A Role for the Extracellular Signal-regulated Kinase and p38 Mitogen-activated Protein Kinases in Interleukin-1β-stimulated Delayed Signal Transducer and Activator of Transcription 3 Activation, Atrial Natriuretic Factor Expression, and Cardiac Myocyte Morphology*

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Dominic C. H. Ng†, Carin S. Long‡, and Marie A. Bogoyevitch§,

From the †Department of Biochemistry, University of Western Australia, Crawley 6009, Australia, the ‡Cardiology Section, Denver Health Medical Center and the University of Colorado, Denver, Colorado 80204, and the ¶Western Australian Institute for Medical Research, Perth, Western Australia 6000, Australia

We have demonstrated that two hypertrophic agents, interleukin-1β (IL-1β) and leukemic inhibitory factor (LIF), altered cardiac myocyte morphology with striking similarity and prompted us to investigate the common actions of these cytokines. We compared the phosphorylation/activation of signal transducer and activator of transcription 3 (STAT3), extracellular signal-regulated kinase (ERK), p38MAPK, and c-Jun N-terminal kinase mitogen-activated protein kinases (MAPKs). The phosphorylation of STAT3 by IL-1β was delayed (>60 min), whereas the response to LIF was rapid (<10 min) and transient. We confirmed that IL-1β potently stimulated all three MAPK subfamilies. In contrast, LIF promoted strong activation of ERKs, marginal activation of p38MAPK, and no c-Jun N-terminal kinase activation. To test the roles of ERKs and p38MAPK, myocytes were pre-treated with PD98059 and SB203580. Either inhibitor alone prevented STAT3 phosphorylation, implicating ERKs and p38MAPK in the delayed STAT3 response to IL-1β. The interplay of MAPKs and STAT3 phosphorylation in regulating IL-1β-stimulated hypertrophy was investigated by evaluating the effect of MAPK inhibitors on atrial natriuretic factor (ANF) expression and myocyte morphology. The specific inhibition of either ERK or p38MAPK attenuated the IL-1β- or LIF-stimulated ANF expression by up to 70%. Inhibition was not further increased in the presence of both inhibitors. Furthermore, although individual inhibition of ERK or p38MAPK did not affect morphology, co-treatment with both inhibitors abrogated the hypertrophic morphology stimulated by IL-1β but not by LIF. Taken together, our data indicate that the activation of ERK and p38MAPK is essential in regulating a delayed STAT3 phosphorylation as well as changes in ANF expression and morphology that follow IL-1β treatment. Thus, the role of MAPKs in the hypertrophic response can be dictated at least partly by the nature of the hypertrophic agent employed.

The molecular mechanisms underlying in the development of left ventricular cardiac hypertrophy (LVH) have been investigated extensively in recent years. LVH develops as a compensatory mechanism in the adult animal in response to increased volume or pressure load (1). Several of the molecular and cellular aspects of LVH are also observed in vitro in primary cultures of neonatal rat cardiac myocytes (2). Thus, there has been extensive study of neonatal cardiac myocytes cultured in vitro as a robust model for elucidating the molecular mechanisms underlying LVH development in the adult animal. Importantly, many of the predictions that have been made using this model have been confirmed in vivo (2–4). The discovery of the importance of gp130 in the development of the hypertrophic morphology is one striking example (5, 6). Similarly, predictions of the role of other signaling intermediates in hypertrophy have been confirmed by transgenic overexpression in vivo (7–9). Established markers of LVH both in vivo and in vitro include the re-expression of various fetal genes such as atrial natriuretic factor (ANF), the reorganization of actin, and an increase in individual myocyte size. These changes can be induced in vitro by a diverse range of chemical stimuli including growth factors, G-protein-coupled receptor (GPCR) agonists, and cytokines (3).

One such proinflammatory cytokine is interleukin (IL)-1β. Elevated IL-1β levels have been detected after acute myocardial infarction and in hypertrophic hearts under mechanical overload (10, 11). These correlations have lead to direct testing of the effects of IL-1β on cultured cardiac myocytes. This has confirmed that IL-1β can also induce cardiac hypertrophy in this in vitro model (12–14).

