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Sequence of the cDNA of a Human Dihydrodiol Dehydrogenase Isoform (AKR1C2) and Tissue Distribution of its mRNA.

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Human liver contains three isoforms (DD1, DD2 and DD4) of dihydrodiol dehydrogenase with 20α- or 3α-hydroxysteroid dehydrogenase activity; the dehydrogenases belong to the aldo-keto reductase superfamily. cDNA species encoding DD1 and DD4 have been identified. However, four cDNA species with more than 99% sequence identity have been cloned and are compatible with a partial amino acid sequence of DD2. In this study, we isolated a cDNA clone encoding DD2, which was confirmed by comparison of the properties of the recombinant and hepatic enzymes. Expression of mRNA species for the five similar cDNA species was examined by RT-PCR and restriction endonuclease digestion. All liver samples examined here expressed only one mRNA species corresponding to the newly identified cDNA for DD2, but not mRNA transcripts corresponding to the other cDNA species.

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Evidence for the Presence of Multiple Forms of Sph Kinase in Human Platelets.

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The intracellular distribution of sphingosine (Sph) kinase activity was examined in human platelets. A large portion (72%) of total activity was found to be associated with the membrane fraction, and the membrane-associated fraction had higher specific activity compared with the cytosolic enzyme. Most of the membrane-associated activity could be extracted with 1 M NaCl. The cytosolic activity was unstable upon heat treatment, whereas the NaCl-extractable fraction was stable under the same conditions. When subjected to Mono Q column chromatography, the cytosolic fraction produced two activity peaks and the NaCl-extractable fraction gave a single peak. These three Sph kinase activities showed different responses to stimulation by β-octyl-glucoside and inhibition by *N,N*-dimethylsphingosine and L-*threo*-dihydrosphingosine, suggesting the presence of multiple enzyme forms in human platelets.

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Identification of a Principal mRNA Species for Human 3α-Hydroxysteroid Dehydrogenase Isoform (AKR1C3) That Exhibits High Prostaglandin D_2 11-Ketoreductase Activity.

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Human 3α-hydroxysteroid dehydrogenase exists in four isoforms, which belong to the aldo-keto reductase (AKR) superfamily and are named AKR1C1-1C4. The properties of the AKR1C3 have not been fully characterized. In addition, a cDNA that shows more than 99% homology with AKR1C3 cDNA has been cloned from human myeloblasts. We have expressed and purified a recombinant enzyme (designated as DBDH) from this cDNA. DBDH exhibits high prostaglandin D_2 11-ketoreductase activity. The recombinant AKR1C3 prepared by site-directed mutagenesis of DBDH also showed the same properties as DBDH. Analyses of expression of mRNA for DBDH and AKR1C3 by RT-PCR indicated that only one mRNA species for DBDH is expressed in 33 human tissue samples. These results suggest that principal gene of AKR1C3 in the human has a coding region represented by DBDH cDNA.

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Properties and Tissue Distribution of Mouse Monomeric Carbonyl Reductase.

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We previously cloned a cDNA for mouse cerebellum carbonyl reductase which shows more than 81% homology to the cDNAs for monomeric carbonyl reductases of the rat, rabbit and human, and for pig 20β-hydroxysteroid dehydrogenase. In the present study, we expressed the recombinant monomeric enzyme (34 kDa and p*I* 8.3) from the cDNA and compared its properties with the recombinant human enzyme. The mouse and human enzymes showed similar functional properties, although they differed in kinetic constants for carbonyl substrates and in inhibitor sensitivity. Both enzymes lacked glutathione S-transferase activity. Western blot and RT-PCR analyses showed that the enzyme protein and its mRNA are expressed in various mouse tissues.