Catecholamines stimulate interleukin-6 synthesis in rat cardiac fibroblasts

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Catecholamines stimulate interleukin-6 synthesis in rat cardiac fibroblasts. Am J Physiol Heart Circ Physiol 281: H14–H21, 2001.—Proinflammatory cytokines have been implicated in the pathophysiology of different heart diseases. Recent evidence suggests that interleukin-6 (IL-6) may play a role in mechanisms leading to cardiac hypertrophy. In addition, catecholamines are known to induce cardiac hypertrophy. In the present study, we examined whether cardiac fibroblasts may be a potential source of IL-6 production in the rat heart and whether catecholamines can modulate the IL-6 synthesis. Only a small amount of IL-6 mRNA was detected in unstimulated rat cardiac fibroblasts. However, a 50-fold increase of IL-6 mRNA was found after stimulation with norepinephrine (NE). Addition of carvedilol, a α- and β-adrenergic receptor antagonist, prevented almost completely the NE-induced synthesis of IL-6 mRNA. Phenylephrine, an α-adrenergic agonist, and isoproterenol, a β-adrenergic agonist, also induced an increase in IL-6. However, the stimulation via β-receptors led to a more pronounced elevation. These data show that NE increases IL-6 expression in rat cardiac fibroblasts and that IL-6 may play an important autocrine/paracrine role in cardiac disease states associated with hypertrophy.

Recent evidence suggests that proinflammatory cytokines are capable of modulating cardiovascular function by a variety of mechanisms, including promotion of left ventricular remodeling (31, 35), induction of contractile dysfunction (10, 45), and uncoupling of myocardial β-adrenergic receptors (11, 16).

The cardiovascular effects of IL-6 are not well studied. There are some clinical data showing that serum levels of IL-6 were elevated in patients with mild or moderate heart failure (28) as well as in patients with acute myocardial infarction (21, 27). In a rat model of myocardial infarction, not only the IL-6 gene expression but also the tumor necrosis factor-α (TNF-α) and IL-1β gene expression were increased in the infarcted region (34). These results suggest that IL-6 may be both an acute-phase reactant and a chronic marker of inflammation associated with myocardial damage. A couple of reports have described a hypothesis that IL-6 may exert a negative inotropic effect and an intracellular Ca2+ concentration-lowering effect through nitric oxide-cGMP pathways in cultured chicken embryonic ventricular myocytes, in isolated hamster papillary muscles (10, 24), and in adult rat cardiac myocytes (38). Recent evidence suggests that IL-6 may play a role in mechanisms of heart hypertrophy: transgenic mice overexpressing IL-6 and IL-6 receptor developed hypertrophy of ventricular myocardium (19). In another model of cardiac hypertrophy due to pressure overload in rats, IL-6 was significantly increased (36).

In the present study we tested the effect of norepinephrine (NE) on IL-6 synthesis in rat cardiac fibroblasts, because it had been previously described that NE induced left ventricular hypertrophy in a rat model (46). We examined whether fibroblasts of rat hearts are able to synthesize IL-6 and whether NE can modulate IL-6 expression in these cells. We found that the abundance of IL-6 mRNA was low in rat cardiac fibroblasts and that catecholamines led to a marked increase in IL-6 mRNA mainly via β-adrenergic receptors.

MATERIALS AND METHODS

Cultivation of cardiac fibroblasts. Cardiac fibroblasts from adult female Sprague-Dawley rats (200–250 g body wt) were isolated using a modification of a previously described proto-