Despite intense study, the molecular mechanisms involved in the development of cardiac hypertrophy elicited by any class of stimulus remain unclear. However it is generally accepted that at least two distinct families of signal transduction intermediates are activated after stimulation by hypertrophic agonists. The first group is the mitogen-activated protein kinase (MAPK) family of serine/threonine kinases (15). This group has three subfamilies: the extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), and the p38 MAPKs (p38MAPK). Because of their potent activation by several stress

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† To whom correspondence should be addressed: Cell Signaling Laboratory, Dept. of Biochemistry, University of Western Australia, 35 Stirling Hwy., Crawley, Western Australia 6009, Australia. Tel.: 61-8-9380-1348; Fax: 61-8-9380-1148; E-mail: marieb@cyllene.uwa.edu.au.

§ The abbreviations used are: LVH, left ventricular hypertrophy; ANF, atrial natriuretic factor; GPCR, G-protein-coupled receptor; IL, interleukin; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; JAK, Janus kinase; STAT, signal transducer and activator of transcription; LIF, leukemia inhibitory factor; BSA, bovine serum albumin.
stimuli and inflammatory cytokines, the JNKs and p38MAPK have been also been called the “stress-activated” protein kinases (SAPks) (15). Substantial controversy surrounds the relationship between MAPK activation and cardiac hypertrophy. JNKs and p38MAPK have been implicated in hypertrophy stimulated by GPCR agonists such as phenylephrine and endothelin (16–18). Conversely, ERKs have been implicated by the overexpression of constitutively active components of the ERK pathway leading to ANF expression, actin reorganization, and cellular growth (7, 19, 20) and that antisense to ERK MAPKs could prevent hypertrophy in response to phenylephrine (21). There has continued to be conflicting evidence for the importance for the ERKs in GPCR agonist-stimulated hypertrophy (22–24). However, two recent in vivo studies have highlighted the critical role of MAPKs in the cardiac hypertrophic response. Specifically, transgenic overexpression of the dual-specificity phosphatase MKP-1 to inhibit p38MAPK, JNK, and ERK activation could limit hypertrophic growth, whereas transgenic overexpression of the ERK activator, MAPK/ERK kinase-1, could promote the hypertrophic response (7, 25).

The second group of potentially important signal transducers in hypertrophy comprises the Janus kinases (JAKs) and their downstream transcription factor effectors, the signal transducers and activators of transcription (STATs). The JAK/STAT pathway, specifically that involving STAT3, has been implicated in signal transduction by the gp130-signaling family of cytokines (5). The functions of STAT3 are apparently cell type-specific and range from growth arrest of myeloid cells to the maintenance of proliferation of embryonic stem cells (26, 27). A role for STAT3 in regulating cardiac hypertrophy has been suggested by the complete abrogation of LIF-induced ANF mRNA expression in myocytes expressing dominant negative STAT3 and enhanced ANF expression after overexpression of wild-type STAT3 (5). As yet, no cardiac-specific transgenic overexpression of JAKs has addressed the role of these protein kinases in the development of cardiac hypertrophy in vivo, but STAT3 overexpression indicates that this transcription factor mediates cardiac hypertrophy and cardiac cell protection (28).

The question of cross-talk between the MAPKs and the STATs and the possible roles of such cross-talk in the regulation of cardiac hypertrophy have also not been addressed. In general, it would seem that any investigation into the role of STATs in hypertrophy has been predominantly restricted to gp130-signaling cytokines. However, possible autocrine/paracrine signaling loops involving gp130-signaling cytokines have been proposed recently to mediate the response to the GPCR agonist, angiotensin II (29). Furthermore, much has been made of the particular phenotype of the cardiac myocyte in response to gp130-signaling cytokines. Specifically, these cells have been described as taking on a more elongated appearance when compared with GPCR-stimulated cells (30). This morphology is reminiscent of the elongated cells seen in the end-stage failing heart (31). Although the specific morphology seen in IL-1β-treated cells had not similarly been elaborated on in previous reports, its similarity to that seen with that of gp130-linked agonists prompted the present study evaluating in more detail the signaling pathways stimulated by IL-1β that lead to hypertrophy.

