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Induction of UCP2 Gene Expression by LPS: A Potential Mechanism for Increased Thermogenesis during Infection

Raffaella Faggioni, Judy Shigenaga, Arthur Moser, Kenneth R. Feingold, and Carl Grunfeld
Department of Medicine, University of California, San Francisco, and Metabolism Section, Medical Service, Department of Veterans Affairs Medical Center, San Francisco, California 94121

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UCP2 has been proposed to regulate thermogenesis and energy expenditure. To identify potential mechanisms underlying the increased energy expenditure and heat production during infection, we investigated whether LPS and cytokines might increase UCP2 mRNA levels in mice. LPS (100 μg, i.p.) increased the expression of UCP2 mRNA in liver (28-fold) and muscle and white adipose tissue (5-fold). In liver, both IL-1β (1 μg, i.p.) and TNF (5 μg, i.p.) increased UCP2 mRNA levels, 4- and 3-fold respectively, whereas in muscle and fat tissue, an increase was detectable after TNF, but not IL-1β. Indomethacin (10 mg/kg, i.p.) administered immediately before LPS markedly reduced (70%) the ability of LPS to increase UCP2 mRNA in liver, but not in muscle or adipose tissue. These results suggest a role for UCP2 in the heat production and increased energy expenditure that occurs during infection.

Uncoupling proteins (UCP) are a family of proton channels located in the inner mitochondrial membrane. UCP1 was the first identified member of the family and is primarely located in brown adipose tissue (BAT) (1). UCP1 produces heat by dissipating the proton gradient formed during mitochondrial respiration and has been proposed to play a role in regulated thermogenesis and energy expenditure. UCP1 is controlled by the sympathetic nervous system and mediates cold- and diet-induced thermogenesis in rodents (2-4). However, due to the low levels of BAT in adult humans, the contribution of UCP1 to thermoregulation in humans is questionable.

Recently, two other uncoupling proteins have been described. UCP2 is widely expressed in a variety of tissues in humans and rodents, including white adipose tissue (WAT), skeletal muscle and liver (5, 6). UCP2 mRNA levels are upregulated in WAT in response to fat feeding, suggesting regulation by diet (6). A third uncoupling protein homologue, UCP3, is preferentially expressed in skeletal muscle in human and skeletal muscle, WAT and BAT in rodents (7, 8). Little is known about the regulation of UCP2 and UCP3 in liver and muscle.

Fever and increased energy expenditure are part of the acute phase response to infection and inflammation (9). The development of fever is largely mediated by increased thermogenesis and is induced by endogenous pyrogenic cytokines, such as IL-1, TNF and IL-6 (10). Injection of LPS induces a febrile response similar to that seen during infection, due to the induction of endogenous pyrogens and this pyrogenic response is prevented by cyclooxygenase inhibitors (11). During fever, cytokines raise the set point of the hypothalamic thermoregulatory center. The critical event appears to be the cytokine-induced production of prostaglandins in the brain, which activates efferent pathways to both increase heat production and decrease heat dissipation in the periphery (9). Increased resting energy expenditure also occurs during chronic infections such as HIV infection, even in the absence of fever (12).

To identify the potential molecular mechanisms underlying the increase in energy expenditure and heat production during infection, we investigated whether LPS and cytokines might increase UCP2 mRNA levels. Because prostaglandins mediate many of the metabolic responses induced by LPS and cytokines, we also studied the role of prostaglandins in UCP2 induction by LPS.

MATERIALS AND METHODS

Materials. [(−32P]dCTP (3,000 Ci/mmol) was purchased from New England Nuclear (Boston, MA); LPS (a phenol-extracted preparation from *E. coli* serotype O55:B5) was from Difco Laboratories (Detroit, MI); Human TNF-α with a specific activity of 5×10^7 U/mg was provided by Genentech, Inc. (South San Francisco). Recombinant human IL-1β with a specific activity of 10^9 U/mg was provided by Immunex (Seattle, WA). Multiprime DNA labelling system kits were purchased from Amersham Life Sciences International (Arlington Heights, IL); Minispin Sephadex G-50 columns were from Worthington Biochemical Corporation (Freehold, NJ); Nytran+ nylon membranes were from Schleicher and Schuell (Keene, NH). Oligo (DT) cellulose type 77F was from Pharmacia LKB Biotechnology AB (Upsala, Sweden). The cDNA for UCP2, prepared as described previously (5), was generously provided by Dr. Louis Tartaglia (Millennium Pharmaceutical, Cambridge, MA).

