

Metoprolol Mitigates Ischemic Heart Remodeling and Fibrosis by Increasing the Expression of akap5 in Ischemic Heart

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Research Article

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Abstract

Background The purpose of this study was to verify whether metoprolol regulates AKAP5 expression and test the role of AKAP5 post-injury in mitigating cardiac infarction-associated tissue remodeling and fibrosis.

Methods and results Sprague-Dawley (SD) rats underwent coronary artery ligation (CAL), which was followed immediately with metoprolol daily. HW/BW ratio and cardiac expression of COL1 and COL3 were increased in rats following CAL compared with shams. Treatment with metoprolol post-injury was associated with a decrease in HW/BW ratio and COL1/COL3 expression compared to uncontrol rats. CAL resulted in decreased cardiac AKAP5 expression compared to the control group, while metoprolol treatment restored levels compared to baseline shams. Cardiac expression levels of NFATc3/p-NFATc3 and GATA4 were modest at baseline and increased with injury, whereas metoprolol suppressed gene expression to below injury-associated changes. Immunoprecipitation indicated that AKAP5 could bind and regulate PP2B.

Conclusions The results indicate that metoprolol mitigates ischemic cardiac remodeling and fibrosis, which mechanism of mitigating remodeling likely to improve cardiac AKAP5 expression and AKAP5-PP2B interaction.

Introduction

The incidence of heart failure (HF) continues to rise as the population ages (1, 2). Unfortunately, HF is minimally symptomatic even with real cardiac structural changes such as left-ventricular (LV) hypertrophy, LV systolic or diastolic dysfunction, and valvular disease. The prognosis for advanced HF is poor. Therefore, early intervention is likely to be more impactful at reducing morbidity and mortality.

LV remodeling (LVR) is common after ischemic myocardial injury and manifests as changes in ventricular thickness and size. This compensation mechanism initially minimizes cardiac dysfunction but, over time, proves inadequate at maintaining cardiac function (3). Post-ischemic cardiac remodeling is worsened by chronic activation of the neuroendocrine system and unrestrained extracellular matrix deposition (4, 5). A number of cytokines and hormones promote this process, including hyperactive beta-adrenocortical hormones, and are found increased in animal models and people with cardiac remodeling (6, 7). AKAP5, also known as akap79/150, includes a variety of proteins of different genera encoded by homologous gene AKAP5, including bovine akap75, human akap79, and rat and mouse AKAP150 (8). Under normal physiological condition, akap79/150 can affect cardiac myocytes. The signal transduction and sensitize recycling of adrenaline receptor (9), regulation of the cardiac hypertrophy signal (10) and the coupling of excitation contraction, relaxation and intracellular calcium circulation (10) (11, 12) in cardiac cells are involved in ensuring the normal function and morphology of the heart. In arrhythmia, AKAP150 acts in two ways: it intensifies arrhythmia caused by calcium channel related gene

mutation(13) and it may have a therapeutic effect on arrhythmia caused by potassium channel related gene mutation (14, 15).

β 1-adrenergic receptor (β -AR) stimulation *in vitro* and *in vivo* increased the expression and activity of matrix metalloproteinases 2 and 9 (MMP-2, MMP-9) in cardiac myocytes,(16) it also induced cardiomyocyte apoptosis (7, 17, 18). In addition, β 1-AR-mediated increase in cyclic adenosine monophosphate was greater in AKAP5 null myocytes(19). There are few studies on the role of AKAP5 in cardiac function, especially there are few studies on the role of AKAP5 to regulate the myocardial remodeling and fibrosis after myocardial infarction. The focus of this article is on whether β -AR blockers regulate the expression of AKAP5 and the downstream signal protein after myocardial infarction.

However, how or if AKAP5 participates in the regulation of β 1-ARs in ischemic cardiac remodeling is minimally studied. Herein, we tested the hypothesis that therapy with a selective β 1-AR blocker metoprolol can ameliorate ischemic cardiac remodeling by alerting AKAP5 levels.

Materials And Methods

Animals

Adult male Sprague Dawley (SD) rats (~200 g/animal) were obtained from the Zhejiang Experimental Animal Center of Zhejiang University (Hangzhou, China; License No. [SCXK (Zhen) 20140001]). The animals were raised in the Central Laboratory Animal Room of the Rocky Mountain Hospital of Wannan Medical College (Wuhu, China). All animal experimental protocols used in this study were approved by the Ethics Committee of Yanjishan Hospital of Wannan Medical College and met the guidelines for the use of live animals.