In the present study, we observed striking similarities in morphology after 24 h of exposure to IL-1β or LIF. We confirmed that IL-1β potently and rapidly stimulated all three MAPK subfamilies (ERK, JNK, and p38MAPK) but for the first time demonstrated its capacity to elicit a delayed STAT3 phosphorylation. Using specific chemical inhibitors, we have demonstrated for the first time a role for ERK and p38MAPK in regulating this delayed STAT3 response. Furthermore, ANF expression induced by IL-1β depended on ERK or p38MAPK activity, whereas the morphological changes typical of hypertrophy required the activity of both ERK and p38MAPK. This implicates both ERKs and p38 MAPKs in the development of hypertrophy in response to IL-1β and also emphasizes that it is possible to discriminate between the morphological and transcriptional changes that typify the hypertrophic response.

EXPERIMENTAL PROCEDURES

Materials—Antibodies to STAT3, ERK1/2, and p38MAPK were from Santa Cruz Biotechnology. Antibodies to phosphoSTAT3, phospho-ERK, phospho-p38MAPK, and ANF were from Upstate Biotechnology, Promega, New England Biolabs, and Peninsula, respectively. Horseradish peroxidase-labeled secondary antibodies and chemiluminescence reagents were from Pierce. Fluorescent probes were from Molecular Probes. The Vectashield mounting medium was from Vector Laboratories. IL-1β, PD98059, and SB203580 were from Calbiochem. Penicillin/streptomycin and sera were from Life Technologies, Inc.

Primary Cultures of Myocytes—Cardiac ventricular myocytes were isolated from 1 day-old Harlan Sprague Dawley rats (32). Briefly, ventricular cells were isolated by collagenase digestion and then pre-plated to deplete nonmyocyte cells. Cardiac myocytes were then plated on gelatin-coated 60-mm dishes in Dulbecco’s modified Eagle’s medium/ Medium 199 (4:1 v/v) containing 10% (v/v) horse serum, 5% (v/v) fetal calf serum, and penicillin/streptomycin (100 units/ml). The serum was removed after 18 h, and then cells were starved for 24 h prior to treatment with LIF (10 ng/ml), ATP (10 mM), or phenylephrine (100 μM). Where indicated, cells were pretreated with chemical inhibitors (PD098059 10 μM, SB203580 10 μM) for 60 and 30 min, respectively. In all cases, we ensured that the inhibitors were not toxic at the doses and times used.

Western Blotting—Protein extracts from 4 × 106 myocytes were prepared using lysis cells in 20 mM Hepes, pH 7.7, 2.5 mM MgCl2, 0.1 mM EDTA, 20 mM β-glycerophosphate, 10 mM NaCl, 0.05% (v/v) Triton X-100, 500 μM dithiothreitol, 100 μM Na3VO4, 20 μM leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride, and 20 μg/ml aprotinin. After 10 min on ice, cell debris was removed by centrifugation (14,000 × g, 10 min, 4 °C). The protein concentration was determined by a Bio-Rad protein concentration assay. Western blot analysis with phospho-specific antibodies followed manufacturer instructions. Proteins were visualized by enhanced chemiluminescence using Supersignal reagents. Membranes were then stripped and reprobed with antibodies for the appropriate total protein as appropriate.

JNK Activity Assay—Glutathione S-transferase-c-Jun (1–135) was used to purify JNK from myocyte protein extracts (33). The kinase reaction was performed in 20 mM Hepes, pH 7.6, 20 mM MgCl2, 0.1 mM dithiothreitol, 500 μM Na3VO4, and 20 μM ATP supplemented with 1 μCi of [γ-32P]ATP/assay at 30 °C for 25 min. The reaction mixture was resolved by SDS-polyacrylamide gel electrophoresis, visualized by autoradiography, and incorporated [32P] quantitated by Cerenkov counting.

Immunocytochemistry—After treatment, the cells were fixed in 4% (w/v) paraformaldehyde, permeabilized with 0.2% (v/v) Triton X-100 in PBS, and blocked with 10% (v/v) fetal calf serum in PBS. Actin was stained with tetramethylrhodamine isothiocyanate-phallolidin (1:400 in 5 mg/ml Triton X-100, pH 7.4/1 mM EDTA).