Animals and treatments. Four- to six-week-old C57BL/6 female mice were purchased from Jackson Laboratory, Bar Harbor, ME.

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The animals were housed five per cage in a temperature-, humidity- and light-controlled chamber set at 24 ± 1°C, on a 12h:12h light-dark cycle, and were provided with rodent chow (Simonsen Laboratories) and water ad libitum. LPS or cytokines were administered intraperitoneally and tissues were collected. Control mice were injected with sterile, pyrogen-free saline. Subsequently, food was withdrawn from both control and treated animals because LPS and cytokines can induce anorexia.

All animal protocols were approved by the Animal Studies Committee of the SF VAMC.

Isolation of RNA and Northern blotting. Poly-A or total RNA was isolated from liver, spleen, muscle or fat tissue by a modification of the method of Chomczynski and Sacchi (13). For fat tissue, lipid extraction with $CHCl_3$ was performed immediately after homogenization; the aqueous phase was then acidified and subjected to the standard acid/phenol/chloroform extraction. Poly A+ RNA was isolated using oligo dT cellulose. Gel electrophoresis, transfer, Northern blotting and densitometry were performed as previously described (14). A murine *UCP2* gene cDNA probe, generated as previously described (5), was used. Because we and others have shown that LPS and cytokines increase mRNA levels of "housekeeping" genes such as actin and cyclophilin, two mRNAs commonly used for normalizing data, we loaded equal amounts of total (fat) or poly-A (muscle, liver and spleen) RNA (10 μg determined by spectrophotometry) and assessed uniformity of sample application by ultraviolet visualization of the acridine orange-stained ribosomal RNAs in the gel before electrophoretic transfer. We have previously reported that the effects of LPS or cytokines are specific for individual mRNAs in individual tissues both in terms of the direction of the change and the order of magnitude of the change. In liver or adipose tissue, specific mRNAs may be either increased or decreased to varying degrees ranging from greater than 30 fold increases to 95% decreases (15). For example, treatment of hamsters with LPS or cytokines decreases the level of CETP mRNA and increases the level of leptin mRNA in adipose tissue (16, 17). The blots were exposed to x-ray films for various durations to ensure that measurements were done on the linear portion of the curve, and the bands were quantified by densitometry. However, the large induction of UCP2 in the liver blot 16 h after LPS treatment, may lead to an understimation of the LPS induction at that time.

RESULTS

To explore whether an inflammatory stimulus induced UCP2 mRNA levels, C57BL/6 mice were treated with LPS (100 μg, i.p.) or saline. Liver, muscle, white adipose tissue and spleen were collected 16 h after LPS or saline treatment and UCP2 mRNA levels were determined by Northern blot analysis. As shown in Fig.1A and B, the expression of UCP2 mRNA was stimulated by LPS in liver, muscle and white adipose tissue. The strongest induction was detected in liver, in which a greater than 28-fold increase above the basal levels was observed, whereas a 5-fold induction was detected in muscle and white adipose tissue. In contrast, LPS did not increase UCP2 mRNA above the control levels in spleen.

The kinetics of UCP2 mRNA induction by LPS (100 μg, i.p.) in liver is shown in Fig. 2. LPS administration increased UCP2 mRNA levels by 10 fold 12 h after LPS, reaching a maximal induction at 16h (25-30 fold increase). UCP2 mRNA levels were still significantly elevated 24h after LPS but by 32h had returned to baseline levels.

