Phospholipase C/Protein Kinase C Pathway Mediates Angiotensin II–Dependent Apoptosis in Neonatal Rat Cardiac Fibroblasts Expressing AT1 Receptor

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Abstract: Cardiac fibroblasts are the major non-myocyte cell constituent in the myocardium, and they are involved in heart remodeling. Angiotensin II type 1 receptor (AT_1R) mediates the established actions of angiotensin II (Ang II), and changes in its expression have been reported in cardiac fibroblasts after myocardial infarction. However, the AT_1R-dependent signaling pathways involved in cardiac fibroblast death remain unknown. Using adenovirus, we ectopically expressed AT_1R in cultured neonatal rat cardiac fibroblasts and investigated the role of the phospholipase (PLC)/protein kinase C (PKC) pathway on Ang II-dependent death. Ang II induced cardiac fibroblast death characterized by an early loss of mitochondrial membrane potential, increased Bax/Bcl-2 ratio, caspase-3 activation, and DNA fragmentation. All these effects were prevented by the AT_1R antagonist losartan, PLC inhibitor U73122, and PKC inhibitor Gö6976. We conclude that Ang II stimulates the intrinsic apoptotic pathway in cultured cardiac fibroblasts by the AT_1R/PLC/PKC signaling pathway.

Key Words: angiotensin II, AT_1 receptor, fibroblast, apoptosis, cell death

INTRODUCTION

Cardiac fibroblasts are the major non-myocyte cell constituent in the myocardium and are involved in tissue remodeling.[1] Angiotensin II (Ang II) induces cardiac fibroblast proliferation and collagen accumulation. Two major receptors exist for Ang II, AT_1R and AT_2R.[2] AT_1R mediates the established actions of Ang II, and its expression in cardiac fibroblasts far exceeds that in other cardiac cells.[3,4] AT_2R functions are less clear, but current theories support a role in opposing AT_1R actions.[5] In adult or neonatal rat cardiac fibroblasts, activation of AT_1R by Ang II activates phospholipase C (PLC) and later, protein kinase C (PKC).[1,2]

Current evidence suggests that Ang II regulation of apoptosis in the heart is cell type-specific and subtype receptor-specific. Ang II increases neonatal cardiomyocyte apoptosis depending on AT_1R activation.[6] In contrast, cultured neonatal cardiac fibroblasts undergo AT_1R-dependent proliferation in response to Ang II.[7] However, recent data indicate that fibroblasts from different tissues have different sensitivity to apoptosis inducers.[8] The increased resistance of cardiac fibroblasts to apoptosis has been explained by a lower ratio of pro/anti-apoptotic Bcl-2 proteins.[9,10] The antiapoptotic members of the Bcl-2 protein family inhibit the release of mitochondrial apoptotic factors, whereas the proapoptotic members trigger their release. Mitochondria play a central role in apoptosis; the loss of mitochondrial membrane potential ($\Delta\psi_m$) is thought to contribute to cell death by disruption of normal mitochondrial function. Interaction of members of the Bcl-2 protein family regulates $\Delta\psi_m$, the key event of cytochrome c release into the cytoplasm.[11]

Changes in the expression of AT_1R have been reported in different pathophysiological conditions, especially in cardiac fibroblasts after myocardial infarction.[12] This study is part of an effort to further examine cardiac fibroblast death in conditions where AT_1R is upregulated as seen after myocardial infarction. Therefore, we tested the hypothesis that the intrinsic mitochondrial pathway of cell death is triggered by Ang II in cultured neonatal rat cardiac fibroblasts expressing AT_1R, involving the participation of the PLC-PKC.

METHODS

Isolation and Culture of Cardiac Fibroblasts

The rats were from the Animal Breeding Facility of the Faculty of Chemical and Pharmaceutical Sciences, University of Chile. All studies conformed to the Guide for the Care and Use of Laboratory Animals published by the US National

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Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by our Institutional Ethics Review Committee. Neonatal rat cardiac fibroblasts were prepared from hearts of 1- to 3-day-old Sprague-Dawley rats as described previously.[13] Fibroblasts were cultured in DMEM 10% fetal bovine serum (FBS) and used for all experiments at the second passage. A total of 20×10^3 fibroblasts/cm^2 were plated on plastic dishes and serum-deprived for 24 hours before experiments.

