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TRYPTOPHAN PYRROLASE, TRYPTOPHAN AND TYROSINE TRANSAMINASE CHANGES DURING ALLYLISOPROPYLACETAMIDE-INDUCED PORPHYRIA IN THE RAT*

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Abstract—Changes in liver tryptophan pyrrolase (EC 1.11.1.4) and in tyrosine and tryptophan transaminase (EC 2.6.1.5) activities after induction of experimental porphyria with allylisopropylacetamide (AIA) were studied in female rats of the Long–Evans strain. All three enzymic activities increased after a single injection of AIA (400 mg/kg) to intact animals. AIA treatment also increased tryptophan pyrrolase in adrenalectomized but not in hypophysectomized animals. Peak levels of enzyme were reached in about 8 hr after drug administration to intact animals. The magnitude of the change in intact animals was enhanced by a 16-hr fast preceding the AIA treatment.

Acute administration of AIA also produced behavioral changes and increased urinary excretion of δ-aminolevulinic acid and porphobilinogen. Behavioral, enzymic and excretory changes produced by the drug progressively diminished in magnitude with continued drug treatment.

ACUTE intermittent porphyria is an inborn error of metabolism in man, characterized by gastrointestinal and neuropsychiatric symptoms,[1] and is particularly striking in being associated with increased enzymic activity (δ-aminolevulinic acid synthetase),[2] a higher incidence in females, and a delay in symptom appearance until puberty. Although increased urinary excretion of the porphyrin precursors, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG),[3] and increased activity of hepatic ALA synthetase have been found in porphyric patients,[2] the relationship between these changes and the disease symptoms is unknown.

A number of drugs (e.g. Sulphanol,[4] Sedormide,[5] hexachlorobenzene[6] and sulfanilamidopyrimidine[7]) and compounds of diverse structure disturb porphyrin biosynthesis in humans and induce pseudoporphyria in various animals.[8] The effects of these agents on porphyrin biosynthesis vary. Since porphyrin disturbance produced by allylisopropylacetamide (AIA), a drug related to the barbiturates, resembles that in human genetic acute intermittent porphyria,[9, 10] we have employed it in this study. AIA has been shown to induce an 8-fold increase in ALA synthetase activity in mitochondria of embryonic chick liver in tissue culture[11] and to increase activity of this

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enzyme in rat liver within 1–2 hr after a single dose (400 mg/kg of body weight).[12] Rats treated with this agent excrete large amounts of PBG in the urine.[9, 13]

The AIA induction of the ALA synthetase is thought to be dependent on DNA-mediated synthesis of RNA, and the increased enzymatic activity is considered to reflect increased synthesis *de novo* of the enzyme[11] because the induction can be blocked both by inhibitors of protein synthesis, such as puromycin and DL-*p*-fluorophenylalanine, and by inhibitors of DNA-directed RNA synthesis, such as actinomycin D and 5-fluorouracil.[12]

In addition to its effect on ALA synthetase activity, AIA has been reported to influence the activity of the heme- and steroid-sensitive enzyme, tryptophan pyrrolase (L-tryptophan: H_2O_2 oxidoreductase; EC 1.11.1.4).[14–16] Little is known, however, about the influence of AIA on other steroid-sensitive enzymes.

In this paper we report some changes in some inducible liver enzymes after administration of AIA to rats, the time dependency of these changes, and the effects of fasting, adrenalectomy and hypophysectomy.

EXPERIMENTAL

Animals. Female Long–Evans rats weighing 90–150 g were obtained from Simonsen Laboratories, Inc., Gilroy, Calif., or bred within our laboratory from Simonsen stock. They were maintained on Purina laboratory chow and water *ad lib.*; some were fasted 16 hr before injection, as indicated in the Results section. Bilateral adrenalectomies were performed on sixteen animals 7 days before treatment with AIA. The completeness of adrenalectomy was estimated both by visual inspection at the time of sacrifice and by serum corticoid levels. Transaural hypophysectomies were performed on three animals 5 days before treatment and twelve animals with pharyngeal hyophysectomies were purchased from Simonsen Laboratories and used 3 days after surgery. Serum and adrenal corticoids were determined to ascertain completeness of the surgery.

