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# Sexual Differences in Galactose Metabolism: Galactosyl Ceramide Galactosidase and other Galactosidases in Mouse Kidney

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Male and female mice were compared at two ages, 15 and 50 days, with respect to the activities of three galactosidases in kidney. No sex difference in enzyme activity was seen in the young mice, but appreciable differences were found in the older animals. The male kidneys had about one-third higher specific activities of cerebroside β-galactosidase and nitrophenyl β-galactosidase, but there was no difference with nitrophenyl α-galactosidase. A listing and discussion of other galactose-metabolizing enzymes influenced by sex differences is presented.

Measurements of galactolipid concentration and synthetase activity in mouse kidney have shown marked differences between males and females. Kidney homogenates from C3H/He male mice exhibited about six times the rate of galactosylation of glucosyl ceramide (Hay & Gray, 1970) and homogenates from C57/BL male mice galactosylated ceramide about four times as fast (Coles & Gray, 1970). The latter transferase activity could be increased greatly in female mice by treatment with testosterone (Gray, 1971). In contrast, the galactosyltransferase which forms triglycosyl ceramide from lactosyl ceramide was about 70% more active in females. Morell & Costantino-Ceccarini (1973) investigated the transferase which makes galactosyl ceramide in kidney and found that there was little difference in this activity between young male and female mice, but that a great increase in its activity took place in the males at weaning.

The above findings suggested to us that there might be a general relationship between sex and enzymes involved in galactose metabolism. We investigated this question further by studying several galactosidases of mouse kidney, i.e. the enzymes that hydrolyse galactosyl ceramide, nitrophenyl α-D-galactoside and nitrophenyl β-D-galactoside. The latter enzymes, which might be classified as aryl galactosidases, are now known to be composites of several galactosidases.

## *Experimental*

Swiss Webster mice, 18 to 31 of each sex, were divided into four groups for each sex. The mice were decapitated, the kidneys were removed, and the kidneys of each group were pooled for homogenization in 24vol. of cold water. This step was done with a Teflon–glass homogenizer of the Potter–Elvehjem type. Samples of the homogenate were immediately taken for assay of the cerebroside-cleaving enzyme and the remainder was frozen. The next day the homogenate was thawed and assayed for the two other enzymes.

Cerebroside galactosidase activity was measured by the method of Radin & Arora (1971) with some changes in the medium necessary to yield activities that were proportional to kidney weight. Each incubation tube contained 0.25ml of an emulsion made from 68.7nmol (50μg, sp. radioactivity 450c.p.m./nmol) of stearoyl psychosine ([$^3$H]galactose-labelled), 175μg of oleic acid (as the Tris salt) and 2mg of crude sodium taurocholate. Also present were 50μmol of sodium citrate, pH4.5, and kidney homogenate plus water to make a total volume of 0.5ml. The suspension was shaken at 37°C, then 0.5ml of water was added and the radioactive galactose was isolated and counted for radioactivity (Braun *et al.*, 1970). Samples corresponding to 2 and 4mg of kidney were used, derived from the pooled kidneys of four to nine mice. Four pools were used for each age/sex group. Since the results obtained with 2 and 4mg were very similar, all eight values were averaged and treated equally in calculating the standard deviations and *t*-test probabilities.

The nitrophenyl galactosidases were assayed as described by Hajra *et al.* (1966). A standard curve was made by using *p*-nitrophenol in 0.2M-$Na_2CO_3$. For the α-galactoside, 8 and 16mg of tissue were assayed; for the β-galactoside, 2 and 4mg were assayed. Here too the observed activities at the two concentrations were very similar and all eight values were treated equally.

## *Results and discussion*

The observed specific activities for 15-day-old mice did not differ when male and female mice were compared. The activities observed for cerebroside galactosidase were 51.4±2.6nmol/min per g wet wt. of kidney in females and 50.6±2.0 in males. The activities for α-galactosidase were 139±19 and 133±13,

and the corresponding activities for β-galactosidase were 520±39 and 523±108.

Significant differences in the specific activities were observed in 50-day-old mice, which are sexually fairly mature. The activities for galactosyl ceramide hydrolase were 32.0±4.5nmol/min per g and 41.8±5.2 in the female and male mice respectively. Thus the males had a specific activity that was 31% higher ($P<0.01$). The α-galactosidase activities differed only slightly, being 55±2.6 and in females 58±3.3 in males. However, aryl β-galactosidase was like cerebroside hydrolase in being 34% more active in males ($P<0.01$). The actual activities were 351±70 and 470±40nmol/min per g of kidney. All three enzymes decreased in specific activity with age, but to different degrees in the two sexes.

