JAK2/STAT3, Not ERK1/2, Mediates Interleukin-6-induced Activation of Inducible Nitric-oxide Synthase and Decrease in Contractility of Adult Ventricular Myocytes*  

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Interleukin (IL)-6 decreases cardiac contractility via a nitric oxide (NO)-dependent pathway. However, mechanisms underlying IL-6-induced NO production remain unclear. JAK2/STAT3 and ERK1/2 are two well known signaling pathways activated by IL-6 in non-cardiac cells. However, these IL-6-activated pathways have not been identified in adult cardiac myocytes. In this study, we identified activation of these two pathways during IL-6 stimulation and examined their roles in IL-6-induced NO production and decrease in contractility of adult ventricular myocytes. IL-6 increased phosphorylation of STAT3 (at Tyr705) and ERK1/2 (at Tyr204) within 5 min that peaked at 15–30 min and returned to basal levels at 2 h. Phosphorylation of STAT3 was blocked by genistein, a protein tyrosine kinase inhibitor, and AG490, a JAK2 inhibitor, but not PD98059, an ERK1/2 kinase inhibitor. The phosphorylation of ERK1/2 was blocked by PD98059 and genistein but not AG490. Furthermore, IL-6 enhanced de novo synthesis of iNOS protein, increased NO production, and decreased cardiac contractility after 2 h of incubation. These effects were blocked by genistein and AG490 but not PD98059. We conclude that IL-6 activated independently the JAK2/STAT3 and ERK1/2 pathways, but only JAK2/STAT3 signaling mediated the NO-associated decrease in contractility.

IL-6, a pro-inflammatory cytokine, is produced during the acute phase of the immune response by macrophages, T cells, B cells, and non-immune cells such as endothelial cells (1). After binding to its receptor, IL-6 elicits numerous effects including antibody induction, hematopoiesis, thrombocytopoiesis, and acute-phase protein synthesis (1, 2). Significant increases in serum levels of IL-6 and its mRNA and protein expression in cardiac tissues have been reported in patients with several cardiac diseases including congestive heart failure (3–5), myocarditis (3), septic cardiomyopathy (6), myocardial infarction (7–9), cardiac myxoma (10), and the cell injury associated with ischemia/reperfusion (11) and cardiopulmonary bypass (12). Thus, IL-6 has been suggested to play an important role in the pathophysiology of these cardiac disorders.

In vitro studies in papillary muscle isolated from hamster heart showed that IL-6 decreases contractility via a nitric oxide (NO)-dependent pathway during a 20-min exposure (13). IL-6 was also shown to decrease peak cytosolic intracellular Ca2+ ([Ca2+]i) and cell contraction of chick embryonic cardiomyocytes within minutes (14). The acute IL-6-induced suppression of cardiac contractility and [Ca2+]i was suggested to result from activation of Ca2+-dependent NOS, presumably a constitutive endothelial isoform (eNOS) (14). In the same study, IL-6 was shown to induce iNOS expression after a 24-h incubation, and it was suggested that the enhanced iNOS is responsible for the chronic effect of IL-6 on the Ca2+ transient and cell contraction (14). However, the signaling mechanism by which IL-6 activates iNOS and decreases cardiac contractility remains undefined.

IL-6 has been shown to activate both the JAK2/STAT3 pathway (15, 16) and the ERK1/2 pathway through gp130, a signal transducing receptor, in non-cardiac cells (15, 17, 18). For example, studies in liver and neuronal cells have shown that IL-6 induces Tyr705 phosphorylation of STAT3, a transcription factor, via activation of JAK2 (17, 19, 20). Activated STAT3 translocates to the nucleus and activates expression of many genes in response to cytokines (21–24). The role of gp130 in cardiac function has also been examined. One study showed that transgenic mice with overexpression of gp130 displayed hypertrophied ventricular myocardium (25). Studies in fetal murine cardiac myocytes infected with adenovirus carrying wild-type or mutated STAT3 cDNA suggested that the STAT3-dependent signaling pathway plays a role in promoting the hypertrophy induced by leukemia inhibitory factor (LIF), a member of IL-6-related cytokines (26). In addition, activation of the JAK/STAT signaling pathway in rat heart has been associated with the cardiac hypertrophy stimulated by cardiotrophin-1, another member of IL-6-related cytokines (27), and with cardiac dysfunction during ischemia and reperfusion (28), myocardial ischemia (29), and acute myocardial infarction (30). Although different isoforms of JAK/STAT have been associated with effects of IL-6-related cytokines in adult rat heart, direct links between IL-6 stimulation, activation of JAK/STAT, and IL-6-induced cardiac inotropic actions have not been established.

