TRYPTOPHAN PYRROLASE AS A FUNCTION OF SEX AND DEVELOPMENT IN MICE

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SUMMARY: The hepatic enzyme tryptophan pyrrolase was assayed from male and female Swiss/ICR mice of different ages, for example, the neonate, 5-day, 10-day, 25-day, 30-day, 35-day, 40-day and 45-day olds. These data show an increase through the 15-20th day followed by a decline in enzymic activity, which drop is succeeded by a rise in activities in both sexes by the 30th day of development. These enhanced activities are steeper in the males compared to the females. Males show a steady level of enzymic activity by day 35, whereas females tend to exhibit, around this time period, an oscillation in enzymic activity. Possible explanations of these results are offered.

INTRODUCTION: Due to its central importance in tryptophan metabolism, tryptophan pyrrolase (L-tryptophan: oxygen oxidoreductase, E.C.1.13.1.12) offers many interesting facets of investigation (1). Although this enzyme has been studied in several mammalian species, data on its developmental profiles are relatively scant as well as disputable (2-8); none yet, to our knowledge, has been reported on mice. Notably lacking in this regard are enzymic activities as a possible function of sex and development. In this paper, we present profiles of hepatic holotryptophan pyrrolase from male and female mice; the nature and the possible significance of these results are discussed.

MATERIALS AND METHODS: Albino Swiss/ICR mice, utilized in these experiments, were obtained from Texas Inbred Mice Company, Houston, Texas. Animals were strictly matched according to their age and body weight. To circumvent circadian oscillation, all animals were sacrificed and livers homogenized between 9-9:30 hours. Livers were carefully extracted (20% homogenate) with 0.14 M KCl-0.0025 N NaOH (9) containing 0.03 ml of mercaptoethanol (10) per 100 ml of the extracting solution, which

also contained 3.0 mg of L-tryptophan. Tryptophan in the amount added, stabilizes the enzyme (11) and mercaptoethanol protects the heme iron from oxidation during enzyme extraction. Homogenates were centrifuged for 45 minutes at 14,000 g and the supernatant submitted to enzymic analysis. One ml reaction mixture contained the following: L-tryptophan, 4.6 μmoles; Na_2HPO_4 buffer, pH 7.5, 100 μmoles; 0.2 ml of the supernatant as described above. These mixtures were incubated in open 50 ml Erlenmeyer flasks on a water bath shaker at 38°C for one hour. Controls run simultaneously contained all the components except L-tryptophan. All reactions were stopped with the addition of trichloroacetic acid (6.7%; 3.0 ml/ml reaction mixture). Samples were then centrifuged and aliquots (2.0 ml of supernatants) were utilized for the estimation of kynurenine (12, 13) as modified by Baglioni (14). One unit of specific enzymic activity reported in this paper refers to μmoles of kynurenine formed/gram liver/1 hr at 38°C. The assay measures endogenous holotryptophan pyrrolase (15), perhaps a closer physiological index of the enzyme in vivo.

RESULTS AND DISCUSSION: Data on the levels of endogenous holotryptophan pyrrolase are presented in Table I and Figure I. The following observations may be made from these values; (i) specific enzymic activities (mean 1.362 units) obtained in 35, 40 and 45-day old males are significantly greater (at .05 level) than those obtained from the rest of the age groups in either sex; (ii) enzyme profiles in both sexes until age 25 days are essentially the same; (iii) there is a sharp drop in enzymic activity in the males from day 20 to day 25 (a 42 percent reduction), whereas a similar process (a 33 percent reduction) in the females seems to be more gradual spanning day 15 through day 30 of development; (iv) there is a steep linear increase (2.6 fold) in the males from day 25 through day 30 which then levels off by day 45; (v) the mean enzymic activity in 132 males (neonates through 45-day old) is 22 percent higher (0.812 units) than the females (0.634 units) of a strictly matched population; (vi) an examination of the data reveals the attainment, in the males, of a steady level of enzymic activity (1.4 units) by day 35; the females tend to show around this time period, an oscillation in enzymic activity between 0.55 and 0.95 units, with a mean of 0.75 units which amounts to 53 percent of the male enzymic activity.

TABLE I

ENDOGENOUS HEPATIC HOLOTRYPTOPHAN PYRROLASE IN MALE AND FEMALE SWISS/ICR MICE

Age (Days)	Specific Activity μmoles kynurenine /gram liver/hour ± standard deviation	Specific Activity μmoles kynurenine /gram liver/hour ± standard deviation
Neonates	0.156±0.062 (30)[1]	0.173±0.052 (30)
5 days	0.281±0.061 (18)	0.233±0.056 (18)
10 days	0.519±0.063 (18)	0.472±0.036 (18)
15 days	0.762±0.081 (15)	0.866±0.249 (15)
20 days	0.906±0.403 (9)	0.764±0.278 (9)
25 days	0.526±0.138 (6)	0.667±0.156 (6)
30 days	0.879±0.011 (6)	0.585±0.097 (6)
35 days	1.360±0.201 (6)	0.930±0.076 (6)
40 days	1.339±0.174 (6)	0.962±0.078 (6)
45 days	1.388±0.246 (18)	0.689±0.093 (18)

[1]Figures in parenthesis indicate the total number of mouse livers assayed for enzymic activity.

