Interleukin-1β-induced fever in young and old Long-Evans rats

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Plata-Salaman, Carlos R., Elizabeth Peloso, and Evelyn Satinoff. Interleukin-1β-induced fever in young and old Long-Evans rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1633–R1638, 1998.—Aging is associated with a blunted or absent fever response to naturally occurring infections or to the peripheral administration of bacterial products and proinflammatory cytokines, including interleukin-1β (IL-1β). Whether old rats also exhibit an attenuated fever response when challenged with direct brain administration of IL-1β is unknown. Here we investigated the fever response of young (3–5 mo) and old (24–26 mo) Long-Evans rats to the intracerebroventricular microinfusion of IL-1β. Core body temperature was monitored by telemetry in freely moving rats. Intracerebroventricularly administered IL-1β induced comparable increases in body temperature in young and old Long-Evans rats. In the two groups, IL-1β-induced fever was similar both in latency to peak fever and maximal fever response, whether the cytokine was administered 2 h after lights on or just before lights off. These data show that old Long-Evans rats are not defective in their capacity to develop a fever in response to brain administration of IL-1β.

cytokine; intracerebroventricular; aging; core temperature; nervous system; immune system

INTERLEUKIN (IL)-1β is a proinflammatory cytokine that induces fever (2, 11, 14). In rodents, when IL-1β is administered intracerebroventricularly, low-nanogram doses are sufficient to induce fever. When IL-1β is administered peripherally, microgram doses are required to induce equivalent fevers. This suggests that the pyrogenic effects induced by low doses of centrally administered IL-1β are due to its direct action in the central nervous system (CNS).

Aging is associated with alterations in the host physiological responses to inflammatory and infectious stimuli (4). Often, elderly patients show a blunted fever or no fever at all in response to infection (18). The same is true in old rodents: peripheral (intraperitoneal, intravenous) administration of bacterial products or proinflammatory cytokines (1, 8, 16, 17, 28), including IL-1β (19, 27), is associated with a blunted fever response. Delayed febrile responses to peripheral immunological challenges have also been shown in old rats (8, 22).

Young rats centrally injected with IL-1β respond with fever (25, 29). However, whether old rats respond with fever after such a procedure is unknown (studies in aged rabbits reported that central injection of a crude supernatant containing IL-1β and other lipopolysaccharide-induced cytokines produced a blunted fever response (6, 15); however, use of a crude preparation containing multiple cytokines precludes any conclusion on the role of IL-1β). If old rats did not become febrile, it would suggest that they do not have the physiological ability to make fever responses. If they did become febrile, it would imply that they have the capacity to develop a fever, but peripheral administration of bacterial products or proinflammatory cytokines including IL-1β does not activate the required sequence to initiate the fever response. Here we report that after central administration of IL-1β, old Long-Evans rats develop fevers just as well as do young rats, although they do not maintain the elevated body temperature for as long.

Body temperature in rats is a diurnal rhythm, lower in the morning than in the evening. In earlier work in young rats, we demonstrated that after central injections of prostaglandin E2, peak body temperatures attained were similar even when the injections were made 12 h apart (7). Those results showed that central injections of prostaglandin E2 raise body temperature to a particular level, independent of either the body temperature at the time of the injection or the phase of the light-dark cycle (7). To determine the generalizability of these results, we administered the IL-1β at two different times, just before lights off and 2–2.5 h after lights on. We report here that peak body temperatures attained were similar at the two different infusion times.

MATERIALS AND METHODS

Subjects and maintenance. Male young (3–5 mo) and old (24–26 mo) Long-Evans rats were used. They were housed individually and maintained on ad libitum powdered rat food (Labdiet, PMI Feeds, St. Louis, MO) and tap water (26). Lights were on from 0700 to 1900, and room temperature was kept at 23 ± 1°C. All rats were handled daily.

