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A new function of the leptin receptor: mediation of the recovery from lipopolysaccharide-induced hypothermia

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SPECIFIC AIMS

Studies of the involvement of the leptin receptor (LR) in lipopolysaccharide (LPS) -induced fever have produced contradictory results; the involvement of the LR in LPS-induced hypothermia has not been studied. By using obese *f/f* Koletsky rats (that lack the LR) and lean *F/?* Koletsky rats (that bear a fully functional LR), the present study attempted to determine the roles of the LR in LPS-induced fever and hypothermia.

PRINCIPAL FINDINGS

1. In LR-deficient *f/f* Koletsky rats, the fever response to LPS is unaffected whereas the hypothermic response is drastically prolonged

Koletsky *f/f* and *F/?* rats were preimplanted with telemetric probes (Mini Mitter, Bend, OR, USA) and jugular catheters, and their body temperature (T_b) responses to i.v. LPS (10 or 100 μg/kg) or saline were studied at a tightly controlled ambient temperature. At thermoneutrality (28°C), *F/?* rats responded to either dose of LPS with fever. The fever response to the lower dose (10 μg/kg) consisted of three consecutive T_b rises (febrile phases), whereas the response to the higher dose (100 μg/kg) lacked the first phase and had a blurred transition between the second and third phases. At either dose, the *f/f* rats responded to LPS similarly to *F/?* rats. Saline was thermally ineffective in both genotypes.

In a cool environment (22°C), the thermal response of the *F/?* rats to LPS was dose dependent: to the lower dose, they responded with small hypothermia followed by two consecutive febrile T_b rises; to the higher dose, they responded with pronounced hypothermia followed by a slowly developing fever (**Fig. 1**). The initial hypothermia was significantly prolonged in the *f/f* rats ($P<1.0\times10^{-4}$ for both doses of LPS); the effect was more dramatic at the higher dose. Saline produced no thermal effect in either genotype. Some *f/f* rats showed sporadic, sharp T_b rises in the course of their hypothermic response to LPS, thus demonstrating full competence of their heat production effectors.

2. The prolonged hypothermic response to LPS of Koletsky *f/f* rats is associated with enhanced inflammatory signaling to the brain

To determine whether enhanced inflammatory signaling to the brain is involved in the prolongation of LPS hypothermia in *f/f* rats, the presence of IκB-α (inhibitor of NF-κB) in the hypothalamus was assessed by Western blot. Tissue samples were collected from *F/?* and *f/f* rats injected with LPS (100 μg/kg, i.v.) or saline in a cool environment. At the time of tissue harvesting (120 min postinjection), the rate of divergence between the hypothermic responses of the *f/f* and *F/?* rats to LPS was maximal (maximum of the first derivative of the difference between the two T_b curves shown in the bottom panel of Fig. 1). IκB-α was detected as a single 41 kDa band (**Fig. 2*A***). Although this band was readily visible in all samples obtained from saline- or LPS-treated *F/?* rats and from saline-treated *f/f* rats, it was invisible or nearly invisible in all samples from LPS-treated *f/f* rats. This finding indicates that IκB-α was degraded (NF-κB signaling enhanced) during LPS hypothermia in the *f/f* rats. The housekeeping protein β-actin was detected as

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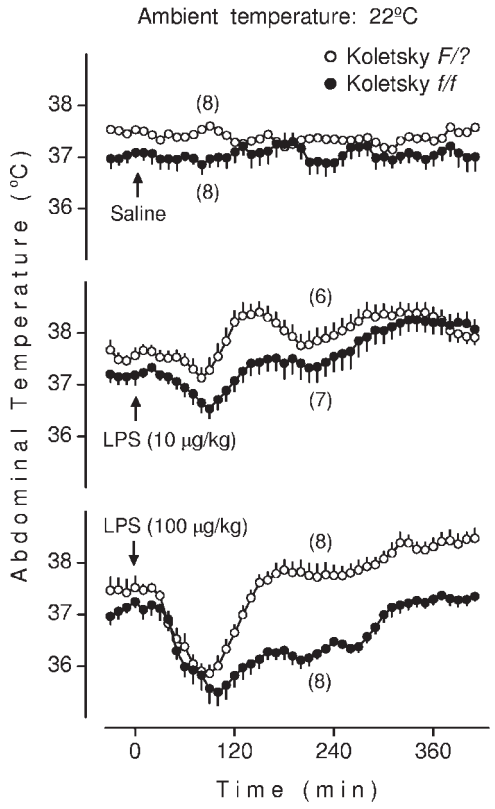

Figure 1. Thermal responses of Koletsky *f/f* (LR-deficient) and *F/?* (LR-bearing) rats to i.v. injection (arrow) of lipopolysaccharide (LPS, doses indicated) or saline in a cool environment. Here and in Fig. 2, the number of rats in each group is shown in parentheses.

an intense single, 42 kDa band; neither the genotype (*f/f* or *F/?*) nor treatment (LPS or saline) affected the expression of this protein.