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col (8, 23). Briefly, rats were anesthetized with ether, and the chest was opened. The heart was immediately arrested by chilling with ice-cold 0.9% saline solution. The ascending aorta was cannulated, and the heart was excised and perfused with 15 ml of sterile Tyrode solution (in mM: 137 NaCl, 5.4 KCl, 0.5 MgCl₂, 1.8 CaCl₂, 11.6 HEPES, and 5 glucose; pH 7.35). The ventricle, free from the atrium, was minced and incubated with 0.1% trypsin and 300 U/ml collagenase type IV (Sigma; Deisenhofen, Germany) in a 37°C shaking water bath. Isolated cells were plated at the end of each 10-min digestion period. After five digestion periods, all isolated cells were combined and resuspended in Dulbecco's modified Eagle's-Ham's F-12 medium (DMEM-Ham's F-12 medium, Biochrom; Berlin, Germany), supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin, and 100 μg of streptomycin (Biochrom) and plated in two wells of a six-well culture plate (Nunc; Roskilde, Denmark). After a 2-h incubation period at 37°C in an atmosphere at 5% CO₂-95% air, the unattached cells were removed and the attached cells (mostly fibroblasts) were grown. Confluent cells were subcultivated after confluence was reached with trypsin-EDTA 10× (Biochrom) diluted 1:10 in phosphate-buffered salt solution (PBS), pH 7.4. The cells were passed every 4 days. All experiments were performed using cells of passages 3 to 4. Cardiac fibroblasts were identified by 1) characteristic morphology, 2) positive immunofluorescence staining with antibodies to vimentin, as previously described (8), and 3) negative immunofluorescence staining with anti-human factor VIII. One day before the experiments were started, the cells were transferred to FCS-free medium to exclude the effects of cytokines possibly present in the FCS.

For stimulation, NE (Sigma) was dissolved at a concentration of 10 mmol/l in DMEM containing 100 mmol/l L-(-)-ascorbic acid (Merck; Darmstadt, Germany) to prevent oxidation and then diluted 1:1,000 in DMEM to obtain a final solution of 10 μmol/l, if not stated otherwise. Metropolol (ME, CIBA-Geigy; Wehr, Germany) was also dissolved at a concentration of 10 mM in DMEM and then diluted 1:1,000 in DMEM to obtain a final solution of 10 μmol/l. Carvedilol (Boehringer Mannheim; Mannheim, Germany) was dissolved at a concentration of 6 mmol/l in DMEM containing 0.5% acetic acid and 5% dimethylformamide and then diluted to obtain the final solutions as indicated. Phenylephrine (PE) and isoproterenol (Iso, Sigma) were dissolved in DMEM at a concentration of 10 mmol/l in DMEM containing 0.5% ACCH solution (NaCl 1.5 M, sodium citrate 0.15 M, pH 7.2, 5 mM EDTA, and 7% SDS) (7) for 2 h at 60°C. cDNA probe for IL-6 (see Generation of the IL-6 cDNA probe by RT-PCR) was labeled using a random priming kit (Boehringer) following the instructions given by the manufacturer. Briefly, 2-5 μg of total RNA were hybridized with radioactive-labeled riboprobes from the PharMingen Multi-Probe Template Sets. After a RNase treatment, remaining “RNase-protected” probes were purified, resolved on denaturing polyacrylamide gels, and quantified by phosphorimaging (Bio-Rad; Munich, Germany). In addition to the samples, a dilution of the labeled probe set of cytokines was loaded on each gel to serve as size markers. The template probes (the markers) have the following size: IL-1a, 432 bp; IL-1β, 390 bp; TNF-β, 351 bp; IL-3, 315 bp; IL-4, 285 bp; IL-5, 255 bp; IL-6, 231 bp; IL-10, 210 bp; TNF-α, 189 bp; IL-2, 171 bp; interferon (IFN-γ), 158 bp; L32, 141 bp, and GAPDH, 126 bp. The protected IL-6 probe contained 202 bp. Data were corrected for the respective GAPDH (protected probe: 97 bp) and L32 (protected probe: 112 bp) values. The relative increase in abundance of IL-6 mRNA was calculated as a x-fold increase over the respective medium control. Because there was never a difference between the x-fold increase data when corrected for GAPDH or for L-32, only the GAPDH corrected values are presented.

Estimation of cell number. To estimate the cell number, a staining procedure was used based on the uptake of crystal violet into the cells and measuring the optical density of extracted dye (41). The adherent cells were washed in serum-free medium and fixed with 1% glutaraldehyde (15 min, room temperature). After being washed three times in distilled water, the cells were air dried at 37°C. Crystal violet (Sigma) was dissolved in 200 mmol/l boric acid, and the pH was adjusted to 9.0 with NaOH; the final concentration of crystal violet was 0.1%. A total of 200 μl of crystal violet solution were added per well. After an incubation for 20 min at room temperature, the solution was removed, and the cells were washed three times with distilled water and air dried at 37°C. The dye was extracted with acetic acid (10% vol/vol). The solution was transferred to a 96-well microtiter plate, and optical density was determined in an ELISA reader (Canberra Packard; Dreieich, Germany) at 570 nm. Standard curves with a defined cell number showed a linear correlation as the hybridization was performed. The filters were exposed to Kodak X-Omat at -80°C for different times, depending on the signal intensity. Relative mRNA levels were determined by densitometric scanning (Elscript 400, Hirschmann Gerätebau; Unterhaching, Germany).

