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Rapid Hepatic Metabolism of 7-Ketocholesterol by 11β-Hydroxysteroid Dehydrogenase Type 1

SPECIES-SPECIFIC DIFFERENCES BETWEEN THE RAT, HUMAN, AND HAMSTER ENZYME*

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The role of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in the local activation of the glucocorticoid receptor by converting inactive 11-ketoglucocorticoids to active 11β-hydroxyglucocorticoids is well established. Currently, 11β-HSD1 is considered a promising target for treatment of obese and diabetic patients. Here, we demonstrate a role of 11β-HSD1 in the metabolism of 7-ketocholesterol (7KC), the major dietary oxysterol. Comparison of recombinant 11β-HSD1, transiently expressed in human embryonic kidney 293 cells, revealed the stereo-specific interconversion of 7KC and 7β-hydroxycholesterol by rat and human 11β-HSD1, whereas the hamster enzyme interconverted 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7KC. In contrast to lysates, which efficiently catalyzed both oxidation and reduction, intact cells exclusively reduced 7KC. These findings were confirmed using rat and hamster liver homogenates, intact rat hepatocytes, and intact hamster liver tissue slices. Reduction of 7KC was abolished upon inhibition of 11β-HSD1 by carbenoxolone (CBX) or 2′-hydroxyflavanone. *In vivo*, after gavage feeding rats, 7KC rapidly appeared in the liver and was converted to 7β-hydroxycholesterol. CBX significantly decreased the ratio of 7β-hydroxycholesterol to 7KC, supporting the evidence from cell culture experiments for 11β-HSD1-dependent reduction of 7KC to 7β-hydroxycholesterol. Upon inhibition of 11β-HSD1 by CBX, 7KC tended to accumulate in the liver, and plasma 7KC concentration increased. Together, our results suggest that 11β-HSD1 efficiently catalyzes the first step in the rapid hepatic metabolism of dietary 7KC, which may explain why dietary 7KC has little or no effect on the development of atherosclerosis.

Several *in vitro* studies demonstrated disturbances of cholesterol metabolism by 7KC[1] (1), including effects on hydroxymethylglutaryl-CoA-reductase and cholesterol 7α-hydroxylase activities (2), inhibition of cholesterol release from cells (3), and down-regulation of low density lipoprotein receptor expression (4). In addition, 7KC exerts multiple effects by inhibiting nitric oxide release, decreasing glucose permeability, disrupting Ca^{2+}-flux and inducing apoptosis in vascular cells (1).

Oxidized cholesterol metabolites play a potential role in the development of atherosclerosis (1, 5, 6). Among other oxysterols, 7KC is found at micromolar concentrations in human macrophage-foam cells and atherosclerotic lesions. In contrast, plasma 7KC concentrations are in the nanomolar range. The major oxysterols present in atherosclerotic plaques are 27-hydroxycholesterol and 7KC, although the direct role of 7KC in the development and progression of atherosclerosis is still unclear. 27-Hydroxycholesterol is produced by sterol 27-hydroxylase, the first enzyme of the alternative pathway from cholesterol to bile acids in the liver (7). 7KC is believed to be formed non-enzymatically by free radical oxidation of cholesterol (8) or absorbed from dietary intake of processed cholesterol-rich food (9, 10).

It was reported that upon administration, 7KC was rapidly metabolized in the liver and only little 7KC reappeared in the circulation, thus questioning the contribution of diet to the high oxysterol concentrations in atherosclerotic lesions (11, 12). Recent evidence suggested that sterol 27-hydroxylase is involved in 7KC metabolism. Patients with cerebrotendinous xanthomatosis have genetic defects in sterol 27-hydroxylase and develop atherosclerosis prematurely (13). They have normal circulating cholesterol levels but increased 7KC. Human monocyte-derived macrophages from healthy individuals but not from patients with cerebrotendinous xanthomatosis converted 7KC to 27-hydroxy-7KC (14). These results indicate an essential role for sterol 27-hydroxylase in the metabolism of 7KC in macrophage-foam cells. However, a recent study in sterol 27-hydroxylase-deficient mice demonstrated efficient hepatic metabolism of 7KC (15), thus indicating that another enzyme must be involved in the initial step of 7KC metabolism in the liver.

In addition to the formation of 7KC by auto-oxidation or its uptake from food, recent evidence from experiments with purified protein and liver microsomal fractions suggest that 7KC may be formed enzymatically from either 7β-hydroxycholesterol or 7α-hydroxycholesterol in various species (16, 17). Song *et al.* (16) purified an enzyme from hamster liver microsomes, which efficiently converted both 7α-hydroxycholesterol and 7β-hydroxycholesterol to 7KC. This enzyme also catalyzed the oxidation of corticosterone to 11-dehydrocorticosterone, and N-terminal sequencing of the purified enzyme revealed high similarity to human and rat 11β-HSD1. However, unlike 11β-HSD1, the purified hamster liver enzyme did not catalyze the

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[1] The abbreviations used are: 7KC, 7-ketocholesterol, 3β-hydroxy-5-cholesten-7-one; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 7β-hydroxycholesterol, 5-cholesten-3β,7β-diol; 7α-hydroxycholesterol, 5-cholesten-3β,7α-diol; 27-hydroxycholesterol, 5-cholesten-3β,27α-diol; CBX, carbenoxolone; HEK-293 cells, human embryonic kidney-293 cells; GC-MS, gas chromatography-mass spectrometry.

reduction of 11-dehydrocorticosterone to corticosterone, and immunochemical analysis with an antibody raised against the purified enzyme suggested the existence of two distinct 11β-HSD-like proteins in hamster liver. Moreover, Robinzon *et al.* (18) recently observed corticosterone inhibitable interconversion of the dehydroepiandrosterone (DHEA) metabolites 7α-DHEA, 7β-DHEA, and 7-keto-DHEA in liver microsomal fractions, further suggesting a potential role of 11β-HSD enzymes in the metabolism of 7-oxysterol compounds.

The role of 11β-HSD1 in the local activation of glucocorticoid receptor by catalyzing the reduction of biologically inactive 11-ketoglucocorticoids (cortisone in humans, 11-dehydrocorticosterone in rodents) to active 11β-hydroxyglucocorticoids (cortisol in humans, corticosterone in rodents) is well characterized (19, 20). Enhanced glucocorticoid levels lead to increased gluconeogenesis and antagonize the metabolic actions of insulin. Transgenic mice overexpressing 11β-HSD1 selectively in adipose tissue have elevated adipose corticosterone concentrations, and they are susceptible to insulin-resistant diabetes, hyperlipidemia, and high arterial blood pressure due to an increased sensitivity to dietary salt and increased plasma levels of angiotensinogen, angiotensin II, and aldosterone (21, 22). Because adipose 11β-HSD1 activity positively correlates to body mass index, percentage of body fat, and waist circumference as well as fasting glucose, insulin levels, and insulin resistance (23–25), 11β-HSD1 is currently considered a promising pharmaceutical target for the treatment of diabetes type 2. Indeed, recent animal studies showed that the administration of a selective 11β-HSD1 inhibitor to diabetic mice reduced blood glucose levels and increased insulin sensitivity (26, 27). In addition to its role in the activation of glucocorticoids, 11β-HSD1 might play a role in the detoxification of reactive ketocompounds such as the potent tobacco carcinogen nicotine-derived nitrosamine ketone (NNK) and the anti-cancer drug oracin (28, 29).

In the present study we tested the hypothesis of whether 11β-HSD1 plays a role in the interconversion of 7-hydroxycholesterol and 7KC. We compared recombinant rat, human and hamster 11β-HSD1 in lysates and intact HEK-293 cells and measured the activities in rat and hamster liver homogenates and in intact rat hepatocytes and hamster liver tissue slices. We also studied the effect of the 11β-HSD inhibitor CBX on 7KC metabolism *in vivo* in rats.

EXPERIMENTAL PROCEDURES

Materials—Cell culture media were purchased from Invitrogen, corticosterone, 11-dehydrocorticosterone, 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7KC were from Steraloids (Wilton, NH), and [1,2,6,7-^{3}H]corticosterone was from Amersham Biosciences. 11-[1,2,6,7-^{3}H]Dehydrocorticosterone was prepared from radiolabeled corticosterone as described earlier (30). 27-Hydroxycholesterol was purchased from Medical Isotopes (Pelham, NH), and [1,2,6-^{3}H]KC was from American Radiolabeled Chemicals, St. Louis, MO. Reagents for derivatization were obtained from Pierce. All other chemicals were from Fluka AG (Buchs, Switzerland) and were of the highest grade available.

