KYNURENINE METABOLISM IN RATS: SOME HORMONAL
FACTORS AFFECTING ENZYME ACTIVITIES

Brenda D. Manning[1] and Merle Mason

Department of Biological Chemistry

The University of Michigan

Ann Arbor, Michigan 48104

SUMMARY

The effects of certain long-term hormonal treatments of male
and female rats on the activities of several enzymes involved in
kynurenine synthesis and degradation were determined to further
rationalize previously observed sex differences. Treatment caus-
ing elevated tryptophan oxygenase activities (corticosteroid ad-
ministration) did not consistently result in changes of kynur-
enine hydrolase and kynurenine aminotransferase activities like
those seen with estrogen treatments. Differences in the latter
enzyme activities caused by sex differences or treatment-induced
changes in estrogen-androgen status were not consistently assoc-
iated with altered oxygenase activities. Castration of male
rats or treatment of female rats with androgen altered the kid-
ney kynurenine aminotransferase activities toward those of the
opposite sex, consistent with the view that differences in an-
drogen-estrogen status during post-natal development satis-
factorily account for the sex differences in enzyme activity.

Kynurenine is formed in liver from tryptophan via N-formyl kynurenine.
The reactions are catalyzed by tryptophan oxygenase (TO) (EC 1.13.1.12) and
kynurenine formamidase (EC 3.5.1.9). Kynurenine is converted to anthranilic
acid by kynurenine hydrolase (KH) (EC 3.7.1.3), to 3-hydroxykynurenine by
kynurenine hydroxylase (EC 1.14.1.2), and to kynurenic acid by kynurenine
aminotransferase (KAT) (EC 2.6.1.7). The KH and KAT catalyze analogous re-
actions of 3-hydroxykynurenine to form 3-hydroxyanthranilic acid and xan-
thurenic acid, respectively. Evidence concerning the intracellular local-
ization of these several enzymes has been cited by Okamoto et al (1). In
rat liver, KH is located in the cytosol, the hydroxylase in the mitochondrial
outer membrane, and KAT in the mitochondrial inner membrane or matrix. A rel-
atively more active KAT is found in rat kidney (2,3), distributed in both the
mitochondrial and cytosolic fractions (4). The control mechanisms determining
the distribution of kynurenine to these various locations and pathways are of
substantial interest but are largely unknown.

Adaptive or developmental variations in enzyme activity are probably
factors in determining the extent of catabolism by the several pathways.
Previous studies have demonstrated a sex difference in the activities of the
rat kidney KAT and the liver KH (5-7). Male KAT activities were depressed
to female levels by diethylstilbestrol treatments and female levels were el-

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[1] Present address: Zoology Department, The University of Michigan, Ann Arbor

evated by ovariectomy (6-7), so it was assumed that the sex differences are
based on differences in estrogen production in the two sexes during post-
natal development. The responses to steroidal estrogens and to androgen
excess or deficiency have not been determined, nor is the relationship of
these effects of gonadal hormones to the well-known actions of corticosteroids
on TO induction known. We have now measured the TO, KH, and KAT activities
in rats subjected to various hormonal regimens and have tried to evaluate
the relationship of the resulting alterations of enzyme activities to those
caused by the sex difference.

MATERIALS AND METHODS

     Albino rats (Sprague-Dawley strain) were maintained on Rockland Rat Diet
ad libitum with a 12 hour light period beginning at 6 a.m. and terminating at
6 p.m.

Tissue Fractionation - The method was like that used previously except that
α-ketoglutarate was omitted from the 0.25 M sucrose solution (7).

Tryptophan Oxygenase Assay - One-gram portions of cold liver, obtained as
described previously (7), were homogenized and assayed as described by Seglen
and Jervall (8).

