Brown fat UCP1 is not involved in the febrile and thermogenic responses to IL-1β in mice

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Okamatsu-Ogura Y, Kitao N, Kimura K, Saito M. Brown fat UCP1 is not involved in the febrile and thermogenic responses to IL-1β in mice. Am J Physiol Endocrinol Metab 292: E1135–E1139, 2007. First published December 12, 2006; doi:10.1152/ajpendo.00425.2006.—The activity of brown adipose tissue (BAT), a site of nonshivering metabolic thermogenesis, has been reported to increase after interleukin (IL)-1β/lipopolysaccharide injection. To clarify the possible contribution of BAT thermogenesis to whole body febrile response, we investigated febrile and thermogenic response to IL-1β using mice deficient in uncoupling protein-1 (UCP1), a key molecule for BAT thermogenesis. In wild-type (WT) mice, IL-1β injection (5 μg/kg ip) increased body temperature (+1.8°C at 20 min), decreased physical activity (−37% at 1 h), and produced a slight and insignificant rise (+15% at 1 h) in oxygen consumption (V̇O₂). V̇O₂ dependent on metabolic thermogenesis (ΔV̇O₂ thermogenesis) calculated by correcting the effect of physical activity was increased after IL-1β injection (726 ± 200 ml h⁻¹ kg⁻¹ at 1 h). Almost the same responses were observed in UCP1-deficient mice, showing 638 ± 87 ml h⁻¹ kg⁻¹ of ΔV̇O₂ thermogenesis at 1 h. In contrast, CL316,243, a selective activator of BAT thermogenesis, increased body temperature, decreased physical activity, and produced a significant rise in V̇O₂ in WT mice, showing 1,229 ± 35 ml h⁻¹ kg⁻¹ of ΔV̇O₂ thermogenesis at 1 h. These changes were not observed in UCP1-deficient mice. These results, conflicting with a previously proposed idea of a role of BAT in fever, suggest a minor contribution of IL-1β to BAT thermogenesis in IL-1β-induced fever. In support of this, we found no effect of IL-1β on triglyceride content and UCP1 mRNA level in BAT, in contrast with apparent effects of CL316,243.

uncoupling protein-1; oxygen consumption; body temperature; knockout mouse; interleukin-1β

PROINFLAMMATORY CYTOKINES, such as interleukin (IL)-1β released by activated immune cells during the host response to infection or inflammation, have potent effects on the brain. When administrated systemically or directly into the brain, these cytokines induce symptoms of illness, including fever, activation of hypothalamic-pituitary-adrenal axis, anorexia, and depressed behavior (3, 7, 12, 17, 26). Similar effects are observed when endogenous cytokines are released in response to administration of lipopolysaccharide (LPS) from gram-negative bacteria.

Fever, an increase in body temperature that occurs in response to infection or inflammation, is generated by a number of physiological processes that cause increases in both heat conservation and production (thermogenesis). The increased thermogenesis in fever was proved by the fact that IL-1β or LPS injection increases whole body oxygen consumption (V̇O₂) (2, 13, 21).

As the peripheral mechanisms responsible for the thermogenesis, the contribution of brown adipose tissue (BAT) has been suggested (5). BAT is a tissue specified for metabolic heat production and has a significant role in cold- and diet-induced thermogenesis (4, 18). BAT thermogenesis is principally dependent on the activation of uncoupling protein-1 (UCP1), which uncouples oxidative phosphorylation in mitochondria to dissipate the electrochemical gradient as heat. The activity of UCP1 is controlled by the sympathetic nerves to BAT, mainly through the β-adrenergic mechanism. The activator of sympathetic nerves, β-adrenergic receptor (AR), also induces lipolysis in BAT (20), and the produced fatty acids are used as substrate for UCP1 thermogenesis (14).

The involvement of BAT thermogenesis in fever was suggested first by Blatteis (2), who demonstrated endotoxin-induced and β-blocker-sensitive rise in BAT temperature in guinea pigs. Subsequently, it was reported that IL-1β/LPS injection increased GDP binding to BAT mitochondria, a marker of UCP1 activity (6, 16, 21). It was also shown that blood flow in BAT was increased after IL-1β/LPS injection in rats (6). Although all of these previous reports suggest an activation of BAT thermogenesis during fever, its contribution to whole body thermogenic and febrile response has not been determined. Moreover, there are some studies that report conflicting roles of BAT thermogenesis in fever. For example, Jennings and Elia (15) showed that V̇O₂ is not changed despite the rise in body temperature after LPS injection in mice. We also failed to detect any stimulatory effect of IL-1β and LPS on norepinephrine turnover in BAT, a biochemical index of sympathetic nerve activity in this tissue (1, 22, 25). Thus, the contribution of BAT thermogenesis in fever is still controversial. To address this, in the present study, we investigated the febrile and thermogenic responses to IL-1β, a major endogenous pyrogen, in UCP1-deficient knockout (UCP1-KO) mice and compared them with those in wild-type (WT) mice.

