The FASEB Journal express article 10.1096/fj.04-2295fje. Published online September 23, 2004.

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

Alexandre A. Steiner,* M. Devrim Dogan,* Andrei I. Ivanov,[†] Shreya Patel,* Alla Y. Rudaya,* David H. Jennings,[‡] Miles Orchinik,[‡] Thaddeus W. W. Pace,[§] Kevin A. O'Connor,[§] Linda R. Watkins,[§] and Andrej A. Romanovsky*

*Systemic Inflammation Laboratory, Trauma Research, St. Joseph's Hospital and Medical Center, Phoenix, Arizona 85013; [†]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia 30322; [‡]School of Life Sciences, Arizona State University, Tempe, Arizona 85287; and [§]Department of Psychology, University of Colorado, Boulder, Colorado 80309

Corresponding author: Andrej A. Romanovsky, Systemic Inflammation Laboratory, Trauma Research, St. Joseph's Hospital and Medical Center, 350 W. Thomas Rd., Phoenix, AZ 85013. E-mail: aromano@chw.edu

ABSTRACT

Obese (*f/f*) Koletsky rats lack the leptin receptor (LR), whereas their lean (*F/?*) counterparts bear a fully functional LR. By using *f/f* and *F/?* rats, we studied whether the LR is involved in lipopolysaccharide (LPS)-induced fever and hypothermia. The body temperature responses to LPS (10 or 100 μg/kg iv) were measured in Koletsky rats exposed to a thermoneutral (28°C) or cool (22°C) environment. Rats of both genotypes responded to LPS with fever at 28°C and with dose-dependent hypothermia at 22°C. The fever responses of the *f/f* and *F/?* rats were identical. The hypothermic response of the *f/f* rats was markedly prolonged compared with that of the *F/?* rats. The prolonged hypothermic response to LPS in the *f/f* rats was accompanied by enhanced NF-κB signaling in the hypothalamus and an exaggerated rise in the plasma concentration of tumor necrosis factor (TNF)-α. The *f/f* rats did not respond to LPS with an increase in the plasma concentration of corticosterone or adrenocorticotropic hormone, whereas their *F/?* counterparts did. The hypothermic response to TNF-α (80 μg/kg iv) was markedly prolonged in the *f/f* rats. These data show that the LR is essential for the recovery from LPS hypothermia. LR-dependent mechanisms of the recovery from LPS hypothermia include activation of the anti-inflammatory hypothalamo-pituitary-adrenal axis, inhibition of both the production and hypothermic action of TNF-α, and suppression of inflammatory (via NF-κB) signaling in the hypothalamus.

Key words: fever • body temperature • systemic inflammation • cytokines • tumor necrosis factor • corticosterone • Koletsky rats

The systemic inflammatory response to bacterial lipopolysaccharide (LPS) is accompanied by either fever or hypothermia, depending on the LPS dose and thermal environment. At thermoneutrality, LPS typically causes fever; in a cool environment, it evokes fever at

low doses, mild hypothermia followed by fever at intermediary doses, and pronounced hypothermia at high, shock-inducing doses (1). There is strong, although circumstantial, evidence that mechanisms of these thermoregulatory responses to LPS involve the leptin receptor (LR) and its endogenous agonist leptin. First, LPS and proinflammatory cytokines increase the expression of the leptin gene and the concentration of leptin in the blood (2–4). Second, activation of the LR by recombinant leptin has multiple effects on the network of pro- and anti-inflammatory cytokines (5–8) and thermoregulation. Administration of leptin activates thermogenesis (9–11) and, at least according to one study (12), produces fever. However, the potential involvement of the LR in LPS hypothermia has never been addressed experimentally, and studies of its involvement in fever (conducted in Zucker rats with *fatty* mutation of the LR) have produced contradictory results (for references and detailed analysis, see ref 13). To further complicate the issue, the LR of *fatty* mutants has been demonstrated to signal normally in some experimental paradigms (14–16).

Unlike the missense *fatty* mutation of Zucker rats, *f* mutation of Koletsky rats is a nonsense point mutation. When homozygous (*f/f*), this mutation results in the obese phenotype with complete absence of the LR (17, 18). If at least one allele of the LR gene is not mutated (*F/?*), a fully functional LR is expressed, which results in the lean phenotype (17, 18). By using *f/f* and *F/?* Koletsky rats, the present study examined the role of the LR in LPS hypothermia and re-evaluated its role in LPS fever.

METHODS

Animals

The experiments were conducted in 110 male Koletsky rats of both *f/f* and *F/?* genotypes (Charles River Genetic Models, Indianapolis, IN). At the time of the experiments, the rats were 9 wk old; the obese (*f/f*) rats weighed 400-420 g; the lean (*F/?*) rats weighed 300-320 g. The rats were caged in a rack equipped with a Smart Bio-Pack ventilation system (model SB4100) and Thermo-Pak temperature control system (model TP2000; Allentown Caging Equipment, Allentown, NJ); the temperature of the incoming air was maintained at 28°C. Standard rat chow (Teklad Rodent Diet “W” 8604; Harlan Teklad, Madison, WI) and tap water were available ad libitum. The room was on a 12:12 h light-dark cycle (lights on at 7:00 AM). The experiments were started between 8:00 and 9:00 AM. The protocols were approved by the St. Joseph’s Hospital Animal Care and Use Committee.