RESULTS

Similarities in the Hypertrophic Response Stimulated by LIF and IL-1β—Neonatal cardiac myocytes have provided a robust model system in which to test the mechanism underlying the development of cardiac hypertrophy. Importantly, the system permits the rapid and reproducible sampling that is required to evaluate the signal transduction events initiating cardiac hypertrophy while also minimizing potentially confounding factors such as cell heterogeneity or inter-animal variation. The hyper-
trophic response of cultured cardiac myocytes is characterized by increased cell size, actin organization, and expression of a number of genes from the “fetal gene program” including ANF (2). Although GPCR agonists stimulate morphological changes in which cells enlarge in length and width, gp130-signaling cytokines stimulate elongation of the cell in absence of width changes (30). These differences in cell morphology have been proposed to reflect potentially important differences in signal transduction pathways as well as differences in the hypertrophic responses to pressure or volume-overload in vivo (30). We were interested in the signal transduction mechanisms employed by IL-1β to elicit a hypertrophic response. We first examined the morphology of unfixed cultured cardiac myocytes stimulated for 24 h with different hypertrophic agents. Fig. 1A (bottom right panel) shows that IL-1β increased myocyte cell size and that this was accompanied by cell elongation with distinct projections. This was similar to the overall morphology of hypertrophic myocytes stimulated by the gp130-signaling cytokine, LIF (Fig. 1A, bottom left panel). In contrast, myocytes stimulated with the α-adrenergic agonist, phenylephrine, were triangular in appearance (i.e. enlarged in length and width) and showed no projections (Fig. 1A, top right panel).

Changes in cell morphology were confirmed in fixed cells stained with tetrachloride isothiocyanate-phalloidin to highlight rearrangement of actin filaments. Again, elongated myocytes with distinct projections were evident with IL-1β and LIF treatment but not in phenylephrine-treated or control cells (Fig. 1B). This confirms previous reports of IL-1β-stimulated cardiac myocyte hypertrophy (12–14). In this same field of cells, we confirmed that IL-1β, LIF, or phenylephrine stimulated the expression of ANF as characterized by prominent perinuclear staining (Fig. 1B). Thus, all three agents tested could stimulate cardiac hypertrophy, with IL-1β and LIF showing striking similarities in overall cell morphology.

IL-1β Is a Potent and Rapid Activator of ERK, p38, and JNK MAPKs—We were interested in how IL-1β stimulated cardiac

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**Fig. 1. Stimulation of hypertrophic myocyte morphology and ANF expression by IL-1β, LIF, and phenylephrine.** Serum-starved myocytes were treated for 24 h with IL-1β (1 ng/ml), LIF (10 ng/ml), phenylephrine (100 μM), or 0.1% (v/v) BSA in PBS as a control. A, morphology of the cells was recorded by photography under phase-contrast microscopy. B, the cells were fixed and stained with phalloidin, anti-ANF antibody, and Hoechst 33258. Cell staining is either shown individually or combined as a triple stain (Triple-Stain). These results are representative of two individual experiments. Each scale bar represents 20 μm.
myocyte hypertrophy and whether LIF and IL-1β could employ similar signal transduction mechanisms despite differences in their receptors (34, 35). There is considerable evidence implicating a role for each of the three subfamilies of the MAPK proteins in hypertrophy (for reviews see Refs. 3 and 15). We confirmed the activation of ERK, p38MAPK, and JNK by IL-1β over a 10–120-min period (36). Specifically, IL-1β stimulated the maximal phosphorylation of the ERKs (2.2 ± 0.2-fold,Fig. 2A) and p38MAPK (5.7 ± 2.1-fold, Fig. 2B) at 10 min, and this decreased by 120 min. In comparison, LIF was also a potent stimulus for ERK phosphorylation (2.3 ± 0.1-fold); however, p38MAPK phosphorylation was only modest (Fig. 2A and B). When JNK MAPK activation was examined, JNK activity was maximally stimulated (3.9 ± 0.4-fold over control levels) after IL-1β treatment for 10 min (Fig. 2C). In contrast, LIF did not activate JNK at any time point examined (Fig. 2C). Thus, despite similar changes to cell morphology, IL-1β and LIF differed in their activation of the MAPK subfamilies.