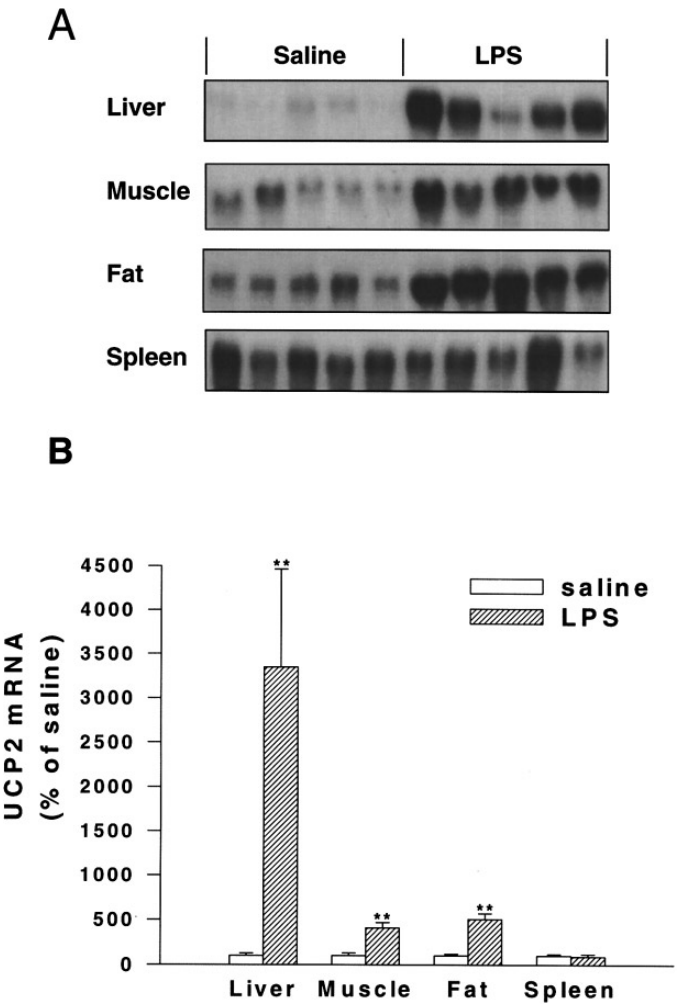

FIG. 1. The effects of LPS on UCP2 mRNA levels in liver, muscle, fat and spleen. (A) Northern blots showing UCP2 mRNA levels after LPS in liver, muscle, fat, and spleen. Mice were administered LPS (100 μg, i.p.) or saline and then fasted. Tissues were removed 16 h after treatment and UCP2 mRNA levels were measured as described in Methods. (B) The data from the blots in panel A are presented as % of UCP2 mRNA levels in saline treated mice. Values are mean ± SEM; n=5 for each group. **, $p<0.01$ vs saline treated mice by Student's t-test.

The dose response curve for LPS induction of UCP2 mRNA at 16h in liver is shown in Fig. 3. Maximal stimulation occurred at 100 μg, with a half maximal stimulatory dose of approximately 2 μg; this range parallels the concentrations of LPS needed for the induction of fever in mice (11).

Administration of LPS induces the synthesis and release of proinflammatory cytokines, such as IL-1 and TNF, which are the endogenous mediators of the pathophysiologic effects of LPS (10). Therefore we next examined the effect of the administration of two known endogenous pyrogens, IL-1β and TNF, on UCP2 mRNA expression in liver, muscle and fat tissue. Mice were treated with IL-1β (1 μg, i.p.), TNF (5 μg, i.p.) or saline