Kynurenine Hydrolase Assay - The incubation mixture (1.2 ml) contained 0.05 M
sodium pyrophosphate buffer, pH 8.0, 10^{-4} M PLP, 1.54 mM L-Kynurenine sulfate,
and 0.4 ml of liver supernatant fraction. The mixture was incubated 30 min-
utes at 37° and the reaction was stopped by the addition of 5 ml of 5% TCA.
The mixture was centrifuged and 3 ml of clear supernatant fluid was mixed with
6 ml of 5% trichloroacetic acid (TCA). The amount of anthranilic acid in the
TCA solution was determined by a modification (2) of the method of Bratten
and Marshall (9) which destroys the diazotized derivative of the residual
kynurenine.

Kynurenine Aminotransferase Assays - Incubation mixtures (0.6 ml), containing
0.10 M imidazol buffer (pH 6.5), 0.1 mM PLP, 5 mM α-ketoglutarate, 3 mM
L-kynurenine sulfate, and 0.1 ml enzyme fraction, were incubated 30 minutes
at 37° and were then deproteinized with 10 ml of 1% boric acid in 95% ethanol.
After 15 minutes the solutions were centrifuged at 5000 x g for 5 minutes.
The clear supernatant fluid was read at 333 and 365 mμ against the boric acid-
ethanol diluent. These values were used to calculate the amount of kynurenic
acid formed (10).

     Earlier experiments with this enzyme have utilized phosphate buffers.
Mason et al. (11) found that imidazol buffer containing 1 mM $CaCl_2$ gave
higher activities than phosphate buffer, apparently because Ca^{++} was required
for optimal penetration of the mitochondrial inner membrane by α-ketoglutarate.
Ca^{++} did not affect the enzyme activity of the supernatant fraction or of ex-
tracts of disrupted mitochondria. Fig. 1 compares activities obtained with
the two buffers. Although the activities measured with phosphate buffer appear
to be underestimated, the discrepancy does not alter the earlier conclusion
that a substantial sex difference in kidney KAT activity is found in rats
weighing over approximately 100 gm. The liver mitochondrial KAT activity
was only slightly greater in the imidazol-$CaCl_2$ buffer.

Activity Units - Both specific activities and activities expressed as μmoles
product formed per hour per gm wet weight tissue per 100 gm body weight were
calculated. For brevity, only the latter activities are presented (± standard
deviations); comparisons of specific activities did not alter the interpre-
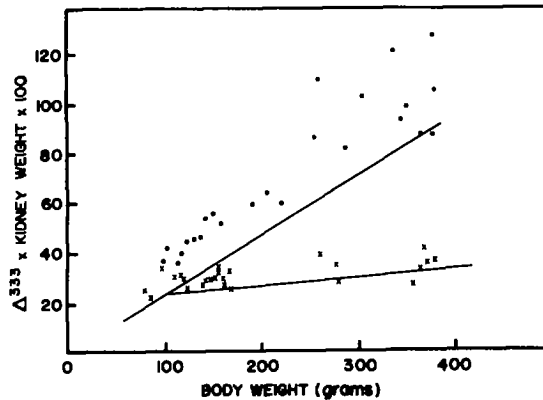
FIG. 1

Comparison of kidney mitochondrial KAT activities of male (o) and female (x) rats as assayed in phosphate buffer (solid lines) and in imidazol-CaCl$_2$ buffer (points). To facilitate comparison with previous reports (7), activity is expressed as the O. D. change (Δ^{333}) resulting from kynurenic acid formation under standard assay conditions.

tations of the data. Since the sex differences and hormonally-induced dif-ferences in enzyme activities generally increased with increasing organ and body weight (see Fig. 1, for example), all data were also plotted as total organ activities vs body weight and were found to show similar trends to those seen in Fig. 1. Such comparisons did not result in interpretations at var-iance with those based on the tables.

RESULTS

Diurnal Rhythms - In 3 separate experiments, utilizing groups of 2 male and 2 female rats at 4 hr intervals for 24 hours, we were unable to detect sig-nificant variations of KAT and KH activities from those seen at 9 a.m. (as in Table III). TO showed the previously-noted (12) 2-fold diurnal increase with a maximum near midnight.

