Role of myofibrillogenesis regulator-1 in myocardial hypertrophy

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CARDIAC HYPERTROPHY is an early milestone over the clinical course of heart failure and an important adaptive mechanism that occurs as a result of various mechanical, hemodynamic, hormonal, and pathological stimuli (19). The heart adapts to increased demands for cardiac work by increasing muscle mass through initiation of a hypertrophic response. However, left ventricular (LV) hypertrophy is a risk factor for congestive heart failure and sudden death. Because cardiac hypertrophy is a highly complex disorder that results from a combination of genetic, physiological, and environmental factors, one of the key events in its pathogenesis and treatment is genetic mutation of contractile proteins (16). The discovery and functional clarification of cardiac hypertrophy-related novel human genes are important for understanding the molecular mechanisms of cardiac hypertrophy. There is still a broad gap, however, between identifying the defective gene and understanding how this defect leads to the cardiac abnormalities.

Recently, we identified a novel homologous gene, myofibrillogenesis regulator-1 (MR-1), from a human skeletal muscle cDNA library (10). The MR-1 gene (GenBank accession no. AF417001) is located on human chromosome 2q35 (GenBank accession no. AC021016) and spans ~2,887 bp of contiguous DNA. The MR-1 gene is composed of three distinct exons between bp 169 and 713. The 5'- and 3'-acceptor splice sites in each of the introns follow the GT-AG consensus sequence for eukaryotic genes. Exon 1 (212 bp) encodes the whole 5'-untranslated region plus the first 22 amino acids. Exon 2 (169 bp) encodes the next 56 amino acids, whereas exon 3 (713 bp) contains the final 64 codons and an extensive 3'-untranslated region of ~182 bp. This gene encodes a 142-amino acid protein with a hydrophobic transmembrane structure between amino acids 75 and 92. Bioinformatic analysis revealed that MR-1 has no homologous gene in the known human gene database. A computer search of an expressed sequence tag database with the MR-1 amino acid sequence identified MR-1 orthologs in mammals such as mice, rats, cows, and pigs, but no detectable homologs were present in invertebrates such as fugu, zebras, Drosophila, Caenorhabditis elegans, sea, yeast, fungi, Plasmodium falciparum, and all 278 known microbial genomes. These findings support the hypothesis that the MR-1 gene exists only in mammals. Further immunohistochemical staining revealed that the MR-1 protein is located mainly on the cytoplasm in fibroblast cells, as well as on the nuclear membrane in the human HeLa cell (11, 12). Northern blot and serial analysis of gene expression revealed that the transcription level of MR-1 in human tissues is especially high in skeletal muscle and myocardium (10).

Muscle consists of myocytes full of strands called myofibrils, which are in turn made up of contractile units called sarcomeres. Sarcomeres mainly consist of two kinds of filamentary protein: actin and myosin. These protein molecules slide over one another telescopically as the sarcomere contracts and relaxes. Yeast two-hybrid screening and in vitro GST pulldown assay revealed that MR-1 interacts with three proteins involved in muscle contraction (myosin regulatory light chain, myomesin, and β-enolase) and several cell signal transduction-related proteins (10). The interaction of MR-1 with sarcomeric structural proteins involved in muscle contraction and its presence in human myocardial myofibrils indicate that MR-1 is involved in the regulation of contractile proteins in the myocardium.
myocardium and might be associated with cardiac hypertrophy. To test this hypothesis, MR-1 expression in hypertrophied myocardium and cardiomyocytes was compared with that in sham-operated (SO) myocardium and control cardiomyocytes of rats. Also the effects of rat MR-1 (rMR-1) RNA interference (RNAi) on angiotensin II (ANG II)-induced hypertrophy were studied in cultured cardiomyocytes from neonatal rats.

**METHODS**

**In Vivo Studies**

Male Sprague-Dawley rats (90–100 g body wt) were housed four per cage at 23°C with a 12:12-h light-dark cycle, fed Purina rat ration, and allowed free access to water. All studies were approved by the Council for Animal Research, Health Center, Peking University. All animals were obtained from the Experimental Animal Center at PLA General Hospital.

Rats underwent abdominal aortic stenosis (AAS, n = 12) or SO (n = 12) as described by Woodwiss et al. (20). Tail systolic blood pressure (SBP) was measured 3, 7, 14, 21, and 28 days after AAS with a tail-cuff blood pressure analyzer (Manoreter-Tachometer KN-210). At 28 days after AAS, mean arterial pressure (MAP) and heart rate (HR) were measured for 3 consecutive minutes in anesthetized rats. For measurement of these parameters, the right carotid artery was cannulated and connected to a pressure transducer in-line with a Grass polygraph.