3. Cytokine responses to LPS are differentially affected in Koletsky *f/f* rats

Levels of proinflammatory cytokines interleukin (IL) -1β, IL-6, and tumor necrosis factor (TNF)-α and of the anti-inflammatory cytokine IL-10 were measured by ELISA in the plasma of the *F/?* and *f/f* rats 120 min after administration of LPS (100 μg/kg, i.v.) or saline in a cool environment. Except for TNF-α, all cytokines were detectable in the plasma of saline-treated rats of either genotype (Fig. 2*B*). The *F/?* rats responded to LPS with a marked surge of TNF-α (424% over the 5 pg/mL detection limit, $P<8.1\times10^{-3}$) and sizable increases in the plasma levels of all other cytokines: the LPS-saline difference in the cytokine concentration was 319% ($P<1.1\times10^{-2}$) for IL-1β, 196% ($P<2.0\times10^{-2}$) for IL-6, and 215% ($P<4.6\times10^{-3}$) for IL-10. For IL-1β, IL-6, and IL-10, the LPS-saline differences observed in the *f/f* rats were similar in value and statistical significance to those seen in the *F/?* rats. However, the TNF-α response (a 1239% surge over the detection limit) was strongly exaggerated in *f/f* rats compared with *F/?* rats ($P<3.2\times10^{-3}$).

4. The prolonged hypothermic response to LPS of Koletsky *f/f* rats is associated with blockade of the glucocorticoid response

The hypothalamo-pituitary-adrenal (HPA) axis normally exerts an anti-inflammatory action. To determine whether an altered activity of the HPA axis contributed to the prolongation of LPS hypothermia in *f/f* rats, we measured plasma levels of corticosterone by ELISA and adrenocorticotropic hormone (ACTH) by radioimmunoassay. Blood samples of the LPS- or saline-treated *F/?* and *f/f* rats were collected at the same time point as other tissue samples (viz., the hypothalamic samples for assessing inflammatory signaling and blood samples for measuring cytokine responses). During LPS hypothermia, the *F/?* rats exhibited a strong activation of the HPA axis, as evident by significantly ($P<4.0\times10^{-2}$) higher levels of the pair corticosterone-ACTH in LPS-treated than in saline-treated rats (Fig. 2*C*). The *f/f* rats

