Dexamethasone treatment of post-MI rats attenuates sympathetic innervation of the infarct region

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El-Helou V, Proulx C, Gosselin H, Clement R, Mimee A, Villeneuve L, Calderone A. Dexamethasone treatment of post-MI rats attenuates sympathetic innervation of the infarct region. J Appl Physiol 104: 150–156, 2008. First published October 4, 2007; doi:10.1152/japplphysiol.00663.2007.—Sympathetic fiber innervation of the damaged region following injury represents a conserved event of wound healing. The present study tested the hypothesis that impaired scar healing in post-myocardial infarction (post-MI) rats was associated with a reduction of sympathetic fibers innervating the infarct region. In 1-wk post-MI rats, neurofilament-M-immunoreactive fibers (1.116±250 μm²/mm²) were detected innervating the infarct region and observed in close proximity to a modest number of endothelial nitric oxide synthase-immunoreactive scar-residing vessels. Dexamethasone (Dex) treatment (6 days) of post-MI rats led to a significant reduction of scar weight (Dex + MI 38±4 mg vs. MI 63±2 mg) and a disproportionate nonsignificant decrease of scar surface area (Dex + MI 0.54±0.06 cm² vs. MI 0.68±0.06 cm²). In Dex-treated post-MI rats, the density of neurofilament-M-immunoreactive fibers (125±47 μm²/mm²) innervating the infarct region was significantly reduced and associated with a decreased expression of nerve growth factor (NGF) mRNA (Dex + MI 0.80±0.07 vs. MI 1.11±0.08; P<0.05 vs. MI). Previous studies have demonstrated that scar myofibroblasts synthesize NGF and may represent a cellular target of Dex. The exposure of 1st passage scar myofibroblasts to Dex led to a dose-dependent suppression of [3H]thymidine uptake and a concomitant attenuation of NGF mRNA expression (untreated 3.47±0.35 vs. Dex treated 2.28±0.40; P<0.05 vs. untreated). Thus the present study has demonstrated that impaired scar healing in Dex-treated post-MI rats was associated with a reduction of neurofilament-M-immunoreactive fibers innervating the infarct region. The attenuation of scar myofibroblast proliferation and NGF mRNA expression may represent underlying mechanisms contributing to the diminished neural response in the infarct region of Dex-treated post-MI rats.

dexamethasone; neural remodeling; myocardial infarction; myofibroblasts; nerve growth factor

FOLLOWING DAMAGE to either the heart or skin, the enhanced synthesis and secretion of extracellular matrix proteins by smooth muscle α-actin-expressing myofibroblasts play a fundamental role in scar formation and healing (14, 20, 27). Recently, both sensory and sympathetic innervation were identified as integral events implicated in cutaneous wound healing, and sympathetic fiber innervation was likewise reported in the damaged region of both the dog and rat heart following an ischemic insult (4, 9, 10, 12, 17, 20, 28, 31). During cutaneous wound healing, myofibroblast-derived synthesis and release of the neurotrophin nerve growth factor (NGF) was postulated as the underlying mechanism propagating sensory and sympathetic innervation (14, 31). In the infarct region of post-myocardial infarction (MI) dogs, NGF protein and mRNA levels were significantly increased 3 days following surgery and remained elevated for at least 1 mo after MI (31). Unfortunately, the cellular source of NGF in the infarcted dog heart was not identified. However, consistent with the paradigm described during cutaneous wound healing, NGF expression was detected in myofibroblasts isolated from the infarct region of 1-wk post-MI rats (9). Collectively, these data suggest that fiber innervation represents an obligatory feature of wound healing across a range of tissue types facilitated in part via the local synthesis and release of NGF by infiltrating myofibroblasts.

It has been well established that corticosteroid therapy during the acute phase following coronary artery ligation leads to impaired scar formation (2, 26). In some cases, scar rupture was reported in post-MI patients receiving corticosteroids (26). An underlying mechanism attributed to the action of corticosteroids was inhibition of the inflammatory response during the early phase of scar formation (2, 13, 26). It remains presently unknown whether additional integral events implicated in scar formation and healing are likewise targeted by corticosteroids. Thus the present study tested the hypothesis that impaired scar healing in dexamethasone-treated post-MI rats was associated with a concomitant reduction of sympathetic fibers innervating the infarct region and attributed at least in part to the inhibition of scar myofibroblast growth and NGF expression.