MATERIALS AND METHODS

Animals. UCP1-KO (ucp1−/−) mice on a congenic background of C57BL/6J were generated by backcross matings of heterozygous (+/−) mice on a mixed 129/SvPas and C57BL/6J background with C57BL/6J mice 15 times, kindly given by Dr. L. Kozak (Pennington Biomedical Research Center, Baton Rouge, LA). All WT (ucp1+/+) mice were C57BL/6J. Mice were housed in plastic cages placed in an air-conditioned room at 26°C with a 12:12-h light-dark cycle (lights

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on at 0700–1900 and given free access to laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and tap water. Male UCP1-KO and WT mice (10–70 wk old) weighing 20–40 g were used. All experiments were performed at 26°C. The experimental procedures and care of animals were approved by the Animal Care and Use Committee of Hokkaido University.

UCP1 mRNA induction and triglyceride content in BAT. WT mice were injected with recombinant mouse IL-1β (5 μg/kg; PeproTech, London, UK) or PBS intraperitoneally twice at 1000 and 1300. A β3-AR agonist, CL316,243 [CL (0.1 mg/kg); American Cyanamid, Pearl River, NY], was injected once at 1000. Food was deprived at 1000. At 1600, all mice were killed by cervical dislocation, and interscapular BAT was quickly removed and weighed.

Tissue specimens were homogenized in Tris-EDTA buffer (10 mM Tris and 1 mM EDTA), pH 7.4. Triglyceride (TG) was extracted in chloroform-methanol (1:2, vol:vol), and concentration was measured enzymatically with a kit (TG test WAKO; Wako Pure Chemical, Tokyo, Japan) and expressed as milligrams TG per whole BAT.

Another tissue specimen was transferred into RNAlater (Invitrogen, Carlsbad, CA). Total RNA was extracted according to the manufacturer’s protocol using TRIzol (Invitrogen). UCP1 mRNA level was measured quantitatively by real-time RT-PCR using respective cDNA fragment as a standard and expressed as relative to β-actin mRNA level. Briefly, 2 μg of total RNA were reverse transcribed with an oligo(dT) 15-adaptor primer and Moloney murine leukemia virus reverse transcriptase (Invitrogen). Real-time PCR was performed on a fluorescence thermal cycler (LightCycler system; Roche Diagnostics, Mannheim, Germany) using SYBR Green I (Roche Diagnostics) as a double-strand DNA-specific dye according to the manufacturer’s protocol. Primers used were 5'-GTG AAG GTC AGA ATG CAA GC-3' and 5'-AGG GCC CCC TTC ATG AGG TC-3' for mouse UCP1, 5'-TCG TTAC CAC AGG CAT TGT GAT-3' and 5'-TGC TCG AAG TCT AGA GCA AC-3' for mouse β-actin.

Body temperature. Fever was induced by intraperitoneal injection of IL-1β (5 μg/kg) to WT and UCP1-KO mice. PBS and CL (0.1 mg/kg) were used in control experiments. Mice were injected with the agents between 1200 and 1400 to eliminate the influence of diurnal variations in body temperature and plasma hormonal levels.

Body temperature was measured in conscious mice by use of a telemetry system (StarMedical, Tokyo, Japan). A transmitter (10 T-T, 15.5 W × 9.0 D × 4.7 H mm; StarMedical) was implanted into the peritoneal cavity of a mouse under anesthesia with 75 mg/kg ketamine in 1 mg/kg medetomidine. After a 7-day recovery period, each mouse was transferred to a plastic cage, and signals from the transmitter were monitored by a receiver (IMT-10 RT; StarMedical) placed under the cage. After a 2-h adaptation period, IL-1β, CL, or PBS were injected intraperitoneally and body temperature was measured at 10-min intervals for 1 h.

