Fever and behavioral thermoregulation in young and old rats

MARIA FLOREZ-DUQUET, ELIZABETH PELOSO, AND EVELYN SATINOFF
Department of Psychology and Program in Neuroscience, University of Delaware, Newark, Delaware 19716-2590

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Florez-Duquet, Maria, Elizabeth Peloso, and Evelyn Satinoff. Fever and behavioral thermoregulation in young and old rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R1457–R1461, 2001.—At standard laboratory ambient temperatures (T_a) of 20–24°C, peripheral injections of lipopolysaccharide (LPS) reliably produce fever in young rats. In contrast, old rats may show a blunted fever, no fever, or even hypothermia after LPS. In the present study we hypothesized that old rats might use behavioral thermoregulation to help them develop a fever. Young and old rats were implanted with temperature transmitters. At least 1 wk postoperatively they were placed in a thermally graded alleyway (T_a 10–40°C). On the third and sixth day they were taken out of the gradient, placed at an T_a of 23°C, injected intraperitoneally with LPS or saline, and left at 23°C for 3 h. At the end of that time, all young rats had become febrile, whereas the old rats had not. When the rats were replaced in the thermal gradient, the young animals continued to develop a fever that was similar to fever in young rats left at 23°C. The old animals chose significantly warmer positions in the thermal gradient than did the young animals and only then became febrile. Although there was a tendency for the young rats to prefer higher T_a after LPS than after saline, these differences were not significant. However, the differences in the old rats were significant. These results suggest that the LPS had increased the thermal set point in the old rats, but they could develop febrile responses only at the warm T_a they selected.

AGING; LIPOLYPOSACCHARIDE

AGED HUMANS OFTEN HAVE difficulty mounting adequate fever responses when they are infected (3, 20). The same is true for aged rodents. There are many reports of blunted fever in old animals after peripheral injections of endotoxin (lipopolysaccharide (LPS)) or proinflammatory cytokines (e.g., Refs. 4, 9, and 19). Most studies investigating the effects of age on thermoregulation have focused primarily on physiological aspects of thermal homeostasis. Indeed, aged organisms may have deficits in shivering (1) and nonshivering (2, 8) thermogenesis when they are in a cold environment. Although heat production and heat loss mechanisms have not been measured in old animals after LPS, it is quite possible that these peripheral thermoregulatory mechanisms are inadequate to raise body temperature (T_b). This is unlikely, because we have recently shown that after central injections of interleukin (IL)-1β and PGE_2 at an ambient temperature (T_a) of 23°C, old rats get as high a fever as do young rats (12, 15).

Thermoregulatory behavior can play a significant role in fever production and, indeed, may be crucial in individuals whose capacity for physiological thermoregulation is limited. For example, in infants, who have little insulation and very high heat loss, heat production mechanisms are inadequate to raise T_b.

Satinoff et al. (14) found that infant rabbits did not develop fever after endotoxin injection if they were kept in incubators. However, they developed adultlike fevers when they were allowed to behaviorally regulate by choosing very warm positions in a thermally graded alleyway. Their data demonstrate that the drive toward fever was present in the infants, but could only be expressed behaviorally. Similar results have been observed in many ectothermic species with no capacity at all for physiological thermoregulation (see Ref. 11 for review).

In the present paper, we hypothesized that old rats that do not become febrile after LPS injection at room temperature might resemble infant rabbits. That is, the old rats might develop a fever if they were allowed to thermoregulate behaviorally. Indeed, this was the case, and we report here that old rats that do not develop fevers after peripheral LPS injections when they are kept at T_a 23°C develop fevers by selecting a warm T_a in a thermally graded alleyway.

MATERIALS AND METHODS

Animals and surgery. Subjects were young (3–5 mo) and old (24–29 mo) male and female Long-Evans rats (n = 21). Animals were maintained in their home cages at T_a 23 ± 1°C on a 12:12-h light-dark cycle (lights on at 7 AM, i.e., 3 h before the LPS injection). Food and water were available ad libitum. Young rats were anesthetized with a solution of ketamine-HCL (87 mg/kg body wt) and xylazine (13 mg/kg body wt) and implanted intraperitoneally with a battery-operated biotelemetry device (model VM, Mini-Mitter, Sunriver, OR). Old rats were anesthetized with 80% of this dose and similarly implanted with transmitters.

