RESEARCH LETTER

Adrenergic-Thyroid Hormone Interactions Drive Postnatal Thermogenesis and Loss of Mammalian Heart Regenerative Capacity

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ewborn mice possess a robust but transient capacity for heart regeneration that is lost when cardiomyocytes permanently exit the cell cycle and binucleate during the first week after birth.¹ We discovered that the increasing levels of circulating thyroid hormones (THs) observed during the acquisition of endothermy promote postnatal cardiomyocyte cell cycle arrest and limits heart regenerative potential.² In this study, we describe novel interactions between adrenergic receptor (AR) and TH signaling regulating thermogenesis, cardiomyocyte proliferation, and heart regeneration.

Diploid cardiomyocyte abundance, a proxy for heart regenerative potential, is inversely correlated with the standard metabolic rate, which is defined as basal metabolic rate divided by animal body mass to the ³/₄ power.² Basal metabolic rate is directly proportional to the blood volume flow rate required for oxygen and nutrient transport.³ Thus, the >10-fold increase in standard metabolic rate during the acquisition of endothermy² would require similar elevations in blood flow and cardiac function. These cardiac adaptations may impede cardiomyocyte proliferative and regenerative capacity.

We hypothesize that elevations in cardiac function necessary to support mammalian thermogenic pathways drive postnatal cardiomyocyte cell cycle arrest, polyploidization, and loss of heart regenerative potential. Sympathetic nerves signal through α -ARs and β -ARs and are critical regulators of thermogenesis.⁴ Mice administrated with both α -AR and β -AR inhibitors,

phenoxybenzamine (α -ARi) and propranolol (β -ARi), have reduced body temperatures (Figure [A]), similarly to those treated with propylthiouracil, which inhibits TH synthesis (Figure [A]). Treatment with both AR inhibitors and propylthiouracil together has the most profound effect in reducing body temperature (Figure [A]). These results suggest that AR and TH signaling interactions drive postnatal thermogenesis.

We next determined whether AR and TH signaling regulate cardiomyocyte number and proliferation. Certain chemical combinations decrease animal bodyweight and heart weight, but not the heart weightto-bodyweight ratio (Figure [B]). We quantified total cardiomyocyte number in postnatal day 14 hearts using stereology. Combined treatment with α/β -AR inhibitors and propylthiouracil increases total cardiomyocyte numbers (Figure [C]) and cardiomyocyte cell cycle activity (Figure [D]). In addition, treatment with the inhibitor cocktail increases retention of mononucleated diploid cardiomyocytes (59%), substantially higher than that observed in control animals (5.3%) and those treated with just α/β -AR blockers (16%) or propylthiouracil (21%; Figure [E]). Consistent with a recent report that β-AR signaling promotes cardiomyocyte cytokinesis failure,⁵ we observed that inhibition of β -AR signaling alone increases diploid cardiomyocyte abundance (Figure [E]). These results suggest that AR and TH signaling interactions also promote postnatal cardiomyocyte cell cycle arrest and polyploidization.

Key Words: cell cycle
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Nonstandard Abbreviations and Acronyms

AR	adrenergic receptor
тн	thyroid hormone

To test whether combined AR and TH signaling inhibition enhances cardiomyocyte regeneration after injury, we treated mice with pathway inhibitors and then occluded the coronary artery to induce a myocardial infarction at postnatal day 14 (Figure [F]). Combined pathway inhibition increases cardiomyocyte cell cycle activity in the border zone, overall cardiomyocyte 5-ethynyl-2'-deoxyuridine incorporation, and diploid 5-ethynyl-2'-deoxyuridine-positive cardiomyocytes indicating successful cardiomyocyte cell division (Figure [G and H]). Cardiomyocyte-specific inactivation of TH signaling alone improved cardiac function after ischemia-reperfusion injury² but not after myocardial infarction (data not shown). Myocardial infarction may cause more cardiomyocyte loss than ischemia-reperfusion injury and likely requires robust cardiomyocyte proliferation to support regeneration. Thus, we investigated cardiac regenerative capacity in mice treated with the inhibitor cocktail. At 28 days after a myocardial infarction, we observed a significant increase in cardiac ejection fraction and reduction in cardiac fibrosis in these animals (Figure [I and J]). In addition, RNA-sequencing and differential gene expression analysis revealed that genes involved in cell cycle regulation were significantly upregulated in whole postnatal day 14 hearts after

combined inhibition of AR and TH signaling (Figure [K]), suggesting that these pathways are upstream regulators of cardiomyocyte cell cycle. These data suggest that combined α/β -AR and TH inhibition extends postnatal cardiac regenerative capacity in part by promoting cardiomyocyte cell division. It is possible that combined pathway inhibition also affects inflammation, angiogenesis, revascularization after injury, cardiac metabolism, and heart size which may contribute to enhanced cardiac regeneration.

