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SELECTIVE ANDROGEN INSENSITIVITY OF HEPATIC DRUG-METABOLIZING ENZYMES IN SENESCENT MICE

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Abstract—The normal sexual dimorphism in murine hepatic hexobarbital metabolism (i.e. females > males) was found to be absent in senescent animals. Hexobarbital metabolism, expressed as microsomal activity of hexobarbital hydroxylase and hexobarbital-induced sleep time, in senescent male mice was similar to that in females, but significantly greater than that found in young adult males. No age-related changes in hexobarbital metabolism were observed in intact females. In addition, experiments involving gonadectomies and testosterone administration indicated that both male and female senescent mice were insensitive to the normally repressive effects of androgens on hexobarbital hydroxylase. In contrast, the sexual dimorphism in the activity of *p*-nitrophenol UDP-glucuronosyltransferase was maintained in the senescent mice as well as the usual responsiveness to testosterone regulation. Furthermore, the growth-promoting effects of androgen on the kidneys and seminal vesicles were similarly expressed in young and old mice. Thus, our results suggest the development of an age-dependent and selective insensitivity of hexobarbital hydroxylase to androgenic regulation in the aging mouse.

Many investigators have examined how drug metabolism, and in particular the hepatic monooxygenase system, is affected by the aging process. Although most of these studies have been conducted on the rat, this may not be the most suitable species to use as a model for age-related changes in drug metabolism. First of all, the magnitude of the sexual dimorphism in the metabolism of xenobiotic compounds observed in the rat [1, 2] is not representative of other species, which exhibit more subtle sex differences or no dimorphism at all [1, 3–10]. In fact, the profound decline in monooxygenase activities observed in aging male rats [11, 12] is the result of a disappearance of this dimorphism due to alterations in the population of sex-dependent isozymes of cytochrome P-450. That is, the more active, male-specific form of cytochrome P-450 is replaced by the less active, female-specific form in senescent male rats [13]. Second, the dramatic decrease in plasma testosterone levels seen in the senescent male rat [13–16] is also not characteristic of other species, which exhibit a more subtle decrease in testosterone levels, if any at all [17–25]. This large fall in plasma testosterone is believed to be responsible for the decline in the androgen-dependent, male isozymes and the concomitant decrease in drug-metabolizing capacity observed in old male rats [13, 26]. Because of the peculiarities of the rat noted above, we decided to examine drug metabolism in the senescent mouse.

Previous experiments in this laboratory have shown that mice exhibit a sexual dimorphism in the levels of hepatic monooxygenases which is opposite to that observed in the rat. Compared to females, male mice have a smaller V_{max} (maximal velocity) for hepatic microsomal aminopyrine-*N*-demethylase and hexobarbital hydroxylase, with a correspondingly longer hexobarbital-induced sleep time [7, 27]. Furthermore, we have found that testicular androgens, acting through the hypothalamus and pituitary, produce the sexual dimorphism observed in mice by repressing hepatic monooxygenase activities [7, 27]. In contrast, in the rat, androgens act through the neuroendocrine axis to induce hepatic monooxygenases [1, 2].

To investigate age-related changes in drug metabolism in the mouse, we examined the ability of testosterone to control hexobarbital hydroxylase activity in young adult and senescent, male and female animals of the Crl:CD-1(ICR)BR strain. In addition to hexobarbital hydroxylase, the activity of hepatic UDP-glucuronosyltransferase was also measured to allow comparison of this phase II conjugation enzyme with the monooxygenase in the young and old animals, and because there is a sexual dimorphism in the activity of this enzyme which is opposite to that of the monooxygenase, that is the male has a higher level of activity than the female [7].

METHODS

Male and female Crl:CD-1(ICR)BR mice (Charles River Breeding Laboratories Inc., Wilmington MA), referred to as Crl:CD-1 mice, were purchased at 28–29 weeks of age (retired breeders) and maintained in our facilities for the senescent age group. Male and female Crl:CD-1 mice were purchased at 7 weeks of age for the young age group. The animals were housed on hardwood bedding in plastic cages and

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were given water and commercial mouse diet *ad lib*. The animals were kept in air conditioned quarters, 20–23°, with a photoperiod of 12 hr light : 12 hr dark.

