Transcription Factor NF-κB Is Necessary for Up-regulation of Type 1 Angiotensin II Receptor mRNA in Rat Cardiac Fibroblasts Treated with Tumor Necrosis Factor-α or Interleukin-1β*

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Tumor necrosis factor-α (TNF-α) and interleukin-1β up-regulate type 1 angiotensin II receptor (AT₁) mRNA and protein in cultured neonatal rat cardiac fibroblasts. The use of pharmacologic inhibitors and a degradation-resistant mutant IκB-α demonstrated that the transcription factor nuclear factor-κB (NF-κB) is necessary for cytokine-induced AT₁ up-regulation. The increase in AT₁ mRNA with TNF-α treatment is slow, reaching significance by 6–12 h and peaking by 24–48 h. Electrophoretic mobility shift assays revealed that NF-κB nuclear translocation was maintained for >24 h with a single dose of TNF-α. Since prolonged NF-κB activation appeared necessary to maximize AT₁ up-regulation, the mechanism of persistent NF-κB activation was studied further. Stimulation with TNF-α induced a >10× increase in IκB kinase (IKK) activity that quickly diminished by 20 min. IκB-α and IκB-β proteins were degraded during this time, and IκB-α was resynthesized subsequently by NF-κB-dependent transcription. However, IκB isoforms and IKK activity did not return completely to unstimulated values during a 12-h time course. These results suggest that low but persistent IKK activity and IκB degradation lead to prolonged NF-κB nuclear translocation and maximal AT₁ up-regulation in the continued presence of TNF-α.

After a MI¹ there is extensive remodeling of the extracellular matrix of the heart, which includes formation of a replacement scar at the infarct site and deposition of fibrous tissue in noninfarcted zones of myocardium (1, 2). Angiotensin II, an octapeptide found throughout the heart following experimental MI (3), stimulates processes related to extracellular matrix remodeling. These effects are mediated through the G-protein-coupled AT₁ receptor. Binding of angiotensin II to AT₁ stimulates cultured cardiac fibroblasts to proliferate and produce extracellular matrix proteins (4–6). Administration of angiotensin-converting enzyme inhibitors or AT₁ blockers inhibits extracellular matrix remodeling and reduces mortality in experimental animal models of MI (7–12). Angiotensin-converting enzyme inhibitors also increase survival of humans after a MI (13, 14). Thus, effects mediated by AT₁ appear to be critical determinants of both extracellular matrix remodeling and the clinical course of patients post-MI.

Increases in AT₁ receptors have been demonstrated in the peri-infarction zone and in remote noninfarcted segments of myocardium after a MI (15–17). However, the mechanisms responsible for this up-regulation are not understood. Only a few agents such as dexamethasone and growth hormone have been shown to increase AT₁ levels transcriptionally (18, 19). Furthermore, most studies of AT₁ regulation have been performed using vascular smooth muscle cells or kidney cells, and little is known about AT₁ regulation in cardiac fibroblasts. We have demonstrated previously that AT₁ mRNA and protein is up-regulated in cultured neonatal rat cardiac fibroblasts by TNF-α and IL-1β but not by other endogenous mediators found in the post-MI heart (20). These studies showed that AT₁ mRNA increases were sustained for at least 24–48 h following a single application of TNF-α and that increased gene transcription was likely involved. Proinflammatory cytokines such as TNF-α and IL-1β are increased in the rat heart following experimental MI (21–23). Since cytokines could be responsible for post-MI increases in AT₁ receptor density, we sought to determine the mechanisms and signaling pathways involved in AT₁ up-regulation by TNF-α and IL-1β.

