Altered expression of nuclear hormone receptors and coactivators in mouse heart during the acute-phase response

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Feingold, Kenneth, Min Sun Kim, Judy Shigenaga, Art Moser, and Carl Grunfeld. Altered expression of nuclear hormone receptors and coactivators in mouse heart during the acute-phase response. Am J Physiol Endocrinol Metab 286: E201–E207, 2004; 10.1152/ajpendo.00205.2003.—Severe sepsis results in the decreased uptake and oxidation of fatty acids in the heart and cardiac failure. Some of the key proteins required for fatty acid uptake and oxidation in the heart have been shown to be downregulated after endotoxin (LPS) administration. The nuclear hormone receptors, peroxisome proliferator-activated receptor (PPAR) and thyroid receptor (TR), which heterodimerize with the retinoid X receptor (RXR), are important regulators of fatty acid metabolism and decrease in the liver after LPS administration. In the present study, we demonstrate that LPS treatment produces a rapid and marked decrease in the mRNA levels of all three RXR isoforms, PPARα and PPARβ, and TRα and TRβ in the heart. Moreover, LPS administration also decreased the expression of the coactivators CREB-binding protein (CBP)/p300, steroid receptor coactivator (SRC)-1, SRC-3, TR-associated protein (TRAP)220, and PPARγ coactivator (PGC)-1, all of which are required for the transcriptional activity of RXR-PPAR and RXR-TR. In addition, the mRNA levels of the target genes malic enzyme, Spot 14, sarcoplasmic reticulum Ca2+-ATPase, or SERCA2, the VLDL receptor, fatty acyl-CoA synthetase, fatty acid transporter/CD36, carnitine palmitoyltransferase Iβ, and lipoprotein lipase decrease in the heart after LPS treatment. The decrease in expression of RXRα, β, and γ, PPARα and -δ, and TRα and -β, and of the coactivators CBP/p300, SRC-1, SRC-3, TRAP220, and PGC-1 and the genes they regulate, induced by LPS in the heart, could account for the decreased expression of key proteins required for fatty acid oxidation and thereby play an important role in cardiac contractility. These alterations could contribute to the myocardial dysfunction that occurs during sepsis.

SEVERE SEPSIS RESULTS IN CARDIAC FAILURE, and this effect can be mimicked by endotoxin (LPS) administration (11, 48, 50, 56). Initially, during sepsis and after LPS administration, there is a decrease in systemic resistance and either normal or increased cardiac output (11, 48, 50, 56). However, with time, cardiac output decreases due to a decrease in both contractility and heart rate (11, 48, 50, 56). Similar effects on cardiac function are observed with the administration of TNF and IL-1 (7, 42, 44, 47), cytokines that mediate the effects of LPS (7, 15).

For the heart to function as a pump, constant generation of ATP is required, which is formed by the oxidation of fatty acids, glucose, or lactate (59). Fatty acid oxidation produces the most energy per molecule, and because of the high-energy demands of the heart, fatty acids are the primary fuel for cardiac ATP generation (58, 59). The oxidation of fatty acids by cardiac myocytes involves a large number of enzymes and transporters. One of the initial steps is the hydrolysis of triglycerides contained in lipoprotein particles to generate free fatty acids by the enzyme lipoprotein lipase (LPL) (1). Free fatty acids generated by LPL, and also circulating free fatty acids, then enter the cell either by diffusion or by fatty acid transporters, either FAT/CD36 or FAT protein (FATP) (10, 19, 35, 60). Alternatively, lipoprotein particles can be endocytosed into the cell, once inside the cell, the lipoprotein triglycerides are hydrolyzed to fatty acids by lysosomal enzymes (27). Within the cell, fatty acids are transported by heart-type fatty acid-binding protein (H-FABP) (9) and are rapidly esterified to fatty acyl-CoA by fatty acyl-CoA synthetase (FACS) (45). Transport of fatty acids into the mitochondria is regulated by carnitine palmitoyltransferase I (CPT I). Two isoforms of this enzyme are expressed in the heart, with CPT Iβ accounting for most of the activity (64). Within the mitochondria, the fatty acids undergo β-oxidation, which generates the ATP required for cardiac function.

In association with the decrease in cardiac output during sepsis and after LPS administration, most, but not all, investigators have observed a reduction in the uptake and oxidation of fatty acids and the accumulation of triglycerides in the myocytes (29, 33, 34, 36, 55, 63). Our laboratory and others (2, 21, 37–39) have shown that the expression of many of the proteins that play key roles in fatty acid oxidation, such as LPL, FATP, FAT/CD36, FACS, and H-FABP, is decreased in the heart after LPS and/or cytokine administration and that these decreases could account for the reduction in cardiac fatty acid oxidation. The mechanisms by which these changes in cardiac metabolism occur are not known.