Tryptophan Oxygenase Activities in Males and Females - To avoid complications arising from diurnal variations of TO, all subsequent experiments were done with animals killed at 9 a.m. TO activities of male and female rats are shown in Table I. No significant sex differences were seen.

Effects of Exogenous Estrogens on Enzyme Activities - Neither oral estradiol-17β (0.5 µg per ml. drinking water) nor estradiol benzoate injections (10 µg daily) caused significant effects on TO activities of males (Tables II and III). In contrast, the estradiol benzoate resulted in 2-fold increases in TO activities in adult females. The KH activities of estradiol benzoate-injected males were depressed to the normal female levels but oral estradiol had no significant effect on the KH levels. Kidney supernatant and mitochondrial KAT (s-KAT and mKAT) activities of males were depressed to female levels with both methods of treatment. The kidney m-KAT levels of females injected with estradiol benzoate were slightly lower than those of control females. Neither treatment affected the liver m-KAT activities.

TABLE I

Comparison of Tryptophan Oxygenase Activities

in Males and Females

Group I (80-144 gm)		Group II (217-305 gm)	
No. Rats	Enzyme Activity	No. Rats	Enzyme Activity
Male	6	31.9 ± 4.9	Male	20	24.4 ± 5.3
Female	5	33.2 ± 7.8	Female	17	23.5 ± 3.8

TABLE II

Effects of Estradiol-17β Treatment on Enzyme Activities

Male rats (70 ± 5 g) were maintained with or without estradiol-17β in the
drinking water (0.5 µg per ml) and were sacrificed at various times after
7 to 40 days of treatment. Numbers of animals are shown in parentheses
below column headings.

	Enzyme Activities		
	Control Males (6)	Estradiol- Treated Males (5-7)	Control Females (6)
TO	15.9 ± 2.3	15.3 ± 7.9	___
KH	30.2 ± 2.5	28.5 ± 3.5	23.7 ± 2.5
Kidney s-KAT	29.9 ± 1.4	18.3 ± 1.8[a]	16.7 ± 2.5
Kidney m-KAT	20.0 ± 2.5	10.8 ± 1.9[a]	10.4 ± 1.6
Liver m-KAT	78.7 ±10.6	68.9 ± 7.7	74.9 ± 6.6

[a] Significantly lower than control males at $P > 0.001$.

Effects of Exogenous Glucocorticoids on Enzyme Activities - Cortisol 21-phos-
phate was added to the drinking water of male rats at dosages of 100, 50, 10,
and 0 µg per ml. Growth was retarded at the highest dosage. TO activity was
increased 3-fold at that level but was not elevated significantly at the lower
dosages, at which growth retardation was minimal (Table IV). KH was increased
significantly at all 3 dosages. Only at the highest dosage was KAT activity
significantly altered, the liver m-KAT and kidney s-KAT activities being
slightly higher.

Effects of Androgens on Enzyme Activities - The effects of castration of males
and the administration of testosterone propionate to females are shown
in Table V. The liver KH and kidney m-KAT activities were significantly de-
creased in the castrates relative to the sham-operated controls. The TO,
kidney s-KAT, and liver m-KAT activities were not significantly altered.

    Testosterone propionate-injected female rats (1 mg daily for 5 to 18
days) showed elevated kidney s-KAT and m-KAT activities relative to controls
receiving vehicle alone. The liver m-KAT and KH activities were not affected.

TABLE III

Effect of Estradiol Benzoate (EB) Injections on Enzyme Activities

Male (135 ± 5g) and female (210 ± 5g) rats were injected daily with 0.1 ml
corn oil subcutaneously or with 0.1 ml of EB in corn oil (10 µg daily) and
were sacrificed after 16 to 30 days of treatment. Number of animals are
shown in parentheses below headings.