Reagents and equipment

Bicinchoninic Acid (BCA) Protein Quantification Kits were from the Bey time Institute of Biotechnology (Nanjing, China). Antibodies to AKAP5, NFATc3, p-NFATc3, and GATA4 were from Santa Cruz Biotechnology (Dallas, Texas, US). Antibody to PP2B was from Cell Signaling Technology (CST; Beverly, Massachusetts, US), and COL1 and COL3 were from Abcam (Cambridge, UK). The chemiluminescence imager was from Tanon Science & Technology Co., Ltd. (Shanghai, China), and the microplate reader from Bio-Rad Laboratories, Inc. (Hercules, California, US).

Myocardial-infarction model

50 adult male Sprague Dawley (SD) rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g) and connected to a RM6240 Multi-path Physio meter (Chengdu Instrument Factory, Chengdu, China). After tracheal intubation, animals were mechanically ventilated (Beijing Zhoushan Electronic Technology Co., Ltd., Beijing, China). Using sterile techniques, the thoracic cavity was opened between the third and fourth ribs on the left margin of the sternum, and a ligature was placed on the root of the left anterior descending coronary artery. Wounds were closed in layers. Sham animals

underwent all procedures except coronary ligation. Animals were observed until fully recovered. Finally, five rats were survived in each group.

Study design

The study groups with interventions were as follows. Overall, 50 rats were randomly divided into three groups for follow-up experiments, (1) a sham-operated control group (sham group) fed normal rat chow; (2) an injury group that underwent coronary-artery ligation; and (3) a treatment group that underwent ligation and were treated with metoprolol (20 mg/kg/d) next day for 8 weeks. The sham and model groups were treated the same amount of saline vehicle for 8 weeks. The rats were given intraperitoneal injections of appropriate amounts of pentobarbital sodium for sedation and analgesia for three consecutive days after the operation. The rats were euthanized by intravenous injection of 100mg/kg of phenobarbital.

Western blot

On study completion, animals were humanely euthanized, and the hearts were excised. Tissue was homogenized in lysis buffer, agitated on wet ice for 30 mins, centrifuged at 12000 rpm, and then the supernatant was reserved. Measurement of total protein concentration was conducted using the BCA method. To detect protein concentration, we performed gel electrophoresis by loading equivalent amounts of protein in each group. After protein transfer, blots were incubated in bovine serum albumin at room temperature for 2 h. Following PBS washing, the corresponding primary antibodies were added and kept at 4°C for 14 h. After washing, blots were incubated with the secondary antibody at room temperature for 1 h, washed and exposed to a chemiluminescence agent. ImageJ software (National Institutes of Health, Bethesda, Maryland, US) was employed to quantitate protein band expression.

Co-immunoprecipitation

Cardiac lysate was incubated with AKAP5 antibody or rabbit anti-mouse immunoglobulin G and incubated at 4°C for 14 h. Protein A/G agarose beads were added to the mixtures and incubated at 4°C for 4 h followed by centrifugation at 3000 rpm for 3 min. The supernatant was discarded, and beads were washed and boiled in water for 5 min. Samples were then subjected to Western blot as detailed above.

Statistical analysis

SPSS version 18.0 was used for statistical analysis. Statistical differences among groups were assessed using two-tailed Student's t-test (two experimental groups) or ANOVA (three or more groups). $p < 0.05$ was considered statistically significant. The results are expressed the means \pm SD, $n \geq 3$.

Results

Characterization of the ischemic cardiac model

Gross observation found that prior to coronary ligation, hearts appeared bright red in color, and electrocardiogram waveforms were normal (**Fig. 1A**). After ligation, blanching of the cardiac wall was

observed in the distribution of the left anterior descending coronary. Marked changes were also seen in the ECG. Specifically, the J point of lead II and the ST-T segment were uniformly elevated (**Fig. 1B**). These later results are consistent with ischemic myocardial infarction.

Ischemia-mediated increase in cardiac weight is ameliorated by metoprolol

The ratios of heart weight to body weight (HW/BW) were determined by weighing heart samples from each group of rats and comparing them with the weight of the rats before sacrifice. Coronary ligation resulted in a significant increase in HW/BW ratio; this was attenuated in animals treated with metoprolol ($P < 0.05$, $n = 5$; **Fig. 2**).