Implantation of brain cannulas. Rats were anesthetized with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg). A 23-gauge guide cannula was implanted into the third cerebral ventricle at the following stereotaxic coordinates: −2.1 mm anteroposterior and 0.0 mm lateral with respect to the bregma and 7.5–8.0 mm dorsoventral from the brain surface, as in previous studies (20). An incision was made through the dura mater with a dental hook. The superior sagittal sinus was carefully pulled to one side, and the 23-gauge guide cannula was gently lowered. Once the cannula was in position, the retraction of the sinus was released and the cannula was anchored with dental acrylic. The location of the cannula tip in the third ventricle was verified by the free outflow of cerebrospinal fluid (CSF)
through the guide cannula. A sterile 29-gauge stainless steel obturator was used to ensure that the cannula remained patent.

Intracerebroventricular microinfusion. At least 14 days postoperatively, the first microinfusions were made into the third ventricle. The third ventricle was chosen because of its proximity to the hypothalamus and the importance of hypothalamic brain regions in thermoregulation. Intracerebroventricular microinfusions (10 µl/rat) were at the rate of 1 µl/60 s using a Harvard infusion pump (Harvard Apparatus, South Natick, MA). The first intracerebroventricular microinfusion was done between 0900 and 0930 (i.e., 2.5 h after lights on) and between 1830 and 1900 (i.e., 0.5 h to just before lights out). All rats received a control infusion of heat-treated IL-1β followed 3 or more days later by infusion of intact IL-1β. These two infusions were repeated at least 2 wk later at the other time of day. The first active IL-1β infusion was in the early evening.

Recombinant human IL-1β (4.0 ng/rat; R and D Systems, Minneapolis, MN)1 was used for all studies. This dose was selected based on our previous studies showing that it induces significant anorexia in rats (21, 26). The same IL-1β lot and stock solutions were used for all experiments. IL-1β was dissolved in sterile physiological saline (0.15 M NaCl) containing 2.0 µg/10 µl BSA (J. R. H. Biosciences, Lenexa, KS) [2.0 µg/10 µl is equivalent to the concentration of albumin normally present in the CSF (3)]. BSA was added because of its properties as a stabilizing agent and carrier protein for cytokines (21). Heat treatment and verification of IL-1β inactivity were done as in previous studies (26). Each test solution was dissolved in a 10-µl volume and had a pH of ~7. To avoid nonspecific adsorption of IL-1β on the experimental tools, we siliconized all such materials. After an experiment was completed, rats were anesthetized with CO2 and decapitated and the position of the cannula tip in the third ventricle was verified.

Measurement of body temperature. Body temperature was measured by a biotelemetry system (Mini-Mitter, Sunriver, OR) using precalibrated transmitters implanted intra-abdominally at the time of the intracerebroventricular cannulation. The transmitter output (accuracy of ±0.1°C, frequency in Hz) was monitored by an antenna in the receiver board placed under each rat’s cage. The output signals were fed into a consolidation matrix processor connected to a PC-based analog-to-digital conversion system (DataCol version 3 data acquisition system) and were converted into degrees Celsius as in our previous studies (28). The body temperature of each undisturbed rat was monitored continuously at 5-min intervals. After the rats were killed, the transmitters were recalibrated to verify calibration temperatures.

Data analyses. Results are expressed as means ± SE. Experiments showed that the intracerebroventricular infusion of heat-treated IL-1β increased body temperature (Fig. 1, A and B). Therefore, to obtain the net effect of IL-1β, we subtracted the body temperature changes induced by the heat-treated (inactive) IL-1β from those induced by the active IL-1β. This subtraction was done on an individual basis, that is, within a rat, for all time points before generation of the data in Fig. 2, A and B.

Statistical analysis compared the preinfusion level (mean values for 120-min period before infusion or baseline) to those obtained after infusion of test solutions for the period shown in Figs. 1 and 2. We also compared the young versus old immune response profiles. Data were analyzed using ANOVA, with treatment and body temperature changes as sources of variation, followed by post hoc tests for pairwise comparisons (Student-Newman-Keuls test). The Kruskal-Wallis test was applied (followed by post hoc tests) when data did not pass the normality (Kolmogorov-Smirnov) test. Differences were considered to be significant only for P < 0.05.