Surgery

Two surgical procedures were performed: venous catheterization and implantation of a temperature transmitter in the peritoneal cavity. These procedures were performed while the animals were under ketamine-xylazine-acepromazine (55.6, 5.5, and 1.1 mg/kg, respectively, ip) anesthesia and antibiotic (enrofloxacin, 1.12 mg/kg sc) protection. A silicon catheter was passed into the superior vena cava through the jugular vein and exteriorized at the nape. A temperature transmitter (series 4000 E-Mitter; Mini Mitter, Bend, OR) was implanted via a midline laparotomy and fixed to the abdominal wall with sutures. On *days 1* and *3* postsurgery, the venous catheter was flushed with heparinized (50 U/ml) saline. The experiments were performed on *day 5*.

Experimental setup and thermometry

The abdominal temperature, a measure of deep body temperature (T_b), was recorded by telemetry (Mini Mitter). Telemetry receivers (model ER-4000) were positioned inside a climatic chamber (Forma Scientific, Marietta, OH) and connected to a computer. Cages with the rats were placed on top of the receivers. The chamber was set to either 22.0°C (cool environment) or 28.0°C (thermoneutral environment for rats under similar caging conditions; ref 19). The venous catheter of each rat was extended with a length of PE-50 tubing filled with saline, and the extension was passed through a wall port and connected to a syringe filled with the drug of interest. This setup permitted injection of drugs from outside the chamber without disturbing the rats.

Tissue harvesting

Each rat was anesthetized with ketamine-xylazine-acepromazine (5.56, 0.55, and 0.11 mg/kg, respectively, iv). Within 2 min from injection of the anesthetizing cocktail, arterial blood (6 ml) was collected by cardiac puncture and transferred to heparin- and EDTA-containing Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ). The tubes were centrifuged (3,000 *g*, 10 min, 4°C), and the plasma was transferred to cryogenic vials and stored at -80°C. Immediately after blood collection, the rat was perfused through the left ventricle (right atrium cut) with 100 ml of saline. The brain was removed, and the entire hypothalamus was dissected, frozen under liquid nitrogen, and stored at -80°C.

Immunoassays

Interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and IL-10 were measured in heparin plasma using ELISA kits (R&D Systems, Minneapolis, MN). Adrenocorticotropic hormone (ACTH) was measured in EDTA plasma by radioimmunoassay (20) using the Rb7 primary antibody (gift of Dr. W. C. Engeland, University of Minnesota, Minneapolis, MN). Corticosterone was assayed in heparin plasma by ELISA (Assay Designs, Ann Arbor, MI). In each assay, all samples were run simultaneously, in duplicate. The detection limits of the assays were as follows: ~5 pg/ml for IL-1β, TNF-α, and IL-10; 14 pg/ml for IL-6; 10 pg/ml for ACTH; and 27 pg/ml for corticosterone.

Western blot

The activity of the transcription factor NF-κB in hypothalamic samples was determined by the presence of its inhibitor IκB-α (β-actin was assayed as a "housekeeping" protein). A reduced content of IκB-α is associated with NF-κB activation (21). The samples were homogenized in a lysis buffer (0.25 M Tris-HCl, pH 6.8, 2% SDS, 25% glycerol) containing protease inhibitors: PMSF (1 mmol/l), aprotinin (50 μg/ml), leupeptin (100 μmol/l), pepstatin (2 μmol/l), NaF (30 mmol/l), and EDTA (5 mmol/l). The homogenate was cleared by centrifugation (14,000 *g*, 10 min, 6°C), and the supernatant was subjected to electrophoresis in SDS-polyacrylamide gel followed by a transfer to a nitrocellulose membrane (Bio-Rad, Hercules, CA). The membrane was treated with 5% milk in Tris-buffered saline (pH 8.0) containing 0.1% Tween 20. It was incubated with a rabbit polyclonal anti-IκB-α antibody (1:1,000; Cell Signaling, Beverly, MA) or anti-β-actin antibody (1:1,000; Sigma-Aldrich, St. Louis, MO) overnight at 4°C and then with

a horseradish peroxidase-conjugated secondary antibody (anti-rabbit IgG, 1:10,000; Jackson Immunoresearch, West Grove, PA) for 30 min at room temperature. The blot was developed using enhanced chemoluminescence reagent (Amersham, Buckinghamshire, UK) and X-ray film (Hyperfilm ECL; Amersham).

Drugs

LPS (*Escherichia coli* 0111:B4) was purchased from Sigma-Aldrich. Recombinant rat TNF-α was purchased from Biocarta (San Diego, CA). According to the manufacturer, the endotoxin level in the TNF-α preparation was <0.1 ng/μg. Hence, the dose of endotoxin contaminant injected with the dose of TNF-α used (80 μg/kg iv) was <8 ng/kg, at least two orders of magnitude lower than the minimal pyrogenic dose in the rat (22).

Data analyses

The T_b responses were compared across genotypes (*f/f vs. F/?*), treatments (LPS or TNF-α vs. saline), and time points by a three-way ANOVA. The cytokine levels were compared across genotypes and treatments by a two-way ANOVA. The pairs ACTH level-corticosterone level were compared across genotypes and treatments by a two-way ANOVA for repeated measures. The statistical analyses were performed using Statistica AX'99 (StatSoft, Tulsa, OK). The results are reported as means ± SE.