Cloning of Hamster 11β-HSD1—The liver from a Golden Syrian Hamster (Charles River Laboratories) was snap-frozen in liquid nitrogen followed by extraction of total RNA from 100 mg of tissue using Trizol reagent according to the instructions by the manufacturer (Invitrogen). Hamster 11β-HSD1 was cloned by reverse transcription of 5 μg of total RNA with primer XRT_{20} (5′-TATTCTAGACACCTGAGCA-CGT_{20}-3′) and subsequent PCR amplification using *Taq* polymerase:*Pfu* polymerase (10:1), primer XR (5′-TATTCTAGACACCTGAGCA-CG-3′), and a degenerate primer (5′-AAAGGATCCGCCTCCCTGTCT-GATG(C/G)(A/C)(C/T)TT(T/C)ATG-3′) based on comparison with the sequences upstream of the initiation codon of 11β-HSD1 from other species. A single DNA product was obtained, cleaved with BamHI and XbaI, and cloned into Bluescript vector. The sequences of all 10 clones analyzed were identical. The cDNA insert with or without a C-terminal FLAG epitope tag that was attached by PCR, was inserted into the pcDNA3 expression vector.

Preparation of Liver Homogenates—The livers from male Sprague-Dawley rats or, alternatively, from Golden Syrian hamsters, were removed, immersed in phosphate-buffered saline at 4 °C, cut in small pieces, and homogenized in buffer TG1 (20 mM Tris-HCl, pH 7.4, 1 mM EGTA, 1 mM EDTA, 1 mM $MgCl_2$, 100 mM NaCl, 20% glycerol) (5 ml/g) using a Potter-Elvehjem homogenizer. The homogenates were subjected to a single centrifugation step at 10,000 × *g* for 10 min at 4 °C. The supernatants were adjusted to a protein concentration of 0.1 mg/ml, and activities were determined immediately.

Isolation of Rat Hepatocytes—Male Sprague-Dawley rats (200–250 g body weight) were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital. Heparin (liquemin), 1 ml/kg, was injected into the vena cava followed by perfusion for 15 min with 16 ml/min of prewarmed, oxycarbon-aerated buffer containing 10 mM HEPES, pH 7.4, 143 mM NaCl, 7 mM KCl, and 0.2 mM EGTA. The liver was then perfused for 20 min with 10 ml/min of a buffer containing 50 mM HEPES, pH 7.6, 100 mM NaCl, 7 mM KCl, 5 mM $CaCl_2$, and 0.1% (w/v) collagenase (*Clostridium histolyticum* type IV, Sigma). The liver was removed and incubated in a buffer containing 10 mM HEPES, pH 7.4, 0.8 mM Na_2HPO_4, 135 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4$, 1.2 mM $CaSO_4$, and 5 mM glucose, the capsule was opened, and hepatocytes were harvested. Cells were incubated with slight shaking for 10 min at 37 °C. Hepatocytes were filtered through a 100-μm pore size nylon mesh to separate from biliocytes, washed twice in the same buffer, and centrifuged for 2 min at 50 × *g*. Cells were finally suspended in steroid-free Dulbecco's modified Eagle's medium, and enzymatic activities were measured immediately.

Preparation of Intact Hamster Liver Slices—Hamster livers were removed, rinsed thoroughly with Hanks' buffered salt solution, and cut in thin slices of ~300 μm with parallel razor blades similar to the method for precision-cut liver slices described by Dogterom and Rothuizen (31). Slices were rinsed with Hanks' buffer, and activities were measured immediately without adding NADPH. When the slices were lysed, no conversion of 7KC was detected unless NADPH was added to the reaction.

Determination of Enzyme Activities—HEK-293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum followed by transient transfection with rat 11β-HSD1 expression plasmid (32). Cells were washed three times 24 h post-transfection with steroid-free (double charcoal-treated) medium and grown for another 24 h. Cells were then detached with phosphate-buffered saline, centrifuged for 3 min at 150 × *g*, and resuspended in the appropriate volume of TG1 buffer. Cells were lysed by sonication, and activities were determined immediately.

11β-HSD1 activities were measured as described recently, with minor modifications (30). Briefly, lysates were incubated in TG1 buffer containing 500 μM $NADP^+$ or NADPH, 30 nCi of [1,2,6,7-^{3}H]corticosterone or 11-[1,2,6,7-^{3}H]dehydrocorticosterone, and various concentrations of unlabeled steroids ranging from 20 nM to 2 μM. Samples in a final volume of 20 μl were incubated at 37 °C for 10–30 min, reactions were stopped, and steroids were separated by thin-layer chromatography (TLC) and analyzed by scintillation counting. When measuring activities in intact cells, steroid-free medium was used and cofactor was omitted.

7-Hydroxydehydrogenase and 7-oxoreductase activities in cell lysates or liver homogenates were measured in buffer TG1 in the presence of various concentrations of 7α-hydroxycholesterol, 7β-hydroxycholesterol, or 7KC ranging from 50 nM to 2.5 μM and the corresponding cofactor in a final reaction volume of 1 ml. Reactions were incubated for 10–30 min at 37 °C and stopped by adding 7 ml of dichloromethane. Intact transfected HEK-293 cells or rat hepatocytes were incubated in 1 ml of steroid-free medium containing the corresponding cholesterol metabolite and stopped as mentioned above. Samples were then subjected to derivatization and analysis by gas chromatography-mass spectrometry (GC-MS). Alternatively, the reduction of 7KC in intact rat hepatocytes or in intact hamster liver slices was measured by adding 50 nCi of radiolabeled 7KC as a tracer followed by determination of the conversion after separation of 7-oxycholesterols by TLC.

Thin-layer Chromatography—Radiolabeled corticosterone and 11-dehydrocorticosterone were separated by TLC (SIL G-25 UV254, Macherey-Nagel, Oensingen, Switzerland) using a solvent system of 9:1 (v/v) chloroform:methanol. A solvent system 2:3 (v/v) hexane:ethyl acetate was used to separate radiolabeled 7KC, 7α-hydroxycholesterol, and 7β-hydroxycholesterol. 7KC was detected under UV light, whereas 7α-hydroxycholesterol and 7β-hydroxycholesterol were visualized using a molybdate spray (100 ml of 10% H_2SO_4, 5 g of $(NH_4)Mo_7O_{24}{\cdot}6H_2O$, 0.1 g of $Ce(SO_4)_2$).

Animal Experimentation—Written approval for the present experiments was obtained from the Ethical Committee for Animal Research of

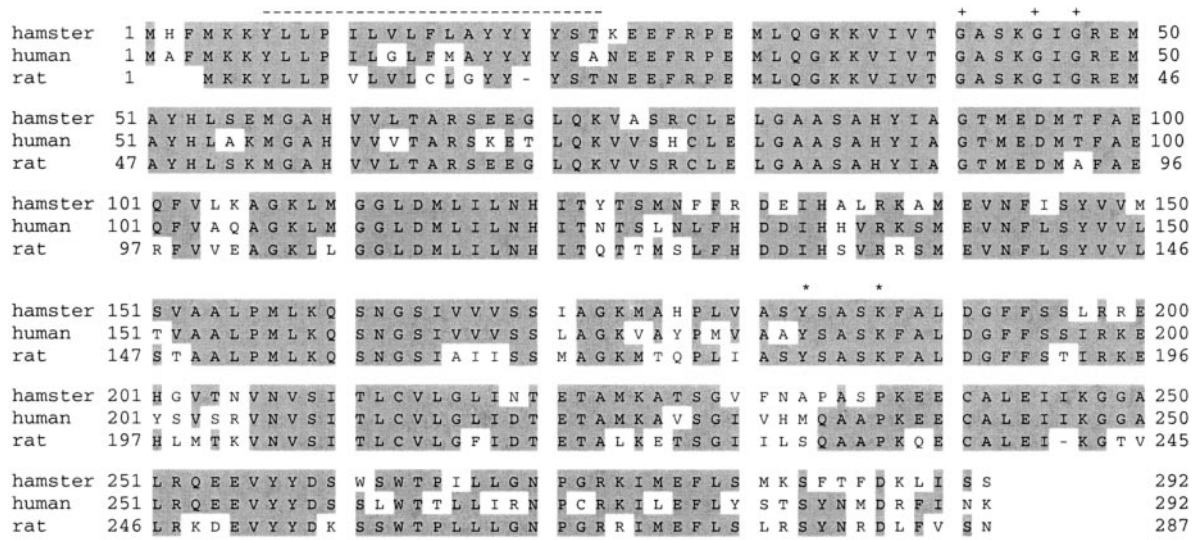

FIG. 1. **Alignment of the protein sequences of hamster, human, and rat 11β-HSD1 (accession numbers: AY519498, NM_ 005525, and NM_017080).** Identical residues are *shaded*. The conserved tyrosine and lysine residues in the catalytic center (*), the transmembrane helix (—), and the conserved glycine residues of the Rossmann-fold in the cofactor binding region (+) are indicated.