	Enzyme Activities			
	Control Males (6)	EB-treated Males (6)	Control Females (3)	EB-treated Females (3)
TO	24.1 ± 7.9	24.5 ± 3.9	23.1 ± 2.8	47.9 ± 5.8^a
KH	55.0 ± 4.1	41.1 ± 1.7^b	37.7 ± 1.3^b	38.7 ± 0.9^b
Kidney s-KAT	36.4 ± 1.8	29.8 ± 3.9^b	26.6 ± 0.6^b	23.6 ± 3.9^b
Kidney m-KAT	33.2 ± 1.2	19.6 ± 2.7^b	17.7 ± 0.8^b	13.1 ± 1.5^c
Liver m-KAT	89.9 ± 9.8	92.5 ± 6.7	85.2 ± 9.3	88.8 ± 13

^{a}Significantly higher than control females at P>0.001
^{b}Significantly lower than control males at P>0.001
^{c}Significantly lower than control females at P>0.02

TABLE IV

Effects of Cortisol 21-phosphate Treatments on Enzyme Activities

Male rats (150-170 g) were given drinking water containing 0, 10, 50, or 100 µg
cortisol 21-phosphate per ml and were sacrificed 5 to 26 days after treatment
began. Numbers of animals are shown in parentheses below headings.

	Enzyme Activities			
	Dosage of cortisol 21-phosphate			
	0 µg (22-24)	100 µg (5-6)	60 µg (4)	10 µg (13)
TO	24.0 ± 6.3	99.6 ± 17.5^a	26.9 ± 16.9	27.4 ± 6.7
KH	31.5 ± 3.1	41.7 ± 4.0^a	40.0 ± 5.5^a	37.6 ± 3.8^a
Kidney s-KAT	28.8 ± 2.3	31.6 ± 2.7^b	30.0 ± 3.4	28.6 ± 2.4
Kidney m-KAT	20.9 ± 4.1	24.0 ± 3.2	19.2 ± 2.3	19.4 ± 2.9
Liver m-KAT	83.1 ± 14.9	114.6 ± 2.6^a	88.0 ± 14.8	90.4 ± 18.6

^{a}Significantly elevated above controls at P<0.001
^{b}Significantly elevated above controls at P<0.02

DISCUSSION

    Our studies, like others (12), detected a nocturnal increase in TO ac-
tivities but they did not detect a diurnal variation of KH or KAT activities.
In animals sacrificed at 9 a.m., no significant sex difference in the TO ac-
tivities was found (Table I). Another report (13) also showed no difference,
although another (14), using suboptimal assay conditions, reported a difference.

TABLE V

Effects of Castration of Males and Androgen-Treatment
of Females on Enzyme Activities

Male rats (95 ± 5g) were orchiectomized and the controls received similar
incisions without orchiectomy. Animals were sacrificed 43 to 48 days after-
ward. Female rats (90-100 g) were injected daily with 0.1 ml corn oil or
with 0.1 ml corn oil containing 1 mg testosterone propionate and were sac-
rificed after 5 to 18 days of treatment. Numbers of animals are shown under
column headings.

	Enzyme Activities			
	Control Males (4)	Castrated Males (4)	Control Females (6)	Androgen-Treated Females (6)
TO	32.8 ± 2.8	25.7 ± 4.2	—	—
KH	56.1 ± 2.2	46.1 ± 4.6^a	40.1 ± 5.1	41.7 ± 6.4
Kidney s-KAT	32.2 ± 2.3	33.3 ± 9.1	25.3 ± 3.0	33.4 ± 5.5^c
Kidney m-KAT	35.5 ± 4.9	24.3 ± 5.8^b	14.9 ± 1.7	23.3 ± 4.3^d
Liver m-KAT	94.5 ± 4.8	87.5 ± 6.4	81.1 ±14.3	84.1 ± 8.5

^{a}Significantly lower than male controls at $P<0.01$
^{b}Significantly lower than male controls at $P<0.05$
^{c}Significantly higher than female controls at $P<0.02$
^{d}Significantly higher than female controls at $P<0.01$

The two methods of estrogen treatment did not yield the same effects on
enzyme levels (Tables II and III), possibly because they resulted in different
levels of active circulating estrogens during the treatment period. Our find-
ing that estradiol benzoate injection resulted in 2-fold increases in the TO
activity of adult females (Table III) confirms a previous report (15). Depres-
sion of the male kidney KAT activities by estradiol benzoate treatment demon-
strates for the first time that steroidal estrogens can prevent the development
of the higher activities typical of males.