The mice were anesthetized with a mixture of ketamine (10 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.), and bilateral and sham gonadectomies were performed as previously described [6, 7]. Twenty days after surgery, the animals were given daily subcutaneous injections of testosterone propionate (5 mg/kg) or an equivalent volume of corn oil diluent for 17 consecutive days. Hexobarbital (125 mg/kg, i.p.)-induced sleep times were measured on day 10 of injection according to a previously described method [6]. The mice were killed by decapitation on the morning following day 17 of hormone or diluent treatment. At the time of their sacrifice, the animals of the young age group were 13–14 weeks of age and those of the senescent age group were 70–76 weeks of age. Since the mean life span of this strain has been reported to be 64 weeks for males and 74 weeks for females [28] or a 50% mortality at 69 weeks of age for males and 78 weeks of age for females [29], the animals in the old age group can be considered senescent.

Hepatic microsomes were prepared from the 100,000 *g* pellet as previously described [6, 27]. Microsomal protein content was determined by the method of Lowry *et al.* [30] using bovine serum albumin as a standard. Hepatic microsomal hexobarbital hydroxylase was assayed by our modification [6, 27] of the radioenzyme procedure of Kupfer and Rosenfeld [31], which measures the microsomal conversion of radioactive hexobarbital, 5-cyclohexenyl-3,5-dimethyl[2-^{14}C]barbituric acid (Du Pont NEN Research Products, Boston, MA), to 3-hydroxyhexobarbital. Kinetic data were obtained with six different hexobarbital concentrations (75–500 μM) and a microsomal suspension (1.0 to 3.0 mg microsomal protein/ml) prepared from the livers of individual mice. Hepatic microsomal UDP-glucuronosyltransferase was assayed by the method of Winsnes [32], which measures the amount of *p*-nitrophenol (PNP) substrate consumed by this enzyme. Kinetic data were obtained with six different PNP concentrations (25–600 μM), and a microsomal suspension (1.0 to 5.0 mg microsomal protein/ml) was prepared from the livers of individual mice and activated with Triton X-100 (0.25%).

Michaelis constants (K_m) and maximal velocities (V_{max}) were calculated for both enzymes from linear regressions of the data using the method of Hofstee [33]. The correlation coefficients for all of the Hofstee plots were positive and greater than 0.95.

Groups were compared for statistically significant differences by three-way analysis of variance and the two-tailed Student's *t*-test.

RESULTS

The kidney weights of the males and females of both age groups were responsive to androgen stimulation (Fig. 1). Orchiectomy produced decreased kidney weights in both young and old males, while testosterone propionate treatment increased kidney weights in castrated males and females of both age groups. A similar pattern was seen in the seminal vesicle weights (Fig. 2). In young males, gonadectomy decreased the seminal vesicle weights, and testosterone propionate treatment was able to restore the seminal vesicle weights to that of the intact animals. While orchiectomy did not produce a significant decrease in seminal vesicle weights in the senescent males, testosterone propionate administration increased the weights in the castrated mice above that of the intact senescent males, indicating that the accessory glands of senescent animals were also responsive to androgens. Castration and testosterone treatment only produced significant body weight changes in the young males (Fig. 3). Orchiectomy prevented body weight gain in the