RESULTS

LPS-induced fever is unaffected in LR-deficient *f/f* Koletsky rats

At thermoneutrality, the *F/?* rats responded to LPS with fever (Fig. 1). As seen in other rat strains, the fever response to the lower dose (10 μg/kg) consisted of three consecutive T_b rises (febrile phases; ref 23), 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 (1, 22). At either dose, the *f/f* rats responded to LPS similarly to the *F/?* rats. The basal T_b of the *f/f* rats did not differ from that of the *F/?* rats. In either genotype, saline produced no thermal effect.

LPS-induced hypothermia is drastically prolonged in Koletsky *f/f* rats

In a cool environment, 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. 2). The initial hypothermia was significantly ($P<1.0\times10^{-4}$, for both doses of LPS) prolonged in the *f/f* rats; the effect was more dramatic at the higher dose. The basal T_b of the *f/f* rats was ~0.5°C lower than that of the *F/?* rats ($P<1.6\times10^{-2}$). In either genotype, saline produced no thermal effect. Importantly, some *f/f* rats showed sporadic, sharp T_b rises in the course of their hypothermic response to LPS (Fig. 3), thus demonstrating full competence of their heat-production effectors.

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. Tissue samples were collected from *F/?* and *f/f* rats that had been injected with LPS (100 μg/kg iv) 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 Fig. 2, bottom panel). IκB-α was detected as a single 41 kDa band (Fig. 4*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 an intense single, 42 kDa band; neither the genotype (*f/f* or *F/?*) nor the treatment (LPS or saline) affected the expression of this protein.

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

The levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α and of the anti-inflammatory cytokine IL-10 were measured in the plasma of the *F/?* and *f/f* rats at 120 min after administration of LPS (100 μg/kg iv) 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. 4*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 the *f/f* rats compared with the *F/?* rats ($P<3.2\times10^{-3}$).

Prolonged hypothermic response to LPS of Koletsky *f/f* rats is associated with blockade of the glucocorticoid response

Activation of the hypothalamo-pituitary-adrenal (HPA) axis normally exerts an anti-inflammatory action (24). To determine whether an altered activity of the HPA axis might have contributed to the prolongation of LPS hypothermia in *f/f* rats, we measured the plasma levels of corticosterone and ACTH. Blood samples of the LPS- or saline-treated *F/?* and *f/f* rats were collected at the same time point as other tissue samples in the present study (i.e., 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 saline-treated rats (Fig. 4*C*). Similar to *fatty* Zucker rats (25), the *f/f* Koletsky rats tended ($P<2.2\times10^{-1}$) to have elevated basal ACTH-corticosterone levels. Remarkably, though, the *f/f* rats did not respond to LPS with activation of the HPA axis.

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 the *f/f* rats. The thermoregulatory responses of the *F/?* and *f/f* rats to TNF-α (80 μg/kg iv) in a cool environment were compared. As expected (26), the *F/?* rats responded to TNF-α with a mild hypothermia followed by fever (Fig. 5). The hypothermic response to TNF-α was drastically ($P<1.0\times10^{-4}$) prolonged in the *f/f* rats, and no fever was observed.

DISCUSSION

The major finding of the present study is that LR-deficient *f/f* Koletsky rats respond to LPS in a cool environment with markedly prolonged hypothermia. Because the basal T_b of the *f/f* rats in a cool environment was slightly lower than that of their *F/?* counterparts, it is tempting to explain the prolongation of the hypothermic response of the *f/f* rats to LPS as being due to impaired thermogenic ability. Such an explanation, however, does not agree with the data obtained. Indeed, we found that the *f/f* rats often exhibited short-lived, sharp T_b rises in the course of LPS hypothermia (Fig. 3). Because these T_b rises were as sharp as (or even sharper than) those in the *F/?* rats, and because T_b during some of these rises easily reached its normal, pre-LPS level, we conclude that thermogenic reserves of the *f/f* rats were not exhausted. Importantly, the results described above were obtained using young adult (9 wk) Koletsky rats. These results are consistent with previous studies showing that young adult (7-10 wk) *fatty* Zucker rats are capable of mounting vivid thermogenic responses to a variety of stimuli (e.g., severe cold exposure or norepinephrine administration) but, nonetheless, have a lowered basal T_b in a cool environment (27–31). Atrophy of the brown adipose tissue and severely impaired thermogenic responses occur only in older (>10 wk) *fatty* Zucker rats (32). It is, therefore, plausible to propose that the mechanisms of the drastic prolongation of LPS hypothermia in *f/f* rats lay upstream of the formation of thermoeffector drive. Several potential upstream mechanisms were assessed in the present study.

During LPS hypothermia, NF-κB signaling was enhanced in the hypothalamus of the *f/f* rats compared with the *F/?* rats. Potent activators of NF-κB (that may be enhanced in the absence of the LR) are the proinflammatory cytokines IL-1, IL-6, and TNF-α (21). These cytokines activate the NF-κB pathway in several tissues, including the brain (33), and many biological activities of these cytokines, including the effects on their own production, are mediated by this signaling pathway (34). It can be, therefore, expected that at least some cytokine responses to LPS are enhanced in *f/f* rats.