A Delayed Phosphorylation and Nuclear Translocation of STAT3 After IL-1β Exposure—The JAK/STAT pathway has been suggested recently to be involved in regulating hypertrophy in vivo as well as in vitro (5, 28, 37). Specifically, STAT3 has been shown to play a major role in the hypertrophic signaling pathways activated by the gp130-signaling cytokines LIF and cardiotrophin-1 (5, 37) and in addition was shown to induce myocardial hypertrophy in the hearts of transgenic mice when overexpressed (28). We confirmed that LIF stimulates a potent, rapid, and transient phosphorylation of Tyr-705 of STAT3 (10.5 ± 2.8 maximal-fold increase at 10 min over control), which is required for activity (Fig. 3A). Surprisingly, IL-1β also stimulated STAT3 phosphorylation, but this was delayed (60 min) and was less robust (3.7 ± 0.8-fold increase at 60 min, 1.7 ± 0.3-fold increase at 120 min, Fig. 3A). When data from three separate experiments were compared, the maximal STAT3 phosphorylation in response to 1 ng/ml IL-1β was 35% of the response to 10 ng/ml LIF or 72% of the response to 1 ng/ml LIF (2).

The phosphorylation of STAT3 promotes its dimerization and translocation to the nucleus where it can bind DNA and
regulate transcription (38). By immunocytochemical staining, we confirmed STAT3 translocation to the nucleus of myocytes after LIF treatment for 10 min (Fig. 3B). Treatment with IL-1β for 10 or 30 min failed to promote nuclear accumulation of STAT3 protein. Only after treatment of myocytes with IL-1β for 60 min could modest STAT3 translocation be observed (Fig. 3B). Therefore, the time course and extent of STAT3 nuclear translocation correlated with the levels and time course of STAT3 phosphorylation by LIF or IL-1β.

**IL-1β-stimulated STAT3 Phosphorylation Is Regulated by either ERK or p38MAPK**—The MAPKs regulate a variety of different biological processes through their actions in the cell cytoplasm and nucleus (15). There have been recent suggestions of cross-talk between the MAPK pathways and STAT3 (39). We therefore investigated whether the rapid activation of ERK and p38MAPK might regulate the delayed STAT3 phosphorylation by IL-1β. Two chemical inhibitors, PD90859 (10 μM, a specific MAPK/ERK kinase/ERK inhibitor) and SB203580 (10 μM, a direct inhibitor of p38MAPK), were employed (40, 41). We found that either PD98059 or SB203580 alone attenuated IL-1β-stimulated STAT3 phosphorylation by 72 ± 9% (p < 0.01) and 65 ± 8% (p < 0.01), respectively (Fig. 4, A and B). This implicates a role for both ERK and p38MAPK in regulating STAT3 activation by IL-1β. In control experiments, we confirmed that each inhibitor was specific for its particular MAPK pathway at the doses employed.²

The IL-1β-stimulated STAT3 Phosphorylation Requires de Novo Protein Synthesis—To our knowledge, there have been no previous reports of the involvement of STAT3 in the IL-1β signaling pathway (42). However, because of the delayed response (>60 min) observed in Fig. 3A, it might be expected that IL-1β stimulates STAT3 activation through indirect mechanisms such as the synthesis of secondary signaling molecules. The presence of the protein synthesis inhibitor, cycloheximide (20 μM), blocked stimulation by IL-1β, with STAT3 phosphorylation levels inhibited by 76 ± 12% (p < 0.01). However, cycloheximide did not alter the total level of STAT3 (Fig. 5). As expected, cycloheximide had no effect on LIF-stimulated STAT3 phosphorylation, thereby confirming the specificity of the effect (Fig. 5). Thus, the delayed STAT3 activation by IL-1β required de novo protein synthesis.