Generation of the IL-6 cDNA probe by RT-PCR. To detect IL-6 mRNA with Northern blotting, a cDNA probe for IL-6 was made by RT-PCR using primers corresponding to the IL-6 DNA sequence. Primers (gift from Dr. K. Thalmeier, Munich, Germany) generated a 617-bp fragment from the IL-6 sequence: sense (22 mer), 5’ GCC TTC CCT ACT TCA CAA GTC C 3’; antisense (22 mer), 5’ CTG ACC ACA GTG AGG AAT GTC C 3’. This fragment was cloned in the pGEM-T Vector (see Fig. 3).

Total RNA was isolated from rapidly frozen rat heart using the method described above. First-strand cDNA synthesis and PCR were performed as previously described (41). Amplification was done in a DNA Thermal cycler as the following: initial denaturation at 94°C for 5 min, 30 cycles of amplification (94°C for 1 min, 65°C for 1 min, 72°C for 1 min), and final extension at 73°C for 5 min. DNA fragments were purified by separation in 1% agarose gel, cloned in the pGEM-T Vector (Promega; Heidelberg, Germany), and sequenced from both strands.

Ribonuclease protection assay. Ribonuclease protection assay was performed using a RiboQuantkit obtained from Pharmingen (Hamburg, Germany) following the instructions given by the manufacturer. Briefly, 2-5 μg of total RNA were hybridized with radioactive-labeled riboprobes from the PharMingen Multi-Probe Template Sets at 56°C for 12-16 h. After a RNase treatment, remaining “RNase-protected” probes were purified, resolved on denaturing polycrylamide gels, and quantified by phosphorimaging (Bio-Rad; Munich, Germany). In addition to the samples, a dilution of the labeled probe set of cytokines was loaded on each gel to serve as size markers. The template probes (the markers) have the following size: IL-1α, 432 bp; IL-1β, 390 bp; TNF-β, 351 bp; IL-3, 315 bp; IL-4, 285 bp; IL-5, 255 bp; IL-6, 231 bp; IL-10, 210 bp; TNF-α, 189 bp; IL-2, 171 bp; interferon (IFN-γ), 158 bp; L32, 141 bp, and GAPDH, 126 bp. The protected IL-6 probe contained 202 bp. Data were corrected for the respective GAPDH (protected probe: 97 bp) and L32 (protected probe: 112 bp) values. The relative increase in abundance of IL-6 mRNA was calculated as a x-fold increase over the respective medium control. Because there was never a difference between the x-fold increase data when corrected for GAPDH or for L-32, only the GAPDH corrected values are presented.
between the optical density (570 nm) and the cell number in the range from $1 \times 10^4$ to $1 \times 10^5$ cells.

**Determination of IL-6 protein.** Cardiac fibroblasts were seeded in 12-well culture plates and incubated with different stimuli. After various times, cell supernatants were removed, and IL-6 activity was determined by measuring the growth rate of the IL-6-dependent B9 cells (DSMZ; Braunschweig, Germany). Cells of the hybridoma line B9 (1) were cultured in RPMI containing l-glutamine, 2-mercaptoethanol ($5 \times 10^{-5}$ M), recombinant rat IL-6 (50 pg/ml) (recIL-6), and 10% FCS (B9 medium). Cells were centrifuged twice in B9 medium without recIL-6, adjusted to $2 \times 10^4$ cells/ml, and 100 µl of this cell suspension were cultured for 72 h at 37°C in 96-well microtiter plates (Greiner; Frickenhausen, Germany) with 10 µl of various dilutions of supernatants from stimulated fibroblasts. The proliferation of the cells was assessed by a MTT-based Cell Proliferation Kit I (Boehringer Mannheim) following the instructions given by the manufacturer. A standard proliferation curve was obtained with recombinant rat IL-6 (BioConcept; Umkirch, Germany), absorbance values were compared with those of the standard curve, and the concentration of IL-6 was extrapolated. Values were expressed in picograms per milliter of supernatant of $\sim 6 \times 10^5$ fibroblasts measured from crystal violet staining.