Treatment. AIA* was dissolved in propylene glycol and saline (4:1) and administered i.p. at a dose of 158 or 400 mg/kg of body weight. In long-term treatment, the rats received one daily injection in the morning. Some of the animals were kept in metabolic cages and urine was collected to verify the production of experimental porphyria. Animals were normally sacrificed at noon to minimize effects of diurnal rhythms. However, animals sacrificed at 8 a.m. and 10 a.m. were used to establish the 6- and 8-hr points in the time course studies.

Analytical methods. At the appropriate time, animals were decapitated, blood was collected, the organs were quickly removed, rinsed, blotted free of excess moisture, weighed and placed in proper medium for assay. Adrenals were homogenized in 5 ml of 20% ethanol–saline. Livers were homogenized in 5 vol. of cold, neutral 0·15 M KCl and centrifuged at 105,000 *g* for 30 min at 4°. The liver supernatant fractions were analyzed for tryptophan pyrrolase according to Knox,[17] with and without addition of hematin to a concentration of 0·15 μM, and tryptophan transaminase and α-ketoglutarate-tyrosine transaminase activities were determined by the enol–borate–tautomerse method of Lin *et al.*[18] Tyrosine transaminase was determined both with and without addition of pyridoxal phosphate to a concentration of 0·135 mM.

* Generously supplied by Dr. W. E. Scott of Hoffman-LaRoche.

Liver protein was determined by the method of Lowry as described by Layne,[19] and urinary ALA and PBG were determined according to a modified Mauzerall and Granick method.[20]

RESULTS

The effect of two different doses of AIA given to fed and 16-hr fasted animals on the activity of the liver enzymes, tryptophan pyrrolase, tryptophan transaminase and tyrosine transaminase is shown in Table 1. Animals were sacrificed 4 hr after the AIA injection. Activities of these enzymes were essentially unaltered 4 hr after administration of 158 mg AIA/kg of body weight to fed animals, but all three activities increased when this dose was given to fasted rats.

Similarly, 4 hr after treatment, fed rats given 400 mg AIA/kg of body weight showed a significant change in tryptophan pyrrolase activity and only marginal changes in the

TABLE 1. EFFECTS OF ALLYLISOPROPYLACETAMIDE (AIA) ON ENZYME INDUCTION IN FED AND IN 16-hr FASTED LONG–EVANS FEMALE RATS SACRIFICED 4 hr AFTER ADMINISTRATION OF AIA*

AIA (mg/kg body wt.)	Tryptophan pyrrolase† (μmoles/hr/g protein)		Tryptophan transaminase (μmoles/min/g protein × 10^2)		Tyrosine transaminase† (μmoles/min/g protein)	
	Fed	Fasted	Fed	Fasted	Fed	Fasted
0	20 ± 6	21 ± 5	13 ± 1	17 ± 1	13 ± 2	12 ± 1
158	20 ± 3	34 ± 13	15 ± 4	24 ± 6	19 ± 8	37 ± 11
400	30 ± 6	53 ± 7	19 ± 2	29 ± 6	26 ± 4	40 ± 11

* Three rats in each group. Enzyme activity is expressed in mean ± standard error.
† Activity measured in presence of cofactor.

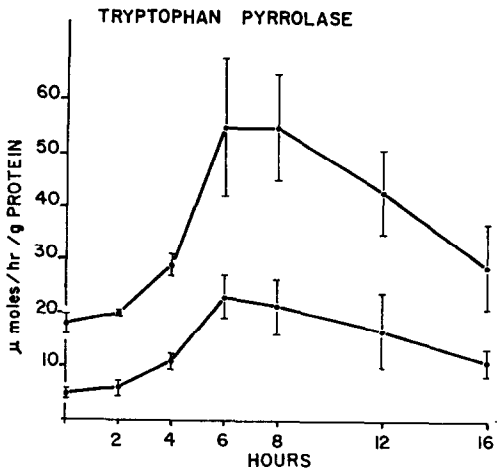

FIG. 1. Hepatic tryptophan pyrrolase activity after injection of allylisopropylacetamide (400 mg/kg) into female Long–Evans rats. Solid circles are enzyme activity with hematin added; the open circles are activity without added hematin. Standard errors are indicated.