The transcription factor NF-κB is a known regulator of genes that control apoptosis, inflammation, and cell division. It is composed of homodimers and heterodimers of the NF-κB/Rel family of proteins but often consists of a heterodimer of p65 and p50. In unstimulated cells, NF-κB is retained in the cytoplasm bound to a family of inhibitory IκB proteins. Of these, IκB-α and IκB-β predominate in a variety of cell types. Stimulation of cells with cytokines such as TNF-α or IL-1β leads to rapid activation of the IκB kinases (IKK1 and IKK2 of which the latter seems to be indispensable) that then phosphorylate IκB on serine residues (Ser32 and Ser36 on human IκB-α). Phosphorylation leads to ubiquitination of IκB on lysine residues (Lys41 and Lys38 on human IκB-α) and degradation by the 26 S proteasome. After removal of IκB, free NF-κB can translocate to the nucleus and affect transcription by binding consensus DNA sequences (for reviews, see Refs. 24–26). We now provide evidence that NF-κB activation is required for increased expression of the AT₁ gene by the cytokines TNF-α and IL-1β in cultured neonatal rat cardiac fibroblasts. In addition, a single

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§ The abbreviations used are: MI, myocardial infarction; AT₁, type 1 angiotensin II receptor; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; NF-κB, nuclear factor-κB; IκB, inhibitor of κB; IKK, IκB kinase; EMSA, electrophoretic mobility shift assay; ATF-2, activating transcription factor-2.
dose of TNF-α persistently activates cardiac fibroblast signaling pathways that sustain NF-κB nuclear translocation for at least 24 h and maximize AT1 mRNA up-regulation.

**EXPERIMENTAL PROCEDURES**

**Reagents**

Oligodeoxynucleotides were synthesized by GENSET Oligos (La Jolla, CA). Pharmacologic inhibitors were purchased from Calbiochem-Novabiochem Corp. Enzymes for molecular biology were from Life Technologies, Inc. GST-1x-Ba-a(1–54) was a generous gift from the Signal Research Division of Delgene (San Diego, CA). Primary antibodies for immunoblotting and immunoprecipitation were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Radioisotopes were purchased from PerkinElmer Life Sciences Inc.

**Isolation of Cardiac Nonmyocytes (Fibroblasts) from Neonatal Rats**

Neonatal rat cardiac fibroblasts were isolated from the hearts of 1–2-day-old Sprague-Dawley rats as described previously (27). Second passage cells were used for all experiments. Fibroblasts were grown on tissue culture plates in medium (Dulbecco's modified Eagle's medium high glucose, Life Technologies, Inc.) supplemented with 10% fetal bovine serum and penicillin/streptomycin/FG (Life Technologies, Inc.) (30). Oligodeoxynucleotides were synthesized by GENSET Oligos (La Jolla, CA). Phenylmethylsulfonyl fluoride was added to the medium at 25 μM 0.1% DMSO, 0.1% DMSO (42 °C, 20 min with wash at high stringency (0.2 SSC, 0.1% SDS, 65 °C, 20 min per wash)). Damp membranes were exposed to a Storage Phosphor Screen and then visualized on the Storm 860 fluorescent scanner (Molecular Dynamics, Sunnyvale, CA). Signal intensities were calibrated using the software supplied with the scanner (ImageQuant). The probe for AT1 mRNA was the −14 kbp EcoRI fragment of plasmid pATg (provided by Dr. David Prichnow, Oregon Health Sciences University). To standardize loading between slots, membranes were stripped and reprobed with a DNA representing nucleotides 2837–4436 of human 28S rRNA.

**Immunoblotting**

Cells were washed with phosphate-buffered saline, placed on ice, lysed with cytoplasmic extraction buffer (10 mM Tris-HCl (pH 7.9), 60 mM KCl, 1 mM EDTA, 0.4% Igepal CA-630, 1 mM NaCl, 10 μg/ml leupeptin, 100 μl/liter aprotinin, 0.1 mg/ml phenylmethylsulfonyl fluoride) for 5 min and scraped from the plate. The mixture was microcentrifuged at 2500 rpm for 3 min at 4 °C, and the supernatant (cytoplasmic extract) was collected. The pellet was washed by gentle resuspension in cytoplasmic extraction buffer and centrifuged again. Nuclear extraction buffer (50 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 420 mM NaCl, 25% glycerol, 10 μg/ml leupeptin, 100 μl/liter aprotinin) (42 °C, 20 min) was added to the pellet and vortexed for 1 min. The mix was allowed to stand on ice for 10 min and then microcentrifuged at maximum speed for 10 min at 4 °C. The supernatant (nuclear extract) was collected. If phosphoproteins were to be analyzed, extraction buffers also contained 0.5 mM sodium orthovanadate and 5 mM β-glycerophosphate to inhibit phosphatases.

