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Sex difference in allopurinol oxidizing enzyme activity in mouse liver supernatant fraction

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A PREVIOUS report from this laboratory showed that there are marked sex differences in acute toxicity of allopurinol in rats and mice, females having higher LD_{50} values than males.[1] It was observed that more [^{14}C]compounds accumulated in the kidney in male mice than females after intraperitoneal administration of [^{14}C]allopurinol.[1] In order to obtain more data to explain the above findings, we studied the rate of oxidation of allopurinol in both sexes of mice by determining allopurinol oxidizing enzyme activity of liver supernatant. The results are reported here.

6-[^{14}C]allopurinol was synthesized according to the method of Elion *et al.*[2] Mice of both sexes were decapitated, the liver was immediately removed and blotted to remove blood. The tissues were weighed and homogenized in 0·05 M potassium phosphate buffer, pH 7·8, (1 g of liver + 5 ml of buffer), in a glass homogenizer with a Teflon pestle for 1 min. The homogenate was centrifuged at 600 *g* at 4° for 20 min and the supernatant was centrifuged again at 105,000 *g* at 4° for 60 min. The supernatant was used as an enzyme preparation. Allopurinol oxidizing enzyme activity was assayed by [^{14}C]oxipurinol formation from [^{14}C]allopurinol. The assay mixture contained 0·05 M potassium phosphate buffer, pH 7·8 (containing 0·005% Versene Fe-3), 4·8 mM [^{14}C]allopurinol and 50 μl of mouse liver supernatant in a final volume of 125 μl. The reaction was started by the addition of the enzyme and the mixture was incubated at 37° in a shaking water bath. The reaction was stopped after 30 min by the addition of 20 μl of 20% trichloroacetic acid. The precipitated protein was removed by centrifugation and the supernatant was applied to a 2 × 40 cm Toyo No. 51 chromatographic paper. Chromatograms were developed in butanol: formic acid: water (77:11:12, by vol) for 14–16 hr. Regions of strips containing allopurinol and oxipurinol were viewed under ultraviolet light and were cut out. The radioactivity was counted in a liquid scintillation counter, Beckman model LS-150. The scintillation solvent contained 5 g of 2,5-diphenyloxazole and 300 mg of 1,4-bis [2-(4-methyl-5-phenyloxazolyl)]-benzene per litre of toluene. Enzyme activity was expressed as μmoles of oxipurinol formed /100 mg of protein/hr. Protein was determined by the method of Lowry *et al.*[3]

As shown in Fig. 1, there was a remarkable sex difference in allopurinol oxidizing enzyme activity in mice. In males, the activity increased after 4 weeks of age and reached almost maximum at 6 weeks of age. The enzyme activity remained constant in females. Adult males exhibited about 3·5 times greater oxidizing activity than females. The age at which the sex difference of the enzyme activity appeared may reflect the period in development in which secretion of sex hormones begin. This result tempted us to investigate the effect of castration and the effect of sex hormone injections in mice of both sexes.

Figure 2 shows the influence of castration on allopurinol oxidizing enzyme activity in adult mice of either sex. In females the enzyme activity was not altered after castration. However, in males the enzyme

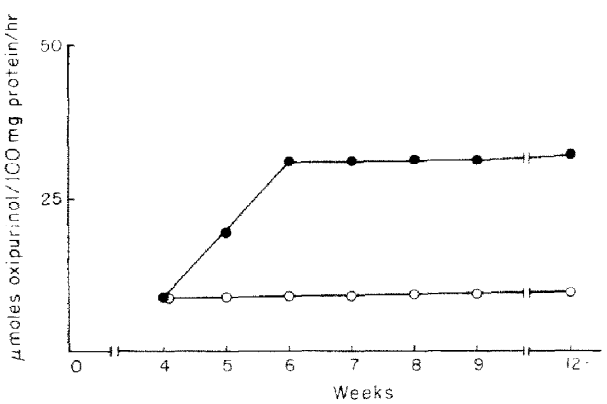

FIG. 1. Changes in allopurinol oxidizing enzyme activity of mouse liver supernatant during maturation. The assay procedure is described in the text. Each point represents the mean of four animals. Closed circles: males; open circles: females.

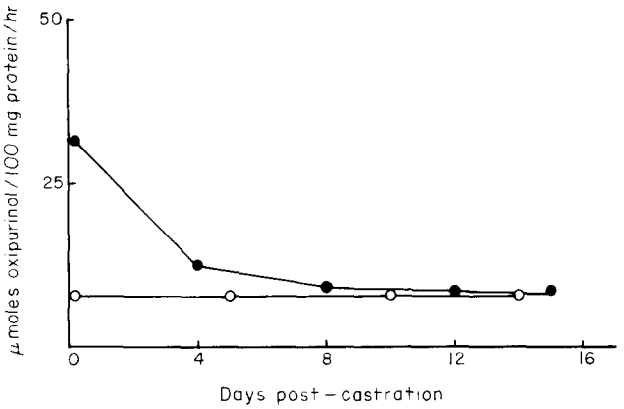

FIG. 2. Influence of castration on allopurinol oxidizing enzyme activity in liver supernatant fraction of mice. The assay procedure is described in the text. Each point represents the mean of two animals. Closed circles—males; open circles—females.