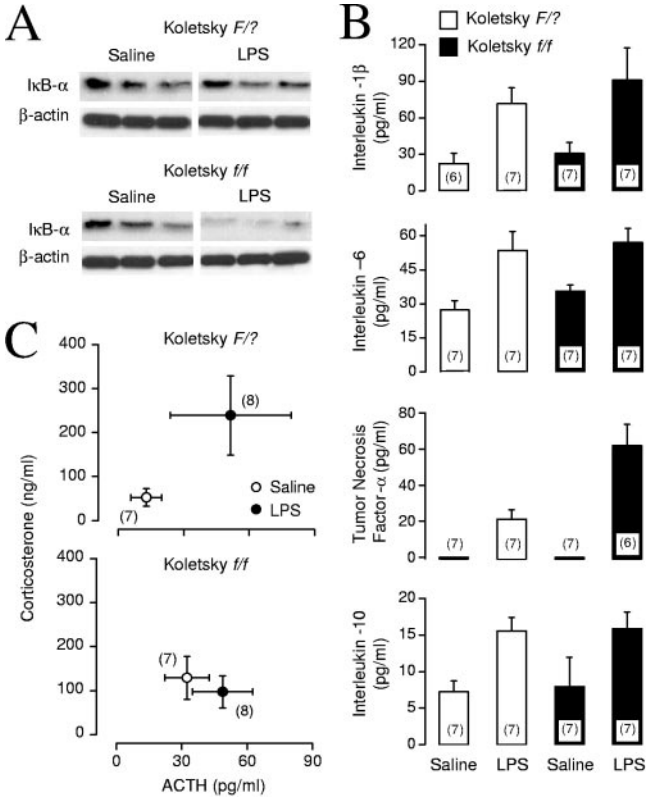

Figure 2. Effects of i.v. LPS (100 μg/kg) or saline in Koletsky *f/f* and *F/?* rats in a cool environment (22°C). *A)* The hypothalamic contents of IκB-α (a decrease in the content of IκB-α indicates NF-κB activation) and β-actin (used as a housekeeping protein). *B)* Plasma concentrations of proinflammatory (interleukin-1β, interleukin-6, and tumor necrosis factor-α) and anti-inflammatory (interleukin-10) cytokines. *C)* The relationship between plasma concentrations of corticosterone and ACTH. Samples of plasma and hypothalamic tissue were collected 120 min after administration of LPS or saline. This time point corresponds to the maximal rate of divergence between the hypothermic responses of the *f/f* and *F/?* rats to LPS. Note that the level of tumor necrosis factor-α in all saline-treated rats (*B*) is below the detection limit (5 pg/mL).

tended ($P<2.2\times10^{-1}$) to have elevated basal corticosterone-ACTH levels. Remarkably, the *f/f* rats did not respond to LPS with activation of the HPA axis.

5. TNF-α-induced hypothermia is drastically prolonged in Koletsky *f/f* rats

We also tested whether the mechanisms of LPS hypothermia downstream of TNF-α production were affected in *f/f* rats. The thermoregulatory responses of the *F/?* and *f/f* rats to recombinant rat TNF-α (80 μg/kg, i.v.) in a cool environment were compared. As expected, the *F/?* rats responded to TNF-α with a mild hypothermia followed by fever. The hypothermic response to TNF-α was drastically ($P<1.0\times10^{-4}$) prolonged in the *f/f* rats and no fever was observed.

CONCLUSIONS AND SIGNIFICANCE

The present study identifies a new role of the leptin-LR system: mediation of the recovery from LPS hypothermia. LR-dependent mechanisms of the recovery from LPS hypothermia include activation of the HPA axis, which has a pleiotropic anti-inflammatory action. Via this mechanism (and possibly additional ones), the leptin-LR system counteracts LPS hypothermia at several levels: production of TNF-α, hypothermic action of TNF-α, and inflammatory (via NF-κB) signaling in the hypothalamus. These findings are summarized in **Fig. 3**.

The present study also shows that Koletsky *f/f* rats respond to LPS with normal fevers, which agrees with our earlier observation that the febrile response to LPS in a thermoneutral environment is preserved in *fatty* Zucker rats bearing a dysfunctional LR. Taken together, these findings provide strong evidence against the popular view that the leptin-LR system is essential for the fever response.

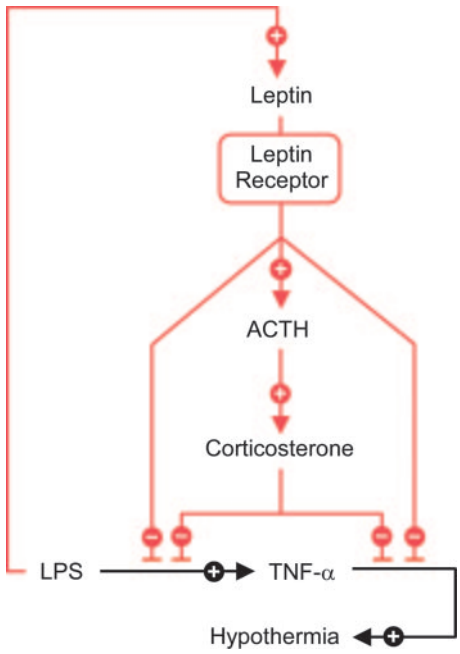

Figure 3. Proposed LR-mediated mechanisms (red) of the recovery from LPS-induced hypothermia. (+) activation; (–) inhibition.

That the LR is involved in LPS-induced hypothermia, but not fever, shows that this receptor is less important in mild and more important in severe systemic inflammation. In both laboratory and clinical settings, less severe forms of systemic inflammation are accompanied by fever, whereas the most severe forms, those associated with high mortality rates (e.g., septic shock), are accompanied by hypothermia. In fact, severe sepsis is a condition in which the leptin-LR system has been proposed to play a protective role, and several observations in humans and laboratory animals suggest that the leptin-LR system should be studied as a potential target in the therapy of severe systemic inflammation. FJ