Protein concentrations of extracts were quantitated with a Bio-Rad protein assay (catalog no. 500-0006). Equal amounts of protein were electrophoresed on a Bio-Rad Mini-PROTEAN II apparatus using the discontinuous SDS-polyacrylamide gel electrophoresis system of Laemmli (28). Resolved proteins were transferred electrophoretically to polyvinylidene difluoride membrane (Immobilon-P, Millipore) in 192 ml glycine, 25% methanol. The membrane was allowed to air-dry, then wetted in methanol, and floated in distilled water for 1–2 min. At this point, protein bands could be seen as “shiny” areas on a dull membrane background to verify equal loading between lanes. The wetted membrane was immobiloblotted using ECF Western blotting Reagent Packs (Amersham Biosciences, Inc.) following the instructions of the manufacturer. Fluorescent bands were visualized on the Storm 860 scanner in blue fluorescence mode with a photomultiplier tube voltage of 600–700. Band intensities were quantified using the software supplied with the scanner.

**EMSA**

Nuclear extracts were prepared from cultured fibroblasts as described for Western blotting. Gel-shift assays for NF-κB were performed as described by Jobin et al. (29). Briefly, a double-stranded synthetic oligonucleotide corresponding to the NF-κB consensus sequence in the B-cell κ light chain enhancer region (5′-AGTTGAGGG-GACCTTCCCCAGGC-3′) (30) was radiolabeled with T4 polynucleotide kinase and [γ-32P]ATP and purified with a QiAquick Nucleotide Removal kit (Qiagen). Nuclear extract (2 μg protein) was incubated with the radiolabeled probe. Bound probe and free probe were separated by electrophoresis on a nondenaturing polyacrylamide gel. The gel was dried and exposed either to film (Kodak BioMax MS) or a Storage Phosphor Screen.

**Packaging of Replication-deficient Adenovirus-5 (Ad-IxBA-M)**

Ad-IxBA-M—Adenovirus expressing a degradation-resistant mutant of IκBα (Ad-IxBoM) was a generous gift from Dr. Inder Verma of the Salk Institute (La Jolla, CA). Construction of the mutant has been described elsewhere (31).

**Ad-Less-Luc (Promoterless Luciferase)**—An adenovirus containing a promoterless luciferase gene was used to determine the effect of adenoviral infection alone on cardiac fibroblasts. For this purpose, a promoterless luciferase gene was excised from the plasmid pGL3-Basic (Promega, Madison, WI) by digestion with BglII and BamHI and ligated into the NotI sites of the adenoviral shuttle vector pACCMV-pLpA5R (see synthetic linker-adapters. A clone that had the luciferase gene inserted in a positive orientation with respect to the adenoviral Genome was selected. Adenovirus was produced by recombination between the shuttle vector and the full-length adenovirus-5 vector pJM17 as described by Goméz-Foríx et al. (32).

**Adenoviral Infection of Cardiac Fibroblasts**

Fibroblasts were grown to confluence in 6-cm tissue culture plates. Two extra plates were seeded to determine cell number via hemacytometer counting. Fibroblasts were infected with adenovirus (at a multiplicity of infection of 5 plaque-forming units/fibroblast) in 3 ml of Dulbecco’s modified Eagle’s medium with 2% fetal bovine serum (heat-inactivated at 65 °C for 15 min) for 18 h. The medium was replaced with Dulbecco’s modified Eagle’s medium with 0.5% fetal bovine serum, and the cells were allowed to recover for 6 h. Cells were then treated and processed according to the protocol of each experiment.