Metoprolol attenuates matrix protein expression in ischemic hearts

Protein expression levels of matrix genes COL1 and COL3 were greater in ischemia hearts compared to sham hearts. Metoprolol treatment was associated with a decrease in matrix protein expression in ischemic hearts ($P < 0.05$, $n = 5$, **Fig. 3**).

Metoprolol corrects ischemia-driven changes in cardiac AKAP5, p-NFATc3/NFATc3 and GATA4

Ischemia was associated with a decrease in cardiac AKAP5 compared to sham which was attenuated by metoprolol ($P < 0.05$, $n = 5$, **Fig. 4A, B**). In contrast, ischemia was associated with decreased cardiac p-NFATc3/NFATc3 and increased GATA4 compared to sham, and metoprolol suppressed these changes ($P < 0.05$, $n = 5$, **Fig. 4C-F**).

Cardiac AKAP5 and PP2B immunoprecipitated in heart tissue

Adrenergic receptors control cardiac function and size. In turn, AKAPs, and specifically AKAP5, regulate β -AR activity. PP2B is widely expressed in the heart and elsewhere, and is reported to complex with various AKAPs. (20) Consistent with this, AKAP5 and PP2B were co-localized by immunoprecipitation in cardiac samples, and this relationship appeared to change under ischemia and metoprolol treatment (**Fig. 5A**). Western blots of protein levels found in samples employed in AKAP5. (**Fig. 5B**).

Discussion

In pre-clinical models, heart injury and subsequent cardiac remodeling are commonly induced models via several techniques such as aortic-arch constriction (21) ischemia(22) toxic agents(23) and hypertension. Ischemic cardiac remodeling is characterized by organ hypertrophy, fibrosis, and functional deterioration (24). Ischemic cardiac hemodynamic changes can induce heart failure via systolic dysfunction (25). This process is potentiated by reflex sympathetic-nerve activation and the release of catecholamines such as norepinephrine and epinephrine (26). While initially supporting ventricular contractility and heart rate to maintain cardiac output(27) long-term sympathetic-nerve activation is detrimental (28, 29).

Herein, we established a model of acute cardiac ischemia and tested the role of adrenergic blockade to revert the morphologic and gene-associated changes. The present study demonstrates, for the first time, that metoprolol can restore cardiac expression levels of AKAP5, and suppress the levels of p-NFATc3/NFATc3 and GATA4, and enhance cardiac AKAP5 and PP2B protein-protein interaction in rat chronic myocardial infarction model. The results indicate that treatment with metoprolol mitigates ischemic cardiac remodeling and fibrosis, which mechanism of mitigating remodeling likely to improve cardiac AKAP5 expression and AKAP5-PP2B interaction. Metoprolol can be selectively antagonized β -1-adrenoceptor. Studies have shown that metoprolol can reduce the level of pro-inflammatory cytokine TNF- α and IL-1(30, 31). Similarly, metoprolol has a positive therapeutic effect on MI rats by inhibiting the expression of proto oncogene protein (32, 33). That is, β adrenergic blockade was tested against established cardiac changes. While loss of adrenergic support may alter function to some degree, in this study, we found this treatment reverted or limited adverse cardiac remodeling and fibrosis. At the same time, changes in key genes involved in adrenergic signaling were improved. This included reversion of ischemia-mediated changes in cardiac AKAP5, p-NFATc3 and GATA4. Therefore, it is possible that β adrenergic blockade altered the interaction between AKAP5 and the general dephosphorylating enzyme PP2B (calcineurin). PP2B also altered NFAT activity (34) and is implicated in cardiac remodeling. Ventricular remodeling after myocardial infarction, mainly includes hypertrophy and fibrosis of myocardial cells. The representative proteins of fibrosis are COL-1 and COL-3. In the experiment, protein expression of COL-1 and COL-3 was detected in the hearts of three groups of mice. COL-1 and COL-3 were significantly higher than in the control and COL-1 and COL-3 were significantly higher than in the model group, indicating that metoprolol can slow myocardial fibrosis (35-37).

This study has several limitations. First, characterization of the ischemic model was morphologic and not correlated with functional outcomes. It would be useful to have also determined systolic and diastolic contractility, chamber volume and cardiac output. Second, changes in gene protein levels do not equate with protein function. It will be useful to assess if target proteins had altered activity under the conditions of the model. Third, immunoprecipitation, while showing adherence, does not represent true molecular binding. Such interactions are further supported with advanced fluorescent microscopy.