Table 1. Effect of norepinephrine on IL-6 mRNA in cardiac fibroblasts

<table>
<thead>
<tr>
<th>Incubation Time, h</th>
<th>Norepinephrine 5 µM</th>
<th>Norepinephrine 10 µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>6.31 ± 0.37(2)</td>
<td>7.32</td>
</tr>
<tr>
<td>1.0</td>
<td>24.48 ± 5.79(4)*</td>
<td>31.04</td>
</tr>
<tr>
<td>2.0</td>
<td>39.42 ± 6.02(10)*</td>
<td>49.38 ± 6.23(3)*</td>
</tr>
<tr>
<td>4.0</td>
<td>15.52 ± 3.52(4)†</td>
<td>19.49 ± 1.77(2)</td>
</tr>
<tr>
<td>6.0</td>
<td>10.25</td>
<td>ND</td>
</tr>
<tr>
<td>8.0</td>
<td>8.86</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are given as x-fold increases (means ± SE) compared with untreated cells; the number of experiments is given in parentheses. IL-6, interleukin-6. *Significantly different from untreated cells with $P < 0.05$; †Not significantly different from untreated cells; ND, not determined.

**RESULTS**

**Effect of NE on cytokine gene expression by cardiac fibroblasts.** In a first set of experiments, the effect of NE on gene expression of various cytokines in rat cardiac fibroblasts was tested by the RNase Protection Assay. Ten different cytokines were tested with the same RNA sample. Unstimulated cardiac fibroblasts expressed a weak or undetectable message for IL-1α and -β, TNF-β, IL-3, IL-4, IL-6, IL-10, TNF-α, IL-2, and IFN-γ. After NE (10 µmol/l) was added, an increase in abundance of only IL-6 mRNA was obtained within 1–2 h (Fig. 1). Enhanced signals for IL-6 mRNA were first detected at 30 min upon stimulation, peaked at 2 h, and returned near control levels after 8 h (Table 1). Depending on different donor hearts, the number of cell passages, and also cell density, a 16- to 71-fold increase of IL-6 mRNA was seen after stimula-

![Fig. 1. Cytokine mRNA synthesis in rat cardiac fibroblasts after stimulation with norepinephrine (NE).](http://ajpheart.physiology.org/)

![Fig. 2. Dose-dependent induction of IL-6 mRNA synthesis by NE.](http://ajpheart.physiology.org/)
tion with 10 μM NE for 2 h compared with unstimulated cells. The same kinetics, but lower values, were obtained after addition of 5 μmol/l NE (data summarized in Table 1). The other cytokine mRNAs were not detectable even after prolonged periods of culture time.

The NE-induced stimulation was dose dependent. With 40 μmol/l NE, a 110-fold increase of IL-6 mRNA was observed within 2 h compared with unstimulated cells. Because the relative increase in the abundance of IL-6 mRNA depends on culture conditions, Fig. 2 shows the results of a representative experiment.

**Effect of NE on IL-6 mRNA.** To investigate the NE-induced IL-6 mRNA in more detail, Northern blot analysis was done using a rat cDNA sequence specific for IL-6 (Fig. 3). In unstimulated fibroblasts, only weak IL-6 mRNA was detected, whereas an increase in abundance of IL-6-specific mRNA was seen after adding NE (5 μmol/l) (Fig. 3). Northern blot analysis of total RNA extracted from NE-stimulated cells revealed two size classes of IL-6 mRNA (32): a “major” class of 1.2–1.3 kb and a “minor” class of 2.4 kb. As assessed by quantitative densitometry of the autoradiographs, the major class was approximately two to three times more abundant than the minor class.

**Analysis of NE-induced IL-6 synthesis via α- and β-adrenergic receptors.** To assess whether the NE-induced increase in IL-6 mRNA is mediated by adrenergic receptors, the effect of carvedilol, a α- and β-antagonist, was tested. Carvedilol inhibited almost completely the NE-induced increase in abundance of IL-6 mRNA (Fig. 4, data of different experiments are summarized in Table 2). Different concentrations of carvedilol were tested; maximum inhibition was already seen at a concentration of 5 μmol/l and was not further influenced with increased doses of carvedilol. Carvedilol alone had no effect on IL-6 mRNA.

