Role of Tryptophan Pyrrolase in Endotoxin Poisoning

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Using substrate induction as a tool, we attempted to determine the role of tryptophan pyrrolase in the response to endotoxin in mice. Previous results have shown that the administration of the LD₅₀ of endotoxin lowers tryptophan pyrrolase activity. α-Methyltryptophan was found to maintain tryptophan pyrrolase activity above control levels in endotoxin-poisoned mice without increasing survival. 5-Hydroxytryptophan, by contrast, lowered tryptophan pyrrolase activity but did not sensitize mice to endotoxin. These results suggest that tryptophan pyrrolase per se does not play a unique role in survival of mice poisoned with endotoxin. This enzyme, however, may reflect the fate of other liver enzymes inducible by adrenocorticoids. In mice given concurrent injections of tryptophan and endotoxin, tryptophan pyrrolase activity was elevated to a level intermediate between that of normal mice and that of mice given tryptophan alone. The mice injected with tryptophan and endotoxin also had about the same mortality as mice given endotoxin alone. Mice treated with tryptophan 4 hr after endotoxin, at a time when the substrate did not fully elevate tryptophan pyrrolase activity, died convulsively and in larger numbers than those given endotoxin alone. This effect was reversed by prior treatment with cyproheptadine, an antiserotonin drug. These results indicate that the depression of tryptophan pyrrolase activity previously observed in vitro after injection of endotoxin reflects an actual decrease in the in vivo activity of the enzyme.

The activity of tryptophan pyrrolase, the adaptive liver enzyme regulating the biosynthesis of nicotinamide adenine dinucleotide from tryptophan, is significantly depressed 4 hr after endotoxin poisoning in mice (1, 2). Cortisone, known to protect mice against endotoxin lethality (2, 10, 14), maintains tryptophan pyrrolase activity at essentially normal levels under these conditions (2). This information led Berry and co-workers (1, 2) to postulate that a primary metabolic effect of endotoxin may be to impair enzymatic regulation, thereby upsetting metabolic homeostasis in the host. Accordingly, protection by cortisone would reflect the ability of this material to maintain more normal enzymatic homeostasis in the poisoned animal.

Since the metabolic imbalances previously observed in endotoxin-poisoned mice (3, 4) could conceivably be correlated with the diminished activity of a single enzyme, tryptophan pyrrolase, it became important to determine the exact significance of the fluctuations of this enzyme in endotoxin poisoning. The experiments to be described are designed to answer two basic questions: (i) is maintenance of tryptophan pyrrolase activity essential for mice to survive endotoxin poisoning, and (ii) does the depression of tryptophan pyrrolase activity as determined by an in vitro assay reflect a true decrease in the in vivo catabolic potential of the enzyme, or is the depression of the enzyme activity an in vitro artifact?

Such a study is feasible because tryptophan (6, 18, 19), α-methyltryptophan (5, 16), and 5-hydroxytryptophan (9) have been shown to influence tryptophan pyrrolase activity both in vivo and in vitro. Although the effect of these compounds may not be limited to tryptophan pyrrolase alone (8, 12, 17), it is reasonable to assume that their influence on inducible enzymes is much more limited than that of adrenocorticoids.

Materials and Methods

Endotoxin. Heat-killed cells of Salmonella typhimurium strain SR-11 were used as endotoxin in all experiments and were prepared as described by Berry and Smythe (4). Appropriate dilutions of the stock

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material were made in isotonic nonpyrogenic saline (Baxter Laboratories, Morton Grove, Ill.). Injections were given intraperitoneally in 0.5-ml volume.

Protection experiments. Mice were injected according to the schedule noted in the footnotes of the tables. Food and water were available at all times. Results were tabulated at 8, 24, and 48 hr after the initial injection unless otherwise specified.