Adult Cardiac Fibroblast Isolation

Animal handling conforms to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23). Male Sprague-Dawley rats (250 g) were anesthetized with ketamine-xylazine (66 mg/kg and 1.6 mg/kg IP, respectively). Adult cardiac fibroblasts (ACF) were isolated by cardiac retrograde aortic perfusion as described previously with a few modifications.[14] Briefly, the heart was digested with a collagenase-hyaluronidase (1:1), and cells were centrifuged at 500 rpm for 1 min. The cell pellet (cardiomyocytes) was discarded, and the supernatant, containing mainly ACF, was centrifuged at 1000 rpm for 10 min and resuspended in M199 medium containing 10% FBS. ACFs were seeded in non-treated culture dishes for 2 hours. The cells were then washed with PBS to eliminate cell debris and nonadherent cells. ACFs were used at passage 2.

Adenoviral Transduction of Cultured Cardiac Fibroblasts

The adenovirus (Ad) expressing AT_1R (Ad-AT_1R) is a bicistronic vector coexpressing green fluorescent protein (GFP); the adenoviral vector expressing only GFP (Ad-GFP) was used as a transfection control.[15] Cells were cultured in DMEM 10% FBS for 24 hours and then switched to DMEM/F-12 medium containing Ad-AT_1R or Ad-GFP for 24 hours; the cells were rinsed and exposed to different stimuli or control medium (DMEM/F-12). Antagonists (losartan or PD123319; 10 μM each one) or inhibitors (Gö6976 100 nM a PKC inhibitor or U73122 1 μM a PLC inhibitor) were added to cultures 1 hour before treatment with Ang II 100 nM.

[^{125}I]Sar-Ile-Ang II Binding Assay

[^{125}I]Sar-Ile-Ang II (Perkin Elmer, Boston, MA) binding assay was performed on membrane fractions obtained from control cardiac fibroblasts or Ad-AT_1R–transduced cells. Plasma membrane was prepared as described previously.[4] Non-labeled Ang II (from 1 nM to 1 mM), AT_1R antagonist losartan (10 μM), and AT_2R antagonist PD123319 (10 μM) were used to verify the identity of the angiotensin receptor.

Western Blot Analysis

Cell proteins were extracted with a protease inhibitor cocktail-containing lysis buffer. Aliquots were resolved on 12% SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with primary antibodies against vimentin (1:1,000), Bax (1:500), Bcl-2 (1:1,000), caspase-3 (1:1,000), and GFP (1:1,000) at 4°C overnight. Bound antibodies were detected by horseradish peroxidase secondary antibody and visualized by ECL.

Cell Viability Assay and $\Delta\psi m$ Analysis

Cell viability was determined by methyl tetrazolium assay (MTT) and by Propidium Iodide (PI, 25 μg/mL) incorporation in stimulated cells and detected by flow cytometry in a Becton Dickinson FACSort. $\Delta\psi_m$ analysis was performed in treated cells that were incubated for 1 hour with tetramethylrhodamine methyl ester (TMR; 10 μg/mL), washed with phosphate buffered saline (PBS), trypsinized, re-suspended in 200 μL of DMEM 10% FBS, and analyzed by flow cytometry in a Becton Dickinson FACSort. Results were analyzed with WinMDI software. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) was used as control.

Detection of DNA Fragmentation by Flow Cytometry

After the treatment, cells were collected, permeabilized with methanol for 24 hours, and treated with RNAase for 2 hours, and 2 μL propidium iodide (PI, 25 μg/mL) was added before flow cytometry analysis. A total of 5000 cells/sample were analyzed.

Statistical Analyses

Data are mean ± SEM of at least 3 independent experiments. Student t test was used for comparisons between 2 groups, and one-way ANOVA was used for multigroup comparisons followed by a Tukey post hoc test for multigroup comparisons. Significance was defined as $P < 0.05$.

RESULTS

Characterization of AT1R Expression in Cultured Cardiac Fibroblasts

Our first aim was to investigate the optimal multiplicity of infection (MOI) for Ad-GFP and Ad-AT_1R to be used in the experiments in which the AT_1R density increases without a significant fibroblast death. To increase the density of AT_1R, cardiac fibroblasts were transduced with the bicistronic Ad-GFP-AT_1R and with Ad-GFP as control. Transduction efficiency was verified by Western blot for the N-terminal Hemaglutinin epitope (data not shown) and GFP expression using epifluorescent microscopy (data not shown) and cell flow cytometry. Figures 1A and 1B show an increase in GFP positive cells that were transduced with increasing MOI of Ad-AT_1R and Ad-GFP. Using the MOI 300 for Ad-GFP, in which more than 95% of cells were green when evaluated by epifluorescent microscopy, and for Ad-AT_1R all MOI assayed (from 37.5 to 600), we show that 100 nM Ang II reduced cardiac fibroblast viability, being significant from MOI 150. Ad-GFP did not induce a loss in cell viability. However, with Ad-AT_1R a significant loss of cell viability was found at the most highest MOI (Figure 1C).