the University of Berne, Switzerland. Male Sprague-Dawley rats (200–250 g body weight) obtained from Charles River Laboratories, were fed standard laboratory chow *ad libitum*. Groups of five animals each received 2 mg/kg of body weight of 7KC by gavage feeding. Animals were sacrificed after 1, 2, 4, 6, or 8 h, and plasma and tissues were harvested. To study the effect of the 11β-HSD inhibitor CBX, animals were intraperitoneally injected with 15 mg/kg of body weight of CBX 1 h before administration of 7KC and 2 and 5 h after 7KC administration, respectively. For analysis of plasma electrolytes and steroid hormones, groups of four animals were treated as above and sacrificed 6 h after 7KC administration. Plasma aldosterone and progesterone were measured by radioimmunoassay using the coat-a-count procedure as described by the manufacturer (Diagnostic Products Corp., Los Angeles, CA). Other steroid metabolites were determined by GC-MS as described previously (33).

Analysis of 7-Oxycholesterols by GC-MS—After removal, tissues were washed from contaminating blood with phosphate-buffered saline, blotted on filter paper, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis. 7-Oxycholesterols were analyzed according to the method described by Iuliano *et al.* (34) with modifications. Tissues (100–250 mg) were cut into small pieces and homogenized in a solution containing 10 ml of chloroform:methanol (2:1, v/v), 0.01% butylated hydroxytoluene (v/w) and transferred to a tube containing 2 ml of 0.9% NaCl (H_2O), methanol (19:1) and 100 ng of 6α-hydroxycholestanol (5α-cholestan-3β,6α-diol) as the internal standard. Plasma samples (1 ml), obtained from EDTA anti-coagulated blood, were treated as above but without homogenization. After mixing, samples were centrifuged at 750 × *g* for 5 min at 4 °C. The organic phase was washed twice with 4 ml of methanol, 0.9% NaCl (1:1, v/v) and centrifuged, and the organic phase was subjected to alkaline hydrolysis (saponification) with 0.35 M KOH under an argon atmosphere for 2 h at 25 °C. The reaction mixture was adjusted to pH 7.0 with phosphoric acid and centrifuged, and the organic phase was washed twice with 2 ml of 0.9% NaCl (H_2O), methanol (19:1). The organic phase was transferred to a new tube containing 100 μl of 1% butylated hydroxytoluene in pyridine. For the external standard 125 ng of stigmasterol was added followed by evaporation of the solvent. Next, samples were loaded on a silica cartridge (Supelco), and cholesterol was removed with 0.5% 2-propanol in cyclohexane. Oxidized cholesterol metabolites were eluted with 30% 2-propanol in cyclohexane into a tube containing 100 μl of 1% butylated hydroxytoluene in pyridine, the solvent was evaporated under nitrogen, and the sample was converted to trimethylsilyl ether by adding 100 μl of pyridine and 100 μl of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide. Samples were incubated under argon for 1 h at 60 °C. 200 μl of cyclohexane were added to the residue, and the sample was subjected to GC-MS analysis.

Enzyme activities from cell lysates or intact cells were measured in 1-ml reaction volumes and stopped by the addition of 7 ml of dichloromethane. The internal standard medroxyprogesterone (0.5 μg) was added, and samples were mixed for 20 min at 4 °C followed by centrifugation for 5 min at 2000 × *g*. The organic phase was transferred to new tubes, and dichloromethane was evaporated at 25 °C under argon. For the external standard 0.5 μg of stigmasterol was added. The solvent was evaporated, and 100 μl of 2% methoxamine-HCl in pyridine was added to protect keto groups followed by incubation for 1 h at 60 °C. After evaporation of the solvent, 100 μl of trimethylsilylimidazole was added to protect hydroxyl groups followed by incubation for 16 h at 100 °C. After cooling to 25 °C, 500 μl of cyclohexane:pyridine:hexamethyldisilazane (98:1:1) was added, and samples were purified on Lipidex-5000 columns. Another 2.5 ml of the above mixture were added, samples were collected, and the solvent was evaporated. Cholesterol metabolites were dissolved in 200 μl of cyclohexane, sonicated for 1 min, and subjected to GC-MS analysis on a Hewlett Packard gas chromatograph 6890 equipped with a mass-selective detector 5973 by selected ion monitoring).

RESULTS

Cloning of Hamster Liver 11β-HSD1—To test whether 11β-HSD1 accepts both glucocorticoids and 7-oxycholesterols as substrates and whether the hamster 7α-hydroxycholesterol dehydrogenase purified by Song *et al.* (16) is indeed 11β-HSD1, we compared the activities of rat, human, and hamster 11β-HSD1. The coding region of 11β-HSD1 was cloned from liver RNA of a Golden Syrian hamster. PCR amplification yielded a single fragment (accession number AY519498) encoding a protein of 292 amino acids. Fig. 1 shows ~90% identity between hamster, rat, and human 11β-HSD1. The N-terminal sequence of hamster 11β-HSD1 is identical to the peptide sequence of the 7α-hydroxycholesterol dehydrogenase purified by Song *et al.* (16), demonstrating that their purified protein is indeed 11β-HSD1 (16).

Comparison of Recombinant Rat, Human, and Hamster 11β-HSD1 Activities—Recombinant rat, human, and hamster 11β-HSD1 were expressed in HEK-293 cells, and activities in cell lysates were determined. Untransfected cells neither interconverted glucocorticoids nor 7-oxycholesterols. 11β-HSD1 from all three species efficiently catalyzed both the oxidation of corticosterone and the reduction of 11-dehydrocorticosterone (Table I). The activities in the three species were comparable, except that human 11β-HSD1 catalyzed the oxidation of corticosterone with ~3-fold lower apparent K_m and 3-fold lower apparent V_{max}. This was also observed when activities were measured in intact cells (Table II). The reduction of 11-dehydrocorticosterone in intact cells expressing recombinant 11β-HSD1 was comparable in all three species.

Lysates expressing recombinant rat or human 11β-HSD1 stereospecifically oxidized 7β-hydroxycholesterol to 7KC (Table I). The hamster enzyme efficiently oxidized both 7α-hydroxy-

TABLE I

Oxidation and reduction by lysates from HEK-293 cells expressing recombinant 11β-HSD1

Enzymatic activities of lysates from HEK-293 cells transiently transfected with either human, rat or hamster 11β-HSD1 were determined by measuring the reduction of 11-dehydrocorticosterone and 7KC in the presence of NADPH or the oxidation of corticosterone and 7-hydroxycholesterols in the presence of $NADP^+$ as described under "Experimental Procedures." Data represent the mean ± S.D. and were obtained from at least four independent experiments. ND, measurements were below the detection limit. NA, not analyzed.

Species	K_m [a]	V_{max} [a,b]	V_{max}/K_m
	nM	*nmol × h⁻¹ × mg⁻¹*	*h⁻¹ × mg⁻¹ × 10⁻³*
Oxidation of corticosterone			
Human	305 ± 48	1.1 ± 0.3	3.6
Rat	1170 ± 136	3.2 ± 0.6	2.7
Hamster	1296 ± 127	3.0 ± 1.1	2.3
Reduction of 11-dehydrocorticosterone			
Human	314 ± 42	1.4 ± 0.2	4.5
Rat	307 ± 31	1.4 ± 0.5	4.6
Hamster	396 ± 82	1.5 ± 0.6	3.8
Oxidation of 7α-hydroxycholesterol			
Human	NA	ND	NA
Rat	NA	ND	NA
Hamster	486 ± 62	1.4 ± 0.3	2.9
Oxidation of 7β-hydroxycholesterol			
Human	739 ± 67	1.6 ± 0.3	2.2
Rat	751 ± 196	2.4 ± 0.8	3.2
Hamster	729 ± 69	1.5 ± 0.4	2.1
Reduction of 7-ketocholesterol			
Human	487 ± 50	0.64 ± 0.06	1.3
Rat	420 ± 67	0.61 ± 0.20	1.5
Hamster	384 ± 44	0.63 ± 0.15	1.6

[a] Apparent K_m (nM) and apparent V_{max} (nmol × min^{-1} × mg of total $protein^{-1}$) were calculated using the Eadie-Hofstee equation assuming first order rate kinetics.

