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Species differences among various rodents in the conversion of 7α-hydroxycholesterol in liver microsomes

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ABSTRACT

Our previous study demonstrated that there are species differences among vertebrates in their conversion of 7α-hydroxycholesterol (7HC) to 7-ketocholesterol (7KC). To examine this further, we investigated the differences in the products of 7α-hydroxycholesterol in various species of male muroid rodents. Adult male Syrian hamsters (*Mesocricetus auratus*), dwarf hamsters (*Phodopus rovolovskii*), Djungarian hamsters (*Phodopus sungorus*), Chinese hamsters (*Cricetulus griseus*), rat-like hamsters (*Tscherskia triton*), and hispid cotton rats (*Sigmodon hispidus*) were used. Microsomal fractions were prepared from their livers, and the activities of the enzymes that participate in the dehydrogenation of 7α-hydroxycholesterol were determined by measuring the products using high-performance liquid chromatography. 7α-hydroxycholesterol was converted to both 7α-hydroxy-4-cholesten-3-one (7HCO) and 7-ketocholesterol in all of the hamsters tested. However, in the rat-like hamster and the hispid cotton rat, 7α-hydroxycholesterol was converted mostly to 7α-hydroxy-4-cholesten-3-one, as also observed in the rat (*Rattus norvegicus*).

The results suggest that microsomal enzyme activity in the conversion of 7α-hydroxycholesterol to 7-ketocholesterol varies considerably, even within the subfamily Cricetinae and the family Muridae.

1. Introduction

In mammals, 7α-hydroxycholesterol (7HC) is the first intermediate product of cholesterol in the bile acid metabolic pathway [1,2]. It is then further converted into 7α-hydroxy-4-cholesten-3-one (7HCO) in the liver by the microsomal enzyme 3β-hydroxy-Δ^5-C27-steroid dehydrogenase [3,4]. 7-Ketocholesterol (7KC), one of the oxysterols, is distributed in various tissues in the body, including the aorta, brain, kidney, and liver [5]. Structures of these steroids are shown in Fig. 1. 7KC is not only an autoxidation product of cholesterol, 7HC, and 7β-hydroxycholesterol, but is also produced from 7HC and 7β-hydroxycholesterol enzymatically [4,6,7]. In our recent comparative study of the conversion of 7HC and 7β-hydroxycholesterol into 7KC in various animals, we found species differences [8]. 7HC is converted not only to 7HCO, but also to 7KC in the Syrian hamster and the chicken. Conversion of 7β-hydroxycholesterol to 7KC was observed in all of the vertebrates tested.

In this study, inter/intra-subfamily differences in the conversion of 7αHC were investigated in six muroid rodents with special reference to hamsters and rats.

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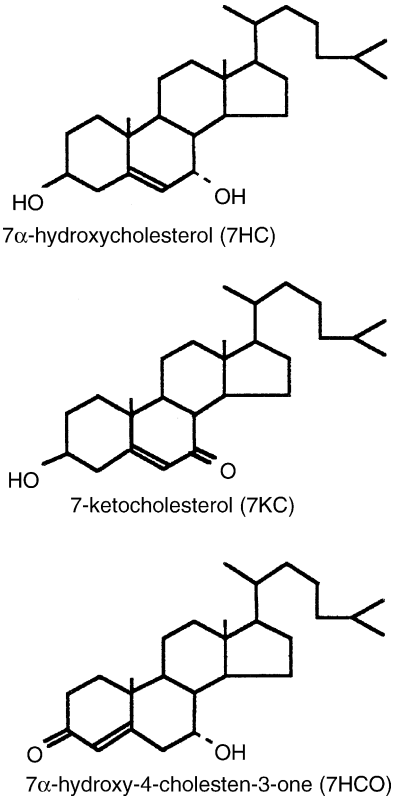

Fig. 1 – Structures of 7α-hydroxycholesterol (7HC), 7α-hydroxycholest-4-ene-3-one (7HCO), and 7-ketocholesterol (7KC).

