Biochemical Pharmacology, Vol. 19, pp. 2833–2838. Pergamon Press, 1970. Printed in Great Britain

# GLUCOSE EFFECT ON TRYPTOPHAN OXYGENASE AND TYROSINE AMINOTRANSFERASE INDUCTION AFTER ALLYLISOPROPYLACETAMIDE-INDUCED PORPHYRIA IN THE RAT*

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(*Received* 30 *January* 1970; *accepted* 24 *March* 1970)

**Abstract**—Tryptophan oxygenase, EC 1.11.1.4, and tyrosine aminotransferase, EC 2.6.1.5, were increased 4 hr after i.p. injection of 400 mg/kg of allylisopropylacetamide (AIA) to intact fasted female Long–Evans rats. Prior consumption of glucose markedly attenuated these enzymic responses to AIA.

GLUCOSE is known to repress synthesis of several bacterial enzymes, and to inhibit the dietary induction by casein hydrolysate of the rat liver enzymes, threonine dehydrase, ornithine δ-transaminase and tyrosine transaminase.[1,2] More recently, glucose administration has been shown to inhibit elevations in δ-aminolevulinic acid synthetase activity in animals treated with drugs that produce experimental porphyria,[3] and a carbohydrate-rich diet has been reported to depress excretion of porphyrin precursors in patients with acute intermittent porphyria.[4,5]

In an earlier study,[6] we found that administration of allylisopropylacetamide (AIA) to rats reproduces many of the biochemical features of acute intermittent porphyria. Such treatment also elevated tyrosine aminotransferase activity in intact animals. We also confirmed earlier findings[7,8] that tryptophan oxygenase activity in rat liver is induced by AIA in both intact and adrenalectomized animals. Since heme is a cofactor for tryptophan oxygenase, it seemed possible that glucose feeding would affect the inductive action of AIA on tryptophan oxygenase by inhibiting δ-aminolevulinic acid synthetase and thereby decreasing heme availability for cofactor saturation of tryptophan oxygenase.

In the present paper, we report the effects of glucose pretreatment on induction of tryptophan oxygenase and tyrosine aminotransferase by allylisopropylacetamide.

* This investigation was supported in part by Public Health Service International Postdoctoral Research Fellowship F05TW-1462, USPHS Grants AM 08775 and HD 01058, and by the Swedish Medical Research Council Grant B70-21R-2510-02. The authors are indebted to R. Wallace and C. Wang for technical assistance.

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## EXPERIMENTAL PROCEDURE

*Animals.* Female Long–Evans rats weighing 100–180 g, bred in our laboratory from Simonsen Laboratory stock or purchased from Simonsen Laboratory, were maintained at 23° on a 6 p.m. to 6 a.m. dark–light cycle. Some groups were adrenalectomized 3–4 days before the experimental day; completeness of adrenalectomy was established both by visual inspection and by serum corticoid levels. Intact animals were given water, *ad lib.*, and adrenalectomized animals saline, *ad lib.*

Rats referred to as "glucose-fed" were provided 15 per cent glucose in water in place of their drinking water at 4 p.m. the day before AIA treatment. Adrenalectomized rats were provided with 15 per cent glucose in saline. In most of these experiments, food was removed from the cage at that time. In two separate experiments glucose-water was substituted for water at 4 a.m. or 7 a.m. on the experimental day.

Glucose solutions were given in water bottles equipped with ball bearings in the outlet tube to prevent leakage of the relatively dense liquids. Consumption was estimated by the measured difference between the initial and final volume of solution.

*Drug treatment.* AIA* was dissolved in propylene glycol–saline (4:1) and injected i.p. at 8 a.m. at a dose of 400 mg/kg of body weight. Controls were injected with equal volumes of the propylene glycol–saline mixture. All animals were killed 4 hr after injection, near noon; therefore, any effects of diurnal rhythms were minimized.

*Analytical methods.* After decapitation of the rats, the liver was quickly removed, rinsed with saline, blotted free of excess moisture, weighed, homogenized in 5 vol. of cold, neutralized 0·15 M KCl, and centrifuged at 105,000 *g* for 30 min at 4°. The supernatant fractions were analyzed for tryptophan oxygenase activity according to Knox,[9] with and without addition of hematin to a concentration of 0·15 $\mu$M. Tyrosine aminotransferase activity was determined by the enol–borate–tautomerase method of Lin, *et al.*[10] in the presence of 0·135 mM pyridoxal phosphate.

Protein in the supernatant fraction was determined by the method of Lowry, as described by Layne.[11] Enzyme activities are expressed as micromoles per hour per gram of protein for tryptophan oxygenase and as micromoles per minute per gram of protein for tyrosine aminotransferase. Adrenal and serum corticoids were determined according to Givner and Rochefort.[12] Statistical comparisons between groups were made using the Student *t*-test.