At 28 days after AAS, anesthetized rats were weighed, and plasma angiotensin peptide concentration was assayed by the method of Cassis et al. (2). Then the rats underwent median thoracotomy for removal of the heart, and the LV was dissected and weighed for detection of LV hypertrophy assessed by the ratio of LV wet weight to whole heart weight (LV/HW) or LV wet weight to body weight (LV/BW). A longitudinal slice of the anterior LV wall was cut and immersed in neutral and tamponade formalin (10%). The tissue was washed, dehydrated, and embedded in paraffin. Serial sections (6 μm) were incubated at 4°C overnight with a 1:500 dilution of rabbit anti-rMR-1 polyclonal antibody and then incubated for 1 h at 37°C with a 1:500 dilution of goat anti-rabbit immunoglobulin (10). Antibody-biotin conjugate was detected with a 3,3'-diaminobenzidine kit according to the manufacturer’s instructions. The immunohistochemically stained slides were viewed with a light microscope, and digital gray-scale images were acquired with a charge coupled device camera with constant settings. Images were collected and analyzed with Microcomputer Imaging Device software (Imaging Research). Gray-scale values within the regions of interest were plotted as histograms, and minimum, maximum, and mean pixel intensity values were calculated with conventional software. Data are expressed as intensity units compared with values for which IgG was used as the primary antibody and as a control for comparison.

**Plasmid Construction**

With the use of bioinformatics analysis, two pairs of primers were designed and synthesized according to human MR-1 homologous sequences on rat genome chromosome 9. GenBank accession no. NW_047816.1 Olig1 and Olig2 (5'-ATGCCGGCGGTTAGCT-GCT-3' and 5'-TCAGGTCTGTAHTCCAGACCCAC-3', respectively) were used for the first RT-PCR; Olig3 and Olig4 (5'-ATTAGATCTCATGGCGGCTGTAGCTGCT-3' (Olig3), with the EcoRI site underlined, and 5'-ATATAAGCTTTCCAGGTCTG-TACTCCAGACCCAC-3' (Olig4), with the HindIII site underlined) were used for the second PCR. A 0.65-kb RT-PCR fragment was amplified from the total RNA of cardiomyocytes. A total of 0.1 μl of this product was used as a template for the second PCR. The RT-PCR was carried out in a volume of 25 μl containing 2.4 mM MgSO4, incubated for 30 min at 50°C, and then subjected to 40 cycles of 15 s at 94°C, 30 s at 55°C, and 1 min at 72°C on a thermal cycler (model PTC-200, MJ Research, Waltham, MA). The PCR was conducted at 94°C for 4 min followed by 30 cycles at 94°C for 30 s, 55°C for 50 s, and 72°C for 1 min, with a final extension at 72°C for 5 min.
Plasmids were constructed using standard techniques (6). Each constructed plasmid was sequenced for verification of accuracy. The prediction of MR-1 mRNA secondary structures from the sequence we cloned was based on the principle of minimizing free energy using the RNA structure 3.71 program. Figure 1 shows the structure with the minimum thermodynamic free energy calculated: −176.5 kcal/mol. The red regions represent the three double-stranded RNA (dsRNA) delivering plasmids pSi-1, pSi-2, and pSi-3 targets (Fig. 1). With the use of these recombinant plasmids, three RNAi were constructed from the pSilencer 3.0-H1 (Ambion, Austin, TX), each targeting different regions of MR-1 (Table 1).

**Semiquantitative RT-PCR Assay**

Total RNA was extracted using Trizol reagent. After transfection of the cultured cardiomyocytes with the RNAi plasmids for 24 h, a semiquantitative RT-PCR was performed for detection of trigger-silencing efficiency. This method enables quantitation on the basis of semiquantitative RT-PCR was performed for detection of trigger-silencing efficiency. The prediction of MR-1 mRNA secondary structures from the sequence we cloned was based on the principle of minimizing free energy using the RNA structure 3.71 program. Figure 1 shows the structure with the minimum thermodynamic free energy calculated: −176.5 kcal/mol. The red regions represent the three double-stranded RNA (dsRNA) delivering plasmids pSi-1, pSi-2, and pSi-3 targets (Fig. 1). With the use of these recombinant plasmids, three RNAi were constructed from the pSilencer 3.0-H1 (Ambion, Austin, TX), each targeting different regions of MR-1 (Table 1).