Recent studies have demonstrated that nuclear hormone receptors, especially Class II receptors that heterodimerize with RXR, play a critical role in regulating fatty acid oxidation by the heart. For example, activation of PPARα increases the expression of many of the genes required for fatty acid oxidation, including FATP, FAT/CD36, H-FABP, acyl-CoA synthetase (ACS), and CPT Iβ (for review see Refs. 4, 31). Moreover, in PPARα-deficient (PPARα−/−) mice, the expression of the genes that regulate fatty acid oxidation in the heart is decreased compared with wild-type mice (16, 32). As expected, myocardial uptake and oxidation of fatty acids are greatly reduced in PPARα−/− mice (16, 32).

The thyroid hormone receptor (TR) also plays an important role in regulating cardiac function. Two different genes encode TR, and recent studies with null mice have shown that, in
TRα-deficient mice, both heart rate and contractility are decreased, whereas in TRβ-deficient mice no impairment is noted (20, 25, 65). Activation of TR stimulates the expression of genes involved in fatty acid metabolism, such as malic enzyme (ME), Spot 14 (S14), and the VLDL receptor (VLDLR), as well as genes important for myocardial contractility, such as myosin heavy chain-α and sarcoplasmic reticulum Ca2+-ATPase (SERCA2) (12, 14, 26, 43). LPS administration and cytokine treatment reduce the expression of myosin heavy chain-α and SERCA2 in rats (23, 49).

For nuclear hormone receptors such as PPARα and TR to activate gene transcription, they interact with coactivators, such as CREB-binding protein (CBP)/p300, steroid receptor coactivator (SRC)-1, SRC-2, SRC-3, TR-associated protein (TRAP)220, and PPARγ coactivator-1 (PGC-1), which link the transcription factors with the transcriptional regulatory complex regulating gene expression (66). Recently, PGC-1 was shown to be expressed in tissues such as brown adipose tissue, muscle, and heart, where there are a large number of mitochondria and a high oxidative capacity (51). In cardiac tissue, PGC-1 has been shown to interact with PPARα and stimulate the expression of genes required for fatty acid oxidation and to promote mitochondrial biogenesis (30).

Recent studies by our laboratory (5, 6) have demonstrated that LPS and cytokine administration decreases mRNA and/or protein levels of RXRα, β, and γ, PPARα, and TRα and β in the liver. Moreover, the expression of many of the genes regulated by RXR-PPAR and RXR-TR is reduced in the liver (5, 6). Thus a reduction in hepatic RXR levels, alone or in combination with decreases in PPARs and TR, could be a mechanism for coordinately inhibiting the expression of multiple genes during sepsis and/or following LPS administration. On the basis of studies demonstrating the important role of nuclear hormone receptors in regulating fatty acid metabolism in the heart, the decrease in heart fatty acid oxidation during infection and showing a reduction of nuclear hormone receptors in the liver by LPS treatment, we hypothesized that LPS treatment would also produce a decrease in RXR, PPARα, and TR α in the heart that could contribute to the decrease in the expression of the proteins that play a key role in the changes in cardiac fatty acid metabolism that occur during sepsis. In addition, we postulated that the expression of key coactivators, including CBP/p300, SRC-1, SRC-2, SRC-3, TRAP220, and PGC-1, would also decrease during sepsis.

METHODS

Animals. Six-week-old female C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were maintained on a normal light-dark cycle and provided with rodent chow and water ad libitum. Anesthesia was induced with halothane. Mice were injected intraperitoneally with 100 μg of LPS (Escherichia coli 55:5B; Difco Laboratories, Detroit, MI) in saline or with saline alone. Except where indicated, food was withdrawn immediately after the injection of LPS, because LPS induces anorexia in rodents (22). Hearts were removed at the time indicated in the text after treatment. The dose of LPS used in this study was nonlethal, since the half-maximally lethal dose (LD50) for LPS in rodents is ~10 μg/250 g body wt (17).