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The body weights of the four groups were: young females, 277.6 ± 21.3 g (range 232–376); young males, 495.4 ± 46 g (range 370–577); old females, 526.4 ± 41 g (range 411–658); old males, 715.8 ± 59.2 g (range 495–820).

Experimental design. At least 1 wk postoperatively, animals were placed in a thermally graded alleyway. At 10 AM on day 3, one-half the rats were injected intraperitoneally with LPS (50 μg/kg) or an equivalent volume of saline. They were placed in their home cage at 23°C for the 3 h immediately following injections, after which they returned to the gradient. The injection procedure was repeated on day 6 in the thermal gradient, with rats that had been injected with LPS given saline, and vice versa. All rats were left in the gradient for 2 more days. Thus each rat served as its own control. Other groups of young and old rats were injected with the same dose of LPS or saline and left in their home cages.

Thermal gradient. Two identical thermal gradients were used. Each consisted of an aluminum floor and wall (75 cm long × 12 cm wide × 15 cm high) with a hinged Plexiglas top. The floor was extended 30.5 cm beyond the walls on each end for heating and cooling. Heating tape was wrapped on one end and the other end was bathed in a circulating solution of chilled ethylene glycol. The gradient floor temperatures varied from 10 to 40°C. Position sensors and thermocouples were placed at 5-cm intervals along the floor of the gradient. The temperature gradient was linear, and no animals chose to stay at either the coldest or hottest gradient positions.

LPS. Purified lyophilized phenol extract of Escherichia coli endotoxin (0111:B4, Sigma, St. Louis, MO, Catalog No. L-2630) was dissolved in sterile saline (American Pharmaceutical Partners, Los Angeles, CA) aliquoted, and frozen at −20°C. LPS was injected intraperitoneally at a dose of 50 μg/kg body wt. Injection of an equivalent volume of sterile saline was used as a control.

Data collection and analysis. T\(b\) of rats in their home cages were monitored continuously using a peripheral processor (Datacol III System) connected to a personal computer. Temperature-dependent transmitter pulse frequencies were converted to T\(b\) by the Datacol system. The data were stored on hard disk every 5 min. When animals were in the thermal gradient, T\(b\) data were collected in the same manner via a loop antenna placed in the gradient and connected to the Datacol receiver. While animals were in the thermal gradient, selected T\(b\) (T\(sal\)) was recorded every minute via a second data acquisition system (Dasylab, Amherst, NH) and stored on disk. Thirty-minute averages of T\(b\) and T\(sal\) were used in the analysis. Repeated-measures ANOVA was used to test for significant main effects. Student’s t-test or Bonferroni t-test was used to assess post hoc differences between the groups. Significance level was set at \(P < 0.05\).

RESULTS

\(T_b\) response to LPS. Figure 1 shows the time course of \(T_b\) changes in young and old rats before and after LPS injection. Because there were no significant sex differences, males and females are grouped together within each age group. Preinjection \(T_b\) (measured at time 0) were similar in both groups both before the saline injection (young, 37.5 ± 0.2°C; old, 37.7 ± 0.2°C; Fig. 1A) and before the LPS injection (young, 37.5 ± 0.2; old, 37.4 ± 0.2; Fig. 1B). Note the stress-induced hyperthermia immediately after the injection, which is significantly higher in young animals (\(P < 0.001\)).

After 3 h in their home cages, the rats were returned to the thermal gradient. The rats that had received saline showed no change in \(T_b\) and this continued for the duration of the time in the gradient. After LPS, all young rats had elevated \(T_b\) 3 h postinjection vs. preinjection \(T_b\) at time 0 (38.4 ± 0.2°C vs. 37.5 ± 0.1°C, \(P < 0.001\)). After LPS, the mean \(T_b\) of the old rats was 37.7 ± 0.2°C at 3 h postinjection vs. 37.4 ± 0.3°C at time 0 (not significant). When the young rats were returned to the thermal gradient, \(T_b\) continued to increase and remained significantly elevated for the next 6 h. Their mean \(T_b\) reached a peak of 38.7 ± 0.2°C at about 6 h postinjection.