## RESULTS

The intact, fed rats consumed about 35 ml water and about 45 ml of 15 per cent glucose in the 20-hr period prior to sacrifice. As can be seen in Table 1, this glucose consumption depressed basal levels of tryptophan oxygenase and tyrosine aminotransferase. The level of enzyme activity after AIA treatment was also lowered by glucose feeding to animals fasted 20 hr (Table 1). Glucose feeding to intact animals also decreased the degree of heme saturation of the tryptophan oxygenase apoenzyme. Glucose-fed, AIA-treated animals had higher liver weights and lower hepatic protein concentrations than treated animals not receiving glucose. However, the differences in enzymic activities were not simply due to enzyme dilution, since activities measured per total liver also were significantly lower for the glucose-treated animals (Table 2).

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

TABLE 1. EFFECT OF GLUCOSE FEEDING ON ALLYLISOPROPYLACETAMIDE (AIA)-INDUCED ENZYME ACTIVITY IN FED OR 16-hr FASTED LONG–EVANS FEMALE RATS SACRIFICED 4 hr AFTER ADMINISTRATION OF AIA*

| Diet | Treatment | Surgery | Glucose 15% in drinking water (hr) | Tryptophan oxygenase (μmoles/hr/g protein) | | Tyrosine aminotransferase (μmoles/min/g protein) |
|---|---|---|---|---|---|---|
| | | | | Without hematin | With hematin | |
| Fed | | | | 3 ± 0·7 | 16 ± 5 | 9 ± 3 |
| Fed | | | 16 | 1 ± 0·3 | 7 ± 3 | 5 ± 2 |
| Fasted | | | | 7 ± 2 | 21 ± 5 | 12 ± 1 |
| Fasted | | | 16 | 2 ± 0·5 | 5 ± 1 | 3 ± 0·6 |
| Fasted | AIA | | | 31 ± 3 | 53 ± 6 | 42 ± 4 |
| Fasted | AIA | | 1 | 20 ± 1 | 41 ± 4 | 10 ± 0·6 |
| Fasted | AIA | | 4 | 11 ± 2 | 23 ± 1 | 6 ± 1 |
| Fasted | AIA | | 16 | 4 ± 1 | 9 ± 2 | 8 ± 2 |
| Fasted | | Adrex | | 5 ± 0·1 | 14 ± 1 | 11 ± 1 |
| Fasted | | Adrex | 16 | 3 ± 0·8 | 12 ± 0·1 | 5 ± 1 |
| Fasted | AIA | Adrex | | 17 ± 2 | 25 ± 2 | 13 ± 1 |
| Fasted | AIA | Adrex | 16 | 6 ± 1 | 15 ± 2 | 6 ± 0·8 |

* AIA was given in a dose of 400 mg/kg of body weight. Controls were given carrier alone. At least four rats were in each group. Enzyme activity is expressed as mean ± standard error. Adrex = adrenalectomy.

As observed previously,[6] AIA administration to adrenalectomized animals was found to induce tryptophan oxygenase but not tyrosine aminotransferase. This indicates that the AIA induction of tyrosine aminotransferase is either secondary to AIA activation of the adrenal glands or involves an interaction between inducer and adrenal hormones. Glucose pretreatment was effective in lowering basal levels of tryptophan oxygenase and tyrosine aminotransferase in adrenalectomized animals

TABLE 2. BODY WEIGHT, LIVER WEIGHT, LIVER PROTEIN CONTENT AND ENZYMIC ACTIVITIES IN LONG–EVANS FEMALE RATS SACRIFICED 4 hr AFTER ADMINISTRATION OF AIA*

| Type of tissue or enzyme | Units | AIA alone ($n = 5$) | AIA + glucose ($n = 5$) | Difference P |
|---|---|---|---|---|
| Body weight | g | 138 ± 2·9 | 145 ± 6·1 | < 0·1 |
| Liver weight | g | 5·28 ± 0·2 | 6·93 ± 0·3 | > 0·01 |
| Protein in liver supernatant | mg/ml | 17·9 ± 0·4 | 14·5 ± 0·6 | > 0·01 |
| Enzyme activity/total liver | | | | |
| Tryptophan oxygenase | | | | |
| Without hematin | μmoles/hr | 14·3 ± 1·6 | 3·62 ± 0·8 | > 0·001 |
| With hematin | μmoles/hr | 33·1 ± 6·1 | 7·62 ± 1·8 | > 0·01 |
| Tyrosine aminotransferase | μmoles/min | 23·6 ± 4·9 | 4·7 ± 1·1 | > 0·01 |

* AIA was given in a dose of 400 mg/kg of body weight. Glucose-treated rats were provided with drinking water containing 15 per cent glucose for 16 hr prior to injections. All figures are expressed as mean ± standard error.

and in diminishing the absolute level of the tryptophan oxygenase response to AIA. AIA treatment did not alter the depression in basal tyrosine aminotransferase activity caused by glucose in these animals. Although it is clear from these data that the lowering of basal enzyme levels is independent of adrenals, it was conceivable that the lowering of the induced levels of activity in intact animals could be due to glucose blockade of adrenocortical activation. Adrenal steroid levels in AIA-treated animals were $16 \pm 6$ μg/g of adrenal (mean ± S.D.) as compared to $14 \pm 8$ μg/g in AIA-treated animals prefed glucose. The corresponding values for serum corticords were $67 \pm 16$ and $37 \pm 12$ μg/100 ml, respectively, both of which are significantly higher than control levels but not significantly different from each other. Further, because glucose ameliorated the magnitude of AIA induction in adrenalectomized animals, any glucose effect on adrenocortical activation is probably incidental.