[b] For calculation of apparent V_{max} the amount of 11β-HSD1 protein was compared by densitometric analysis of Western blots.

TABLE II

Oxidation and reduction of glucocorticoids and 7-oxycholesterols in intact HEK-293 cells expressing recombinant 11β-HSD1

Enzymatic activities in intact HEK-293 cells transiently expressing either human, rat, or hamster 11β-HSD1 were determined by measuring the reduction of 11-dehydrocorticosterone and 7KC or the oxidation of corticosterone and 7-hydroxycholesterols in steroid-free medium as described under "Experimental Procedures." Data represent the mean ± S.D. and were obtained from at least four independent experiments.

Species	K_m [a]	V_{max} [a,b]	V_{max}/K_m
	nM	*nmol × h⁻¹ × mg⁻¹*	*h⁻¹ × mg⁻¹ × 10⁻³*
Oxidation of corticosterone			
Human	165 ± 12	0.45 ± 0.11	2.7
Rat	367 ± 52	0.97 ± 0.30	2.6
Hamster	321 ± 48	1.2 ± 0.4	3.7
Reduction of 11-dehydrocorticosterone			
Human	594 ± 127	0.51 ± 0.16	0.9
Rat	681 ± 75	0.57 ± 0.21	0.8
Hamster	726 ± 118	0.56 ± 1.2	0.8
Oxidation of 7α-hydroxycholesterol			
Human	NA	ND	NA
Rat	NA	ND	NA
Hamster	NA	ND	NA
Oxidation of 7β-hydroxycholesterol			
Human	NA	ND	NA
Rat	NA	ND	NA
Hamster	NA	ND	NA
Reduction of 7-ketocholesterol			
Human	564 ± 62	0.21 ± 0.04	0.37
Rat	776 ± 154	0.14 ± 0.04	0.18
Hamster	549 ± 72	0.20 ± 0.05	0.36

[a] Apparent K_m (nM) and apparent V_{max} (nmol × min^{-1} × mg of total $protein^{-1}$) were calculated using the Eadie-Hofstee equation assuming first order rate kinetics.

[b] For calculation of apparent V_{max} the amount of 11β-HSD1 protein was compared by densitometric analysis of Western blots.

cholesterol and 7β-hydroxycholesterol to 7KC, with a slight preference for 7α-hydroxycholesterol. All three species efficiently catalyzed the reduction of 7KC, whereby rat and human 11β-HSD1 acted stereo-specifically, forming exclusively 7β-hydroxycholesterol, whereas hamster 11β-HSD1 led to the formation of both 7α-hydroxycholesterol (60%) and 7β-hydroxycholesterol (40%). Untransfected HEK-293 cells or cells transfected with rat or human 11β-HSD2 were unable to reduce 7KC.

Intact cells expressing rat, human, or hamster 11β-HSD1 efficiently catalyzed the reduction of 7KC; however, in contrast to lysates, 11β-HSD1 did not act as 7-hydroxycholesterol dehydrogenase in intact cells (Table II). The rat and human enzymes were stereo-specific, whereas hamster 11β-HSD1 led to the formation of 7α-hydroxycholesterol and 7β-hydroxycholesterol (60 and 40%, respectively). As observed with lysates, apparent K_m values were similar, whereas V_{max} of the reduction of 7KC was about 30% that of the reduction of 11-dehydrocorticosterone. A graphic plot of the activities from rat 11β-HSD1 suggests that the enzyme follows first order rate kinetics (Fig. 2).

Comparison of Endogenous Rat and Hamster Liver 11β-HSD1 Activities—We then compared 11β-HSD1-dependent activities under endogenous conditions using rat and hamster

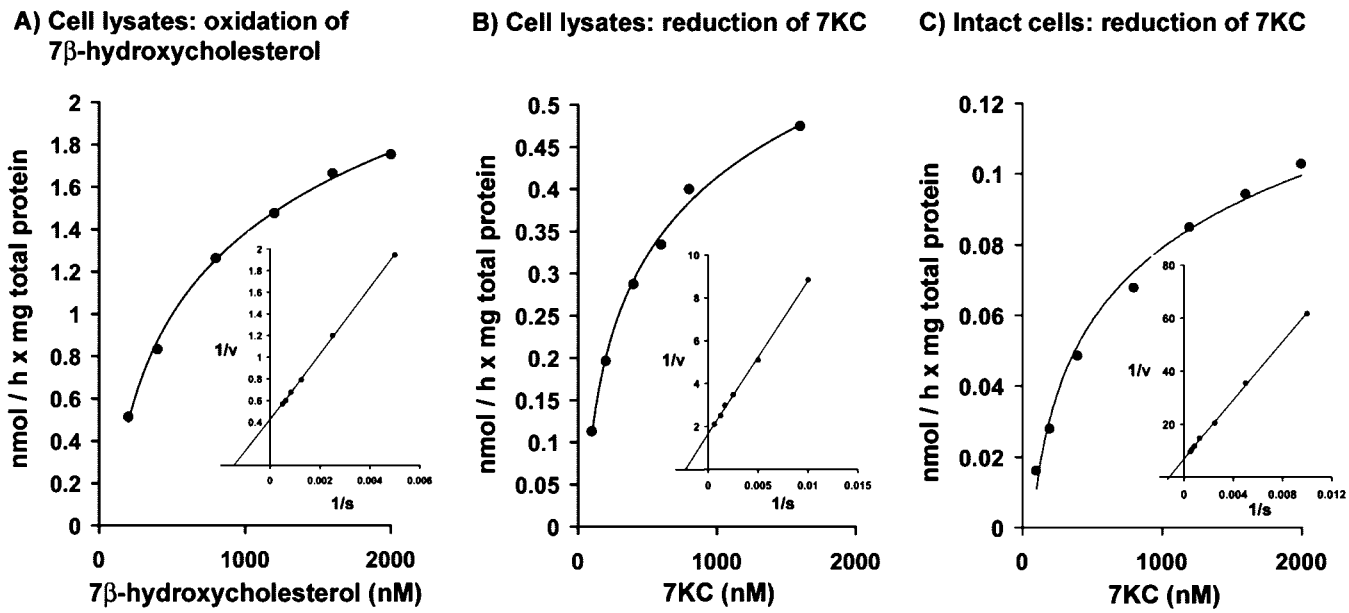

FIG. 2. **Metabolism of 7-oxycholesterols by recombinant rat 11β-HSD1.** Enzymatic activities of recombinant rat 11β-HSD1 were determined in lysates or in intact HEK-293 cells as described under "Experimental Procedures." The *insets* show Lineweaver-Burk plots of the concentration-dependent oxidation of 7β-hydroxycholesterol in lysates (*A*) or reduction of 7KC in lysates (*B*) or in intact cells (*C*). Representative experiments are shown.

liver homogenates. The results presented in Table III confirmed those obtained with lysates from HEK-293 cells expressing recombinant 11β-HSD1 (Table I), except that V_{max} values were higher. The oxidative activities remained relatively stable during homogenization and subsequent centrifugation. In contrast, a loss of reductase activities was observed, which was more pronounced for 7KC compared with 11-dehydrocorticosterone. A single centrifugation step was used after homogenization of the liver, and activities were determined immediately because a time-dependent loss of the reductase activities was observed. Isolated microsomes retained oxidative activities and some reductase activity for 11-dehydrocorticosterone, whereas reduction of 7KC was completely abolished under the conditions applied (not shown).

Next, we measured activities in isolated intact rat primary hepatocytes and in intact hamster liver tissue slices. In both systems no oxidative activities could be detected with 7β-hydroxycholesterol or 7α-hydroxycholesterol as substrates even after prolonged incubation and at various concentrations. Intact hepatocytes catalyzed the stereospecific reduction of 7KC to 7β-hydroxycholesterol (Fig. 3, *A* and *B*), and intact hamster liver slices converted 7KC to 7α-hydroxycholesterol and 7β-hydroxycholesterol (62 and 38% after 30 min) (Fig. 3, *C* and *D*), comparable with the results obtained with recombinant 11β-HSD1 in the HEK-293 expression system (Table II). Fig. 3 shows that the reduction of 7KC in intact rat hepatocytes and hamster liver slices was almost completely blocked by adding 10 μM nonselective 11β-HSD inhibitor CBX or by 200 μM concentrations of the selective 11β-HSD1 inhibitor 2′-hydroxyflavanone (30). These results together with the findings from experiments with transfected HEK-293 cells indicate that the reduction of 7KC to 7-hydroxycholesterol in the liver is predominantly catalyzed by 11β-HSD1.

