Induction of tryptophan oxygenase and tyrosine aminotransferase in mice

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SEVERAL WORKERS have reported specialized observations in mice on the activities in liver of tryptophan oxygenase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12) (1, 2, 18, 19) and tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) (1, 2, 14, 18). These are enzymes that in the rat have been used extensively for the study of enzyme inductions (8). The present investigation was undertaken to provide the necessary basis for extending such studies in mice. It was necessary to determine the optimal conditions for the assay of the activities of these enzymes in this species, to affirm the existence of both the hormonal and co-factor types of inductions, and to seek any marked differences with regard to these enzymes among four commonly used inbred strains of mice. The results show that the assays developed for rat liver are applicable to mouse liver, that both enzymes are induced in the same ways as they are in the rat, and that there are significant strain differences in the extent of the inductions.

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RESULTS

It was first necessary to establish the conditions for accurate measurement of the tryptophan oxygenase and tyrosine aminotransferase of mouse liver in vitro. In rat liver, tryptophan oxygenase exists in two inactive forms, the heme-free apoenzyme and the oxidized holoenzyme, both of which become active upon incubation of the particle-free fraction of liver with methemoglobin, ascorbate, and tryptophan (17). The tryptophan oxygenase of C57BL mouse liver was found to have similar properties. In liver supernatants obtained from normal or hydrocortisone-injected mice very little activity was seen without preliminary incubation or when globin was added to the complete mixture to prevent conjugation (Table 1). Globin was without effect if added to the actual assay, i.e., to the conjugated enzyme. As in the case of rat liver (17), the SH-group reagent, N-ethylmaleimide, also prevented conjugation (Table 1). Thus, the enzyme of mouse liver was mostly in the unconjugated form in untreated or hydrocortisone-treated mice. However, 2.5 hr after the injection of tryptophan, the enzyme became conjugated (active in presence of globin during the preliminary incubation) (Table 1). This increased conjugation lasted less than 5 hr, and so, in preparations obtained 5 hr after the injection of tryptophan, the presence of globin or of N-ethylmaleimide during the preliminary incubation prevented full activation of the tryptophan oxygenase.

The method used for the measurement of tyrosine aminotransferase in rat liver (14) was found to be suitable for mouse livers. Fresh preparations had to be used since storage at 5 C for 24 hr or at 25 C for 30 min caused significant loss of activity.

To establish a suitable test system for the comparison of both enzyme inductions in the different strains of mice, the response of tryptophan oxygenase and tyrosine aminotransferase to different doses of hydrocortisone at different times were measured in male C57BL mice (Figs. 1 and 2). With the smaller dose of 0.25 mg of hydrocortisone, tyrosine aminotransferase reached a maximum at 2.5 hr and returned to normal at 5 hr, while the tryptophan oxygenase reached its maximum at 5 hr (Fig. 1). With observations at 5 hr, tyrosine aminotransferase responded more quickly, both enzymes appeared to be equally sensitive to the doses of hydrocortisone. More hydrocortisone was apparently required to maintain the elevated level of tyrosine aminotransferase until the tryptophan oxygenase reached its maximum. For the purpose of comparing the magnitude of responses of the different strains, both enzymes were measured 5 hr after the higher dose of hydrocortisone (2.5 mg), when both enzymes were maximally induced. The response to tryptophan was also measured 5 hr after injection, although the enzyme had become unconjugated by this time (Table 1). The enzyme measurements in the different strains of mice are shown in Table 2.

The basal levels of tyrosine aminotransferase and tryptophan oxygenase are comparable to those in rats and did not show any marked strain differences (Table 2). In intact males of the four strains of mice studied, tryptophan oxygenase and tyrosine aminotransferase were induced by both hydrocortisone and tryptophan. These increases were highly significant, with the possible exception of the induction of tryptophan oxygenase by tryptophan in strain C5H (P < 0.05). Intact females of two strains were also induced by hydrocortisone. As in rats (15), the response of tyrosine aminotransferase to