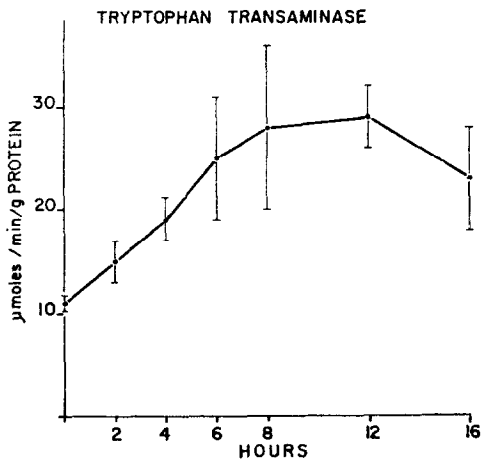

FIG. 2. Hepatic tryptophan transaminase activity after injection of allylisopropylacetamide (400 mg/kg) into female Long–Evans rats. Enzyme activity was measured with pyridoxal phosphate added. Standard errors are indicated.

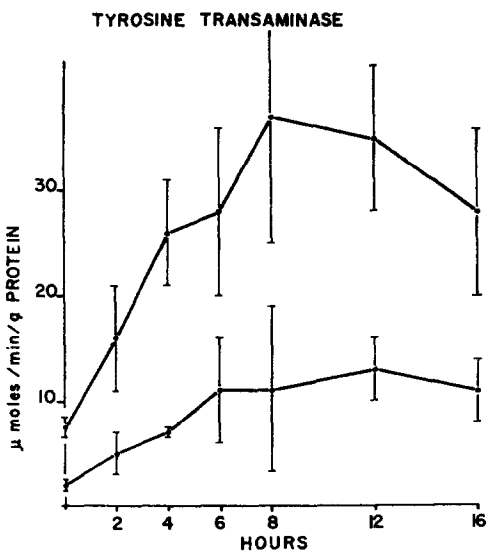

FIG. 3. Hepatic tyrosine transaminase activity after injection of allylisopropylacetamide (400 mg/kg) into female Long–Evans rats. Solid circles are enzyme activity with pyridoxal phosphate added; the open circles are activity without added pyridoxal phosphate. Standard errors are indicated.

other enzymes, while a marked increase in all three liver enzymes was observed 4 hr after administration of this dose to 16-hr fasted rats. Dietary state thus markedly alters sensitivity to the enzyme-inducing effects of this agent.

The time course of the changes in enzyme activities after AIA treatment in fed rats is given in Figs. 1, 2 and 3. Tryptophan pyrrolase activity reaches a maximum level about 6 hr after injection of AIA and both transaminase activities peak around 8 hr. In all three cases, peak values represented a 3-fold elevation over baseline.

These enzymic effects of acute AIA administration appear to diminish with more chronic treatment. As can be seen in Table 2, after 4, 10 or 12 days of continuous injection, induction could no longer be observed.

This pattern of enzymic changes was paralleled by changes in excretion of PBG in the urine and by alterations in behavior. Acute AIA treatment resulted in increased urinary excretion of ALA and PBG, which peaked on the second day of treatment and then declined with some marginal fluctuations upon continuous treatment.

TABLE 2. EFFECT OF AIA ON LIVER ENZYME ACTIVITY IN LONG-TERM TREATMENT OF LONG–EVANS FEMALE RATS*

Days of treatment	Type of treatment	Tryptophan pyrrolase† (μmoles/hr/ g protein)	Tryptophan transaminase (μmoles/min/g protein × 10^2)	Tyrosine transaminase† (μmoles/min/g protein)
4	AIA	33 ± 4	10 ± 1	11 ± 3
	Controls	25 ± 3	14 ± 1	10 ± 2
10	AIA	19 ± 2	8 ± 1	18 ± 5
	Controls	18 ± 2	12 ± 1	15 ± 3
21	AIA	16 ± 2	12 ± 3	13 ± 2
	Controls	9 ± 2	16 ± 2	11 ± 1

* AIA was given daily in a dose of 400 mg/kg of body weight. Control rats were given carrier alone. At least four rats were in each group. All rats were fed *ad lib*. Enzyme activity is expressed in mean ± standard error.

† Activity measured in presence of cofactor.

TABLE 3. EFFECT OF AIA ON ENZYME INDUCTION IN ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS*

Types of animals	Type of treatment	Tryptophan pyrrolase† (μmoles/hr/ g protein)	Tryptophan transaminase (μmoles/min/g protein × 10^2)	Tyrosine transaminase† (μmoles/min/g protein)
Adrenal-ectomized	AIA	25 ± 2	13 ± 1	13 ± 1
	Controls	14 ± 1	9 ± 1	11 ± 1
Hypophys-ectomized	AIA	32 ± 3	19 ± 2	19 ± 2
	Controls	32 ± 4	22 ± 2	23 ± 3

* AIA was given in a dose of 400 mg/kg of body weight. Control rats were given carrier alone. The values are expressed as mean ± standard error.