The \(T_b\) of old rats did not increase above preinjection levels at any time after LPS in the home cage. When the old animals were returned to the thermal gradient, \(T_b\) increased and was significantly elevated above pre-
Injection levels after 30 min (3.5 h, 38.3 ± 0.2°C, \( P < 0.001 \)). Mean \( T_b \) reached a peak of 39.0 ± 0.2°C at 6.5–7 h postinjection. Old male and female rats that were injected with LPS (50 \( \mu \)g/kg) and left in their home cages at \( T_a \) 23°C did not develop fevers (Fig. 2). None of the rats injected with saline developed fevers (data not shown).

\( T_{sel} \) in the thermal gradient. The old animals selected significantly warmer floor temperatures than did the young animals after either saline or LPS injection (Table 1, \( P < 0.001 \)).

Figure 3 shows \( T_{sel} \) of young and old rats from −3 to 9 h postinjection. In neither age group was any \( T_{sel} \) time point different from the time 0 baseline for that age. From −3 to 0 h one can see that the \( T_{sel} \) of the young rats rises (as their \( T_b \) declines normally, Fig. 3A), whereas the \( T_{sel} \) of the old rats stays the same, even though their \( T_b \) also declines normally (Fig. 3B). At all time points, old rats selected higher gradient positions than did young rats after either saline or LPS.

The previous figure compared the \( T_{sel} \) of young and old rats. Figure 4 compares the \( T_{sel} \) with respect to whether the rats were injected with saline or LPS. Although there is a trend for the \( T_{sel} \) of the young rats to be higher after LPS than after saline, because of the variability of the means, this difference was not significant. However, the old rats did choose a higher \( T_a \) after LPS than after saline (\( P < 0.05 \)). The preinjection \( T_{sel} \) of the rats, starting right after lights on, also clearly show that the young rats were choosing higher \( T_{sel} \), whereas the old rats were not.

**DISCUSSION**

These data demonstrate that at \( T_a \) 23°C young rats injected with LPS developed febrile responses after 3 h; old rats similarly treated either had blunted fever or no fever. However, 3 h postinjection, when the old rats were placed in a thermal gradient, they chose warm positions and then became febrile. Our data suggest that 1) after LPS, the drive toward fever is present in old rats but 2) the old animals could not reach the higher \( T_b \) at a \( T_a \) of 23°C. At the higher \( T_a \), however, the old rats reached a peak fever higher than that of the young rats (Fig. 1B) at the time when the febrile responses of the young rats were already abating.

**Table 1. Selected temperature on day of saline injection and day of LPS injection**

<table>
<thead>
<tr>
<th>Injection</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old saline</td>
<td>32.1 ± 1.1</td>
<td>32.8 ± 0.3</td>
</tr>
<tr>
<td>Old LPS</td>
<td>32.4 ± 1.1</td>
<td>33.6 ± 0.4</td>
</tr>
<tr>
<td>Young saline</td>
<td>29.4 ± 1.1</td>
<td>27.3 ± 0.6</td>
</tr>
<tr>
<td>Young LPS</td>
<td>27.7 ± 1.1</td>
<td>29.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 21 \) rats. LPS, lipopolysaccharide.
The delayed defervescence in the old rats agrees with the observation of Sapolsky et al. (13) that once a response is elicited in old rats, it may take longer to return to baseline. For instance, when 24-mo-old rats were injected intravenously with ACTH, the corticosterone response was dampened and recovery was slower (16). The amount of tumor necrosis factor (TNF-α) produced after challenge with endotoxin was 20 times higher in old mice than in young ones in the presence of elevated corticosterone levels (10).

