Thermoregulatory and metabolic changes during fever in young and old rats

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Submitted 1 May 2003; accepted in final form 25 July 2003

Buchanan, Jessica B., Elizabeth Peloso, and Evelyn Satinoff. Thermoregulatory and metabolic changes during fever in young and old rats. Am J Physiol Regul Integr Comp Physiol 285: R1165–R1169, 2003. First published July 31, 2003; 10.1152/ajpregu.00238.2003.—We injected old and young rats with lipopolysaccharide (LPS; 50 μg/kg ip) at two ambient temperatures (Tₐ; 21 and 31°C). Young rats mounted equivalent fevers at both Tₛ [peak body temperatures (Tₚ) of 38.3 and 38.7°C, respectively]. The Tₚ of old rats was not different from baseline (37.3°C) after LPS at Tₐ, 21°C, whereas, at 31°C, their Tₚ rose to a mean peak of 38.4°C. We also measured the associated thermoregulatory responses by use of calorimetry. At 21°C, young rats developed a fever by increasing both O₂ consumption and heat conservation. Old rats did not become febrile, and O₂ consumption fell by 15%. Heat loss was the same in old and young rats. At 31°C, young and old rats developed similar fevers with similar increases in heat production and conservation. Our results suggest that the lack of LPS fever in old rats at 21°C is due mainly to the lowered metabolic rate.

In the present study, we investigated thermoregulatory responses in old and young rats after peripheral administration of LPS. We measured whole body heat production and total heat loss at two different Tₛ, 21 and 31°C. We found that heat production decreased dramatically in the old rats at 21°C. These results suggest that the blunted fever seen in old rats at lower Tₛ is most likely due to a decrease in heat production.

METHODS AND MATERIALS

Animals and surgery. Subjects were young (male n = 6, female n = 6, 3–6 mo) and old (male n = 7, female n = 6, 24–28 mo) Long-Evans rats. Animals were maintained in basin cages (56 × 32 × 20 cm) at a Tₐ of 23 ± 1°C on a 12:12-h light-dark cycle (lights on at 7:00 AM). 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, Bend, OR). Old rats were anesthetized with 80% of this dose and similarly implanted with transmitters. Tₚ was measured continuously from time of implantation until the end of the experiment.

Experimental design. At least 1 wk postoperatively, a rat was placed in the calorimeter (see below) without food or water for at least 4 h for acclimatization. The next day, after 3 h in the calorimeter (at 10:00 AM), the rat was injected (ip) with either LPS or saline at a Tₐ of either 21 or 31 ± 1°C. The rat remained in the calorimeter for a further 7 h. At least 3 days after the first injection, the procedure was repeated with the alternate injection at the same Tₐ. At least 3 wk later, the experiment was repeated with the same rat at the other Tₐ. Injection and Tₐ exposure were counterbalanced to account for any order effects. Rats were weighed before being placed in the calorimeter on each injection day. At the time of injection, vaginal smears were obtained from the young female rats to determine the stage of the estrous cycle.

All 12 young rats were tested at both Tₛ. Three old male and four old female rats received injections at both Tₛ. Four old males and two old females received injections at only one Tₐ. Old rats at a Tₐ of 21°C include the seven old rats that received injections at both Tₛ plus two old males and one old female that were tested at a Tₐ of 21°C only. Old rats at a Tₐ of 31°C include the seven old rats that received injections at both Tₛ plus two old males and one old female that were tested at a Tₐ of 31°C only.

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LPS. Purified lyophilized phenol extract of *Escherichia coli* endotoxin (0111:B4; Sigma, St. Louis, MO) was dissolved in sterile saline, aliquoted, and frozen at −20°C. LPS was injected (ip) at a dose of 50 μg/kg. Injection of an equivalent volume of saline was used as a control.