METHODS

Myocardial infarct model, hemodynamics, and isolation of scar-derived cells. MI was induced in male Sprague-Dawley rats (9–11 wk old; Charles Rivers) following ligation of the left anterior descending coronary artery as previously described (9). Dexamethasone (0.1 mg/kg) was added to normal rat chow 24 h post-MI and continued for 6 days. During the 1-wk protocol, the dosage was adjusted according to changes in body weight. Left ventricular contractility was examined at 1 wk postinfarction by a microtip pressure transducer catheter (model SPR-407, 2F, Millar Instrument, Houston, TX), as previously described (9). Data were analyzed with the program IOX version 1.8.9 (Emka Technologies; Falls Church, VA). In both MI and dexamethasone-treated MI groups, we were unable to acquire hemodynamic data from three rats. Following hemodynamic measurements, the heart was removed and separated into the right ventricle, left ventricle (or noninfarcted left ventricular free wall), septum, and scar. The left ventricular free wall and right ventricle were immediately weighed and stored at −80°C. In MI rats, infarct region was excised and stored at −80°C. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
weighed, and the surface area was calculated by planimetry, as previously described (21). The use and care of laboratory rats was according to the Canadian Council for Animal Care and approved by the Animal Care Committee of the Montreal Heart Institute. Scar myofibroblasts were isolated from the infarct region of 1-wk post-MI rats, and the in vitro experiments described in the present study were performed on 1st passage cells, as previously described (6).

Real-time PCR. Real-time PCR was performed by standard methodology on total RNA isolated from the left ventricle of normal rats, the noninfarcted left ventricle of 1-wk post-MI rats, and 1st passage scar myofibroblasts, as previously described (10). Real-time PCR was performed according to the manufacturer’s instructions employing the molecule SYBR Green (Applied BioSystems). Primers for each gene were obtained from distinct exons that span an intron employing the program Ensembl Genome Browser (www.ensembl.org). The sequence specificity of each primer was verified with the program Blast derived from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The primers used were the following: rat ANP, forward 5′-AGGATGGCTTATATCGTGTA-3′ and reverse 5′-CTTCTGTTCCTCTCACC-3′; and reverse 5′-AGTTTGCTGTTATCTTCGTA-3′; rat nerve growth factor, forward 5′-CAGCTTCTCTCAGGCGACT-3′ and reverse 5′-GAGTCCTCCTTGCGACATGTT-3′; and rat β-actin, forward 5′-CCCTAAGGCCAACGGTGA-3′ and reverse 5′-GAGGGCATACGGGACACACAG-3′.

