Epinephrine stimulates IL-6 expression in skeletal muscle and C2C12 myoblasts: role of c-Jun NH2-terminal kinase and histone deacetylase activity

Robert A. Frost, Gerald J. Nystrom, and Charles H. Lang

Departments of Cellular and Molecular Physiology, and Surgery, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

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Frost, Robert A., Gerald J. Nystrom, and Charles H. Lang. Epinephrine stimulates IL-6 expression in skeletal muscle and C2C12 myoblasts: role of c-Jun NH2-terminal kinase and histone deacetylase activity. Am J Physiol Endocrinol Metab 286: E809–E817, 2004. First published January 13, 2004; 10.1152/ajpendo.00560.2003.—Although an individual’s genetic makeup is a major determinant of muscle mass, other influences, such as hormones, cytokines, nutrition, and exercise can also modulate muscle size. IL-6 is an important inflammatory cytokine. Mice that overexpress IL-6 fail to thrive and/or have reduced skeletal muscle mass. The purpose of the present study was to determine whether the stress hormone epinephrine increases inflammatory cytokine expression in skeletal muscle and muscle cells. Infusion of epinephrine in vivo for 2 h increased IL-6 protein (15-fold) and mRNA (40-fold) in skeletal muscle but not in liver. Epinephrine had a similar effect in C2C12 muscle cells, where the hormone increased IL-6 protein and mRNA in a dose- and time-dependent manner. Epinephrine-stimulated IL-6 expression was attenuated by the α-adrenergic receptor antagonist phentolamine and completely blocked by either the β1/2-adrenergic receptor antagonist propranolol or the β2-receptor ICI-118551. The transcriptional inhibitor DRB and the synthetic glucocorticoid dexamethasone also blocked epinephrine-induced IL-6. SP-600125 (a JNK inhibitor) and SB-202190 (a p38 MAP kinase inhibitor) completely blocked epinephrine-induced IL-6. Trichostatin A (a histone deacetylase inhibitor) blocked both endotoxin- and epinephrine-induced IL-6 expression. These data suggest that epinephrine induces IL-6 synthesis in skeletal muscle in vivo and myocytes in vitro. Epinephrine utilizes predominantly the β1/2-adrenergic receptors to stimulate IL-6 synthesis. Endotoxin and epinephrine synergize to increase IL-6 mRNA expression. Optimal IL-6 synthesis may require both stress kinase and histone deacetylase activity.

A major physiological response to stress in vertebrates is the secretion of endogenous catecholamines by the adrenal medulla (8). Epinephrine and norepinephrine modify heart rate, blood flow, and blood pressure to maximize the classical “fight or flight” response. Yet, the prolonged stress of traumatic injuries, such as burns and bacterial and viral infections, can also result in enhanced secretion of the catecholamines (28, 65). In these circumstances, prolonged sympathetic stimulation may have potentially deleterious effects. For example, prolonged exposure to epinephrine creates pressure overload in the heart and primes the liver for an exaggerated acute-phase response (61). Epinephrine does not appear to alter muscle protein breakdown directly but may function in conjunction with other stress hormones to accelerate protein breakdown during critical illness (4). Moreover, the sepsis-induced insulin resistance in skeletal muscle is mediated in part by catecholamines (33). Loss of skeletal muscle is associated with an increased morbidity and mortality in a variety of wasting conditions (11, 59, 60).

Epinephrine can have direct effects on cells or may stimulate the expression of secondary mediators such as inflammatory cytokines. Both acute and chronic bouts of exercise are associated with an increase in the blood concentration of not only epinephrine and norepinephrine but also interleukin-6 (IL-6) (29, 48, 56). IL-6 is synthesized locally within skeletal muscle itself. This is evidenced by the nearly 20-fold increase in IL-6 output from a leg undergoing dynamic knee extension exercise compared with the output from the contralateral resting limb (57). IL-6 and other cytokines are elevated not only with exercise but also with inflammation (2, 14), cardiovascular disease (9, 12), and aging (53). IL-6 may contribute to the muscle wasting that is associated with each of these pathophysiological conditions either directly or by indirectly affecting other hormones. For instance, IL-6 may alter either the amount or the signaling of anabolic hormones such as growth hormone and insulin-like growth factor I (IGF-I) (20, 39).