Significant differences (.05 level; Factorial Analysis of Variance with Duncan Multiple Range Test) of the above mean values are shown by "greater than" symbols in the following fifteen series. A "slash" between two values indicates non-significant difference.

Series I: 1.388/1.360/1.339 > 0.962; Series II: 0.962/0.930/0.906/0.879/0.866/0.764/0.762/0.689/0.667; Series III: 0.962 > 0.585; Series IV: 0.930/0.906/0.879/0.866/0.764/0.762/0.689/0.667/0.585; Series V: 0.930/0.906 0.526/0.519; Series VI: 0.879/0.866/0.764/0.762/0.689/0.0.667/0.585/0.526/0.519; Series VII: 0.879/0.866 0.472; Series VIII: 0.764/0.762/0.689/0.667/0.585/0.526/0.519/0.472; Series IX: 0.764/0.762/0.689/0.667 > 0.281/0.233; Series X: 0.585/0.526/0.519/0.472/0.0.281/0.233; Series XI: 0.585 > 0.173; Series XII: 0.526/0.519/0.472/0.281/0.233/0.173; Series XIII: 0.519/0.472/0.281/0.233/0.173; Series XIV: 0.526/0.519 > 0.156; Series XV: 0.472/0.281/0.233/0.173/0.156.

The foregoing may be explained due to the onset of sexual maturity in mice which is a more complex process in the female than in the male. Sexual maturity is marked by a number of interactions among the hypothalamus,

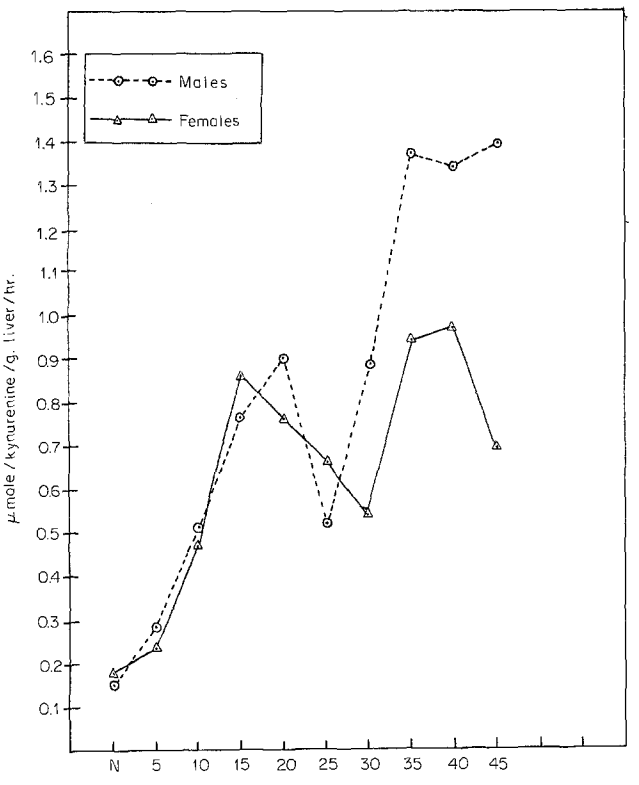

FIGURE I: Graphic Presentation of Hepatic Tryptophan Pyrrolase In Male and Female Mice During Development

anterior pituitary, gonadal and placental hormones, the latter (placental) factors, however, being inoperative in males as well as non-pregnant females. Circulating estrogens in sexually mature females could affect hepatic tryptophan pyrrolase. Figure I shows a cyclic trend in enzymic activity which does seem to coincide with sexual maturity and the onset of estrous cycle with an usual periodicity of 4 to 5 days (16). On the contrary, higher enzymic activities, essentially non-fluctuating, are attained in the males by day 35. Excess estrogens, transported into the liver, are metabolized by microsomal hydroxylases which vie, far more efficiently, for some common reaction components (i.e. O_2, heme) with tryptophan pyrrolase. Interestingly enough, cortisol

and corticosterone, which are chemically akin to estrogens, require a specific α_1 globulin (named transcortin, M.W. 52,000) for their transport; plasma levels of transcortin are, in turn, controlled by thyroid hormones and estrogens. Thus, circulating cortisol and corticosterone, which enhance the transcription of m-RNA for apotryptophan pyrrolase, could be affected by estrogens. A possible regulation, at single or multiple points of control, is thus visualized. Experiments are in progress in trying to answer some of these questions.

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