**IKK Kinase Assay**

Neonatal rat fibroblasts were assayed for IKK kinase activity as described previously (33, 34) with minor modifications. Immunoprecipitation was performed using a rabbit polyclonal antibody against IKKα/β (Santa Cruz Biotechnology, Inc., catalog no. sc-7607) on 200 μg of total extracted protein (determined using a Bio-Rad protein assay, catalog no. 500-0006). Kinase assays contained 2.5 μCi of [γ-32P]ATP, 2.5 μM cold ATP, and 1 μg GST-IκBα(1–54) (34). Polyacrylamide gels were fixed in 50% methanol, 10% acetic acid for 2 h and then soaked overnight in several changes of water. Dried gels were exposed to a Storage Phosphor Screen and visualized on the Storm 860 fluorescent scanner. Signal intensities were quantified using the software supplied with the scanner.

**Statistical Analyses**

Data were collected from at least three independent experiments. Quantitative data are expressed as the mean ± S.E. Statistical significance was determined by one-way analysis of variance followed by
Inhibitor concentrations were as follows: pyrrolidine dithiocarbamate bars, caspase inhibitor, had no significant effect on AT1 up-regulation (Fig. 1). SB202474, an inactive pyridinyl imidazole compound, significantly reduced cytokine-induced AT1 mRNA up-regulation (20). IL-1β increased AT1 mRNA levels significantly with TNF-α treatment (Fig. 1). When considered collectively, all of the compounds that inhibited AT1 up-regulation have been shown to inhibit NF-κB activity by inhibiting nuclear translocation, DNA binding, or transcriptional competency (37–40). In addition, the mitogen-activated protein kinase kinase inhibitor PD98059 increased AT1 mRNA levels significantly with TNF-α treatment (Fig. 1). Funakoshi et al. (41) have demonstrated that cytokine-induced NF-κB activation can be augmented by PD98059 in T88G cells. Therefore, the screen of pharmacologic compounds suggested the involvement of NF-κB, but additional evidence using a more selective inhibitor was needed.

To confirm the role of NF-κB, cultured cardiac fibroblasts were infected with an adenovirus expressing a mutant (Ser32,36→Ala32,36) IκB-α (Ad-IκBm) that cannot be phosphorylated and degraded and thus sequesters NF-κB in the cytoplasm (31). Ad-IκBm completely blocked NF-κB nuclear translocation induced by IL-1β, while a negative control adenovirus (Ad-Less-Luc) had no effect (Fig. 2A, upper panel). In contrast, Ad-IκBm did not affect phosphorylation of the transcription factors c-Jun and ATF-2, which is an indirect measure of mitogen-activated protein kinase activation (Fig. 2A, middle and lower panels). Thus, IκB-α appeared to specifically inhibit NF-κB activation in the fibroblasts. When tested on AT1 expression, Ad-IκBm completely blocked IL-1β-induced AT1 mRNA up-regulation, while Ad-Less-Luc had no significant effect (Fig. 2B). Therefore, the use of the signaling mutant and pharmacologic inhibitors provides strong evidence that the transcription factor NF-κB is necessary for cytokine-induced up-regulation of AT1 mRNA.

\[ p \leq 0.05 \]

\[ p < 0.01 \]
NF-κB and Cytokine-induced AT₁ Up-regulation in Fibroblasts

Gurantz et al. (20) demonstrated that AT_1 mRNA was elevated for 24–48 h in neonatal rat cardiac fibroblasts after a single administration of TNF-α to the culture medium. Assuming that the half-life of AT_1 mRNA in these cells is ~18 h (42), NF-κB would need to be activated for at least 24 h to increase AT_1 mRNA as observed. As shown in Fig. 3A, nuclear translocation of NF-κB occurred within 1 h of a single dose of 50 ng/ml TNF-α and persisted for at least 24 h as measured by EMSA; NF-κB was barely detected in the nuclei of fibroblasts that were not stimulated with cytokine. To verify the identity of the band in Fig. 3A as NF-κB, it was competed with excess nonradiolabeled κB probe (Fig. 3B, lane 3) and supershifted by an anti-p65 antibody (Fig. 3B, lane 4). Nonspecific rabbit IgG was unable to supershift the complex (Fig. 3B, lane 5).