Immunofluorescence staining of tissue. In a separate series of experiments, the heart from MI (n = 3) and dexamethasone-treated MI (n = 3) rats was excised, immersed directly in 2-methylbutanol (temperature maintained at −80°C), and stored at −80°C. Immunofluorescence on cardiac tissue (cryostat sections of 14-μm thickness) was performed as previously described (10). In both groups, cardiac weight, infarct size, and hemodynamics were not assessed. Sympathetic fibers were identified in the infarct region via neurofilament-M immunoreactivity (rabbit polyclonal 1:500, Chemicon), and endothelial cells were identified by endothelial nitric oxide synthase (eNOS) staining (mouse monoclonal anti-eNOS 1:100, BD Transduction Laboratories). The nucleus was identified with ToPro3 (1.5 μM; emission wavelength 661 nm) staining following RNase digestion. Secondary antibodies used were a goat anti-mouse conjugated to horseradish peroxidase (1:500; Jackson ImmunoResearch Laboratories), a goat anti-rabbit IgG conjugated to Alexa-488 (1:500; InVitrogen; emission wavelength 520 nm) and a goat anti-mouse conjugated to Alexa-546 (1:500; InVitrogen; emission wavelength 661 nm) staining following RNase digestion. Secondary antibodies used were a goat anti-rabbit IgG conjugated to Alexa-488 (1:500; InVitrogen; emission wavelength 520 nm) and a goat anti-mouse conjugated to Alexa-546 (1:500; InVitrogen; emission wavelength 661 nm). A fluorescence signal was not detected when the tissue was incubated with conjugated secondary antibody alone. Immunofluorescence was visualized with either a ×10- or ×63-oil 1.4-NA DIC plan apochromat objective mounted on a Zeiss Axiosvert 100 M confocal microscope in a field of 0.7–0.84 mm². To determine sympathetic fiber density, two transverse sections from the heart of each MI and dexamethasone-treated MI rat were used, and neurofilament-M-immunoreactive fibers were examined throughout the entire infarct region. The program LSM 5 Image Browser (Zeiss) was used to calculate sympathetic fiber density (μm²) and was subsequently normalized to the scar surface area (mm²; consisting of projections (LSM 510 software; Zeiss) derived from a Z-stack (voxel size of 143 × 143 × 250 nm in XYZ) of a 14-μm-thick cryosection. The Z-stack image was subsequently deconvolved (Huygens Professional 3.0; SVI), and the final image was reconstructed with the LSM 510 software.

DNA synthesis. First passage scar myofibroblasts were treated with dexamethasone for 24 h, and DNA synthesis was assessed by the addition of 1 μCi/ml of [3H]thymidine (ICN Biomedicals) for 4–6 h before the end of the treatment protocol. Cells were washed twice with PBS (4°C), and cold 5% trichloroacetic acid was added for 1 h to precipitate DNA. The precipitates were washed twice with cold water and resuspended in 0.4 M NaOH. Aliquots were counted in a scintillation counter.

Western blot. Scar tissue was lysed in a buffer containing 10 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM sodium vanadate, 1% Triton X-100, 0.5% nonidet P-40, and 1 μg/ml of leupeptin and aprotonin. The homogenate was centrifuged for 10 min, and the supernatant was frozen and stored at −80°C. The Bio-Rad assay was used to determine protein content. The lysate (100 μg) was subjected to SDS-polyacrylamide gel (10%) electrophoresis, and protein was subsequently transferred to a PVDF membrane (Perkin Elmer Life Sciences, Boston, MA). Immunoblotting was performed as previously described (9). Antibodies used were a mouse monoclonal anti-smooth muscle α-actin (1:500; Sigma); a rabbit polyclonal anti-growth associated protein 43 (GAP43, 1:1,000; Chemicon); and a mouse monoclonal anti-GAPDH (1:10,000; Ambion). The secondary antibody used was a goat anti-mouse conjugated to horseradish peroxidase (1:10,000 Santa Cruz Biotechnology). The immunoreactive protein signal was visualized by an ECL detection kit (Perkin Elmer). Films were scanned with a laser densitometer utilizing the program Quantity One (Bio-Rad Laboratories). Smooth muscle α-actin and GAP43 protein expression were normalized to GAPDH protein content.

Statistics. Data were presented as means ± SE, and n represents either the number of rats used per experiment or independent preparations of cells. Morphological and hemodynamic data and gene expression were evaluated by a two-way ANOVA, and a significant difference was determined by the Neuman-Keuls post hoc test. Scar weight, surface area, neurofilament-M fiber density, protein content, and NGF mRNA expression in the infarct region were evaluated with a Student’s unpaired t-test. In 1st passage scar myofibroblasts, NGF mRNA expression was evaluated with a Student’s paired t-test as untreated and dexamethasone-treated cells were matched from the same preparation and repeated in three separate experiments. A value of P < 0.05 was considered statistically significant.