In summary, the present study revealed that adrenergic blockade can resolve established ischemic cardiac remodeling and alter protein levels of key signaling intermediators, which mechanism of mitigating remodeling likely to improve cardiac AKAP5 expression and AKAP5-PP2B interaction. Further research is needed to determine how and to what extent AKAP5 targets cardiomyocyte remodeling in the context of HF.

Declarations

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Conflict of interest

The authors declare that they have no competing interests.

Ethical approval All animal experimental protocols used in this study were approved by the Ethics Committee of Yanjishan Hospital of Wannan Medical College and met the guidelines for the use of live animals.

Consent to participate Not applicable.

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Figures

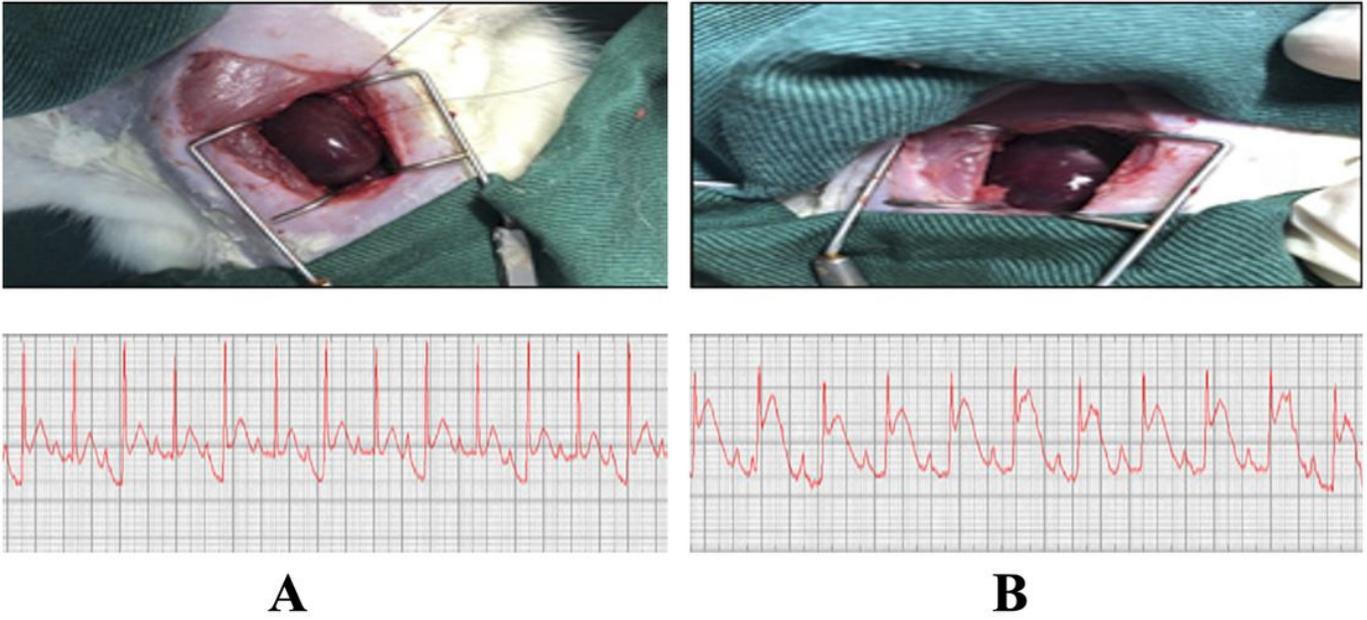


Figure 1

Ligation of the anterior descending coronary induces ischemia. Whole heart images showing heart color and ECG tracings before (A) and after myocardial infarction (B). Representative hearts and tracing are shown from a total of four animals.