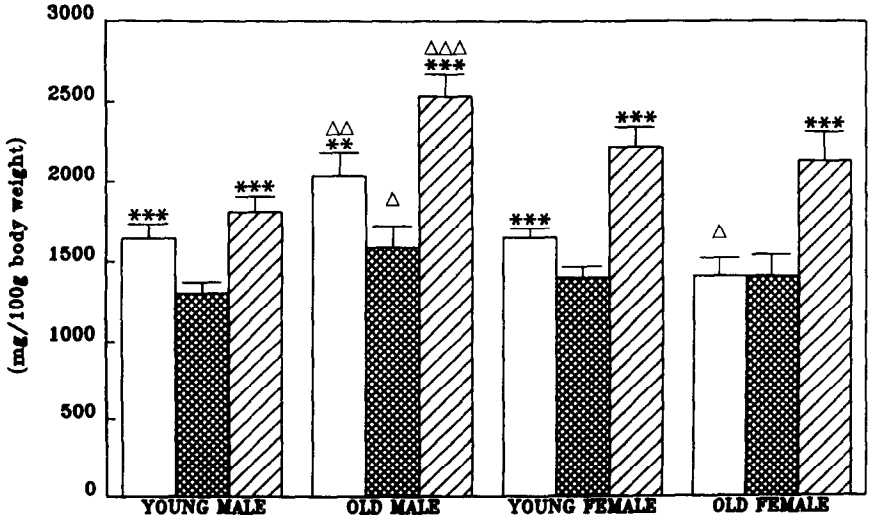

Fig. 1. Kidney weights in sham-gonadectomized (□), gonadectomized (▩), and testosterone-treated, gonadectomized (▨) animals. Values are means ± SEM for eight mice. (**)$P < 0.01$ and (***)$P < 0.001$: significantly different from gonadectomized animals of the same age and sex; (Δ)$P < 0.05$; (ΔΔ)$P < 0.01$; (ΔΔΔ)$P < 0.001$: significantly different from young animals of the same sex and treatment group.

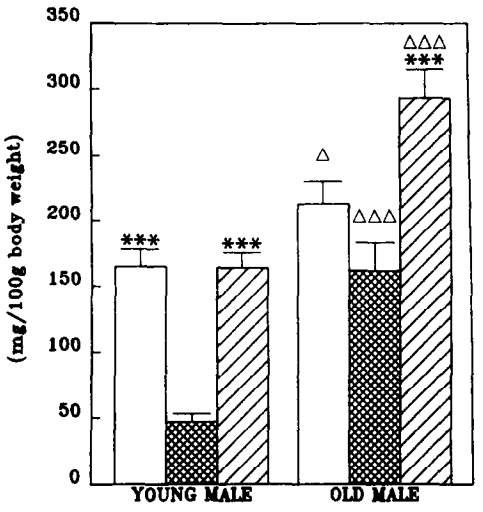

Fig. 2. Seminal vesicle weights in sham-gonadectomized (□), gonadectomized (▩), and testosterone-treated, gonadectomized (▨) animals. Values are means ± SEM for eight mice. (***)$P < 0.001$: significantly different from gonadectomized animals of the same age and sex; (△)$P < 0.05$ and (△△△)$P < 0.001$: significantly different from young animals of the same sex and treatment group.

young animals, and this weight gain was restored by testosterone administration. Since the senescent animals were no longer growing, it was not surprising that castration or testosterone administration did not produce an effect on body weight.

As shown previously in this laboratory [27], the young intact animals exhibited an androgen-dependent sexual dimorphism in the V_{max} for hepatic hexobarbital hydroxylase (Fig. 4). The V_{max} for this enzyme in the young intact male was significantly ($P < 0.05$) lower than that of the young intact female. Orchiectomy elevated the V_{max} in young animals, while testosterone treatment decreased the V_{max} of the monooxygenase in young gonadectomized males and females. In contrast to the young animals, no sexual dimorphism was observed in the activity of hexobarbital hydroxylase in the senescent animals. There was no significant difference in the activity of hexobarbital hydroxylase in the old males and females, whose values were both similar to that found in the young intact females. Neither orchiectomy nor testosterone administration produced any significant changes in the activity of hexobarbital hydroxylase in the senescent animals. The K_m values for this enzyme showed no significant differences between any of the experimental groups (data not shown).

The hexobarbital-induced sleep times correlated quite well with the V_{max} values for hexobarbital hydroxylase (Fig. 5). The young intact males had sleep times that were approximately 50% longer than those of the young intact females ($P < 0.001$). Orchiectomy produced a significant decrease in the sleep time of the young males, while testosterone treatment increased the sleep time in the young gonadectomized animals of both sexes. No significant difference was observed between the sleep times of the old males and old females, which were both comparable to the value reported for the young intact females. In agreement with the hexobarbital hydroxylase activity, orchiectomy and testosterone administration had no effect on the sleep times of the senescent animals.