It has been suggested (16) that estrogens promote the excretion of ele-
vated levels of tryptophan catabolites by causing increased glucocorticoid
levels in the blood (17), which is known to induce the TO. The effects of
large doses of estradiol benzoate on TO activity (Table III and ref. 15) are
consistent with this view. However, the treatment with estradiol-17β did
not cause elevated TO activities although it did depress the KH activity.
In view of these results, and since there was no sex difference in TO activ-
ities (Table I), even though female rats are reported (18) also to have
higher blood estrogen and corticosteroid levels than males, there does not
appear to be a consistent linkage of elevated TO activities with elevated
estrogen levels.

From a similar point of view, one may question whether the depression
of KH and KAT activities by estrogen treatments might be mediated by effects
on corticosteroid levels. Our experiments (Table IV) show that cortisol 21-
phosphate, even at levels which induced the TO, did not result in depressed
KAT activities and actually increased the KH activities at all dosages. It
thus seems more likely that increased corticosteroid levels would antagonize
the depressing effects of estrogens on the enzyme activities rather than
mediate them.

Considering the dissimilarities of the effects of estrogen and cortico-
steroid treatments on the various enzyme levels, our experiments do not sup-
port the view that estrogens alter these activities by causing elevated cor-
ticosteroid levels or through metabolic adaptations resulting from TO induc-
tion. It seems reasonable however to suggest that the estrogens may have
acted on the enzymes partly through their effects on the production or func-
tion of androgens. Interference with androgen production is indicated in
the estradiol benzoate experiments (Table III); the average testicular weight
of treated rats was less than one-half that of controls. Since castration of
males caused depressed KH and KAT activities (Table V) and androgen treatment
of females caused elevated levels, the depressed activity seen with estrogen
treatment may be attributed to androgen deficiency. Such an interpretation
would be more consistent with other observations concerning the effects of
steroids on enzyme activities; the elevation by androgens is typical and the
depression by estrogens is atypical. Whatever the molecular mechanism, the
actions of androgens, estrogens, and orchiectomy, as reported here, and of
ovariectomy, as previously reported (6), are all consistent with the view that
the post-natal estrogen-androgen status influences these enzyme activities and
may be a factor in the observed sexual differences.

The significance of the sexual differences and of the hormone sensitiv-
ity of the KAT and KH activities in metabolic function is unknown. It is
possible that the differences may be involved in sex differences and/or hor-
monally-caused alterations in excretion of tryptophan catabolites (for recent
review, see ref. 19). If so, the KH activities are probably more significant
than the KAT activities, since hormone-sensitivity of the latter enzyme is
observed only in the kidney. The relatively high kidney KAT activity (2,3)
has seemed anomalous in view of the negligible levels of certain other enzymes
of tryptophan catabolism in that organ. Recent experiments (20), which dem-
onstrate the probable identity of KAT with α-aminodipate aminotransferase,
implicate that enzyme in lysine catabolism. This function would rationalize
its anomalous presence in kidney if other lysine catabolic enzymes can be
demonstrated there. The recent demonstration of lysine-α-ketoglutarate reduc-
tase and saccharopine dehydrogenase in the kidneys of human adults (21) sug-
gests the presence of such enzymes in mammalian kidneys. If so, the sex dif-
ferences and hormonal responsiveness of kidney KAT may possibly influence
lysine catabolism in that organ.

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