Studies in neonatal rat cardiac myocytes have also shown that ERK1/2 is activated by LIF via a gp130-dependent process (31) and that the ERK1/2 activation is associated with cardiac...
hypertrophy (32). In contrast, LIF-induced hypertrophy occurred only in fetal cardiac myocytes infected with a wild-type STAT3 construct and with the level of ERK1/2 activation showing no difference from those cells infected with mutated STAT3 cDNA (26). Thus, the functional role of gp130-associated activation of ERK1/2 and JAK/STAT in IL-6-induced cardiac effects remains unclear.

In this study, we first demonstrated that IL-6 activated both the JAK2/STAT3 and ERK1/2 pathways in adult ventricular myocytes. We also showed that protein expression of iNOS and the JAK2/STAT3 and ERK1/2 pathways in adult ventricular myocytes were first detectable after 2 h of IL-6 stimulation. Such treatment with IL-6 also decreased the contractile function of ventricular myocytes. Most importantly, we found that the IL-6-induced activation of iNOS and decrease in contractility were mediated by JAK2/STAT3, but not by ERK1/2.

EXPERIMENTAL PROCEDURES

Myocyte Isolation—Single ventricular myocytes were isolated from hearts of adult (3–6-month-old) male Sprague-Dawley rats using enzymatic dissociation as described previously (33). Isolated cells were plated into Petri or culture dishes (Falcon) containing a serum-free, phenol-red-free culture medium and cultured overnight. Myocytes were then treated with 10 ng/ml IL-6 for various periods of time. The use of animals was carried out under a protocol approved by the Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

Western Blot Analysis—Protein preparation and immunoblotting were carried out as described previously (34). Briefly, control and treated myocytes (0.25 × 10^6 cells/group) were lysed in ice-cold lysis buffer containing 20 mM HEPES, 250 mM sucrose, 2 mM dithiothreitol, 2 mM EDTA, 2 mM EGTA, 10 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, 20 μg/ml leupeptin, and 10 μg/ml aprotinin. The concentration of total protein was determined using a Bradford assay (Bio-Rad). 20–40 μg of each protein sample were separated electrophoretically on 10% SDS-polyacrylamide gels and electrotransferred onto nitrocellulose membranes. After blocking with Tris buffer (Bio-Rad) the membranes were then stripped and reprobed with anti-iNOS antibodies (1:1,000 dilution; Cayman Co., Ann Arbor, MI) and anti-STAT3 antibodies (1:1000 dilution; Cell Signaling Technology Inc., Beverly, MA), monoclonal anti-phospho-ERK antibodies (1:1,000 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), or polyclonal anti-iNOS antibodies (1:1,000 dilution; Cayman Co., Ann Arbor, MI) overnight at 4 °C. Immunoblots were detected using enhanced chemiluminescent kits (SuperSignal; Pierce) and analyzed with a densitometer (Bio-Rad). The membranes were stripped and reprobed with polyclonal anti-STAT3 antibodies (1:2,000 dilution; Santa Cruz Biotechnology, Inc.) or polyclonal anti-ERK antibodies (1:2,000 dilution; Santa Cruz Biotechnology, Inc.).

Immunocytochemistry—Myocytes were plated onto sterile multi-chamber slides (Nalgene Nunc Corp., Naperville, IL) in serum-free culture media overnight at 37 °C. After treatment with 10 ng/ml IL-6, cells were washed twice with PBS, fixed with 4% paraformaldehyde for 10 min, and permeabilized with PBS containing 0.1% Triton X-100. Cells were then incubated for 20 min in PBS containing 10% goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA), washed with PBS, and incubated for 60 min with polyclonal anti-iNOS antibodies (1:100; Cayman Co., Ann Arbor, MI) in PBS containing 1.5% goat serum at room temperature. After three washes with PBS (5 min each), cells were incubated for 45 min in PBS containing fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (1:100; Jackson ImmunoResearch Laboratories) and 1.5% goat serum in a dark chamber and then examined using an epifluorescence microscope (Zeiss).