*Measurement of T*<sub>b</sub>. *T*<sub>b</sub> of rats in their home cages was monitored using a peripheral processor (Datacol III System) connected to a personal computer (PC). Temperature-dependent transmitter pulse frequencies were converted to *T*<sub>b</sub> by the Datacol system and stored on hard disk. When animals were in the calorimeter, the transmitter frequencies were received via a loop antenna and collected in the same manner. *T*<sub>b</sub> was recorded every 5 min until the end of the experiments.

*Measurement of heat loss and heat production.* Nonevaporative heat loss was measured in a calorimeter (30 × 15 × 15 cm, model Sec A; Thermonetics, La Jolla, CA). Heat generated by the animal passed through the walls of the calorimeter and was measured by thermoelectric sensors distributed throughout the walls of the calorimeter. The sensors operate on the gradient layer principle in which the flow of heat transferred to a solid results in a measurable temperature gradient in the direction of heat flow. The temperature difference across the gradient layer was measured by thermopiles, the output of which is a measure of the heat flow. The output signal (a direct current potential) was recorded on a PC and then converted to calories per hour. Evaporative heat loss was calculated from the airflow and the temperature and humidity of air that were measured by inlet and outlet micrometeorimeters (Thermonetics). Airflow, humidity, and temperature values were entered into Akton Psychrometric Chart software (Akton, Concord, CA) to yield evaporative heat loss in British thermal units.

Heat production was estimated by *O*<sub>2</sub> consumption and calculated from measurements of *O*<sub>2</sub> content and airflow. Room air was drawn through the calorimeter at 2 ml/min. Expired air was desiccated by drawing it through a canister of 8-mesh Drierite (W.A. Hammond Drierite, Xenia, OH). Airflow was controlled with a Matheson mass flow controller. A fraction of the dried air was drawn through an *O*<sub>2</sub> analyzer (Applied Electrochemistry, S-3A) that provided continuous recording of percent *O*<sub>2</sub> values. At 1-min intervals, these values were fed into a PC and converted into milliliters of *O*<sub>2</sub> per minute using the formula

\[
|O_{2, \text{in}} - O_{2, \text{out}}/100| \times F
\]

where *O*<sub>2, in</sub> is the baseline *O*<sub>2</sub> percentage going into the chamber, *O*<sub>2, out</sub> is the *O*<sub>2</sub> percentage coming out of the chamber, and *F* is airflow rate of air into the chamber (in ml/min). This value was then multiplied by the caloric equivalent of *O*<sub>2</sub> (0.4837 cal/ml) to yield calories per hour.

All heat production and heat loss values were converted into watts and divided by kg<sup>0.75</sup>. Nonevaporative and evaporative heat loss were added together to yield total heat loss.

*Data analysis.* Results are presented as means ± SE; 1-min *O*<sub>2</sub> consumption and heat loss and 5-min *T*<sub>b</sub> data were averaged over 30 min. *O*<sub>2</sub> consumption and heat loss data are presented as percent change from time 0. Baseline measurements were designated as the 30-min average before each injection. Statistical differences were analyzed using analysis of variance (ANOVA) followed by Tukey's post hoc tests. Separate ANOVAs with repeated measures were run on the rats that received injections at both *T*<sub>b</sub>s. Results from analyses with and without rats that received treatment at only one *T*<sub>b</sub> were similar. Unless otherwise stated, statistical significance was taken from the analysis that included all animals. Significance level was set at *P* < 0.05.

**RESULTS**

**Baseline values.** The young females weighed significantly less than any of the other three groups at both *T*<sub>b</sub>s. At 31°C, the old females weighed significantly less than did the old males. After 2.5 h in the calorimeter, baseline *T*<sub>b</sub>, *O*<sub>2</sub> consumption, and heat loss were measured for 30 min (Table 1). Baseline *T*<sub>b</sub> values for the four groups were equivalent, both between groups and between *T*<sub>b</sub>s. *O*<sub>2</sub> consumption values were higher in the young rats at 21 than 31°C, but in the old rats *T*<sub>b</sub> made no difference: values were equally low at both *T*<sub>b</sub> 21 and 31°C. Resting heat loss values for both young and old rats were equivalent and both were lower at 21 than 31°C. Nonevaporative heat loss accounted for 30–40% of total resting heat loss in all groups of rats at 21°C, and 8–10% at 31°C. Changes in nonevaporative heat loss detected by the calorimeter include vasomotor changes and any behavioral methods of heat loss and conservation.