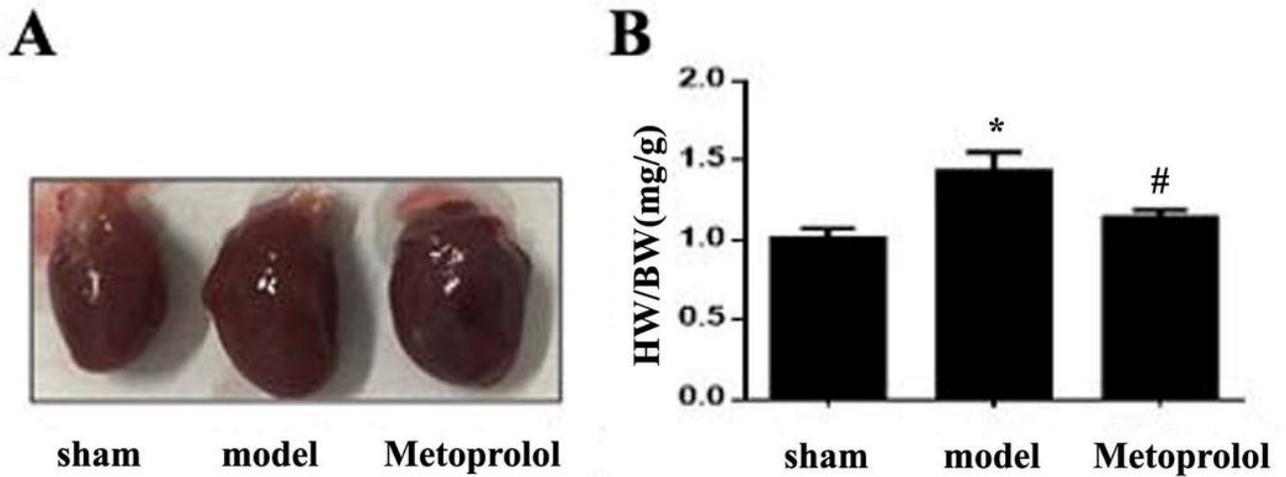


Figure 2

Ischemia-mediated increase in cardiac weight is ameliorated by metoprolol. Rats were subject to ischemia \pm metoprolol, and HW/BW ratios were determined. (A) Morphologic appearance of rat hearts in each group. (B) Calculated HW/BW ratios in each group of rats. * $P < 0.05$ indicates comparison with the sham group; # $P < 0.05$ indicates comparison with the metoprolol-treated group.