To determine the α- and β-adrenergic selectivity for NE-mediated IL-6 synthesis, various agonists and antagonists were tested. Both the β-selective agonist Iso and the α-agonist PE induced an increase in abun-

**Fig. 3.** IL-6 mRNA determined by Northern blotting. Left: RT-PCR product (lane 1, lane M shows DNA 100-bp marker). Right: Northern blot using the PCR product as probe. Rat cardiac fibroblasts were cultivated in the presence of medium alone (−) or 5 μmol/l NE (+) for 2 h. Total RNA was then isolated and IL-6 specific mRNA was determined by Northern blotting. The size of the two IL-6 mRNA bands is indicated on right.

**Fig. 4.** Effect of carvedilol (CA) and metoprolol (ME) on NE-induced IL-6 mRNA. Left panel: effect of CA. Cells were cultivated in the presence of medium alone (lane 2; CA−, NE−), 10 μmol/l CA alone (lane 3; CA+, NE−), 10 μmol/l NE in combination with 10 μmol/l CA (lane 4; CA+, NE+), or in the presence of 10 μM NE (lane 5; CA−, NE+) for 2 h. Total RNA was isolated and cytokine mRNA was determined by RPA. First lane represents a dilution of the labeled probe set to serve as a size marker. Protected IL-6-specific mRNA band, two protected L32-specific mRNA bands, and three GAPDH bands are shown on the right side of the panel. Right panel: effect of ME. Cells were cultivated in the presence of medium alone (lane 1; ME−, NE−), 10 μmol/l metoprolol (lane 2; ME+, NE−), 10 μmol/l NE in combination with 10 μmol/l ME (lane 3; ME+, NE+), or in the presence of 10 μmol/l NE (lane 4; ME−, NE+) for 2 h. Total RNA was isolated and cytokine mRNA was determined by RPA. Last lane represents a dilution of the labeled probe set to serve as a size marker. Protected IL-6-specific mRNA band, two protected L32-specific mRNA bands, and three GAPDH bands are shown on the left side of the panel.
dance of IL-6 mRNA, whereas no other cytokine mRNA was upregulated (Fig. 5). The effect of Iso was more prominent; when fibroblasts were stimulated with 10 μmol/l Iso, a 29-fold ($\pm 1.5; n = 3; P < 0.05$) increase was observed after 2 h. Addition of 10 μmol/l PE led to an increase in IL-6 mRNA of about 12-fold ($\pm 1.63; n = 3; P < 0.05$) over unstimulated cells. To see whether stimulation of cardiac fibroblasts with either of these agonists or with NE affected IL-6 mRNA synthesis with the same kinetic, cells were cultivated with PE, Iso, or NE for different periods of time. In these experiments, the most abundant increase in IL-6 mRNA was also found after 2 h of stimulation (Fig. 5).

Consistent with an effect of Iso mediated through β-adrenergic receptors, the effect of NE-induced IL-6 mRNA was attenuated by the β-antagonist ME (Fig. 4; Table 2). Various concentrations of ME were tested: the maximum of inhibition was already obtained at 10 μM ME and could not further be influenced with 30 μM ME. Within this range of ME, an up to 91% inhibition of NE-induced IL-6 mRNA synthesis was obtained.

### Table 2. Effect of carvedilol and metoprolol on norepinephrine-induced IL-6 mRNA in rat cardiac fibroblasts

<table>
<thead>
<tr>
<th>Fibroblasts Stimulated for 2 h</th>
<th>NE (10 μM)</th>
<th>CA (10 μM) + NE (10 μM)</th>
<th>CA (10 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 mRNA</td>
<td>43.7 ± 4.63*(4)</td>
<td>4.8 ± 0.99†(4)</td>
<td>1.1 ± 0.17‡(4)</td>
</tr>
</tbody>
</table>

Data are given as x-fold increases (means ± SE) compared with untreated cells; the number of experiments is given in parentheses. CA, Carvedilol; ME, metoprolol; NE, norepinephrine. *Significantly different from untreated cells with $P < 0.05$; †$P < 0.05$ calculated for the differences between IL-6 mRNA after stimulation with NE alone and NE in combination with the blocker; ‡Statistically not different from untreated cells.

