Abstract

Metabolic pathways involved in the conversion of cholesterol to cholic and chenodeoxycholic acids have been investigated in bile fistula patients treated with a number of labeled potential bile acid intermediates. The findings of the present report indicate that the human liver has the capacity to synthesize both primary bile acids via multiple routes from cholesterol and 7α-hydroxycholesterol. Evidence has been obtained for the existence of a major pathway to chenodeoxycholic acid via the 26-hydroxylation of 7α-hydroxycholest-4-en-3-one. Cholic acid is synthesized preferentially via pathways from 5β-cholestan-3α,7α-diol and a pathway from cholesterol not involving an initial 7α-hydroxylation.

The generally accepted view of how cholesterol is transformed into cholic and chenodeoxycholic acids within the hepatocyte is based, to a large extent, on studies carried out in the rat (3). The key steps in the reaction sequence involve an initial 7α-hydroxylation of cholesterol to form 7α-hydroxycholesterol; a reaction which is rate limiting for bile acid synthesis. The 7α-hydroxycholesterol intermediate is then converted to the bifurcation compound 7α-hydroxycholest-4-en-3-one. This latter intermediate can be 12α-hydroxylated to form 7α,12α-dihydroxycholest-4-en-3-one, which is committed to cholic acid, or it can be reduced to 5β-cholestan-3α,7α-diol and subsequently 26-hydroxylated and then oxidized to chenodeoxycholic acid. Evidence in support of the existence of similar pathways in human liver has been obtained from in vivo studies in bile fistula patients (1, 2, 4, 5). When 7α-hydroxycholesterol, 7α-hydroxycholest-4-en-3-one, dihydroxycoprostanic acid, and trihydroxycoprostanic acid were administered to these patients, they were found to be efficiently converted to primary bile acids.

Several years ago we attempted to more critically examine each stage of the bile acid biosynthetic pathway in humans with a view toward elucidating the feedback regulation sites. The initial compound tested was labeled 5β-cholestan-3α,7α,26-triol, which, according to the prevailing view, should have been converted almost exclusively to chenodeoxycholic acid since the side chain was oxidized. However, it was found (6) to be converted efficiently to both cholic and chenodeoxycholic acids. This unexpected deviation from the conceptualized version of the bile acid pathway formed the basis for the present series of investigations concerned with elucidation of the metabolic pathways from cholesterol to cholic and chenodeoxycholic acids in humans.

Experiments

Labeled Compounds. The labeled bile acid intermediates were synthesized and purified as described previously (7). The following labeled compounds have been administered to bile fistula patients and normal subjects: 7α-hydroxycholesterol; 12α-hydroxycholesterol; 26-hydroxycholesterol; 7α-hydroxycholest-4-en-3-one; 5β-cholestan-3α,7α-diol; 7α,26-dihydroxycholest-4-en-3-one; 5β-cholestan-3α,7α,26-triol; 5β-cholestan-3α,7α,12α-triol; 5β-cholestan-3α,7α,12α,26-tetrol; dihydroxycoprostanic acid; trihydroxycoprostanic acid; varanic acid; and 5β-cholestan-3α,7α,25-triol.

Other labeled compounds (cholesterol, cholic acid, chenodeoxycholic acid, and deoxycholic acid) were obtained from commercial sources (New England Nuclear, Boston, Mass., and Amersham/Searle Corporation, Arlington Heights, Ill.) and purified by thin-layer chromatography.

Patients. Initial bile acid pathway studies were carried out in patients with complete bile fistulas. The T-tube was allowed to drain externally for 5 to 7 days to achieve a maximal constant rate of bile acid synthesis prior to the start of the experiments. The intermediates were solubilized in a solution of human blood albumin, passed through a 0.22-μm Millipore filter, and administered i.v. to the patients. Bile was then collected at frequent intervals (20 to 60 min) over the next 24 hr. No radioactivity was found in the bile after 24 hr, and most patients then received one or more additional labeled intermediates on ensuing days.

Methods

Bile was extracted with 2:1 chloroform:methanol and partitioned with 1/5 volume of water. Bile acid composition, mass, and radioactivity were determined by a combination of thin-layer chromatography and gas-liquid chromatography as described earlier (6, 7).