We also show that 100 nM Ang II reduces adult cardiac fibroblast viability, being significant from MOI 225. At MOI 600, Ad-AT_1R, but not Ad-GFP, induces a significant loss of cell viability (Figure 1D).

The presence of AT_1R in cultured cardiac fibroblasts was confirmed by radioligand binding assays with [^{131}I]Sar-Ile-Ang II. The basal number of AT_1R at MOI 0 was 0.121

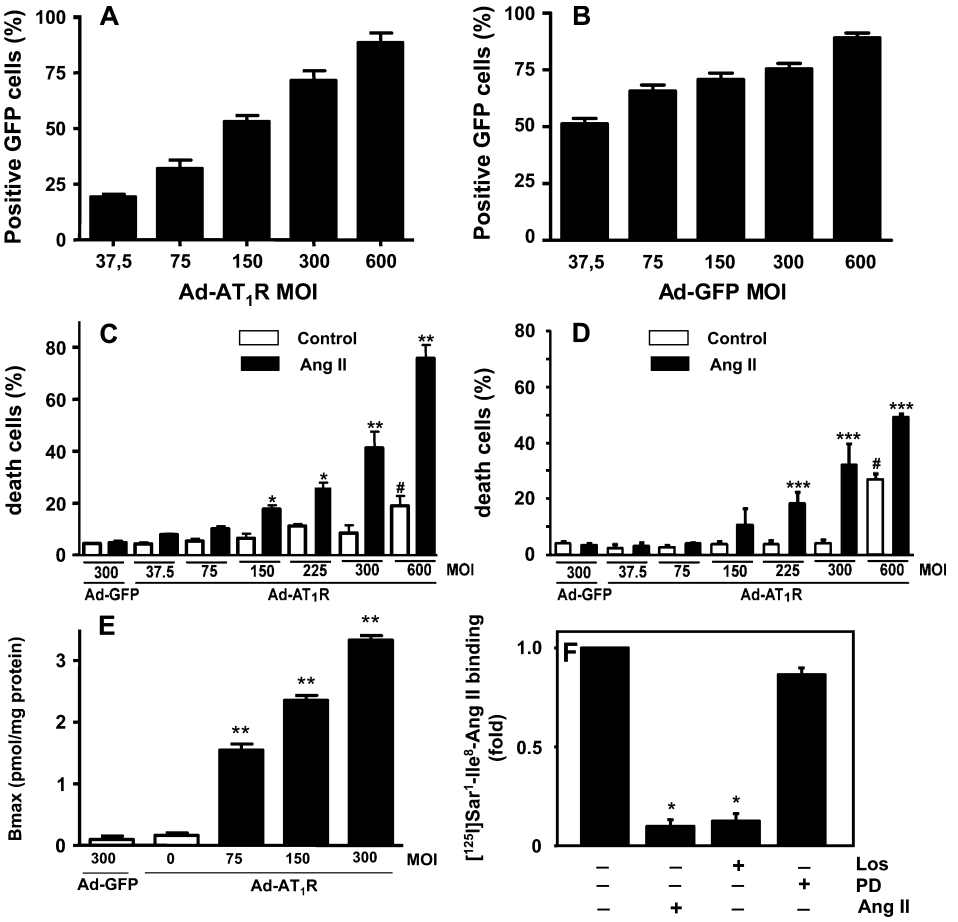

FIGURE 1. Characterization of AT_1R in cultured cardiac fibroblasts. (A, B) Cells were transduced with adenovirus expressing green fluorescence protein (Ad-GFP) or AT1R (Ad-AT1R), respectively, at a multiplicity of infection (MOI) from 37.5 to 600 for 24 hours. GFP-positive cells were evaluated by flow cytometry. Neonatal (C) and adult (D) cardiac fibroblasts were transduced with Ad-GFP at MOI 300 and Ad-AT1R at all MOI used (from 37.5 to 600); cells were stimulated with 100 nM Ang II or saline (control) for 24 hours, and cell viability was evaluated by PI incorporation analyzed by flow cytometry. $^*P < 0.05$ versus Ad-GFP + Ang II, $^{**}P < 0.01$ versus Ad-GFP + Ang II; $^{\#}P < 0.05$ versus Ad-GFP. (E) Bmax quantification at different MOI of Ad-AT1R. Membrane fractions of nontransduced cardiac fibroblast, Ad-GFP, and Ad-AT1R–transduced cardiac fibroblasts were obtained, and binding and competition studies were carried out using [125]Sar1-Ile8 Ang II (9 nM), and dose dependent displacements were done with Ang II (1 nM to 1 μM). $^{**}P < 0.01$ versus Ad-GFP. (F) Competition binding for the AT1R using [125]Sar1-Ile8 Ang II (9 nM) displaced with Ang II (1 mM), Losartan (Los, 1 μM), and PD123319 (PD, 10 μM), respectively. $^*P < 0.05$ versus Ad-GFP. The assays were done as described in Methods. Results shown are mean ± SEM of 3 separate experiments.