RESULTS

Cardiac morphology and left ventricular function in 1-wk post-MI rats and the effect of dexamethasone. Ligation of the anterior descending coronary artery resulted in the loss of cardiac tissue, leading to scar formation (Table 1). Absolute left ventricular weight and left ventricular-to-body weight (LV/BW) ratio were reduced in 1-wk post-MI rats (Table 1). Left ventricular systolic pressure, the rate of contraction, and

Table 1. Body and heart weights of sham-operated and 1-wk post-MI rats and the effect of dexamethasone

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>LV, mg</th>
<th>LV/BW, mg/g</th>
<th>RV, mg</th>
<th>RV/BW, mg/g</th>
<th>Scar Weight, mg</th>
<th>Scar Surface Area, cm²</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>315±5</td>
<td>501±36</td>
<td>1.58±0.09</td>
<td>161±21</td>
<td>0.51±0.03</td>
<td></td>
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<tr>
<td>MI (n = 11)</td>
<td>314±3</td>
<td>471±36</td>
<td>1.34±0.02*</td>
<td>163±17</td>
<td>0.53±0.02</td>
<td>63±2</td>
<td>0.68±0.06</td>
</tr>
<tr>
<td>DEX + sham operated (n = 8)</td>
<td>228±3*</td>
<td>484±10</td>
<td>2.13±0.09*</td>
<td>147±4</td>
<td>0.64±0.02*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX + MI (n = 11)</td>
<td>229±2*</td>
<td>418±22</td>
<td>1.83±0.06*</td>
<td>130±4*</td>
<td>0.56±0.02‡</td>
<td>38±4‡</td>
<td>0.54±0.06</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = no. of rats examined. DEX, dexamethasone; MI, myocardial infarction; BW, body weight; LV, left ventricle; RV, right ventricle. *P < 0.05 vs. sham operated. †P < 0.05 vs. MI. ‡P < 0.05 vs. DEX + sham operated. Data were evaluated by a 2-way ANOVA, and a significant difference was determined by the Neuman-Keuls post hoc test. Scar weight and surface area were evaluated with a Student’s unpaired t-test.

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the relaxation were decreased, whereas left ventricular end-diastolic pressure was modestly increased in 1-wk post-MI rats compared with sham-operated rats (Table 2). In dexamethasone-treated sham-operated and MI rats, a significant reduction of body weight was observed compared with nontreated rats. In dexamethasone-treated post-MI rats, a nonsignificant reduction (21%) of scar area surface was detected compared with untreated post-MI rats. By contrast, a disproportionate and significant decrease (40%) of scar weight was observed in dexamethasone-treated post-MI rats compared with untreated post-MI rats. However, dexamethasone administration to post-MI rats normalized the latter effect was attributed primarily to the significant loss of body weight. Despite an overall smaller infarct, left ventricular end-diastolic pressure remained depressed in dexamethasone-treated MI treated post-MI rats compared with untreated rats. However, dexamethasone-treated post-MI rats were comparable to untreated post-MI rats. Furthermore, an increase in LV/BW ratio was observed in dexamethasone-treated post-MI rats compared with untreated rats. However, molecular remodeling of the noninfarcted left ventricle and the effect of dexamethasone. To assess the hypertrophic response of the noninfarcted left ventricle, a semiquantitative measure of the putative marker ANP was performed (3, 9). In 1-wk post-MI rats, ANP mRNA was significantly increased in the noninfarcted left ventricle (Fig. 1). In dexamethasone-treated sham-operated rats, ANP mRNA expression was similar to sham (data not shown). Following the administration of dexamethasone to post-MI rats, elevated ANP mRNA levels were significantly attenuated in the noninfarcted left ventricle (Fig. 1). Likewise, a reactive fibrotic response was evident in the noninfarcted left ventricle of 1-wk post-MI rats, as reflected by the increased expression of collagen \( \alpha_1 \) type 3 mRNA (Fig. 1). Dexamethasone treatment had no effect on mRNA expression in sham-operated rats (data not shown) but normalized collagen \( \alpha_1 \) type 3 mRNA levels in the noninfarcted left ventricle of post-MI rats (Fig. 1).