Fig. 5. Effects of NE, phenylephrine (PE), and isoproterenol (Iso) on IL-6 mRNA. Left panel: an RPA is shown. Cells were cultivated for 2 h in presence of 10 μmol/l NE (lane 2), 10 μmol/l PE (lane 3), 10 μmol/l Iso (lane 4), or in the presence of control medium (CO) (last lane). Total RNA was isolated and cytokine mRNA was determined by RPA. First lane represents a dilution of the labeled probe set to serve as a size marker. Protected IL-6-specific mRNA band, two protected L32-specific mRNA bands, and three GAPDH bands are shown on the right side of the panel. Right panel: time course of the effect of PE, Iso, and NE on IL-6 mRNA. Cells were incubated with 10 μmol/l NE, 10 μmol/l PE, or with 10 μmol/l Iso for the time periods indicated. Total RNA was isolated and cytokine mRNA was determined by RPA. Relative increase in abundance of specific cytokine mRNA was calculated by phosphorimaging, and data were corrected for the value obtained for GAPDH. Data represent x-fold increase over unstimulated cells.
Effect of NE on IL-6 protein synthesis. To examine whether the increase in IL-6 mRNA also resulted in an increased protein synthesis, IL-6 protein was determined in the cell supernatant by means of a bioassay. Cardiac fibroblasts were stimulated with NE (10 μmol/l) and for comparison fibroblasts were stimulated with TNF-α (10 ng/ml) and with platelet-derived growth factor (PDGF) (10 ng/ml) for various periods of time. The cell supernatants were harvested and added to B9 cells. With supernatants from unstimulated cells and from cells stimulated with TNF-α, only minimal proliferation was induced, whereas NE led to a 10-fold increase and PDGF led to a 3- to 4-fold increase in IL-6 activity compared with cells that had been cultured with medium alone (Fig. 6). In another set of experiments, IL-6 protein was detected by SDS-PAGE and Western blotting (data not shown).

DISCUSSION

It is well known that catecholamines induce cardiac hypertrophy (46, 20). It was shown that DNA content increased in rat hearts after NE treatment (46). Because cardiomyocytes are end-differentiated cells, this effect could either be the result of polyploidy or the proliferation of fibroblasts. Fibroblasts are also responsible for the accumulation of extracellular matrix proteins, which was also observed during cardiac hypertrophy (8, 5, 42). Therefore, fibroblasts seem to be the cell type in the heart that modulates a variety of cardiac functions. For example, it has been shown that angiotensin II stimulates cardiac myocyte hypertrophy via paracrine release of TGF-β1 and endothelin-1 from cardiac fibroblasts in a neonatal rat cell culture model (15). With regard to recent findings that proinflammatory cytokines are capable of modulating cardiovascular function, we investigated whether cardiac fibroblasts in culture expressed cytokines, in particular IL-6, and whether NE can modulate this cytokine synthesis. Among a variety of cytokines tested, only a weak amount of IL-6 mRNA was detected in unstimulated cells. Upon incubation of fibroblasts with NE, a rise in IL-6 mRNA was seen. There was a variation of the extent of stimulation, from 10-fold as a minimal increase to a maximal increase of 71-fold, when independent experiments were compared. An average 49-fold increase was seen after 2 h. The fact that primary cultures of cells of different donor rats were used might explain the differences. In addition, although not yet studied in detail, cell density and number of passages appear to affect IL-6 mRNA synthesis. Whether the increase of IL-6 mRNA is transcriptionally mediated or regulated on the mRNA level remains to be determined. These data were in line with first in vivo data showing that IL-6 mRNA and protein were increased in rat hearts after l-isoproterenol treatment (29) and within hours after administration of NE (2). However, fibroblasts were perhaps not the only cell type participating in the increased IL-6 mRNA of hypertrophied rat hearts. Cardiac myocytes have been shown to express IL-6, for example, after hypoxic stress (44) or after stimulation with cytokines (17).

