Jefferson

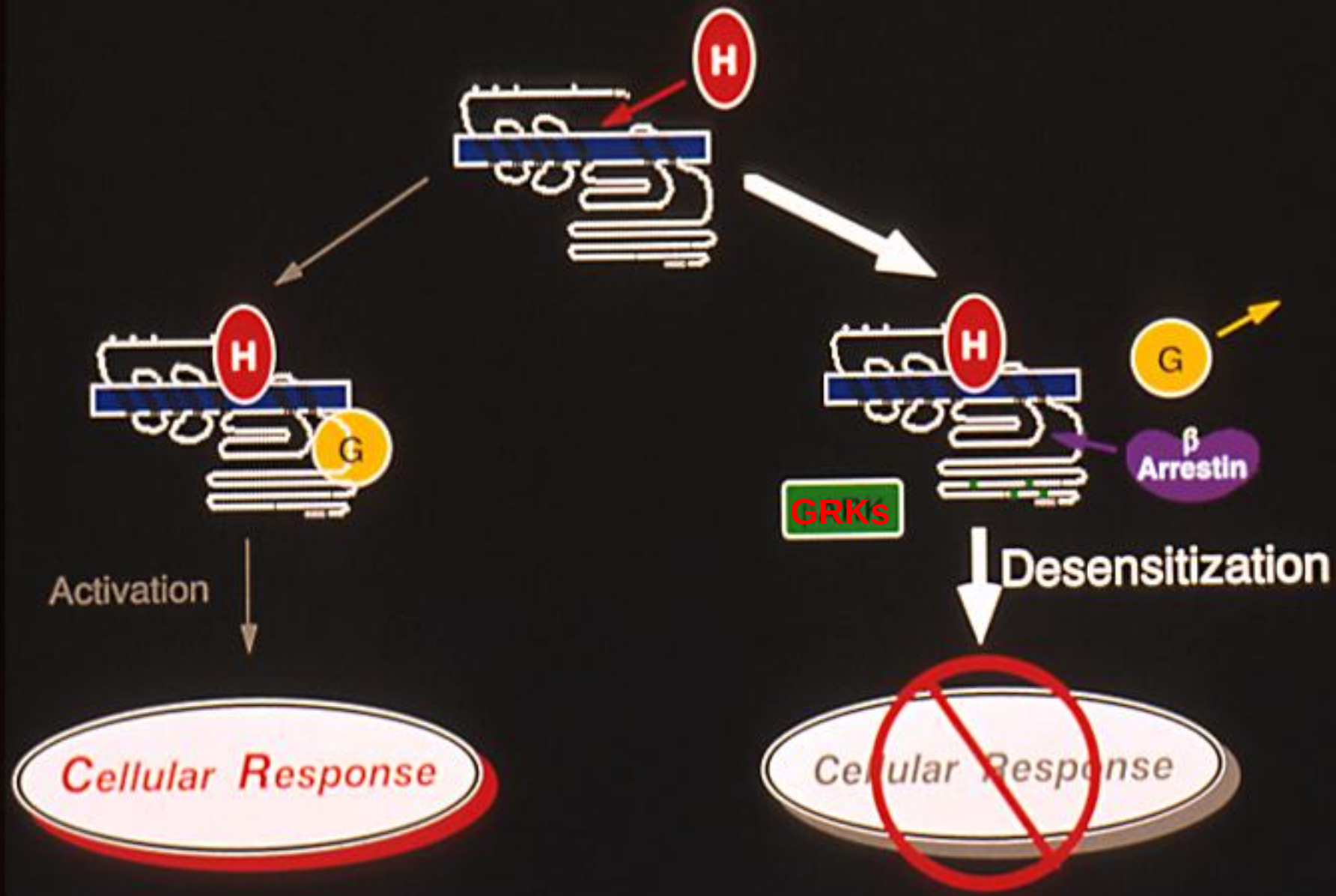
Center for Translational Medicine

GRK2 Inhibition as a Treatment for Heart Failure: Moving from Bench to Bedside

Wally Koch, PhD.

W.W. Smith Professor of Medicine

November 13, 2010


H
H
G
Activation
Cellular Response
H
G
β
Arrestin
GRKs
Desensitization
Cellular Response

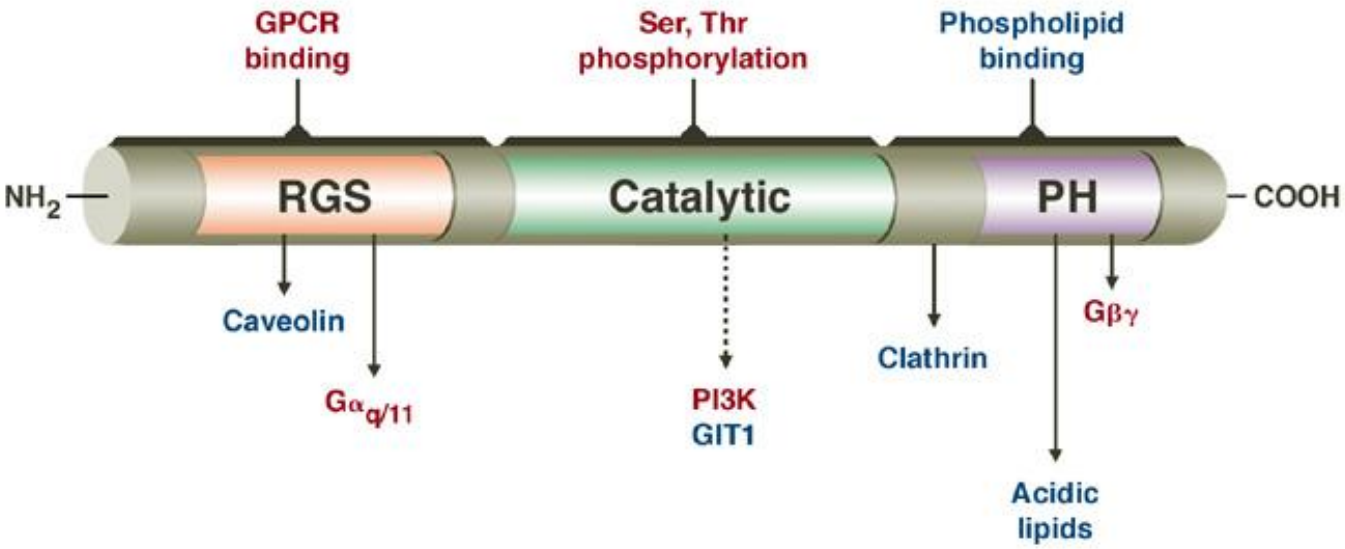
Jefferson

Center for Translational Medicine

G Protein-Coupled Receptor Kinases (GRKs)

Serine/ Threonine Kinases

3 classes: GRK1 (Rhodopsin Kinase), GRK7
GRK2 (βARK1), GRK3 (βARK2)
GRK4, **GRK5**, GRK6



Adapted from Pao CS and Benovic JL, *Science's STKE* 8 October 2002, pp. pe42

Jefferson

Center for Translational Medicine

Heart Failure Therapeutics

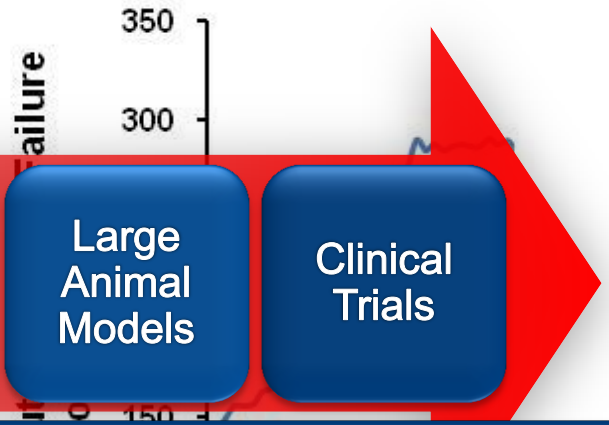
Translational Medicine Approach to Advance HF Therapeutics

Clinical Finding → Therapeutic Design → *Ex vivo* Testing → Transgenic Models → Small Animal Models → Large Animal Models → Clinical Trials

Drug Class	Examples	Discovery
ACE Inhibitors	Captopril, Lisinopril, Enalapril	1979
Aldosterone Antagonists	Spironolactone, Eplerenone	1956
Vasodilators	Hydralazine, Isosorbide Dinitrate	18th (
Digitalis	Digoxin	
Diuretics	Furosemide, HCTZ, Amiloride	18th (

ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult

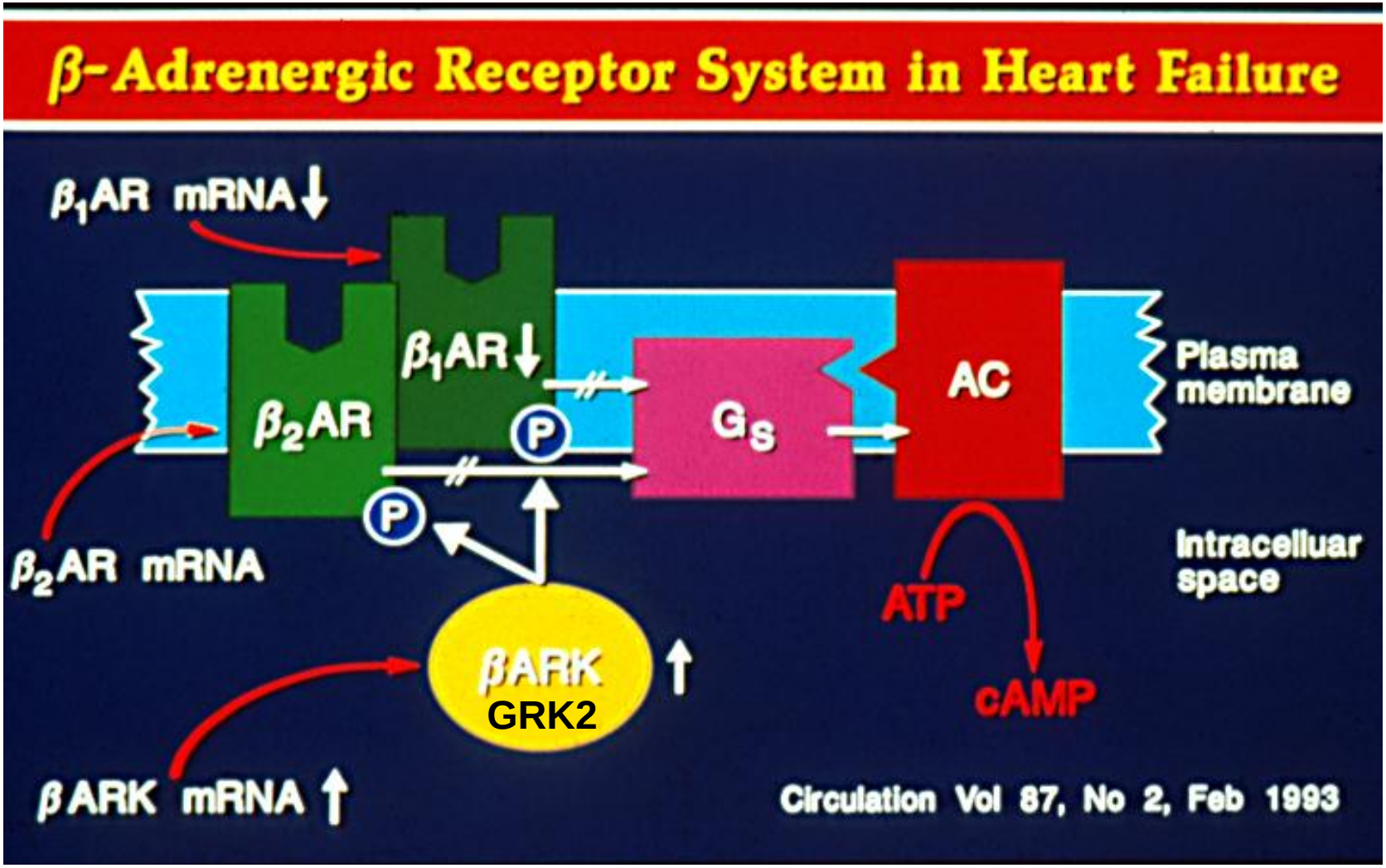
Young HFSA



Alterations of β-Adrenergic Receptor Signal Transduction

Table 1: Alterations in β-AR Signaling Pathways in Chronic HF (12)

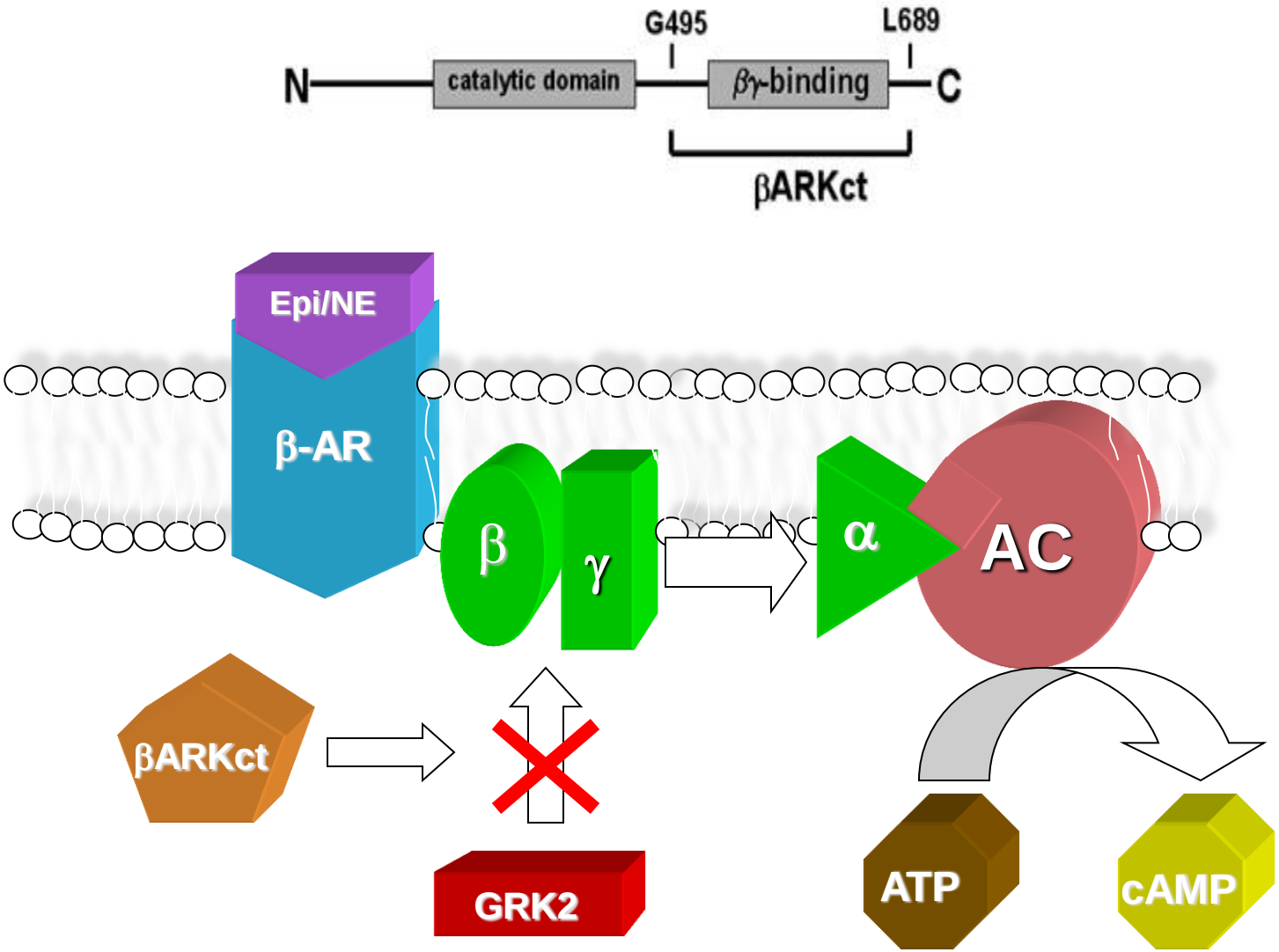
Molecule	Change
β1-AR	**↓, uncoupled**
β2-AR	NC, uncoupled
GRK2	**↑ levels, activity**
GRK3	NC
GRK5	**↑ levels**
Arrestin2	NC
Arrestin 3	NC
Gi	↑
Gs	NC


β-Adrenergic Receptor System in Heart Failure
β1AR mRNA
β1AR
β2AR
P
P
Gs
AC
Plasma membrane
β2AR mRNA
Intracellular space
ATP
cAMP
βARK
GRK2
βARK mRNA
Circulation Vol 87, No 2, Feb 1993