pmol/mg protein, and the maximum AT_1R number reached at MOI 300 was 3.256 pmol/mg protein; in transduced cells with Ad-AT_1R at MOI 300, the number of AT_1R was increased more than 20-fold, and Ad-GFP did not modify cell number AT1R (Figure 1E). These binding sites were specifically displaced by Ang II and by AT_1R-specific antagonist losartan (Los) but not by AT_2R-specific antagonist PD123319 (PD) (Figure 1F).

Taken together, these results confirm the specificity and high expression of AT_1R in Ad-AT_1R–transduced cardiac fibroblasts.

Ang II Stimulates Death of Ad-AT1R–Transduced Cardiac Fibroblasts

To determine the effect of Ang II on cardiac fibroblast death, control (nontransduced) and Ad-AT_1R–transduced cardiac fibroblast were incubated with 1 nM to 10 μM Ang II for 24 hours and analyzed for their viability using the MTT method. Ang II did not show any cytotoxicity in control cells (Figure 2, upper panel), but Ang II treatment significantly decreased cell viability of all Ad-AT_1R–transduced cells in a dose-dependent manner (Figure 2, bottom panel). The viability of Ad-AT_1R–transduced cells decreased below the initial number after 24 hours with 1 nM Ang II, indicating a cytotoxic effect of Ang II.

Ang II Triggers Cell Death in Ad-AT1R–Transduced Cardiac Fibroblasts by Activation of the Intrinsic Apoptotic Pathway

To determine the involvement of the mitochondria-mediated pathway in Ang II-induced apoptotic cell death, we measured changes in $\Delta\psi_m$. Ang II (100 nM) treatment of Ad-AT_1R–transduced cells induced a rapid dissipation of $\Delta\psi_m$, which was significant from 6 hours; CCCP was used as internal control (Figure 3A). No changes in $\Delta\psi_m$ by Ang II were observed in Ad-GFP cells treated at any time.

To investigate whether Ang II–induced apoptosis is a consequence of the altered expression of Bcl-2 protein family, Western blotting for Bcl-2 and Bax was performed (Figure 3B, upper panel). In Ad-AT_1R–transduced cells, Ang II (100 nM for 18 hours) did not induce a decrease of the anti-apoptotic Bcl-2, but it did trigger a significant 2-fold increase of Bax (Figure 3B, bottom panel). Bax/Bcl-2 ratio increased 4-fold over control in Ad-AT_1R–transduced cells exposed to Ang II. No changes in Bcl-2 and Bax levels by Ang II were observed in Ad-GFP cells.

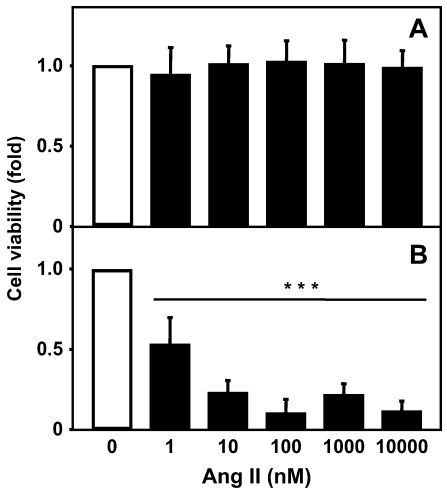

FIGURE 2. Effect of Ang II on cardiac fibroblast viability. (A) Serum-starved non-transduced cells were exposed to increasing concentration of Ang II (1 to 10000 nM) for 24 hours. (B) Cells were transduced with Ad-AT1R at MOI 300 and were stimulated with Ang II (1 to 10000 nM) for 24 hours. Cellular viability was evaluated using MTT assay. Results are expressed as fold over fibroblast without Ang II (control) and represent the mean ± SEM of 3 to 5 separate experiments. ***$P < 0.001$ versus nontreated cells.