RNA isolation and Northern blot analysis. Total RNA was purified from snap-frozen whole heart tissue by use of Tri-Reagent (Sigma, St. Louis, MO). Twenty micrograms of total RNA were electrophoresed on a 1% agarose-formaldehyde gel, electrotransferred to Nytran membrane (Schleicher and Schuell, Keene, NH), and hybridized with cDNAs labeled with [α-32P]dCTP (NEN Life Science Products, Boston, MA) by use of the random priming technique. mRNA levels were detected by exposure of the membrane to X-ray film and were quantified by densitometry. RXRα cDNA was a gift from Dr. D. Bikle (University of California, San Francisco, CA). RORβ and RORγ cDNAs were kindly provided by Dr. D. Mangelsdorf (University of Texas Southwestern Medical Center, Dallas, TX). PPARα, -β, and -γ cDNAs were kindly provided by Dr. A. Bass (University of California, San Francisco, CA). FACS cDNA was a gift from Dr. J. Strauss (University of Pennsylvania Medical Center, Philadelphia, PA). Serum amyloid A (SAA) cDNA was a gift from Dr. J. Sipe (NIH, Bethesda, MD). SERCA 2 cDNA was a gift from Dr. W. Dillman (UCSD, San Diego, CA). The following mouse probes were generated by RT-PCR, starting from total RNA from mouse liver (ME, S14, TRβ1, CPT 1β, GLUT1, GLUT4, hexokinase (HK1 and HKII), and using the following primers: ME: 5′-CCA CCA GCG CGG CTA CCT GCT GAC GCC GGA-3′ (upper), 5′-CCT CTC ACT CCT CGG TGC AGC AGC CGG ATG-3′ (lower) (33); S14, 5′-ATG CAA GTG CTA ACC AGG AAA CGC-3′ (upper), 5′-AGA AGT GCA GTC GGA ACT GGG C-3′ (lower) (35); TRβ1, 5′-GCC TGC GAC AAC AGG AAC CCC CGT-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33); RXRα, 5′-GCC TTG GAG AAC AAG CAC ACC-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33); RXRβ, 5′-GCC TTG GAG AAC AAG CAC ACC-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33); RXRγ, 5′-GCC TTG GAG AAC AAG CAC ACC-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33). Glut1, 5′-ATG CAA GTG CTA ACC AGG AAA CGC-3′ (upper), 5′-AGA AGT GCA GTC GGA ACT GGG C-3′ (lower) (33); S14, 5′-ATG CAA GTG CTA ACC AGG AAA CGC-3′ (upper), 5′-AGA AGT GCA GTC GGA ACT GGG C-3′ (lower) (33); TRβ1, 5′-GCC TGC GAC AAC AGG AAC CCC CGT-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33); RXRα, 5′-GCC TTG GAG AAC AAG CAC ACC-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33); RXRβ, 5′-GCC TTG GAG AAC AAG CAC ACC-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33); RXRγ, 5′-GCC TTG GAG AAC AAG CAC ACC-3′ (upper), 5′-AGC GAT GTC CTT CCT GGC ACC AGT GGG G-3′ (lower) (33).

Statistical analysis. Differences between the experimental and control groups were analyzed using the unpaired t-test.

RESULTS

Figure 1 shows the effect of LPS administration on the mRNA levels of RXR, PPAR, and TR in the heart 4 and 16 h after treatment. At 4 h, RXRα, TRα, TRβ, PPARα, and PPARβ mRNA levels decreased, whereas the mRNA levels of RXRβ and RXRγ isoforms were not significantly altered (Fig. 1A). However, by 16 h, all three RXR isoforms were markedly decreased after LPS treatment (Fig. 1B). RXRα, the most abundant isoform, decreased by 72%, whereas RXRβ and RXRγ decreased by 60 and 75%, respectively, after LPS treatment. The levels of TRα, TRβ, PPARα, and PPARβ mRNA decreased by 81, 75, 74, and 53%, respectively, at 16 h after LPS treatment. PPARγ mRNA was not detectable in the heart in either control or LPS-treated mice.
Figure 2 shows the effect of different doses of LPS on RXR, TR, and PPAR mRNA levels in the heart 16 h after LPS treatment. For most of these nuclear hormone receptors, half-maximal inhibition occurred at \( \frac{100}{1101} \) g LPS/mouse, which is greater than the concentrations of LPS that inhibited the expression of these same nuclear hormone receptors in the liver (5, 6).