Jefferson

Center for Translational Medicine

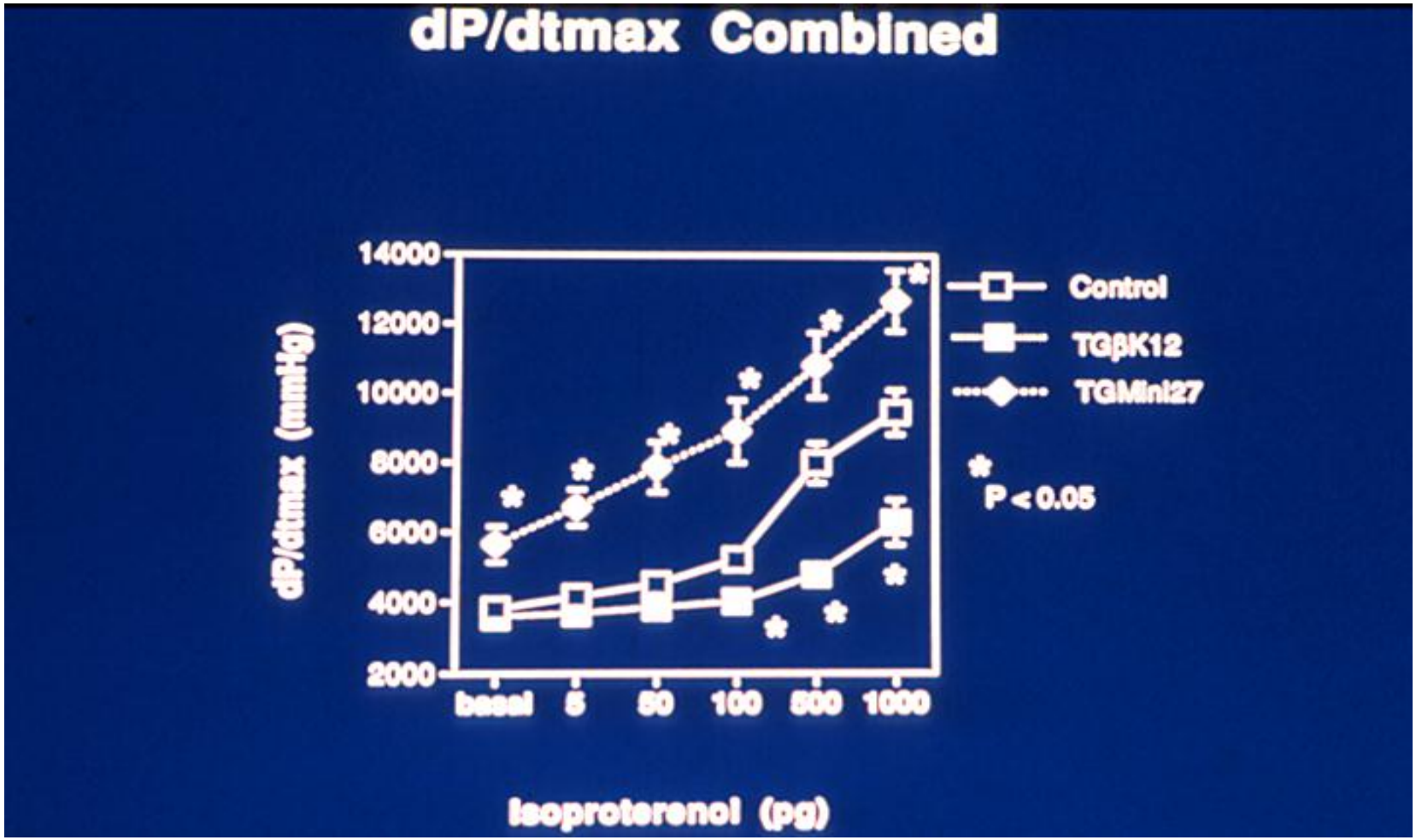
The βARKct as a GRK2 Inhibitor



Jefferson

Center for Translational Medicine

GRK2 and βARKct Transgenic Mice



Koch et al., Science, 1995

Jefferson

Center for Translational Medicine

βARKct rescues several different murine models of HF

Murine model	Result of βARKct cross	Reference
MLP$^{-/-}$ Knockout	Complete functional rescue with restored βAR responsiveness	1
Transgenic Cardiac CSQ Overexpression	Rescue of cardiac function with smaller cardiac dimension and also improved survival	2
Transgenic Cardiac Expression of a Mutant Myosin Heavy Chain (HCM)	Rescue of function, prevention of hypertrophy and dimensions and improved exercise tolerance	3
Transgenic Cardiac Overexpression of MCP-1	Hypertrophy prevented	4
Transgenic Cardiac Overexpression of dominant-Negative mutant of CREB ($CREB_{A133}$)	Only βAR signaling improved with no functional or mortality rescue	5

1. **Rockman et al.** 1998 PNAS 95:7000-7005.
2. **Harding et al.** 2001 PNAS 98:5809-5814.
3. **Freeman et al.** 2001 J Clin Invest 107:967-974.
4. **Khouri et al.** 2002 J Amer Coll Cardiol 39:I-164.
5. Eckhart et al. 2002 J Mol Cell Cardiol 34:669-677.

