Effect of Tryptophan and Selected Analログues on Body Temperature of Endotoxin-poisoned Mice

ROBERT J. MOON AND L. JOE BERRY

Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010

Received for publication 9 November 1967

The effect of endotoxin on the body temperature of mice was studied in animals housed without bedding at an environmental temperature of 15 C. Rectal temperatures were measured during the initial 3 to 5 hr of exposure. Doses of endotoxin ranging from 0.01 to 1 LDD₃₀, as determined for mice maintained at 25 C, produce a hypothermia in proportion to dose. Concurrent injection of tryptophan magnified this response in a dose-dependent manner. Cyproheptadine, an antiserotonin drug, antagonized both the hypothermia produced by serotonin alone, and the augmentation of hypothermia produced by tryptophan in endotoxin-poisoned mice. α-Methyltryptophan, an analogue of the amino acid that is known to induce tryptophan pyrrolase, also antagonized the increased hypothermia produced by tryptophan. These data support a previous suggestion that inhibition of tryptophan pyrrolase in endotoxin-poisoned mice has the effect of funneling injected tryptophan into the serotonin pathway.

In a recent investigation in this laboratory, serotonin was implicated as a mediator of certain biological responses to endotoxin in mice (Moon and Berry, submitted for publication). Mice were found to be sensitive to an injection of tryptophan, the amino acid precursor of serotonin, for several hours after administration of endotoxin; this sensitization was prevented by cyproheptadine, an antiserotonin drug. In addition, severe hypoglycemia, over and above that caused by endotoxin alone, was produced when mice were injected with either tryptophan or serotonin 4 hr after the bacterial poison. The effect of these compounds was not seen in animals pretreated with cyproheptadine.

Berry has shown (1) that alteration in body temperature of mice in response to endotoxin is dependent upon the environmental temperature at which the animals were housed. Mice respond to endotoxin with a hyperthermia when exposed to temperatures greater than 30 C and with a hypothermia when placed in an environment of 25 C or less. The mechanisms underlying these fluctuations in body temperature of endotoxin-poisoned mice are poorly understood, but there is some suggestion that peripheral vascular effects play an important role in thermoregulation of an animal as small as the mouse.

The present study was undertaken with two objectives in mind: (i) to determine the degree of serotonin involvement in the hypothermia produced in endotoxin-poisoned mice housed at 15 C, and (ii) to provide evidence for the participation of serotonin in the endotoxin-induced sensitivity to tryptophan. Garattini previously demonstrated the ability of cyproheptadine to antagonize the hypothermia produced by serotonin in mice (6), and this antiserotonin compound was employed as an additional tool in these investigations.

MATERIALS AND METHODS

Endotoxin. Heat-killed (pasteurized) cells of Salmonella typhimurium, strain SR-11, suspended in isotonic nonpyrogenic saline (Baxter Laboratories, Morton Grove, Ill.) served as the source of endotoxin in all experiments. The dosages shown in the various figures refer to the LD₃₀ for mice housed at a temperature of 25 C. The LD₃₀ of endotoxin for mice exposed to 15 C is approximately two-thirds the dose at 25 C (1).

Temperature control. All experiments were carried out for either 3 or 5 hr in a Modulab (Lab-Line Instrument Co., Melrose Park, Ill.) maintained at 15 ± 0.5 C. Mice were singly housed without bedding in special Plexiglas cages as described by Previte and Berry (10). Humidity was not controlled.

Body temperature measurements. The rectal temperature of mice was measured with a Telethermometer (model 477A Yelllow Springs Instrument Co., Yellow Springs, Ohio) equipped with a small rectal probe (YSI no. 402). The probe was inserted a distance of approximately 1 cm, and the temperature was recorded 20 to 30 sec later. In an effort to avoid temperature changes associated with circadian rhythms (7), experiments, with only a few exceptions, were started
between 1:30 and 2:00 PM and were continued for a 3- to 5-hr period. All data represent the average temperature of either 5 or 10 mice. Results are expressed as the average change in body temperature of a group of animals compared to their temperature at the start of the experiment.

Mice. Female Swiss-Webster albino mice, purchased from Dierolf Farms (Boyertown, Pa.), were used when they weighed 23 ± 2 g. They were housed until the beginning of each experiment, under conditions previously described (3), in an air-conditioned room maintained at 25 ± 2 °C.

