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The alpha-1A adrenergic receptor agonist A61603 reduces cardiac polyunsaturated fatty acid and endocannabinoid metabolites associated with inflammation in vivo

Monte S. Willis, MD, PhD^{1,2,3,*}, Amro Ilaiwy, MD^{4,5,*}, Megan D. Montgomery, PhD⁶, Paul C. Simpson, MD⁶, and Brian C. Jensen, MD^{1,7}

¹McAllister Heart Institute, University of North Carolina, Chapel Hill, NC USA

²Department of Pathology & Laboratory Medicine, University of North Carolina, Chapel Hill, NC USA

³Department of Pharmacology, University of North Carolina, Chapel Hill, NC USA

⁴Sarah W. Stedman Nutrition and Metabolism Center, Duke Molecular Physiology Institute, Duke University Medical Center, Durham, NC, USA

⁵Division of Endocrinology, Metabolism, and Nutrition, Department of Medicine, Duke University Medical Center, Durham, NC, USA

⁶VA Medical Center and University of California, San Francisco, CA, USA

⁷Department of Internal Medicine, Division of Cardiology University of North Carolina, Chapel Hill, NC, USA

Abstract

Introduction—Alpha-1-adrenergic receptors (α 1-ARs) are G-protein coupled receptors (GPCRs) with three highly homologous subtypes (α 1A, α 1B, and α 1D). Of these three subtypes, only the α 1A and α 1B are expressed in the heart. Multiple pre-clinical models of heart injury demonstrate cardioprotective roles for the α 1A. Non-selective α 1-AR activation promotes glycolysis in the heart, but the functional α 1-AR subtype and broader metabolic effects have not been studied.

Objectives—Given the high metabolic demands of the heart and previous evidence indicating benefit from α 1A activation, we chose to investigate the effects of α 1A activation on the cardiac metabolome in vivo.

Corresponding authors: Brian C. Jensen MD, UNC Division of Cardiology, 160 Dental Circle, CB 7075, Chapel Hill, NC 27599-7075, 919-843-5214 (p), 919-966-1743 (f), bcjensen@med.unc.edu, Paul C. Simpson MD, VA Medical Center (111-C-8), 4150 Clement St, San Francisco, CA 94121, 415-221-4810 x3200 (p), 415-379-5570 (f), paul.simpson@ucsf.edu.
*contributed equally

Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with Ethical Standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Methods—Mice were treated for one week with a low, subpressor dose of A61603, a highly selective and potent α 1A agonist. Cardiac tissue and serum were analyzed using a non-targeted metabolomics approach.

Results—We identified previously unrecognized metabolic responses to α 1A activation, most notably broad reduction in the abundance of polyunsaturated fatty acids (PUFAs) and endocannabinoids (ECs).

Conclusion—Given the well characterized roles of PUFAs and ECs in inflammatory pathways, these findings suggest a possible role for cardiac α 1A-ARs in the regulation of inflammation and may offer novel insight into the mechanisms underlying the cardioprotective benefit of selective pharmacologic α 1A activation.

Keywords

alpha-1A adrenergic receptor; agonist; endocannabinoid; arachidonic acid; fatty acid elongation; anti-inflammatory

Introduction

Adrenergic receptors (ARs) are G-protein coupled receptors (GPCRs) that are activated endogenously by catecholamines and play critical roles in regulating cardiac function. The β 1-AR is the most abundant AR in the heart and chronic activation of β 1-ARs by catecholamines contributes to the pathobiology of heart failure. α 1-ARs are less abundant, but play adaptive and protective roles in the heart, including induction of physiological cardiac hypertrophy, enhanced cardiac contractility, and protection against multiple forms of cardiac injury.¹

Pharmacologic approaches have been used for decades to elucidate downstream effects of non-selective cardiac α 1-AR activation. The endogenous catecholamines norepinephrine and epinephrine activate α 1-ARs as well as β -ARs, whereas the synthetic drug phenylephrine is a non-selective α 1-AR agonist used widely in experimental settings. Studies using these agents have identified important roles for α 1-ARs in regulation of glucose metabolism in skeletal muscle², liver³, pancreas⁴, and adipocytes⁵. Non-selective α 1-AR activation in the heart increases glucose metabolism^{6,7} and decreases fatty acid oxidation.⁸ This metabolic substrate switch from fatty acid to glucose is a critical component of the heart's adaptation to stress^{9,10}. Despite the central role of catecholamines in cardiac biology, very little is known about their global metabolic effects in the heart. One previous study investigated the effects of β -AR stimulation on the rat cardiac metabolome¹¹, but the broad metabolic effects of α 1-AR stimulation have not been investigated.

There are three highly homologous subtypes of α 1-ARs: α 1A, α 1B, and α 1D, with variable tissue expression throughout the body. The α 1A and α 1B are expressed in the rodent and human heart, whereas the α 1D is found in the coronary vasculature and mediates vasoconstriction^{12,13}. Transgenic mouse models and pharmacologic approaches have begun to delineate distinct roles for the cardiac α 1-AR subtypes, indicating that the α 1A is cytoprotective in vitro and cardioprotective in vivo¹⁴. Transgenic mice overexpressing the

α 1A in cardiomyocytes demonstrate enhanced contractility and functional recovery after ischemic challenge and pressure overload.¹⁵⁻¹⁷

Recent studies have expanded these observations using the potent and highly selective α 1A agonist, A61603 (N-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydro naphthalen-1-yl] methanesulfonamide hydrobromide)¹⁸ in multiple pre-clinical studies¹⁹⁻²¹. A61603 protects against cardiomyocyte death due to multiple cytotoxic exposures in vitro²², prevents doxorubicin-induced cardiotoxicity in vivo²³, and restores right ventricular contractility in a mouse model of right heart failure¹⁹. Importantly, these beneficial effects were achieved using a dose of A61603 that did not increase blood pressure, suggesting that vascular α 1-ARs were not activated, and reinforcing the possibility that selective α 1A-AR activation might represent a novel approach to treating heart muscle disease²⁴. It is unknown whether metabolic alterations contribute to these beneficial effects.