The kidneys grow with increasing age, especially in male mice. At 15 days the average female kidney weighed 60mg and the male kidney weighed 62mg. By 50 days the corresponding weights were 193 and 265mg. This change accentuates the differences in enzyme activities when they are calculated on the basis of total organ weight. The total cerebroside galactosidase activity rose by 100% in the females but by 250% in the males. The total aryl α-galactosidase activity rose by 29% in the females and by 92% in the males. Aryl β-galactosidase activity rose by 113% in the females and by 286% in the males.

Our finding that cerebroside galactosidase activity is higher in sexually mature male mice than in females matches the similar finding by Gray (1971) with cerebroside synthetase. A similar matching had been seen with the changes resulting from brain maturation in rats, in which cerebroside synthetase (Brenkert & Radin, 1972) showed a developmental pattern which preceded, but paralleled, the developmental pattern of cerebroside hydrolase (Bowen & Radin, 1969). We offer the tentative suggestion that sex hormones can influence galactosyltransferases in a relatively direct fashion, whereas the corresponding galactosidases are influenced by the hormones only indirectly, being induced by the increased concentrations of galactosides formed by the transferases. This sensitivity of hydrolases to substrate concentration was demonstrated for aryl β-galactosidase (and related hydrolases) in rats that were injected with erythrocyte stroma (Kampine *et al.*, 1967). The stroma presumably acted to raise the concentration of glycolipids in the spleen, thereby inducing increases in the activities of the appropriate hydrolases.

We surveyed the literature for relationships between galactose and sex and uncovered an unexpected number of findings, not all of which could be attributed to galactosyltransferases. The relationships found were the following. (1) Some of the α-galactosidases appear to be determined by a gene on the X chromosome, judged by the inheritance seen in Fabry's disease (Sweeley & Klionsky, 1966; Mapes & Sweeley, 1973). (2) Diets high in galactose are toxic to animals, with female chicks exhibiting twice the mortality rate of male chicks (Parkhurst & Mayes, 1972). These workers also found that female chicks on a normal diet have an activity in liver of UDP-glucose–β-D-galactose 1-phosphate galactosyltransferase that is 40–50% lower than that in the male, although galactokinase and UDP-galactose 4-epimerase activities are the same in both sexes. (3) The rate of urinary excretion of galactosylglucosyl ceramide seems to be about 5 times higher in women, although galactosyl ceramide excretion may be lower in women (Philippart *et al.*, 1971). (4) The oxidation of galactose by liver slices from female rabbits is stimulated by up to 326% by sex hormones added *in vitro* (Pesch & Topper, 1958). The progesterone stimulation was produced also in the liver cytosol fraction, whereas in kidney slices and other tissues there was 30–50% inhibition. Oxidation of [$^{14}$C]galactose was very slow in galactosaemic children, but progesterone produced a marked oxidation of the sugar (Pesch *et al.*, 1960). (5) When rats are fed on a high-galactose diet they develop cataracts. Males are much more sensitive to this phenomenon (J. Sidbury, personal communication). Administration of progesterone slows the development of cataracts in male rats (Pesch *et al.*, 1960). (6) Aryl β-galactosidase from cattle can be separated into two fractions by affinity chromatography. The proportion of each type depends markedly on the particular organ, but also markedly on the animal's sex (J. Distler & G. W. Jourdian, personal communication of preliminary findings). (7) Last but not least is the well-known dependence on sex of the synthesis of galactosylglucose in mammary glands.

This survey should not be taken to indicate that all galactose-metabolizing enzymes are affected by sex differences, since there are reports to the contrary. It does suggest that more attention ought to be paid to the matter and that therapists who treat genetic disorders involving galactose might do well to investigate sex hormones as adjuncts. Where an organ transplant is to be used to compensate for a genetic lack, it may be important to choose a donor of the correct sex. It is possible that the developmental changes seen in some galactose-metabolizing enzymes are influenced by progesterone from the mother, either during the foetal stage or during the nursing stage. Evidence for such an effect has been given for ornithine aminotransferase (Herzfeld & Greengard, 1969).

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