Jefferson

Center for Translational Medicine

HF Rescue with βARKct Gene Therapy in Rats

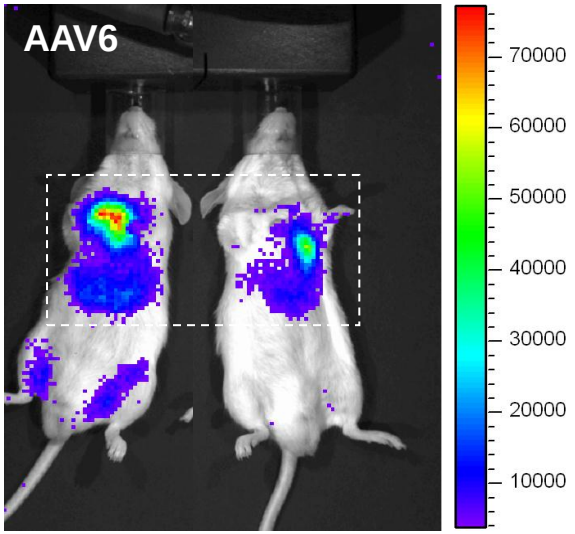
Saline

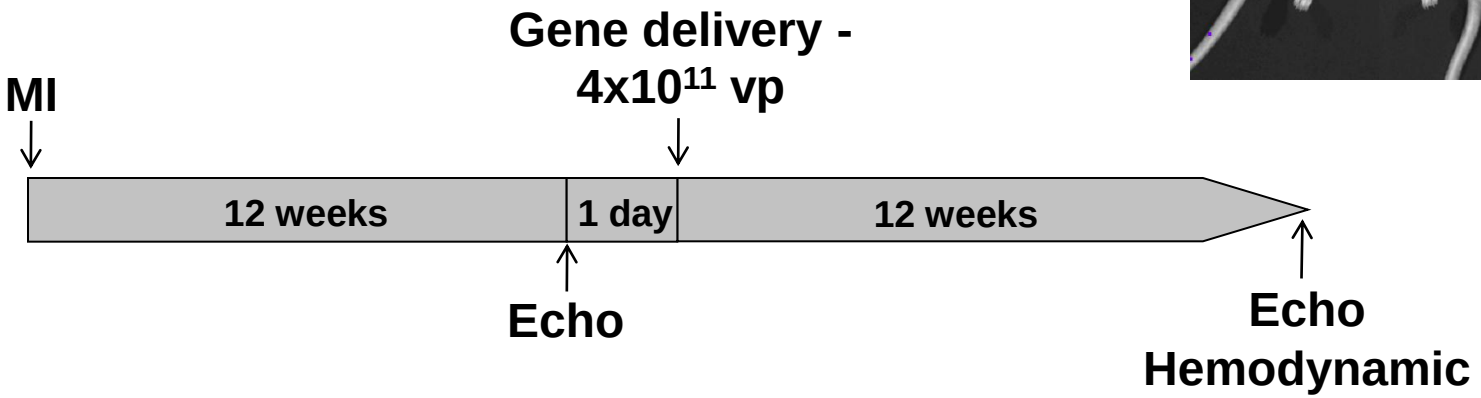
rAAV6-GFP

rAAV6-βARKct Metoprolol

rAAV6-βARKct

rAAV6-GFP Metoprolol

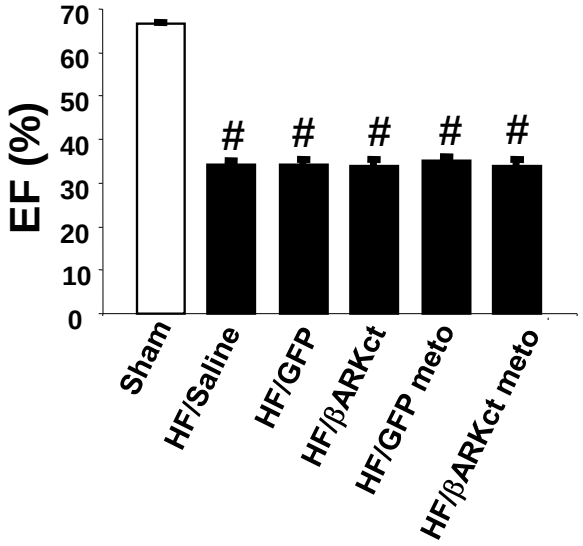


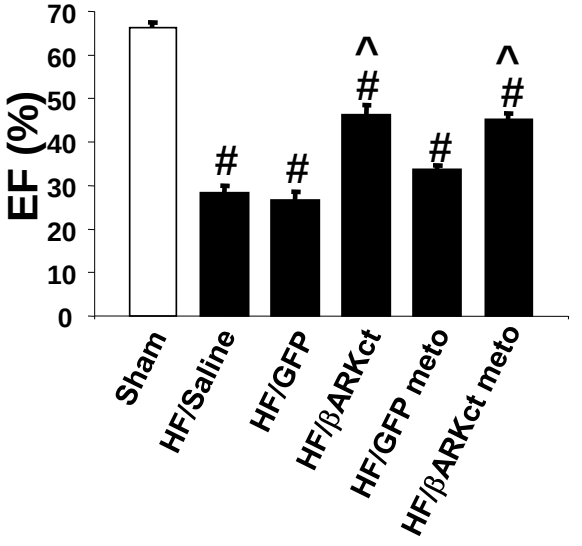


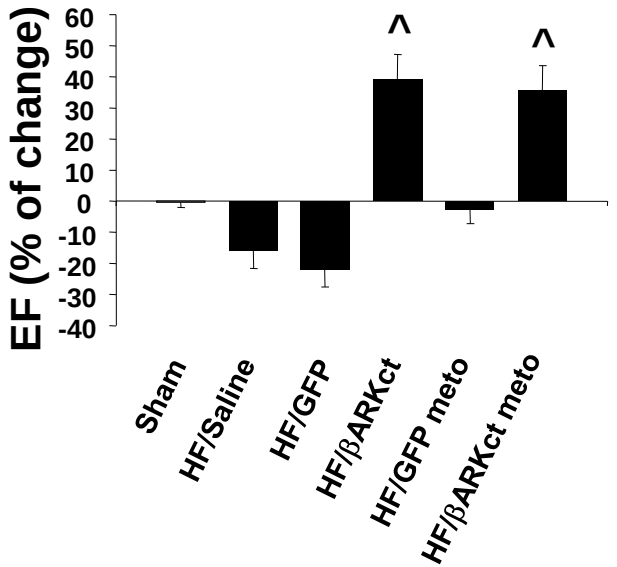
Jefferson

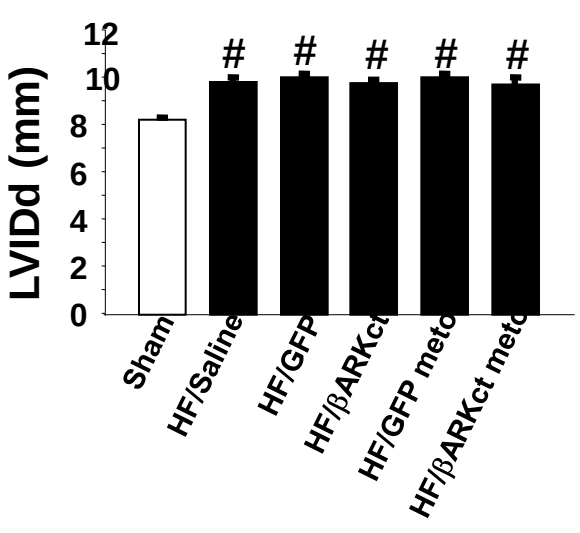
Center for Translational Medicine

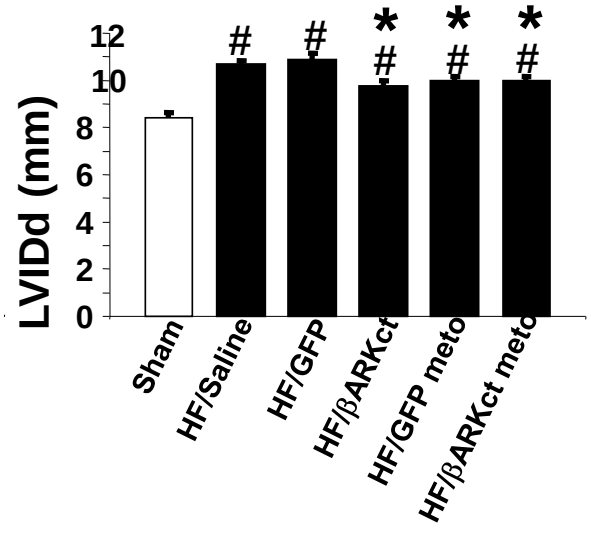
Rengo et al., Circulation, 2009

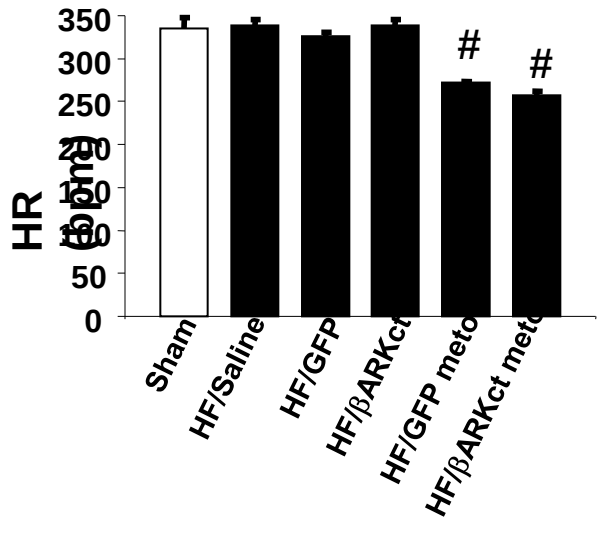












Jefferson

Center for Translational Medicine

Potential Actions of the βARKct as a Gβγ Sequestrant

Additional Gβγ – GRK2 Inhibitory Actions

- **Enhanced signaling through other GPCR's**
- **Non-GPCR actions of GRK2 (i.e. cytoskeleton proteins)**
- **MAP Kinase signaling (Gβγ-dependent = GRK2-dependent)**

Novel GRK2 + Gβγ Binding Partner

Gβγ - PI3-Kinase	**Activity is inhibited in βARKct mice after TAC**

Other Gβγ-Mediated Signaling

Doubtful

Gβγ - AC	**Cardiac AC's (V and VI) not regulated by Gβγ**
Gβγ - PLCβ	**Gq-PLC activity not altered in βARKct mice**
	Gi/Gs - PLC activity weak or absent in the heart

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12974–12979, November 1996
Cell Biology

Essential role of β-adrenergic receptor kinase 1 in cardiac development and function

(gene cloning/knock out/phosphorylation/thin myocardium syndrome)

MOHAMED JABER*†, WALTER J. KOCH‡, HOWARD ROCKMAN§, BRADLEY SMITH¶, RICHARD A. BOND‖, KATHLEEN K. SULIK**, JOHN ROSS, JR.§, ROBERT J. LEFKOWITZ*††, MARC G. CARON*†‡‡, AND BRUNO GIROS*†§§

*Howard Hughes Medical Institute Laboratories, and Departments of †Cell Biology, ††Medicine and Biochemistry, ¶Radiology, and ‡Surgery, Duke University Medical Center, Durham, NC 27710; §Department of Medicine, University of California at San Diego, La Jolla, CA 92093; ‖Department of Pharmacological and Pharmaceutical Sciences, University of Houston, TX 77204; and **Birth Defects Center, University of North Carolina, Chapel Hill, NC 27599

