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Discontinuous Variation in Hepatic Uridine Diphosphate Glucuronyltransferase toward Androsterone in Wistar rats A Regulatory Factor for *In Vivo* Metabolism of Androsterone*

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Glucuronidation plays an important role in the detoxication of steroid hormones. Hepatic microsomal UDP-glucuronyltransferase (EC 2.4.1.17) catalyzes the transfer of glucuronic acid from UDP-glucuronic acid to various steroids. Considerable evidence, from studies with several steroid substrates, has been accumulated to demonstrate the multiplicity of action of UDP-glucuronyltransferase. However, the mechanisms responsible for their regulation remain unclarified.

Recent studies from this laboratory showed marked variations in biliary metabolites of androsterone and testosterone in female Wistar rats. Additional studies with androsterone glucuronide and androsterone sulfate suggest that hepatic UDP-glucuronyltransferase may be principally responsible for large variations in the metabolism *in vivo* of androsterone and testosterone in female rats.

The present study was designed to compare the glucuronidation of androsterone and testosterone by liver microsomes from Wistar rats and other animals. The study includes effects of Triton X-100 on the enzyme activity and determination of kinetic parameters.

The present study demonstrates that male and female Wistar rats give discontinuous variation in hepatic microsomal UDP-glucuronyltransferase activities toward androsterone but not toward testosterone. The rats with a high level of transferase activity toward androsterone (HG) and the rats with a low level of transferase activity (LG) were found approximately in the ratio 5: 4. In contrast, Sprague-Dawley rats, Hartley guinea pigs and CF1 mice did not show such diversity. It is of interest to note the existence of strain differences between Wistar and Sprague-Dawley rats.

The microsomal UDP-glucuronyltransferase is latent, probably buried inside the microsomal vesicle and can be activated by physical, chemical or enzymatic perturbation of the membrane structure. Triton X-100 is one of the typical xenobiotic activators. However, activation of the transferase with Triton X-100 rather amplified the diversity between HG and LG microsomes.

The observed low activity of the LG microsomes could not be ascribable to a high level of microsomal UDP-glucuronic acid pyrophosphatase which hydrolyzes UDP-glucuronic acid, because the LG microsomes formed testosterone glucuronide similar to the extent

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of the HG microsomes. Quantitative recovery of the incubated androsterone glucuronide from the assay medium containing the LG microsomes indicates that β-glucuronidase, the other microsomal enzyme which may affect the glucuronidation, was not involved in the hydrolysis of androsterone glucuronide formed in the assay medium.

Though kinetic data may lead to multiple interpretations with the restricted membrane-bound enzyme and nonpolar substrates, comparison of the apparent K_m and V_{max} values obtained from native and activated microsomes revealed several interesting features. With androsterone and testosterone as substrates, male and female rats gave similar K_m values for the respective HG or LG microsomes, while the V_{max} exhibited sex differences, with females showing greater enzyme activity than males. The K_m values were slightly increased by detergent treatment, although marked increases in V_{max} were observed except for the V_{max} of androsterone in female LG microsomes. The results indicate that the affinity of the transferase toward androsterone and testosterone was not appreciably affected by the detergent and that the detergent might change the microsomal membranes, by which means the formerly latent enzyme may become accessible to the substrate leading to an increase of the velocity. The most striking aspect is that the apparent K_m for androsterone was much higher in the LG microsomes than in the HG microsomes, whereas the apparent K_m values for UDP-glucuronic acid were quite similar between the HG and LG groups. On the other hand, the apparent K_m values for testosterone were slightly higher in the LG microsomes than in the HG microsomes. These results imply that the low affinity of UDP-glucuronyltransferase toward androsterone must be responsible for the poor glucuronidation of anrosterone in the LG microsomes. The low transferase activity of the LG microsomes can be interpreted as a defective transferase toward androsterone and/or abnormal microsomal membrane microenvironment of the transferase. The different kinetic behaviours of the transferase toward androsterone and testosterone in the HG and LG microsomes suggest the existence of multiple steroid UDP-glucuronyltransferases. However, the apparent multiplicity of the transferase may be caused by the existence of a single enzyme in several different lipid-stabilized conformations. Zakim and Vessey suggest that the microsomal lipids may affect the activity of UDP-glucuronyltransferase by concentrating the substrate and restricting the orientation of the substrate at the active site of the enzyme. We demonstrated that the transferase toward androsterone was competitively inhibited by testosterone in the HG microsomes. However, this does not necessarily mean that androsterone and testosterone can be glucuronidated at the same enzymatic active site. It is difficult to distinguish between these mechanisms from the present study. Purification of the transferase is necessary before its heterogeneity can be established.

The hereditary deficiency of UDP-glucuronyltransferase toward bilirubin and several substrates is well known in Gunn rats, the mutant strain of Wistar rats. In humans, Crigler-Najjar syndrome and Gilbert's disease are characterized by a genetically determined

defect or insufficiency of bilirubin glucuronidation. Our colony of Wistar rats may be another example of UDP-glucuronyltransferase insufficiency of genetic origin. Further studies with this experimental model system may be of interest for obtaining insight into the regulatory mechanism and heterogeneity of UDP-glucuronyltransferase.