Decrease in $\Delta\psi_m$ and increase in Bcl-2/Bax results in the loss of selective permeability of mitochondrial membrane, releasing apoptogenic proteins and triggering caspase activation. To test whether caspases are involved in Ang II–induced apoptosis, caspase-3 proteolysis was analyzed using Western blotting. In Ad-AT_1R–transduced cells, Ang II induced procaspase 3 activation at 18 hours (Figure 3B). Moreover, Ang II (100 nM for 18 hours) also induced DNA fragmentation in Ad-AT_1R–transduced fibroblasts, as determined by PI staining followed by FACScan analysis (Figure 3C). No changes by Ang II were observed in Ad-GFP cells.

AT1R-Phospholipase C-Protein Kinase C Signaling Pathway Mediates Ang II–Induced Apoptosis in Ad-AT1R–Transduced Cardiac Fibroblasts

To investigate the role of the AT_1R-PCL-PKC signaling pathway on cardiac fibroblast apoptosis stimulated by Ang II, Ad-AT_1R–transduced cells were preincubated with or without Los, U, Gö, or PD. Figure 4 shows that the effects of Ang II in cell viability, $\Delta\psi$m, Bax and Bcl-2 protein levels, caspase 3, and DNA fragmentation were significantly blocked by Los, U, and Gö, but not by PD.

DISCUSSION

Expression of AT1R in Cardiac Fibroblasts

AT_1R subtype has been detected in human, neonatal, and adult rat cardiac fibroblasts.[16–18] [^{125}I]Sar-Ile-Ang II binding assays showed that only AT_1R was present in cultured neonatal cardiac fibroblasts with values of Kd and densities similar to those previously reported.[15] In our work, the upregulation of AT_1R in transduced cardiac fibroblasts was monitored by radioligand binding. The density of AT_1R in the Ad-AT_1R–transduced cardiac fibroblasts was more than 20-fold more than the mock transduced cells (controls). Both in non-transduced and transduced cells, losartan but not PD123319 blocked [^{125}I] Sar-Ile-Ang II binding, showing that AT_1R was specific, saturable, and reversible. Changes in the expression of AT_1R have been reported in different pathophysiological conditions, especially after myocardial infarction.[12,19,20] Schorb et al[16] showed that cardiac fibroblasts had a single high-affinity (IC50, 1.0 nM) Ang II binding site (B_{max}, 778 fmol/mg protein) that was coupled with cell proliferation. In contrast, our results show that a strong increase of AT_1R is linked to cell death rather than cell proliferation. We have titrated the number of AT_1Rs by increasing the MOI of the Ad-AT_1R, and they correlate with cell viability, thus we found that the lowest threshold of AT_1R needed to result in Ang II–mediated apoptosis was 20-fold more than control levels. Our results also show that in adult cardiac fibroblasts, Ang II induces a significant apoptosis at MOI 225; meanwhile, this peptide triggers neonate cardiac fibroblast death from MOI 125. These data suggest that the lowest threshold to trigger apoptosis is similar in both cell types.

Ang II/AT_1R in Mitochondria-Dependent Cardiac Fibroblast Apoptosis

Little is known about the physiologic stimuli leading to fibroblast apoptosis. It has been reported that serum withdrawal, irradiation, and loss of adhesion to the extracellular matrix can induce apoptosis in cultured primary fibroblasts.[21,22] In addition, Mayorga et al[8] showed that serum starvation, etoposide, and staurosporine induce cardiac fibroblast apoptosis. Our data show that Ang II also has cytotoxic effects in cultured neonatal cardiac fibroblasts only when the density and affinity of AT_1R is increased 20-fold. The ability of Ang II to cause significant reduction of $\Delta\psi_m$ at as early as 6 hours of incubation supports mitochondria-mediated apoptotic cell death. Moreover, during apoptosis, the intracytosolic balance of members of the Bcl-2 protein family is critical for maintaining the integrity of the mitochondrial membrane. The Bax/Bcl-2 protein ratio regulates apoptotic susceptibility in most cell types by regulating the mitochondrial release of cytochrome-c, which is an important event in the execution of apoptosis in response to different stimuli. The upregulation of Bax[9] or downregulation of Bcl-2[10] have pro-apoptotic effects on fibroblasts, and both have been suggested to play a role in the resolution of wound healing. A 2-fold increase in Bax level was found in Ad-AT_1R–transduced cardiac fibroblasts treated with Ang II, although Bcl-2 remained unaltered. The exact mechanism of Bax increase and whether mitochondrial proteins take part in membrane permeabilization still remain to be characterized. Taken together, experimental evidence suggests the involvement of Bax and caspase-3 activation in the induction of DNA fragmentation. Ang II induces apoptosis in cardiomyocytes, vascular smooth muscle cells and endothelial cells.[6,23–25] The mechanism by which Ang II induces apoptosis in these cells remains unclear,