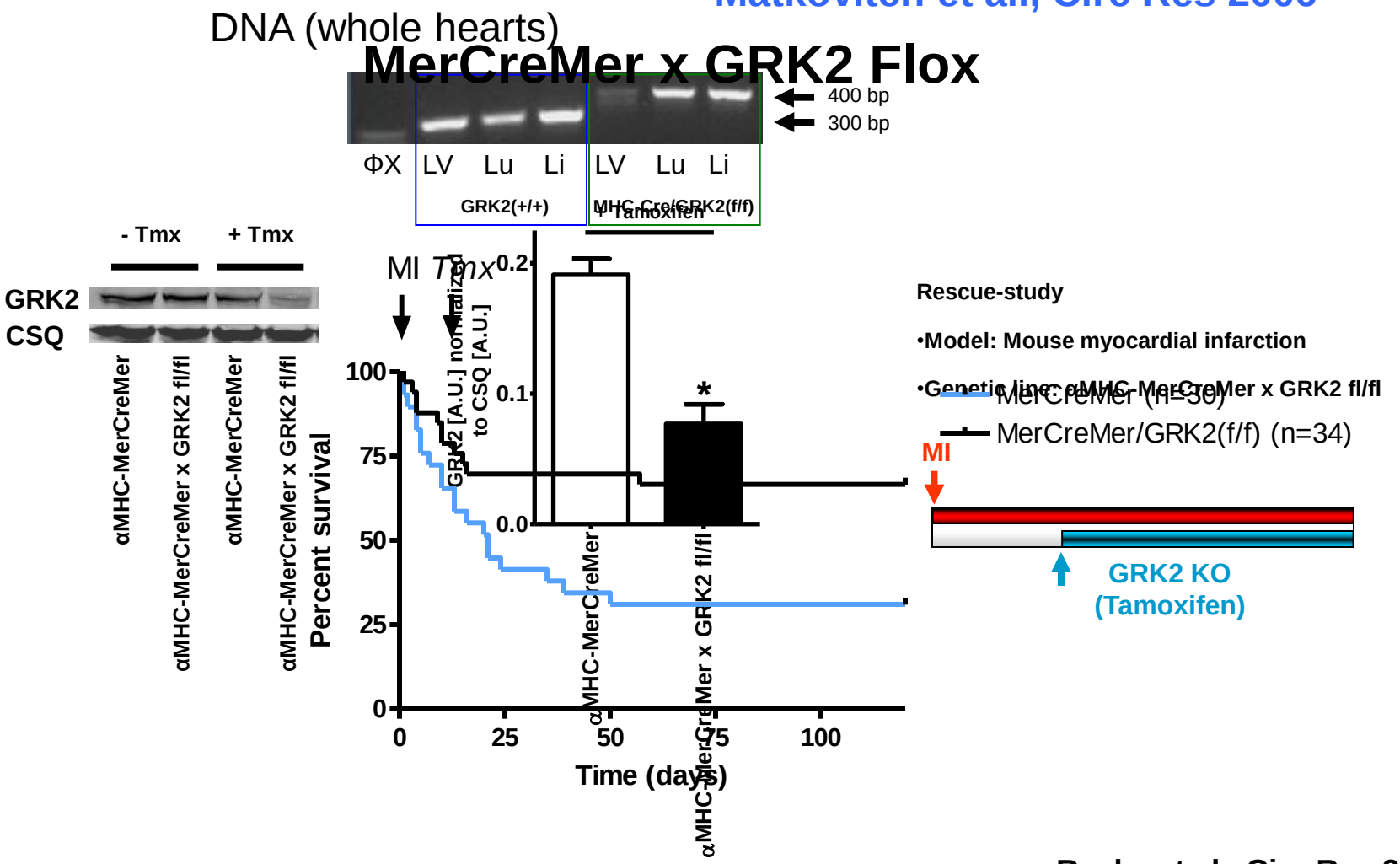
Communicated by Gordon G. Hammes, Duke University Medical Center, Durham, NC, August 26, 1996 (received for review July 10, 1996)