RESULTS

In the morning, the mean body temperatures of both groups of rats for the 2-h preinfusion period were similar (Fig. 1A): 37.46 ± 0.19°C for young rats and 37.45 ± 0.21°C for old rats receiving active IL-1β. In the evening, body temperatures were slightly higher than in the morning, but there was no difference between the two groups (Fig. 1B): 37.67 ± 0.16°C for young rats and 37.65 ± 0.10°C for old rats receiving IL-1β.

In the morning, body temperatures began to rise ~30 min preinfusion, which is when the door to the animal room was opened and preparations for the infusions were begun. For the first 2 h postinfusion, at both times of day, the body temperature responses to IL-1β of the old rats were indistinguishable from those of the young rats in both latency to peak fever and initial fever height (Fig. 1, A and B). After approximately the first 2 h, the body temperature of the young rats declined, whereas the body temperature of the old rats remained high for an additional 2 h. The old rats were also more responsive to the heat-inactivated IL-1β injections, from 120 min postinfusion at both times of day.

The way the data are presented in Fig. 1, A and B, suggests that the old rats were more responsive to the IL-1β infusion, because their body temperature remained significantly higher than that of the young rats from 120 to 240 min postinfusion. However, this is misleading, because old rats were also more responsive to the heat-inactivated IL-1β preparation. Therefore, the proper comparison may be that shown in Fig. 2, A and B. This figure shows the net changes in body

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1 We estimated that a dose of 4.0 ng IL-1β/rat administered intracerebroventricularly will be at the interface between the pathophysiological and supraphysiological range. The rat’s normal CSF volume is ~300–400 µl. We estimate that the concentration of IL-1β in the CSF, if not metabolized, will be 100–133.3 pg/10 µl after a dose of 4.0 ng IL-1β. Furthermore, considering the rat’s normal rate of CSF turnover and secretion to be ~0.7% of the total volume/min [based on a CSF production rate of 1.99 ± 0.16 µl/min (mean ± SD from 3 studies; see Ref. 26)], the concentration of IL-1β 120 and 240 min after 4.0 ng IL-1β administration would be ~43 and 18.5%, respectively, of the initial amount; that is, 43.2 and 18.8 pg/10 µl for a volume of 400 µl and 57.6 and 24.8 pg/10 µl for a volume of 300 µl at 120 and 240 min, respectively. In these calculations, C1 = 10S(1 - K)T/V, where C1 is the concentration after time T (min), 10 is 10 µl, S is the amount of test substance administered (here a 4.0 ng IL-1β), K is the volume of CSF exchanged every minute (here a constant 0.7% of the volume of CSF/min), V is the volume of CSF, and T is the time (min) elapsed after administration. Considering enzymatic IL-1β degradation and binding and uptake mechanisms, a smaller amount of IL-1β than that calculated might be bioavailable after intracerebroventricular administration. Therefore, the amount of IL-1β administered in the present study is in the pathophysiological-supraphysiological range observed in the CSF during infections of the CNS: for example, 40% of patients with bacterial meningitis exhibit >10 pg IL-1β/10 µl CSF, with some patients exhibiting >20–40 pg IL-1β/10 µl CSF (13).
temperature before and after infusions of active and inactive IL-1β in old and young rats in the morning and evening for 12 h postinfusion. Figure 2 makes it clear that there are no differences between young and old responses for the first 240 min.