Recently, we demonstrated that lipopolysaccharide (LPS) stimulates IL-6 mRNA expression not only in immune tissues but also in rat and mouse skeletal muscle (18, 36). In addition, LPS directly stimulates the expression of IL-6 mRNA in mouse and human skeletal muscle cells in vitro (16, 18). Because other stress hormones such as catecholamines are elevated during stress, infection, and critical illness and these hormones can stimulate IL-6 expression (7, 56), but this relationship has not been established in skeletal muscle, we examined whether epinephrine regulates IL-6 directly in this tissue. Our present data establish that epinephrine increases IL-6 mRNA and protein in skeletal muscle but not liver. Subsequent studies using C2C12 myocytes were performed to address the mechanism by which epinephrine increases IL-6. Adrenergic receptor agonists and antagonists were used to identify the receptor type through which epinephrine regulates IL-6 synthesis. In addition, MAP kinase and histone deacetylase inhibitors were used to demonstrate the important role of these enzymes in IL-6 expression.
MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 175–200 g were purchased from Charles River Breeding Laboratories (Cambridge, MA). Rats were acclimated for 1 wk in a light-controlled room (12:12-h light-dark cycle) under constant temperature and were 50 days old on the day of the experiment. This corresponds to a sexually mature but young adult in human terms. Water and standard rat chow were provided ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State University College of Medicine and adhere to the National Institutes of Health guidelines for the use of experimental animals.

Experimental protocols. The day before each experiment, rats were anesthetized with an intramuscular injection of ketamine and xylazine (90 and 9 mg/kg, respectively), and sterile surgery was performed to implant catheters in the carotid artery and jugular vein, as previously described (23, 37). Because xylazine can act as an α-adrenergic agonist and attenuate the expression of inflammatory genes (24), the animals were returned to individual cages, fasted overnight, and provided water ad libitum. Any confounding effects of xylazine would be expected to have dissipated because the anesthetic has a relatively short half-life in vivo (1, 51). Experiments were started the next day, and rats were conscious and unrestrained throughout the protocol. Epinephrine was diluted in 0.9% saline containing 1% ascorbic acid and infused intravenously at a rate of 0.2 μg·kg⁻¹·min⁻¹. This dose of epinephrine was selected on the basis of our previous studies to achieve plasma epinephrine concentrations that are comparable to that seen in catabolic conditions (23). Two hours after the start of the infusion, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), and blood was collected into heparinized syringes as previously described (35). The gastrocnemius muscle, heart, and liver were rapidly excised and weighed. Tissue was frozen between aluminum blocks precooled to the temperature of liquid nitrogen. The frozen tissues were later powdered in liquid nitrogen with a mortar and pestle. Blood was collected in heparinized syringes and centrifuged (13,000 g for 1 min at 4°C). All tissue and plasma samples were stored at −70°C.

Cell culture. The C2C12 mouse myoblast cell line was purchased from the American Type Culture Collection (Manassas, VA) and used for all studies. Cells were grown in 100-mm Petri dishes (Becton Dickinson, Franklin Lakes, NJ) and cultured in minimal essential medium containing 10% bovine calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (25 μg/ml) (all from Mediatech, Herndon, VA). Cells were grown to confluence and switched to fresh serum-containing medium before addition of epinephrine, LPS, or other agents. Experiments were performed with lipopolysaccharide B derived from Escherichia coli 026:B6 (DIFCO Laboratories, Detroit, MI). A variety of compounds were used to characterize the response to epinephrine and LPS, including phenolamine, propranolol, ICI-118551, 5,6-dichloro-1-ß-ß-thio-ribofuranosyl benzimidazole (DRB), dexamethasone, H-89, SP-600125, SB-202190, and PD-98059. In addition, some cells were treated with trichostatin A or histone deacetylase inhibitor III (all from Calbiochem, San Diego, CA).