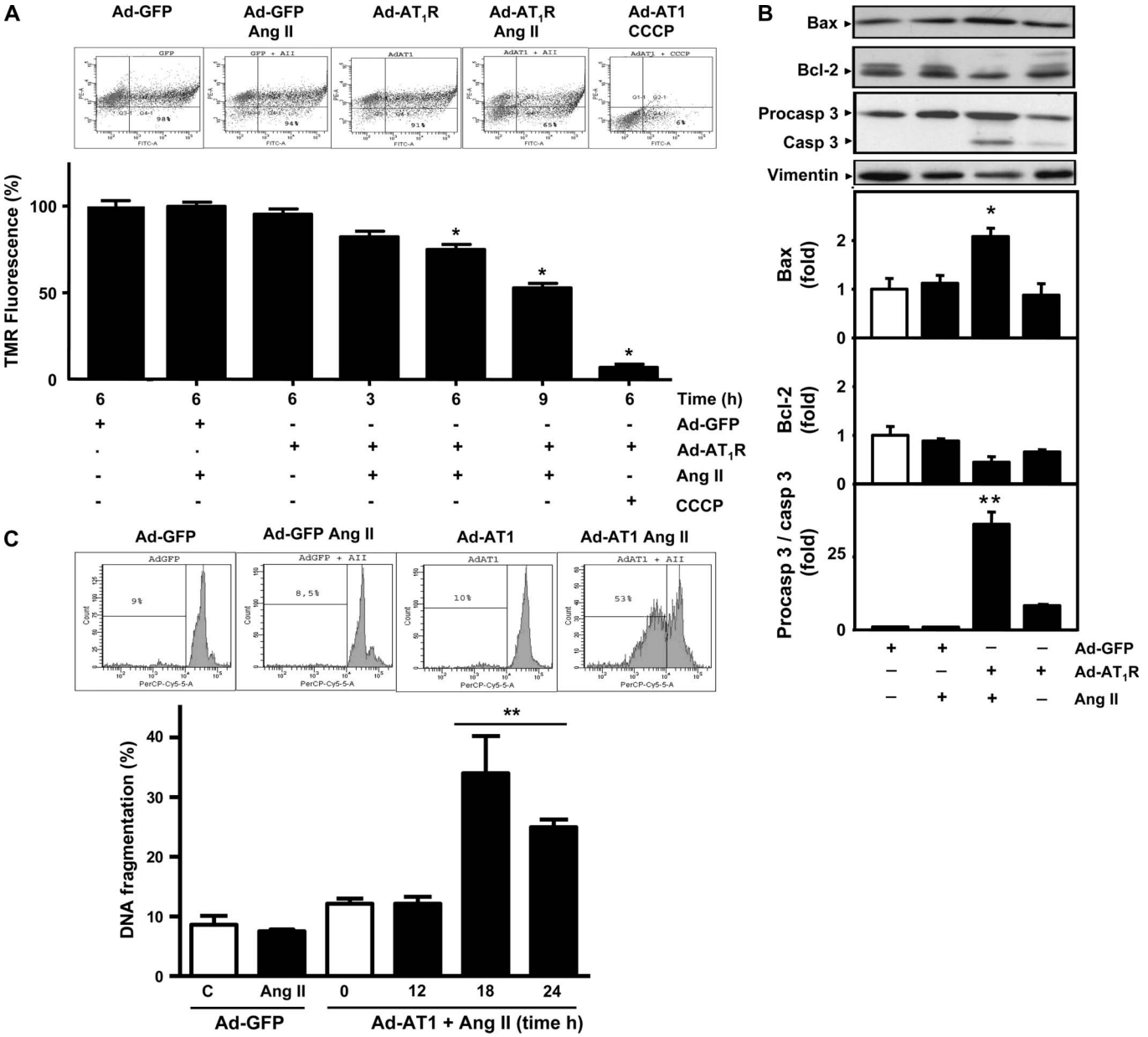

FIGURE 3. Increased expression of AT1R results in Ang II-dependent cardiac fibroblast apoptosis. (A) Mitochondrial potential of Ang II-treated Ad-GFP and Ad-AT1R–transduced cells were measured by TMR staining. Mitochondrial potential was measured after 3, 6, and 9 hours of treatment with Ang II (100 nM). CCCP (40 nM) were used as mitochondrial uncoupler; at the top of the figure, the FACS images at 6 hours are shown. *$P < 0.05$ versus Ad-GFP+Ang II. (B) Ad-GFP and Ad-AT1R–transduced fibroblasts were treated with 100 nM Ang II for 18 hours. Bax, Bcl-2, and caspase-3 (Casp 3) and procaspase-3 (Procasp 3) ratio were determined by Western blot; *$P < 0.05$ and **$P < 0.01$ versus Ad-GFP+Ang II. (C) Apoptotic activation on Ad-AT1R–transduced cells treated with Ang II. Cells transduced with Ad-GFP and Ad-AT1R were stimulated with Ang II, stained with PI, and analyzed by flow cytometry as described in Methods. DNA fragmentation was measured 12, 18, and 24 hours after 100 nM Ang II treatment. On the top of the figure, the FACS images at 18 hours are showed. Results are expressed as fold over control and represent the mean ± SEM of 3 to 5 separate experiments. **$P < 0.01$ versus Ad-GFP+Ang II.

although different reports have shown that both AT_1R and AT_2R are involved. Our results regarding the role of Ang II in Ad-AT_1R cardiac fibroblast death were unexpected, and they differed from those reported in literature.[1,7,16,26] These differences could be essentially explained by the increase in the number of AT_1R and by qualitative and/or quantitative changes in the activation of Ang II-dependent signaling pathways such as calcium, PKC, etc in cultured neonatal cardiac fibroblasts. Further studies should be done to explore these possibilities.