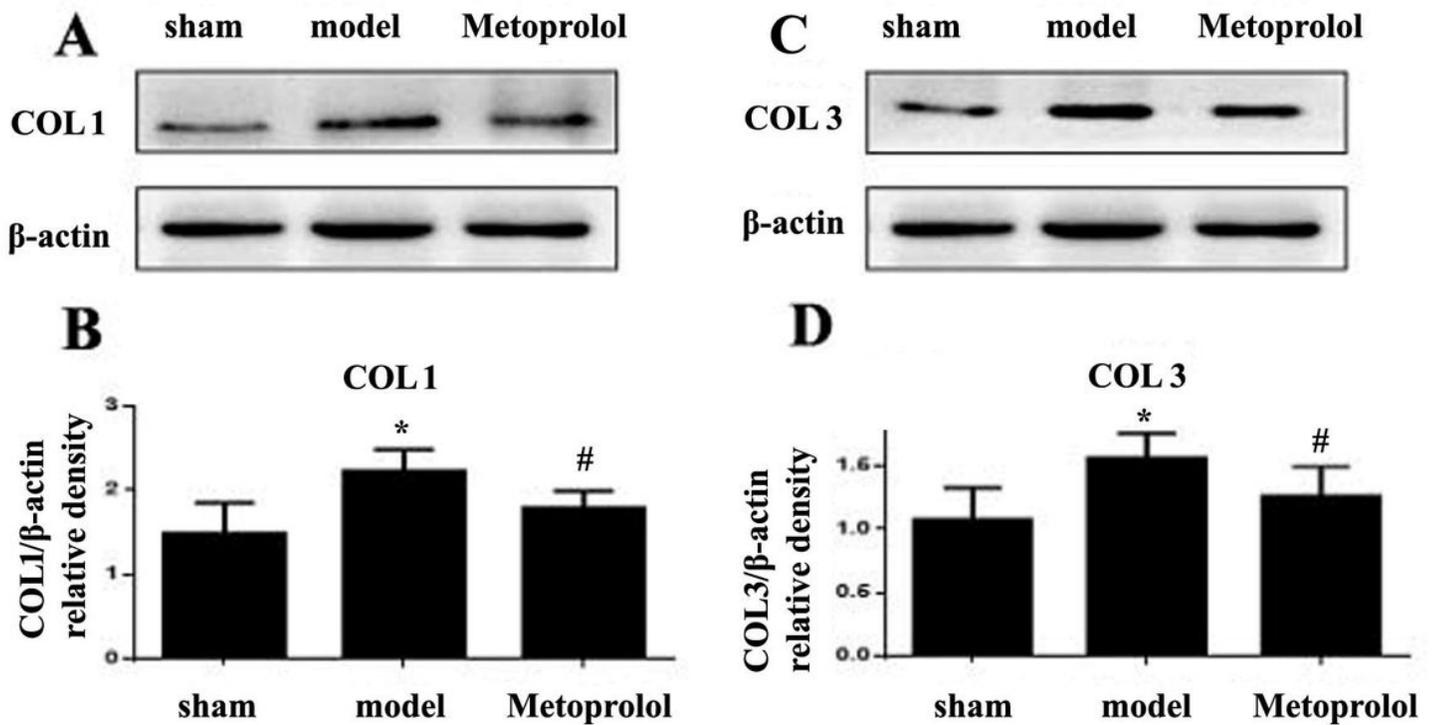


Figure 3

Metoprolol attenuates matrix protein expression in ischemic hearts. Changes in expression levels of COL1 and COL3 proteins. (A, C) Western blot expression of COL1 and COL3 and respective densitometry (B, D). * $P < 0.05$ indicates comparison with the sham group; # $P < 0.05$ indicates comparison with the metoprolol-treated group.

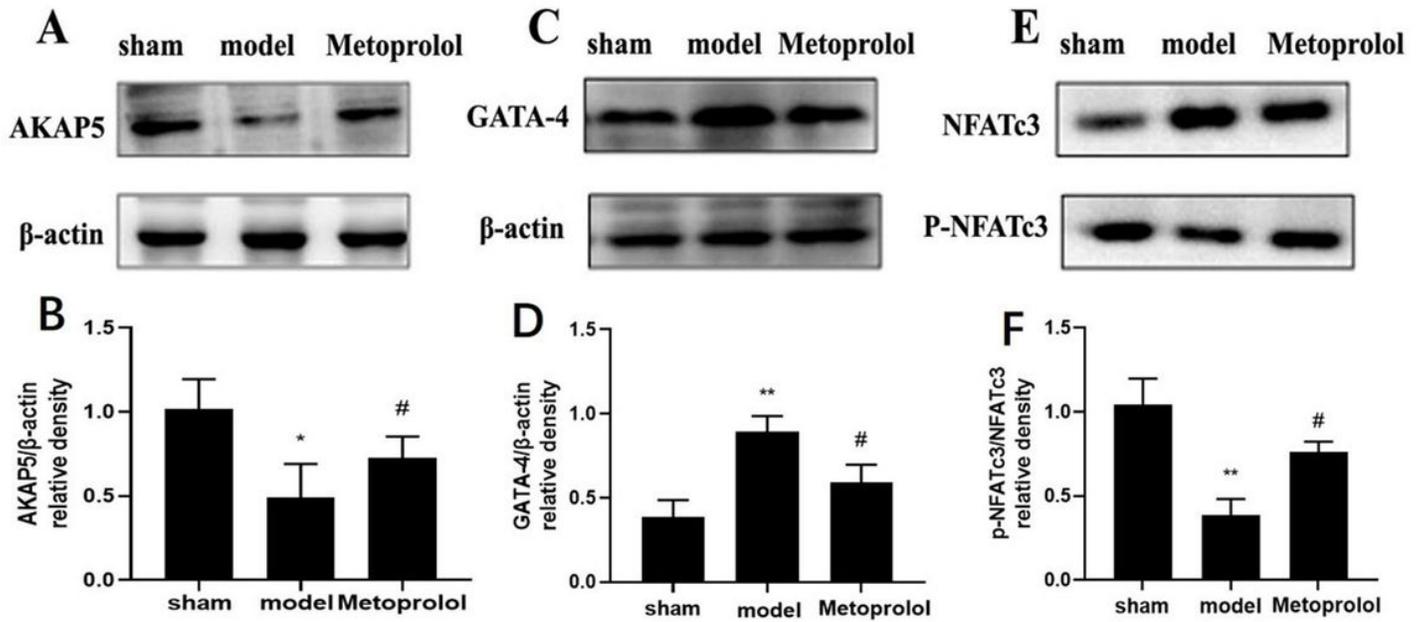


Figure 4

Metoprolol corrects ischemia-driven changes in cardiac AKAP5, NFATc3 and GATA4.

Changes in AKAP5, NFATc3, and GATA4 expression during myocardial remodeling. (A, C, E) Protein expression of AKAP5, NFATc3, and GATA4 were detected by Western blot. (B, D, F) Densitometry analysis of respective blots. ** $P < 0.01$ indicates comparison with the sham group; # $P < 0.05$ indicates comparison with the metoprolol-treated group.