In the present study, mice were treated with A61603 then heart tissue and serum were analyzed using a non-targeted metabolomics approach to discover whether broad metabolic adaptations may underlie the cardioprotective effects of activating the α 1A-AR in vivo.

Materials and Methods

Animals, experimental design, drug delivery, and harvest

Mice were 12-week old C57Bl6J males. All mice underwent implantation of subcutaneous osmotic minipump (Alzet) that delivered A61603 10ng/kg/d to 8 mice and vehicle (100 μ M vitamin C, 0.9% NaCl) to 8 mice for one week. Mice were fed the usual animal care diet per the San Francisco VA Animal Care facility. Mice were sacrificed under deep isoflurane anesthesia, blood was collected by cardiac puncture, clotted, and 100-200 μ L serum was snap frozen in liquid nitrogen. The heart was dissected, the atria and great vessels were excised, and the ventricles were allowed to beat in cold PBS to clear blood. Excess liquid was blotted then the heart was snap-frozen in liquid nitrogen and shipped to Metabolon (Durham, NC).

Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectroscopy (UPLC-MS/MS)

All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Samples were processed as previously described^{25,26}, sample extract reconstituted in solvents containing a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. The extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 μ m) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall

higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5 mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH amide 2.1x150 mm, 1.7 μ m) using a gradient consisting of water and acetonitrile with 10mM ammonium formate, pH 10.8. The MS analysis alternated between MS and data-dependent MSⁿ scans using dynamic exclusion. The scan range varied slightly between methods but covered 70-1000 m/z.

Raw data were extracted, peak-identified by comparison to library entries of purified standards or recurrent unknown entities based on authenticated standards that contain the retention time/index (RI), mass to charge ratio (m/z), and chromatographic data (including MS/MS spectral data). Biochemical identifications are based on retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores are based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. The QC and curation processes were designed to ensure accurate and consistent identification of true chemical entities, and to remove those representing system artifacts, misassignments, and background noise. Peaks were quantified using area-under-the-curve. For studies spanning multiple days, a data normalization step was performed to correct variation resulting from instrument inter-day tuning differences. All data used in this analysis has been archived in the UCSD Metabolomics Workbench (<http://www.metabolomicsworkbench.org/>), accession # (Pending Assignment/Uploaded 17 April 2016).

Metabolomic Statistical Analyses

Metaboanalyst (v3.0) run on the statistical package R (v2.14.0) used metabolite peak areas (as representative of concentration)^{27,28}. These data were first normalized to a pooled average sample from the relevant vehicle-treated group, and scaled using Pareto scaling feature. To detect systemic metabolic signature of A61603 treatment, a Pairwise One-Way Analysis of Variance (ANOVA) and Fisher LSD post-hoc test across the tissues (heart, serum) and experimental groups (vehicle treated, A61603-treated) were performed using Metaboanalyst v3.0. ANOVA significant metabolites (FDR<0.05) were matched to metabolomics pathways using the Pathway Analysis and enrichment analysis features in Metaboanalyst 3.0. Only metabolites identified and detected in all groups were included in the one-way ANOVA. Up to three missing values in each group were imputed. If four or more values of each metabolite were missing in a given group, the entire metabolite was removed from the analysis. Only metabolites significantly altered between tissue-matched control and A61603 treatment groups were included. All data from this study are available in Supplemental Table 1. Heat maps were generated using the GENE- E software (<http://www.broadinstitute.org/cancer/software/GENE-E/index.html>) and Microsoft Excel 2016. All data are shown as mean $\pm$ SEM, unless otherwise indicated.

Results

Heart Tissue

We compared heart tissue from mice treated for 7 days with the selective $\alpha 1A$ -AR agonist, A61603, or vehicle. We used a very low A61603 dose, 10 ng/kg/d, that has no detectable effect on blood pressure, heart rate, heart size, or heart function, but does increase phosphorylation of cardiac ERK, a canonical $\alpha 1A$ signaling partner. (data not shown) All comparisons used a one-way ANOVA to identify significantly altered metabolites. Of the 503 total metabolites identified in heart, 381 were adequate for the one-way ANOVA (Supplemental Figures 1 and 2), and 30 were ANOVA-significant with a false discovery rate (FDR) < 0.05 (Figure 1A). Metaboanalyst recognized 23 of these 30 metabolites and applied pathway analysis to identify biosynthesis of unsaturated fatty acids as the most affected pathway (Figure 1B, $p=1.29E^{-11}$, $FDR=1.06E^{-9}$). Metaboanalyst enrichment analysis for pathways identified alpha linoleic acid and linoleic acid metabolism, ammonia recycling, and urea cycle with the lowest p values, enriched 3 to 10 fold (Figure 1C). Enrichment analysis for location identified the peroxisome with the lowest p values, enriched 1.5-2.0 fold expected (data not shown).