Effect of dexamethasone on sympathetic fiber innervation of the infarct region, GAP43, and nerve growth factor expression. The disproportionate reduction of infarct weight vs. surface area in dexamethasone-treated post-MI rats supports the premise that various cellular events implicated in scar formation and healing were adversely influenced. We and others have demonstrated that infarct formation was associated with sympathetic fiber innervation (9, 28, 31). To evaluate the neural component of scar formation, neurofilament-M fiber density was determined. In the infarct region of untreated 1-wk post-MI rats (\( n = 3 \)), neurofilament-M-immunoreactive fibers were evident with a calculated density of 1,116 ± 250 \( \mu \)m²/mm² (normalized to scar area) (Figs. 2 and 3). Furthermore, neurofilament-M fibers were detected in close proximity to a modest number of eNOS-immunoreactive vessels residing in the infarct region (Fig. 2). In dexamethasone-treated MI rats, impaired scar healing was associated with a marked and significant (\( P < 0.05 \)) reduction of neurofilament-M fiber density in the infarct region (125 ± 47 \( \mu \)m²/mm²; \( n = 3 \)) compared with nontreated MI rats (Fig. 3). Consistent with these data, the expression of GAP43, a marker of axonal growth (31), was significantly (\( P < 0.05 \)) reduced in the infarct region of dexamethasone-treated post-MI rats (0.37 ± 0.01; \( n = 4 \)) compared with untreated post-MI rats (0.83 ± 0.02; \( n = 4 \)) (Fig. 4). Last, the loss of sympathetic fiber innervation in the scar following dexamethasone treatment may be attributed at least in part to an attenuation of neurotrophin expression. NGF mRNA expression was significantly reduced in the infarct region of dexamethasone-treated post-MI rats compared with untreated post-MI rats (Fig. 5).

Effect of dexamethasone on myofibroblast growth and NGF mRNA expression. Myofibroblasts, characterized by smooth muscle \( \alpha \)-actin expression, play a seminal role in scar healing (27). In addition to the synthesis and secretion of extracellular matrix proteins required for scar formation, myofibroblasts represent a cellular source of the neurotrophin NGF (9). Thus, we tested the hypothesis that impaired sympathetic fiber innervation of the dexamethasone-treated post-MI rats was attrib-
uated at least in part to an attenuation of myofibroblast proliferation and/or reduced expression of NGF. In dexamethasone-treated post-MI rats, smooth muscle α-actin protein content in the infarct region was significantly (P < 0.05) reduced (0.29 ± 0.01; n = 4) compared with the scar region of untreated MI rats (0.41 ± 0.013; n = 4) (Fig. 4). The latter data suggest that dexamethasone may have attenuated myofibroblast proliferation. However, the concomitant expression of smooth muscle α-actin in the vasculature of scar-residing vessels limited the interpretation of the data. To directly ascertain the effect of dexamethasone on myofibroblast proliferation and NGF mRNA expression, cells were isolated from the scar of 1-wk post-MI rats. Dexamethasone treatment of 1st passage scar myofibroblasts (n = 3) caused a dose-dependent suppression of [3H]thymidine uptake (Fig. 6). In parallel, the 24 h exposure of 1st passage scar myofibroblasts to dexamethasone (10−8 M) significantly reduced NGF mRNA expression (Fig. 5).

**DISCUSSION**

Numerous studies have reported that corticosteroid therapy administered early after coronary artery occlusion leads to abnormal infarct formation (2, 26). In some cases, impaired wound healing of the damaged heart following corticosteroid treatment led to cardiac rupture (26). In the present study, dexamethasone administration 1 day following complete coronary artery occlusion in the rat heart and continued for 6 days led to inadequate scar healing and was associated with a marked reduction of neurofilament-M fiber innervation of the infarct region. The latter effect may be related in part to dexamethasone-mediated inhibition of myofibroblast proliferation and the concomitant attenuation of NGF mRNA expression.

