

Fig. 5. A: real-time RT-PCR of fibrillin-1 mRNA. Standard curves for fibrillin-1 and ribosomal protein S16 were assessed by real-time RT-PCR (insets representing dilution curves). B: quantification of myocardial fibrillin-1/S16 mRNA content expressed as *n*-fold increase relative to control animals (run in duplicate). *n* = 6–10 (rats) or 3 (mice) per group, respectively. Ct, control. **P* < 0.05 vs. control animals.

Immunohistochemical evidence of fibrillin-1 accumulation at sites of microscopic scarring and perivascular fibrosis of coronary arteries appeared in both the right and left ventricles. Fibrillin-1 staining was particularly intense in reparative fibrotic areas (Fig. 2G, solid arrow). Elastin accumulation was often observed in the center of scars in the DOCA-salt myocardium (Fig. 2H, solid arrow) and in the ANG II-infused rat

heart (not shown). Fibrillin-1 was distributed evenly throughout the scars without apparent association with elastin.

In 1B normotensive transgenic mice, compared with controls (Fig. 4, A and B), we clearly observed an increased perivascular and interstitial fibrillin-1 deposition ($1.09 \pm 0.28\%$ to $4.11 \pm 0.69\%$; *P* < 0.05) (Fig. 3 and Fig. 4, C and D). In 2C hypertensive ANG II-producing mice (Fig. 4, E and F), we detected a sixfold increase of fibrillin-1 immunoreactivity from $1.09 \pm 0.28\%$ in control mice to $7.77 \pm 0.83\%$. These two lines showed no evidence of cardiac necrosis despite dramatically increased cardiac ANG II content (60). Moreover,

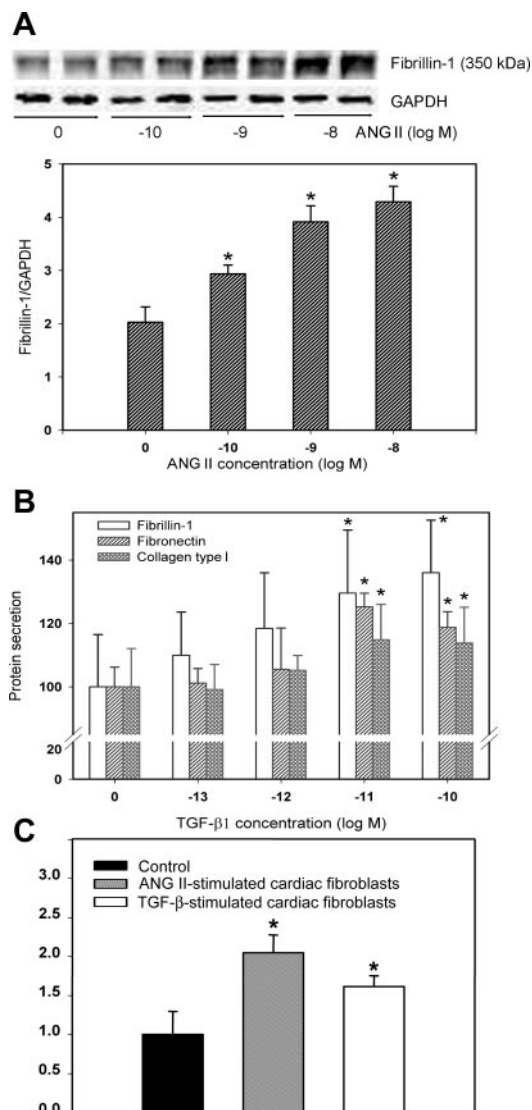


Fig. 6. Fibrillin-1 expression in cultured cardiac fibroblasts. Quiescent cardiac fibroblasts were stimulated for 48 h by increasing concentrations of ANG II (10^{-10} – 10^{-8} mol/l) (A) or TGF-β1 (10^{-13} – 10^{-10} mol/l) (B). SDS-soluble fibrillin-1 was analyzed by Western blot analysis (A); fibrillin-1, collagen type I, and fibronectin deposition were measured by an in-cell-based assay (B). Data were normalized to GAPDH (A) or β-actin (B). *n* = 4 in A and *n* = 6 in B. Results are representative of 3 experiments. **P* < 0.05 vs. quiescent cells. Quantification of myocardial fibrillin-1/S16 mRNA content by real-time RT-PCR (C) is expressed as *n*-fold increase relative to nonstimulated cardiac fibroblasts (run in duplicate). Quiescent cardiac fibroblasts were stimulated for 48 h with ANG II (10^{-8} mol/l) or TGF-β1 (1.5×10^{-10} mol/l). *n* = 4 experiments. **P* < 0.05 vs. quiescent cells.