Cytokine-induced fever in obese (fa/fa) and lean (Fa/Fa) Zucker rats

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Plata-Salaman, Carlos R., Elizabeth Peloso, and Evelyn Satinoff. Cytokine-induced fever in obese (fa/fa) and lean (Fa/Fa) Zucker rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1353–R1357, 1998.—In earlier work, we reported that genetically obese (fa/fa) Zucker rats exhibited significantly greater anorexia than did lean (Fa/Fa) Zucker rats to intracerebroventricular infusion of interleukin (IL)-1β. Here, we investigated the fever response of obese (fa/fa) and lean (Fa/Fa) Zucker rats to intracerebroventricular microinfusion of IL-1β as well as to the following other cytokines: IL-2, IL-6, and tumor necrosis factor-α (TNF-α). Core body temperature was monitored by a radiotelemetry system in freely moving rats. The results show that 1) both IL-1β and IL-6 induce fever in obese and lean rats; 2) IL-1β induces a significantly higher fever response in obese rats than it does in lean rats; 3) IL-6 induces a significantly higher fever response in lean rats than it does in obese rats; 4) IL-2 induces a moderate fever response in lean but not obese rats; 5) TNF-α induces a similar fever response in obese and lean rats; and 6) the fevers induced by each effective cytokine have different time courses. Thus obese (fa/fa) and lean (Fa/Fa) Zucker rats show differential responsiveness to the intracerebroventricular microinfusion of various classes of cytokines. This suggests that genetic obesity in the fa/fa Zucker rat is associated with differential cytokine action on thermoregulatory mechanisms.

interleukin; tumor necrosis factor; intracerebroventricular; core temperature; nervous system; immune system

IMMUNOMODULATORY CYTOKINES affect many physiological and behavioral systems, including food intake and thermoregulation. Recently, we reported that genetically obese (fa/fa) Zucker rats exhibited significantly greater anorexia than did lean (Fa/Fa) Zucker rats to intracerebroventricular infusion of interleukin (IL)-1β (15). The same differential sensitivity to IL-1β was found in obese yellow mice (17).

Many cytokines also induce fever (1, 6, 9). In rodents, when the cytokines are administered intracerebroventricularly, nanogram doses are sufficient to induce fever. When they are administered peripherally, microgram doses are required to induce equivalent fevers. This suggests that the pyrogenic effect induced by low doses of cytokines administered intracerebroventricularly is due to their direct action in the central nervous system (CNS).

In the present paper, we examined whether the differential responsiveness to IL-1β (and other cytokines) between obese and lean Zucker rats that is evident in food intake also occurs during fever. Using a radiotelemetry system for continuous core body temperature monitoring, we report on the fever responses to the acute intracerebroventricular microinfusion of IL-1β, IL-2, IL-6, and tumor necrosis factor-α (TNF-α). These compounds represent four classes of cytokines that induce neurological manifestations differentially (13). The results show that obese and lean Zucker rats exhibit dissimilar fever responses to IL-1β and IL-6, whereas they respond in a like manner to TNF-α. IL-2 is moderately effective in producing fever responses only in lean rats.

MATERIALS AND METHODS

Subjects and maintenance. Male obese (fa/fa) and lean (Fa/Fa) Zucker rats (11 wk old at the beginning of the study) were purchased from the Animal Model Core, Department of Nutrition, University of California at Davis. They were housed individually and maintained ad libitum on semipurified powdered rat food (D 11714; Research Diets, New Brunswick, NJ) and tap water (15). Lights were on from 0700 to 1900, and room temperature was kept at 23 ± 1°C. All rats were handled daily. After several days of adaptation to their home cages, brain cannulas were implanted.