NO Production—NO production was assessed by measuring nitrate and nitrite (NOx) in culture media and cell lysates. Myocytes were incubated in serum-free and phenol-red-free culture medium in the absence and presence of 10 ng/ml IL-6 for 2, 4, and 24 h before being centrifuged at 100,000 × g for 10 min. The supernatant (culture media) was removed and kept at −20 °C until the NOx assay. The pellets were resuspended in 500 μl of PBS (pH 7.4), half of which was used to determine NOx. The other half was sonicated and centrifuged at 10,000 × g for 20 min. The resulting supernatant was centrifuged at 100,000 × g for 15 min at 4 °C, and the final supernatant was filtered through a 10-kDa molecular mass cut-off filter (Fisher Scientific, Pittsburgh, PA) at 12,000 × g for 30 min. These cell lysates were kept at −20 °C until the NOx assay. The concentration of NOx in cell lysates and culture media was determined using a colorimetric assay kit with a detection limit of 2.5 μM (Cayman Co., Ann Arbor, MI) according to the manufacturer’s instructions. Nitrate/nitrite concentrations were averaged from duplicate or triplicate measurements and reported as nmol NOx per mg cell protein. Results of treated groups were then normalized to each untreated control.

Measurements of Cell Shortening (CS)—After the designated exposure duration, contraction of ventricular myocytes was elicited by field stimulation (2-ms duration, 1.5-fold threshold voltage) at 0.5 Hz in normal Tyrode’s solution containing the following (in mM): 140 NaCl, 5.4 KCl, 1 CaCl2, 0.8 MgCl2, 10 HEPES/Tris, and 5.6 glucose. CS was monitored using an edge motion detector (Crestec Electronics, Sandy, UT) as described previously (35). The voltage signal was calibrated to determine actual motion (μm). Post-rest potentiation (PRP), which has been used to assess cardiac Ca2+ handling and contractile function (36, 37), was measured as the first contraction after given rest intervals (e.g. for 30 s (PRP30) or 60 s (PRP60)). The relative amplitude of PRP CS was presented by normalizing its peak amplitude to that of steady-state CS before the rest interval.

Chemicals—Recombinant rat IL-6 was purchased from Pepro Tech Inc (Rocky Hill, NJ); the nitrate/nitrite colorimetric assay kit was obtained from Cayman Co. Cycloheximide, genistein, PD98059, and AG490 were purchased from Calbiochem.

Statistical Analysis—In biochemical assays, all treated groups were normalized to each time control and presented as means ± S.E. Statistical significance (p < 0.05) was evaluated by Student’s t test or analysis of variance with Duncan’s multiple range test.

RESULTS

IL-6-induced Phosphorylation of STAT3 in Adult Rat Ventricular Myocytes—Although activation of JAK/STAT signaling has been associated with gp130-related stimulation by LIF (26) and cardiotoxin (1-27), there were no data demonstrating a direct link of this pathway to IL-6 stimulation in cardiac myocytes. Thus, we first examined whether IL-6 activates the JAK2/STAT3 pathway in adult ventricular myocytes. Fig. 1A shows that phosphorylation of STAT3 at Tyr705 was detected in time controls (C) and myocytes treated with 10 ng/ml IL-6 (I) for indicated times was detected with anti-phospho-STAT3 (Tyr705) antibodies (top panel). After stripping phosphorylation-specific antibodies, total protein levels of STAT3 in the same samples were detected using anti-STAT3 antibodies (lower panel). B, phosphorylation level of STAT3 in each group was presented as a ratio of phosphorylated to total STAT3 (p-STAT3/STAT3), which was then normalized to each untreated time control. Data represent mean ± S.E. for three to four experiments. *p < 0.05, compared with time control.
exposure to IL-6, respectively. The level of phosphorylation returned to basal levels after 2 and 4 h of treatment, and a second increase (≈ 2-fold) was observed at 24 h (Fig. 1B). Total STAT3 protein levels remained relatively constant during the 24-h stimulation (lower panel in Fig. 1A). These results demonstrate that IL-6 activates a STAT3 signaling in adult rat ventricular myocytes.

Fig. 2 shows the effects of three different kinase inhibitors on the phosphorylation of STAT3 after 30 min of exposure to 10 ng/ml IL-6. Cells were pretreated for 30 min with these inhibitors before IL-6 exposure. IL-6-induced phosphorylation of STAT3 at Tyr705 was blocked by AG490 and genistein but not by PD98059. B, combined data from three experiments showing p-STAT3/STAT3 relative to time controls. These inhibitors alone had no significant effect on STAT3 phosphorylation. *, p < 0.05, compared with control; #, p < 0.05, compared with PD98059 alone.