RNA isolation and ribonuclease protection assay. Total RNA, DNA, and protein were extracted from C2C12 cells or tissues in a mixture of phenol and guanidine thiocyanate (TRI-Reagent; Molecular Research Center, Cincinnati, OH), using the manufacturer’s protocol (Pharminigen, San Diego, CA). Protected RNAs were separated using a 5% acrylamide gel (19:1 acrylamide-bisacrylamide). Gels were transferred to blotting paper and dried under vacuum on a gel dryer. Dried gels were exposed to a phosphor-imager screen (Molecular Dynamics, Sunnyvale, CA), and the resulting data were quantified using ImageQuant software and normalized to the mouse ribosomal protein L32 mRNA signal in each lane.

Western blot analysis and IL-6 ELISA. Cell extracts were electrophoresed on denaturing polyacrylamide gels and electrophoretically transferred to nitrocellulose with a semidy blotter (Bio-Rad Laboratories, Melville, NY). The resulting blots were blocked with 5% nonfat dry milk for 1.5 h and incubated with antibodies against IκB-α, as previously described (18). Unbound primary antibody was removed by washing with Tris-buffered saline containing 0.05% Tween-20, and blots were incubated with anti-rabbit or anti-mouse immunoglobulin conjugated with horseradish peroxidase (HRP). Blots were briefly incubated with the components of an enhanced chemiluminescence detection system (Amersham, Buckinghamshire, UK). Dried blots were used to expose X-ray film for 1–3 min.

Conditioned medium from C2C12 cells was collected at various time points and frozen at −20°C until assay. Cells and tissues were homogenized in PBS containing Tween-20, Triton X-100, and a protease inhibitor cocktail (0.05, 0.2, and 0.5%, respectively). Mouse IL-6 in plasma, conditioned medium, and tissue and cell extracts were measured with a sandwich ELISA consisting of two anti-mouse IL-6 antibodies and a streptavidin- and HRP-linked secondary antibody (Pharminigen). Conditioned medium was diluted with an equal volume of assay diluent, whereas plasma was diluted 1:12 before assay. Antigen and antibody complexes were detected with tetramethylbenzidine (TMB, an HRP substrate), and the reaction was stopped with 2 N H2SO4. Ninety-six-well plates were read at the absorption maximum for TMB (450 nm).

Statistics. Values are means ± SE. Unless otherwise noted, each experimental condition was tested in triplicate, and each experiment was repeated twice. Data were analyzed by one-way analysis of variance followed by a Student-Newman-Keuls test for multiple comparisons. Statistical significance was set at P < 0.05. Results from individual experiments were averaged for each group. For animal studies, the number of rats per group was 5 (control) and 5 (LPS), and each study was repeated twice.

RESULTS

Epinephrine infusion increases IL-6 protein and mRNA in rat skeletal muscle. Two hours after start of the intravenous infusion of epinephrine, IL-6 protein and mRNA in the gastrocnemius were dramatically increased (15- and 40-fold, respectively; Fig. 1, A-C). The increase in IL-6 in muscle from individual rats did not correlate with changes in IL-6 protein in the plasma, which were at a level that was much less than were present in skeletal muscle (≤60 pg/ml) or IL-6 mRNA in the liver, which did not show an increase in expression (control 100 ± 3 vs. epinephrine 85 ± 25). This suggests that IL-6 is made locally in the skeletal muscle proper. Epinephrine also increased IL-1β mRNA (5-fold; Fig. 1B) and TNF-α mRNA (3-fold; Fig. 1) in skeletal muscle. The changes in cytokine mRNAs were independent of changes in the level of mRNA for two housekeeping genes (L32 and GAPDH).

Epinephrine induces IL-6 expression via β-adrenergic receptors. Because epinephrine was a potent stimulus for IL-6 in skeletal muscle in vivo, we examined whether the hormone stimulated IL-6 in C2C12 myocytes. Epinephrine increased IL-6 protein and mRNA in a dose-dependent manner (Fig. 2). IL-6 synthesis occurred with an ED50 of 10 ng/ml of epinephrine with maximal stimulation at a dose of 100 ng/ml (Fig. 2A).
Epinephrine (100 ng/ml) increased IL-6 mRNA and protein concomitantly, with maximal mRNA expression occurring 2 h after addition of the hormone. IL-6 mRNA expression was transient and returned to baseline between 7 and 11 h. Norepinephrine also significantly increased IL-6 synthesis in C2C12 cells to a degree similar to that of epinephrine (50- to 100-fold).