**Calculation of Cardiomyocyte Size**

Neonatal cardiomyocytes were plated on 24-well culture plates at a density of 1 × 10^4 cells per well with maintenance medium for 24 h. Serum-starved cardiomyocytes were randomly distributed into the seven groups described above, but the ANG II incubation was 48 h, instead of 24 h as in the incorporation experiment. The surface area of cardiomyocytes was assessed by the method of Kodama et al. (9).

**Preparation of Whole Cell Extracts From Cardiomyocytes and Western Blotting Analysis**

The proteins were extracted from cardiomyocytes or myocardium instead of 24 h as in the incorporation experiment. The surface area of cardiomyocytes was assessed by the method of Kodama et al. (9).

**Chemicals**

DNA restriction endonucleases, T4 ligase, and the LA-PCR ThermoScript One-Step System from Invitrogen (Carlsbad, CA), chemicals used in internal standard in hypotrophy study (4). Two pairs of competitive primers were synthesized: P1 (5′-TCTTCCGGGGGATTCGCGG-3′) and P2 (5′-CGATGGCCAGGCAGTCCCCAC-3′), corresponding to 203- to 388-nt positions of the rMR-1 open reading frame (ORF) sequence, respectively. P3 (5′-GCCCCAGCCAGGGCCGTT-3′) and P4 (5′-GCCACAGGTTCCATACCC-3′) corresponded to nt 110–189 of rat β-actin (GenBank accession no. NM_031144). The RT-PCR was the same as that described above. Amplification yielded a 186-bp rMR-1 fragment and a 710-bp β-actin fragment. The products were analyzed on 1.5% agarose gel stained with ethidium bromide. The gels were scanned by an Alpha 5500 scanner, and the band intensities were evaluated using AlphaEase FC3.1.2 software. The relative level of rMR-1 mRNA was obtained from the ratio of its individual band intensity to internal standard band (i.e., β-actin) intensity.

**Incorporation of [3H]Leu**

Neonatal cardiomyocytes were plated on 24-well culture plates at a density of 1 × 10^4 cells per well with maintenance medium for 24 h. Cells were randomly distributed into the following groups: 1) control group, i.e., untransfected cardiomyocytes incubated in a cell incubator for 50.5 h; 2) ANG II group, i.e., untransfected cardiomyocytes cultured for 36 h and then incubated for 24 h with ANG II (10 −7 mol/l); 3) losartan + ANG II group, i.e., untransfected cardiomyocytes cultured for 35.5 h and preincubated with losartan (10 −7 mol/l) for 30 min before 24 h of ANG II treatment; 4) MR-1 RNAi + ANG II group, i.e., pSi-1-transfected cardiomyocytes cultured for 36 h before 24 h of ANG II treatment; 5) MR-1 RNAi group, i.e., pSi-1-transfected cardiomyocytes cultured for 50 h; 6) pSilencer 3.0-H1 transfection group, i.e., cardiomyocytes transfected with pSilencer 3.0-H1 and cultured for 50 h; and 7) pSilencer 3.0-H1 transfection + ANG II group, i.e., cardiomyocytes transfected with pSilencer 3.0-H1 and cultured for 36 h before 24 h of ANG II treatment. Then the cardiomyocytes were incubated in DMEM containing 12.5 μCi/ml [3H]Leu for 24 h, and [3H]Leu uptake was determined according to the methods described by Liu et al. (13).