To obtain more information about the structure of the IL-6 mRNA, Northern blot analysis was performed. Again, in unstimulated rat cardiac fibroblasts only a weak amount of IL-6 mRNA was detected, whereas stimulation with NE revealed a marked increase in IL-6 mRNA. As described by others (32), two IL-6-specific mRNA bands were detected, one of about 1.2 kb and another of about 2.4 kb length. As assessed by quantitative densitometry, the 1.2-kb mRNA was two to three times more abundant than the 2.4-kb mRNA. Human and murine IL-6 mRNA appears as a single band in the 1.2- to 1.3-kb size range on Northern blots. The presence of a separate 2.4-kb mRNA is a unique property of rat cells. The reason for this phenomena is not the presence of two different IL-6 genes, but is the consequence of the existence of alternative polyadenylation sites. The murine and human IL-6 genes also contain two polyadenylation sites, but the distance between these sites is shorter than in rats because of the longer version of exon 5 of the rat IL-6 gene, which results in a 1.2-kb longer mRNA (32). Whether the mRNA species generated by different polyadenylation have different structural or functional properties, such as mRNA folding, mRNA half-life, transport, or translation efficiency is not known to date.

Since it was previously described that stimulatory effects of catecholamines are not always mediated by adrenergic receptors (22, 14), it was examined whether the NE-induced increase in IL-6 mRNA was mediated by adrenergic receptors. As shown in Fig. 4, carvedilol, an α- and β-antagonist, inhibited almost completely the NE-induced increase in abundance of IL-6 mRNA, indicating that the stimulatory effect of
catecholamines was clearly mediated by adrenergic receptors. To determine whether it is caused by α- or β-adrenergic receptors, the effect of PE and Iso was tested. Both agonists increased the IL-6 mRNA with the same kinetics as did NE, but Iso led to a more pronounced increase than PE in our culture conditions. Consistent with these data is that addition of ME, a selective β-receptor antagonist, markedly attenuated the NE-induced increase in IL-6 mRNA. So it seems that the catecholamine-induced increase in IL-6 mRNA may be mainly mediated by β-adrenergic receptors. Because PE also increased the IL-6 mRNA, further experiments are required to assess a possible synergistic effect when both receptors are stimulated.

It is well known and recently confirmed (4) that β-blockers have a beneficial effect on ventricular function and remodeling in patients with mild or moderate heart failure. Because it is also known that serum levels of IL-6 were elevated in these patients, it might be possible that the beneficial effect of β-blockers is partially due to the inhibition of cytokine expression in the heart, especially IL-6.

The increase in mRNA also resulted in an increase in IL-6 protein. In the cell supernatants, up to a 10-fold increase in IL-6 protein was seen within 8 h compared with that seen in unstimulated cells. There was a variation with regard to the extent of stimulation when independent experiments were compared. These differences may be explained by the fact that a bioassay was used as a test system and that fibroblast cell density may affect IL-6 protein synthesis (13). To the best of our knowledge, this is the first report showing that rat cardiac fibroblasts are able to synthesize IL-6. For comparison, the effect of PDGF and TNF-α was also tested. These two substances are well-known stimuli of the IL-6 synthesis in fibroblasts from other organs and other cell species (9, 13, 40). Under our culture conditions, however, TNF-α did not stimulate IL-6 protein release in rat cardiac fibroblasts. PDGF had a moderate effect, whereas the most pronounced increase was obtained with NE.

In a number of pathophysiological conditions leading to cardiac hypertrophy, the activity of the sympathetic nervous system is enhanced, resulting in the increased release of NE from the sympathetic nerve endings within the myocardium (33). Furthermore, administration of catecholamines can induce cardiac hypertrophy (20, 46). However, the biological or pathophysiological role of the NE-induced IL-6 synthesis in this context is not well known. Because it was recently described that IL-6, mostly in combination with the soluble IL-6 receptor, increased different extracellular matrix proteins (3), as well as the expression of collagenases and tissue inhibitors of metalloproteinases in different cell types (12, 26), IL-6 may participate in remodeling of the extracellular matrix. The growth-promoting effect of IL-6 is also well documented (1, 13, 47) so that this cytokine may be involved in the induction of cell proliferation observed during NE-induced cardiac hypertrophy (46). In addition, the fact that neonatal mice as well as adult cat cardiomyocytes enlarged in response to a combination of IL-6 and a soluble form of IL-6 receptor (19, 37) also emphasizes a possible role of IL-6 in cardiac hypertrophy.

Taken together, IL-6 appears to be a possible mediator of cardiac hypertrophy; possible sources of IL-6 in vivo are cardiomyocytes (17, 44) and, as we have now shown, also fibroblasts.

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