Implantation of brain cannulas. Under intraperitoneal anesthesia (100 mg/kg ketamine and 5 mg/kg xylazine), a 23-gauge guide cannula was implanted into the third ventricle at the following stereotaxic coordinates: −2.1 anteroposterior and 0.0 lateral with respect to the bregma and 7.5–8.0 dorsoventral from the brain surface, as in previous studies (15). An incision was made through the dura mater with a dorsal 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 into 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). Infusions were done at the same time between 1830 and 1900 (i.e., before lights out). All rats were infused with IL-1β, and a subgroup was infused with all four cytokines. The order of the infusions in this subgroup was IL-1β, TNF-α, IL-6, and IL-2. At least 1 wk separated each infusion. In a previous study (15), we found that the feeding responses of obese and lean Zucker rats were similar if the rats received the sequence TNF-α followed by IL-1β or vice versa. Those
data showed that the sequence for IL-1β and TNF-α does not alter the responsiveness of the animals to the subsequent treatment. We do not know if the same applies to IL-6 and IL-2 in the sequence used here. However, in each case, baseline body temperatures were not significantly different between obese and lean rats, and for all rats the circadian rhythms of body temperature were similarly consistent before each cytokine administration.

The groups of obese (fa/fa) and lean (Fa/Fa) Zucker rats received a control infusion of heat-treated cytokine, followed by infusion of intact cytokine. Cytokines used (R&D Systems, Minneapolis, MN) were as follows: recombinant human IL-1β (4.0 ng/rat), recombinant murine IL-2 (100 ng/rat), recombinant murine IL-6 (100 ng/rat), and recombinant murine TNF-α (100 ng/rat). Cytokine doses were selected based on our previous studies and were those that induced significant anorexia in Wistar (IL-1β, IL-2, IL-6, and TNF-α) and obese and lean Zucker rats (IL-1β and TNF-α; see Refs. 13, 15, 18). The same cytokine lot and stock solutions were used for all experiments. Cytokines were dissolved in sterile physiological saline (0.15 M NaCl) containing 2.0 µg/10 µl bovine serum albumin (BSA; J. R. H. Biosciences, Lenexa, KS; 2.0 µg/10 µl is equivalent to the concentration of albumin normally present in the CSF). BSA was added because of its properties as stabilizing agent and carrier protein for cytokines (15). Heat treatment and verification of cytokine inactivity were as in previous studies (18). Each test solution was administered in a 10-µl volume and had a pH of ~7. To avoid nonspecific adsorption of the compounds on the experimental tools, all such materials were siliconized. After an experiment was completed, rats were deeply anesthetized with CO2 and decapitated, and the position of the cannula tip in the third ventricle was verified.

Measurement of body temperature and activity. Body temperature and activity were 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 data were collected using the DataCol version 3 data acquisition system (20). The body temperature and activity of each undisturbed rat were monitored continuously at 5-min intervals. After death, the transmitters were recalibrated to verify calibration temperatures.

Data analyses. Results are expressed as means ± SE. The data presented correspond to the body temperature after subtracting values of heat-treated (inactive) cytokine infusion from active cytokine infusion for each rat. This presentation is used because initial experiments showed that the intracerebroventricular injection of each heat-treated cytokine increased body temperature. This effect was likely due to the psychological stress of the procedure, since the increase was similar after each heat-treated cytokine infusion in both obese and lean rats. Therefore, to obtain the net effect of a cytokine, we subtracted the body temperature changes induced by the heat-treated cytokine from those induced by the active cytokine. This subtraction was done on an individual basis, that is, within a rat, for all time points, before generation of the data in Figs. 1-4. The data obtained in this study suggest that graphing subtracted data between control and tests of interest provides a highly accurate determination of a net change in body temperature.

Statistical analysis compared the preinfusion level (values for the 60-min period before infusion or baseline) with those obtained after infusion of test solutions and obese versus lean profiles. Data were analyzed using analysis of variance 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. Probability was two-tailed.