2. Experimental

2.1. Materials

7α-Hydroxycholesterol and 7-ketocholesterol were purchased from Steraloids (Wilton, NH). 7α-Hydroxy-4-cholesten-3-one was synthesized according to the method described by Shimasue [9], with a slight modification. Hexane, isopropanol methanol (of high-performance liquid chromatography (HPLC) grade) and glycerol were purchased from Wako Pure Chemical Industries (Osaka, Japan). β-NAD^+ and β-$NADP^+$ were purchased from Oriental Yeast (Tokyo, Japan). Other reagents were of the highest grade commercially available.

2.2. Experimental design

Male Syrian hamsters (*Mesocricetus auratus*) weighing 80–95 g, male dwarf hamster (*Phodopus rovolovskii*) weighing 20–30 g, male Djungarian hamsters (*Phodopus campbelli*) weighing 23–35 g, male Chinese hamsters (*Cricetulus griseus*) weighing 22–35 g, male rat-like hamsters (*Tscherskia triton*) weighing 140–160 g, and hispid cotton rats (*Sigmodon hispidus*) weighing 75–95 g were donated by the Department of Bio-resources, Division of Biotechnology, Frontier Science Research Center, University of Miyazaki (Miyazaki, Japan). Animals were housed in groups of 2–4 animals per cage, with a bedding of wood chips and kept in a temperature-controlled environment (23 ± 1 °C with 50 ± 10% relative humidity) with a 12-h light:12-h dark cycle (lights on 8:00 a.m.). They were fed a commercial rat chow, CE-2 (Clea Japan, Tokyo, Japan) ad libitum, and were acclimated to this food and the light-dark cycle for 1 week before the experiments. The rat (*Rattus norvegicus*) was used as the control for comparison. The research protocol was approved by the Animal Study Committee of the University of Miyazaki, and all of the experiments were carried out under the guidelines for animal experiments of the University of Miyazaki.

Animals were anesthetized with diethyl ether. The livers were removed quickly, perfused with 0.9% NaCl and then homogenized with 4 volumes of 0.25 M sucrose containing 5 mM Tris–HCl and 1 mM EDTA, pH 7.4. The homogenate was centrifuged at 9000 × *g* for 15 min in the cold. The supernatant was centrifuged at 105,000 × *g* for 60 min. The precipitate was washed and recentrifuged at 105,000 × *g* for 60 min. The microsomal precipitate was suspended in 100 mM Tris–HCl buffer, pH 7.4, containing 20% glycerol, 1 mM EDTA, and 0.5 mM dithiothreitol (DTT).

2.3. Equipment and chromatographic conditions

A high-performance liquid chromatograph (HPLC) produced by Shimadzu (Kyoto, Model LC-8A) was used. The HPLC column (Wakosil 5SIL, 4.6 mm × 250 mm) was obtained from Wako Pure Chemical Industries (Osaka, Japan) equipped with an UV–vis detector set at 240 nm and an integration system (Shimadzu; Model Chromatopack C-R3A).

2.4. Enzyme assay

Enzyme activity was measured as described previously [8,10]. A typical assay mixture contained 0.1 M of potassium phosphate buffer, pH 7.4, 1 mM EDTA, 0.1–0.2 mg of microsomal protein, and 50 nmol of 7α-hydroxycholesterol dissolved in 10 μl of isopropanol, and 0.5 μmol of β-NAD^+, or β-$NADP^+$, in a final volume of 1.0 ml. Incubation was carried out at 37 °C for 5 min and stopped by adding 1 ml of methanol. Steroids were extracted with 5 ml of *n*-hexane. An aliquot of the extract was evaporated, dissolved in isopropanol, and injected into the HPLC. In all incubations, a sample without substrate served as the control. A mixture of *n*-hexane and isopropanol (80:20, v/v) was used for the elution of steroids, at a flow rate of 1 ml/min at room temperature. Effluent absorbance was monitored at 240 nm. Using a Shimadzu integrator–recorder, C-RIS, peak areas were measured.

To distinguish whether a non-enzymatic product of 7-ketocholesterol was present or not, we assayed both heated and unheated microsomal suspensions. Heated microsomes were prepared by boiling at 80 °C for 5 min.

2.5. Other methods

Protein concentrations were determined by the method of Lowry et al., using bovine serum albumin as the standard [11].

2.6. Statistics

Data are presented as the mean ± standard deviation of the mean (S.D.). Statistical analyses were performed using