Chemicals. Serotonin was purchased from Nutritional Biochemical Corp. (Cleveland, Ohio) as serotonin creatinine sulfate. All quantities referred to in the paper are the weight of this substance, and the use of the term serotonin in the text refers to this material. α-Methyltryptophan was supplied through the courtesy of Earl Pierson, Merck, Sharp and Dohme, West Point, Pa. Cyproheptadine hydrochloride (Periactin HCl) was obtained from Merck, Sharp and Dohme, West Point, Pa. Injection of all substances was via the intraperitoneal route, and the solvent for the chemicals employed was nonpyrogenic Baxter saline.

Results

Effect of serotonin on the body temperature of mice exposed to 15 °C. Figure 1 shows the effect of graded doses of serotonin on the body temperature of mice exposed to 15 °C. Serotonin was administered intraperitoneally in doses of 0.5, 1.0, and 2.5 mg per mouse, and both the magnitude and duration of the hyperthermia were more or less proportional to the amount of serotonin administered.

Effect of cyproheptadine on the hyperthermia produced in mice at 15 °C given graded doses of serotonin. The hyperthermia produced by serotonin was effectively reduced in mice 4 hr after pretreatment with 0.5 mg of cyproheptadine (Fig. 1). The dose of cyproheptadine employed in this and subsequent experiments was large. Several different doses between 100 μg and 1.0 mg were tested in preliminary experiments, and the quantity finally employed was chosen because it yielded the most consistent results. No toxic effects were noted at this dose level, and mice pretreated with cyproheptadine alone were able to maintain a normal body temperature when placed at 15 °C.

Effect of graded doses of endotoxin on the temperature response in mice at 15 °C. The body temperatures of mice exposed to 15 °C after an injection of one of several different doses of endotoxin are presented in Fig. 2. The uppermost line represents untreated mice and shows that only a slight decrease in mean body temperature occurred during the 5-hr period. In contrast to the controls,
mice given endotoxin had a drop in mean body temperature of 1 to 3 °C within the first 30 min postinjection, and those receiving the larger doses (0.2 to 1 LD₅₀) had the greater hypothermia. This early decrease in temperature was followed by a plateau that lasted from 30 to 90 min. Beyond this time, the temperature again began to drop at a rate dependent upon the amount of endotoxin administered. The biphasic nature of the hypothermic response to endotoxin is in contrast with the linear decline in temperature elicited by serotonin (Fig. 1).

Effect of cyproheptadine on body temperature of endotoxin-poisoned mice exposed to 15°C. The effect of pretreatment for 4 hr with 0.5 mg of cyproheptadine on the hypothermia produced by endotoxin is shown in Fig. 3. At the two lower doses, the antiserotonin drug eliminated the decrease in body temperature produced by endotoxin alone, but its effectiveness diminished as the dose of endotoxin increased. Accordingly, with 0.2 LD₅₀ of endotoxin, only the initial drop and rise in temperature failed to occur. At the 1 LD₅₀ level (not shown here), the antiserotonin agent failed to alter in any detectable way the sequence of changes in body temperature seen in mice given this dose of endotoxin alone.

Effect of tryptophan on the body temperature of endotoxin-poisoned mice. If tryptophan is converted to serotonin when administered to endotoxin-poisoned mice, as suggested in the introduction, then the amino acid should magnify in a dose-dependent manner the hypothermia produced by endotoxin. The curves in Fig. 4 show that this occurred. All mice were injected with 0.02 LD₅₀ of endotoxin and either no tryptophan or 5, 10, or 20 mg. It is clear from the data that injection of tryptophan resulted in a greater de

![Temperature Response to Endotoxin in Mice at 15°C.](chart)

**Fig. 2.** Mean change in body temperature of mice during the first 5 hr of individual exposure to 15°C. Each animal received either 0, 0.01, 0.02, 0.2, or 1 LD₅₀ of endotoxin. Each value is the average for either 5 or 10 animals.

- Endotoxin alone
- Endotoxin 4 hours after Cyproheptadine (0.5mg)

![Temperature Change](chart)

**Fig. 3.** Mean change in body temperature of mice during the first 3 hr of individual exposure to 15°C. Each animal received 0.01, 0.02, or 0.2 LD₅₀ of endotoxin (●) or 0.5 mg of cyproheptadine 4 hr before 0.01, 0.02, or 0.2 LD₅₀ of endotoxin (○). Each value is the average for five animals.
crease in body temperature than that produced by endotoxin alone. Control injections of only tryptophan at each of the dose levels employed had no effect on the temperature of cold-exposed mice (data are not presented).