RESULTS

Characteristics of body temperature and activity in obese and lean Zucker rats. The entrained patterns of body temperature in the two groups of male Zucker rats were very similar to those described previously for obese and lean female Zucker rats (10). Mean daily body temperature was lower in obese rats than in lean rats (37.13 ± 0.03°C, average of 5 days before any manipulation). The amplitude of the circadian rhythm was also lower (1.6 vs. 2.0 °C) for the same 5-day period. There were no differences in the daily mean activity between the two groups (16 for obese vs. 17 for lean).

Effects of intracerebroventricular administration of IL-1β on body temperature. IL-1β induced a significant increase in body temperature in both obese (H1 degree of freedom = 15.2, P < 0.0001) and lean (H1 = 7.5, P = 0.006) rats (Fig. 1). From baseline (average for 1 h before infusion), body temperature increased from 60 to 420 min in obese and from 60 to 300 min in lean rats (P < 0.01 for each hour). IL-1β-induced fever was significantly stronger in obese than in lean rats [F(1, 14) = 18.7, P < 0.0001, power of performed test (ppt) = 0.99]. The differential responsiveness of obese and lean rats to IL-1β action extended from 120 to 420 min. However, the latency to peak body temperature was shorter in the lean rats.

Effects of intracerebroventricular administration of IL-2 on body temperature. The difference between the curves of lean and obese rats was significant [F(1, 13) = 10.22, P = 0.006]. The differential responsiveness of obese and lean rats to IL-1β was the same for IL-2.
40) = 24.5, \( P < 0.0001 \). IL-2 induced a moderate but significant increase in body temperature in lean \( F(1, 25) = 21.6, \ P < 0.0001 \) but not obese \( F(1, 25) = 0.4, \ P = 0.5 \) rats (Fig. 2).

Effects of intracerebroventricular administration of IL-6 on body temperature. IL-6 increased body temperature above baseline in both obese (from 120 to 300 min, \( P < 0.05 \)) and lean (from 60 to 420 min, \( P = 0.0002 \)) rats (Fig. 3). The difference between the fever response in obese and lean rats was significant \( F(1, 40) = 50.6, \ P < 0.00001, \ ppt = 1.0 \), with lean rats being more responsive from 70 to 420 min \( P < 0.02 \) for each hour.

Effects of intracerebroventricular administration of TNF-\( \alpha \) on body temperature. TNF-\( \alpha \) induced a similar increase in body temperature in both obese and lean rats (Fig. 4). The difference in body temperature from baseline was significant from 70 to 230 min.

**DISCUSSION**

Our data demonstrate that obese (fa/fa) and lean (Fa/Fa) Zucker rats show differential thermoregulatory responsiveness to central infusions of four cytokines. Obese rats were much more responsive to IL-1\( \beta \) than were lean rats; the opposite was true for IL-6. Both groups responded similarly to TNF-\( \alpha \), whereas IL-2 caused moderate fever responses only in lean rats. Thus the data show that genetic obesity is associated with distinct responsiveness to cytokine-induced fever. However, dose responses of cytokine-induced fever will be needed to determine the profiles of this differential sensitivity between obese and lean Zucker rats.

Obese Zucker (fa/fa) rats exhibit greater responsiveness to IL-1\( \beta \) in several behavioral models. For example, obese rats show a significantly stronger anorexia in response to the central administration of IL-1\( \beta \) than do lean controls (15). Thus greater responsiveness to IL-1\( \beta \) action in obese rats includes fever and anorexia. The weaker fever response to IL-6 in obese rats suggests that genetic obesity is associated with differential cytokine action on thermoregulatory mechanisms. The results with IL-1\( \beta \) and TNF-\( \alpha \) are similar with respect to fever and anorexia, i.e., IL-1\( \beta \) produces higher fever and more anorexia in obese rats, and TNF-\( \alpha \) has the same effect on fever or food intake in obese and lean rats.