V02 and physical activity. V02 was measured with an O2 metabolism measuring system (model MM102R; Muramachikikai, Tokyo, Japan) in a transparent chamber (50 W × 150 D × 150 H mm) with an air flow of 0.6–0.7 l/min. V02 of each mouse was measured for 1 min at 3-min intervals for 1 h. Cumulative V02 for each mouse was calculated and expressed as milliliters O2 per kilogram body weight. Each mouse was set into the chamber ≥6 h before the start of measurement for adaptation. Mice had free access to food and water during the measurement. At the same time, physical activity of each mouse was continuously recorded by a video recording system (digital video camera NV-GS50K and DVD video recorder DMR-E90H; Panasonic, Osaka, Japan) and assessed later by an investigator who was blind to the treatments. The video records were checked for behaviors such as locomotion, rearing, grooming, and feeding. If one of these behaviors was observed during a 1-min period, physical activity was scored as one count. Behavioral change was checked at 3-min intervals corresponding to the V02 measurement. Cumulative physical activity for 1 h was calculated, giving the maximum possible score over 1 h of 20 counts.

The ΔV02 thermogenesis was calculated as previously reported (19). In brief, by the use of correlation between total V02 and physical activity of the data from PBS-injected mice, the V02 dependent on physical activity (V02 activity) and basal V02 with no physical activity (V02 basal) was calculated. ΔV02 thermogenesis was obtained as ΔV02 thermogenesis = V02 – V02 basal – V02 activity.

Statistical analysis. All values are expressed as means ± SE. Statistical analysis was performed using analysis of variance followed by post hoc testing by the Fisher’s protected least significant difference multiple range test and analysis of covariance.

RESULTS

UCP1 induction and TG content in BAT. To investigate whether BAT stimulation is parallel with fever induction we examined the effects of IL-1β injection on TG content and UCP1 mRNA level in BAT. Since β3-AR stimulation is established to activate BAT thermogenesis, we used CL, a highly specific β3-AR agonist, in a control experiment. As predicted, CL decreased BAT weight (56% of PBS-injected control) and TG content (29%) (Fig. 1, A and B), and it induced UCP1 mRNA expression remarkably (Fig. 1C). On the other hand, IL-1β injection failed to produce any change in tissue weight, TG content, and UCP1 expression.
Body temperature. Body temperature response to IL-1β, CL, or PBS injection was monitored for 1 h in conscious mice (Fig. 2). Body temperature prior to injection was 36.2 ± 0.26 and 35.9 ± 0.40°C in WT and UCP1-KO mice, respectively, and did not differ between the genotypes. Intraperitoneal injection of PBS caused a small and insignificant increase within 10 min of injection and decreased thereafter in both genotypes. In WT mice, CL injection increased body temperature significantly compared with PBS control, with a maximal rise of 1.19 ± 0.14°C at 20 min (Fig. 2A). In UCP1-KO mice, however, body temperature response was not different between CL- and PBS-injected groups. On the other hand, IL-1β injection caused a remarkable increase both in WT and UCP1-KO mice with a maximal rise of 1.82 ± 0.25 and 1.76 ± 0.40°C at 20 min, respectively (Fig. 2B). There was no difference in the response of body temperature to IL-1β injection between WT and UCP1-KO mice.

VO₂ and physical activity. To investigate whether IL-1β increases thermogenesis, VO₂ and physical activity were measured for 1 h after the injection. VO₂ in control mice with PBS injection was not different between the genotypes (2,775 ± 184 and 2,889 ± 129 ml·h⁻¹·kg⁻¹ in WT and UCP1-KO mice, respectively) (Fig. 3A). The physical activity of PBS-injected mice tended to be lower in UCP1-KO mice than WT mice (16 ± 1 and 12 ± 1 count/h in WT and UCP1-KO mice), but the difference was insignificant (P = 0.092; Fig. 3B). CL injection increased VO₂ significantly in WT mice (+27%) but decreased slightly in UCP1-KO mice (−11%). CL injection suppressed physical activity both in WT (−63%) and UCP1-KO mice (−27%), but the effect was statistically significant only in WT mice. IL-1β injection produced a slight and insignificant increase of VO₂ in WT mice (+15%) but little or no response in UCP1-KO mice (+5%). In contrast, IL-1β injection substantially suppressed physical activity (−37% in WT and −42% in UCP1-KO mice).

ΔVO₂ thermogenesis. Since the change of physical activity largely affects the energy expenditure, ΔVO₂ thermogenesis was calculated. The correlation between physical activity and VO₂ thermogenesis was calculated. Analysis of covariance revealed no difference between the genotypes.