Comparison of the amounts of 7KC and 7-hydroxycholesterol after incubation for 30 min (Fig. 3, *A* and *C*) and 90 min (Fig. 3, *B* and *D*) shows that 7KC disappeared more rapidly than 7-hydroxycholesterol content increased, suggesting that intact liver cells further metabolized 7-hydroxycholesterol. In addition, ~25% of 7KC was metabolized to substrates other than 7β-hydroxycholesterol after 90 min in the presence of either CBX or 2′-hydroxyflavanone. Therefore, 7KC seems to be mainly metabolized by 11β-HSD1, but other enzymes such as sterol 27-hydroxylase are likely to be involved in the metabolism of 7KC.

11β-HSD1-dependent Reduction of 7KC to 7β-Hydroxycholesterol in Vivo in Rats—To study the role of 11β-HSD1 in the metabolism of 7KC *in vivo*, we administered 7KC (2 mg/kg body weight) by gavage feeding to male Sprague-Dawley rats and determined the amount of 7-oxycholesterols in plasma and liver. 7KC rapidly appeared in the liver, reaching the highest concentrations of about 850 ng/g of tissue 6 h after administration (Fig. 4*A*). In livers from rats treated with 15 mg/kg of body weight CBX, 7KC concentrations tended to increase more rapidly than in untreated animals and reached ~2-fold higher concentrations after 6 and 8 h. The observed tendency of CBX-dependent accumulation of 7KC was paralleled by a reduced formation of 7β-hydroxycholesterol in CBX-treated rats with maximal levels of 700 ng/g of tissue after 6 h (Fig. 4*B*). In contrast, in the absence of CBX, 7β-hydroxycholesterol concentrations increased rapidly to a maximal level of 1550 ng/g of tissue after 6 h followed by a decline to 1000 ng/g of tissue after 8 h. Despite large interindividual differences, the values of 7β-hydroxycholesterol in the absence of CBX were significantly higher than the corresponding values in the presence of the inhibitor at 2, 4, and 6 h after the administration of 7KC ($p < 0.05$).

The comparison of the concentrations of 7KC and 7β-hydroxycholesterol in the absence or presence of CBX revealed a positive correlation of 7KC to 7β-hydroxycholesterol in the *in vivo* experiment with a highly significant shift from 7β-hydroxycholesterol to 7KC in the presence of the 11β-HSD inhibitor CBX (7β-hydroxycholesterol:7KC, absence *versus* presence of CBX; $p < 10E-10$)(Fig. 4, *C* and *D*). The ratio of 7β-hydroxycholesterol to 7KC was significantly higher in the absence of CBX than in its presence ($p < 0.05$) at all time points except at time 0.

Upon administration of 7KC, plasma concentrations of 7β-hydroxycholesterol increased to 200 ng/ml after 2 h, stayed more or less constant for another 4 h, and then declined (Fig. 5*A*). CBX caused an increase in plasma 7KC concentrations with maximal levels of 330 ng/ml after 6 h. The increase in 7KC

TABLE III

Metabolism of glucocorticoids and 7-oxycholesterols by rat and hamster liver homogenates

Enzymatic activities were determined by measuring the reduction of 11-dehydrocorticosterone and 7KC in the presence of NADPH or the oxidation of corticosterone and 7-hydroxycholesterols in the presence of $NADP^+$ as described under "Experimental Procedures." Data represent the mean ± S.D. and were obtained from at least four independent experiments.

Substrate	Cofactor 400 μM	K_m[a]	V_{max}[a]	V_{max}/K_m
		nM	*nmol × h⁻¹ × mg⁻¹*	*×10⁻³*
Rat				
Corticosterone	$NADP^+$	1041 ± 212	12.6 ± 2.9	12.1
11-Dehydrocorticosterone	NADPH	363 ± 40	7.7 ± 2.3	21.2
7α-Hydroxycholesterol	$NADP^+$	ND	ND	ND
7β-Hydroxycholesterol	$NADP^+$	767 ± 205	14.0 ± 3.5	18.3
7KC	NADPH	572 ± 157	2.2 ± 0.9	3.8
Hamster				
Corticosterone	$NADP^+$	1188 ± 203	4.8 ± 1.7	4.0
11-Dehydrocorticosterone	NADPH	411 ± 101	2.2 ± 1.2	5.4
7α-Hydroxycholesterol	$NADP^+$	410 ± 108	6.2 ± 1.9	15.1
7β-Hydroxycholesterol	$NADP^+$	663 ± 85	3.4 ± 1.1	5.1
7KC	NADPH	460 ± 70	3.2 ± 0.8	7.0

[a] Apparent K_m (nM) and apparent V_{max} (nmol × min^{-1} × mg of total $protein^{-1}$) were calculated using the Eadie-Hofstee equation assuming first order rate kinetics.

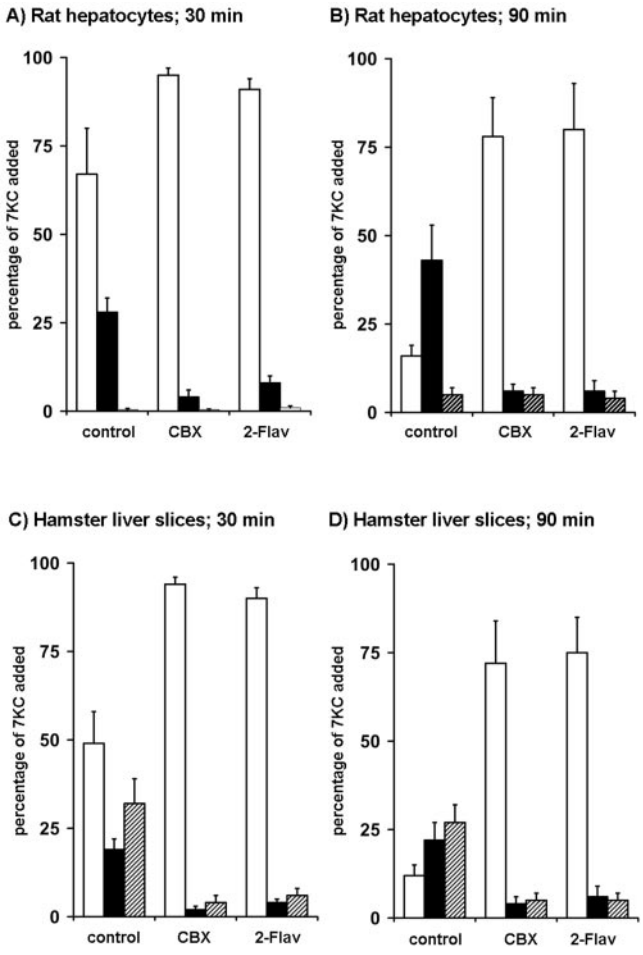

FIG. 3. **Metabolism of 7KC in intact rat hepatocytes and in intact hamster liver tissue slices.** Freshly isolated rat hepatocytes (*A* and *B*) or hamster liver slices (*C* and *D*) were preincubated for 15 min with 10 μM CBX or 200 μM 2′-hydroxyflavanone (*2-Flav*) as indicated followed by incubation in steroid-free medium with 800 nM 7KC for 30 min (*A* and *C*) or 90 min (*B* and *D*) at 37 °C. 7-Oxycholesterol metabolites were analyzed by GC-MS as described under "Experimental Procedures." *White bars*, 7KC; black bars, 7β-hydroxycholesterol; *hatched bars*, 7α-hydroxycholesterol. Results are expressed as a percentage of initially supplied 7KC.

concentration was significant ($p < 0.05$) after 8 h. CBX treatment also reduced the occurrence of 7β-hydroxycholesterol in plasma, although these differences were not significantly different (Fig. 5*B*). The ratio of 7β-hydroxycholesterol to 7KC was significantly lower after 4, 6, and 8 h in animals cotreated with CBX compared with animals treated only with 7KC (Fig. 5, *C* and *D*). These results suggest reduced removal of 7KC from plasma, most likely due to reduced metabolism in tissues expressing 11β-HSD1.