In the presence of TNF-α some cell types transiently activate NF-κB, while others maintain a persistent activation (43–46). Neonatal rat cardiac fibroblasts belong to the latter group according to Fig. 3A. To study the mechanism of persistent NF-κB activation in these cells, the protein levels of IκB isoforms α and β were analyzed by immunoblotting. During TNF-α treatment IκB-α was degraded and resynthesized rapidly, reaching a minimum by 15 min and then rebounding by 60 min (Fig. 4). In contrast IκB-β was degraded and resynthesized more slowly, reaching a minimum by 60 min and rising slightly over 12 h (Fig. 4). Despite evidence of resynthesis, both IκB isoforms did not return to time 0 values during the 12-h time course (Fig. 4B), an effect that required the continued presence of TNF-α. For example, after 6 h of treatment with TNF-α, IκB-α was 79.5% and IκB-β was 38.4% of that in untreated cells (Fig. 5A, lanes 3 and 7). However, if TNF-α was removed from the medium after 1 h of the 6-h incubation, IκB-α was 136.4% and IκB-β was 70.7% (Fig. 5A, lanes 4 and 8). During this period of cytokine removal, p65 protein levels dropped in the nucleus as assessed by immunoblotting (Fig. 5B, compare lanes 11 and 12). These data are consistent with the hypothesis that sustained NF-κB activation is caused by a persistent degradation of IκB isoforms that depends on the continued presence of TNF-α. Sustained NF-κB activation appears necessary to maximize AT_1 mRNA increases since removal of TNF-α during a 24-h incubation prevents AT_1 mRNA up-regulation (Fig. 5B).

To study further the mechanism of persistent IκB degradation, IKK kinase assays were performed. IKK is the proximal kinase that phosphorylates IκB, thus regulating IκB ubiquitination and degradation (25). Stimulation of neonatal rat car-

![Image](https://example.com/image1.jpg)

**Fig. 3.** Nuclear translocation of NF-κB is sustained in cardiac fibroblasts treated with a single application of TNF-α. A, time course of NF-κB activation. Confluent fibroblasts were stimulated either with or without 50 ng/ml TNF-α for the indicated time (h). Nuclear extracts were prepared and subjected to EMSA for NF-κB. B, specificity of gel shift. Nuclear extracts were prepared from fibroblasts stimulated with 50 ng/ml TNF-α for 3 h. EMSA was performed either with (lanes 2–5) or without (lane 1) nuclear extract. To determine its specificity, binding was also performed in the presence of 100-fold excess nonradiolabeled κB probe (lane 3), 150 ng/µl rabbit polyclonal anti-p65 IgG (Santa Cruz Biotechnology, Inc., catalog no. sc-372) (lane 4), or 150 ng/µl normal rabbit IgG (lane 5).

![Image](https://example.com/image2.jpg)

**Fig. 4.** IκB isoform levels are diminished in cardiac fibroblasts treated with a single application of TNF-α. Confluent fibroblasts were stimulated with 50 ng/ml TNF-α for the indicated times (h). Cytoplasmic extracts were prepared and subjected to immunoblotting for IκB isoforms α and β (10 µg of total protein loaded per lane). Primary antibodies were obtained from Santa Cruz Biotechnology, Inc. and used at 1:200 dilution (IκB-α, catalog no. sc-371; IκB-β, catalog no. sc-945). A shows representative immunoblot images. Band intensities from three independent experiments were quantified and plotted in B (mean ± S.E.).