None of the young female rats was in estrus on the days of injection. Because there were no significant sex differences at either *T*<sub>b</sub>, data from males and females were grouped for both ages.

**Young vs. old rats after LPS at 21°C.** Starting about 90 min after LPS injection, young rats showed febrile responses leading to a maximum fever of 38.3 ± 0.2°C by 330 min (Fig. 1A; *P* < 0.001). After LPS, old rats showed a small but significant rise in *T*<sub>b</sub> compared with saline (*P* < 0.001). However, this rise did not significantly differ from baseline *T*<sub>b</sub>. *O*<sub>2</sub> consumption rose immediately in the

### Table 1. Baseline measurements in young and old rats

<table>
<thead>
<tr>
<th>Animals</th>
<th><em>T</em>&lt;sub&gt;b&lt;/sub&gt;, °C</th>
<th><em>O</em>&lt;sub&gt;2&lt;/sub&gt; Consumption, W/kg&lt;sup&gt;0.75&lt;/sup&gt;</th>
<th>Heat Loss, W/kg&lt;sup&gt;0.75&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>493.5 ± 13</td>
<td>37.3 ± 0.1</td>
<td>6.23 ± 0.42*</td>
</tr>
<tr>
<td>31</td>
<td>475.5 ± 14.19</td>
<td>37.6 ± 0.1</td>
<td>4.76 ± 0.28</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>315.16 ± 24.17†‡</td>
<td>37.2 ± 0.1</td>
<td>6.54 ± 0.17*</td>
</tr>
<tr>
<td>31</td>
<td>338.83 ± 25.31†‡</td>
<td>37.5 ± 0.2</td>
<td>5.96 ± 0.3</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old males</td>
<td>21</td>
<td>581.6 ± 48.7</td>
<td>37.0 ± 0.2</td>
</tr>
<tr>
<td>31</td>
<td>585.6 ± 29.4</td>
<td>37.2 ± 0.1</td>
<td>4.69 ± 0.55‡</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>1.41 ± 0.19*</td>
</tr>
<tr>
<td>Old females</td>
<td>21</td>
<td>473.6 ± 27.7</td>
<td>37.3 ± 0.2</td>
</tr>
<tr>
<td>31</td>
<td>454.8 ± 24.14‡§</td>
<td>37.2 ± 0.2</td>
<td>5.00 ± 0.32‡</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>4.18 ± 0.43</td>
</tr>
</tbody>
</table>

Values, except for ambient temperature (*T*<sub>a</sub>), are means ± SE. Baseline measurements in young and old rats at 21 and 31°C, *T*<sub>b</sub>, body temperature. *P* < 0.001, difference between ambient temperatures; †*P* < 0.01, difference between young males and females; ‡*P* < 0.001, difference between young and old rats; §*P* < 0.05, difference between old males and females.
In the young rats, O2 consumption stayed elevated for 6 h, whereas in the old rats, it fell by a maximum of 15% and only returned to baseline levels toward the end of the time in the calorimeter (Fig. 1B; P < 0.001 compared with saline, P < 0.001 compared with young). Heat loss decreased in the same way in both groups in response to LPS (Fig. 1C). In summary, at 21°C, old rats had a blunted fever response to LPS that was accompanied by a drop in O2 consumption.

Young vs. old rats after LPS at 31°C. The fever response of the young and old rats did not differ at this Ta (Fig. 2). After LPS, young rats showed febrile responses leading to a maximum fever of 38.7 ± 0.2°C by 300 min (P < 0.001). Old rats showed febrile responses leading to a maximum fever of 38.4 ± 0.2°C by 300 min (P < 0.001). O2 consumption was not significantly different between young and old rats at this Ta, nor was heat loss. In summary, at 31°C there were no metabolic differences between young and old rats and their fevers were similar.

