Matrix metalloproteinases mediate β-adrenergic receptor-stimulated apoptosis in adult rat ventricular myocytes

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Menon, Bindu, Mahipal Singh, and Krishna Singh. Matrix metalloproteinases mediate β-adrenergic receptor-stimulated apoptosis in adult rat ventricular myocytes. Am J Physiol Cell Physiol 289: C168–C176, 2005. First published February 23, 2005; doi:10.1152/ajpcell.00606.2004.—Changes in the synthesis and activity of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are associated with myocardial remodeling. Here we measured the expression and activity of MMPs and TIMPs, and tested the hypothesis that increased MMP activity plays a proapoptotic role in β-adrenergic receptor (β-AR)-stimulated apoptosis of adult rat ventricular myocytes (ARVMs). β-AR stimulation (isoproterenol, 24 h) increased mRNA levels of MMP-2 and TIMP-1 while it decreased TIMP-2 mRNA levels as analyzed by real-time PCR. Western blot analysis, immunocytochemical analysis, in-gel zymography, and MMP-2 activity assay confirmed β-AR-stimulated increases in MMP-2 protein levels and activity. Inhibition of MMPs using GM-6001 (a broad-spectrum inhibitor of MMPs), SB3CT (inhibitor of MMP-2), and purified TIMP-2 inhibited β-AR-stimulated apoptosis as determined by Tdt-mediated dUTP nick end labeling staining. Treatment with active MMP-2 alone increased the number of apoptotic cells. This increase in MMP-2-mediated apoptosis was inhibited by GM-6001 and SB3CT pretreatment. Coimmunoprecipitation studies indicated increased physical association of MMP-2 with β1-integrins after β-AR stimulation. Inhibition of MMP-2 using SB3CT or stimulation of β1-integrins signaling using laminin inhibited the increased association of MMP-2 with β1-integrins. β-AR stimulation increased poly-ADP-ribose-polymerase cleavage, which was inhibited by inhibition of MMP-2. These data suggest the following: 1) β-AR stimulation increases MMP-2 expression and activity and inhibits TIMP-2 expression; 2) inhibition of MMPs, most likely MMP-2, inhibits β-AR-stimulated apoptosis; and 3) the apoptotic effects of MMP-2 may be mediated, at least in part, via its interaction with β1 integrins and poly-ADP-ribose-polymerase cleavage.

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and dissociated in the same buffer containing trypsin (0.02 mg/ml) and DNase (0.02 mg/ml). The cell mixture was filtered and sedi-
mented through a 6% bovine serum albumin cushion to remove nonmyocyte cells. The cell pellet was resuspended in DMEM supple-
mented with creatine (5 mM), t-carnitine (2 mM), taurine (5 mM), and 0.1% penicillin-streptomycin, and plated at a density of 30–50
cells/mm² on 100-mm tissue culture dishes (Fisher Scientific) or coverslips precoated with laminin (1 µg/cm²). The investigation con-
forms to the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication No.
85-23, Revised 1996). The animal protocol was approved by the University Committee on Animal Care.

**Cell treatment.** ARVMs, cultured for 24 h, were treated with isoprotoreno (Iso; 10 µM, Sigma) in the presence of ascorbic acid
(100 µM) for 24 h to study expression of MMP-2, MMP-9, TIMPs, MT1-MMP, activity of MMPs and TIMP-2. To measure PARP cleavage,
ARVMs were treated for 6 h. To study apoptosis, cells were pretreated with GM-6001 (2 µM) or its negative control for 60 min, SBSCT (1 nM, Calbiochem) or TIMP-2 (50 ng/ml, Calbiochem) for 30 min, followed by treatment with Iso (10 µM) or active MMP-2 (1 nM; Calbiochem) for 24 h. To stimulate β₁-integrin signaling, cells were pretreated with laminin (10 µg/ml) for 30 min.