**IL-1β-stimulated Hypertrophic Morphology and ANF Expression Is Regulated by ERK and p38MAPK in Combination**—Numerous studies have reported a role for MAPKs in regulating features of cardiac myocyte hypertrophy (16, 17, 19, 23, 24). We were interested in whether the potent activation of ERK and p38MAPK, and subsequent STAT3 activation, could be involved in signaling to changes in morphology or gene expression as exemplified by increased ANF expression. In 24 h, IL-1β or LIF stimulated an increase in ANF expression, with 24 ± 2% and 57 ± 3% of respective cells positively stained compared with 2% of untreated cells (Fig. 6A). This is in general agreement with the levels of ANF expression induced by other hypertrophic stimuli. For example, MAPK/ERK kinase-1 overexpression and phenylephrine and endothelin-1 stimulation results in ANF positively stained myocytes that range from 20 to 75% of total cells (7, 43–45). SB203580 (10 μM) pretreatment of IL-1β-stimulated cells partially inhibited ANF positively stained cells to 14 ± 2% (Fig. 6A). The MAPK/ERK kinase-specific inhibitor (PD98059, 10 μM) pretreatment proved more potent, significantly reducing the percentage of positively stained cells after IL-1β to 7 ± 1% (Fig. 6A). LIF stimulation of ANF expression was similarly inhibited by identical doses of SB203580 (45 ± 2% of total cells stained) and PD98059 (16 ± 1% of total cells stained).
Despite the usual association of ANF expression and cardiac hypertrophy, the sole use of ANF as a hypertrophic marker has, however, been questioned in some recent studies. When a transgenic mouse model of hypertrophic cardiomyopathy was examined, it was clear that hypertrophy could occur in the absence of increased ventricular levels of ANF and that increased levels of this ANF could also occur in the absence of detectable cardiac hypertrophy (46). Similarly, in deoxycorticosterone acetate salt-induced hypertension there was no direct correlation between hypertrophy and ANF expression (47). This lack of association has also been suggested by studies in the neonatal cultured myocyte model that have dissected the intracellular signaling events leading to hypertrophy. Specifically, transcriptional and morphological changes that typify the hypertrophic response can be separated (48). We therefore sought further evidence for the role of MAPKs in the morphological responses of hypertrophy.

When we examined IL-1β-stimulated hypertrophic morphology, we found that the chemical inhibitors, individually, did not have an effect (Fig. 6C). Cardiac myocytes treated with IL-1β remained elongated with projections in the presence of PD98059 or SB203580. However, IL-1β-treated myocytes failed to develop the elongated morphology when pretreated with PD98059 and SB203580 simultaneously (Fig. 6C). In the presence of both inhibitors, the myocytes resembled control cells. These results demonstrate that ERK and p38MAPK must be coordinately responsible for the regulation of IL-1β-stimulated hypertrophic myocyte morphology. In contrast, the co-treatment with these inhibitors did not affect LIF-stimulated morphological changes (Fig. 6D). This highlights the differences in the mechanisms used by IL-1β and LIF to produce similar long-term cellular changes.

**DISCUSSION**

Elevated levels of proinflammatory cytokines such as IL-1β have been implicated in the initiation and progression of various disease states of the heart (10, 11). IL-1β has also been shown to stimulate hypertrophy in the in vitro model of cultured neonatal cardiomyocytes (12–14). However, the earliest events in intracellular signaling transduction utilized by IL-1β to initiate these cardiac-specific changes are largely unknown. In this study, we have demonstrated that the hypertrophic cell morphology stimulated by IL-1β exposure was very similar to that stimulated by the gp130 receptor-signaling cytokine, LIF, and not the GPCR agonist, phenylephrine (Fig. 1). This is in agreement with previous separate reports describing the morphological changes associated with either LIF or IL-1β stimulation but not directly comparing both (14, 30).

Although the pathways mediating LIF-stimulated hypertrophy remain controversial, both the MAPKs and the STATs have been implicated (37, 49). Because of the striking similarities in cell morphology, we therefore asked whether these same pathways could be activated by IL-1β as well as LIF. This revealed a number of interesting features in intracellular signaling profiles shared by these cytokines. When examining the MAPK activation, we observed that LIF selectively activated ERK and p38MAPK but failed to activate JNK MAPKs (Fig. 2). In contrast, we confirmed that IL-1β rapidly activated all three MAPK subfamilies (Fig. 2) (38). As expected (5), LIF stimulated phosphorylation and translocation of STAT3 to the nucleus (Fig. 3). Although there have been no previous reports of links between IL-1β and STAT activation, we found that IL-1β stimulated a delayed and lower level of phosphorylation of STAT3 accompanied by its delayed nuclear localization (Fig. 3). This indicated that the two different receptor types shared the activation of ERKs, p38MAPK, and STAT3 but not JNKs and that

![Fig. 4. IL-1β-stimulated STAT3 phosphorylation requires ERK or p38MAPK](#)

![Fig. 5. Cycloheximide attenuates IL-1β-stimulated STAT3 phosphorylation.](#)
there were distinct differences in the extent and kinetics of activation of STAT3.