DISCUSSION

The present findings confirm prior results from this laboratory demonstrating that after LPS, old rats mount poor febrile responses at a Ta of 21°C and good febrile responses at 31°C compared with young rats. Here we add that the poor fever was accompanied by a 15% drop in O2 consumption, which did not occur after LPS at a Ta of 31°C. Indeed, at the warmer Ta, old and young rats responded to LPS in a similar manner. This suggests that the decrease in O2 consumption is responsible for the blunted fever seen in old rats at cooler Ta's.

O2 consumption is a measure of whole body heat production. It reflects the contribution of both shivering and nonshivering thermogenesis (accomplished through activation of brown adipose tissue; BAT). It is generally lower in warm environments, as was seen here in the young rats but not in the old ones (Table 1). After LPS injections, young rats increased their O2 consumption by >15% at 21°C and by 12% at 31°C. These results support findings from other laboratories in which O2 consumption at Ta's between 22 and 26°C increased 12–20% during fever (3, 4, 10).

In contrast, old rats decreased their O2 consumption by 15% at a Ta of 21°C. This was completely unexpected, and several reasonable hypotheses fail to explain it. For instance, the amount of functional BAT...
declines with age, which could result in a decrease in nonshivering heat production (5, 11). Scarpace et al. (17) reported that blunted fevers in old rats might be due to a failure to increase BAT thermogenesis after pyrogen. We did not measure BAT activity directly, but it would necessarily be reflected in the O2 consumption values, and, indeed, resting O2 consumption levels were lower in our old rats. However, a failure on the part of old rats to increase BAT activity would not explain the decrease in O2 consumption we report here. At best, it should leave O2 consumption unchanged, not reduced, after LPS.

Given that old rats prefer warm Ta (6), 21°C is most likely a mild cold stress. One might assume, therefore, that the old rats may already be close to their maximum heat-producing capabilities. However, from our own studies (unpublished data) as well as others (8, 12), we know that old rats exposed to cold can increase O2 consumption by as much as 41%. Therefore, the possibility that old rats simply cannot increase heat production at 21°C is not tenable.

Another possibility is that old rats are more sensitive to LPS than are young rats. This would mean that a dose that generally causes fever in young rats could cause endotoxin shock in old rats. Endotoxin shock is characterized by a decrease in O2 consumption and increased peripheral vasodilation, hypothermia, and cold-seeking behavior (16). However, despite showing decreased O2 consumption after 50 μg/kg of LPS, our old rats showed no other symptom of shock. None of the old rats became hypothermic, none preferred a cooler Ta (6), and none showed increased heat loss (reflecting peripheral vasodilation). Indeed, old rats decreased heat loss as well as did young rats after LPS. Further, a lower dose of 25 μg/kg of LPS did not cause fever in old rats at a Ta of either 21 or 31°C (unpublished data).

A last possibility for the decreased O2 consumption is that the cytokines induced peripherally by LPS injection are not penetrating the blood-brain barrier as well in old rats and that they therefore have much higher levels of peripheral cytokines than do young rats. LPS induces macrophages to produce and release proinflammatory cytokines (specifically, IL-1β, IL-6, and tumor necrosis factor-α) that are responsible for a number of peripheral and central effects. McLay et al. (13) reported that IL-1β does not cross the blood-brain barrier in aged mice nearly as readily as it does in young ones. In turn, this may lead to increased levels of peripheral cytokines. Indeed, there are several reports of increased peripheral cytokines after LPS in old ro-

Fig. 2. Same as in Fig. 1 but for rats at 31°C. LPS 31 and Sal 31, LPS and saline at 31°C, respectively. There were no differences between young and old rats in any of the measurements.
dents compared with young (2, 7, 19, 20). We do not know whether high levels of peripheral cytokines can cause a decrease in metabolic rate, but this is certainly a question worth investigating.

**DISCLOSURES**

Research was supported by National Institute of Mental Health Grant RO1-MH-41138 (to E. Satinoff).

**REFERENCES**