Jefferson

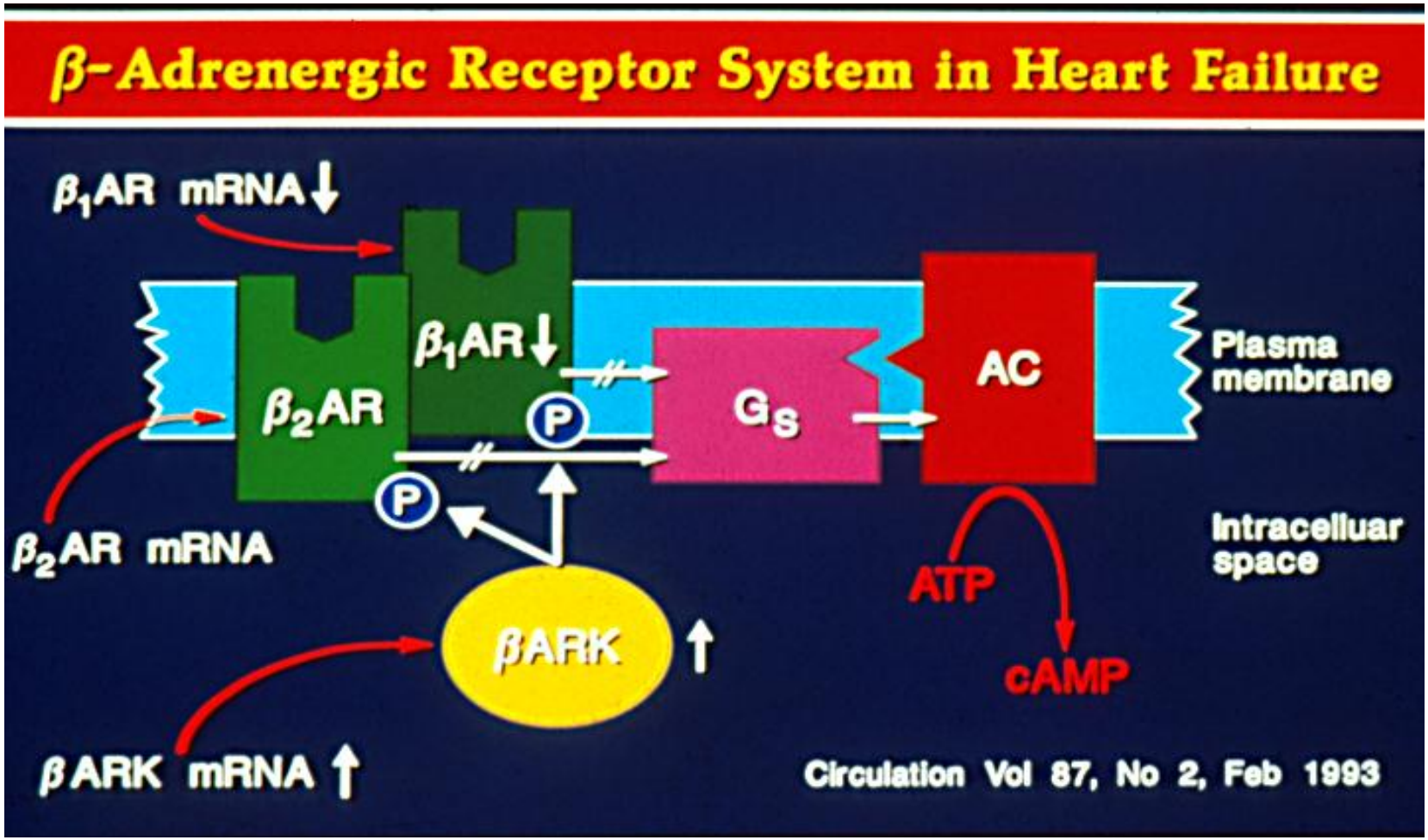
Center for Translational Medicine

Conditional GRK2 Knockout Mouse

Matkovitch et al., Circ Res 2006



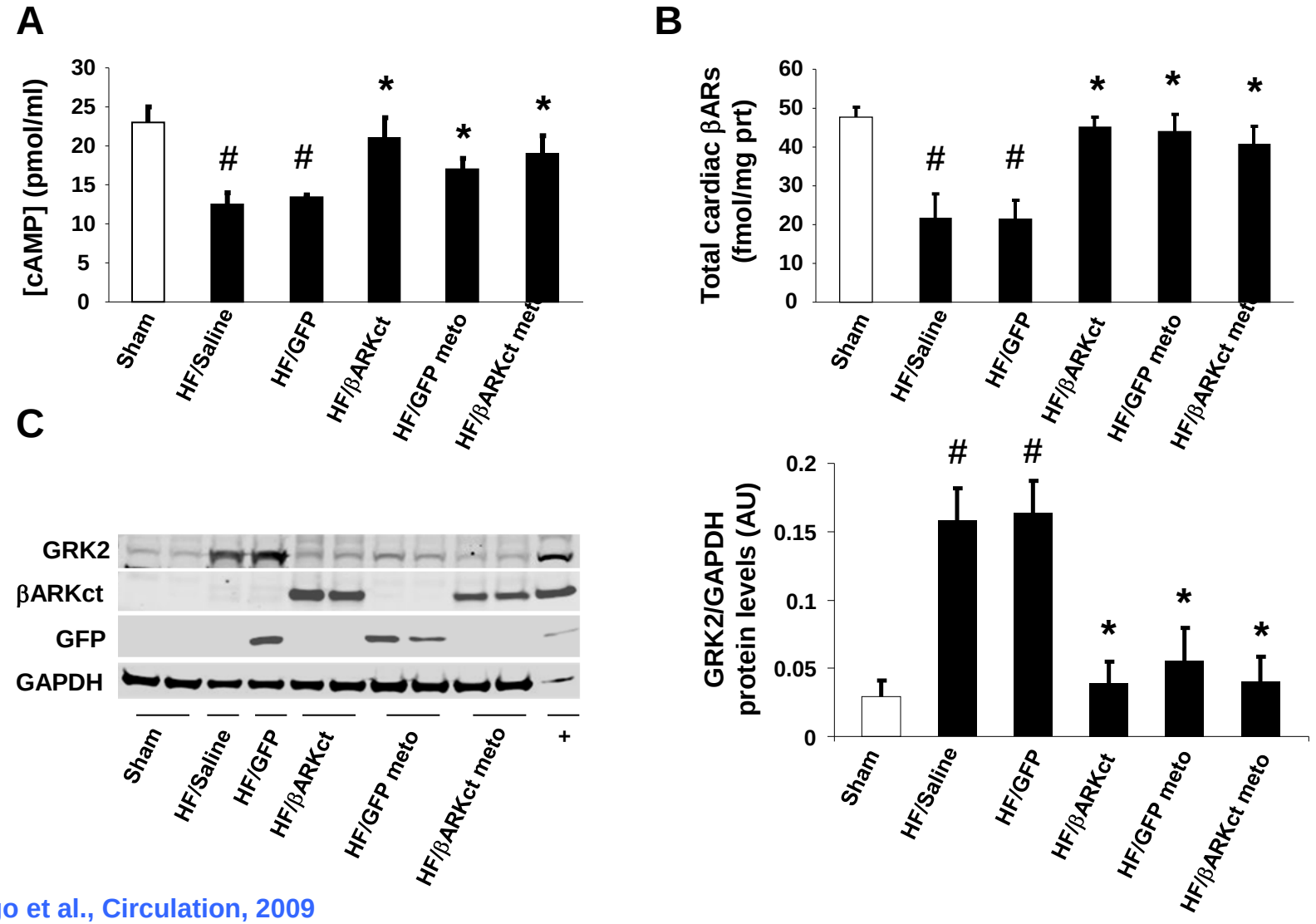
Raake et al., Circ Res 2008


β-Adrenergic Receptor System in Heart Failure
β1AR mRNA
β1AR
β2AR
P
P
Gs
AC
Plasma membrane
β2AR mRNA
Intracellular space
ATP
cAMP
βARK
βARK mRNA
Circulation Vol 87, No 2, Feb 1993