![Image](https://example.com/image3.jpg)

**Fig. 5.** The continued presence of TNF-α is required to suppress IκB levels, maintain p65 nuclear translocation, and up-regulate AT₁ mRNA in cardiac fibroblasts. Confluent fibroblasts were stimulated with or without 50 ng/ml TNF-α for the indicated times. A, cytoplasmic IκB isoforms and nuclear p65. Cytoplasmic extracts were prepared and immunoblotted for IκB-α and IκB-β as described in Fig. 4. Nuclear extracts were also prepared and subjected to immunoblotting for p65 (2.5 µg of total protein loaded per lane; primary antibody from Santa Cruz Biotechnology, Inc., catalog no. sc-372, used at 1:200 dilution). B, AT₁ mRNA. Total RNA was isolated from the cells, and AT₁ mRNA was quantified by slot-blotting as described under “Experimental Procedures.” All plots were derived from three independent experiments (mean ± S.E.).
NF-κB and Cytokine-induced AT₁ Up-regulation in Fibroblasts

![Graph](image)

**DISCUSSION**

Most of the effects of angiotensin II that are involved in extracellular matrix remodeling are mediated by the AT₁ receptor whose density is increased in the hearts of experimental animals post-MI (15, 16). Previous work from our laboratory demonstrated that cytokines such as TNF-α and IL-1β, but not other factors in the post-MI heart, increase AT₁ mRNA levels in cultured neonatal rat cardiac fibroblasts (20). The present studies were performed to elucidate the signal transduction pathways involved in this cytokine-induced AT₁ up-regulation. The major finding is that activation of the transcription factor NF-κB is required for AT₁ mRNA up-regulation to occur. Furthermore, in response to TNF-α stimulation, persistent activation of the kinases that lead to degradation of inhibitory IκB proteins appears responsible for the sustained nuclear translocation of NF-κB that maintains AT₁ mRNA up-regulation.

Many signaling pathways are activated upon binding of TNF-α or IL-1β to its receptor (for reviews, see Refs. 36 and 47). We screened several pharmacologic compounds that are known to inhibit such signaling pathways to determine which might be responsible for AT₁ up-regulation. The profile of inhibition of the pharmacologic compounds was nearly identical for TNF-α and IL-1β, suggesting that both cytokines up-regulate AT₁ by utilizing a common pathway. All compounds that demonstrated significant inhibition of cytokine-induced AT₁ mRNA up-regulation were known to affect the functioning of NF-κB. In contrast, compounds that altered other signaling pathways failed to inhibit AT₁ up-regulation. Since both TNF-α and IL-1β activate NF-κB downstream of receptor engagement (25, 35) the observation that both cytokines produced similar pharmacologic profiles is not surprising.

To verify the necessity of NF-κB in AT₁ up-regulation, a degradation-resistant mutant IκB-α was utilized that more specifically blocked NF-κB. For these experiments it was necessary to use IL-1β since cardiac fibroblasts that had been infected with Ad-IκB-αM were killed when treated with TNF-α at concentrations and durations known to increase AT₁ mRNA levels. TNF-α-induced apoptosis has been well documented in other cell types in which NF-κB has been inhibited (31, 48, 49). Preventing transcription of NF-κB-dependent antiapoptotic genes unmasks the proapoptotic effects of TNF-α in these situations. However, IL-1β treatment of IκB-αM-expressing fibroblasts did not induce apoptosis. In these cells both NF-κB nuclear translocation and AT₁ mRNA up-regulation were completely blocked.

Although the present studies indicate that the trans-acting factor NF-κB is required for cytokine-induced up-regulation of AT₁ mRNA in rat cardiac fibroblasts, the cis-acting DNA elements in the AT₁A gene that are responsible for this effect have yet to be identified. Preliminary computer analysis of the AT₁A 5′-flanking region (GenBank™ accession number S64602) has revealed two putative NF-κB binding sites at −365 and −2540 (50). Studies to ascertain the involvement of these putative sites are ongoing.