Epinephrine increased IL-6 protein sixfold, and this response was blunted by the α2-adrenergic antagonist phentolamine and completely blocked by the α2-adrenergic antagonist propranolol. β2-adrenergic receptor agonist, also stimulated IL-6 mRNA and protein (Fig. 3, C and D). Epinephrine- and clenbuterol-induced IL-6 expression was completely blocked by the β2-adrenergic receptor antagonist ICI-118551, whereas the antagonist had no effect on LPS-induced IL-6 expression (Fig. 3D).

Epinephrine and LPS synergistically induce IL-6 gene expression. Epinephrine is known to affect transcription, RNA stability, mRNA translation, processing, and secretion. We examined whether ongoing transcription was necessary for the ability of epinephrine to stimulate IL-6 expression. Pretreatment with the transcriptional inhibitor DRB partially blocked epinephrine-induced IL-6 expression (Fig. 4A). Epinephrine-induced IL-6 expression was also blocked by the synthetic glucocorticoid dexamethasone (Fig. 4B). We also examined whether dibutyl cAMP increased IL-6 expression, because epinephrine is known to stimulate cAMP production in vitro. Epinephrine and cAMP increased IL-6, but neither response was blocked by the protein kinase A inhibitor H-89 (Fig. 4C).
Because stress hormones often activate MAP kinases, we examined whether selective MAP kinase inhibitors could block epinephrine-induced IL-6 expression. Epinephrine-induced IL-6 expression was completely inhibited by a JNK inhibitor (SP-600125) and a p38 MAP kinase inhibitor (SB-202190) but not by a MEK/ERK inhibitor (PD-98059) (Fig. 5, A, B, and C, respectively).

LPS increases epinephrine release from the adrenal gland in vivo, and the plasma concentration of epinephrine is elevated during infection. Therefore, we examined whether LPS and epinephrine have an additive or a synergistic effect on IL-6 expression in C2 C12 myoblasts. LPS and epinephrine individually stimulated IL-6 protein synthesis and secretion 10- to 16-fold (Fig. 6A). When cells were treated with LPS and epinephrine simultaneously, they acted synergistically and increased IL-6 protein in the media nearly 100-fold. A similar synergistic relationship between LPS and epinephrine was observed when IL-6 protein was measured in cell extracts (Fig. 6B) and IL-6 mRNA was measured by RPA (Fig. 6C). The biological activity of epinephrine in these experiments was maximal, as supplementing the cell cultures with additional epinephrine at later time points did not further increase IL-6 synthesis (data not shown). LPS- and epinephrine-induced IL-6 expression are JNK dependent, as the combination of the two agents was completely inhibited by SP-600125 (Fig. 6D). The p38 and MEK kinases also contribute to the synergistic activation of IL-6 synthesis, as inhibitors to these kinases partially blocked LPS- and epinephrine-stimulated IL-6 synthesis. The synergistic effect of LPS and epinephrine may be specific to IL-6 expression, because epinephrine did not increase either IκB protein degradation or IκB mRNA expression above that seen with LPS alone (Fig. 7, A and B). Epinephrine also did not act synergistically with LPS to increase either of two other LPS-inducible genes, SOCS-3 and TNF-α (Fig. 7, C and D).

Histone deacetylase inhibitors block LPS- and epinephrine-induced IL-6 gene expression. Optimal gene transcription relies not only on the recruitment of transcription factors but also on transcriptional coactivators and loosening of chromatin via
the phosphorylation and acetylation of histone proteins. Because histone deacetylase activity can be both positively (42) and negatively (62) associated with IL-6 expression, we examined whether histone deacetylase inhibitors could block LPS, epinephrine, or the combined effects of the two compounds on IL-6 expression. Trichostatin A and histone deacetylase inhibitor III dose-dependently attenuated LPS-stimulated IL-6 expression with an ED50 of 0.01 and 0.1 \( \mu M \), respectively (Fig. 8A). Trichostatin A also inhibited epinephrine-stimulated IL-6 expression and significantly blunted the synergistic effect of LPS and epinephrine on IL-6 protein and mRNA (Fig. 8, B and C). Inhibition with histone deacetylase III yielded identical results (data not shown). In comparison, trichostatin A did not block LPS-induced IκB-\( \alpha \) mRNA expression (Fig. 8D).

