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RESEARCH LETTER

Monoamine Oxidases Desensitize Intracellular β_1 AR Signaling in Heart FailureYing Wang¹, Meimi Zhao, Qian Shi, Bing Xu, Chaoqun Zhu, Minghui Li, Vaseem Mir, Donald M. Bers, Yang K. Xiang¹

Desensitization of β_1 AR (β_1 adrenergic receptor) and depressed cardiac contractility are hallmarks of heart failure (HF). Therefore, clinical drugs have been primarily aimed at rescuing β_1 ARs at the plasma membrane in therapy. This paradigm has been challenged by emerging evidence of functioning intracellular β_1 ARs at the sarcoplasmic reticulum (SR).¹ The SR- β_1 AR regulates local PKA (protein kinase A) phosphorylation of PLB (phospholamban) and excitation-contraction coupling. Thus, enhancing β_1 AR signaling at the SR represents an appealing approach for effectively improving contractility in HF. We found that elevation of MAO-A (monoamine oxidase A) in HF prevents local β_1 AR-PKA-PLB signaling at the SR. Inhibition of MAO-A rescues local β_1 AR signaling, phosphorylation of PLB, and excitation-contraction-coupling in HF.

We applied chronic intraperitoneal injection of β -agonist isoproterenol to induce HF. We examined SR- β_1 AR association with SERCA2a (sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a) using proximity ligation assay (Figure [A]). HF adult ventricular myocytes (AVMs) displayed more proximity ligation assay signals between β_1 AR and SERCA2a than non-HF cells, suggesting an increased β_1 AR association with SERCA2a in failing hearts.¹ There was minimal proximity ligation assay signal between β_1 AR and ryanodine receptor 2 in non-HF and HF AVMs (Figure [A]). Failing hearts usually have low catecholamine contents associated with contractile dysfunction.² Catecholamines are imported via OCT (organic cation transporter)¹ and degraded by MAOs in hearts.^{1,2} Transcriptomic analysis of patients with dilated cardiomyopathy⁴ revealed significant downregulation of β_1 AR (ADRB1) and upregulation of MAO-A, but no change in MAO-B, OCT3, and COMT

(catechol-O-methyltransferase, another catecholamine degradation enzyme; Figure [B]).

We employed fluorescence resonance energy transfer-based AKAR3 (A kinase activity reporter 3)¹ to assess the impacts of MAO-A on local PKA activity at the plasma membrane or SR (Figure [C]). In non-HF AVMs, epinephrine-induced robust increases in PKA activity at the plasma membrane and SR. MAOi (MAO-A inhibitor) clorgyline enhanced PKA activity only at the SR. In HF AVMs, epinephrine-induced negligible PKA activation, and MAOi selectively enhanced PKA activation at the SR. These observations imply that the upregulated MAO-A in HF limits local β_1 AR-PKA activation. Inhibition of MAO-A rescues intracellular β_1 AR-PKA signaling at the SR.

Activation of SR- β_1 AR promotes PKA phosphorylation of PLB, a SERCA2a regulator, to enhance Ca^{2+} transients. MAOi selectively enhanced PKA phosphorylation of PLB in non-HF AVMs after stimulation with epinephrine but not dobutamine, a nonsubstrate for MAO (Figure [D]). In HF AVMs, MAOi enhanced epinephrine-induced increases in PKA phosphorylation of PLB at the SR but not L-type Ca^{2+} channel at the plasma membrane (Figure [D]).

Norepinephrine, epinephrine, and dobutamine promoted little contractile response in HF AVMs. MAOi significantly rescued norepinephrine and epinephrine but not dobutamine-induced excitation-contraction (E-C) coupling (Figure [E]). Collectively, our results indicate inhibition of MAO-A restores SR-localized β_1 AR-PKA-PLB signaling and excitation-contraction-coupling in HF.

The expression of MAO-A is increased by HF-associated pathological stresses including inflammation, aging,

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Figure. Elevated monoamine oxidase A impairs intracellular β 1AR signaling in failing myocytes.

A, Proximity labeling assay of adult ventricular myocytes (AVMs) co-stained with anti- β 1AR/SERCA2a (sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase 2a) or anti- β 1AR/RyR2 (ryanodine receptor 2) antibodies. Representative 3-dimensional images were randomly selected and quantified with Image J. **B**, Volcano plot of transcriptome mRNA from human heart failure (HF) patients with dilated cardiomyopathy relative to non-HF patients (GSE3586). Dot-plot shows the mRNA expression of MAO-A (monoamine oxidase A) (Continued)

Figure Continued. from non-HF and HF patients. **C**, Schematic of intracellular sarcoplasmic reticulum (SR)- β_1 AR signaling in non-HF and HF AVMs. AVMs expressing PKA (protein kinase A) biosensors anchored on the plasma membrane and SR were pretreated with MAOi (MAO-A inhibitor; clorgyline, 5 μ mol/L, 5 min) before stimulation with EPI (epinephrine, 1 μ mol/L). Traces show time courses of the changes in fluorescence resonance energy transfer (FRET) YFP/CFP (yellow fluorescent protein, YFP emission intensity divide Cyan fluorescent protein, CFP emission intensity) ratio. Dot-plots show maximal increases in FRET ratio. **D**, Detection of PKA phosphorylation of PLB (phospholamban) at serine 16 (pS16) and LTCC (L-type Ca^{2+} channel) $\text{Ca}_v1.2$ at serine 1928 (pS1928) after stimulation with EPI (1 μ mol/L) or dobutamine (1 μ mol/L) in the presence of MAOi. **E**, HF AVMs were preloaded with Ca^{2+} indicator Fluo-4 (2 μ mol/L), paced at 1 Hz, and pretreated with MAOi. Ca^{2+} transient (CaT) and sarcomere shortening (SS) were recorded in response to EPI, NE, or DOB. Dot-plots show maximal changes in SS, CaT amplitude (F/F₀), and Ca^{2+} decay (Tau). Dot-plots show mean $\pm$ SEM of the number of AVMs from mice (indicated). For **D**, *P* values were obtained in paired comparisons only after a significance found in a nonparametric Kruskal-Wallis test. All other data passed Shapiro-Wilk normality test. *P* values were obtained after 2-way ANOVA analysis followed by Tukey test (**A**, **C**, and **E**) or Student *t* test (**B**). AU indicates arbitrary unit; and DOB, dobutamine.

and reactive oxidative species. The expression of MAO-A is also regulated by hormones such as thyroid and estrogen, which affects SR calcium handling and cardiac contractility.⁴ Our data indicate that MAO-A inhibitors may hold promise in rescuing SR- β_1 AR signaling and enhancing cardiac contractility while reducing oxidative stress in HF.²

Data Availability

The methods, data, and materials are available upon request. C57BL/6J male mice (2–4-month-old) were randomly assigned for intraperitoneal injection of saline or ISO (30 mg/kg per day, 14 days) and blinded for data analysis. Animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and the protocols approved by the University of California Davis Institutional Animal Care and Use Committee (IACUC).

ARTICLE INFORMATION

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Disclosures

None.

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