**Real-time PCR.** Total RNA was isolated as described previously by Singh et al. (42). The RNA was reverse transcribed using superscript reverse transcriptase kit (Invitrogen). The mRNA levels of MMP-2, MMP-9, MT1-MMP, activity of MMPs and TIMP-2 were quantified using real-time PCR (iCycler, BioRad). The forward and reverse
primers used were 5'-CTGATAACCTGGATGCAGTCGT-3' (MMP-2); 5'-TTCAAGGACCGTT-CGGTATT-3' and 5'-CTTCTGAGCGGTACCCAACTTA-3' (MMP-9); 5'-GACAGGACCGAAGATA-AGTGATGTGA-3' (MT1-MMP); 5'-TCTGCGACTCTTTGGTCTT-GCTAT-3' and 5'-CCACAGCATCGAATCTT-3' (TIMP-1); 5'-GGATTCCGGAGAATGACATCTT-3' and 5'-CGCCCTCTCTGGCAATAGA-3' (TIMP-2); 5'-GTCCTACAGCCATTTGACT-CTT-3' and 5'-GTACAGGCGCTGAGTACGC-3' (TIMP-4) and 5'-TGACACCAACCTGCTTA-3' and 5'-GGATGCAAGGTATGTTCGT-3' (GAPDH). The PCR conditions for MMP-2, MMP-9, TIMP-2, and TIMP-4 were 50 cycles of denaturation (94°C, 18 s), annealing and elongation (68°C for 45 s), and for MT1-MMP and GAPDH were 50 cycles of denaturation (94°C for 18 s), annealing (65°C for 20 s) and elongation (72°C for 18 s). Reactions are characterized by comparing threshold cycle (Ct) values. Samples with a high starting copy number show an increase in the fluorescence early in the PCR process resulting in a low Ct number, whereas a lower starting copy number results in higher Ct numbers. Initial characterization of GAPDH expression using RT-PCR followed by agarose gel electrophoresis and real-time PCR indicated no significant change in the intensity of the GAPDH signal in ARVMs after β-AR stimulation. Therefore, mRNA levels were normalized relative to GAPDH values.

**In-gel zymography.** The conditioned media were lyophilized to dryness and the pellet was resuspended in water (referred as concen-
trated conditioned media), and protein content was measured with the use of Bradford assay (Bio-Rad). MMP activity in the concentrated conditioned media containing 10 µg of protein was measured using gelatin in-gel zymography (51). Clear and digested regions were stained with Ponceau S to confirm equal loading of proteins in the samples. After being destained, the membranes were stained with Ponceau S to confirm equal loading of proteins in the samples. After being destained, the membranes were incubated overnight in the TBST blocking buffer, composed of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20, containing 5% nonfat dry milk. The membranes were then incubated with primary antibodies diluted in blocking buffer. After being washed with TBST, the membranes were incubated with a peroxidase-conjugated secondary antibody. The immune complexes were detected using chemiluminescence reagents (Pierce Biotechnology).

**Western blot analysis.** To study PARP cleavage, cells were lysed in 150 µl of extraction buffer (100 µl of 25 mM Tris·HCl, pH 8, containing 50 mM glucose, 10 mM EDTA, 1 mM PMSF and 50 µl of 50 mM Tris·HCl, pH 6.8, containing 6 M urea, 6% 2-mercaptoetha
ol, 3% SDS, and 0.003% bromophenol blue). The lysates were then sonicated for 60 s at 180 V and incubated at 65°C for 15 min before loading on a 7.5% gel. For MMP-2 protein, concentrated conditioned media (50–100 µg) were resolved by 10% SDS-PAGE (Bio-Rad). Proteins from the gels were electrotheroetically transferred to a PVDF membrane (Hybond-P, Amersham Biosciences). The membranes were stained with Ponceau S to confirm equal loading of proteins in the samples. After being destained, the membranes were incubated overnight in the TBST blocking buffer composed of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20, containing 5% nonfat dry milk. The membranes were then incubated with primary antibodies diluted in blocking buffer. After being washed with TBST, the membranes were incubated with a peroxidase-conjugated secondary antibody. The immune complexes were detected using chemiluminescence reagents (Pierce Biotechnology). The levels of active MMP-2 in the concen-
trated conditioned media containing 20 µg of protein were measured using MMP-2 activity assay kit according to the manufacturer’s instructions (Amersham Biosciences).