Of the 7 metabolites not recognized by Metaboanalyst for pathway analysis using multiple synonymous chemical names (i.e. *N-Palmitoyl taurine**, 1-(1-enyl-stearoyl)-GPE, *Palmitoylethanolamide**, *N-Stearoyltaurine**, *Oleoyl Ethanolamide**, 1-palmitoyl GPG, 10-Nonadecenoic acid, 15-Methylpalmitate), 4 are involved in the endocannabinoid (EC) Pathway. The EC system consists of cannabinoid receptors and their endogenous neuromodulatory lipid ligands that collectively play long-recognized roles in the neurological and immunological systems²⁹ and recently have been identified in the heart³⁰. We found a roughly 50% decrease in the abundance of the biologically active EC metabolites oleoyl ethanolamide, palmitoyl ethanolamide, N-stearoyltaurine, and N-palmitoyltaurine after treatment with A61603 (Figure 2).

Review of specific metabolites within these pathways revealed a 25% decrease in cardiac arachidonic acid, an omega (ω)-6 polyunsaturated fatty acid (PUFA) (Figure 2 Figure 3B). Similarly, we found decreases of ~25-45% in eight metabolites involved in the synthesis of $\omega 3$ (Figure 3A) and $\omega 6$ fatty acids (Figure 3B). Biosynthesis of PUFAs, including arachidonic acid, occurs through multiple desaturation and elongation steps (Figures 3 and B), each controlled by distinct enzymes.³¹ Our findings suggest that activation of the $\alpha 1A$ broadly reduces $\omega 3$ and $\omega 6$ fatty acid synthesis, possibly through regulation of one or more of these desaturases and elongases.

There was no clear signal that $\alpha 1A$ activation affected glucose metabolism, though pyruvate levels were consistently and significantly decreased in hearts of A61603-treated mice (Figure 1A). Collectively, these findings suggest that A61603-treatment significantly affects the biosynthesis of biologically active PUFAs and EC receptor ligands.

Serum

Of the 543 total metabolites identified in serum, 381 were adequate for one-way ANOVA (Supplemental Figures 4 and 5), and 34 of them were ANOVA significant with an FDR <

0.05 (Figure 4A). Pathway analysis of these 34 metabolites identified pyrimidine metabolism as the most likely affected pathway (Figures 4B and 4C, $p=0.026$, FDR =1). The pyrimidine salvage pathway (Figure 5) recovers core pyrimidine components which are recycled for use as nucleic acids or for recovery of nitrogen to the general nitrogen metabolism³². Further analysis of metabolites in the pyrimidine salvage pathway revealed that A61603 treatment is associated with significant decreases in serum levels of deoxycytidine, deoxyuridine, and ureidopropionate (Figure 5). All three of these metabolites are intermediates in β -alanine synthesis, but A61603 treatment did not affect abundance of β -alanine in either serum or heart.

Of note, treatment with A61603 had no effect on serum glucose, cholesterol, or creatinine levels—three commonly used markers of cardiovascular risk in the clinical setting. Collectively, these findings suggest relatively minimal systemic effects from the administration of an $\alpha 1A$ agonist.

Heart and Serum

We next determined if there were A61603-altered metabolites in the serum that might reflect A61603-altered metabolites in the heart and might serve as a circulating marker of cardiac response to A61603. Since there were no metabolites found in our initial stringent cut-off of an FDR<0.05, we decreased the stringency to FDR<0.10 and identified A61603-induced metabolites compared to the vehicle controls (Figure 6A). Only 5-hydroxy-lysine overlapped in both compartments (Figure 6A, top row).

Since both A61603-induced heart and serum 5-hydroxy-lysine levels were significantly different by FDR (defined as <0.10), we next compared these groups using one-way ANOVA (Figure 6B). There was a statistically significant change in A61603-treated heart compared to its vehicle control ($p<0.02$)(Figure 6B), and the A61603-treated serum had a p value trending to significance ($p=0.128$)(Figure 6B). 5-Hydroxylysine is a hydroxylated derivative of the amino acid lysine present in certain collagens and belongs to a class of organic compounds known as hydroxyl-fatty acids³³. While the changes in the serum reflected those in the heart, a more sensitive marker or set of serologic markers would be needed to follow A61603 activity in the heart. As such, the metabolite subset recognized in the current study did not identify a reliable serum metabolite marker for the cardiac effects of A61603.

Discussion

In this study, we used non-targeted metabolomics analysis to identify previously unrecognized links between pharmacological $\alpha 1A$ -AR activation and biosynthesis of PUFAs and ECs in the heart. These findings expand our limited understanding of adrenergic regulation of the cardiac metabolome and generate novel hypotheses regarding the activity of cardiac $\alpha 1A$ -ARs. In particular, PUFAs and ECs both play central roles in inflammation, suggesting that regulation of inflammation may contribute to the demonstrated cardioprotective effects of the selective $\alpha 1A$ agonist, A61603.

We discovered that systemic treatment with A61603 broadly reduced the cardiac content of ω 3, ω 6, and ω 9 PUFAs without affecting serum PUFA levels. PUFAs play critical and complex roles in the heart, acting as both biosynthetic intermediates and signaling molecules³⁴. Observational studies have linked higher serum ω 3-PUFA levels and dietary PUFA intake with lower mortality due to cardiovascular disease³⁵. However, the biological relevance of PUFA abundance in cardiac tissue is uncertain and rodent models have demonstrated that dietary PUFA content is not correlated with heart content³⁶. Previous reports indicate that treatment with PUFAs leads to a decrease in α 1-AR mediated contractility in cultured cardiomyocytes^{37,38}, but the role of cardiac α 1-ARs in regulating PUFA biosynthesis has not been explored.