Effect of cyproheptadine on the augmentation of hypothermia produced by tryptophan in endotoxin-poisoned mice. Figure 5 shows that, after 4 hr of pretreatment with cyproheptadine, the severity of hypothermia produced in mice given both endo-

![Graph showing the effect of cyproheptadine on body temperature](image)

**FIG. 4.** Mean change in the body temperature of mice during the first 3 hr of individual exposure to 15°C. Each animal received 0.02 LD<sub>50</sub> of endotoxin plus 0, 5, 10, or 20 mg of tryptophan. Each value is the average for either 5 or 10 animals.

- No Cyproheptadine
- 4 hrs. pretreatment with Cyproheptadine (0.5mg)

![Graph showing the effect of tryptophan and cyproheptadine](image)

**FIG. 5.** Mean change in body temperature of mice during the first 3 hr of exposure to 15°C. Each animal received 0.02 LD<sub>50</sub> of endotoxin plus 0, 5, or 10 mg of tryptophan (●) or 0.5 mg of cyproheptadine 4 hr before 0.02 LD<sub>50</sub> of endotoxin plus 0, 5, or 10 mg of tryptophan (○). Each value is the average for five animals.
toxin and tryptophan was less than that in mice that had not received the antiserotonin drug. These results are consistent with the hypothesis that tryptophan becomes converted into serotonin under the conditions of the experiments.

**Effect of 5-hydroxytryptophan on the body temperature of normal mice and mice given 0.02 LD50 endotoxin.** 5-Hydroxytryptophan is the immediate biosynthetic precursor of serotonin (5-hydroxytryptamine) in the metabolic conversion of tryptophan to serotonin. It is commonly used in place of serotonin in research on this biogenic amine. It was considered important, therefore, to determine the influence of this compound on body temperature of mice housed at 15 C. A single dose of 5 mg of 5-hydroxytryptophan produced a hypothermia similar to that seen after approximately one-tenth the amount of serotonin (by weight) and of the same order of magnitude as 0.02 LD50 of endotoxin. This is made evident by a comparison of the data of Fig. 6 with the appropriate curves in Fig. 1 and 2. 5-Hydroxytryptophan (Fig. 6) also augments the hypothermia produced by endotoxin in a manner similar to that obtained with tryptophan (Fig. 4 and 5).

**Effect of cyproheptadine on the hypothermia produced by 5-hydroxytryptophan in endotoxin-poisoned mice.** Cyproheptadine pretreatment reduced the hypothermia produced by a concurrent injection of 0.02 LD50 of endotoxin and 5 mg of 5-hydroxytryptophan (Fig. 7). The antiserotonin drug had little effect, however, on the body temperature of mice given only 5-hydroxytryptophan (data are not presented). In such animals, the drop in body temperature was more transitory than it was after an injection of either endotoxin or serotonin (cf. Fig. 1 and 6). Possibly 5-hydroxytryptophan is cleared or metabolized more rapidly than the other substances. On the other hand, cyproheptadine in all of the situations investigated, had a minimal effect on those changes in body temperature that occurred during the first hour of exposure to 15 C.

**Effect of pretreatment with α-methyltryptophan on the increased hypothermia in mice given endotoxin and tryptophan.** The increased sensitivity of endotoxin-poisoned mice to tryptophan may depend, as suggested above, on a decreased activity of liver tryptophan pyrrolase produced by

---

*Fig. 6. Mean change in body temperature of mice during the first 3 hr of individual exposure to 15 C. Each animal received either 0.02 LD50 of endotoxin ( ), 5 mg of 5-hydroxytryptophan (○) or 0.02 LD50 of endotoxin plus 5 mg of 5-hydroxytryptophan ( ). Each value is the average for either 5 or 10 animals.*