Indeed, the LPS-induced rise in plasma TNF-α was greatly enhanced during the prolonged hypothermic responses of the *f/f* rats. An exaggerated TNF-α response to LPS has also been reported to occur during fasting in mice (7). Such an exaggerated response is accompanied by a decrease in the plasma level of leptin and is reversed by administration of leptin (7). Because TNF-α plays a major role in the maintenance of hypothermia associated with systemic inflammation (35–37), the enhanced TNF-α response to LPS of *f/f* rats likely accounts for their prolonged hypothermic response. It has been shown that administration of TNF-α substantially increases the duration (but not the magnitude) of LPS hypothermia in mice (35), whereas neutralization of TNF-α by its soluble receptor antagonist (TNF-ra) reduces the duration (but not

the magnitude) of LPS hypothermia in rats (36). It has also been reported that the duration of the hypothermic response to cecal ligation and puncture (an experimental model of sepsis) is considerably shortened in transgenic mice lacking the two forms (p55 and p75) of the TNF-α receptor (37).

The biological effect of a cytokine depends not only on its plasma level but also on the responsiveness of tissues to its action. The responsiveness to TNF-α is affected by the absence of the LR. For example, Takahashi et al. (38) have shown that TNF-α-induced mortality is exaggerated in mice lacking the long form of the LR (or its ligand, leptin). Therefore, it was important to determine the sensitivity of the *f/f* rats to the hypothermic action of TNF-α. We have found that *f/f* rats respond to a moderate dose of TNF-α with a substantially longer hypothermia than *F/?* rats.

We have also assessed the activity of the HPA axis by measuring the plasma levels of ACTH and corticosterone. Like *fatty* Zucker rats bearing a dysfunctional LR (25) and *db/db* mice lacking the long isoform of the LR (39), the *f/f* rats lacking the LR tended to have elevated basal levels of ACTH and corticosterone. The mechanisms of such an abnormality are not completely understood but may include interruption of leptin signaling at several levels of the HPA axis, including the hypothalamus (40, 41) and adrenals (42). Despite the elevated basal levels of ACTH and corticosterone, the *f/f* rats failed to develop activation of the HPA axis during LPS hypothermia. The revealed irresponsiveness of the HPA axis to LPS in LR-deficient *f/f* rats agrees with the fact that LPS does not activate the HPA axis in leptin-deficient *ob/ob* mice (39) and that corticosterone responses of wild-type mice or rats to LPS (7, 43) and of wild-type mice to IL-1β (44) are enhanced by leptin. The leptin-LR system is involved in the release of corticotropin-releasing factor (a major activator of ACTH release) by hypothalamic neurons (45). Indeed, several agents that normally cause the release of corticotropin-releasing factor with the consequent activation of the HPA axis do not produce such effects when administered to *fatty* Zucker rats (46, 47).

Because activation of the HPA axis has a pleiotropic anti-inflammatory action, the lack of such activation in *f/f* rats may contribute to the prolongation of their hypothermic response to LPS via several mechanisms, including an enhancement of the plasma TNF-α response, increase of tissue responsiveness to TNF-α, and exaggeration of inflammatory (e.g., via NF-κB) signaling in the hypothalamus. It has been shown that administration of endogenous (e.g., corticosterone) and exogenous (e.g., dexamethasone) glucocorticoids suppresses LPS-induced surge in plasma TNF-α, whereas adrenalectomy or hypophysectomy exaggerates this response (48, 49). In addition to this action upstream of TNF-α production, glucocorticoids have been shown to suppress inflammation by acting downstream of TNF-α production, as is evident from their ability to inhibit TNF-α-induced cytotoxicity and mortality (48, 50). Glucocorticoids are also known to prevent LPS-induced activation of NF-κB signaling in the brain (51) and suppress hypothermia associated with the systemic inflammatory response to an anti-CD3 antibody (52).

Whereas the hypothermic response to LPS in a cool environment is drastically prolonged in *f/f* rats, their fever response to LPS at thermoneutrality is completely normal. This finding agrees with the fact that the febrile response of *fatty* Zucker rats to LPS is normal in a neutral environment (13). Hence, although exogenous leptin has been shown to increase T_b in one (12),

but not in another (53), study, the LR and its endogenous ligand(s) are likely to mediate a T_b rise not during LPS fever but during the recovery from LPS hypothermia. It can also be speculated that the attenuated fevers of *fatty* Zucker rats in a cool environment reported by Dascombe et al. (54) and others (reviewed in ref 13) were due to a prolongation of an obvious or a hidden hypothermic component of the overall thermoregulatory response to LPS and the cytokines used.

Our finding 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. Both in the laboratory and clinical setting, less severe forms of systemic inflammation are accompanied by fever, whereas the most severe forms, those that are associated with high mortality rates (e.g., septic shock), are accompanied by hypothermia (55, 56). In fact, severe sepsis is a condition in which the leptin-LR system has been proposed to play a protective role. The plasma level of leptin is substantially increased in survivors of severe sepsis and septic shock (57, 58). Obese patients are frequently leptin resistant (59) and more likely to develop severe sepsis (60). LPS-induced mortality is increased in leptin-deficient mice, whereas treatment with leptin affords protection against lethal doses of LPS (39). All these data suggest that the leptin-LR system should be studied as a potential target in the therapy of severe systemic inflammation.