Although infarct wall thickness was not directly measured in the present study, the significant reduction of scar weight and disproportionate nonsignificant decrease of scar surface area following dexamethasone treatment were consistent with scar thinning. Furthermore, the modest reduction of overall infarct size in dexamethasone-treated post-MI rats was not associated with an amelioration of either left ventricular systolic pressure or the rate of left ventricular contraction and relaxation. In fact, the hemodynamic profile was comparable to that observed in untreated post-MI rats. Thus persistent ventricular dysfunction in dexamethasone-treated post-MI rats may be secondary to the inadequate healing of the ischemic region. Consistent with this concept, interventions begun early following coronary artery occlusion that led to increased infarct wall thickness were associated with improved left ventricular function (7, 15). By contrast, the modest increase of left ventricular end-diastolic pressure detected in untreated post-MI rats was normalized following dexamethasone administration. This result may be related in part to the suppressive action of dexamethasone on reactive fibrosis as elevated collagen α1 type 3 mRNA levels were normalized in the noninfarcted left ventricle of treated post-MI rats.

Previous studies have reported a reduction of body weight gain following corticosteroid treatment (25). A similar observation was reported in the present study, and the loss of body weight in dexamethasone-treated MI rats prevented an interpretation regarding the status of the hypertrophic response of the noninfarcted left ventricle, as measured by the LV/BW ratio. In this regard, ANP mRNA expression was used as a surrogate measure to establish a hypertrophic response of the noninfarcted left ventricle, as reflected by endothelial nitric oxide synthase (eNOS; red fluorescence) immunoreactivity of endothelial cells. eNOS immunoreactivity was not detected in the vascular wall, and ToPro3 (blue fluorescence) was used to stain the nucleus. Furthermore, the background Alexa-488 fluorescence signal (green) was modestly increased to highlight the vascular wall. B represents an additional example of neurofilament-M-immunoreactive fiber in close proximity to small-caliber vessels (denoted by *) in the infarct region containing eNOS-immunoreactive endothelial cells.
A seminal feature of cutaneous wound healing is sympathetic and sensory fiber innervation (12, 17, 20). In the ischecically damaged canine and rat myocardium, sympathetic fibers were likewise reported innervating the infarct region (9, 28, 31). Consistent with previous studies, neurofilament-M fibers were detected in the infarct region of 1-wk post-MI rats and observed in close proximity to a modest number of eNOS-immunoreactive scar-residing vessels. A similar paradigm was observed in the damaged skin as sensory fibers were reported adjacent to blood vessels immunoreactive to von Willebrand factor (20). Functionally, it has been demonstrated that either chemical or surgical denervation of sensory and/or sympathetic fibers delays and/or attenuates the healing process (1, 5, 17–19, 24). The underlying mechanism attributed to the latter effect may be related in part to a decrease of de novo blood vessel formation secondary to the loss of locally secreted neurotrophins (e.g., brain-derived neurotrophic factor and/or nerve growth factor) (8, 11). Thus it is tempting to speculate that sympathetic fibers detected in close proximity to blood vessels in the infarct region of the ischemically damaged heart.

Fig. 3. Dex and sympathetic fiber innervation of the infarct region. A: in untreated post-MI rats, numerous neurofilament-M fibers (indicated by arrow) were detected innervating the infarct region. B: phase-contrast photo of A. C: in Dex-treated post-MI rats, neurofilament-M fiber immunoreactivity was significantly reduced in the infarct region. D: phase contrast photo of C.

Fig. 4. Smooth muscle α-actin (SM α-actin) and growth associated protein 43 (GAP43) protein expression in the infarct region and effect of Dex. In Dex-treated post-MI rats (Dex + MI), SM α-actin and GAP43 protein levels in the infarct region were significantly decreased compared with untreated post-MI rats (MI). SM α-actin and GAP43 protein levels were normalized to GAPDH protein content.
In the infarcted canine heart, an increased expression of NGF was identified in the viable myocardium and infarct region. Consistent with this finding, expression of GAP43, a putative marker of axonal growth, was markedly reduced in the infarct region of dexamethasone-treated post-MI rats (31). Thus, based on the postulated relationship between neurogenesis and angiogenesis, de novo blood vessel formation in the infarct region of dexamethasone-treated post-MI rats may have been likewise compromised because of the significant reduction of innervating sympathetic fibers. However, this relationship cannot be directly established in the present model as a direct angio-static action of dexamethasone was previously reported during tumor angiogenesis and following cutaneous injury and was attributed at least in part to an inhibition of VEGF expression (29, 30).