**DISCUSSION**

Previous studies have shown that IL-6 is synthesized not only by immune tissues, such as the spleen and the liver, but also by adipose tissue (64), heart (45), and skeletal muscle (57). The molecular mechanisms regulating IL-6 mRNA expression in skeletal muscle have remained largely obscure due to a lack of a reliable model system for examining positive and negative regulators of IL-6 expression. Recently, our laboratory and others \((18, 21)\) have shown that myocytes express IL-6 mRNA and that its abundance can be positively regulated by LPS and inflammatory cytokines. It is likely that a number of other serum hormones also influence IL-6 synthesis by skeletal muscle and muscle cells \((40, 41, 56, 66)\).

A prime candidate for positively regulating IL-6 in skeletal muscle is endogenous catecholamines, because these hormones are elevated during stress and after exercise. In humans, exercise increases IL-6 protein in skeletal muscle fibers as detected by immunohistochemistry \((49)\). IL-6 protein is uniformly expressed in different fiber types, including types I, IIa, and 2x \((49)\). Finally, infusion of epinephrine stimulates muscle IL-6 synthesis in rats and humans \((56)\).

The present study demonstrates that epinephrine infusion stimulates the concomitant expression of IL-6 protein and mRNA in vivo in rat skeletal muscle. In addition, epinephrine increases IL-1\( \beta \) and TNF-\( \alpha \) mRNA in the same tissue, suggesting a generalized inflammatory response similar to that found in rats and mice injected with LPS \((18, 36)\). The increased IL-6 mRNA and protein in skeletal muscle were of comparable magnitude, suggesting that epinephrine increases IL-6 predominantly by first increasing the pool of IL-6 mRNA available for translation. In addition, because IL-6 protein was elevated in muscle from individual rats in which plasma IL-6 was at the lower end of the assays detection range, it is likely that muscle itself is the source of IL-6 in this model.

The cell type(s) that synthesize IL-6 in muscle in response to epinephrine in our model are unknown, but recently Penkowa et al. \((49)\) have shown that IL-6 is made by muscle fibers in response to exercise. Muscle injury also increases IL-6 mRNA, and this tends to be present in damaged muscle fibers and satellite cells as detected by in situ hybridization \((27)\). Because skeletal muscle is composed of multiple cell types, we have used C2C12 myoblasts as a model system to examine the mechanism by which epinephrine and LPS regulate IL-6 mRNA in skeletal muscle. These cells resemble satellite cells that are resident in adult muscle, but they can also differentiate into mature myotubes \((5)\). In addition, we have found that the C2C12 cell line is responsive to multiple proinflammatory molecules, cytokines, and hormones. We \((19)\) have previously shown that both myoblasts and myotubes express IL-6 mRNA in response to LPS but that myotubes secrete less IL-6 protein.

Epinephrine stimulated IL-6 expression with an ED50 of \( \sim 10 \text{ng/ml} \). Although this concentration is significantly higher than that used in most in vivo studies that mimic stress \((13)\), it is similar to the concentration used in cell culture studies to elicit a response to epinephrine \((31, 64)\). Epinephrine also stimulated IL-6 synthesis via \( \beta_2 \)-adrenergic receptors in all three cell types. In myocytes, the bulk of the IL-6 response to epinephrine is mediated by the \( \beta_2 \)-adrenergic receptor, because it can be mimicked by clenbuterol (a \( \beta_2 \)-specific agonist), and inhibited by ICI-118551 (a \( \beta_2 \)-specific antagonist). Additional regulation may also occur via \( \alpha \)-adrenergic receptors, because phenotolamine blunted the epinephrine-induced increase in IL-6 protein. Further studies with \( \alpha \)-adrenergic specific agonists and...
antagonists will be necessary to delineate the contribution of this receptor to IL-6 synthesis in muscle and muscle cells.