CONCLUSIONS

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.

ACKNOWLEDGMENTS

We thank M. Milovancevic for technical assistance. The study was funded in part by a National Institute of Neurological Disorders and Stroke Grant NS-41233 (A. A. Romanovsky). M. D. Dogan's present address: Omega-Contract Research Organization, Ankara, Turkey 06700. A. Y. Rudaya's permanent address: Institute of Physiology, National Academy of Sciences, Minsk, Belarus 220072.

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Received May 25, 2004; accepted July 30, 2004.

Fig. 1

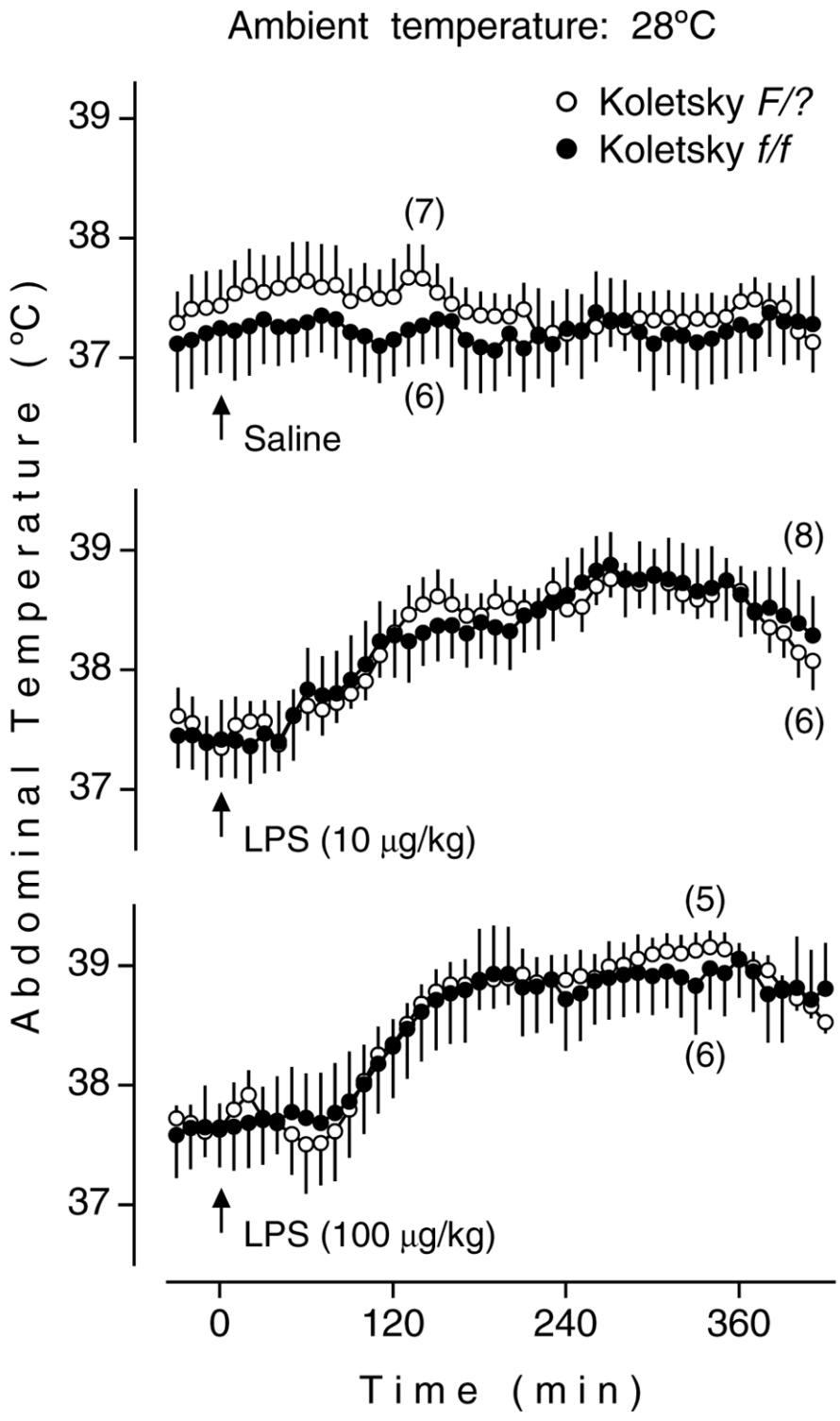

Figure 1. Thermal responses of Koletsky *f/f* (LR-deficient) and *F/?* (LR-bearing) rats to intravenous injection (arrow) of lipopolysaccharide (LPS, doses indicated) or saline at thermoneutrality. Here and in **Figs. 2**, **4**, and **5**, the number of rats in each group is shown in parentheses.