In the infarcted canine heart, an increased expression of NGF was identified in the viable myocardium and infarct region and postulated to participate in sympathetic fiber growth (31). Consistent with this finding, NGF mRNA was likewise detected in the infarct region of 1-wk post-MI rats. Furthermore, in dexamethasone-treated post-MI rats, NGF mRNA levels in the scar were significantly reduced and may represent an underlying mechanism contributing in part to the reduction of sympathetic fiber innervation of the infarct region. Within the scar, myofibroblasts, characterized by the expression of smooth muscle α-actin, participate in scar healing via the synthesis and secretion of extracellular matrix proteins and represent an important cellular source of NGF (9, 27). Moreover, infiltrating myofibroblasts detected during cutaneous wound healing likewise synthesize and secrete the neurotrophin NGF (14). Thus, on the basis of these data, the decreased expression of NGF mRNA in the infarct region of dexamethasone-treated post-MI rats may represent a direct effect of the corticosteroid on either myofibroblast proliferation and/or gene expression. In the present study, smooth muscle α-actin protein content was decreased in the infarct region of dexamethasone-treated post-MI rats. However, it remains equivocal whether the latter finding was attributed exclusively to a reduction of the myofibroblast population as scar-residing vessels represent an additional source of smooth muscle α-actin. Nonetheless, the in vivo finding provided the impetus to directly examine the effect of dexamethasone on scar myofibroblast growth and gene expression in an in vitro setting. The treatment of 1st passage scar myofibroblasts with dexamethasone caused a dose-dependent inhibition of \[^{[3]}H\]\text{thymidine} uptake. Furthermore, the growth-suppressing action of dexamethasone was associated with a concomitant attenuation of NGF mRNA expression. Consistent with the latter finding, previous studies have reported that dexamethasone treatment reduced NGF expression in eosinophils and 3T3-L1 adipocytes (22, 23). Collectively, these data demonstrate that scar myofibroblasts represent a cellular target of dexamethasone, and the concomitant attenuation of proliferation and NGF mRNA expression may have contributed in part to the significant loss of neurofilament-M fibers innervating the infarct region. Moreover, the inadequate reparative fibrotic response observed in dexamethasone-treated post-MI rats may be further related to the antiproliferative action on scar myofibroblasts, independent of sympathetic fiber innervation.

The seminal finding of the present study was that impaired scar healing in dexamethasone-treated post-MI rats was associated with a reduction in the density of neurofilament-M fibers innervating the infarct region. The latter effect may be related

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**Fig. 5.** Effect of Dex on nerve growth factor (NGF) mRNA expression in the infarct region and isolated scar myofibroblasts. A: Dex treatment of post-MI rats (Dex + MI; n = 6) significantly reduced NGF mRNA expression in the infarct region compared with untreated post-MI rats (MI; n = 7). Data were evaluated by a Student’s unpaired t-test. B: in 1st passage scar myofibroblasts (n = 3), Dex (10^{-8} M) treatment for 24 h significantly reduced NGF mRNA expression. Data were evaluated by a Student’s paired t-test. *P < 0.05 vs. untreated post-MI rats or untreated scar myofibroblasts.

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**Fig. 6.** Suppressive action of Dex on DNA synthesis in isolated scar myofibroblasts. Dex treatment of 1st passage myofibroblasts (n = 3) caused a dose-dependent decrease of \[^{[3]}H\]\text{thymidine} uptake. *P < 0.05 vs. untreated myofibroblasts as assessed by a 1-way ANOVA.
at least in part to dexamethasone-mediated attenuation of myofibroblast proliferation and NGF mRNA expression. These observations are compatible with the findings reported in the damaged skin that nerve fiber innervation and myofibroblasts are integral events required for the rapid and efficient healing. Further studies are required to ascertain the relative contribution of sympathetic fibers in scar formation and healing in the ischmically damaged heart.

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