We found decreases in ostensibly beneficial ω 3 PUFAs such as docosapentanoic acid (DPA) and docosahexanoic acid (DHA)³⁹, as well as more cardiotoxic PUFAs such as the ω 9 PUFA, erucic acid^{40,41}. Arachidonic acid (AA), among the most extensively studied of PUFAs, was decreased to 0.74-fold relative abundance in the hearts of mice treated with A61603. Previous studies have reached variable conclusions about the function of AA in the heart, in part because AA metabolites play conflicting roles—some beneficial⁴² and some maladaptive⁴³. AA is released from membrane phospholipids by phospholipases. α 1-AR stimulation activates phospholipase C⁴⁴ and phospholipase D^{45,46}, which cleave phospholipids to diacyl glycerol (DAG) and phosphatidic acid respectively. These lipid products are then enzymatically converted to AA. In our study, A61603 treatment increased total DAG content by 24% ($p < 0.05$). The observed decrease in AA content could potentially result from regulation of DAG lipase—the enzyme that converts DAG to AA—or through an increase in conversion of AA to downstream metabolites.

PUFAs have not been studied widely in injured heart tissue. PUFA abundance in the heart is not affected by activation of the angiotensin I receptor⁴⁷, and treatment with the β -AR agonist isoproterenol increases AA 3.4-fold and the ω 6 PUFA linoelaidic acid 2.5-fold¹¹. Chronic activation of both the angiotensin I receptor and the β -AR lead to maladaptive cardiac hypertrophy and heart failure. Consistent with these findings, AA and DHA increase in failing human heart tissue as well as hypertrophied and failing rat hearts⁴⁸. In contrast, sustained α 1A activation leads to a decrease in PUFA levels and cardioprotection, though the mechanistic connection between these findings is uncertain. Collectively, these studies illustrate the complexity of ascribing inherent benefit or harm to tissue PUFA levels.

Our results also indicate that pharmacological activation of the α 1A-AR leads to decreased levels of ECs in the heart. ECs are metabolites in the AA biosynthetic pathway, formed by activation of fatty acid amid hydrolase⁴⁹. ECs participate in the regulation of cellular metabolism and inflammation through activation of CB1, CB2, and TRPV1 channels. The effects of ECs in the heart are either beneficial⁵⁰ or maladaptive^{51,52}, depending on context and selective receptor activation⁵³.

Cardioprotective mechanisms induced by EC modulation include enhanced $\text{Na}^+/\text{Ca}^{2+}$ exchange current⁵⁴, activation of HSP72, PKA, PLC, PKC, eNOS, iNOS, and ERK1/2⁵⁵⁻⁵⁷, and regulation of myosin heavy chain isoform switching⁵⁰. Interestingly, α 1-AR activation regulates each of these processes in the heart as well, suggesting that the EC system may

participate broadly in the mechanisms underlying α 1-mediated cardioprotection. Of particular significance to our current study, ECs have been shown to decrease responsiveness to AR agonist stimulation in the vasculature^{58,59}, to mediate α 1-induced glutamate neurotransmission in the central nervous system⁶⁰, and to regulate peripheral norepinephrine release⁶¹. However, our study is the first to show an effect of α 1-AR activation on EC biosynthesis.

Given the complex functions of both PUFAs and the EC system in the heart, it is unclear how our findings might help to explain the cardioprotective effects of A61603. However, one reasonable hypothesis is that α 1A activation regulates inflammation, insofar as many of the metabolites that were reduced in A61603-treated hearts are involved in inflammatory pathways. ECs are well-recognized mediators of systemic inflammatory diseases such as rheumatoid arthritis⁶¹, and also regulate inflammation in the heart⁶². PUFAs regulate T lymphocyte function⁶³ and modify inflammatory diseases⁶⁴ and AA and its metabolites provide critical positive and negative regulation of systemic and cardiac inflammation⁶⁵. Inflammation is central to the biology of acute myocardial injury⁶⁹ and to the pathophysiology of chronic heart failure⁷⁰ and α 1A activation is protective in both settings. The role of inflammation in the heart is complex, however, and inflammatory mediators released from both circulating cells or any of the resident cell cardiac types play both adaptive and maladaptive roles.