Tryptophan pyrrolase assay. The in vitro assay of tryptophan pyrrolase was performed by the method of Knox and Auerbach (13) as modified by Berry and Smythe for application to mice (1, 2). Approximately 1 g of liver (wet weight) was homogenized in 7 volumes of ice-cold 0.14 M KCl containing 0.0025 M NaOH. A 2-ml portion of the homogenate was transferred to a 25-ml Erlenmeyer flask containing 0.6 ml of l-tryptophan (0.13 M), 20 μg of the cofactor hematin (Nutritional Biochemicals Corp., Cleveland, Ohio), 2 ml of 0.2 M phosphate buffer (pH 7), and water to a total volume of 8 ml. Tryptophan was omitted from the control flask. The reaction mixture was incubated for 1 hr under an atmosphere of oxygen in a water-bath shaker (model 2156, Research Specialties Co., Berkeley, Calif.) at 37 C. The reaction was stopped by the addition of 4 ml of 15% metaphosphoric acid, and the resulting precipitate was removed by filtration. A sample of the filtrate was neutralized with 2 ml of 0.5 N NaOH. The kynurenine formed was measured spectrophotometrically at 360 μM in a Hitachi-Perkin Elmer 139 UV-Vis spectrophotometer (Arthur H. Thomas Co., Philadelphia, Pa.). The quantity of product was estimated from a standard curve, and the results are expressed as micromoles of kynurenine formed per gram (dry weight) of liver per hour.

Chemicals. L-Tryptophan, 5-hydroxytryptophan, and serotonin creatinine sulfate were purchased from Nutritional Biochemicals Corp. Most of the α-methyltryptophan was generously supplied by Merck, Sharp and Dohme, Rahway, N.J., through the courtesy of Earl Pierson. A limited amount of this material was purchased from Sigma Chemical Co., St. Louis, Mo. Cyproheptadine was also supplied by Merck, Sharp and Dohme.

Mice. Female Swiss-Webster mice (Dierolf Farms, Boyertown, Pa.) were employed in all experiments. Handling procedures were similar to those described previously (3).

RESULTS

Effect of single or repeated injections of tryptophan on the activity of tryptophan pyrrolase. To ascertain the unique significance of tryptophan pyrrolase in the response of mice to endotoxin, it is necessary to produce changes in activity of the enzyme by means that are more specific than those resulting from administration of adrenocorticoids, such as cortisone or hydrocortisone.

Tryptophan pyrrolase is substrate-inducible, as shown for rats (6, 18, 19) and for mice (1), but the rise in its activity after a single intraperitoneal injection of 20 mg of l-tryptophan reaches a peak at 2 hr and is back to normal levels at 4 hr (Fig. 1, dashed line). There is evidence in the literature that other hormonally inducible enzymes respond to tryptophan (8, 12, 17), but it is assumed that such changes are more limited in scope and degree than those associated with hydrocortisone. Since mice given the LD₅₀ of endotoxin usually die between 18 and 36 hr, a single injection of tryptophan produces an elevation in tryptophan pyrrolase that is likely to be too transitory to alter mortality.

It was considered necessary, therefore, to

![Graph showing tryptophan pyrrolase activity in mice](http://jb.asm.org/)

**Fig. 1.** Tryptophan pyrrolase activity (micromoles of kynurenine per gram, dry weight, of liver per hour) in livers of mice given single or repeated intraperitoneal injections of 20 mg of l-tryptophan. Mice that received repeated injections of nonpyrogenic isotonic sodium chloride solution served as controls.
In an effort to avoid some of the undesirable side-effects caused by intraperitoneal administration of such large amounts of tryptophan, mice were given the amino acid subcutaneously at intervals of 2 hr for a total of five injections. There was a linear increase in tryptophan pyrrolase activity with time (Fig. 2, upper curve). The maximal activity occurred at the time of the final injection, and 4 hr later it was almost back to normal.

*Activity of tryptophan pyrrolase in mice simultaneously administered tryptophan and endotoxin.* Endotoxin-poisoned mice injected with tryptophan have a markedly different response to the bacterial poison than do animals given no amino acid. Administration of the LD$_{50}$ of endotoxin at the same time as the first of five subcutaneous injections of tryptophan resulted in a total enzyme activity that was significantly less in poisoned mice than in those given tryptophan alone.

A comparison of the two curves in Fig. 2 makes this relationship evident.

*Survival of endotoxin-poisoned mice given tryptophan or other selected amino acids.* It was observed in the course of the experiments just described that mice injected with the LD$_{50}$ of endotoxin followed by a series of injections of tryptophan began to die convulsively after 6 to 8 hr (three or four injections). Convulsive death does not normally occur with endotoxin alone, nor do fatalities occur so quickly. A single injection of 20 mg of tryptophan given concurrently with slightly less than the LD$_{50}$ of endotoxin failed to alter the lethality of the bacterial poison (Table 1). When mice were pretreated with endotoxin for 4 hr and then given tryptophan (15 mg per mouse), large numbers died in convulsions within 8 hr (Table 1). A similar response was obtained when the tryptophan was given via either the intraperitoneal or subcutaneous route.