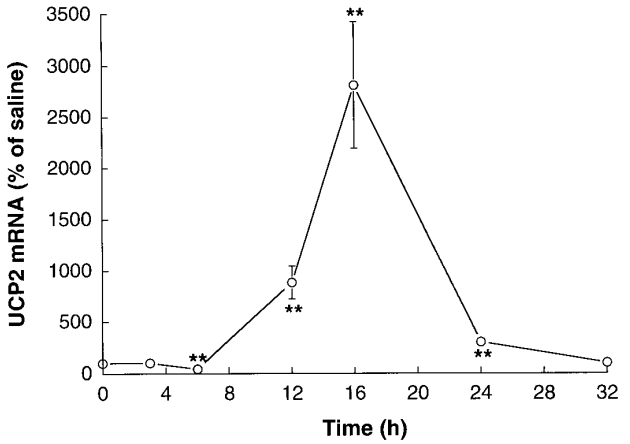

FIG. 2. LPS time course curve. Mice were administered saline or LPS (100 μg, i.p.), then the liver was removed at different time points and UCP2 mRNA levels were measured as described in Methods. Animals were fasted after LPS or saline treatment. Values are mean ± SEM; n=5 for each group. * $p<0.05$; ** $p<0.01$ compared to each point vs mice simultaneously treated with saline by Student's t-test.

and tissues were collected 12 h after treatment. As shown in Table 1, IL-1β and TNF increased UCP2 mRNA levels 4- and 3-fold respectively in liver. In muscle and fat tissue, a 2-fold increase was detectable after TNF, whereas no significant changes were observed after IL-1β.

Many of the effects of LPS and/or cytokines, including fever, are mediated by prostaglandins (18). To demonstrate potential mediation by eicosanoids on the LPS induction of UCP2 mRNA, we next examined the effect of the cyclooxygenase inhibitor and antipyretic drug indomethacin on the ability of LPS to stimulate UCP2

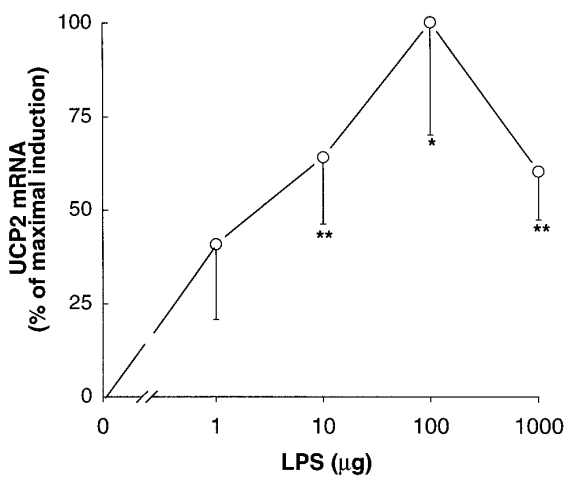

FIG. 3. LPS dose response curve. Mice were administered saline or the dose of LPS indicated on the abscissa, then liver was removed at 16 h and UCP2 mRNA levels were measured as described in Methods. All animals were fasted after treatment. Values are mean ± SEM; n=5 for each group. * $p<0.05$; ** $p<0.01$ vs saline treated mice by unpaired ANOVA with Bonferroni as post hoc test.

TABLE 1

UCP2 mRNA Induction by Il-1β and TNF in Liver, Muscle, and Fat Tissue

	Saline	IL-1β	TNF
Liver	100 ± 26	455 ± 265*	308 ± 46**
Muscle	100 ± 6	120 ± 19	200 ± 26*
Fat	100 ± 20	119 ± 17	200 ± 26**

Note. Values are mean ± SEM; n = 5 for each group. *$p < 0.05$; **$p < 0.01$ vs saline by unpaired ANOVA.

mRNA expression. As shown in Fig. 4, a single injection of indomethacin (10 mg/kg, i.p.) immediately before LPS administration resulted in a marked reduction (70%) of the ability of LPS to increase UCP2 mRNA in liver. In a similar experiment, where UCP2 mRNA levels were measured 12 h after LPS administration, indomethacin reduced LPS induction of UCP2 mRNA in liver by 80% (data not shown), indicating that in liver the induction of UCP2 mRNA by LPS is principally mediated by prostaglandins. Indomethacin treatment did not affect the induction of UCP2 mRNA levels by LPS in muscle and adipose tissue (data not show).