The length of glucose pretreatment required to block the inductive effects of AIA in intact rats was also examined. Glucose feeding for only 4 hr prior to AIA treatment was sufficient to diminish the response to inducer (Table 1). Prefeeding glucose for only 1 hr lowered AIA-mediated induction of tyrosine aminotransferase but did not block the induction of tryptophan oxygenase activity.

## DISCUSSION

The present results indicate that glucose prefeeding lowers basal levels of tryptophan oxygenase and tyrosine aminotransferase activity in fed and fasted intact rats and in fasted adrenalectomized rats. Further, such treatment decreases the magnitude of response to the inducer, AIA. However, AIA induction is not abolished and the relative increase in activity after AIA injection is proportional to the relative basal levels before injections.

Animals consuming glucose-water have heavier livers and a lower liver protein concentration than animals drinking water. However, the lower enzyme levels in the glucose-fed group do not appear to be simply an artifact of enzyme concentration, since enzyme activity per total liver differs between groups in the same manner as activity per unit of protein. Further, preliminary results indicate that AIA increases incorporation of [$^3$H]leucine into total soluble liver protein and that this incorporation is not decreased by prior glucose feeding. Thus, the difference in the absolute level of enzyme after AIA treatment to glucose-fed animals cannot easily be explained on the basis of a general inhibition of protein synthesis by glucose.

Glucose prefeeding for 1 hr before AIA is sufficient to lower the response of tyrosine aminotransferase activity to AIA, and 4-hr glucose feeding prior to AIA injection lowers tryptophan oxygenase activity as well, indicating a differential sensitivity of the systems forming the enzymes to the inhibitory effects of glucose. This may be simply a dose-related phenomenon reflecting the difference in glucose consumption in 1 and 4 hr or, alternatively, different mechanisms may be operative for glucose inhibition of tryptophan oxygenase and tyrosine aminotransferase induction.

Indeed, the demonstration that AIA induction of tyrosine aminotransferase is adrenal dependent, while induction of tryptophan oxygenase is not, suggests different inductive mechanisms for these two systems. This is further supported by reports that tyrosine aminotransferase, but not tryptophan oxygenase, is affected by the glucose-sensitive hormones, insulin, glucagon and growth hormone.[13,14] However,

it is difficult to see how these hormones could account for the glucose inhibition of induction, even in the case of tyrosine aminotransferase. Insulin and glucagon are inducers of the enzyme,[13] while growth hormone inhibits induction.[15] Of these, only insulin would be expected to be released by a glucose load. To account for glucose inhibition by hormonal mediation, then, would require that suppression of glucagon release by glucose feeding decreases tonic stimulation of tyrosine aminotransferase more than acute stimulation by insulin increases it. A comparison of the effective doses of these hormones relative to their normal levels does not support this requirement. Minimal and equal inductive effects on tyrosine aminotransferase are elicited by 1 unit of insulin/kg and 300 $\mu$g glucagon/kg.[13] Basal blood levels of these hormones in the rat are not known, but in man they are estimated as $4 \times 10^{-3}$ units of insulin/100 ml of blood[16] and $30 \times 10^{-3}$ $\mu$g glucagon/100 ml.[17] If levels are of the same magnitude in the rat, it is clear that the inducing dose of either hormone is several orders of magnitude greater than resting blood levels and that insulin is at least as effective an inducer as glucagon. Although adrenocorticoids may be synergistic with glucagon,[18] a heightened effectiveness of glucagon cannot be evoked to explain basal suppression of tyrosine aminotransferase by glucose, since this suppression occurs equally well in both intact and adrenalectomized animals.

Direct inhibition of enzyme synthesis by glucose metabolites or glycolytic intermediates must be considered in lieu of hormonal suppression. The recent report that NADPH inhibits tryptophan oxygenase activity by a feedback mechanism[19] provides one possible link between glucose consumption and the activity of this enzyme.

Whatever the inhibiting mechanism for total enzyme activity, the decreased heme saturation of tryptophan oxygenase apoenzyme after glucose feeding in both intact and adrenalectomized rats treated with AIA probably results from glucose inhibition of δ-aminolevulinic acid synthetase activity[3] and consequent decreased heme formation. However, increased heme saturation does not in itself induce tryptophan oxygenase, at least not in the absence of adrenal hormones,[20] so that the inhibitory effect of glucose on AIA-induced tryptophan oxygenase activity is not secondary to a decreased heme formation because of glucose repression of δ-aminolevulinic acid synthetase.

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