This effect did not result when mice pretreated with endotoxin were treated with 20 mg of phenylalanine, histidine, glycine, glutamine, or tyrosine (data are not presented). The effect of tryptophan in combination with endotoxin is not, therefore, common to all amino acids.

These results make it evident that induction of tryptophan pyrrolase with tryptophan is not an effective approach to the study of the specific protective role of the enzyme in endotoxin poisoning; early abnormal convulsive death obscures the issue. Other experiments with tryptophan will be described later.

*Effect of $\alpha$-methyltryptophan on tryptophan pyrrolase activity in normal and endotoxin-poisoned mice.* Civen and Knox (5) and Moran and Sourkes (16) have shown that the non-metabolizable analogue of tryptophan, $\alpha$-methyl-tryptophan, is capable of increasing tryptophan pyrrolase activity in adrenalectomized rats and

![Fig. 2. Tryptophan pyrrolase activity (micromoles of kynurenine per gram, dry weight, of liver per hr) of mice given repeated subcutaneous injections of 20 mg of $\alpha$-tryptophan. One group of animals served as controls (O) and another group (•) was given an intraperitoneal injection of the LD$_{50}$ of endotoxin.](image)

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>No. of survivors/total injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD$_{50}$ of endotoxin..................</td>
<td></td>
</tr>
<tr>
<td></td>
<td>63/70 (90%)</td>
</tr>
<tr>
<td></td>
<td>41/70 (59%)</td>
</tr>
<tr>
<td>LD$_{50}$ of endotoxin + 15 mg of tryptophan</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57/60 (35% )</td>
</tr>
<tr>
<td></td>
<td>35/60 (58%)</td>
</tr>
<tr>
<td>LD$_{50}$ of endotoxin + 15 mg of tryptophan</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28/70 (40%)</td>
</tr>
<tr>
<td></td>
<td>14/70 (20%)</td>
</tr>
</tbody>
</table>

* Time postinjection.  
* Per cent survival.

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in intact rats. Moran and Sourkes also reported that the increased activity of tryptophan pyrrolase as measured in vitro under these conditions reflects an actual in vivo increase in the catalytic activity of the enzyme (16). As shown in Fig. 3, a single subcutaneous injection of 20 mg of α-methyltryptophan increased and maintained tryptophan pyrrolase activity at an elevated level for at least 24 hr. When the LD₅₀ of endotoxin was administered 4 hr after 20 mg of α-methyltryptophan, the increase in activity of the enzyme was not fundamentally changed. It was clearly higher than that seen with endotoxin alone (Fig. 3).

Effect of α-methyltryptophan on the survival of endotoxin-poisoned mice. No significant protection against challenge with 2 LD₅₀ of endotoxin was afforded mice pretreated for 4 hr with 20 mg of α-methyltryptophan (Table 2). These results suggest that a high level of tryptophan pyrrolase activity is not in itself sufficient to protect mice against endotoxin lethality.

Effect of 5-hydroxytryptophan on tryptophan pyrrolase activity. Addition of small quantities of 5-hydroxytryptophan directly to an assay flask so nearly stopped the tryptophan pyrrolase reaction that only questionable optical density readings (<1) could be obtained (Table 3). A similar effect was noted by Frieden (9). Subcutaneous injection of 5 mg of 5-hydroxytryptophan resulted in a pronounced decrease in tryptophan pyrrolase activity (Table 3); the activity was about 50% of normal after 18 hr.

Effect of 5-hydroxytryptophan on survival of mice poisoned with endotoxin. Since maintenance or elevation of tryptophan pyrrolase activity does not seem to alter the survival of mice injected with endotoxin, the effect of lowering the enzyme was next determined (Table 4). Administration of 5-hydroxytryptophan 4 hr before graded doses of endotoxin caused no significant change in the mortality of the mice. This result, in combination with those described above, suggests that maintenance of activity of tryptophan pyrrolase is not in itself essential for the survival of mice challenged with endotoxin, nor does depression of the enzyme render the mice more susceptible to the lethality of the poison.