*Fig. 7. Mean change in body temperature of mice during the first 3 hr of individual exposure to 15 C. Each animal received 0.02 LD50 of endotoxin ( ), 0.02 LD50 of endotoxin plus 5 mg of 5-hydroxytryptophan (○), or 0.5 mg of cyproheptadine 4 hr before 0.02 LD50 of endotoxin plus 5 mg of 5-hydroxytryptophan ( ). Each value is the average for either 5 or 10 animals.*
the bacterial poison (2) that, in turn, results in a funneling of tryptophan into the serotonin pathway. Since \( \alpha \)-methyltryptophan has been found to maintain tryptophan pyrrolase activity in endotoxin-poisoned mice (Moon and Berry, submitted for publication), it should allow more complete catabolism of tryptophan through the tryptophan pyrrolase pathway. This, then, should reduce the quantity of the amino acid diverted to serotonin. That this may occur can be seen from the data presented in Fig. 8. Mice given 4 hr of pretreatment with \( \alpha \)-methyltryptophan and then injected with 0.02 \( LD_{50} \) of endotoxin plus 10 mg of tryptophan maintained higher body temperatures than did animals not pretreated and given the latter two injections. In this experiment, perhaps less serotonin is formed and better thermal regulation is possible. Pretreatment with \( \alpha \)-methyltryptophan had no effect on the hypothermia produced by 0.02 \( LD_{50} \) of endotoxin alone (not shown here).

**DISCUSSION**

Much of the literature concerned with the role of serotonin in an animal's response to endotoxin is contradictory (4, 5). This may be due, in part, to experiments in which the dose range has been too narrow. For example, a constant amount of cyproheptadine produced markedly different temperature responses in mice poisoned with graded amounts of endotoxin (Fig. 3). In the temperature drop observed with lower doses of endotoxin, serotonin (or some biogenic substance antagonized by cyproheptadine) must have contributed to the resulting hypothermia. As the dose of endotoxin increased, cyproheptadine became progressively less effective. The apparent poikilothermic effect produced by endotoxin in mice would appear from these results to be multifaceted. This conclusion hinges on the action of cyproheptadine and seems to be justified despite the fact that its precise mode of action has not been established. Its ability to antagonize a variety of physiological responses produced by serotonin as well as histamine is well documented (10).

The data presented here and elsewhere strongly suggest that certain altered biological responses in endotoxin-poisoned mice given tryptophan involve serotonin. For example, (i) cyproheptadine counteracts the sensitivity of endotoxin-poisoned mice to a delayed injection of tryptophan without altering the original lethality of the bacterial poison (submitted for publication); (ii) cyproheptadine antagonizes the augmentation of hypothermia seen in endotoxin-poisoned mice given either tryptophan (Fig. 5) or 5-hydroxytryptophan (Fig. 6); and (iii) \( \alpha \)-methyltryptophan reduces the increased hypothermia produced by tryptophan in endotoxin-poisoned mice (Fig. 8) but does not protect them against endotoxin lethality (submitted for publication).

The action of \( \alpha \)-methyltryptophan is particularly significant under these conditions because tryptophan pyrrolase activity is maintained at a level above that found in normal mice (submitted for publication), even though endotoxin alone (2) significantly depresses the activity of the enzyme. Moran and Sourkes (8) believe that the increased activity of tryptophan pyrrolase after administration of \( \alpha \)-methyltryptophan, as measured in vitro, reflects an actual in vivo increase in the catabolic potential of the enzyme as well. The ability of \( \alpha \)-methyltryptophan to reduce the hypothermia produced by the combined injection of endotoxin and tryptophan supports two suggestions previously made from this laboratory; first, that inhibition of tryptophan pyrrolase by endotoxin as experimentally determined reflects a true inhibition of the enzyme in vivo, and, second, that \( \alpha \)-methyltryptophan administration results in a greater in vivo activity of tryptophan pyrrolase.

**Fig. 8. Mean change in body temperature of mice during the first 3 hr of individual exposure to 15 C. Each animal received either 0.02 \( LD_{50} \) of endotoxin plus 10 mg of tryptophan (●) or 20 mg of \( \alpha \)-methyltryptophan 4 hr before 0.02 \( LD_{50} \) of endotoxin plus 10 mg of tryptophan (○). Each value is the average for 10 animals.**
in endotoxin-poisoned mice (submitted for publication).

Acknowledgments

This investigation was supported by Training Grant 2E-148 from the National Institute of Allergy and Infectious Diseases, National Science Foundation Grant GB-4019, and by grant AI-07851 from the National Institutes of Health.

Literature Cited