DISCUSSION

UCP1 is known to play an important role in regulated thermogenesis in mice (1). A role for UCP2 in

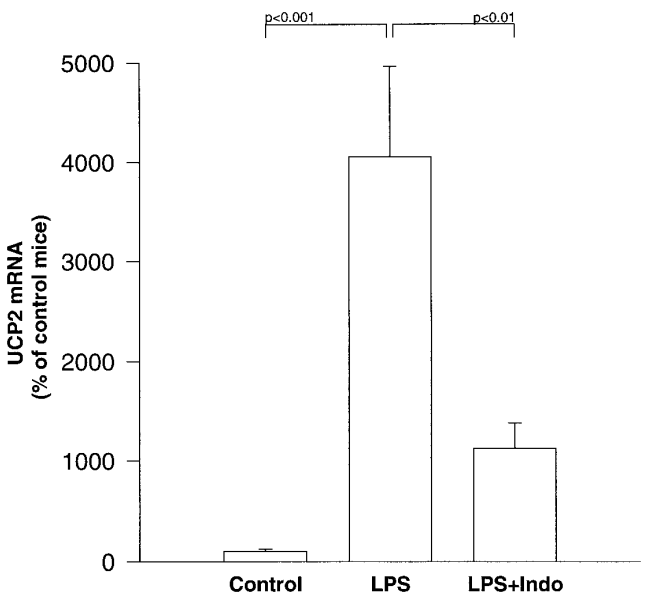

FIG. 4. The effect of indomethacin on UCP2 mRNA induction by LPS in liver. Indomethacin (10 mg/kg, i.p.) or saline was administered immediately before LPS (100 μg, i.p.) or saline then liver was removed at 16 h after LPS or saline and UCP2 mRNA levels were measured as described in Methods. All animals were fasted after treatment. n=5 for each group. Statistical analysis by unpaired ANOVA with Bonferroni as post hoc test.

regulated thermogenesis and energy expenditure has been proposed and its tissue distribution is consistent with this hypothesis (5, 6). Adipose tissue, skeletal muscle and liver contribute to regulated thermogenesis (19-21) and UCP2 mRNA is detectable in all of these tissues. The previously reported increase in UCP2 mRNA in WAT in response to fat feeding suggests regulation by diet (6).

LPS significantly increased UCP2 mRNA levels in liver, muscle and adipose tissue. Although UCP2 protein levels need to be determined, our results suggest a role for UCP2 in the heat production and increase in energy expenditure that occurs during infections. The inhibition by indomethacin of UCP2 induction in the liver suggests an important role for this organ in the UCP2-mediated thermogenesis during inflammation. Interestingly, UCP2 is expressed at high levels in Kupffer cells in the basal state and only a weak signal can be detected in hepatocytes (22) raising the possibility that the induction of UCP2 mRNA observed in liver might occur in Kupffer cells.

In fever, the thermoregulatory center in the hypothalamus activate vasoconstriction, shivering and metabolic activities, which represent mechanisms of heat conservation and production (9). UCP2 induction might be part of the heat production mechanisms. UCP2 mRNA levels are induced to a greater extent in liver than in muscle and fat. However, in rodents after LPS administration, blood flow is shunted away from the muscle while blood flow to liver is increased (23). Thus the dramatic increase in UCP2 in liver, to which blood flow has been increased, could yield efficient production of heat.

Prostaglandins mediate the pyrogenic response to LPS (11) and we have shown that indomethacin treatment blunts the induction of UCP2 mRNA by LPS in liver; these data suggest a correlation between UCP2-mediated heat production in liver and fever. However, indomethacin treatment did not affect the induction of UCP2 mRNA levels by LPS in skeletal muscle and adipose tissue, indicating different regulation in these tissues. On the other hand, it is likely that the liver plays the most relevant role in terms of UCP2-mediated heat production during an acute phase response, as IL-1β, a key endogenous pyrogen, induces UCP2 mRNA only in liver. Therefore, the induction of UCP2 in muscle and adipose tissue might participate in other aspects of the increase in energy expenditure rather than in the generation of fever, which is usually defined as thermogenesis blocked by cyclooxygenase inhibitors (9).

In conclusion, UCP2 mRNA levels are induced by LPS and cytokines and the induction in the liver is inhibited by indomethacin. These results are consistant with a role for UCP2 in the heat production and increase in energy expenditure that occurs during infections.

ACKNOWLEDGMENTS

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