† Activity measured in presence of cofactor.

Profound behavioral changes accompany AIA administration and are reported elsewhere.[21] In brief, however, treated animals show a rapid onset of ataxia with rear leg paralysis and accompanying EEG desynchrony with intermittent bursts of hypersynchrony. An hour after treatment, the EEG showed continuous hypersynchrony during which animals had abnormal posture and open eyes. This pattern lasted for 8–12 hr before return to normalcy.

As with the enzymic changes, repeated daily injections produced a progressive reduction in the duration of the weakness and ataxia. After 10–14 days of treatment, neither ataxia nor weakness was apparent after injection.

Hormonal influences on AIA induction of tryptophan pyrrolase and of tyrosine and tryptophan transaminases were examined in 16-hr prefasted, adrenalectomized rats and in 12-hr prefasted, hypophysectomized rats (Table 3). Only tryptophan pyrrolase enzymic activity was found to be significantly higher in adrenalectomized rats given a single injection of AIA, while AIA administration did not increase tryptophan pyrrolase or transaminase activities in hypophysectomized animals.

DISCUSSION

The results indicate that the AIA induction of the three liver enzymes, tryptophan pyrrolase, tryptophan transaminase and tyrosine transaminase, is more pronounced in fasted than in fed rats. A dose of 400 mg per kg of body weight is, however, sufficient to induce enzymic activity even in fed rats after 4 hr. Peak activities are reached 6–8 hr after the inducer, whereas 4–6 hr is the usual period for peak values after corticoid treatment.

Liver tryptophan pyrrolase activity has previously been reported to be changed in experimental porphyria.[14–16] Marver *et al.*[16] studied tryptophan pyrrolase induction in female rats (120–150 g) given a single AIA injection. From their results they postulated that the tryptophan pyrrolase increase was a secondary effect due to induction of ALA synthetase and enhanced heme formation, which increased heme saturation of pyrrolase apoenzyme, protecting it from degradation and leading to its accumulation. They also suggested that tryptophan pyrrolase induction was not due to adrenal cortical stimulation. Their suggestion is supported by our finding that tryptophan pyrrolase activity significantly increased after i.p. injection of AIA in adrenalectomized rats and the activities of the steroid-sensitive enzymes, tryptophan transaminase and tyrosine transaminase, were unaltered. However, since the degree of heme saturation of induced tryptophan pyrrolase is not markedly elevated by AIA (Fig. 1), it seems unlikely that induction of this enzyme exclusively follows the mechanism suggested by Marver *et al.*[16] Thus, the ratio of activities without hematin addition to that with cofactor addition ranges from 29 per cent for controls to 40 per cent 6 hr after treatment. This range is within the normal variation. Induction is slightly less marked after adrenalectomy, however, so that adrenocortical hormones may contribute to the increased enzyme activity in intact animals.

Tryptophan pyrrolase was not induced in hypophysectomized rats. This suggests that pituitary hormones are essential for the induction of this enzyme. The mechanism for this is not known and has to be sought among a variety of hormone-related factors.

The liver transaminase activities were not elevated in either adrenalectomized or hypophysectomized rats, suggesting that adrenal and pituitary hormones are involved in the AIA-mediated induction of these enzymes.

The absence of enzyme elevation in long-term treated animals implies an adaptation to the inducing agent, which may be due to changed metabolism of the agent or to changes in the inducing system. Urinary excretion of ALA and PBG, as well as the behavioral changes, similarly diminishes with continued treatment, suggesting that altered metabolism of AIA may be involved. In another study from this laboratory, we have shown that epileptoid activity in the EEG induced by AIA in rats with chronically implanted brain electrodes also decreased after repeated daily injections.[21]

Although it is not possible from this study to differentiate between direct drug

effects on induction and secondary effects derived from alterations in porphyrin biosynthesis, further studies on the mechanism and effect of the porphyrin induction and other metabolic disturbances caused by AIA may be valuable in elucidating the pathogenesis of the genetic types of acute porphyria.

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