Jefferson

Center for Translational Medicine

Cardiac βAR Signaling Status

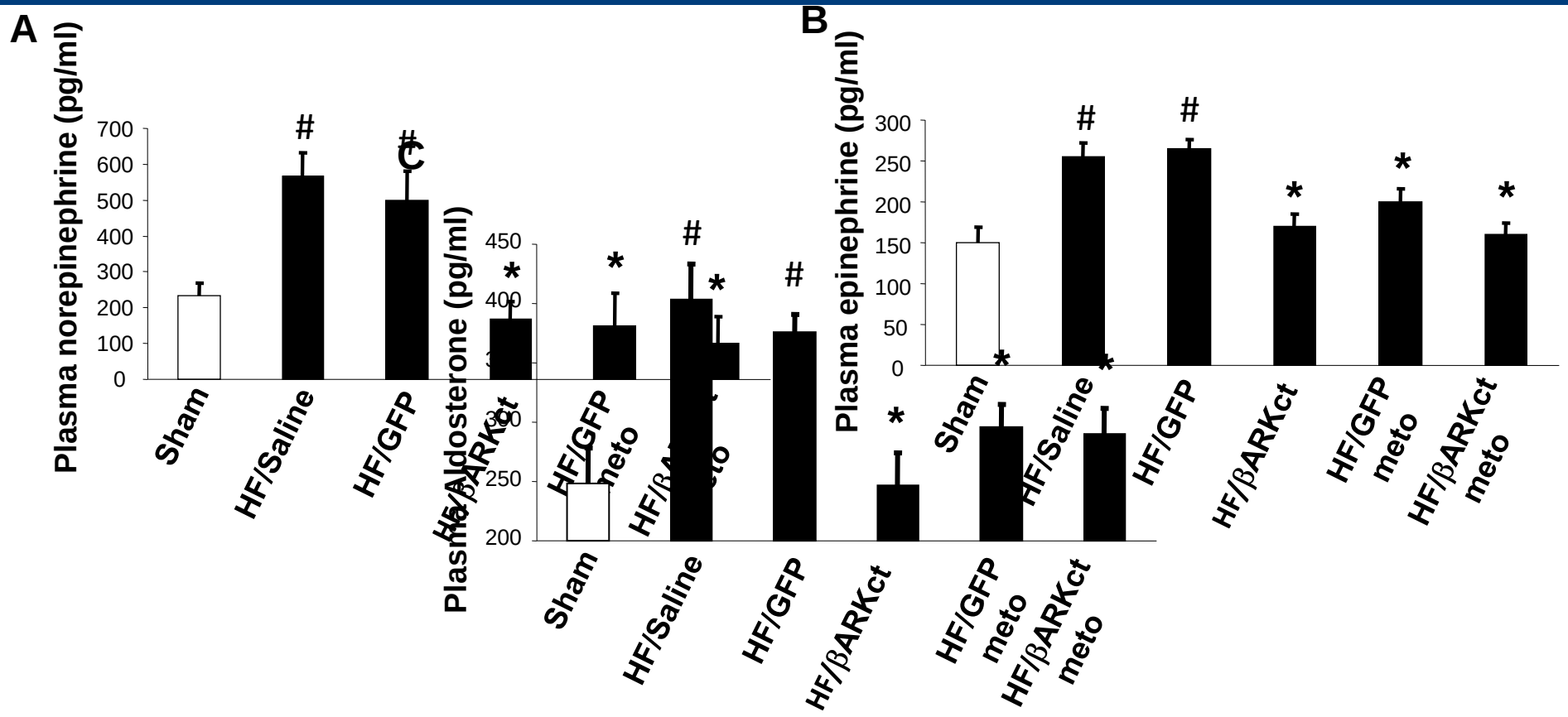


Rengo et al., Circulation, 2009

Jefferson

Center for Translational Medicine

Feedback to Quiet Neurohormonal System



Rengo et al., Circulation, 2009

nature medicine

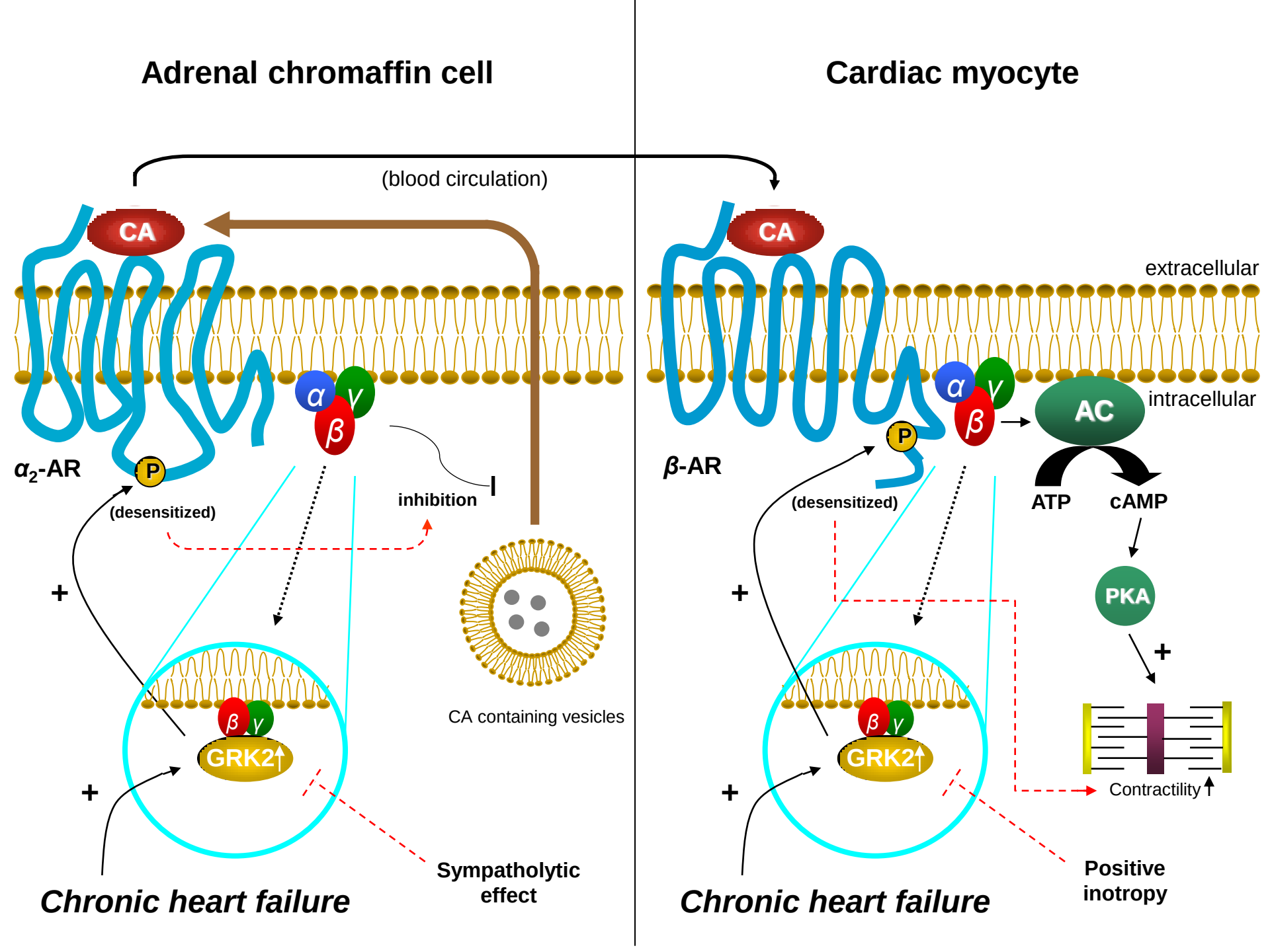
ARTICLES

Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure

Anastasios Lymperopoulos[1,2], Giuseppe Rengo[1,2], Hajime Funakoshi[1], Andrea D Eckhart[1,3] & Walter J Koch[1,2]

[1]Center for Translational Medicine, [2]George Zallie and Family Laboratory for Cardiovascular Gene Therapy and [3]Eugene Feiner Laboratory for Vascular Biology and Thrombosis, Department of Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA. Correspondence should be addressed to W.J.K. (walter.koch@jefferson.edu).

Received 22 September 2006; accepted 19 January 2007; published online 18 February 2007; doi:10.1038/nm1553


Adrenal chromaffin cell
Cardiac myocyte
(blood circulation)
CA
CA
extracellular
intracellular
α
β
γ
α
β
γ
AC
P
P
α2-AR
β-AR
(desensitized)
(desensitized)
inhibition
ATP
cAMP
PKA
+
+
+
+
GRK2↑
GRK2↑
CA containing vesicles
Contractility↑
Sympatholytic effect
Positive inotropy
Chronic heart failure
Chronic heart failure

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association®
Learn and Live℠

Effects of Sustained-Release Moxonidine, an Imidazoline Agonist, on Plasma Norepinephrine in Patients With Chronic Heart Failure

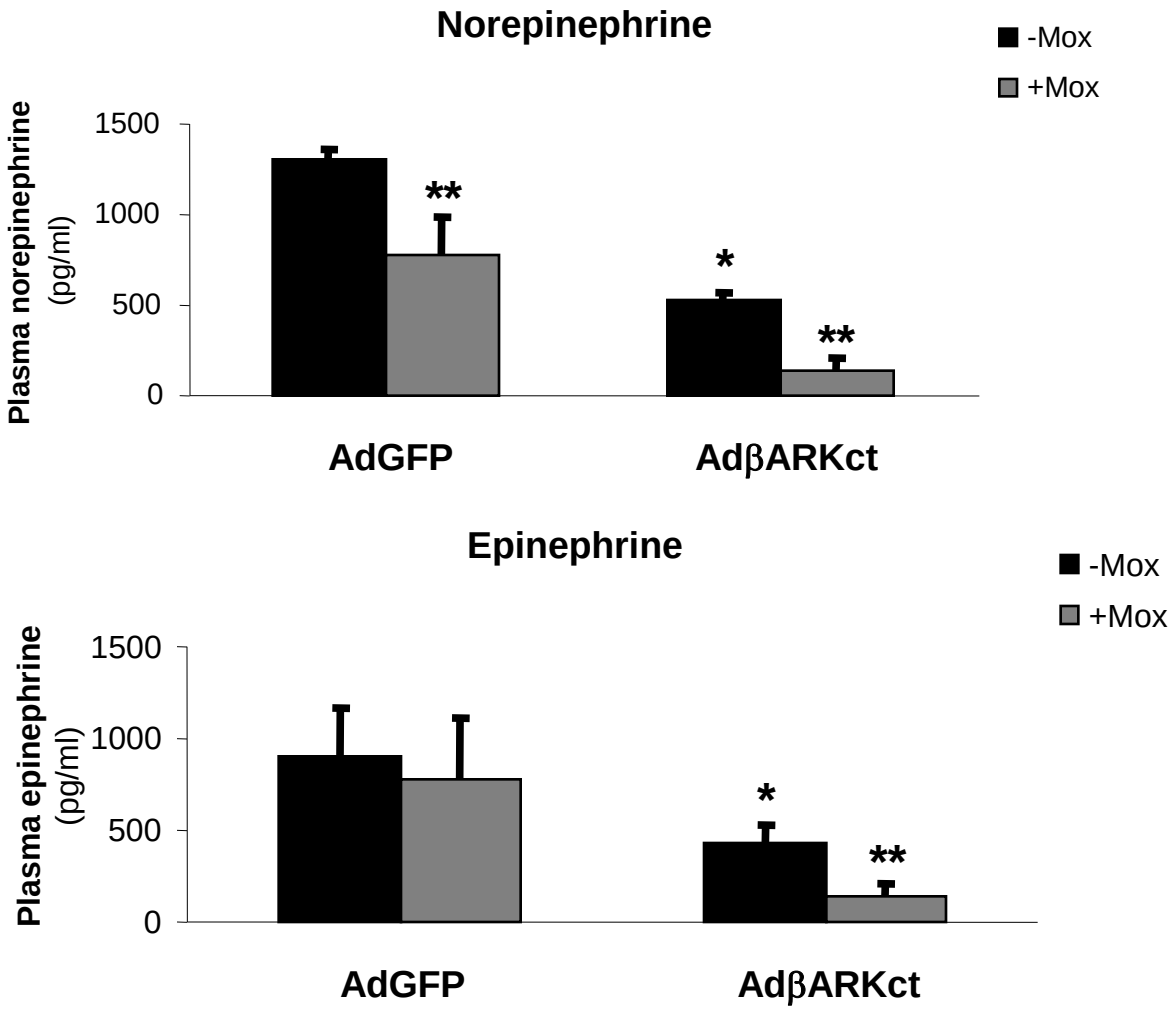
Karl Swedberg, MD; Michael R. Bristow, MD; Jay N. Cohn, MD; Henry Dargie, MD; Matthias Straub, MD; Curtis Wiltse, PhD; Theressa J. Wright, MD; for the Moxonidine Safety and Efficacy (MOXSE) Investigators

Background—In chronic heart failure, sympathetic activation is increased. Moxonidine acts on central nervous system receptors to decrease sympathetic activation. We investigated the dose-response relationship of a new sustained-release (SR) preparation of moxonidine and the plasma concentration of norepinephrine in patients with chronic heart failure.

Methods and Results—A total of 268 patients with chronic heart failure in NYHA functional class II to IV on optimal standard therapy were randomized to placebo or 1 of 5 doses of moxonidine SR: 0.3, 0.6, 0.9, 1.2, or 1.5 mg BID. After a dose-titration phase (7 weeks), patients were followed up for another 12 weeks at their maximally tolerated dose. Blood samples for plasma norepinephrine were collected at baseline and weekly during the initial 7 weeks, at week 19, and at the end of the study. At baseline and 7 and 19 weeks, sampling was also done 4 hours after the dose. After the active phases of the study, plasma norepinephrine was evaluated for an additional 3 days. A marked, statistically significant dose-related decrease in plasma norepinephrine was observed for predose levels as well as 4 hours after the dose at week 19. At the highest dose (1.5 mg BID), the trough reduction in norepinephrine was 52%. These reductions were accompanied by a modest decrease in heart rate, a modest increase in left ventricular ejection fraction, and a dose-related increase in adverse events.