In addition to decreasing the mRNA levels of these nuclear hormone receptors, LPS treatment resulted in a marked decrease in the mRNA levels of several coactivators, including CBP/p300, SRC-1, SRC-3, TRAP220, and PGC-1 (Figs. 3 and 4). PGC-1 is a key coactivator for both PPAR\( \alpha \) and TR; it allows for the stimulation of fatty acid oxidation and mitochondrial biogenesis (30, 62). Although LPS treatment resulted in a marked decrease in the mRNA levels of these nuclear hormone receptors and coactivators in the heart, it should be noted that the mRNA levels of other genes are increased in the heart after LPS. For example, we observed that SAA mRNA levels increased from virtually no expression to a readily apparent band (Fig. 4). Thus LPS administration results in a specific and marked suppression of both nuclear hormone receptors and their coactivators in the heart.

In the experiments described above, the animals were fasted after LPS administration because LPS administration decreases food intake. To determine the effect of fasting per se on the mRNA levels of nuclear hormone receptors in the heart, we next compared animals either fed or fasted for 16 h. Fasting resulted in a decrease in the mRNA levels of PPAR\( \alpha \) and PPAR\( \delta \) and RXR\( \alpha \) but did not significantly affect the mRNA levels of TR\( \alpha \) or TR\( \beta \) (Table 1). Similarly, PGC-1 mRNA levels were also unchanged by fasting (Table 1).
We next examined the effect of LPS treatment on the mRNA levels of proteins that play important roles in cardiac fatty acid metabolism and function and are known to be regulated by PPAR and TR. Three genes that are regulated by PPARα and play key roles in fatty acid oxidation are FACS, FAT/CD36, and CPT Iβ. As shown in Fig. 5, the mRNA levels of FACS, FAT/CD36, and CPT Iβ are all decreased ~70% by 16 h after LPS administration. ME, S14, and VLDLR are regulated by TR and are involved in various aspects of fatty acid metabolism. As shown in Fig. 5, the mRNA levels of these proteins in the heart are also decreased after LPS administration (ME decreased 32%, S14 decreased 42%, and VLDLR decreased 86%). SERCA2 is regulated by TR and is important for cardiac contractility. As shown in Fig. 5, LPS treatment results in a 58% decrease in SERCA2 mRNA levels in the heart. Finally, because of its pivotal role in catalyzing the hydrolysis of triglycerides in lipoproteins to fatty acids, we determined the effect of LPS on LPL mRNA levels. As shown in Fig. 5, LPL mRNA levels were decreased 48% after LPS treatment. Thus a variety of genes important in fatty acid metabolism and cardiac contractility, which are regulated by PPAR and TR, are decreased by LPS treatment in the heart.

Glucose is an alternative source of energy for the heart. We therefore next determined the effect of LPS on the mRNA levels of GLUT1 and GLUT4, key transporters that mediate glucose uptake into cells, and hexokinase (HK)I and HKII, enzymes that catalyze the first step in glucose metabolism, glucose phosphorylation. In LPS-treated animals, GLUT1 mRNA levels are increased (control = 100% ± 6.1, LPS = 155% ± 10.3, P = 0.001, n = 10), whereas GLUT4 mRNA levels are decreased (control = 100% ± 7.5, LPS = 38% ± 5.5, P = 0.001, n = 10). Additionally, LPS treatment results in a decrease in HKI mRNA (control = 100% ± 4.8, LPS = 68% ± 3.4, P = 0.002, n = 10), whereas HKII mRNA levels are not significantly altered (control = 100% ± 6.5, LPS = 85% ± 8.5, n = 10). Thus, although LPS treatment decreased many of the key enzymes and transporters required for fatty acid oxidation, the enzymes and transporters required for glucose oxidation are not uniformly decreased. In fact, GLUT1 expression is increased.

### DISCUSSION

The data presented here describe a potential mechanism by which cardiac function deteriorates during sepsis. They demonstrate that LPS administration, a model of sepsis, produces a rapid and marked decrease in the expression of all three RXR isoforms, PPARα and -δ, TRα and -β, and the coactivators CBP/p300, SRC-1, SRC-3, TRAP220, and PGC-1 in the heart. These results are similar to our previous observations in liver, where we also found that LPS administration resulted in a marked and rapid reduction in RXR, PPAR, and TR levels (5, 6).
Thus sepsis reduces the expression of these nuclear hormone receptors in two crucial organs. In addition, the present study further demonstrates that the mRNA levels of a number of key proteins whose expression is regulated by these nuclear hormone receptors were also decreased by LPS treatment. Specifically, in the present study, we demonstrate that the mRNA levels of ME, S14, SERCA2, VLDLR, FACS, FAT/CD36, CPT Iβ, and LPL decrease in the heart. In previous studies we have shown that LPS administration decreases the expression of FATP and H-FABP in the heart (4, 39).