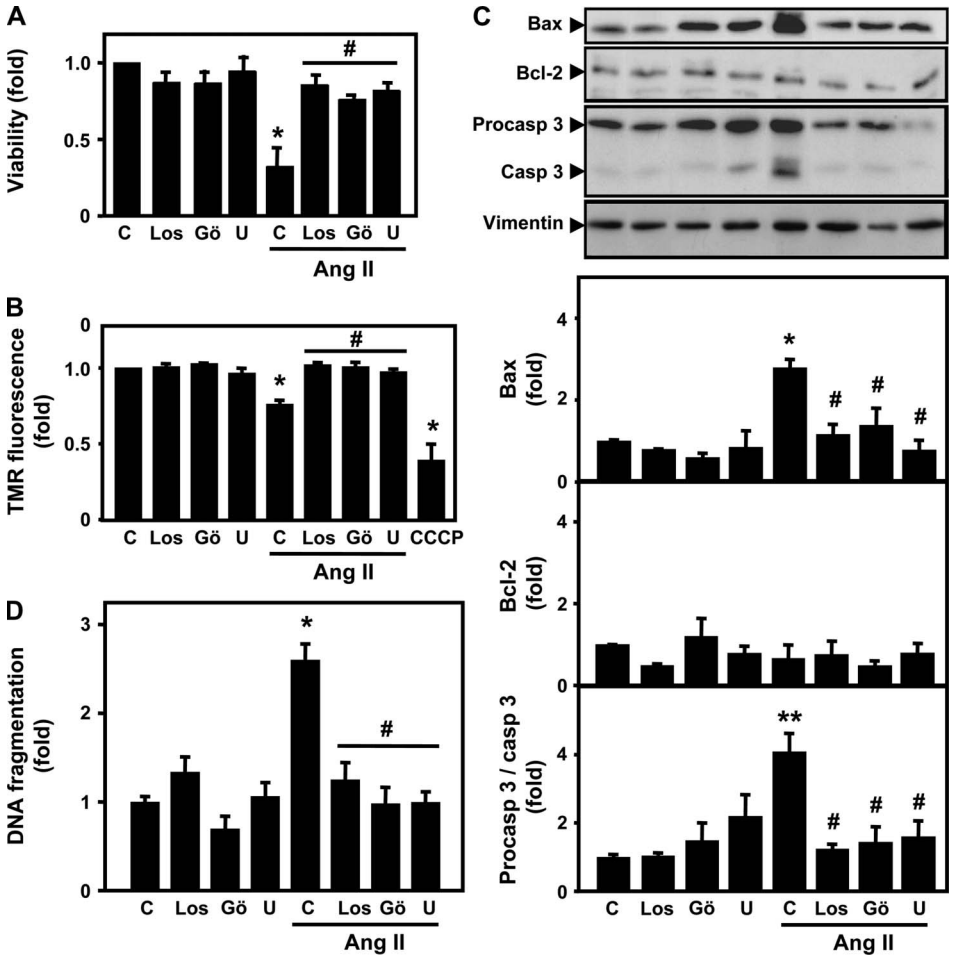

Figure 4. PLC and PKC inhibitor prevent Ang II effect on pro-apoptotic signal activation on AT1R–transduced cardiac fibroblast. Fibroblasts were transduced with Ad-AT1R. (A) Cell viability was determined by MTT assay at 24 hours of treatment with or without Ang II (100 nM). (B) Mitochondrial membrane potential by TMR and flow cytometry as indicated in Methods at 6 hours of treatment with or without Ang II (100 nM). (C) Bax, Bcl-2, and caspase-3 (Casp 3) and procaspase-3 (Procasp 3) ratio were determined by Western blots at 18 hours of treatment with or without Ang II (100 nM). (D) DNA fragmentation was determined by PI staining and flow cytometry as described in Methods at 18 hours of treatment with or without Ang II (100 nM). PD123319 (PD, 10 μM), Losartan (Los, 10 μM), U73122 (U, 1 μM), and Gö6976 (100 nM) were added 1 hour before Ang II stimulation. Results are expressed as relative levels over control and represent the mean ± SEM of 3 to 5 separate experiments. $*P < 0.05$ and $**P < 0.01$ versus cells without Ang II; $^{\#}P < 0.05$ versus cells with Ang II.

PLC/PKC Pathway in Cardiac Fibroblast Apoptosis

Distinct AT_1R signaling pathways may be responsible for different cellular responses such as growth, extracellular matrix component synthesis, or inflammatory/stress response. Crosstalk among the signaling pathways of AT_1R may determine the ultimate response of the cardiac fibroblasts. The PKC family, which comprises at least 10 isoforms with distinct regulation and tissue distribution patterns, has been shown to exert both inhibitory and stimulatory influences on apoptosis. Evidence indicates that the alpha, beta, epsilon, and atypical isoforms are antiapoptotic in their action, whereas the delta and theta isoforms are usually involved in the promotion of apoptosis.[27] We investigated the possible involvement of the PLC and PKC signaling pathways using their respective inhibitors U73122 and Gö6976. These inhibitors had an effect on Ang II–induced apoptosis in Ad-AT_1R–transduced cardiac fibroblasts analyzed by cytoxicity, loss of $\Delta\psi m$, Bax levels, caspase-3, and DNA fragmentation. At present, however, the involvement of other signaling cascades cannot be ruled out. Different molecular effectors activated by PKC may conduct to apoptosis. An increase in intracellular Ca^{2+} levels by excessive Ca^{2+} influx has been implicated in the activation of cell death pathways.