Reduction of 7KC to 7β-Hydroxycholesterol by 11β-HSD1 in Homogenates from Major Organs in the Rat—To assess the relative contribution of 11β-HSD1-dependent reduction of 7KC to 7β-hydroxycholesterol by the major organs in the rat, we determined the reduction of 7KC in homogenates from liver, kidney, heart, and intestine (duodenum) in the absence or presence of the nonselective 11β-HSD inhibitor CBX and the selective 11β-HSD1 inhibitor 2′-hydroxyflavanone. Upon incubation of homogenates for 30 min at 37 °C with 800 nM 7KC, activities of 1.8 and 0.4 nmol × h^{-1} × mg^{-1} were obtained for liver and kidney, respectively, whereby CBX (10 μM) and 2′-hydroxyflavanone (200 μM) inhibited more than 90% of the generation of 7β-hydroxycholesterol. Activities for heart and intestine (duodenum) were below 0.05 nmol × h^{-1} × mg^{-1}.

CBX Does Not Disturb Plasma Electrolytes and Steroid Hormone Levels under the Conditions Used—In the *in vivo* experiment the effect of CBX on 7β-hydroxycholesterol generation was most pronounced 6 h after 7KC administration. To investigate whether the doses of the nonselective 11β-HSD inhibitor CBX applied in the present study significantly increased corticosterone concentrations by inhibition of 11β-HSD2 or interfered with other steroid hormone-metabolizing enzymes, we compared electrolytes, creatinine, and various steroid hormones in the plasma of control rats, animals treated for 6 h with 7KC, CBX, or both compounds. No significant changes were observed in these essential parameters within the time course of the experiment (Table IV). Importantly, the ratio of plasma corticosterone to 11-dehydrocorticosterone, a measure for the combined activities of 11β-HSD1 and 11β-HSD2, was not altered, indicating little or no inhibition of 11β-HSD2 within the relatively short time of treatment.

DISCUSSION

Despite the pro-atherogenic effects of oxysterols and their presence in micromolar concentrations in human macrophage-foam cells and atherosclerotic lesions (1, 5), there is evidence emerging that dietary oxysterols play a rather minor role in atherogenesis due to their rapid hepatic metabolism (11, 12, 35). Sterol 27-hydroxylase was shown to hydroxylate 7KC to 27-hydroxy-7KC in human atherosclerotic lesions and in cultured macrophages (14); however, only a transient increase in

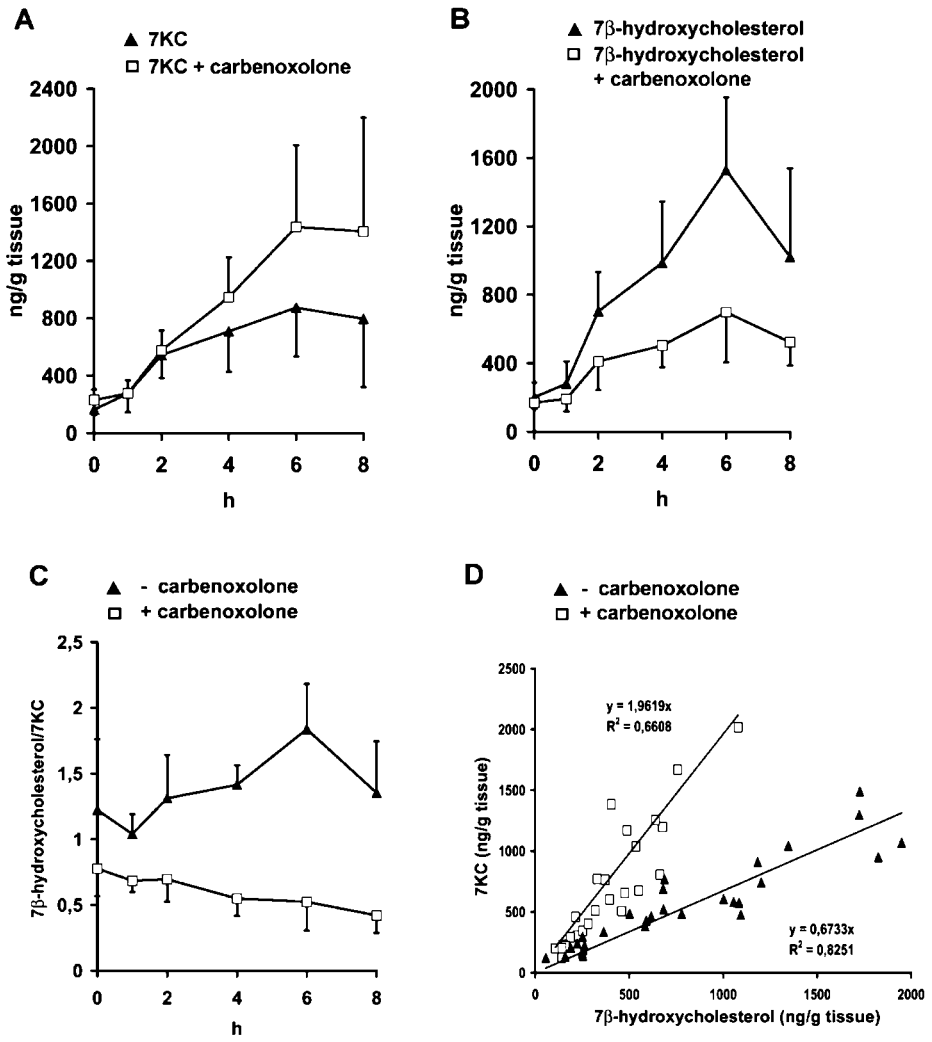

FIG. 4. **Rapid metabolism of 7KC in the liver.** 7KC (2 mg/kg body weight) was administered by gavage feeding to either untreated male Sprague-Dawley rats (200–220 g) (▲) or to animals treated 1 h before 7KC administration and after 2 h and 5 h with 15 mg/kg of the 11β-HSD inhibitor CBX (□). Animals were sacrificed at time 0 or after 1, 2, 4, 6, or 8 h, the livers were removed, and whole liver homogenates were analyzed for the content of 7-oxycholesterols as described under "Experimental Procedures." 7KC concentrations (*A*) and 7β-hydroxycholesterol concentrations (*B*) in livers from untreated (▲) or CBX-treated rats (□) are shown. *C*, ratio of 7β-hydroxycholesterol to 7KC in livers from untreated (▲) and CBX treated rats (□). *D*, plot of 7KC against 7β-hydroxycholesterol content from livers of untreated (▲) and CBX-treated rats (□). Values are expressed as mean ± S.D. (4 or 5 rats/time point).

aortic levels of 7KC was observed in sterol 27-hydroxylase-deficient mice after 7KC administration, which was followed by efficient hepatic metabolism (15). These results suggested the existence of an as yet unidentified mechanism contributing to the clearance of 7KC in the liver.

Here, we describe a novel potential mechanism for the first step in the hepatic metabolism of 7KC after its uptake from food. Together, our results from *in vitro* studies with recombinant 11β-HSD1, endogenous 11β-HSD1 in liver homogenates, or in intact liver cells as well as from *in vivo* experiments with rats demonstrate an important role of 11β-HSD1 in the detoxification of food-derived 7KC in the liver. Although lysates from cells expressing recombinant 11β-HSD1 catalyzed both oxidation of 7-hydroxycholesterol and reduction of 7KC, the enzyme exclusively catalyzed the reduction reaction in intact cells. The stereospecific reduction of 7KC to 7β-hydroxycholesterol by rat and human 11β-HSD1 and to 7α-hydroxycholesterol and 7β-hydroxycholesterol by hamster 11β-HSD1 was comparable in lysates and intact cells expressing recombinant enzymes as well as in homogenates and intact hepatocytes or liver tissue slices expressing endogenous 11β-HSD1. CBX, a nonselective inhibitor of 11β-HSD enzymes, and 2′-hydroxyflavanone, a selective 11β-HSD1 inhibitor (30), both reduced enzymatic activities in HEK-293 cells expressing recombinant 11β-HSD1 and in cells expressing endogenous 11β-HSD1. These results provide strong evidence that 11β-HSD1 acts as a 7KC reductase but not as a 7-hydroxycholesterol dehydrogenase under endogenous conditions. The absolute kinetic parameters obtained from experiments with cultured cells, however, have to be analyzed with caution, since the addition of the free compound to the culture medium may not reflect the physiological situation.