Fig. 2

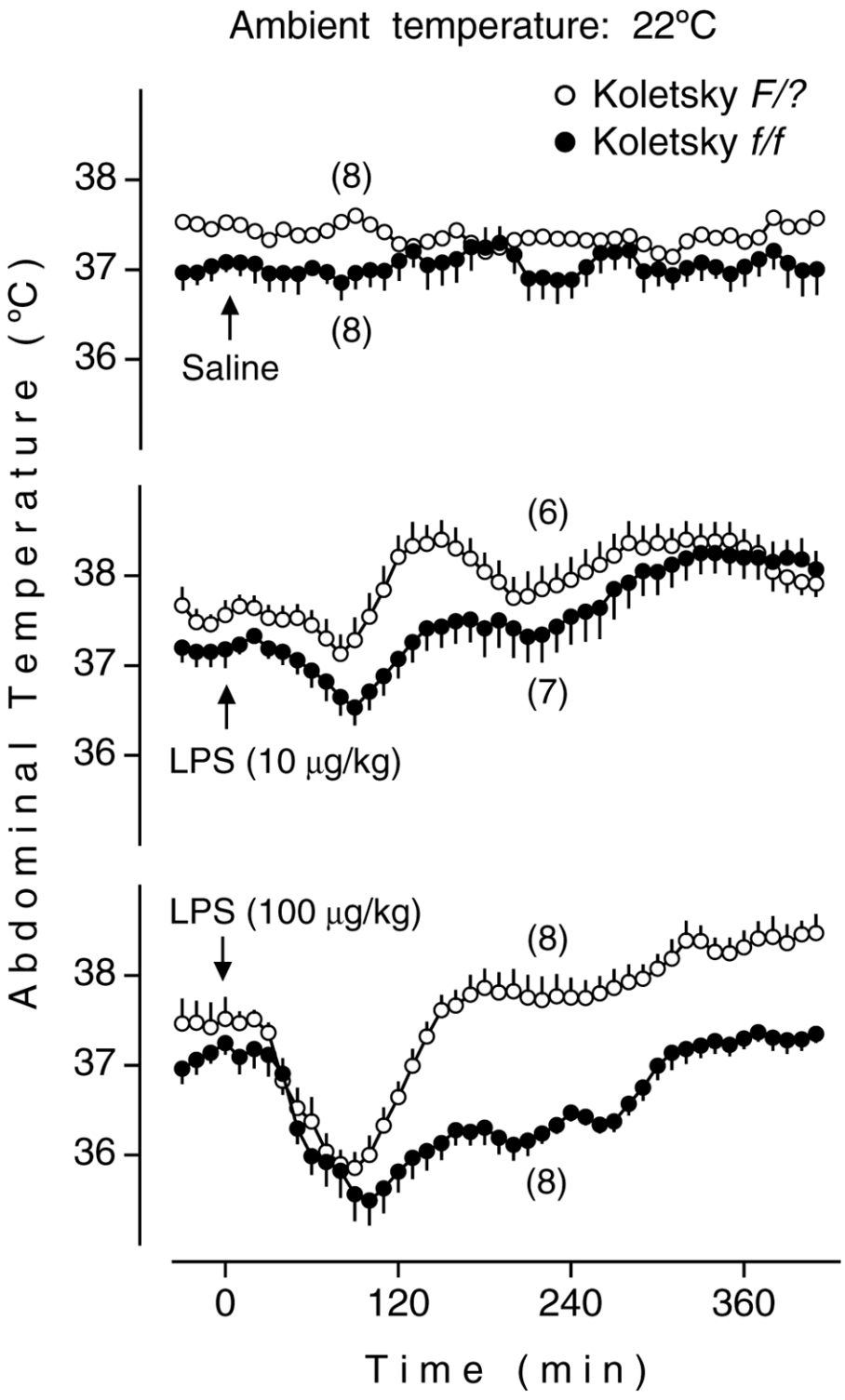

Figure 2. Thermal responses of Koletsky *f/f* and *F/?* rats to intravenous injection (arrow) of LPS (doses indicated) or saline in a cool environment.