**Immunofluorescent labeling.** ARVMs were fixed in 3.7% formal-dehyde and permeabilized using 1% Triton X-100. The cells were then incubated with 10% goat serum for 1 h. After being washed with phosphate-buffered saline, the cells were incubated overnight with monoclonal anti-MMP-2 antibodies (1:100, Chemicon, Temecula, CA). After incubation with FITC-conjugated secondary antibody, the coverslips were mounted, visualized with the use of a fluorescent microscope, and photographed.

**RESULTS**

**β-AR stimulation increases MMP-2 expression and activity.** To study whether β-AR stimulation increases mRNA levels for MMPs (MMP-2 and -9), ARVMs were treated with Iso for 24 h. Real-time PCR analysis of total RNA demonstrated that β-AR stimulation (Iso; 10 µM; 24 h) increases mRNA levels of MMP-2 by 1.6 ± 0.1 fold (P < 0.05; n = 3; Fig. 1A) compared with control. In contrast to MMP-2 mRNA, MMP-9 mRNA levels remained unchanged after β-AR stimulation [1.3 ± 0.4-fold vs. control; P = not significant (NS); n = 3].

Western blot analysis of conditioned media using anti-
MMP-2 antibodies demonstrated increased MMP-2 protein levels (1.9 ± 0.1-fold vs. control; P < 0.05; n = 8; Fig. 1B) after 24 h of β-AR stimulation. To confirm that ARVMs are indeed the source of MMP-2 protein, immunocytochemical analysis was performed. This analysis showed positive immuno-
reactivity for MMP-2 in untreated rod-shaped ARVMs (Fig.
β-Adrenergic receptor (β-AR) stimulation induced morphological changes from rod-shaped to rounded, and the cells exhibited increased immunostaining for MMP-2 protein (Fig. 1C). Negative control in the absence of primary antibodies showed no fluorescence (data not shown).

Gelatin in-gel zymographic analysis of conditioned media demonstrated increased MMP-2 activity (1.7 ± 0.1-fold vs. control; *P < 0.05; n = 12; Fig. 2A) after 24 h of β-AR stimulation. In contrast to MMP-2 activity, MMP-9 activity remained unchanged after β-AR stimulation. Analysis of levels of active MMP-2 in the conditioned media using MMP-2 activity assay indicated increased MMP-2 activity (levels of active MMP-2, ng/20 μg of total protein; CTL, 1.6 ± 0.2; Iso, 2.5 ± 0.3; n = 3; *P < 0.05; Fig. 2B).

β-AR stimulation reduces TIMP-2 mRNA levels and protein expression. MT1-MMP activates MMP-2 (23), whereas TIMP-2 is suggested to inhibit MMP-2 activity (28). TIMP-4 is predominantly expressed in the heart (18), whereas TIMP-1 forms a specific complex with MMP-9 (16). Therefore, we next studied the expression of MT1-MMP and TIMPs (TIMP-1, -2, and -4) at mRNA level using real-time PCR. MT1-MMP mRNA levels remained unchanged after 24 h of β-AR stimulation (1.2 ± 0.3-fold vs. control, *P = NS; n = 3; Fig. 3A). Interestingly, β-AR stimulation significantly reduced TIMP-2 mRNA levels (0.4 ± 0.1-fold; *P < 0.05; n = 3; Fig. 3B) compared with control. β-AR stimulation increased TIMP-1 mRNA levels (2.5 ± 0.3-fold vs. control; *P < 0.05; n = 3; Fig. 3B), whereas there was no significant change in TIMP-4.
mRNA levels. Analysis of conditioned media using Western blot analysis demonstrated a significant decrease in TIMP-2 protein levels after 24 h of H9252-AR stimulation (0.45 ± 0.05-fold vs. control, P < 0.05; n = 3; Fig. 3C).