In the young animals, there was an androgen-dependent sexual dimorphism in the activity of UDP-glucuronosyltransferase (Fig. 6). The young intact males had a significantly higher V_{max} ($P < 0.01$) than

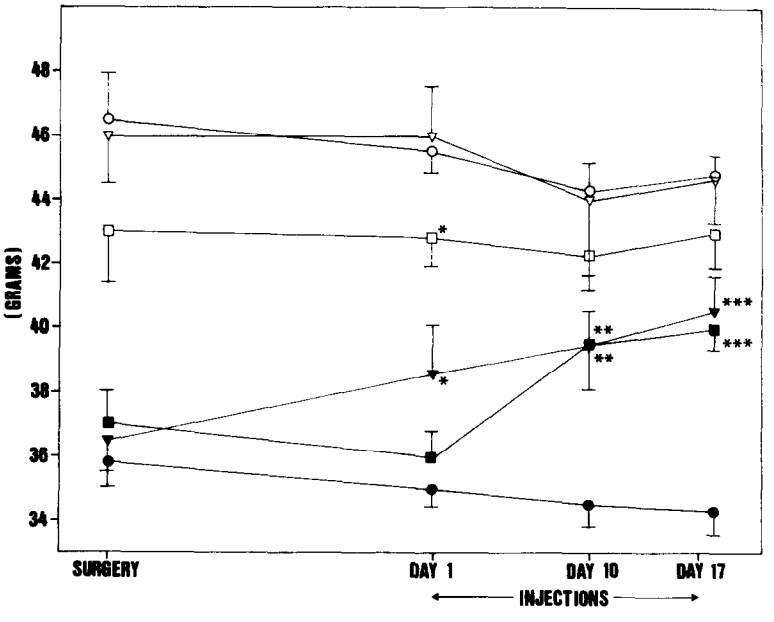

Fig. 3. Body weights of young male (solid) and senescent male (open), sham-gonadectomized (▼, ▽), gonadectomized (●, ○) and testosterone-treated, gonadectomized (■, □) animals. Values are mean ± SEM for eight mice. (*)$P < 0.05$, (**)$P < 0.01$, and (***)$P < 0.001$: significantly different from gonadectomized animals of the same age on the same day of measurement.

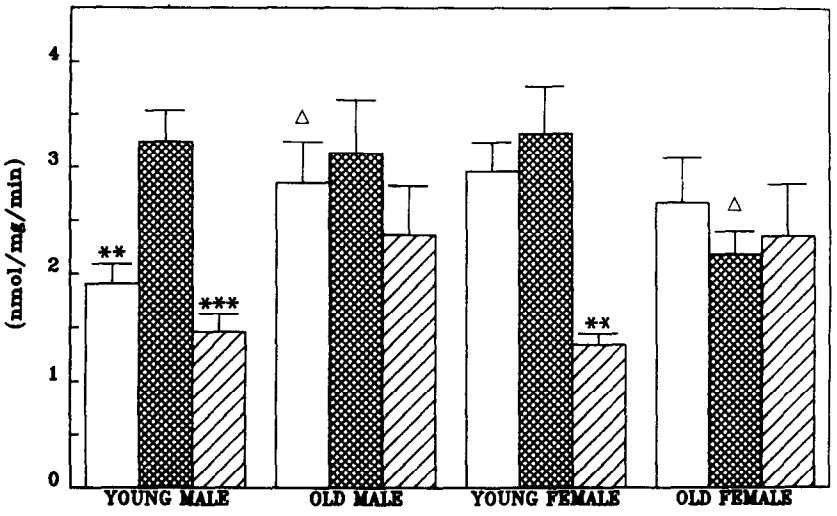

Fig. 4. V_{max} for hexobarbital hydroxylase in hepatic microsomes from sham gonadectomized (□), gonadectomized (▩), and testosterone-treated, gonadectomized (▨) animals. Values are mean ± SEM for eight mice. (**)$P < 0.01$ and (***)$P < 0.001$: significantly different from gonadectomized animals from the same age and sex; (△)$P < 0.05$: significantly different from young animals of the same sex and treatment group.

the young intact females. Orchiectomy produced a decrease in the V_{max} in the young males, and testosterone treatment increased the V_{max} of this enzyme in young, gonadectomized males and females. In the senescent animals, the V_{max} of the intact males was still significantly ($P < 0.01$) higher than that of the intact females. In addition, orchiectomy decreased the V_{max} for UDP-glucuronosyltransferase in the senescent male, while testosterone treatment produced no significant changes in the V_{max} of senescent, gonadectomized animals of either sex. No significant differences in the K_m values for this enzyme were observed between any of the experimental groups (data not shown).