Fig. 3

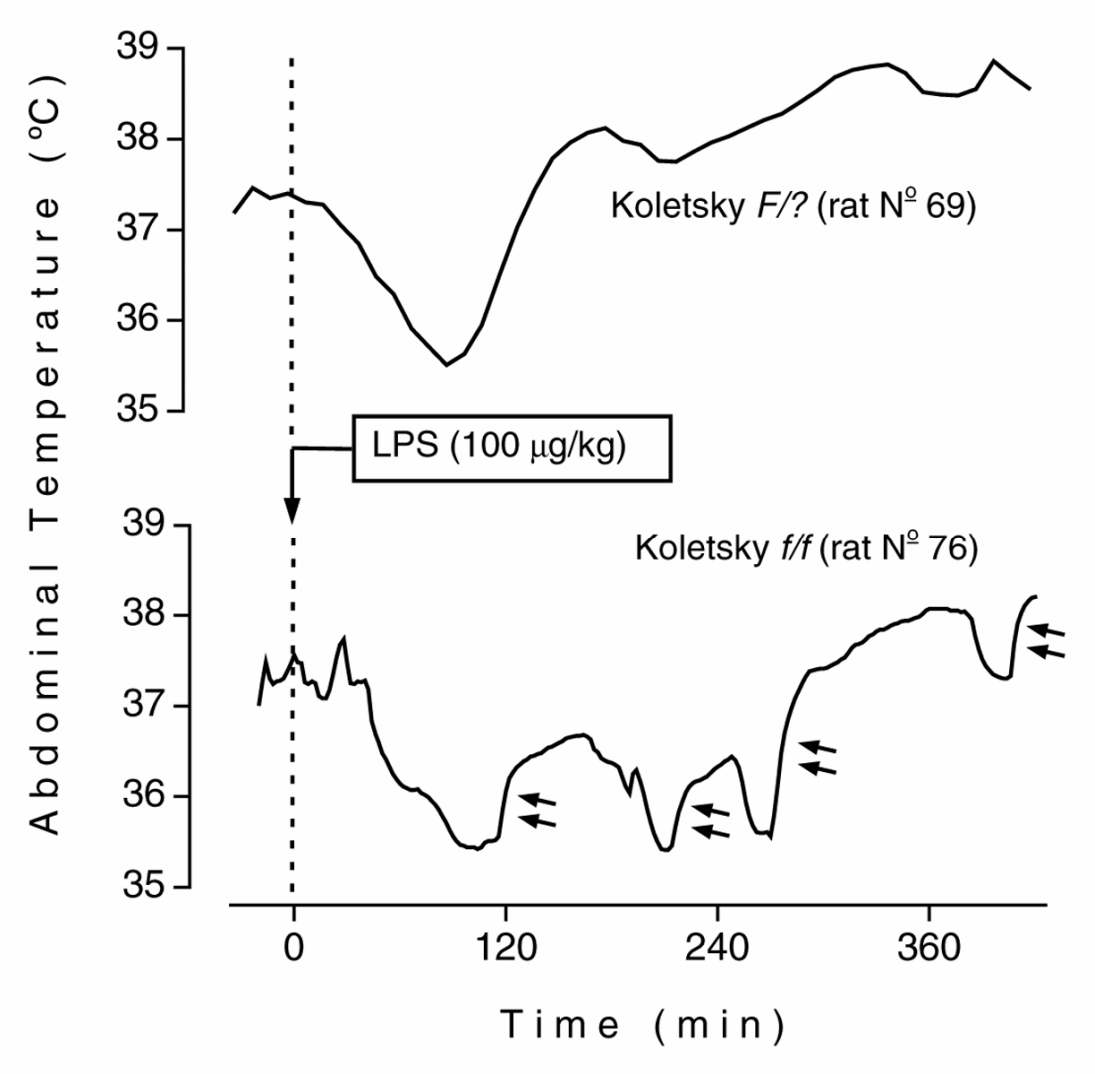

Figure 3. Representative thermal responses of individual Koletsky *f/f* and *F/?* rats to intravenous injection (arrow) of LPS (dose indicated) in a cool environment. Doubled arrows show sporadic, sharp rises in abdominal temperature of the *f/f* rat. For mean curves, see **Fig. 2**.