**Table 1. rMR-1 RNA interference plasmids target sequence and primers designed for each construction**

<table>
<thead>
<tr>
<th>pSi-1</th>
<th>Target Sequence and Primers Designed for Construction</th>
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<tr>
<td>GC Content, %</td>
<td>AAACAGCAGCCTCTCTACAAAGG</td>
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<tr>
<td>pSi-2</td>
<td>rMR-1 ORF 91–111</td>
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<tr>
<td>GC Content, %</td>
<td>AAATACATTCCCAGAAAGAGGG</td>
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<tr>
<td>pSi-3</td>
<td>rMR-1 ORF 343–363</td>
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<tr>
<td>GC Content, %</td>
<td>AAGCTTTTCCAAAAAACAGGAGTTCAAGAGACCCTCTTTCTGGGAATGTATTTTTTGGAAA</td>
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<tr>
<td>pSi-11</td>
<td>5′-AGCTTTTCCAAAAAACACGGGCGAGTATGAGAGCTCTCTTGAAGCTCTCATACTCGCCCGTGCG-3′</td>
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<tr>
<td>pSi-21</td>
<td>5′-GATCCGTACATTCCCAGAAAGAGGTTCAAGAGACCCTCTTTCTGGGAATGTATTTTTTGGAAA-3′</td>
</tr>
<tr>
<td>pSi-31</td>
<td>5′-GATCCGGACGGGGGATGTAGAAGCTCTTCTCATACTGGGCGTTTGGAAA-3′</td>
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**Table 2. Characterization of body weight, heart rate, and tail SBP before and after AAS**

<table>
<thead>
<tr>
<th>Body Wt, g</th>
<th>Heart Rate, min</th>
<th>Tail SBP, kPa</th>
</tr>
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<tbody>
<tr>
<td>103.80±8.23</td>
<td>165.33±22.64</td>
<td>15.13±0.70</td>
</tr>
<tr>
<td>120.27±17.92</td>
<td>196.40±25.91</td>
<td>17.49±1.89</td>
</tr>
<tr>
<td>165.07±21.55</td>
<td>226.33±30.03</td>
<td>17.99±3.62</td>
</tr>
<tr>
<td>205.80±18.87</td>
<td>246.47±28.9</td>
<td>19.48±2.59</td>
</tr>
<tr>
<td>244.60±33.83</td>
<td>280.00±31.7</td>
<td>21.07±2.79</td>
</tr>
<tr>
<td>276.00±47.14</td>
<td>343.33±49.95</td>
<td>21.16±2.59</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 12. SBP, systolic blood pressure; AAS, abdominal aortic stenosis. *P < 0.01 vs. preoperation.
and fetal bovine serum from HyClone (Logan, UT). Rabbit anti-rat MR-1 (rMR-1) polyclonal antibody was generated in our laboratory (11). Other monoclonal antibodies, enhanced chemiluminescence immunodetection kits, and 3,3’-diaminobenzidine kits were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Prestained SDS-PAGE protein standards (broad range) were purchased from Bio-Rad Laboratories (Hercules, CA) and L-[3,4,5-3H]Leu from Amersham (Buckinghamshire, UK). Other chemicals were purchased from Sigma (St. Louis, MO), unless otherwise noted.

Statistical Analysis

Values are means ± SD from at least three independent experiments. Each treatment in each experiment was performed in triplicate. In each experiment,cardiomyocytes were pooled from three different litters, and data from the three experiments were pooled (n = 3). Differences in means between groups were tested by one-way ANOVA. P < 0.05 was considered statistically significant.

RESULTS

Rat MR-1 Expression in Hypertrophied Myocardium

Magnitude of pressure overload. The time course for alterations in tail SBP and HR after 3, 7, 14, 21, and 28 days of pressure overload is presented in Table 2. AAS resulted in a significant increase in tail SBP (P < 0.01) and HR (P < 0.01).

Tail SBP increased by 16%, 19%, 29%, 39%, and 40% and HR by 19%, 37%, 49%, 69%, and 107% in AAS-induced rats at 3, 7, 14, 21, and 28 days, respectively, compared with SO rats.

AAS resulted in a significant increase in MAP, LV pressure, LV/HW, and LV/BW after 28 days of pressure overload. MAP increased by 29% (P < 0.01) and LV pressure by 36% (P < 0.01) 28 days after pressure overload compared with SO rats. LV hypertrophy developed by 28 days of pressure overload, as manifested by an increase in LV/HW and LV/BW by 39% (P < 0.01) and 27% (P < 0.01), respectively (Table 3).

Plasma ANG II concentration increased threefold in the AAS group 28 days after pressure overload compared with SO rats (Table 3).

Protein expression of rMR-1 in myocardium. Although only faint immunoreactivity of rMR-1 was detected in myocardium of SO rats, strong signals were seen in the AAS rats (Fig. 2).

Quantitative image analysis indicated a 110% increase in the area of rMR-1 staining in AAS-induced hypertrophied myocardium (P < 0.05 vs. SO rats).