**Fig. 2. Effects of kinase inhibitors on IL-6-induced tyrosine phosphorylation of STAT3.** Myocytes were treated with 10 μM PD98059, 20 μM AG490, or 10 μM genistein alone for 1 h or 30 min before and during exposure for 30 min to 10 ng/ml IL-6. A, a representative experiment showing that IL-6-induced tyrosine phosphorylation of STAT3 (p-STAT3; top panel) was blocked by AG490 and genistein but not by PD98059. B, combined data from three experiments showing p-STAT3/STAT3 relative to time controls. These inhibitors alone had no significant effect on STAT3 phosphorylation. *, p < 0.05, compared with control; #, p < 0.05, compared with PD98059 alone.

**Fig. 3. Time course of tyrosine phosphorylation of ERK1/2 in response to IL-6 stimulation.** A, phosphorylation of ERK1/2 at Tyr204 (p-ERK1/2; top panel) in the same cell lysates as shown in Fig. 1, which was then immunoblotted for total ERK1/2 (lower panel). B, combined data from three experiments showing level of ERK2 (p-ERK2/ERK2) relative to time controls. *, p < 0.05, compared with control.

**Fig. 4. Effects of kinase inhibitors on IL-6-induced tyrosine phosphorylation of ERK2.** A, an inhibition of IL-6-induced tyrosine phosphorylation of ERK2 by PD98059 and genistein in the same protein samples used for the detection of STAT3 phosphorylation. B, combined data from three experiments showing relative p-ERK2/ERK2 to time controls. *, p < 0.05, compared with control.

**Fig. 5A.** Expression of iNOS Protein Induced by IL-6 in Adult Rat Ventricular Myocytes—Although iNOS expression was detected after 24 h of IL-6 stimulation in chick embryonic heart cells (14), the time course of induction of iNOS has not been examined in adult cardiac myocytes. Fig. 5A shows the time-dependent increase in iNOS protein (~130 kDa) expression in response to 10 ng/ml IL-6 in adult rat ventricular myocytes. iNOS protein was detectable at 1 h but was increased after 2, 4, and 24 h of incubation with IL-6. Fig. 5B, which represents
combined data from five experiments, shows that iNOS protein expression in IL-6-treated myocytes was increased 9-fold, compared with time controls after 2 h of treatment. Similarly, immunocytochemical data in Fig. 5C show that iNOS was evident in a myocyte after 2 h of exposure to 10 ng/ml IL-6, whereas it was barely detectable in 2-h time control myocytes or after 1 h of IL-6 stimulation. The iNOS expression induced by a 2-h incubation with 10 ng/ml IL-1β, a well known inducer of iNOS in cardiac myocytes (38), was used as a positive control in these experiments. Thus, these results suggest that IL-6 induces iNOS protein expression within 2 h of exposure.

We then determined whether de novo protein synthesis is involved in IL-6-induced iNOS protein expression. Fig. 6A shows that total NO\textsubscript{x} concentration in culture medium was increased after a 2-h exposure to 10 ng/ml IL-6 (note that the IL-6-induced NO\textsubscript{x} production was blocked completely by 50 nmol/liter 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine, a potent and selective inhibitor of iNOS) (39). This 2.5-fold increase in NO\textsubscript{x} production was abolished in the presence of 10 μM cycloheximide (CHX), an inhibitor of protein synthesis that was applied 30 min prior to and during the IL-6 treatment. The NO\textsubscript{x} concentration in the 2-h time control was 2.65 ± 0.46 nmol/mg cell protein and was not affected by CHX. Similarly, Fig. 6B shows that under the same conditions of IL-6 treatment, the NO\textsubscript{x} concentration in cell lysates was increased −3.5-fold of the time control after 2 h of incubation. CHX completely blocked this IL-6-induced increase in NO\textsubscript{x} production and had no effect on the basal level (2.18 ± 0.31 nmol/mg cell protein) in cell lysate. These results suggest that de novo protein synthesis and the activity of iNOS are required for IL-6-induced NO production during a 2-h exposure.