Epinephrine increased IL-6 synthesis and secretion at least partially by a transcriptional response, because epinephrine-induced IL-6 expression was blocked by pretreatment with an RNA polymerase II inhibitor (DRB). Epinephrine was also blocked by the synthetic glucocorticoid dexamethasone. Dexamethasone is thought to block IL-6 gene transcription by a mechanism in which the glucocorticoid receptor physically occludes the access of transcription factors and coactivators to the IL-6 promoter (50). We have previously demonstrated that dexamethasone also attenuates LPS-induced IL-6 synthesis

Fig. 6. Epi and LPS synergistically increase IL-6 protein and mRNA synthesis. C2C12 myoblasts were grown as described in MATERIALS AND METHODS and treated with either LPS alone, Epi (1 μM) alone, or a combination of both. Conditioned medium (A) and cell extracts (B) were collected and assayed for IL-6 by ELISA. Additional cells were treated as above, and RNA was isolated in TRI-Reagent and hybridized to a cytokine mRNA RPA template as described in MATERIALS AND METHODS and run on a 5% acrylamide gel. C: the IL-6 band was quantified with ImageQuant software. Data are normalized to L32 mRNA, as described in MATERIALS AND METHODS, and expressed as a fold increase relative to time-matched cells treated with saline alone. D: LPS and Epi synergistically increased IL-6 synthesis; this was inhibited to varying degrees by SP-600125 (SP, 50 μM), SB-202190 (SB, 20 μM), and PD-98059 (PD, 20 μM). Values are means ± SE. Bars with different lowercase letters are significantly different from each other (P < 0.05). Where absent, SE bars are within the bar.

Fig. 7. LPS, but not Epi, alters IκB-α, suppressor of cytokine signaling (SOCS)-3, and TNF-α mRNA. C2C12 myoblasts were grown as described in MATERIALS AND METHODS and treated with either Epi alone (1 μM), LPS alone, or a combination of both. A: cell extracts were isolated and run on an SDS-PAGE gel and probed for IκB-α by Western blotting. Additional cells were treated as above, and RNA was isolated in TRI-Reagent and hybridized to a cytokine mRNA RPA template as described in MATERIALS AND METHODS and run on a 5% acrylamide gel. IκB-α mRNA (B), SOCS-3 (C), and TNF-α (D) bands were quantified with ImageQuant software. Data are normalized to L32 mRNA as described in MATERIALS AND METHODS and expressed as a fold increase relative to time-matched cells treated with saline alone. Values are means ± SE. Bars with different lowercase letters are significantly different from each other (P < 0.05).
both in vitro (18) and in vivo (36); thus the mechanism for inhibiting epinephrine induced IL-6 may be the same. Although adrenergic agonists increase cAMP 20-fold in C2C12 cells (25) and we have shown that cAMP increases IL-6 synthesis, a protein kinase A inhibitor (H-89) had no effect on either epinephrine- or cAMP-induced IL-6 synthesis. These data suggest that other signaling pathways may mediate the ability of epinephrine to increase IL-6.

One possible signaling pathway for the increase in IL-6 is the MAP kinase pathway. Many stressors have been shown to stimulate the activation of the ERK kinases, the p38 kinase, and the JNK kinase (26). The JNK kinase is a particularly attractive candidate because we (19) have previously shown that it mediates the LPS-induced increase in IL-6 in C2C12 cells. β-Adrenergic agonists stimulate the JNK pathway. Nor-epinephrine stimulates JNK phosphorylation in cardiomyocytes (52), and epinephrine stimulates JNK activity in skeletal muscle in vivo (46). Additionally, TNF-α fails to stimulate IL-6 synthesis in embryonic fibroblasts from JNK1/2-null mice (63), and mice deficient in either the JNK1 or the JNK2 pathway have decreased serum levels of IL-6 in response to LPS compared with wild-type mice (43).

Epinephrine inhibits the ability of LPS to stimulate TNF-α and IL-6 in whole blood (3, 55). In contrast, epinephrine synergizes with LPS to induce IL-6 synthesis in human skin microvascular cells (22). We, too, found that epinephrine and LPS synergistically increase IL-6 secretion from C2C12 cells.

Epinephrine and LPS also increased IL-6 mRNA and IL-6 protein content in cell extracts, suggesting that epinephrine does not simply increase secretion of the peptide. We (18) have previously shown that LPS increases IL-6 by a transcriptional mechanism. Epinephrine may increase IL-6 transcription further, as is seen in microvascular cells, or it may stabilize IL-6 mRNA and/or enhance IL-6 translation (22). Interestingly, the JNK pathway appears to be essential for both LPS- and epinephrine-induced IL-6 expression. A JNK inhibitor blocks not only LPS-induced IL-6 synthesis (19) but also that of epinephrine and the combination of LPS and epinephrine.