Results and Discussion

Primary Bile Acid Synthesis. The extent of conversion of several key selected labeled intermediates to cholic and chenodeoxycholic acids is shown in Table 1. The intermediate 7α-hydroxycholesterol was converted efficiently to both cholic and chenodeoxycholic acids. However, in all patients studied, it formed more labeled chenodeoxycholic and less labeled cholic acid than would be expected from the mass (or endogenous synthesis) distribution of the primary bile acids. Similar findings were made with 7α-hydroxycholest-4-en-3-one. These observations suggested the presence of an additional pathway to cholic acid directly from cholesterol. Attempts to elucidate this pathway have thus far been unsuccessful; 26-hydroxycholesterol, 25-hydroxycholesterol, and 12α-hydroxycholesterol

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2 To whom requests for reprints should be addressed.
Conversion of $^3$H intermediates to bile acids in humans

<table>
<thead>
<tr>
<th>No. of</th>
<th>Labeled compounds administered</th>
<th>% of $^3$H activity recovered as primary bile acids</th>
<th>$^3$H (synthesis)</th>
<th>Mass (synthesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>patients</td>
<td>of $^3$H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7a-hydroxycholesterol</td>
<td>81.5 ± 4.2$^b$</td>
<td>1.02 ± 0.13</td>
<td>2.16 ± 0.23</td>
</tr>
<tr>
<td>4</td>
<td>7a-hydroxycholesterol-4-en-3-one</td>
<td>71.9 ± 3.1</td>
<td>0.96 ± 0.15</td>
<td>1.95 ± 0.27</td>
</tr>
<tr>
<td>4</td>
<td>5β-cholane 3a, 7a-diol</td>
<td>76.0 ± 5.4</td>
<td>1.64 ± 0.21</td>
<td>2.01 ± 0.36</td>
</tr>
<tr>
<td>3</td>
<td>7a, 26-dihydroxycholesterol-4-en-3-one</td>
<td>82.4 ± 10.8</td>
<td>0.32 ± 0.03</td>
<td>2.15 ± 0.27</td>
</tr>
<tr>
<td>6</td>
<td>5β-cholane 3a, 7a, 26-triol</td>
<td>55.2 ± 4.2</td>
<td>1.19 ± 0.12</td>
<td>1.92 ± 0.16</td>
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<tr>
<td>3</td>
<td>5β-cholane 3a, 7a, 25-triol</td>
<td>25.9 ± 11.7</td>
<td>3.48 ± 1.37</td>
<td>1.72 ± 0.41</td>
</tr>
<tr>
<td>3</td>
<td>5β-cholane 3a, 7a, 12α-triol</td>
<td>73.0 ± 1.0</td>
<td></td>
<td>1.89 ± 0.22</td>
</tr>
</tbody>
</table>

$^a$ Bile fistula patients.

$^b$ Mean ± S.E.

Chart 1. Proposed multiple pathways to bile acids from cholesterol in humans.

were tested and found to be poorly converted to bile acids. The intermediate 5β-cholane 3a, 7a, 26-triol was efficiently converted to bile acids but favored cholic acid over chenodeoxycholic acid when compared to its parent compound, 7α-hydroxycholesterol-4-en-3-one. This observation was also unexpected since reduction of the Δ4-3-keto compound has been generally accepted as a commitment to chenodeoxycholic acid. Labeled 5β-cholane 3a, 7a, 26-triol and 5β-cholane 3a, 7a, 12α, 26-tetrol were converted efficiently and exclusively to cholic acid. The comparative findings between 7α-hydroxycholesterol-4-en-3-one which favored chenodeoxycholic acid and 5β-cholane 3a, 7a-diol which favored cholic acid suggested the presence of an additional pathway to chenodeoxycholic acid. This possibility was examined by administering 7α, 26-dihydroxycholesterol-4-en-3-one. This compound was found to be very efficient in forming chenodeoxycholic acid in 3 patients. The anomalous behavior of this compound as compared to 5β-cholane 3a, 7a, 26-triol suggests the presence on this pathway of yet an additional intermediate which could perhaps be 3-keto, 7α-hydroxy-5β-cholestanoic acid. A schematic presentation of the present status of the major bile acid pathways in humans is shown in Chart 1. The available information suggests that there are multiple pathways to both cholic and chenodeoxycholic acids from cholesterol and at several points beyond 7α-hydroxycholesterol. There also appear to be several suitable substrates for 7α, 12α, 26-hydroxylation. Thus, human liver has the capacity to form bile acids via several routes in order to efficiently degrade cholesterol to bile acids.

References

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