An autoregulatory feedback loop exists in many cells to avoid prolonged activation of NF-κB that can cause chronic inflammation. After strong activation, IKKs show a rapid loss of activity that is thought to be caused by autophosphorylation of C-terminal serine residues (51). In addition, transcription of the IκB-α gene is NF-κB-dependent (52–54). Upon activation of NF-κB, transcription of IκB-α increases, and IκB-α protein levels quickly rise. IκB-α can resequester NF-κB and remove it from the nucleus via chromosome region maintenance 1-dependent nuclear export (55). Lowered IKK activity and retranscription of IκB-α prevent prolonged activation of NF-κB. Our results indicate that stimulation of cardiac fibroblasts with TNF-α produces a rapid activation of IKK, subsequent degradation of IκB-α/β, and nuclear translocation of NF-κB. IKK activity rapidly declines thereafter, and IκB-α is re-synthesized. However, over a 12-h period both IKK activity and IκB-α/β proteins do not return fully to unstimulated levels. Low but persistent IKK activity and IκB degradation could be responsible for persistence of NF-κB activity in the continued presence of TNF-α. This sustained NF-κB activation appears to be responsible for the sustained increase in AT₁ mRNA that was observed in our previous study (20). Whether a 2–4× elevation of IKK activity is sufficient to maintain degradation of IκB-NF-κB complexes still needs to be addressed. Signal-induced nuclear proteasome activity has been implicated in degrading IκB-α that has re-sequestered NF-κB in 293T and HeLa cells, thus prolonging NF-κB nuclear translocation (55, 56). Lowered but nuclear localized IKK activity may be sufficient to maintain nuclear localization of NF-κB in cardiac fibroblasts.

There also is evidence that, after its signal-induced degradation, IκB-β can be re-synthesized as a hypophosphorylated form that can bind nuclear NF-κB and protect it from resequestration by IκB-α (43, 57, 58). IκB-β is degraded substantially in cardiac fibroblasts by TNF-α treatment (see Fig. 4). Although we have not studied its contribution to prolonging NF-κB activation in these cells, re-transcription of hypophosphorylated IκB-β may play a role. However, this mechanism seems unlikely for two reasons. 1) The effect in cardiac fibroblasts is dependent on the continued presence of cytokine since nuclear p65 levels drop if TNF-α is removed from the culture medium (Fig. 5A).
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2) IκB-α seems to predominate over IκB-β from 1-12 h post-cytokine stimulation in the fibroblasts (Fig. 4A).

There is evidence that AT1 receptor density is increased on cardiac fibroblasts post-MI in the peri-infarction zone and in distant regions of myocardium (15, 16). Following a MI there is up-regulation of AT1 in cardiac fibroblasts, for example, to proliferate, to produce extracellular matrix proteins, and to secrete transforming growth factor-β (4-6, 59). TNF-α-induced up-regulation of fibroblast AT1 receptors has been shown to augment angiotensin II-mediated responses such as production of inositol phosphates (20), collagen synthesis, and tissue inhibitor of metalloproteinases-1 secretion. Therefore, by up-regulating AT1 cytokines appear to enhance cultured cardiac fibroblast properties that would contribute to post-MI fibrosis. This possibility is supported by observations made in transgenic mice with cardiac-specific overexpression of TNF-α. These animals develop a heart failure phenotype that includes increased interstitial fibrosis (60, 61).

AT1 mRNA is increased in cultured neonatal rat cardiac fibroblasts by treating with TNF-α or IL-1β, an effect that requires NF-κB. Sasasamur et al. (62) have shown that IL-1β but not TNF-α up-regulates AT1 mRNA 2-3-fold in rat vascular smooth muscle cells. This contrast exemplifies the cell type-specific regulation of AT1 and may reflect a difference in function of the receptor between vascular smooth muscle cells and fibroblasts.

NF-κB is involved typically in the regulation of genes controlling apoptosis and inflammation. It is well known that NF-κB can up-regulate cell surface adhesion receptors such as E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 that are involved in inflammatory responses (63). That NF-κB can also up-regulate the cell surface AT1 receptor in cardiac fibroblasts suggests an important and novel role of NF-κB in modulating post-MI cardiac remodeling.

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