In an attempt to understand how LPS and epinephrine synergistically increase IL-6 synthesis, we examined whether other LPS-induced genes were also synergistically increased by epinephrine. LPS increased TNF-α and SOCS-3 mRNA in C2C12 cells, but epinephrine did not enhance the expression of either gene. Epinephrine also did not enhance NF-κB activation, as evidenced by an equivalent degradation of IκBα protein and subsequent IκBα mRNA expression by either LPS alone or the combination of LPS and epinephrine. Thus the synergistic effect of LPS and epinephrine appears to be relatively unique to the IL-6 gene.

Various cell types differ in their response to histone deacetylase inhibitors. Histone deacetylase inhibitors enhance LPS-induced IL-6 synthesis in microglial cells and murine fibrosarcoma cells (58, 62) but inhibit IL-6 synthesis in splenocytes treated with LPS and interferon (42). We found that trichosta-
tin A and histone deacetylase inhibitor III were potent inhibitors of LPS-induced IL-6 synthesis. Both inhibitors also blocked epinephrine-induced IL-6 mRNA synthesis as well. Trichostatin A blocked the synergistic effect of LPS and epinephrine on IL-6 mRNA and protein, but the inhibitor did not reduce IL-6 to control levels. Histone deacetylase inhibitors enhance histone acetylation and are thought to activate gene transcription by opening the chromatin structure to allow free access of transcription factors to previously repressed genes (10). However, an increasing number of genes have been shown to be downregulated by histone deacetylase inhibitors (32, 38, 44). Trichostatin A may trigger the acetylation of nonhistone proteins necessary for basal and/or LPS-induced transcription and consequently inhibit their activity. Although trichostatin A may influence Toll-like receptor-4 levels, the inhibitor did not directly affect LPS signaling at the receptor level in C2C12 cells. LPS stimulated IkB-α mRNA expression, even in the presence of trichostatin A, suggesting that receptor signaling is intact in trichostatin A-treated C2C12 cells.

Several lines of evidence suggest that inflammatory cytokines have a negative effect on muscle protein balance due to both a decrease in muscle protein synthesis and an increase in muscle protein degradation (34, 59). Many of the effects of cytokines also appear to be mediated by their ability to reduce the expression of the anabolic growth factor insulin-like growth factor I (IGF-I) (20). Cytokines also alter the bioavailability of IGF-I via IGF-binding proteins (IGFBPs) such as IGFBP-1 (15, 37). Not surprisingly, epinephrine alters IGF-I content in skeletal muscle during chronic heart failure (47). Epinephrine infusion in humans, to a level that mimics stress, also doubles the circulating concentration of IGFBP-1 (13). Hence, cytokines and epinephrine may act synergistically to alter muscle protein balance during inflammation.

A recent emphasis has been placed on the signal transduction pathways that mediate muscle wasting (60). Our findings suggest that inflammatory mediators such as LPS, cytokines such as TNF-α, and stress hormones such as epinephrine may synergize to alter the expression of IL-6 (19), IGF-I (20), and IGFBP-1 (17, 54). Many of these changes appear to be mediated by stress kinases such as JNK and to directly impact on muscle protein balance (6, 30).

In summary, our data show that epinephrine stimulates IL-6 expression in both rat and skeletal muscle in vivo and muscle cells in vitro. Epinephrine utilizes predominantly β2-adrenergic receptors to stimulate IL-6 transcription, and this effect is specific, because β2-adrenergic receptor antagonists inhibit epinephrine- but not LPS-induced IL-6 synthesis. Epinephrine and LPS synergize to increase IL-6 mRNA expression, and optimal IL-6 synthesis requires both stress kinase and histone deacetylation activity. The efficacious nature of stress kinase and histone deacetylase inhibitors at inhibiting IL-6 synthesis in C2C12 myoblasts suggests that these inhibitors have potential usefulness in modulating the inflammatory response in skeletal muscle and other tissues.

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