Fig. 4

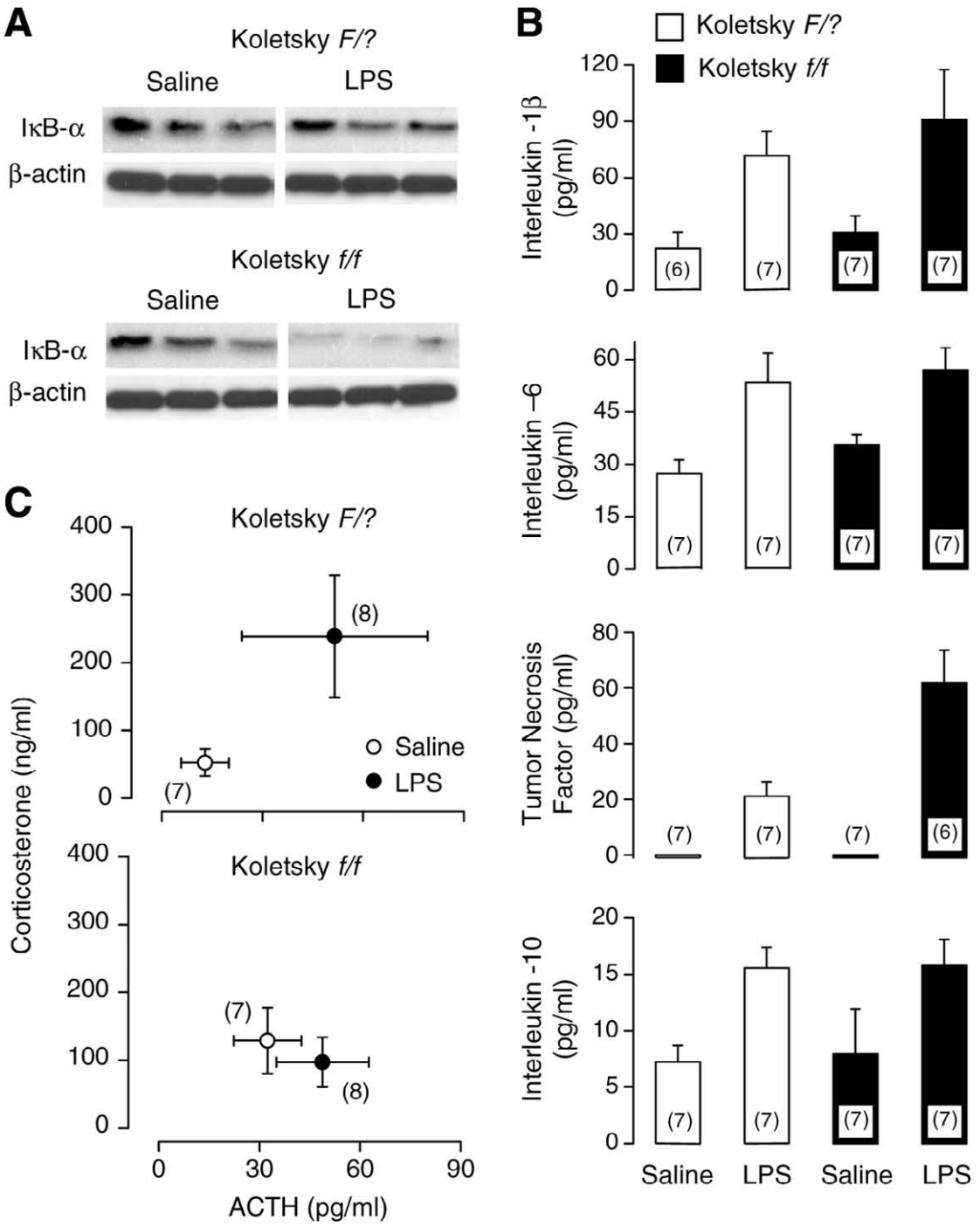

Figure 4. Effects of intravenous LPS (100 μg/kg) or saline in Koletsky *f/f* and *F/?* rats in a cool environment (22°C). ***A***) the hypothalamic contents of IκB-α (a decrease in the content of IκB-α indicates NF-κB activation) and β-actin (used as a "housekeeping" protein). ***B***) plasma concentrations of pro-inflammatory (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 maximal rate of divergence between the hypothermic responses of *f/f* and *F/?* rats to LPS (see **Fig. 2**). Note that level of tumor necrosis factor-α in all saline-treated rats (***B***) is below detection limit (5 pg/ml).

Fig. 5

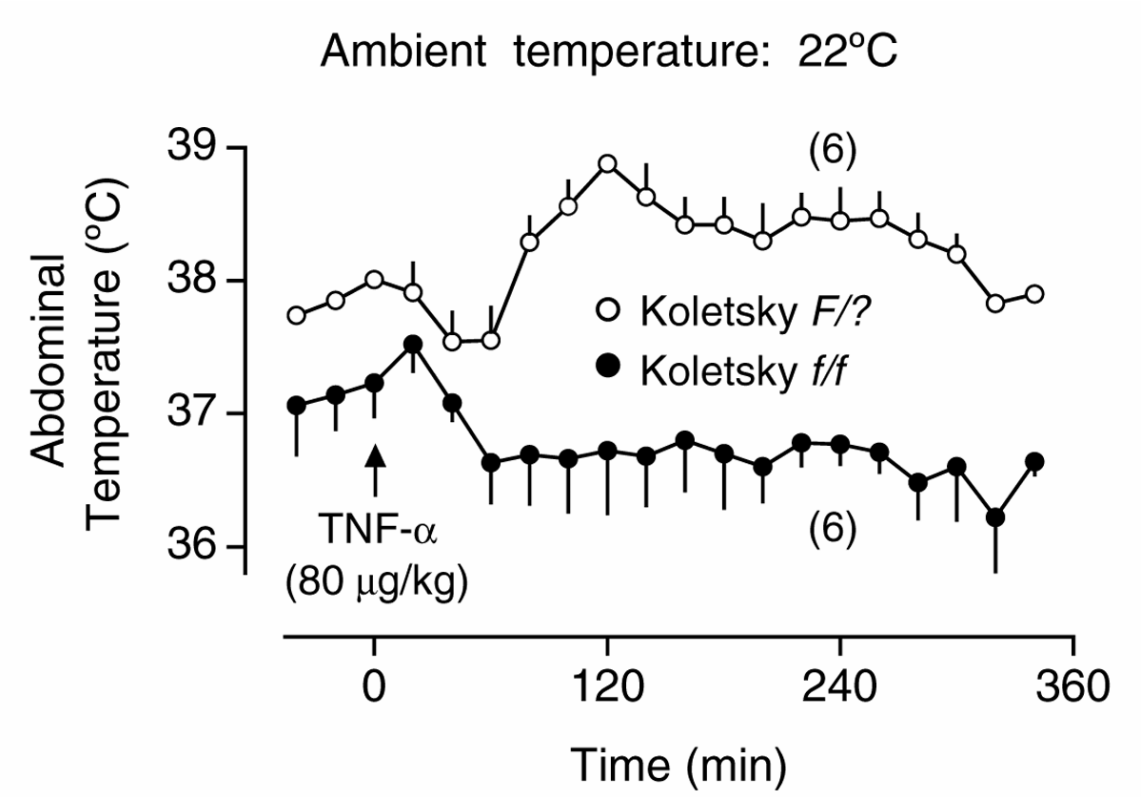

Figure 5. Thermal responses of Koletsky *f/f* and *F/?* rats to intravenous injection (arrow) of recombinant rat tumor necrosis factor (TNF)-α (dose indicated) in a cool environment.