Possible involvement of tryptophan pyrrolase in the sensitization of endotoxin-poisoned mice to tryptophan. Table 5 contains additional data on the effect of tryptophan on tryptophan pyrrolase activity in endotoxin-poisoned mice. Mice were injected with 20 mg of tryptophan at the same time they were injected with endotoxin, and tryptophan pyrrolase activity was measured 1 hr later. The elevation of the enzyme was not significantly different from that in mice injected with tryptophan alone (compare lines 3 and 2, Table 5). When tryptophan was given 4 hr after endotoxin, at a time when mice were sensitized to the amino acid, the increase in tryptophan pyrrolase activity was less than that observed when the two injections were given concurrently. This could be due, in part, to the fact that the activity of the enzyme at the time when the try-

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**Fig. 3.** Tryptophan pyrrolase activity (micromoles of kynurenine per gram, dry weight, of liver per hour) in livers of mice given an intraperitoneal injection of the LD₅₀ of endotoxin (●), 20 mg of α-methyltryptophan subcutaneously (○), or α-methyltryptophan at time-zero and the endotoxin 4 hr later, designated by E (dashed curve).
TABLE 2. Effect of α-methyltryptophan on the survival of endotoxin-poisoned mice

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>No. of survivors/total injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 LRD₅₀ of endotoxin...</td>
<td>15/63 (23.8%)³*</td>
</tr>
<tr>
<td>20 mg of α-methyltryptophan + 2 LRD₅₀ of endotoxin 4 hr later...</td>
<td>20/65 (30.7%)</td>
</tr>
</tbody>
</table>

* Determined 24 hr postinjection.

Per cent survival.

TABLE 3. Effect of 5-hydroxytryptophan on tryptophan pyrrolase activity in vitro and in vivo

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Tryptophan pyrrolase activity¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control.............</td>
<td>21.4 ± 0.8e (10)³</td>
</tr>
<tr>
<td>18 hr after subcutaneous injection of 5 mg of 5-hydroxytryptophan...</td>
<td>11.1 ± 1.8 (8)</td>
</tr>
<tr>
<td>Addition of 1 mg of 5-hydroxytryptophan directly to assay flask...</td>
<td>&lt;1 (6)</td>
</tr>
</tbody>
</table>

* Expressed as micromoles of kynurenine per gram (dry weight) of liver per hour.

Each value is the mean ± standard error.

Number of determinations.

tophan was given, i.e., 3 hr after the endotoxin, was lower than that of the controls. As the curve at the bottom of Fig. 3 shows, the activity of this enzyme is depressed at this time (about 10 units of activity) but not to the same extent that the induction is lowered. Thus, the induction in the poisoned mice could not have been more than about 23 units (33.4 - 10), whereas that in the controls was 37 units (58.4 - 21.4). Pretreatment with endotoxin seems, therefore, to alter the substrate inducibility of tryptophan pyrrolase, and this, in turn, suggests that tryptophan cannot be metabolized as efficiently in such animals. The amino acid must be metabolized through other pathways, including the one to serotonin. This effect can be seen in Table 6 by comparing the data of lines 2 and 3 with those of line 1.

Possible involvement of serotonin in the sensitization of endotoxin-poisoned mice to tryptophan. Administration of serotonin in sublethal doses for mice (1 or 5 mg) results in an increased percentage of deaths in animals when it is given 4 hr after an injection of the LD₅₀ of endotoxin. It was shown above (Table 1) and again in Table 6 that a delayed injection of tryptophan has a similar effect. Pretreatment with cyproheptadine, a drug known to inhibit the action of serotonin

TABLE 4. Effect of 5-hydroxytryptophan on the survival of mice given graded doses of endotoxin

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>No. of survivors/total injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD₅₀ of endotoxin........</td>
<td>4/10</td>
</tr>
<tr>
<td>5 mg of 5-hydroxytryptophan + 1 LRD₅₀ of endotoxin 4 hr later...</td>
<td>6/10</td>
</tr>
<tr>
<td>0.5 LRD₅₀ of endotoxin....</td>
<td>10/10</td>
</tr>
<tr>
<td>5 mg of 5-hydroxytryptophan + 0.5 LRD₅₀ of endotoxin 4 hr later...</td>
<td>8/10</td>
</tr>
</tbody>
</table>

* Time postinjection.