When VO₂ from CL-injected mice in each genotype was superimposed to that of PBS-injected mice, VO₂ of WT mice apparently fell above the regression line, whereas that of UCP1-KO mice was comparable (Fig. 4). In contrast, VO₂ from IL-1β-injected mice apparently fell above the regression line in both genotypes. Thus, ΔVO₂ thermogenesis was calculated at the difference from the regression line (Fig. 5). CL increased ΔVO₂ thermogenesis in WT mice (1,229 ± 35
but not in UCP1-KO mice (71 ± 103 ml·h⁻¹·kg⁻¹), whereas IL-1β increased ΔVO₂ thermogenesis similarly in WT and UCP1-KO mice (726 ± 200 and 638 ± 87 ml·h⁻¹·kg⁻¹ in WT and UCP1-KO mice, respectively). Thus, IL-1β caused a thermogenic response in UCP1-KO mice in the same way as in WT mice.

DISCUSSION

The major objective of this study was to investigate the contribution of BAT thermogenesis to fever using UCP1-KO mice.

First, we examined whether the mouse is an appropriate model for the fever study, because animals other than mice, such as rats or guinea pigs, were used in most previous reports investigating the involvement of BAT thermogenesis in fever (2, 6, 13, 16, 21). IL-1β injection caused a remarkable rise in body temperature in WT mice. However, total VO₂ was not changed. These responses to IL-1β were quite in contrast with those to CL, a highly specific β₃-AR agonist; CL injection both caused a rise in body temperature and increased total VO₂. Considering that β₃-AR is exclusively expressed in brown and white adipocytes, the effect of CL on body temperature is due to the activation of BAT thermogenesis (8, 11, 14).

Fever is generated by a number of physiological processes that cause increases in both heat conservation and production (thermogenesis). In the present study, we found that IL-1β induced fever without any notable effect on total VO₂, suggesting that IL-1β-induced fever is due to an increase in conservation, rather than production, of heat. Consistent with our results, Jennings and Elia (15) reported that LPS injection caused a remarkable rise in body temperature in both rats and mice, but VO₂ was increased only in rats. They proposed that there is a species difference in response to LPS, and in the case of mice, fever is generated mainly by the reduction of the heat loss more than activation of thermogenesis. In fact, the heat loss from the skin, especially that from the tail, has been claimed to play an important role in regulation of body temperature in mice (10, 23).

However, it is to be noted that we also found that physical activity was largely suppressed in IL-1β injected mice. Since total VO₂ is much influenced by physical activity (19), we calculated the VO₂ thermogenesis by correcting the effect of physical activity. This revealed that IL-1β caused a significant increase of thermogenesis (ΔVO₂ thermogenesis) in mice similar to that reported in rats despite no apparent increase in total VO₂. Thus, species differences mentioned above may be caused by the different effects on physical activity.

There have been some reports suggesting the stimulatory effect of IL-1β on BAT thermogenesis, such as increase in GDP binding to mitochondria and blood flow to this tissue (6, 16, 21). In the present study, we reevaluated the effect of IL-1β on some biochemical parameters of BAT. CL induced a remarkable reduction in TG content and a rise in UCP1 mRNA level, consistent with a well-established view of UCP1-dependent degradation of TG in BAT (4, 14). In contrast, IL-1β failed to induce any of these changes. Moreover, we (1, 22, 25) reported previously that IL-1β/LPS increases norepinephrine turnover in spleen and lung, but not in BAT, indicating the absence of activation of sympathetic nerve to BAT in fever. Taken together, these results suggest little or no stimulatory effect of IL-1β on BAT thermogenesis.

To obtain more direct evidence for this idea, we investigated whether the thermogenic effect of IL-1β was affected by the
ablation of ucp1 gene. IL-1β injection increased body temperature in UCP1-KO mice, in the same way as WT mice, confirming similar febrile response in the two types of mice. Moreover, IL-1β showed no apparent effect on total V02 but a suppressive effect on physical activity in UCP1-KO mice, as in WT mice. Finally, ΔV02 thermogenesis calculated by correcting the effect of physical activity in UCP1-KO mice was found to be comparable to that in WT mice. Thus, IL-1β increased thermogenesis and induced fever in UCP1-KO mice in the same manner as in WT mice. In contrast to IL-1β, CL increased thermogenesis and body temperature only in WT mice and not in UCP1-KO mice. These results suggest a minor role of UCP1-dependent BAT thermogenesis in IL-1β-induced thermogenesis and fever.

It is to be noted that the present results do not necessarily rule out the possibility of some compensatory thermogenic mechanism in UCP1-KO mice. In fact, it was reported (9) that UCP1-KO mice can maintain their body temperature by shivering under cold circumstances, whereas WT mice maintain their body temperature mainly by BAT thermogenesis. To clarify a precise role of BAT thermogenesis and shivering in fever, additional studies including the measurement of shivering in UCP1-KO mice are needed.

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