A previous study reported impaired effects of IL-1\( \beta \) on fever in obese Zucker rats (3). The main differences between that study and our own are the method of body temperature measurement (colonic vs. biotelemetry), the number of sampling points (three points vs. continuous monitoring), and the length of sampling (120 min vs. continuous for 24 h). Dascombe et al. (3) concluded that obese Zucker rats were insensitive to the central administration of IL-1\( \beta \), as their colonic temperature did not change significantly from the preinjection levels at 60, 90, and 120 min after IL-1\( \beta \) administration; lean Zucker rats, on the other hand, exhibited a significant increase in colonic temperature at the three time points. Our data (Fig. 1) show that obese rats do respond with fever to IL-1\( \beta \), but there is a longer latency to develop a fever relative to the lean animals.
Although the fever response in the lean rats peaked between 60 and 90 min, the fever response in the obese Zucker rat was just developing by 90 min. Also, the maximal fever height in obese Zucker rats occurred after 120 min. Thus both data sets [Dascombe et al. (3) and ours] may reach different conclusions based on the length of sampling. When considering the data within the initial 120 min [the latest sampling time by Dascombe et al. (3)], we find that both data sets are consistent: the lean rats showed a significant increase in body temperature at 60, 90, or 120 min, whereas the obese rats exhibited a fever response after 90 min.

We have estimated that a dose of 4.0 ng IL-1β/rat will be at the interface between the pathophysiological and supraphysiological range. If we consider the rat's normal CSF volume (~300–400 μl), it is estimated that the concentration of IL-1β in the CSF, if not metabolized, will be 100–133 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 per minute [based on a CSF production rate of 1.99 ± 0.16 μl/min (mean ± SD from 3 studies; see Ref. 18)], we find that the concentration of IL-1β after 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 and 19 pg/10 μl for a volume of 400 μl and 58 and 25 pg/10 μl for a volume of 300 μl at 120 and 240 min, respectively. In these calculations, C_τ = 10 S(1 - K)^T/V, where C_τ is the concentration (C) after time T (min), 10 is equal to 10 μl, S is the amount of test substance administered (here 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 the superscript T is the time (min) elapsed after administration. With the consideration of enzymatic cytokine degradation and binding and uptake mechanisms, a smaller amount of cytokine than that calculated might be bioavailable after the intracerebroventricular administration. Therefore, the amounts of IL-1β administered in the present study are in the pathophysiological-supraphysiological range observed in the CSF during infections of the CNS (8); 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.

The reasons for the distinct response profile between obese (fa/fa) and lean (fa/FA) rats to IL-1β and IL-6 are unknown. Obese and lean rats may have different binding, degrading capacity, clearance, and/or uptake mechanisms for different cytokines. The dissimilar profiles between IL-1β and IL-6 suggest different modes of action or activation of distinct target sites. An enhanced fever response to IL-1β in obese rats may involve a lower activity of CNS antipyretic pathways (e.g., vasopressin) in these animals. Secretion of vasopressin into the hypophysial-portal circulation of obese rats is 35% lower than that of lean rats (16). IL-1β stimulates vasopressin release (7, 21), and vasopressin inhibits IL-1β-induced fever (7). Obese Zucker rats also exhibit abnormal concentrations of hypothalamic neurotransmitters and neuropeptides (e.g., histamine, catecholamines, serotonin, neuropeptide Y; see Refs. 4, 5, 11, 12). IL-1β can modulate all of these endogenous substances (14), and, therefore, one or various alterations exhibited by obese Zucker rats may be associated with an enhanced responsiveness to IL-1β. Evidence also shows that excess adiposity is associated with impairment in host immunological defense mechanisms (19). Obese rodents, for instance, may present decreased immunocompetence and alterations in cell- and humoral-mediated immunities. These immunological alterations could also be associated with an enhanced responsiveness of the obese rodent to the exogenous administration of IL-1β.

In conclusion, the present studies support the hypothesis that genetic obesity in the fa/fa Zucker rat is associated with differential responsiveness to the intracerebroventricular administration of various cytokines and that these effects are centrally mediated.

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