**Role of JAK2/STAT3 Pathway and ERK1/2 Pathway in IL-6-induced Decrease in Cardiac Contractility**—Our preliminary findings showed that IL-6 decreased contractility in adult ventricular myocytes as demonstrated by reductions in PRP and the responsiveness to extracellular Ca\textsuperscript{2+} concentrations (39). Thus, we examined whether the JAK2/STAT3 and/or ERK1/2 pathways are involved in the IL-6-induced negative inotropic effect. Fig. 9A shows representative data of PRP30 and PRP60 in a time-control (upper panel) and an IL-6 treated myocyte (lower panel). The amplitude of PRP30 and PRP60 relative to that of pre-rest steady state is shown in Fig. 9, B and C, respectively. Exposure for 2 h to 10 ng/ml IL-6 reduced PRP30 and PRP60 by −34% (n = 17, p < 0.05) and 32% (n = 16, p < 0.05), respectively. The IL-6-induced decreases in PRP30 and PRP60 were abolished by pretreatment with 20 μM AG490 and 10 μM genistein. In contrast, pretreatment with 10 μM PD98059 had no effect on IL-6-induced iNOS expression. Similar results were observed in five experiments. Fig. 8 shows that IL-6 increased the total NO\textsubscript{x} concentration in cell lysates ~2.4-fold (n = 5, p < 0.05) after 2 h of exposure when compared with time controls. This IL-6-induced increase in NO production was blocked by AG490 and genistein but not by PD98059. Similar results were observed in cell lysate after a 4-h incubation (data not shown). These results suggest that IL-6-induced iNOS protein expression and NO production are mediated by activation of the JAK2/STAT3 pathway, but not the ERK1/2 pathway.

**Fig. 6. Effect of cycloheximide on the IL-6-induced increase in NO production.** Myocytes were treated with and without 10 ng/ml IL-6 for 2 h in the absence and presence of 10 μM CHX, a protein synthesis inhibitor. Some cells were treated with CHX alone for 2 h. A, normalized concentrations of NO\textsubscript{x} in culture media were expressed as ratios of the NO\textsubscript{x} concentration in treated groups to the control value. B, NO\textsubscript{x} concentrations in cell lysates obtained from the same experiments as shown in A. Data represent means ± S.E. from three experiments. *, p < 0.05, compared with the control.
Recently, both IL-6-related cytokines and the JAK/STAT signaling pathway have been suggested to play important roles in cardiac pathophysiology (40). Our preliminary studies suggested that IL-6 decreases contractility of adult rat ventricular myocytes via an iNOS pathway during chronic exposure (39), consistent with findings reported by others using chick cardiomyocytes (14). The mechanism underlying IL-6-induced decrease in cardiac contractility is mediated by activation of the JAK2/STAT3, but not ERK1/2, pathway.

**DISCUSSION**

PRP60 were blocked by 20 μM AG490 and 10 μM genistein but not by 10 μM PD98059. Pretreatment with PD98059, AG490, or genistein alone had no effect on PRP30 or PRP60. The same results were observed after 4 and 24 h of exposure to IL-6 (data not shown). These data suggest that the IL-6-induced decrease in cardiac contractility is mediated by activation of the JAK2/STAT3, but not ERK1/2, pathway.

**Effects of kinase inhibitors on IL-6-induced iNOS protein expression.** Expression of iNOS protein was examined in myocytes after 2 h of exposure to 10 ng/ml IL-6 in the absence and presence of 10 μM PD98059, 20 μM AG490, or 10 μM genistein as shown in Fig. 2. Cell lysate from lipopolysaccharide-treated macrophage 264.7 cells served as positive control. Similar results were observed in four other experiments.

**Effects of kinase inhibitors on IL-6-induced increase in NO production.** Cell lysates were obtained from myocytes pretreated with 10 ng/ml IL-6 for 2 h. Normalized NO concentrations of treated groups were presented as ratio to each control value. The NO production in myocytes treated with kinase inhibitors alone for 2.5 h did not differ from that in time controls. Data represent means ± S.E. from three to eight experiments. *, p < 0.05, compared with control; #, p < 0.05, compared with PD98059 alone.

**Effects of kinase inhibitors on IL-6-induced decrease in post-rest potentiation.** A, representative cell shortening traces of PRP after 30 (PRP30)- and 60-s (PRP60) rest intervals in a time control and a myocyte pretreated with 10 ng/ml IL-6 for 2 h. B, relative PRP30 (top panel) and PRP60 (lower panel) normalized to the pre-rest steady-state amplitude of cell shortening from time controls and myocytes pretreated with PD98059, AG490, or genistein for 30 min before exposure to 10 ng/ml IL-6 for 2 h. These kinase inhibitors alone had no effect on RP30 or PRP60 after 2.5 h of incubation. Data represent means ± S.E. from four to seventeen cells. *, p < 0.05, compared with control; #, p < 0.05, compared with PD98058 alone.