The role of α 1-ARs in regulating cardiac inflammation has not been explored to any significant extent. Transgenic mice overexpressing a constitutively active α 1A have increased IL-6 expression in the heart, but no evidence of increased cardiac inflammation⁶⁶. These mice are protected against ischemia-reperfusion injury, a condition that typically is worsened by inflammation. One other study found that non-selective α 1-AR activation suppresses lipopolysaccharide-induced cardiomyocyte TNF- α expression and improves cardiac dysfunction during endotoxemia through ERK activation and NF- κ B suppression⁶⁷. α 1-ARs are found on monocytes and macrophages, and their activation appears to alter production of inflammatory mediators⁶⁸. Our analysis of the serum of mice treated with A61603 revealed no clear evidence of systemic inflammatory modulation, though reduction in pyrimidine salvage intermediates (Figure 5), would favor a reduction in inflammatory cell function.^{69,70} The role of the α 1A-AR in regulating cardiac inflammation will be the subject of future work in our lab.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Jensen BC, O'Connell TD, Simpson PC. Alpha-1-adrenergic receptors in heart failure: the adaptive arm of the cardiac response to chronic catecholamine stimulation. *Journal of cardiovascular pharmacology*. Apr; 2014 63(4):291–301. [PubMed: 24145181]
2. Hutchinson DS, Bengtsson T. AMP-activated protein kinase activation by adrenoceptors in L6 skeletal muscle cells: mediation by alpha1-adrenoceptors causing glucose uptake. *Diabetes*. Mar; 2006 55(3):682–690. [PubMed: 16505231]
3. Thomas AP, Martin-Requero A, Williamson JR. Interactions between insulin and alpha 1-adrenergic agents in the regulation of glycogen metabolism in isolated hepatocytes. *J Biol Chem*. May 25; 1985 260(10):5963–5973. [PubMed: 2860104]
4. Skoglund G, Lundquist I, Ahren B. Alpha 1- and alpha 2-adrenoceptor activation increases plasma glucagon levels in the mouse. *European journal of pharmacology*. Nov 3; 1987 143(1):83–88. [PubMed: 2891547]
5. Fitzpatrick, D., Purves, D., Neur, Augustine G. *Neuroscience*. 3. Purves, D. Augustine, G. Fitzpatrick, D., et al., editors. Sunderland, MA: 2004. p. 773
6. Clark MG, Patten GS, Filsell OH. Evidence for an alpha-adrenergic receptor-mediated control of energy production in heart. *J Mol Cell Cardiol*. Jun; 1982 14(6):313–321. [PubMed: 6127414]
7. Eger S, Nguyen N, Schwaiger M. Contribution of alpha-adrenergic and beta-adrenergic stimulation to ischemia-induced glucose transporter (GLUT) 4 and GLUT1 translocation in the isolated perfused rat heart. *Circ Res*. Jun 25; 1999 84(12):1407–1415. [PubMed: 10381893]
8. Barger PM, Brandt JM, Leone TC, Weinheimer CJ, Kelly DP. Deactivation of peroxisome proliferator-activated receptor-alpha during cardiac hypertrophic growth. *The Journal of clinical investigation*. Jun 12.2000 105:1723–1730. [PubMed: 10862787]
9. Pascual F, Coleman RA. Fuel availability and fate in cardiac metabolism: A tale of two substrates. *Biochim Biophys Acta*. Mar 16.2016
10. Griffin TM, Humphries KM, Kinter M, Lim HY, Szveda LI. Nutrient sensing and utilization: Getting to the heart of metabolic flexibility. *Biochimie*. May.2016 124:74–83. [PubMed: 26476002]
11. Liu YT, Jia HM, Chang X, Ding G, Zhang HW, Zou ZM. The metabolic disturbances of isoproterenol induced myocardial infarction in rats based on a tissue targeted metabolomics. *Molecular bioSystems*. Nov; 2013 9(11):2823–2834. [PubMed: 24057015]
12. Jensen B, Swigart P, Laden M-E, DeMarco T, Hoopes C, Simpson P. The alpha-1D is the predominant alpha-1-adrenergic receptor in human epicardial coronary arteries. *JACC*. 2009; 54(13):1137–1145. [PubMed: 19761933]
13. Turnbull L, McCloskey DT, O'Connell TD, Simpson PC, Baker AJ. Alpha 1-adrenergic receptor responses in alpha 1AB-AR knockout mouse hearts suggest the presence of alpha 1D-AR. *Am J Physiol Heart Circ Physiol*. Apr; 2003 284(4):H1104–1109. [PubMed: 12595294]
14. O'Connell TD, Jensen BC, Baker AJ, Simpson PC. Cardiac alpha1-adrenergic receptors: novel aspects of expression, signaling mechanisms, physiologic function, and clinical importance. *Pharmacological reviews*. 2014; 66(1):308–333. [PubMed: 24368739]
15. Woodcock EA. Roles of alpha1A- and alpha1B-adrenoceptors in heart: insights from studies of genetically modified mice. *Clin Exp Pharmacol Physiol*. Sep; 2007 34(9):884–888. [PubMed: 17645635]
16. Du XJ, Gao XM, Kiriazis H, et al. Transgenic alpha1A-adrenergic activation limits post-infarct ventricular remodeling and dysfunction and improves survival. *Cardiovasc Res*. Sep 1; 2006 71(4):735–743. [PubMed: 16859660]
17. Zhao X, Balaji P, Pachon R, et al. Overexpression of Cardiomyocyte alpha1A-Adrenergic Receptors Attenuates Postinfarct Remodeling by Inducing Angiogenesis Through Heterocellular Signaling. *Arteriosclerosis, thrombosis, and vascular biology*. Nov; 2015 35(11):2451–2459.
18. Knepper SM, Buckner SA, Brune ME, DeBernardis JF, Meyer MD, Hancock AA. A-61603, a potent alpha 1-adrenergic receptor agonist, selective for the alpha 1A receptor subtype. *J Pharmacol Exp Ther*. Jul; 1995 274(1):97–103. [PubMed: 7616455]