DISCUSSION

The large sexual dimorphism in hepatic monooxygenase activity, and the androgenic control of this dimorphism, have been well established in the rat. Aging studies in rats have shown that this dimorphism disappears with senescence as the level of monooxygenase activity in the male falls to that of the female [11–13, 26]. It has been shown that a change in the population of cytochrome P-450 isozymes from a male-specific form to a female-specific form is responsible for this altered drug-metabolizing capacity in the aged male rat [11–13]. It is believed that the drastic decline in plasma tes-

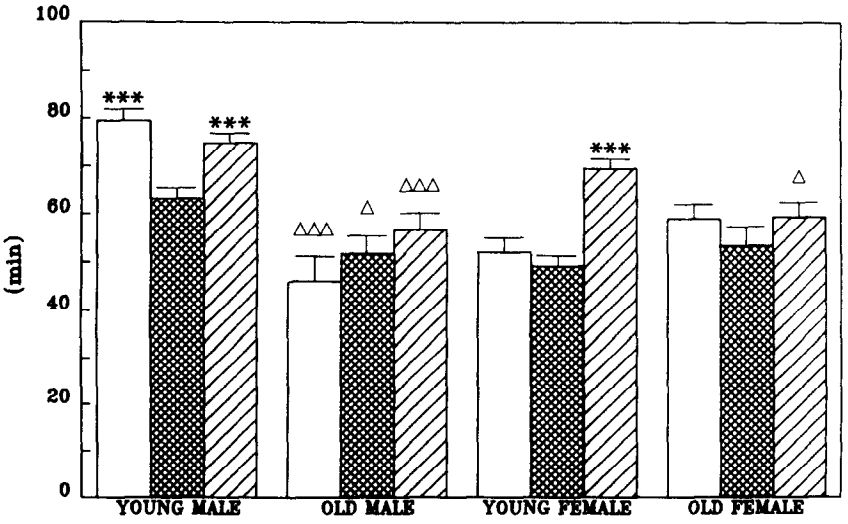

Fig. 5. Hexobarbital-induced sleep times (125 mg/kg) for sham gonadectomized (□), gonadectomized (▩), and testosterone-treated, gonadectomized animals (▨). Values are means ± SEM for eight mice. (***)$P < 0.001$: significantly different from gonadectomized animals of the same age and sex; (△)$P < 0.05$ and (△△△)$P < 0.001$: significantly different from young animals of the same sex and treatment group.

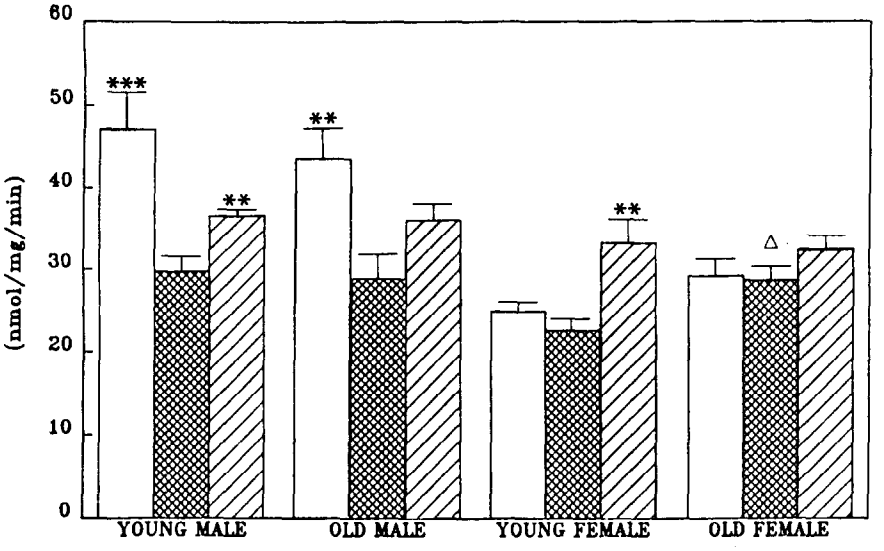

Fig. 6. V_{max} for PNP UDP-glucuronosyltransferase in hepatic microsomes from sham gonadectomized (□), gonadectomized (▩), and testosterone-treated gonadectomized (▨) animals. Values are means ± SEM for eight mice. (**)$P < 0.01$ and (***)$P < 0.001$: significantly different from gonadectomized animals of the same age and sex; (Δ)$P < 0.05$: significantly different from young animals of the same sex and treatment group.

tosterone levels observed in the aging rat is responsible for this changeover in cytochrome P-450 species and the decrease in monooxygenase activities.