The cloning of hamster 11β-HSD1 and its comparison with the rat and human enzyme explains part of the species-specific differences in the interconversion of 7-hydroxycholesterol and 7KC observed by Maeda *et al.* (17). They observed the oxidation of 7α-hydroxycholesterol to 7KC by hamster and chicken but not rat, rabbit, or guinea pig liver microsomes and conversion of 7β-hydroxycholesterol to 7KC in all species except guinea pig. Importantly, Maeda *et al.* (17) report the reduction of 7KC to both 7α-hydroxycholesterol and 7β-hydroxycholesterol in equal amounts by hamster liver microsomes, consistent with the activities of recombinant hamster 11β-HSD1 obtained in the present study. This is, however, in contrast to Song *et al.* (16), reporting only oxidation of 7-hydroxycholesterols. Because the hamster enzyme purified by Song *et al.* (16) did not reduce 11-dehydrocorticosterone, it is possible that reductase activity was lost during microsomal preparation or purification, in line with the present observation of a loss of reductase activity upon preparation of microsomes.

Recent analyses by Prough and coworkers (18, 36) showing the inhibition of the interconversion of 7-oxy-DHEA metabolites by corticosterone suggested the involvement of 11β-HSD enzymes. These studies described both rat and human liver microsomes as converting 7α-hydroxy-DHEA to 7-keto-DHEA, whereby the human enzyme was ~7-fold more efficient. The reverse reaction was also catalyzed; however, at much lower

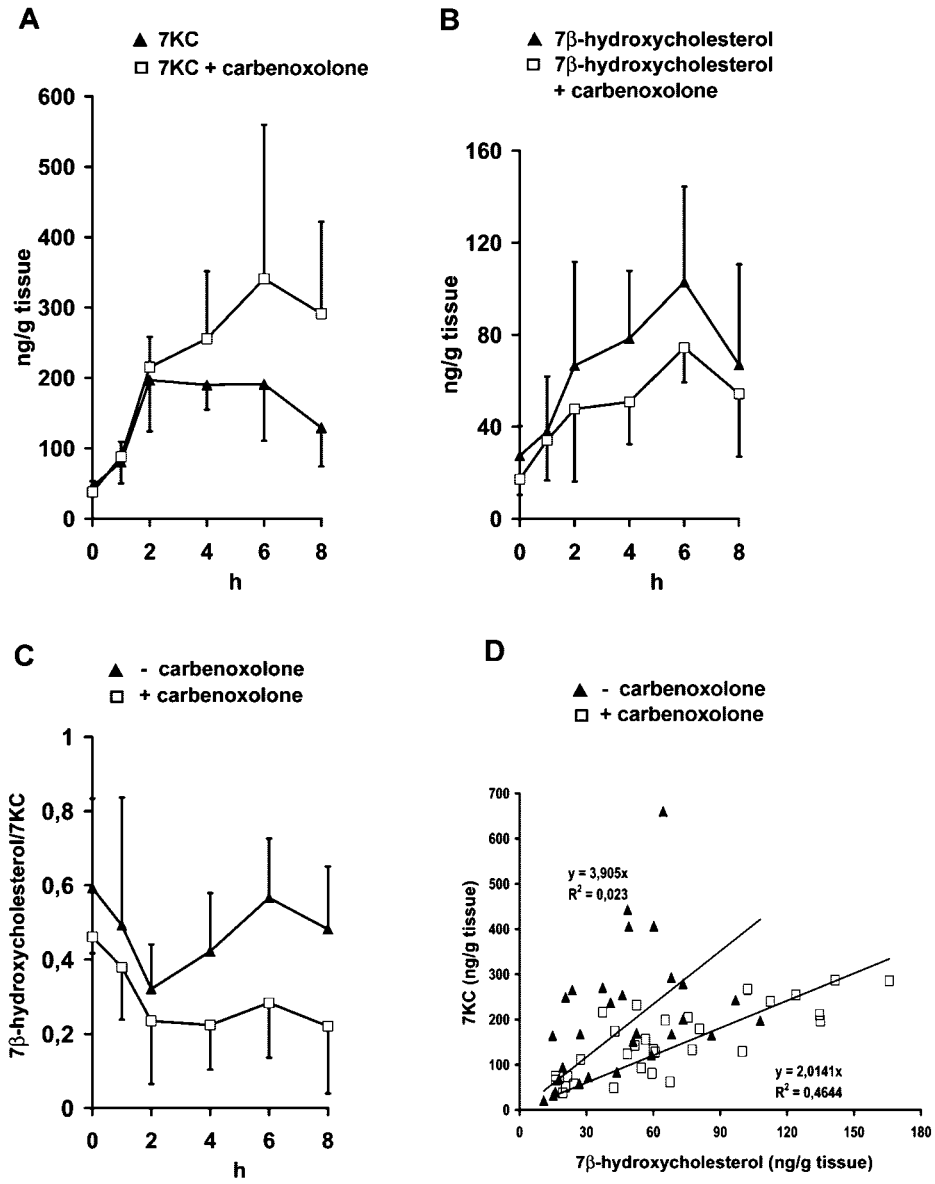

FIG. 5. **Determination of 7KC and 7β-hydroxycholesterol in plasma.** Rats were treated as described in Fig. 3, and the content of 7-oxycholesterols were determined in plasma (see "Experimental Procedures"). Shown are 7KC concentrations (*A*) and 7β-hydroxycholesterol concentrations (*B*) in plasma from untreated (▲) or CBX-treated rats (□). *C*, ratio of 7β-hydroxycholesterol to 7KC in plasma from untreated (▲) and CBX-treated rats (□). *D*, plot of 7KC against 7β-hydroxycholesterol content from plasma of untreated (▲) and CBX-treated rats (□). Values are expressed as mean ± S.D. (4 or 5 rats per time point).

TABLE IV

Plasma concentrations of electrolytes, creatinine, and steroid hormones after CBX treatment

Sprague-Dawley rats received 2 mg/kg 7KC by gavage feeding with or without treatment with 15 mg/kg CBX every 3 h. Six hours after the administration of 7KC animals were sacrificed, plasma was obtained, and concentrations of electrolytes, creatinine, and steroid hormones were determined as described under "Experimental Procedures." Data represent the mean ± S.D. (n = 4).

	Control	CBX	7KC	CBX + 7KC
Na^+ (mM)	126 ± 5	123 ± 6	128 ± 5	129 ± 6
K^+ (mM)	45 ± 14	48 ± 18	39 ± 5	44 ± 17
Creatinine (μM)	16 ± 2	18 ± 3	16 ± 1	19 ± 5
Aldosterone (pM)	549 ± 123	571 ± 100	634 ± 54	421 ± 98
Progesterone (nM)	3.1 ± 1.2	2.9 ± 0.6	2.1 ± 0.6	2.7 ± 0.7
DHEA-sulfate (nM)	13 ± 3	15 ± 5	14 ± 2	12 ± 2
Androsterone[a]	1.02 ± 0.29	0.80 ± 0.22	0.86 ± 0.37	0.81 ± 0.23
Etiocholanolone[a]	0.69 ± 0.41	0.55 ± 0.14	0.59 ± 0.25	0.53 ± 0.16
Corticosterone B (nM)	851 ± 99	762 ± 67	559 ± 33	750 ± 92
11-Dehydrocorticosterone A (nM)	50 ± 20	36 ± 8	27 ± 5	33 ± 4
B/A	17.0	21.0	21.1	22.6

[a] Arbitrary units, relative to an internal standard.

efficiency. In addition, Prough and coworkers (18, 36) report conversion of 7α-hydroxy-DHEA to 7-keto-DHEA by kidney microsomes and suggest that this reaction was catalyzed by 11β-HSD2 or by an as yet unidentified 11β-HSD3. In the present study, we demonstrate that human and rat 11β-HSD1 catalyze the stereospecific interconversion of 7β-hydroxycholesterol and 7KC, whereby only the reduction of 7KC is catalyzed in intact cells. These experiments indicate significant differences between 7KC and 7-keto-DHEA metabolism regarding the isozyme involved and the stereospecificity as well as the tissue specificity of the corresponding reaction. Clearly, experiments with recombinant enzymes and the use of intact

cells are required to elucidate species-specific differences between 7-oxy-DHEA metabolites and 7-oxycholesterols, to reveal the isozyme responsible for the observed activity in the corresponding tissue, and to assess the reaction direction.