Alteration in protein expression of rMR-1 in AAS-induced hypertrophied myocardium was detected by Western blotting. Positive results were followed up with blots made from myocardial proteins (Fig. 3). Blotting of whole cell extracts from

<table>
<thead>
<tr>
<th>Groups</th>
<th>MAP, kPa</th>
<th>LVDP, kPa</th>
<th>LV/HW</th>
<th>LV/BW</th>
<th>Plasma ANG II, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO</td>
<td>23.23±7.32</td>
<td>63.71±10.44</td>
<td>2.26±0.15</td>
<td>3.24±0.29</td>
<td>63.71±10.44</td>
</tr>
<tr>
<td>AAS</td>
<td>29.89±6.40*</td>
<td>86.62±11.32*</td>
<td>3.15±0.38*</td>
<td>4.10±0.38*</td>
<td>86.62±11.32*</td>
</tr>
</tbody>
</table>

Values are means ± SD. MAP, mean arterial pressure; LVDP, left ventricular (LV) developed pressure; LV/HW, LV wet weight-to-heart weight ratio; LV/BW, LV wet weight-to-body weight ratio; SO, sham operated. *P < 0.01 vs. SO.

Fig. 2. A: cardiac cross sections stained immunohistochemically for rMR-1 in myocardium from sham-operated (SO) rats (left) and rats subjected to abdominal aortic stenosis (AAS, right). Brown stain delineates rMR-1 visualized with diaminobenzidine. B: quantitative data of immunohistochemical stains based on quantitative image analysis of immunohistochemically detected rMR-1. Error bars, SD. *P < 0.05 vs. SO.
AAS-induced hypertrophied myocardium resulted in a 242% increase of rMR-1 expression compared with SO myocardium ($P < 0.01$). The results are consistent with those from immunohistochemical stains.

Effects of RNAi on rMR-1 mRNA Expression

Figure 4 shows mRNA expression of rMR-1 24 h after transfection of cardiomyocytes with plasmids targeting different regions of rMR-1. pSi-1, targeting the rMR-1 ORF 91–111 sequence, gave the best target gene-silencing effect and was chosen for the following experiments.

Effects of RNAi on ANG II-Induced Hypertrophy in Neonatal Cardiomyocytes

Surface area of cells. Measurement of the surface area of cardiomyocytes revealed that ANG II caused a 66% increase in cell size compared with controls (2,645.8 ± 129.3 vs. 1,589.3 ± 135.2 $\mu$m$^2$, $P < 0.01$). The ANG II-induced increase in surface area of cells was prevented by losartan, an AT$_1$ receptor inhibitor, and by transfection with the plasmid pSi-1, respectively (Fig. 5). Compared with the ANG II group, incorporation of $[^3]$HLeu was decreased 31% in the losartan + ANG II group (5,736.2 ± 872.1 $cpm/10^6$ cells in ANG II + losartan, $P < 0.01$ vs. ANG II group) and 37% in the rMR-1 RNAi + ANG II group (5,239.4 ± 763.8 $cpm/10^6$ cells in rMR-1 RNAi + ANG II, $P < 0.01$ vs. ANG II group). Transfection with a vector had no effect on the ANG II-induced increase in protein synthesis. As with results of the study of cardiomyocyte surface area, these results indicate that MR-1 may be critical for ANG II-induced hypertrophy of cardiomyocytes.

Protein expression of rMR-1 in cardiomyocytes. Alteration of protein expression of rMR-1 in cardiomyocytes was detected by Western blotting analysis. Blotting of whole cell

Fig. 3. A: Western blot analysis of rMR-1 expression in myocardium from SO and AAS rats. Equal protein loading was routinely verified by stripping the blot and reblotting with an anti-β-actin mouse monoclonal antibody. B: densitometric data from immunoblots in A. Error bars, SD. *$P < 0.01$ vs. SO.

Fig. 4. RT-PCR detection of rMR-1 and β-actin transcription level after transient transfection with plasmids pSi-1, pSi-2, and pSi-3 for 24 h. Lane $M$, 200-bp ladder DNA marker; lanes 1, 2, 3, and 4, RT-PCR products 24 h after transient transfection of cardiomyocytes with vector pSilencer 3.0-H1, pSi-1, pSi-2, and pSi-3, respectively, with total RNAs used as templates. A: representative RT-PCR gel. pSi-1 was most efficient in silencing rMR-1 after plasmid DNA treatment. GAPDH was amplified as an internal control. B: densitometric analysis of mRNA expression for rMR-1 24 h after treatment with the irrelevant small interfering RNA (siRNA, pSi-0) and the active siRNA (pSi-1, pSi-2, and pSi-3) generating plasmid DNAs. pSi-1 treatment dramatically reduced rMR-1 mRNA by $\sim 95%$. *$P < 0.01$ vs. SO.
extracts from ANG II-induced hypertrophied cardiomyocytes resulted in a 66% increase of rMR-1 expression compared with that in whole cell extracts from controls (Fig. 6). The upregulation of rMR-1 expression was abolished in losartan-pre-treated and pSi-1-transfected cardiomyocytes. Transfection with a vector had no effect on rMR-1 protein expression in control and ANG II-stimulated cardiomyocytes (Fig. 6).