TABLE 5. Activity of tryptophan pyrrolase 1 hr after an injection of tryptophan in normal and endotoxin-poisoned mice

<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>Tryptophan pyrrolase activity¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal (fasted) control...</td>
<td>21.4 ± 0.8e (10)³</td>
</tr>
<tr>
<td>2. 20 mg of tryptophan and sacrificed 1 hr later...</td>
<td>58.4 ± 2.5 (8)</td>
</tr>
<tr>
<td>3. Endotoxin (LD₅₀) + 20 mg of tryptophan at time-zero and sacrificed 1 hr later...</td>
<td>58.1 ± 4.5 (8)</td>
</tr>
<tr>
<td>4. Endotoxin (LD₅₀) + 20 mg of tryptophan 3 hr later and sacrificed 4 hr later...</td>
<td>33.4 ± 3.1 (8)</td>
</tr>
</tbody>
</table>

* Expressed as micromoles of kynurenine per gram (dry weight) of liver per hour.

Each value is the mean ± standard error.

Number of determinations.

(20), significantly protects the endotoxin-poisoned mice against the enhanced lethality of a delayed injection of tryptophan (Table 6).

DISCUSSION

The first of the two questions asked in the introduction to this paper must, on the basis of the data presented, be answered in the negative. Tryptophan pyrrolase activity is not immediately related to the ability of the mouse to survive endotoxin poisoning, since the level of the enzyme can be raised or lowered at the time of challenge without altering mortality. This particular enzyme is only one of a number of enzymes induced in mammals in response to adrenocortical stimulation (7, 11). The net result of the metabolic alterations that follow such enzymatic changes is the initiation of events for which the adrenal cortex is responsible if it is to maintain the homeostasis that is necessary for an animal's survival of stress, including that caused by an injection of bacterial endotoxin. It is for this
reason that the adrenalectomized animal is assumed to be so sensitive not only to endotoxin but to all forms of stress. Without hormonally induced enzymes, the homeostatic adjustment to stress becomes impossible, or at least is seriously limited. Because one of the inducible enzymes can be selectively altered without changing an animal's susceptibility to endotoxin, there is no need to assume that the broader concept of enzyme regulation in homeostasis is untenable. 

To establish experimentally the validity of this interpretation of our results requires more knowledge of the specific metabolic cause of death from endotoxin than is available at the present time, and must, therefore, await further insight into the problem. 

The second question asked was whether or not the in vitro assay for tryptophan pyrrolase reflects the true catabolic potential of the enzyme in vivo. The evidence presented above supports an affirmative answer, even though the indirect nature of the experiments requires that the conclusion be a tentative one. The ability of cyproheptadine, an antiserotonin drug, to protect mice against the convulsive type of death that occurs when tryptophan is injected 4 hr after the LD₉₀ of endotoxin strongly suggests that the amino acid is being converted into serotonin. At the time of administration of tryptophan, tryptophan pyrrolase is known to be less active than in the control mice, as judged by an in vitro assay. Convulsive deaths do not occur and cyproheptadine has no effect on mortality when endotoxin and tryptophan are given concurrently. Under these conditions, tryptophan pyrrolase is initially normal.

These interpretations are confirmed and extended in studies on the hypothermia produced by low doses of endotoxin in mice housed at 15 °C (15). An injection of serotonin resulted in a similar degree of hypothermia. Tryptophan alone did not alter the body temperature of mice, but when it was injected into endotoxin-poisoned mice a drop in body temperature greater than that produced by endotoxin alone resulted. The effect of both serotonin and tryptophan was antagonized by cyproheptadine. These results suggest that tryptophan is converted to serotonin in endotoxin-poisoned mice.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


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<table>
<thead>
<tr>
<th>Experimental treatment</th>
<th>No. of survivors/total injected</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>8 hr&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD₉₀ of endotoxin</td>
<td>33/40 (82.5%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD₉₀ of endotoxin + 1 mg of serotonin 4 hr later</td>
<td>14/20 (70%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LD₉₀ of endotoxin + 5 mg of serotonin 4 hr later</td>
<td>13/20 (65%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5 mg of cyproheptadine + LD₉₀ of endotoxin 4 hr later + 15 mg of tryptophan 8 hr later</td>
<td>4/20 (20%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15/20 (75%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Time postinjection. 
<sup>b</sup> Per cent survival.