19. Cowley PM, Wang G, Chang AN, et al. The alpha1A-adrenergic receptor subtype mediates increased contraction of failing right ventricular myocardium. *Am J Physiol Heart Circ Physiol*. Sep; 2015 309(5):H888–896. [PubMed: 26116709]
20. Luo DL, Gao J, Fan LL, Tang Y, Zhang YY, Han QD. Receptor subtype involved in alpha 1-adrenergic receptor-mediated Ca²⁺ signaling in cardiomyocytes. *Acta Pharmacol Sin*. Jul; 2007 28(7):968–974. [PubMed: 17588332]
21. Snabaitis AK, Yokoyama H, Avkiran M. Roles of mitogen-activated protein kinases and protein kinase C in alpha(1A)-adrenoceptor-mediated stimulation of the sarcolemmal Na(+)-H(+) exchanger. *Circ Res*. Feb 4; 2000 86(2):214–220. [PubMed: 10666418]
22. Huang Y, Wright CD, Merkwand CL, et al. An alpha1A-adrenergic-extracellular signal-regulated kinase survival signaling pathway in cardiac myocytes. *Circulation*. Feb 13; 2007 115(6):763–772. [PubMed: 17283256]
23. Dash R, Chung J, Chan T, et al. A molecular MRI probe to detect treatment of cardiac apoptosis in vivo. *Magnetic resonance in medicine*. Oct; 2011 66(4):1152–1162. [PubMed: 21360750]
24. Jensen BC, O'Connell TD, Simpson PC. Alpha-1-adrenergic receptors: targets for agonist drugs to treat heart failure. *J Mol Cell Cardiol*. Oct; 2011 51(4):518–528. [PubMed: 21118696]
25. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. *Anal Chem*. Aug 15; 2009 81(16):6656–6667. [PubMed: 19624122]
26. Lawton KA, Berger A, Mitchell M, et al. Analysis of the adult human plasma metabolome. *Pharmacogenomics*. Apr; 2008 9(4):383–397. [PubMed: 18384253]
27. Xia J, Sinelnikov IV, Han B, Wishart DS. MetaboAnalyst 3.0--making metabolomics more meaningful. *Nucleic Acids Res*. Jul 1; 2015 43(W1):W251–257. [PubMed: 25897128]
28. Xia J, Psychogios N, Young N, Wishart DS. MetaboAnalyst: a web server for metabolomic data analysis and interpretation. *Nucleic Acids Res*. Jul; 2009 37(Web Server issue):W652–660. [PubMed: 19429898]
29. Vasileiou I, Fotopoulou G, Matzourani M, Patsouris E, Theocharis S. Evidence for the involvement of cannabinoid receptors' polymorphisms in the pathophysiology of human diseases. *Expert Opin Ther Targets*. Apr; 2013 17(4):363–377. [PubMed: 23293857]
30. O'Sullivan SE. Endocannabinoids and the Cardiovascular System in Health and Disease. *Handb Exp Pharmacol*. 2015; 231:393–422. [PubMed: 26408169]
31. Guillou H, Zadavec D, Martin PG, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog Lipid Res*. Apr; 2010 49(2):186–199. [PubMed: 20018209]
32. Zrenner R, Riegler H, Marquard CR, et al. A functional analysis of the pyrimidine catabolic pathway in Arabidopsis. *New Phytol*. 2009; 183(1):117–132. [PubMed: 19413687]
33. Schor DS, Verhoeven NM, Struys EA, ten Brink HJ, Jakobs C. Quantification of 3-hydroxyglutaric acid in urine, plasma, cerebrospinal fluid and amniotic fluid by stable-isotope dilution negative chemical ionization gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. Nov 15; 2002 780(1):199–204.
34. van Bilsen M, Planavila A. Fatty acids and cardiac disease: fuel carrying a message. *Acta physiologica*. Jul; 2014 211(3):476–490. [PubMed: 24773697]
35. Mozaffarian D, Lemaitre RN, King IB, et al. Plasma phospholipid long-chain omega-3 fatty acids and total and cause-specific mortality in older adults: a cohort study. *Annals of internal medicine*. Apr 2; 2013 158(7):515–525. [PubMed: 23546563]
36. Cleland LG, Gibson RA, Pedler J, James MJ. Paradoxical effect of n-3-containing vegetable oils on long-chain n-3 fatty acids in rat heart. *Lipids*. Oct; 2005 40(10):995–998. [PubMed: 16382570]
37. Reithmann C, Scheininger C, Bulgan T, Werdan K. Exposure to the n-3 polyunsaturated fatty acid docosahexaenoic acid impairs alpha 1-adrenoceptor-mediated contractile responses and inositol phosphate formation in rat cardiomyocytes. *Naunyn-Schmiedeberg's archives of pharmacology*. Jul; 1996 354(2):109–119.