Inhibition of MMP-2 inhibits β-AR-stimulated apoptosis. To study the role of MMP-2 in β-AR-stimulated apoptosis, ARVMs were pretreated with GM-6001 (a broad spectrum inhibitor of MMPs) and its negative control for 60 min, followed by treatment with Iso for 24 h. As reported previously (10), Iso increased the percentage of TUNEL-positive myocytes (CTL, 5.03 ± 0.6%; Iso, 18.03 ± 0.9%; P < 0.05; n = 4). Pretreatment with GM-6001 (2 μM, 60 min) almost completely inhibited β-AR-stimulated apoptosis (Iso+GM-6001, 7.5 ± 1.2%; P < 0.05 vs. Iso; n = 4; Fig. 4). GM-6001(-), a structurally similar compound, in which the metal binding site hydroxamic acid is replaced by butoxycarbonyl group used as a negative control, failed to inhibit β-AR-stimulated apoptosis (Iso+GM-6001 negative control, 15.8 ± 0.9; P = NS vs. Iso; n = 4; Fig. 4).

To study the role of MMP-2 in β-AR-stimulated apoptosis, ARVMs were pretreated with SB3CT (1 nM). The K_i value of SB3CT for MMP-2 is 13 nM, whereas it is 600 nM for MMP-9 (3, 22). Measurement of levels of active MMP-2 demonstrated that SB3CT at 1 nM concentration completely inhibits β-AR-stimulated increases in active MMP-2 (levels of active

**Fig. 2.** β-AR stimulation increases MMP-2 activity. A: ARVMs were treated with 10 μM Iso for 24 h. Concentrated conditioned media were analyzed by gelatin in-gel zymography. Bottom, the intensity of MMP-2 as fold increase vs. CTL. *P < 0.05 vs. CTL; n = 12. B: levels of active MMP-2 in the concentrated conditioned media were measured using MMP-2 activity assay. *P < 0.05 vs. CTL; n = 3.

**Fig. 3.** Effect of Iso on expression of membrane type 1 (MT1)-MMP and tissue-inhibitor MMPs (TIMPs). A: ARVMs were treated with Iso (10 μM) for 24 h. Total RNA was analyzed by real-time PCR using primers specific for MT1-MMP (A) or TIMPs (B). B: mRNA levels were normalized to GAPDH and are expressed as fold increase vs. control. *P < 0.05, n = 3. C: ARVMs were treated with Iso (10 μM) for 24 h. Concentrated conditioned media underwent Western blot analysis using anti-TIMP-2 antibodies. Bottom, intensity of TIMP-2 as fold increase vs. CTL. *P < 0.05 vs. CTL; n = 3.
MMP-2, ng/20 μg of total protein; Iso, 2.5 ± 0.3; Iso+SB3CT, 0.98 ± 0.24; P < 0.05 vs. Iso; n = 3). Analysis of apoptosis using TUNEL-staining assay indicated that pretreatment with SB3CT almost completely inhibits β-AR-stimulated apoptosis (Iso, 18.03 ± 0.9%; Iso+SB3CT, 6.85 ± 0.3%; P < 0.05 vs. Iso; n = 4; Fig. 5A). Pretreatment of ARVMs with purified TIMP-2 protein (50 ng/ml) also significantly inhibited β-AR-stimulated apoptosis (Iso, 18.03 ± 0.9%; Iso+TIMP-2, 7.2 ± 1.8; P < 0.05 vs. Iso; n = 4; Fig. 5B). Furthermore, treatment of ARVMs with purified active MMP-2 significantly increased the number of apoptotic cells compared with control (CTL, 6.4 ± 1.0; MMP-2, 18.2 ± 1.3; P < 0.05 vs. CTL; n = 6; Fig. 5C). MMP’s inhibitors, GM-6001 and SB3CT, inhibited MMP-2-mediated increases in apoptosis. 