### TABLE 1. Tryptophan oxygenase activities with different conditions of preliminary incubation

<table>
<thead>
<tr>
<th>Treatment of C57BL Mice</th>
<th>Preliminary Incubation</th>
<th>Activity</th>
<th>Activity</th>
<th>Activity</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Standard</td>
<td>With globin</td>
<td>With NEM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \text{wulits/hr per g liver, } 25 \text{ C} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.1</td>
<td>2.6</td>
<td>0.1</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>HC, 5 hr</td>
<td>0.1</td>
<td>9.6</td>
<td>9.7</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Trp, 2.5 hr</td>
<td>11.9</td>
<td>10.0</td>
<td>0.5</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Trp, 5 hr</td>
<td>10.0</td>
<td>0.5</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A pool of livers from three C57BL mice, treated as indicated in the first column, was used for each assay. Doses per 100 g body wt of hydrocortisone phosphate (HC) (given 5 hr before assay) and of tryptophan (Trp) (given 2.5 or 5 hr before assay) were 0.25 mg and 100 mg, respectively. The standard method of preliminary incubation, with methemoglobin, ascorbate, and tryptophan, is described in METHODS. "With globin" indicates the omission of methemoglobin and the presence of 0.5 mg globin/ml in the preliminary incubation. N-ethylmaleimide (NEM, 0.02 mM final concentration) was added 5 min before the preliminary incubation.
hydrocortisone was significantly higher in males than in females and higher in adrenalectomized males than in intact males. Tryptophan oxygenase may also respond to hydrocortisone more in intact males than in females ($P < 0.04$).

It was particularly important that both tryptophan oxygenase and tyrosine aminotransferase were induced by tryptophan in adrenalectomized C57BL mice ($P < 0.01$). The increases were smaller than those in intact mice, in which the secretion of glucocorticoid stimulated by tryptophan contributed to the induction, but they were reproducible evidence of the second type of inductions occurring without corticosteroids. These inductions by substrate- or cofactor-type compounds in adrenalectomized rats have been classified as instances of "cofactor induction," in contrast to the hormonal induction by glucocorticoids (3).

Both enzymes were also measured in AKR and C57BL males at 6, 8, and 14 weeks of age, but no changes with age in behavior of the enzymes were found.

There were a number of significant differences among the strains in the extent of the inductions. The hydrocortisone-induced level of tryptophan oxygenase was significantly higher in strain C57BL than in the other three strains, whereas the hydrocortisone-induced level of tyrosine aminotransferase was lowest in C57BL and highest in DBA. The tryptophan-induced levels of both enzymes followed somewhat the same pattern as the hydrocortisone-induced levels, especially with tyrosine aminotransferase, since the tryptophan given stimulated adrenocortical secretion in these intact mice. Tryptophan had no noticeable effect on the tryptophan oxygenase in C57BL mice. Tryptophan had a particularly marked effect on the tyrosine aminotransferase in DBA mice, as did hydrocortisone. The strain differences mentioned were all statistically significant.

**DISCUSSION**

The assays for both tryptophan oxygenase and tyrosine aminotransferase currently used for rat liver (10, 14) were suitable for assays of preparations from mouse livers. In both species, maximal activity of tryptophan oxygenase required prior conjugation of the apoenzyme with heme and reduction of the holoenzyme to the active form. Few of the activities reported in earlier studies on mice can be compared directly with the values obtained here because of the different conditions used. The tryptophan oxygenase activity equivalent to 0.2 of the present units per gram, reported for untreated C57BL male mice by Wood et al. (19), and the tyrosine aminotransferase activity equivalent to 60 of the present units per gram reported by Lin et al. (14) are similar to the basal values found here.

While both hydrocortisone and tryptophan have been

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**TABLE 2. Tryptophan oxygenase and tyrosine aminotransferase levels in different strains of inbred mice**

<table>
<thead>
<tr>
<th>Treatment of Mice</th>
<th>Tryptophan Oxygenase</th>
<th>Tyrosine Aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DBA</td>
<td>C57BL</td>
</tr>
<tr>
<td>Intact males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.6±0.8 (6)</td>
<td>2.6±0.5 (9)</td>
</tr>
<tr>
<td>HC</td>
<td>3.4±0.8 (6)</td>
<td>9.3±1.7 (11)</td>
</tr>
<tr>
<td>Trp</td>
<td>6.8±1.2 (6)</td>
<td>6.9±1.1 (7)</td>
</tr>
<tr>
<td>Intact females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>3.5±0.9 (6)</td>
<td>1.5±0.6 (6)</td>
</tr>
<tr>
<td>HC</td>
<td>7.3±1.6 (6)</td>
<td>3.6±0.6 (6)</td>
</tr>
<tr>
<td>Adrenalectomized males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.8±1 (7)</td>
<td>71±13 (11)</td>
</tr>
<tr>
<td>HC</td>
<td>4.7±1 (5)</td>
<td>418±122 (5)</td>
</tr>
<tr>
<td>Trp</td>
<td>4.1±0.9 (7)</td>
<td>115±57 (7)</td>
</tr>
</tbody>
</table>