activity decreased progressively and reached the female level at approximately 8 days after castration. These results indicate that the allopurinol oxidizing enzyme activity may be controlled by androgens. Administration of sex hormones following castration supports this suggestion. The results are shown in Fig. 3. The oxidation rate of allopurinol in both sexes of mice increased to the level of that in normal adult males when treated subcutaneously with testosterone propionate for 10 days, while estradiol 17-β treatment showed no influence on the activity in castrated mice of either sex. Moreover, the *in vitro* addition of testosterone propionate to the enzyme preparation from castrated mice caused no changes in the enzyme activity. These results suggest that the most probable mechanism of action of testosterone on allopurinol oxidizing enzyme may involve enzyme induction.

It is widely accepted that the hydroxylation of allopurinol is catalyzed by xanthine oxidase (xanthine–oxygen oxidoreductase, EC 1.2.3.2). Allopurinol is rapidly oxidized *in vivo* to oxipurinol and excreted in the urine,[2] while the hydroxylation of allopurinol by xanthine oxidase *in vitro* is extremely slow. The reasons for this disparity have not been fully elucidated.

Chalmer *et al.*[4] observed that a xanthinuric patient who presumably lacked xanthine oxidase excreted appreciable amounts of oxipurinol in his urine when allopurinol was given by mouth. Other evidence suggests that allopurinol is oxidized by enzyme(s) other than xanthine oxidase at the substrate concentration used in this experiment (unpublished data). In addition, Johns *et al.*[5] and Krenitsky *et al.*[6] recently

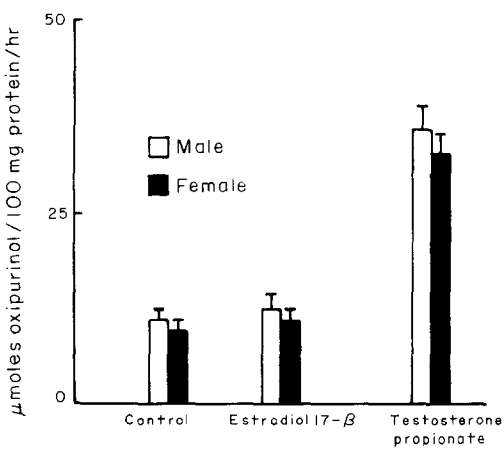

FIG. 3. Effect of testosterone propionate and estradiol 17-β on allopurinol oxidizing enzyme activity in liver supernatant fraction after castration in mice. Daily injections of 0·15 mg testosterone propionate and 0·015 mg of estradiol 17-β in 0·1 ml olive oil were administered s.c. for 10 days, which began 12 days after castration. The assay procedure is described in the text. The values are the means ± standard errors of four animals.

reported that rabbit aldehyde oxidase (aldehyde : oxygen oxidoreductase, EC 1.2.3.1) has the ability to oxidize allopurinol to oxipurinol *in vitro*. Furthermore, Huff *et al.*[7,8] showed that there is a sex difference of aldehyde oxidase activity in mice when *N*-methylnicotinamide was used as substrate. Therefore we are now investigating the relationship between allopurinol oxidizing enzyme and aldehyde oxidase in mice.

Elion *et al.*[2,9] reported that allopurinol is rapidly oxidized *in vivo* to oxipurinol which also inhibits xanthine oxidase. Previously we reported that the ^{14}C-compounds disappear much slower in male than in female mice after labeled allopurinol treatment. Thus it seems that this sex difference in allopurinol oxidizing activity may, to some extent, be concerned with acute toxicity.

Further work is needed to clarify this problem.

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Inability of rat brain homogenate to oxidize amphetamine

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In a recent series of papers in this journal Guha and Mitra[1–3] have reported that homogenates of rat and guinea pig brain catalyze the reduction of neo-tetrazolium chloride (NTC) in the presence of amphetamine. The system has been described as an amphetamine dehydrogenase; the most recent report suggests that tranylcypromine is also actively dehydrogenated by this system.[3] However, the activity of the enzyme system has been estimated by measuring the production of diformazan from NTC. The authors have not determined that a degradation product of amphetamine is produced in the course of the reaction.

Hucker[4] and Parli and McMahon[5] have recently reported on the metabolism of amphetamine with no evidence for a dehydrogenase pathway. Earlier work from this laboratory, using [^3H]labeled amphetamine, had shown that rats convert the drug *in vivo* by *p*-hydroxylation and oxidative deamination.[6] The present communication reports on attempts to measure the production of metabolites of [^3H]amphetamine by the rat brain dehydrogenase system as described by Guha and Mitra.[1–3]