β-AR stimulation increases association of MMP-2 with β1-integrins. Altered cellular localization of MMP-2 and its physical association with β1-integrins is suggested to be a mechanism by which MMPs induce apoptosis in HUVECs (26). Because ARVMs predominantly express β1-integrins (39), therefore, we next examined the physical association of MMP-2 with β1-integrins using coimmunoprecipitation assay. This analysis demonstrated that MMP-2 interacts with β1-integrins at basal levels (Fig. 6). β-AR stimulation increased the level of interaction between β1-integrins and MMP-2 protein by 1.7 ± 0.1 fold (P < 0.05, n = 6; Fig. 6). Pretreatment with SB3CT significantly inhibited β-AR-stimulated interaction of MMP-2 with β1-integrins. 

Activation of β1-integrin signaling inhibits association of MMP-2 and β1-integrins. Previously, we have shown that stimulation of β1-integrin signaling using laminin pretreatment protects ARVMs against β-AR-stimulated apoptosis (11). To determine whether association of MMP-2 with β1-integrins is regulated by the activation state of the β1-integrins, we pretreated ARVMs with laminin (10 μg/ml, 30 min). Coimmunoprecipitation analysis of proteins from the membrane fractions demonstrated that stimulation of β1-integrin signaling decreases the amount of MMP-2 protein communoprecipitated with β1-integrins (fold increase vs. control, Iso, 1.7 ± 0.1; laminin +Iso, 1.1 ± 0.02; P < 0.05 vs. Iso; n = 3–6; Fig. 7). 

SB3CT inhibits β-AR-stimulated increases in PARP cleavage. Activation of caspase-3 has been suggested to cause proteolytic cleavage of PARP (13). β-AR stimulated apoptosis in cardiac myocytes is mediated via JNK-dependent mitochondrial death pathway and activation of caspase. Inhibition of caspase using z-Val-Ala-Asp(OMe)-CH2F inhibits β-AR-stimulated apoptosis (37). To determine whether MMP-2 is involved in PARP cleavage, we analyzed total cell lysates for the presence of 89-kDa PARP fragment by Western blot analysis. This analysis indicated that β-AR stimulation increases the amount of 89-kDa PARP fragment (Fig. 8). Pretreatment with SB3CT inhibited β-AR-stimulated increases in PARP cleavage (fold increase vs. control, Iso, 1.9 ± 0.2; Iso+SB3CT, 0.7 ± 0.04; P < 0.05, n = 3).
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Fig. 6. β-AR stimulation increased physical association of MMP-2 with β1-integrins. ARVMs were pretreated with SB3CT (1 nM) for 30 min, followed by treatment with 10 μM Iso for 24 h. The proteins from the membrane fractions were immunoprecipitated using polyclonal anti-β1-integrin antibodies. The immunoprecipitates were resolved by SDS-PAGE and analyzed by Western blot analysis using monoclonal anti-MMP-2 antibodies. Immunoprecipitation of β1-integrins was confirmed by probing the membrane with anti-β1-integrin antibodies. Bottom, intensity of MMP-2 as fold increase vs. CTL. *P < 0.05 vs. CTL; n = 6, #P < 0.05 vs. Iso; n = 3.

DISCUSSION

Stimulation of β-AR induces apoptosis in cardiac myocytes in vitro and in vivo (43). The present study demonstrates that β-AR stimulation increases the expression and activity of MMP-2, and inhibition of MMP-2 inhibits β-AR-stimulated apoptosis in ARVMs. Increased recruitment and association of MMP-2 with β1-integrins may be a mechanism by which MMP-2 plays a pro-apoptotic role in β-AR-stimulated apoptosis.