In contrast to the rat, much less is known about the sexual dimorphism in hepatic monooxygenase activities in the mouse. In general, the pattern of this sexual dimorphism is one in which the female has greater enzyme activity than the male [7]. Such a pattern has been well established for the Crl:CD-1 strain [3, 4, 27] and has been shown to be due to a pituitary-dependent, androgenic repression of these hepatic enzymes [34]. Several conflicting reports have arisen from studies examining the effects of aging on murine hepatic monooxygenases. Some studies failed to detect any changes in the activities of various drug-metabolizing enzymes in the livers of senescent mice [35, 36]. Other reports have shown an age-related decline in the activities of such enzymes as benzphetamine-*N*-demethylase, ethylmorphine-*N*-demethylase and zoxazolamine hydroxylase with a concomitant increase in the activities of benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin-*O*-deethylase in old male mice [37, 38].

Our results indicate that hepatic hexobarbital hydroxylase activity in the Crl:CD-1 mouse was increased significantly in the senescent male as compared with the young male, whereas the activity of this enzyme showed no age-related changes in the female. Therefore, as is the case in the rat, the sexual dimorphism in the activity of this monooxygenase was abolished by the aging process. Since androgens have a repressive effect on hexobarbital hydroxylase activity in the mouse [7, 27], a decrease in testosterone levels with age may explain the increased activity of this enzyme in old mice observed in this study. However, the fact that the administration of testosterone to senescent, gonadectomized males and females did not result in a reduction of the V_{max} for hepatic hexobarbital hydroxylase or an increase in hexobarbital-induced sleep time, as seen in the young animals, indicates that a simple decline in testosterone levels is not responsible for this increased monooxygenase activity. In addition, the dramatic fall in testosterone levels, which accounts for the age-related changes in hexobarbital hydroxylase activity in the male rat, does not occur in male mice [17–21, 24]. Furthermore, since the kidney weights and seminal vesicle weights clearly responded to testosterone in the senescent animals, it is apparent that there is a selective insensitivity of the hepatic monooxygenase system to androgens, not a generalized lack of response to androgens with aging. This selective androgen insensitivity exhibited in the mouse may not occur in the rat since exogenous testosterone can restore the sexually dimorphic character of the hepatic monooxygenase system in the senescent rat [26].

Our previous studies have shown that androgens repress hepatic monooxygenase activity by regulating pituitary secretion of growth hormone [34, 39]. The fact that kidney and seminal vesicle weights responded to androgens in the senescent animals, while the hepatic monooxygenase system did not, suggests that hypothalamic–pituitary regulation of growth hormone and subsequent control of monooxygenase activity is insensitive to testosterone in the senescent animal. Although age-related changes in growth hormone secretion in the mouse have not yet been elucidated, reports of decreased amplitudes in the pulsatile secretions of growth hormone in the senescent rat [40] and a decrease in the response of LH secretion to estrogen regulation in the mouse [41] suggest that the pituitary may be the source of the altered hexobarbital metabolism in senescent Crl:CD-1 males.

Phase II conjugation enzymes are often overlooked in studies that explore age-related changes in drug metabolism. In our study, we measured PNP

UDP-glucuronosyltransferase activity in hepatic microsomes and, in agreement with other investigators, found an androgen-dependent, sexual dimorphism in the activity of this enzyme in young animals [7, 42]. In addition to the fact that androgens have the opposite effect to that observed in hexobarbital hydroxylase in young animals, this enzyme also differs from the monooxygenase in that this dimorphism persists in senescent mice. Although the old, gonadectomized males and females did not respond to testosterone treatment with an alteration of the V_{max} of PNP UDP-glucuronosyltransferase, castration of the senescent males resulted in a reduction in the V_{max} of this enzyme. Thus, it appears that this isozyme exhibits some responsiveness to androgens during aging. Since the sexual dimorphism in the V_{max} of this conjugation enzyme does not disappear with senescence, it is possible that PNP UDP-glucuronosyltransferase is regulated by a mechanism which is different from the neuroendocrine control of hepatic monooxygenase activities.

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