Oxidized cholesterol products including 7KC are present at high concentrations in processed cholesterol-rich food (9). A recent comparison of 7-oxycholesterol compounds in fresh *versus* broiled olive oil and butter revealed high 7KC concentrations of 30 μM and 7α-hydroxycholesterol and 7β-hydroxycholesterol concentrations of 10 μM in broiled butter compared with concentrations clearly below 0.5 μM in fresh or broiled olive oil and in fresh butter.[2] Upon ingestion, dietary oxysterols are absorbed through the intestine and incorporated into lymph chylomicrons (10, 37, 38) followed by partial hydrolysis of the triacylglycerol core and clearance of the chylomicron remnants by hepatocytes (39). Lyons and Brown (12) observe rapid hepatic metabolism of 7KC when delivered in either acetylated low density lipoprotein or in chylomicron remnant-like emulsion to rats.

Our results from the *in vivo* experiments with rats suggest that the initial step in the metabolism of 7KC, which is incorporated into chylomicrons and transported into the liver, is the stereo-specific conversion to 7β-hydroxycholesterol catalyzed by 11β-HSD1. The rapid hepatic conversion of 7KC to 7β-hydroxycholesterol in rats was significantly reduced by CBX, although the concentration applied (15 mg/kg) did not lead to complete inhibition. CBX also inhibits 11β-HSD2; however, it is not expressed in liver and does not reduce 7KC. Studies *in vivo* in man and in animals provided evidence that CBX predominantly inhibits 11β-HSD1 in the liver but not in adipose tissue (40). Treatment of humans with CBX for 1 week decreased the ratio of urinary tetrahydrocortisol and allo-tetrahydrocortisone to tetrahydrocortisone, indicating inhibition of hepatic 11β-HSD1, and slightly increased the ratio of urinary free cortisol to cortisone, indicating modest inhibition of 11β-HSD2 (41). In the present study, inhibition of 11β-HSD2 in the kidney was not relevant since plasma corticosteroid levels were not altered. In addition, CBX did not alter plasma concentrations of electrolytes or various other steroids, suggesting that the observed effect of CBX on 7KC metabolism is due to inhibition of 11β-HSD1 and not caused by a secondary effect through another steroid-metabolizing enzyme. The interference of other effects of CBX, including inhibition of collagen-α1 expression (42), stimulation of intestinal bicarbonate secretion (43), and action as a gap-junction uncoupler (44) on 7KC metabolism, cannot be fully excluded; however, these effects were either observed after prolonged exposure or at high concentrations (>50 μM in cell experiments; >50 mg/kg in animal experiments).

A comparison of the conversion of 7KC to 7β-hydroxycholesterol by major rat organs showed that the liver has by far the highest activity, followed by 4–5-fold lower activity in kidneys and barely detectable activities in heart and intestine. Because both CBX and 2′-hydroxyflavanone abolished the conversion of 7KC to 7β-hydroxycholesterol in all tissue homogenates examined, these results suggest that this activity is mainly catalyzed by hepatic 11β-HSD1. 2′-Hydroxyflavanone, a selective 11β-HSD1 inhibitor without significant effects on 11β-HSD2, 17β-HSD1, or 17β-HSD2, is not suitable for the inhibition of 11β-HSD1 *in vivo* in rats, because it is a weak inhibitor of rat 11β-HSD1 (IC_{50} of 80 μM) compared with human 11β-HSD1 (IC_{50} of 1.8 μM).[3]

Our finding that 11β-HSD1 stereo-specifically converts 7KC to 7β-hydroxycholesterol in rats is supported by previous studies reporting the generation of 7β-hydroxycholesterol after intraperitoneal or intravenous administration of 7KC to rats (45–47) and by a study from Lyons *et al.* (11) who observed rapid hepatic metabolism upon administration of radiolabeled 7KC. Their TLC analysis revealed the transient occurrence of 7β-hydroxycholesterol but not 7α-hydroxycholesterol, indicating stereospecific conversion of 7KC to 7β-hydroxycholesterol, further metabolism of the latter compound, formation of bile acids, and excretion.

The initial increase in 7KC content observed in the rat liver 6 h after administration (Fig. 4*A*) may reflect hepatic 7KC uptake from the clearance of chylomicrons after intestinal absorption, and the subsequent decline may be explained by hepatic conversion to 7β-hydroxycholesterol, other metabolites including 27-hydroxy-7KC, and uptake by other tissues. Fig. 3 shows that CBX and 2′-hydroxyflavanone both abolished 7KC metabolism in intact rat hepatocytes and hamster liver tissue slices, with only about 25% of 7KC metabolized to other compounds after incubation for 90 min. The rapid decline of 7β-hydroxycholesterol after 6 h likely reflects the rapid metabolism of this compound in the liver (Fig. 4*B*). In intact rat hepatocytes, the decrease in 7KC, and the increase in 7β-hydroxycholesterol concentration was almost equal after 30 min, but after 90 min 7β-hydroxycholesterol content was only about 50% of the amount expected (Fig. 3), indicating further metabolism of 7β-hydroxycholesterol. Similarly, in intact hamster liver slices the decrease in 7KC was accompanied by an increase in 7α-hydroxycholesterol and 7β-hydroxycholesterol, and both compounds seemed to undergo further metabolism.

Sterol 27-hydroxylase-deficient mice have typically decreased fecal bile acid metabolites; however, fecal excretion of 7KC metabolites was increased, indicating an unusual bile acid pathway. Norii *et al.* (48) found that upon *intraperitoneal* administration of 2 mg of radiolabeled 7β-hydroxycholesterol to rats, most of the compound was metabolized and excreted in bile as acidic fraction, with the main metabolites being 3α,7β-dihydroxy-5-cholenoic acid and 3β,7β-dihydroxy-5-cholenoic acid. These authors also reported the excretion of 3-hydroxy-7-oxo-5-cholenoic acid; however, their saponification conditions (130 °C for 3 h) suggest that the 7-oxo compounds were formed by auto-oxidation rather than enzymatically. Ursodeoxycholic acid, the 7β-epimer of chenodeoxycholic acid, was found as a minor metabolite only, indicating that 7β-hydroxycholesterol follows an unusual metabolic pathway. 3β,7β-Dihydroxy-5-cholenoic acid has also been identified as a 7-*N*-acetylglucosamine conjugate and shown to be a minor component in the urine of healthy individuals (49, 50). These unusual bile acid metabolites may be derived from 7β-hydroxycholesterol after its hepatic conversion from 7KC by 11β-HSD1.

It is tempting to speculate that 7β-hydroxycholesterol is further metabolized by sterol 27-hydroxylase to 27-hydroxy-7β-hydroxycholesterol. In addition to 27-hydroxy-7KC, Brown *et al.* observe a large unidentified peak in macrophages incubated with 7KC (14). This peak had a retention time that was very close to a peak assigned to 7β-hydroxycholesterol that was observed in sterol 27-hydroxylase-deficient mice, where as expected 27-hydroxy-7KC was absent. If the unidentified peak is indeed 7β-hydroxycholesterol, one might postulate that the efficient metabolism of 7KC includes, first, the conversion to 7β-hydroxycholesterol by 11β-HSD1, then to 27-hydroxy-7β-hydroxycholesterol by sterol 27-hydroxylase, followed by further metabolism to bile acids. In patients with cerebrotendinous xanthomatosis, lacking 27-hydroxylase, inefficient me-

[2] A. Odermatt and M. Zürcher, unpublished data.
[3] A. Odermatt, unpublished observation.

tabolism may lead to accumulation of 7β-hydroxycholesterol, causing end-product inhibition of 11β-HSD1, which may explain the increased 7KC concentrations in these patients.

Under normal conditions, plasma 7KC concentrations are in the nanomolar range, and metabolites of 7KC are expected to be low. However, in certain disease states or upon intake of high amounts of oxidized cholesterol-rich food, 7KC concentrations increase, and its metabolism is essential to cope with the toxic effects of 7KC. The results from our *in vitro* and *in vivo* experiments provide evidence for an important role of 11β-HSD1 in the initial step of the detoxification of 7KC by the stereospecific reduction of 7KC to 7β-hydroxycholesterol. Moreover, the present findings may have important implications for the appropriate application of specific 11β-HSD1 inhibitors in the therapeutic treatment of obese and diabetic patients. To avoid accumulation of 7KC taken up from food, a pharmaceutical compound acting specifically on 11β-HSD1 in adipose tissue may be of advantage.

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Rapid Hepatic Metabolism of 7-Ketocholesterol by 11β-Hydroxysteroid Dehydrogenase Type 1: SPECIES-SPECIFIC DIFFERENCES BETWEEN THE RAT, HUMAN, AND HAMSTER ENZYME

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