Coker et al. (6) were the first to demonstrate that porcine cardiac myocytes express and secrete MMPs, specifically MMP-2, in vitro. Intracellular staining for MMP-2 in cardiac myocytes in conjunction with sarcomere is also observed in human heart during dilated cardiomyopathy (40). Recently, Kwan et al. (24) demonstrated presence of MMP-2 within the nucleus of rat cardiac myocytes. Endothelin-1 and angiotensin II increased MMP-2 activity in porcine cardiac myocytes, while Iso at 10 mM concentration had no effect with the use of a gelatin in-gel zymography assay (7). Using several different techniques, we provide evidence that Iso at 10 μM concentration increases expression and activity of MMP-2 in ARVMs. Lower concentrations of Iso (1 μM or less) showed no significant change in MMP-2 activity (data not shown). Lower concentrations of Iso only slightly increase the extent of apoptosis in ARVMs (52). In fact, Iso at 1 μM concentration is shown to protect ARVMs against apoptosis (20). We and others have shown that 10 μM concentration of Iso significantly increases apoptosis in ARVMs (10, 52). Collectively, these data suggest that the apoptotic concentration of Iso (10 μM) increases MMP-2 expression and activity in cardiac myocytes. The specific increase in MMP-2, not MMP-9, expression and activity suggests that MMP-2 may be an important factor in determining the interaction of ARVMs with ECM components and their survival. The signaling mechanisms by which β-AR stimulation increases MMP-2 expression are not yet clear. At the level of transcription, activation of mitogen-activated protein kinase (MAPK) superfamily, which includes ERK1/2, JNKs, and p38 kinase, plays an important role in the regulation of MMP expression (25, 32, 49). Our preliminary data suggest involvement of JNKs in the regulation of MMP-2 because SP-600125, an inhibitor of JNK pathway, inhibits β-AR-stimulated increases in MMP-2 protein levels and activity (30).

MMPs are synthesized and secreted as proenzymes. MT1-MMP activates MMP-2 on the cell surface with MT1-MMP-TIMP-2 complex serving as a receptor for pro-MMP-2 (23). In porcine cardiac myocytes, Iso (10 nM) increased MT1-MMP...
abundance within 4 h of treatment (7). In ARVMs, we found no change in MT1-MMP mRNA levels. This may be because MT1-MMP is required for the activation of MMP-2; therefore, an early increase in MT1-MMP may be crucial for the increased activation of MMP-2 observed after 24 h of β-AR stimulation.

The concentration of TIMP-2 is suggested to determine the role of TIMP-2 in the activation of MMP-2. At low concentrations, TIMP-2 may serve as a receptor for pro-MMP-2, whereas at higher concentrations, TIMP-2 may neutralize MT1-MMP and prevent MMP-2 activation (28). Increased expression and activity of MMPs with decreased expression of TIMPs is observed in many pathological situations of the heart (38, 46). The data presented here demonstrate that β-AR stimulation decreases expression of TIMP-2 in ARVMs. The decreased TIMP-2 expression with increased MMP-2 suggests an increase in the MMP-2/TIMP-2 ratio. It is interesting to note that there is no significant change in the expression and activity of MMP-9, whereas the expression of TIMP-1 is significantly increased after β-AR stimulation. TIMP-1 forms a complex with MMP-9 (16).