In the morning, IL-1β increased body temperature above baseline in both young [H (with 1 degree of freedom) = 8.22, \( P = 0.004\)] and old [F (1,12) = 25.6, \( P = 0.0003\), power of performed test (ppt) = 1.0] rats from 80 to 240 min (Fig. 2A). The body temperature response was similar in latency to peak fever and maximal effect observed [F (1,16) = 0.0, \( P = 0.89\)]. After that, the body temperature of the young rats remained significantly above baseline for 540 min [H (with 1 degree of freedom) = 11.2, \( P = 0.0008\)] (Fig. 2A). The old rats exhibited a different profile: after the initial fever response, which lasted up to 240 min, their body temperature decreased below baseline from 260 to 600 min. This differential profile between old and young rats from 260 to 540 min was significant (\( P < 0.001\)).

For the evening infusions, the curves are very similar (Fig. 2B). From baseline, IL-1β induced a significant increase in body temperature during the 80- to 240-min period in both young [F (1,12) = 13.9, \( P < 0.003\), ppt = 0.93] and old [F (1,12) = 45.8, \( P < 0.0001\), ppt = 1.0] rats. The fever profile (including latency to peak fever) was similar in both groups [F (1,16) = 1.58, \( P = 0.23\)]. From 360 to 560 min after IL-1β administration, both young and old rats exhibited a similar tendency to a lower body temperature. However, this was significant only in the old rats (\( P < 0.03\) from 420 to 540 min relative to baseline).

Figure 3, A and B, illustrates that there is no difference between the fever time course profiles after morning or evening infusions for both young and old rats. Preinfusion body temperature is higher in the
evening in both groups. Nevertheless, postinfusion body temperatures in response to IL-1β were equivalent whether IL-1β was administered during the morning or just before nighttime.

**DISCUSSION**

These data demonstrate that old Long-Evans rats are not defective in their capacity to develop a fever in response to the intracerebroventricular administration of IL-1β. The IL-1β-induced fever profile was similar in latency to peak fever and maximal fever height in both old and young rats during the first 4 h postinfusion. The differences between the two groups started at ~260 min postinfusion after the morning IL-1β administration. The young rats maintained the increase in body temperature for 540 min after IL-1β administration, whereas the old rats exhibited a decrease relative to baseline from 260 min. This suggests that although young and old rats have similar responsiveness to brain IL-1β-induced pyrogenesis, their thermoregulatory modulation is not the same.

The mechanisms for the dissimilar time course profile shown in Fig. 2, A and B, are unknown. Young and old rats may have distinct clearance and/or uptake mechanisms for IL-1β or different levels of cytokine antagonists and endogenous antipyretic peptides. Indeed, in humans, concentrations of IL-1β receptor antagonist (an endogenous competitive inhibitor of IL-1β action) are elevated in healthy aged subjects relative to young ones (4, 23). Both old humans and old rodents exhibit higher activity of CNS antipyretic pathways (e.g., vasopressin). For instance, hypothalamic vasopressin mRNA and vasopressin production increase with age (5, 10) and IL-1β stimulates vasopressin release (12, 29, 30). Moreover, there are changes in the hypothalamic-pituitary-adrenal axis that may also contribute to differences between young and old rats. For example, in old rats, once a stress response is elicited, it takes longer to return to baseline (24), and data also suggest that there is a profound dysregulation of the hypothalamic-pituitary-adrenal axis in aging (9).
For both young and old rats, the absolute body temperatures reached were the same in the morning and in the evening, although the baseline body temperatures were lower in the morning. These data agree with earlier work showing similar responses to the central injection of prostaglandin E\(_2\) (7). Thus, after central injections of two pyrogenic compounds, the change in body temperature is controlled to achieve the same regulated fever height.

**Perspectives**

The present studies show that after central administration of IL-1\(\beta\), old rats develop an immediate fever response that is very similar to that of young rats. Therefore, the lack of fever or delayed onset of fever reported previously after peripheral administration of bacterial products or proinflammatory cytokines, including IL-1\(\beta\), in old rats is due to an inability of immunological challenges to activate the required sequence of events from the periphery rather than to an unresponsiveness of brain systems to stimulate the appropriate physiological heat-producing and heat-conserving mechanisms.

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