38. Delerive P, Oudot F, Ponsard B, et al. Hypoxia-reoxygenation and polyunsaturated fatty acids modulate adrenergic functions in cultured cardiomyocytes. *J Mol Cell Cardiol.* Feb; 1999 31(2): 377–386. [PubMed: 10093050]
39. Kang JX, Leaf A. Evidence that free polyunsaturated fatty acids modify Na⁺ channels by directly binding to the channel proteins. *Proceedings of the National Academy of Sciences of the United States of America.* Apr 16; 1996 93(8):3542–3546. [PubMed: 8622972]
40. Beare-Rogers JL, Nera EA, Heggtveit HA. Myocardial alteration in rats fed rapeseed oils containing high or low levels of erucic acid. *Nutrition and metabolism.* 1974; 17(4):213–222. [PubMed: 4462046]
41. Imamura F, Lemaitre RN, King IB, et al. Long-chain monounsaturated Fatty acids and incidence of congestive heart failure in 2 prospective cohorts. *Circulation.* Apr 9; 2013 127(14):1512–1521. 1521e1511–1518. [PubMed: 23487436]
42. Gross GJ, Falck JR, Gross ER, Isbell M, Moore J, Nithipatikom K. Cytochrome P450 and arachidonic acid metabolites: role in myocardial ischemia/reperfusion injury revisited. *Cardiovasc Res.* Oct 1; 2005 68(1):18–25. [PubMed: 15993870]
43. Alsaad AM, Zordoky BN, Tse MM, El-Kadi AO. Role of cytochrome P450-mediated arachidonic acid metabolites in the pathogenesis of cardiac hypertrophy. *Drug metabolism reviews.* May; 2013 45(2):173–195. [PubMed: 23600686]
44. Steinberg SF, Kaplan LM, Inouye T, Zhang JF, Robinson RB. Alpha-1 adrenergic stimulation of 1,4,5-inositol trisphosphate formation in ventricular myocytes. *J Pharmacol Exp Ther.* Sep; 1989 250(3):1141–1148. [PubMed: 2550617]
45. Parmentier JH, Ahmed A, Ruan Y, Gandhi GK, Saeed AE, Malik KU. Calcium and protein kinase C (PKC)-related kinase mediate alpha 1A-adrenergic receptor-stimulated activation of phospholipase D in rat-1 cells, independent of PKC. *J Pharmacol Exp Ther.* Dec; 2002 303(3): 1206–1215. [PubMed: 12438545]
46. Gosau N, Fahimi-Vahid M, Michalek C, Schmidt M, Wieland T. Signalling components involved in the coupling of alpha 1-adrenoceptors to phospholipase D in neonatal rat cardiac myocytes. *Naunyn-Schmiedeberg's archives of pharmacology.* Jun; 2002 365(6):468–476.
47. Mervaala E, Biala A, Merasto S, et al. Metabolomics in angiotensin II-induced cardiac hypertrophy. *Hypertension.* Feb; 2010 55(2):508–515. [PubMed: 20065148]
48. Le CH, Mulligan CM, Routh MA, et al. Delta-6-desaturase links polyunsaturated fatty acid metabolism with phospholipid remodeling and disease progression in heart failure. *Circulation Heart failure.* Jan.2014 71:172–183.
49. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature.* Nov 7; 1996 384(6604): 83–87. [PubMed: 8900284]
50. Duerr GD, Heinemann JC, Suchan G, et al. The endocannabinoid-CB2 receptor axis protects the ischemic heart at the early stage of cardiomyopathy. *Basic research in cardiology.* Jul.2014 109(4): 425. [PubMed: 24980781]
51. Al Kury LT, Voitychuk OI, Yang KH, et al. Effects of the endogenous cannabinoid anandamide on voltage-dependent sodium and calcium channels in rat ventricular myocytes. *British journal of pharmacology.* Jul; 2014 171(14):3485–3498. [PubMed: 24758718]
52. Batkai S, Pacher P, Osei-Hyiaman D, et al. Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. *Circulation.* Oct 5; 2004 110(14):1996–2002. [PubMed: 15451779]
53. Hiley CR. Endocannabinoids and the heart. *Journal of cardiovascular pharmacology.* Apr; 2009 53(4):267–276. [PubMed: 19276990]
54. Li Q, Cui N, Du Y, Ma H, Zhang Y. Anandamide reduces intracellular Ca²⁺ concentration through suppression of Na⁺/Ca²⁺ exchanger current in rat cardiac myocytes. *PLoS One.* 2013; 8(5):e63386. [PubMed: 23667607]
55. Li Q, Shi M, Li B. Anandamide enhances expression of heat shock protein 72 to protect against ischemia-reperfusion injury in rat heart. *J Physiol Sci.* Jan; 2013 63(1):47–53. [PubMed: 23007622]

56. Cappellano G, Uberti F, Caimmi PP, et al. Different expression and function of the endocannabinoid system in human epicardial adipose tissue in relation to heart disease. *Can J Cardiol.* Apr; 2013 29(4):499–509. [PubMed: 22926037]
57. Gonzalez C, Herradon E, Abalo R, et al. Cannabinoid/agonist WIN 55,212-2 reduces cardiac ischaemia-reperfusion injury in Zucker diabetic fatty rats: role of CB2 receptors and iNOS/eNOS. *Diabetes Metab Res Rev.* May; 2011 27(4):331–340. [PubMed: 21309057]
58. Szekeres M, Nadasy GL, Turu G, et al. Endocannabinoid-mediated modulation of Gq/11 protein-coupled receptor signaling-induced vasoconstriction and hypertension. *Molecular and cellular endocrinology.* Mar 5.2015 403:46–56. [PubMed: 25595485]
59. Marichal-Cancino BA, Manrique-Maldonado G, Altamirano-Espinoza AH, et al. Analysis of anandamide- and lysophosphatidylinositol-induced inhibition of the vasopressor responses produced by sympathetic stimulation or noradrenaline in pithed rats. *European journal of pharmacology.* Dec 5; 2013 721(1-3):168–177. [PubMed: 24076186]
60. Haj-Dahmane S, Shen RY. Chronic stress impairs alpha1-adrenoceptor-induced endocannabinoid-dependent synaptic plasticity in the dorsal raphe nucleus. *J Neurosci.* Oct 29; 2014 34(44):14560–14570. [PubMed: 25355210]
61. Lowin T, Straub RH. Cannabinoid-based drugs targeting CB1 and TRPV1, the sympathetic nervous system, and arthritis. *Arthritis Res Ther.* 2015; 17:226. [PubMed: 26343051]
62. Moris D, Georgopoulos S, Felekouras E, Patsouris E, Theocharis S. The effect of endocannabinoid system in ischemia-reperfusion injury: a friend or a foe? *Expert Opin Ther Targets.* 2015; 19(9): 1261–1275. [PubMed: 25936364]
63. Nicolaou A, Mauro C, Urquhart P, Marelli-Berg F. Polyunsaturated Fatty Acid-derived lipid mediators and T cell function. *Frontiers in immunology.* 2014; 5:75. [PubMed: 24611066]
64. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *AmJ Clin Nutr.* Jun; 2006 83(6 Suppl):1505S–1519S. [PubMed: 16841861]
65. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology.* Nov 8; 2011 58(20):2047–2067. [PubMed: 22051327]
66. Perez DM, Papay RS, Shi T. alpha1-Adrenergic receptor stimulates interleukin-6 expression and secretion through both mRNA stability and transcriptional regulation: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB. *Mol Pharmacol.* Jul; 2009 76(1):144–152. [PubMed: 19363165]
67. Yu X, Jia B, Wang F, et al. alpha(1) adrenoceptor activation by norepinephrine inhibits LPS-induced cardiomyocyte TNF-alpha production via modulating ERK1/2 and NF-kappaB pathway. *Journal of cellular and molecular medicine.* Feb; 2014 18(2):263–273. [PubMed: 24304472]
68. Grisanti LA, Perez DM, Porter JE. Modulation of immune cell function by alpha(1)-adrenergic receptor activation. *Current topics in membranes.* 2011; 67:113–138. [PubMed: 21771488]
69. Rudolph FB, Kulkarni AD, Fanslow WC, Pizzini RP, Kumar S, Van Buren CT. Role of RNA as a dietary source of pyrimidines and purines in immune function. *Nutrition.* Jan-Feb;1990 6(1):45–52. discussion 59-62. [PubMed: 1726309]
70. Breedveld FC, Dayer JM. Leflunomide: mode of action in the treatment of rheumatoid arthritis. *Annals of the rheumatic diseases.* Nov; 2000 59(11):841–849. [PubMed: 11053058]