Studies in other cellular models have reported regulation of STAT3 activity by members of the MAPK family, but the results of these studies have produced conflicting results. Thus, some studies have indicated either an enhanced activation of STAT3 by MAPKs or, conversely, an inhibition of STAT3 activation by MAPKs (39, 50, 51). Such conflicting information therefore prompted us to investigate the nature of any cross-talk between the MAPK and JAK/STAT pathways in IL-1β-stimulated cardiac myocytes. We found that specific inhibition of the rapid ERK or p38MAPK activation completely inhibited the delayed STAT3 response to IL-1β (Fig. 4). This implicates ERK and p38 MAPK as positive upstream regulators of STAT3 activation in the response to IL-1β and that the interruption of either pathway alone is sufficient to prevent STAT3 activation. Because there are currently no specific chemical inhibitors of JNK, the role of this third MAPK subfamily could not be
investigated and therefore should not be discounted.

The exact nature of the secondary signaling molecule mediating the delayed STAT3 response induced by IL-1β remains a subject for further investigation but is likely to be a member of the IL-6 family of cytokines that utilizes gp130 and thus activates the JAK/STAT pathway (52–54). In the present study, we instead focused on characterizing the contribution of the MAPKs (and by inference the downstream activation of STAT3) in the ultimate biological response, which in this case was the hypertrophic response elicited by IL-1β. In previous studies, only MAPK activation profiles and their contribution to c-Jun and ATF2 regulation after IL-1β exposure have been examined (36). Thus, we extended such observations to evaluate the effects that inhibiting ERK, p38MAPK, or STAT3 activation may have on hypertrophy as judged by ANF expression and altered cellular morphology. We therefore conducted a series of studies in which we inhibited ERK and p38MAPK either alone or in combination. In this way, we could additionally characterize the relationships between these two major MAPK pathways. Indeed, there already is a large body of evidence implicating each of the three different MAPK subfamilies in regulating ANF expression stimulated by the GPCR agonists (17–19). However, the exact role and degree of functional overlap by each of the MAPK subfamilies remain controversial, and very little is known about the potential function of ERK and p38MAPK in regulating IL-1β-stimulated hypertrophic morphology or ANF expression.

We demonstrated that inhibiting either ERK or p38MAPK alone had a significant inhibitory effect on ANF protein expression, with ERK inhibition having a significantly greater effect (Fig. 6B). This suggests that both ERK and p38MAPK can play a role in regulating fetal gene expression during IL-1β-stimulated hypertrophy. These data are in agreement with several findings reporting inhibition of ANF expression by ERK pathway-specific inhibitors (19, 23) but additionally reveals the importance of p38MAPK.

In the final section of our study, we evaluated the contribution of ERK and p38MAPK to the IL-1β-stimulated hypertrophic morphology and expression of the fetal gene program marker, ANF. In this case, inhibition of either ERK or p38MAPK attenuated the percentage of IL-1β-stimulated cells expressing ANF (Fig. 6B). This is similar to the effects on STAT3 phosphorylation (Fig. 4) and suggests a possible link between STAT3 phosphorylation/activation and ANF expression. In contrast, neither MAPK inhibitor alone had an effect on myocyte morphology (Fig. 6C). Therefore, although ANF expression was ablated by ERK or p38MAPK inhibition alone, cardiac myocytes retained the ability to exhibit hypertrophic morphogenesis in response to IL-1β and LIF. This suggests that ANF expression does not necessarily correlate with hypertrophic morphology and may not in itself be an appropriate index of hypertrophy as it does not necessarily correlate with hypertrophic morphology or ANF expression.

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Mechanism of IL-1β-stimulated Cardiac Hypertrophy

A Role for the Extracellular Signal-regulated Kinase and p38 Mitogen-activated Protein Kinases in Interleukin-1 β-stimulated Delayed Signal Transducer and Activator of Transcription 3 Activation, Atrial Natriuretic Factor Expression, and Cardiac Myocyte Morphology

Dominic C. H. Ng, Carlin S. Long and Marie A. Bogoyevitch

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