Collectively, our results demonstrate that postnatal AR and TH signaling interactions promote mammalian thermogenesis, inhibit cardiomyocyte cell division, and limit cardiac regenerative capacity. It is unlikely that reducing body temperature directly increases cardiomyocyte cell cycle entry because culturing embryonic day18 rat ventricular cardiomyocytes at lower temperatures reduces cardiomyocyte proliferation based on 5-ethynyl-2'-deoxyuridine incorporation assays (37°C: 29.26±0.025%; 31°C: 13.14±0.023%; 25°C: 0.14±0.002%, n=3). Future studies will be necessary to determine whether manipulation of AR and TH signaling beyond postnatal development will improve cardiac regenerative capacity in adult animals. Understanding how mammalian thermogenesis and cardiomyocyte cell cycle withdrawal are linked is likely to yield evolutionary insights into why adult mammals cannot regenerate the heart.

Animal procedures were conducted in accordance with UCSF Institutional Animal Care and Use Committee. The data, analytical methods, and study materials are available on request. CORRESPONDENCE



Figure. Inhibition of adrenergic receptor (AR) and thyroid hormone (TH) signaling delays postnatal body temperature elevation, cardiomyocyte cell-cycle exit, and the loss of cardiac regenerative potential.

A, Body temperatures of neonatal mice treated with saline, α -ARi (phenoxybenzamine; 10 µg·g⁻¹·d⁻¹), β -ARi (propranolol; 40 µg·g⁻¹·d⁻¹), and propylthiouracil (PTU; 0.15% in iodine-deficient diet, ad libitum) individually and in combination. PTU was given to pregnant and nursing female mice through food chow starting at E13.5 and maintained thereafter. α -ARi and β -ARi were subcutaneously injected from postnatal day 1 (P1) to postnatal day 14 (P14). Representative infrared thermography images are shown on the right. **B**, Bodyweights, heart weights, and heart weight-to-bodyweight (HW:BW) ratios after treatment with α -ARi, β -ARi, and PTU at P14. **C** through **E**, Analyses of total cardiomyocyte (CM) number at P14 determined by design-based stereology (as described in Hirose et al²; **C**), proliferating CMs determined by quantification of CM-specific nuclear 4′,6-diamidino-2-phenylindole (DAPI) fluorescence intensity (as described in Hirose et al²; **E**) in animals with indicated treatments. Arrowheads denote cycling CMs in **D** and diploid CMs in **E**, respectively. **F** through **J**, Assessment of cardiac (*Continued*)

Figure Continued. regenerative potential after P14 myocardial infarction (MI). **F**, Schematic presentation of experimental design. **G**, Analysis of CM proliferative activity determined by colocalization of PCM1 with either Ki67 or phosphohistone H3 (pHH3; mitosis marker). **H**, 5-Ethynyl-2'-deoxyuridine (EdU) incorporation and CM ploidy analysis in dissociated CMs 28 days after MI (as described in Hirose et al²). **I**, Analysis of cardiac ejection fraction by echocardiography. **J**, Cardiac fibrosis analysis. Representative images of heart sections (**Left**) at 300 µm (for the first 3 groups) or 250 µm (for the fourth group treated with α ARi, β ARi, and PTU) intervals and quantification of fibrotic area% (**Right**). Fibrotic tissues are stained by Sirius red and viable myocardial cells are stained by Fast green. **K**, Differential gene expression analysis after combined treatment with α -ARi, β -ARi, and PTU in whole P14 hearts (as described Hirose et al²). False discovery rate <0.1 and fold-change >2 were applied as cutoffs. K-means clustering and gene ontology classification of differentially expressed genes. Top upregulated and downregulated pathways are shown. The RNA-sequencing data set has been deposited in Gene Expression Omnibus under accession number GSE174511. All values are reported as mean±SEM. Number of animals (n) analyzed is identified in each figure. Statistical analyses were performed in GraphPad Prism. Two-way repeated-measures ANOVA was used in **A** and **I**. Only the analysis results at P14 are presented in **A** because of the space constraint. Two-way ANOVA was used in **C**, **G**, **H**, and **J**, and 3-way ANOVA was used in **B**, **D**, and **E**. Pairwise comparisons between all pairs of means were performed following ANOVA in multiple comparisons. In all analyses, the Tukey test was used to correct for multiple comparisons. **P*<0.05; ***P*<0.01; ****P*<0.001; ****P*<0.001. Scale bars: 10 µm (**D**), 50 µm (**E**), and 4 mm (**J**).

ARTICLE INFORMATION

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Disclosures

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