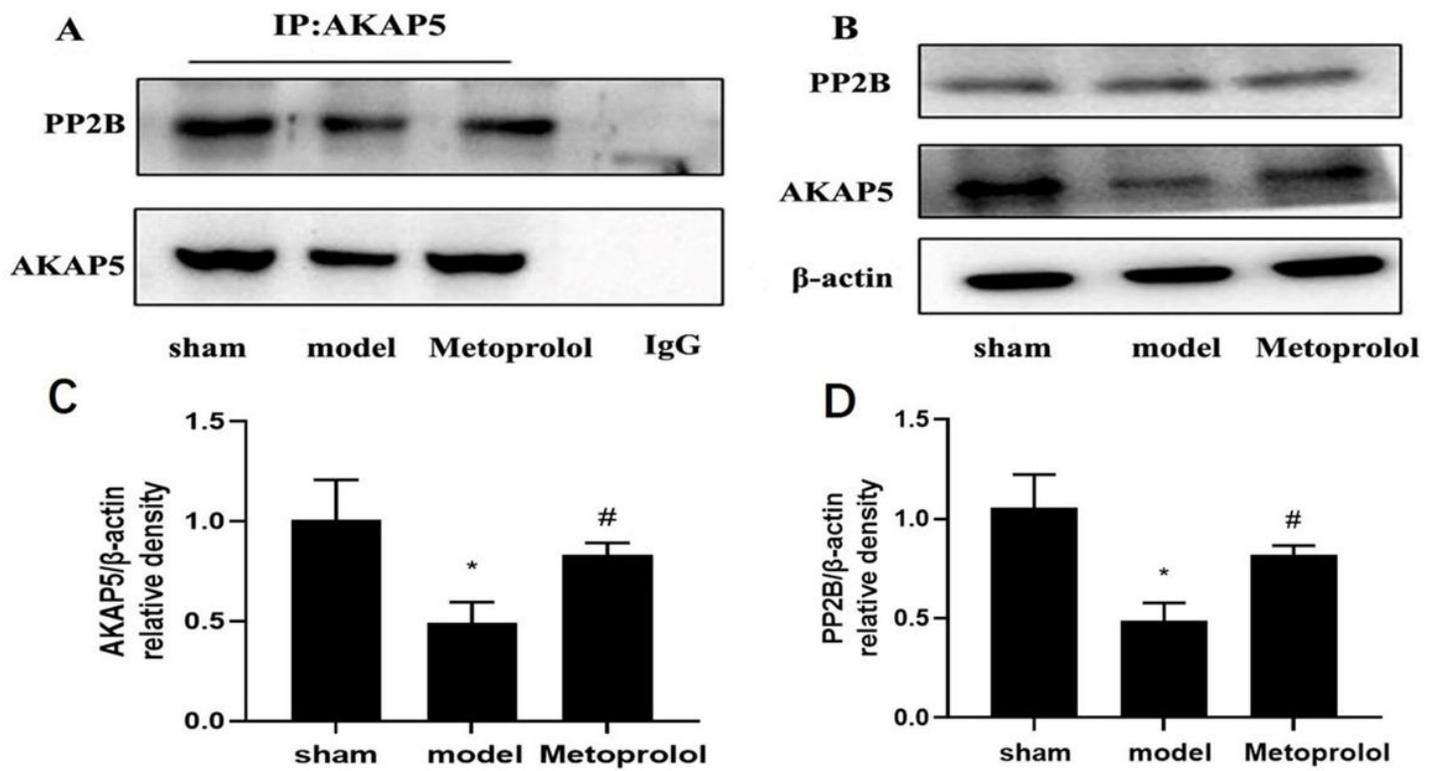


Figure 5

Cardiac AKAP5 and PP2B immunoprecipitated in heart tissue.

Immunoprecipitation pull-down displaying association of AKAP5 and PP2B. (A) In the AKAP5 co-immunoprecipitation experiment, the PP2B band was not noted in IgG control incubated samples. (B) Western blot showing the expression of AKAP5 and PP2B in samples employed in the co-immunoprecipitation experiment. AKAP5 was found to bind and regulate PP2B. * $P < 0.05$ indicates comparison to sham group; # $P < 0.05$ indicates comparison to metoprolol-treated group.