Although the old rats chose warmer positions in the gradient after LPS than after saline, the young rats did not. Although there was a tendency toward higher $T_{sel}$ after LPS, this was not significant. Sugimoto et al. (17) reported similar results in young rats. LPS produced similar fevers whether it was given during the light or dark phase. C. J. Gordon (unpublished data) has seen similar effects in a small sample of young rats; given an injection of LPS (50 μg/kg) in the early part of the light cycle, they do not choose a warmer $T_a$ than do saline-injected controls until light off. Briese (6) reported that there was a significant correlation between the magnitude of the $T_b$ rise in fever after a large dose of LPS (40 mg/rat) and the $T_{sel}$ of male rats. However, neither the age of the rats, nor their initial $T_b$, nor the light cycle was reported. The injections were given at 1730. This could have been near the beginning of the dark cycle, but there is no way of knowing. The same problem occurs in work by Bodurka et al. (5). The rats were injected between 8 and 9 h, and were "exposed to seasonal illumination conditions."

With the exception of the latter two studies, in which the methods are unclear, the results in young rats from three different laboratories with three different protocols are puzzling. Apparently in the light, young rats do not use behavior in a thermal gradient to augment their autonomic responses to achieve fever. This is puzzling because no work is required to choose to stay in a warm position in the gradient. Clearly, more comprehensive studies such as that of Sugimoto et al. (17) need to be done.

Although young animals develop high fevers in response to endotoxin injection, old rats develop low fevers, no fevers, or become hypothermic. The underlying mechanism involved in this observation remains to be elucidated. There are several steps involved in the initiation of fever, and age may impact any or all of these processes.

The pathway leading to fever production is initiated when the infectious agent stimulates the immune system to produce cytokines, some of which (e.g., IL-1β, IL-6, and tumor necrosis factor-α) are proinflammatory and elicit fever. Proinflammatory cytokines cause the synthesis and release of PGE₂ in the brain, a final common pathway in fever induction. Elevated prostaglandin levels lead to an increased thermal set point and a resulting increase in thermogenesis, decrease in heat loss, and elevated $T_b$.

It is unlikely that blunted fever in old individuals is due to attenuated cytokine levels. LPS-induced cytokine levels actually increase with age, both in plasma and in brain. For instance, plasma and cerebrospinal fluid levels of TNF-α were significantly higher in aged rats and mice (7, 9). Old rodents also showed high levels of IL-6 compared with young ones (9, 18). It would appear that the endotoxin-induced pyrogenic signal is present in old rats. However, if the initial pyrogenic signal is present, then why do old rats not develop high fevers? One explanation may be that in an older population, the pyrogenic signal stimulated by the infection may not reach the thermoregulatory control centers in the hypothalamus and as a result there will be no alteration in the thermal set point and thus no fever. Alternatively, in the event that the pyrogenic signal does reach the brain, it is possible that the signal is not transduced into an elevated set point or

Fig. 4. $T_{sel}$ of young (A) and old (B) rats after saline or LPS. In young rats the difference was not significant. In old rats it was $P < 0.05$. Note the increasing $T_{sel}$ of the young rats in the 3 h after lights on.
that the thermoeffector is not capable of increasing
T_h in response to the altered set point.
We have recently tested several of these possibilities. Results from our laboratory have demonstrated that a pyrogenic signal (i.e., PGE_2 or IL-1), when administrated centrally, is appropriately transduced into an elevation in thermal set point in old rats resulting in high fever responses (12, 15). In addition, the febrile temperatures attained in the old rats were equivalent to or higher than those seen in the young febrile rats. The data indicate that 1) a centrally administered pyrogenic signal is appropriately transduced into an elevated set point, 2) the increase in set point leads to the stimulation of thermoeffector, and 3) thermoeffector in old rats are entirely capable of increasing T_h in response to the stimulus. Therefore, it appears that old rats do retain the capacity to respond to central administration of cytokines with fever.

Whereas there are an increasing number of studies investigating potential age-related effects on autonomic aspects of fever production, particularly activation of brown adipose tissue (8), few studies have focused on behavioral aspects of temperature regulation. Here, we demonstrate that in the case of old rats, behavioral selection of T_a also may have a significant impact on fever production. Elucidating the underlying mechanisms involved in the age-related reduction in fever during infection may provide not only a more appropriate and timely diagnosis of infection and augment survival in the elderly, but it will also contribute to our basic and fundamental understanding of the aging process.

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REFERENCES