DISCUSSION

Cardiac hypertrophy is an adaptive response of the heart associated with several pathological situations, including heart failure, myocardial infarction, and cardiac arrhythmias. The development of LV hypertrophy enhances contractility and allows for normalization of cardiac wall stress in response to pressure or volume overload (8). The benefits of this adaptive response of the heart may be offset by detrimental effects on cardiac function and morphology; thus cardiac hypertrophy is an important cause of increased morbidity and mortality.

Several experimental models have been proposed for the study of LV hypertrophy due to pressure overload. One of these is induction of AAS in rats, which leads to systemic hypertension, LV hypertrophy, and activation of the renin-angiotensin-aldosterone system (1, 3). Using a model of AAS-induced cardiac hypertrophy, we assessed cardiac function, blood pressure, and LV hypertrophy in adult Sprague-Dawley rats. In this study, pressure overload induced by suprarenal AAS increased MAP proximal to the site of aortic constriction. The magnitude of the pressure increase was sufficient to produce sustained MAP, demonstrated as increased LV/HW and LV/BW, within 28 days. Our results also demonstrate that the systemic renin-angiotensin system is activated in response to pressure overload and coincides with the appearance of LV hypertrophy in AAS rats. The results agree with findings from previous studies examining the time course for cardiac hypertrophy and function in response to pressure overload (20).

The mechanisms governing the development of cardiac hypertrophy have been extensively studied; however, they are incompletely understood. Investigation of alterations in myocardial contractile protein-related genes during hypertrophy helps in our understanding of the mechanisms and provides a new pathway for treatment of hypertrophy and heart failure. The MR-1 gene encodes a protein that shows interaction with three proteins involved in muscle contraction and several cell signal transduction-related proteins (10). However, the role of MR-1 in cardiac hypertrophy remains unknown. In the present study, immunohistochemistry and Western blot analyses demonstrated that MR-1 protein expression is upregulated in myocardium subjected to AAS-induced cardiac hypertrophy in rats.
To our knowledge, this is the first direct evidence that MR-1 is involved in the hypertrophic response of the heart.

Cardiomyocytes are terminally differentiated cells (17). In response to various extracellular stimuli, cardiomyocytes grow in a hypertrophic manner, characterized by enlarged individual cell size and increased content of contractile proteins (18). ANG II is a major determinant of arterial pressure and volume homeostasis. Moreover, ANG II has been postulated to be the humoral mediator of mechanical stretch-induced cardiac hypertrophy. In this study, we showed that ANG II induced an increase in $[^{3}H]$Leu uptake and cell area in cardiomyocytes. The results also confirmed that ANG II augmented MR-1 protein synthesis in cardiomyocytes, which coincides with results of our in vivo experiments. Pretreatment with losartan, an AT$_1$ receptor inhibitor, prevented the hypertrophic events and the increased MR-1 protein secretion, which indicated that the upregulation of MR-1 protein expression is associated with ANG II-induced cardiac hypertrophy.

RNAi is a process of sequence-specific gene silencing in animals and plants; it is initiated by dsRNA that is homologous in sequence to the targeted genes (7). The specific messenger RNA degradation is mediated by 21- or 22-nt small interfering RNAs (siRNAs) generated by ribonuclease III cleavage from longer dsRNAs (5). In the present study, RNAi was used to further study the relation between MR-1 expression and cardiac hypertrophy. After transfection of neonatal cardiomyocytes with a pSi-1 targeting the rMR-1 ORF 91–111 sequence, we observed a metrically distributed.

**In summary, our results demonstrate for the first time, we believe, the functional role and direct effect of MR-1 in cardiac hypertrophy. The findings may provide a better understanding of the mechanism(s) of cardiac remodeling and a new insight into the development of novel therapeutic strategies in cardiac hypertrophy.**

**REFERENCES**