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**Effects of kinase inhibitors on IL-6-induced decrease in post-rest potentiation.** A, representative cell shortening traces of PRP after 30 (PRP30)- and 60-s (PRP60) rest intervals in a time control and a myocyte pretreated with 10 ng/ml IL-6 for 2 h. B, relative PRP30 (top panel) and PRP60 (lower panel) normalized to the pre-rest steady-state amplitude of cell shortening from time controls and myocytes pretreated with PD98059, AG490, or genistein for 30 min before exposure to 10 ng/ml IL-6 for 2 h. These kinase inhibitors alone had no effect on RP30 or PRP60 after 2.5 h of incubation. Data represent means ± S.E. from four to seventeen cells. *, p < 0.05, compared with control; #, p < 0.05, compared with PD98058 alone.
The time course of iNOS induction by cytokines in cardiac myocytes is not clearly defined. For example, IL-6-induced iNOS expression was detected in chick embryonic heart cells after a 24-h incubation (14). However, IL-6 levels may be increased for relatively short periods after insults such as cardiomyopathic bypass. The present study examined the time course of IL-6-induced iNOS expression in adult ventricular myocytes and found that de novo synthesis of iNOS protein was detected within 2 h of IL-6 stimulation. Such increased iNOS is accompanied by an increase in NO production and a decrease in contractile function. These IL-6-elicted cardiac effects were sustained during 24 h of exposure to the cytokine.

The iNOS gene promoter region has three sites for STAT binding (42). Studies using human LDL-1 cells, a colon epithelial-derived cell line, showed that a cytokine mixture of INF-γ, IL-1β, and tumor necrosis factor-α induced iNOS expression via activation of the JAK2/STAT1α pathway, which was blocked by AG490 (43). INF-γ-induced iNOS expression has also been shown to be paralleled by STAT1α activation in adult rat ventricular myocytes (44). Therefore, it is likely that STAT3 activation also mediates IL-6-induced iNOS protein synthesis. In the present study, we showed that genistein and AG490 block IL-6-induced STAT3 phosphorylation, iNOS protein expression, NO production, and negative inotropy. These results are consistent with the hypothesis that the IL-6-induced JAK2/STAT3 signaling pathway mediates its induction of iNOS, thereby increasing NO production and decreasing cardiac contractility.

The ERK1/2 pathway has been shown to play an important role in the cardiac hypertrophy induced by a variety of stimuli. However, the role of IL-6-induced increases in ERK1/2 phosphorylation in cardiac function is still unclear. For example, gp130-dependent activation of ERK1/2 induced by LIF induces hypertrophy in cardiac myocytes (26). The same study also showed that LIF-induced increase in STAT3-dependent c-fos and atrial natriuretic factor mRNA expression was attenuated by inhibition of ERK1/2 by PD98059, suggesting a cross-talk between JAK2/STAT3 and ERK1/2 pathways (26). In contrast, the present study showed that the iNOS expression, NO production, or negative inotropy induced by IL-6 was not affected by inhibition of ERK1/2. Although the role of IL-6-induced activation of ERK1/2 in cardiac function remains unclear, it is clear that ERK1/2 activation is not involved in the JAK2/STAT3 signaling pathway nor the negative inotropic actions of IL-6 in adult ventricular myocytes. In contrast to our results, activation of ERK1/2 has been shown to be essential to the induction of iNOS expression elicited by a cytokine mixture of INF-γ and IL-1β in adult rat ventricular myocytes (44). This discrepancy may result from activation of different STATs by different cytokines. For example, the binding of INF-γ and IL-1β activates STAT1α, whereas IL-6 activates primarily STAT3. The activation of STAT1α might be associated with both ERK1/2 and JAK2 activation, whereas only JAK2 is involved in STAT3 activation.

In summary, both the JAK2/STAT3 and ERK1/2 pathways are activated transiently by IL-6 in adult rat ventricular myocytes. Only the activation of JAK2/STAT3 mediates the IL-6-induced increase in iNOS protein expression, increase in NO production, and decrease in cardiac contractility. Thus, the IL-6-activated JAK2/STAT3 signaling pathway could account for the cardiac dysfunction observed in many cytokine-associ-ated cardiac disorders such as acute myocardial infarction, ischemia/reperfusion, myocarditis, or cardiomyopathic bypass.