Enzyme activities in intact male and female mice, and adrenalectomized male mice of the different strains are given as the means ± SD for the number of mice shown in parentheses. For abbreviations, see Table 1.
used previously to elevate these two enzymes in intact mice, their induction by tryptophan in adrenalectomized mice does not appear to have been reported. The inductions by tryptophan in adrenalectomized mice, since it excludes the mechanism of induction by glucocorticoids, identifies the occurrence of cofactor-type induction in mice. As in rats, tryptophan oxygenase is inducible by two independent mechanisms, a substrate and a hormone-type mechanism. In the rat, the cofactor-type of induction involves new protein synthesis that is not inhibited by actinomycin (3). It is associated with increased conjugation of the tryptophan oxygenase by hematin and decreased removal of the enzyme (9). Induction by tryptophan of tryptophan oxygenase in the mouse is also preceded in vivo by increased conjugation with hematin (Table 1). However, the time during which the enzyme remains conjugated is shorter in mice than in rats: 5 hr after the administration of tryptophan the mouse liver tryptophan oxygenase was again unconjugated (Table 1), while rat liver tryptophan oxygenase was still largely conjugated at this time (9). Since the concentration of tryptophan determines the degree of conjugation, it is possible that tryptophan is metabolized faster in the mouse than in the rat. Consistent with this, the adrenalectomized mice given 100 mg L-tryptophan/100 g body wt were not apparently affected, while two-thirds of similarly treated adrenalectomized rats died within 6 hr (7).

It is undecided whether tryptophan induces tyrosine aminotransferase in adrenalectomized rats by virtue of being its substrate (6) or whether tryptophan acts indirectly, giving rise to endogenous inducers. The same problem now arises in mice. The induction of tyrosine aminotransferase, even in adrenalectomized rats, is a less specific process than induction of tryptophan oxygenase. The latter has so far been shown to respond only to tryptophan (or certain analogs (9)), whereas tyrosine aminotransferase is induced by a variety of agents (4, 5, 12). Glucagon, secreted in inverse relation to the concentration of blood glucose, is one of these. Thus, any agent that causes hypoglycemia, as tryptophan has been reported to do (16), may induce this enzyme. The hydrocortisone induction of tyrosine aminotransferase in rats also differs from that of tryptophan oxygenase by being enhanced by starvation of glucagon. Therefore, since the two enzymes have different controls, the differences between the strains of mice need not be the same for the two enzymes. Indeed, the strain differences are different for the two enzymes (Table 2).

The injection of tryptophan in intact rats and mice may induce both tryptophan oxygenase and tyrosine aminotransferase by stimulating secretion of glucocorticoids. Tryptophan oxygenase is therefore elevated by tryptophan in intact rats to about the sum of the increases produced separately in adrenalectomized rats by tryptophan and by hydrocortisone (11). The same relationship appears in mice, indicating that tryptophan in intact mice caused the secretion of the maximally effective amount of glucocorticoids.

The four strains compared by the present tests were not qualitatively different, since both enzymes were inducible in each strain. But there were significant quantitative differences in the responses of both enzymes. These were greater differences for these two enzymes than have so far been observed between different strains of rats, as might be expected from the definite genetic differences between the mouse strains tested. In the only other comparative study of these enzymes in inbred strains of mice found in the literature (18, Table VII), only negligible inductions of the two enzymes were observed using less optimal assays.

With regard to hydrocortisone induction, the order of responses of the enzymes in the different strains were:

- tryptophan oxygenase: C57BL > C3H = AKR > DBA
- tyrosine aminotransferase: DBA > C3H = AKR = C57BL

Thus, C57BL and DBA showed a reciprocal type of response of the two enzymes to hydrocortisone; the strain in which one enzyme increased most had the least increase in the other enzyme, and vice versa.

With regard to tryptophan induction, which in these intact mice consists of a summation of substrate induction and induction by glucocorticoids released from the adrenals, the order of the responses was almost similar for the two enzymes:

- tryptophan oxygenase: DBA = C57BL = AKR < C3H
- tyrosine aminotransferase: DBA > C57BL > AKR = C3H

These patterns were similar to that of tyrosine aminotransferase after hydrocortisone, except that strains C57BL and C3H reversed places.

No simple difference can account for the apparent sensitivity to hydrocortisone of the tryptophan oxygenase in C57BL and of the tyrosine aminotransferase in DBA, while both enzymes in C3H are relatively less sensitive to tryptophan than hydrocortisone. However, the patterns observed may provide a basis for selecting strains of mice for particular studies on the nature of the differences between them.

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REFERENCES