Of note is that many of these proteins play crucial roles in regulating lipid metabolism, particularly the oxidation of fatty acids, and a reduction in the expression of these proteins in the heart would result in a decrease in fatty acid oxidation. The heart oxidizes fatty acids to generate ATP to provide energy. Studies have shown that, during sepsis, the ability of the heart to oxidize fatty acids is impaired and it is likely that this decrease in energy production contributes to the cardiac failure that often accompanies sepsis (33, 34, 36, 55). The present study illustrates a mechanism by which sepsis could impair cardiac function. LPS treatment, by reducing the expression of the key transcription factors RXRα, -β, and -γ, PPARα and -δ, and TRα and -β, and key coactivators including PGC-1, decreases the expression of the proteins required for a number of key steps in the uptake and oxidation of fatty acids. A decrease in LPL, VLDLR, FAT/CD36, and FATP would impair the uptake of fatty acids into cardiac cells (1, 10, 19, 27, 35, 60). A decrease in H-FABP might diminish the intracellular movement of fatty acids, and a decrease in FACS would inhibit the activation of fatty acids to fatty acyl-CoA (9, 45). Finally, a decrease in CPT Iβ would limit the transport of fatty acids into mitochondria, which is required for oxidation and ATP generation (64). In contrast to the sepsis-induced decrease in the key enzymes and transporters required for fatty acid oxidation, the changes in the enzymes and transporters required for glucose oxidation were much more variable. Whereas we observed a decrease in GLUT4 and HKI mRNA levels, no change was observed in HKII mRNA levels, and GLUT1 mRNA levels were increased.

TR is well known to regulate the expression of essential proteins in the heart, such as SERCA2 and α-myosin heavy chain (14); LPS and cytokine administration has been shown to decrease SERCA2 (present study in mice and Ref. 23 in rats) and α-myosin heavy chain (49) mRNA levels, which are markers of myopathic remodeling. Whether decreases in these proteins contribute to a decrease in cardiac function during sepsis remains to be determined.

The results of the present study can be compared with those describing other disorders that decrease cardiac contractility and induce cardiac failure. Conditions that induce cardiac hypertrophic growth, such as pressure overload, result in a decrease in PPARα and PGC-1 levels and the expected reduction in the key proteins required for fatty acid oxidation (4, 16, 31). Additionally, hypoxia has been shown to decrease both PPARα and RXRα expression in the heart (24, 52). Moreover, rapid pacing-induced heart failure also decreases RXRα and the oxidation of fatty acids in cardiac cells (46). To the best of our knowledge, the effect of various cardiac conditions on the expression of PPARα has not been determined, but recent studies (18) have shown a role for PPARα in regulating fatty acid oxidation in the heart. Several studies have shown, in both animal models and humans, that the expression of TR is increased by heart failure (13, 54, 57). In contrast, a study by Kinugawa et al. (28) demonstrated a decrease in cardiac TR in humans with a failing left ventricle. The basis for the difference in results between the various studies is unclear. Regardless, in heart failure the expression of many of the genes that are regulated by TR, such as α-myosin heavy chain and SERCA2, is decreased consistent with decreased activity of the TR, as predicted by Kinugawa and colleagues (28, 40, 53). The effect of heart failure on the expression of other coactivators, such as CBP/p300, SRC-1, SRC-3, and TRAP220, remains to be determined.

Studies (for review, see Refs. 8, 41) have demonstrated that proinflammatory cytokines (TNFα and IL-6) are increased in humans with heart failure. Moreover, a recent study (61) has suggested that elevations in serum proinflammatory cytokine levels (IL-6), the production of TNFα by peripheral blood mononuclear cells, and markers of the acute-phase response (serum C-reactive protein) predict an increased risk for the development of heart failure. It is well known that many of the effects of LPS administration are mediated by increases in proinflammatory cytokines. It is therefore possible that, in patients with heart failure, the increases in proinflammatory cytokines could result in a reduction in the levels of expression of RXR, PPAR, TR, and coactivators in the heart, and thereby adversely affect myocardial function.

In summary, the present study demonstrates that LPS administration decreases the expression of RXRα, -β, and -γ, PPARα and -δ, and TRα and -β, and the coactivators CBP/p300, p300, SRC-1, SRC-3, TRAP220, and PGC-1 in the heart, explaining the decreased expression of key proteins required for fatty acid oxidation. These alterations could contribute to the myocardial dysfunction that often occurs during sepsis.

REFERENCES