Non-standard abbreviations

A61603	alpha-1 adrenergic receptor agonist: N-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydro naphthalen-1-yl] methanesulfonamide hydrobromide
α1-AR	alpha1-adrenergic receptor
AA	arachidonic acid
CB1/2	cannabinoid receptor type 1/2

DAG	diacylglycerol
DHA	<u>docosa</u> hexaenoic <u>a</u> cid
DPA	<u>docosa</u> pentaenoic <u>a</u> cid
ECs	endocannabinoids
GPCR	G-protein coupled receptor
PUFA	poly-unsaturated fatty acid

Figure 1. Analysis of non-targeted metabolomics of hearts from vehicle-treated and A61603-treated mice

A. Summary heat map of pairwise One-way ANOVA significant metabolites, determined by Fisher-LSD post hoc test results. Fisher LSD post hoc comparisons were made between A61603-treated and vehicle-treated heart groups (N=8/group). **B.** Pathway enrichment analysis for A61603-treated heart group compared to vehicle-treated heart group, determined by ANOVA significant metabolites in the heart. *a-c* indicates top pathways identified. **C.** Enrichment analysis of cardiac A61603-mediated alterations compared to pathway metabolite sets

Heart ANOVA Significant Metabolites Endocannabinoid Pathway

Figure 2. Functional annotation of A61603-mediated alterations in cardiac metabolites in vivo
Overview of the endocannabinoid pathway and the intermediates altered in vivo by A61603 treatment, determined by One-way ANOVA test results in the heart ($p < 0.05$). Data represent mean $\pm$ SEM. ($N=8/\text{group}$). * $p < 0.05$.

Heart ANOVA Significant Metabolites

A Unsaturated Fatty Acid Biosynthesis (Desaturase and Elongation): N-3 Family

Δ=Carbon Numbers with Saturated/Double bonds

B Unsaturated Fatty Acid Biosynthesis (Desaturase and Elongation): N-6 Family

Figure 3. Functional annotation of N-3 and N-6 unsaturated fatty acid biosynthesis including intermediates altered in heart tissue by in vivo A61603

α1A-mediated alterations in **A.** N-3 poly-unsaturated fatty acids (PUFAs) and **B.** N-6 poly-unsaturated fatty acids (PUFAs) in the heart, determined by One-way ANOVA ($p < 0.05$). (delta) references the specific double bonds present in fatty acids. Data represent mean $\pm$ SEM. (N=8/group). * $p < 0.05$.

Figure 4. Analysis of non-targeted metabolomics of serum from vehicle-treated and A61603-treated mice

A. Summary heat map of pairwise One-way ANOVA significant metabolites, determined by Fisher-LSD post hoc test results (significance defined as FDR<0.05). Fisher LSD post hoc comparisons were made between A61603-treated and vehicle-treated serum groups (N=8/group). Only significantly altered metabolites are shown. Significance was defined as FDR <0.05. **B.** Pathway enrichment analysis for A61603-treated serum group compared to

vehicle-treated serum group. *a-c* indicates top pathways identified. C. Enrichment analysis of A61603-mediated alterations in serum compared to pathway metabolite sets.

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Serum ANOVA Significant Metabolites Pyrimidine Salvage Pathway

Figure 5. Functional annotation of A61603-mediated alterations in serum intermediates from the pyrimidine salvage pathway

Overview of the pyrimidine salvage pathway and the serum intermediates altered in vivo by A61603 treatment, determined by One-way ANOVA. Data are shown as mean ± SEM.

(N=8/group)

Figure 6. A61603 responsive metabolite found in heart and plasma
Metabolites significantly altered by A61603 in A. Heart and serum. A pairwise One-way ANOVA was performed to identify significant metabolites using a Fisher-LSD post-hoc test results against either vehicle-treated heart (left column) or vehicle-treated serum (right column), which included those identified in Figure 1A (heart) or Figure 4A (serum), using a slightly lower stringency (significance defined as FDR<0.10) to identify metabolites altered in both. Data represent mean $\pm$ SEM. (N=8/group). *FDR<0.10, #p<0.02.