[24,25] Increased intracellular Ca^{2+} levels induce mitochondrial membrane potential depolarization, which leads to the opening of the mitochondrial transition pore and the subsequent release of cyt c.[28] Nevertheless, in Ad-AT_1R–transduced cardiac fibroblasts, Ang II induces an excessive Ca^{2+} influx that was not investigated in this study. Further studies are required to clarify this point. However, Zou et al[29] propose that Ang II–evoked signal transduction pathways differ among cell types. In cardiac fibroblasts, Ang II activates ERKs through a pathway that includes the Gβγ subunit of Gi protein, tyrosine kinases, including Src family tyrosine kinases, Shc, Grb2, Ras, and Raf-1 kinase, whereas Gq and PKC are important in cardiac myocytes.

Increased Ang II concentrations play a crucial role in the progressive hemodynamical alterations and cardiac remodeling after myocardial infarction where apoptosis has been strongly implicated. However, circulating and cardiac levels of other neurohumoral stimuli (catecholamines and endothelin-1), growth factors (TGF-β1, PDGF), and nitric oxide (NO) increase or decrease after myocardial infarction, and they may contribute to regulation of cardiac fibroblast proliferation and/or apoptosis. In these regards, catecholamines, endothelin-1, and TGF-β1 stimulate cardiac fibroblast proliferation[30–32] while NO triggers cell death.[33]

Limitations

Other signaling pathways with strong presence in cardiac fibroblasts (ie, adrenergic) that crosstalk with Ang II–induced pathways were not included in this study. We will considerer to evaluate this possibility in the future.

CONCLUSIONS

In Ad-AT_1R–transduced cardiac fibroblasts, Ang II promotes apoptosis via the AT_1R-PLC-PKC signaling pathway. Such apoptosis could have ambiguous consequences for cardiac pathologies where AT_1R is increased (a) with negative effects on cardiac wound healing after myocardial infarction, where a rapid and efficient scar is necessary, or (b) with positive effects when a wound healing process is not necessary and thus to avoid an excessive deposition of extracellular matrix protein and the development of cardiac fibrosis.

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