The inhibition of MMPs attenuates left ventricular remodeling events associated with chronic volume overload and postmyocardial infarction (4, 48). Inhibition of MMPs is shown to regress β-AR-stimulated myocyte hypertrophy in rats (31). Our results show that inhibition of MMPs, specifically MMP-2, plays a protective role in β-AR-stimulated apoptosis, suggesting that increased MMP-2 expression and activity during heart failure may induce cardiac myocyte loss due to apoptosis. Previously, we have shown that stimulation of β1-integrin signaling plays a protective role in β-AR-stimulated apoptosis in ARVMs (11). Here, we demonstrate physical association of MMP-2 with β1 integrins on the surface of ARVMs. β-AR stimulation significantly increased the level of interaction between MMP-2 and β1-integrins. The increased association of MMP-2 with β1-integrins was inhibited by activation of β1-integrin signaling pathway using laminin and inhibition of MMP-2 activity using SB3CT. SB3CT directly binds the catalytic zinc ion of MMP-2. The novel mode of binding of SB3CT to the catalytic zinc reconstructs the conformational environment around the active site metal ion back to that of the proenzyme (22). Therefore, the data presented here suggest that the cell-secreted activated MMP-2, not the newly synthesized intracellular MMP-2, interacts with β1-integrins. Interestingly, activation of β1-integrin signaling pathway using laminin inhibited the interaction of MMP-2 with β1-integrins. Similar observations have been made in HUVECs (26), where recruitment and binding of MMP-2 with β1-integrins is proposed to be a mechanism by which activation of MMP-2 induces apoptosis.

The intracellular mechanism/s by which interaction of MMP-2 with β1-integrins affects apoptosis in ARVMs is not yet clear. Disruption of normal myocyte anchorage to adjacent ECM and cells is proposed to be a mechanism of increased myocyte apoptosis during the transition from hypertrophy to early failure in mice (14). The presence of a 55-kDa extracellular domain fragment of β1-integrins is observed in the ECM of rat heart after 1 mo of aortic stenosis, and in the conditioned media of neonatal cardiac myocytes and fibroblasts (17). Previously, we have shown that β-AR stimulation does not affect expression of β1-integrins (11), and analysis of total cell lysates (prepared using RIPA buffer) exhibited no change in the levels of intact β1-integrin or 55-kDa fragment after 24 h of β-AR stimulation (data not shown). Therefore, another possibility is that recruitment and interaction of MMP-2 with β1-integrins disrupt β1-integrin-mediated intracellular survival signals. In support of this possibility, we found that inhibition of MMP-2 using SB3CT inhibits β-AR-stimulated increases in proteolytic cleavage of PARP. Of note, integrin engagement is suggested to control mitochondrial function in rabbit synovial fibroblasts via Rho GTPase-dependent mechanism (50).

ARVMs are isolated in a manner to eliminate nonmyocyte cell contamination. After collagenase and trypsin digestion, the cell mixture is filtered and sedimented through a 6% BSA cushion to remove nonmyocyte cells. Using propidium iodide staining and morphological examination, we found that the myocyte culture is ~97% pure. The observation that myocytes express MMP-2 and Iso increases MMP-2 expression in cardiac myocytes is supported by our data demonstrating positive immunoreactive staining for MMP-2 in rod-shaped cells (myocytes; Fig. 1C), and increased staining after 24 h of β-AR stimulation. In addition, other studies have shown that cardiac myocytes express and regulate MMPs in vitro and in vivo (6, 7, 24, 40). However, the possibility that small numbers of nonmyocytes are also contributing to the synthesis of MMPs in culture cannot be completely ruled out.

In conclusion, the data presented here demonstrate that β-AR stimulation increases MMP-2 expression and activity while inhibiting TIMP-2 expression. Inhibition of MMP-2 activity inhibits β-AR-stimulated apoptosis. The apoptotic effects of MMP-2 may be mediated via its interaction with β1-integrins. Continued loss of viable myocytes through apoptosis in failing human hearts is proposed to be a mechanism for progressive myocardial failure (8). The results presented here suggest that inhibition of MMP-2 may inhibit or reverse pathological remodeling. Further studies aimed at determining the molecular mechanism by which interaction of MMP-2 with β1-integrins affects β-AR-stimulated apoptosis in cardiac myocytes may have important implications for the regulation of myocyte survival.

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REFERENCES