Conclusions—Plasma norepinephrine was markedly reduced in a dose-related manner by moxonidine SR. This reduction was accompanied by evidence of reverse remodeling, but also by an increase in adverse events. **(*Circulation*. 2002;105: 1797-1803.)**

Key Words: heart failure ■ norepinephrine ■ nervous system, sympathetic ■ drugs



Lymperopoulos and Koch, Nature Medicine, 2007

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 285, NO. 21, pp. 16378–16386, May 21, 2010

Reduction of Sympathetic Activity via Adrenal-targeted GRK2 Gene Deletion Attenuates Heart Failure Progression and Improves Cardiac Function after Myocardial Infarction*

Received for publication, October 21, 2009, and in revised form, March 8, 2010 Published, JBC Papers in Press, March 29, 2010, DOI 10.1074/jbc.M109.077859

Anastasios Lymperopoulos[‡§1], **Giuseppe Rengo**[§2], **Erhe Gao**[§], **Steven N. Ebert**[¶], **Gerald W. Dorn II**[‖], **and Walter J. Koch**[§]

From the [‡]*Department of Pharmaceutical Sciences, Nova Southeastern University College of Pharmacy, Ft. Lauderdale, Florida 33328, the* [§]*Center for Translational Medicine and the George Zallie and Family Laboratory for Cardiovascular Gene Therapy, Department of Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, the* [¶]*Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, Florida 32827, and the* [‖]*Center for Pharmacogenomics, Department of Internal Medicine, Washington University, St. Louis, Missouri 63110*

Jefferson

Center for Translational Medicine

GRK2 Inhibition and KO in Chronic HF Models

Chronic βARKct expression or lowering of expression improves LV function and morphology in rats and mice with HF

Cardiac GRK2 inhibition in HF causes neurohormonal feedback to decrease SNS activity and lowering of aldosterone - indirect effect due to improved contractile function and responsiveness

Inhibition of GRK2 represents a potential new drug class, targeting βAR and other GPCR systems from "the inside out"

Data indicates that βARKct action in HF is primarily via GRK2 inhibition since KO phenotype is similar – still a role for Gβγ vs GRK2

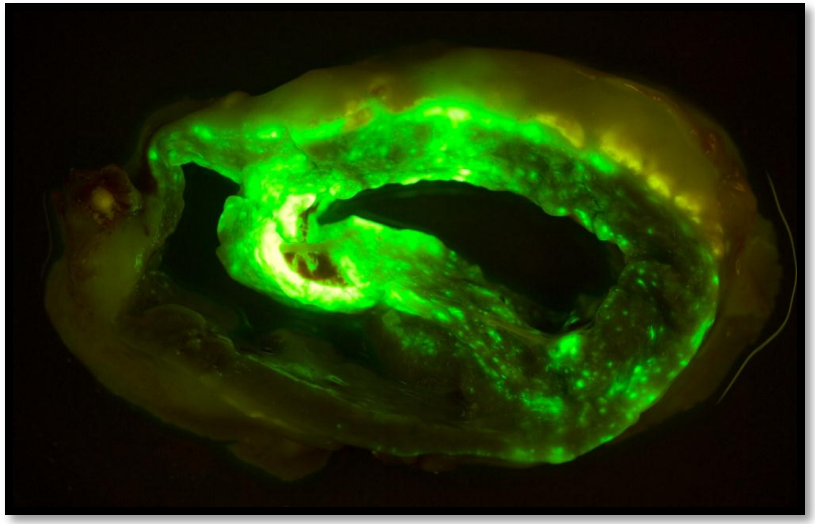
Jefferson

Center for Translational Medicine

Final Translation

- Clinical observation
- Therapeutic Design
- *Ex vivo* testing
- Transgenics
- Small animal models
- **Large animal models**
- **Clinical trials**

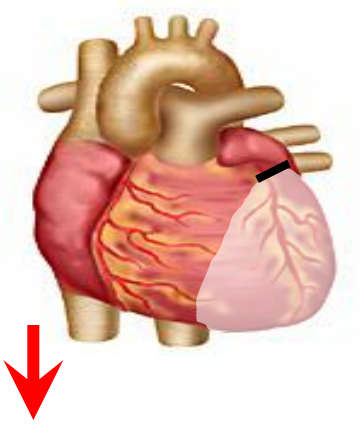
Sheep and pig models of intracoronary gene delivery and HF rescue ongoing in Germany and Philadelphia. – Pre-Clinical efficacy finished in pigs (Raake et al – Monday Oral Presentation) and TOX studies being started.



Jefferson

Center for Translational Medicine

Role of GRK2 in Acute Ischemic Injury



LAD occlusion

Experimental outcomes:

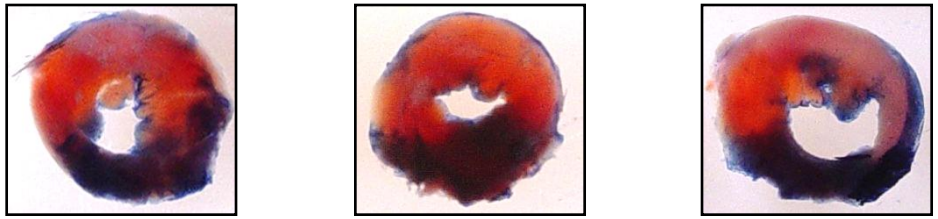
1) Western Blotting
2) Apoptosis (TUNEL, Caspases)
3) Cardiac function (Echo/Hemo)
4) Infarct size (TTC)

Brinks et al, *Circ Res*, 2010

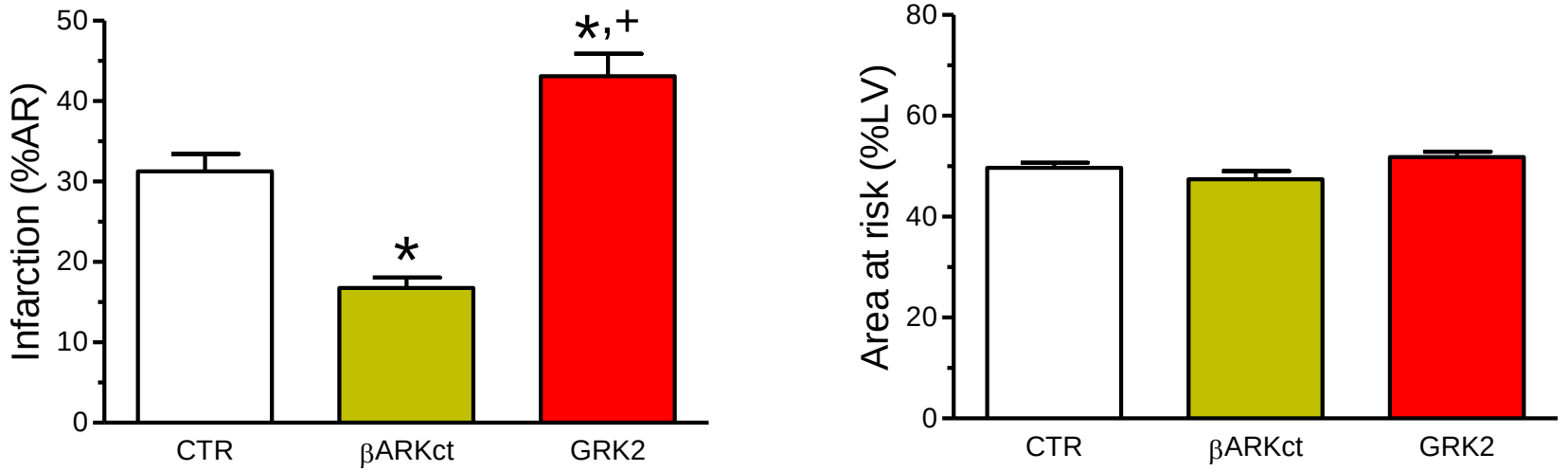
Jefferson

Center for Translational Medicine

GRK2 and βARKct Mice after I/R Injury



CTR βARKct GRK2

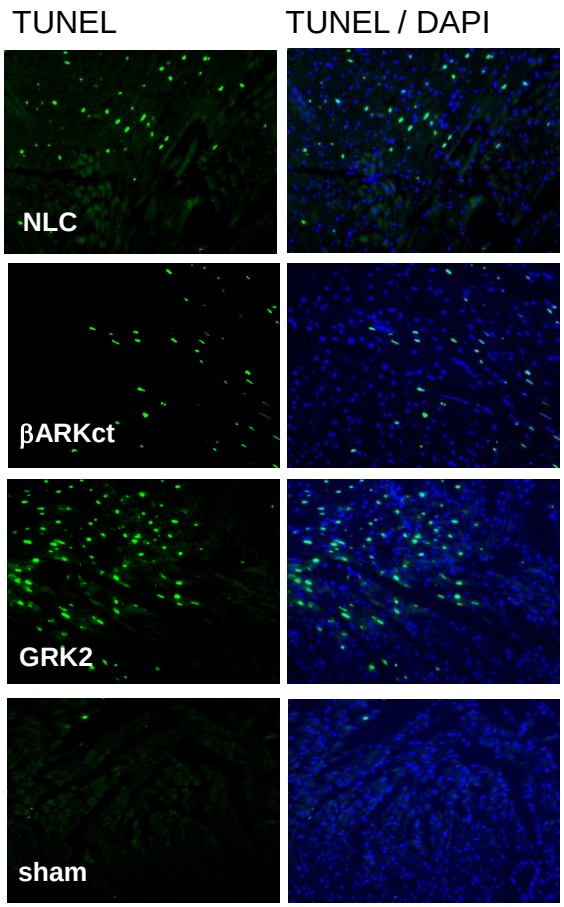


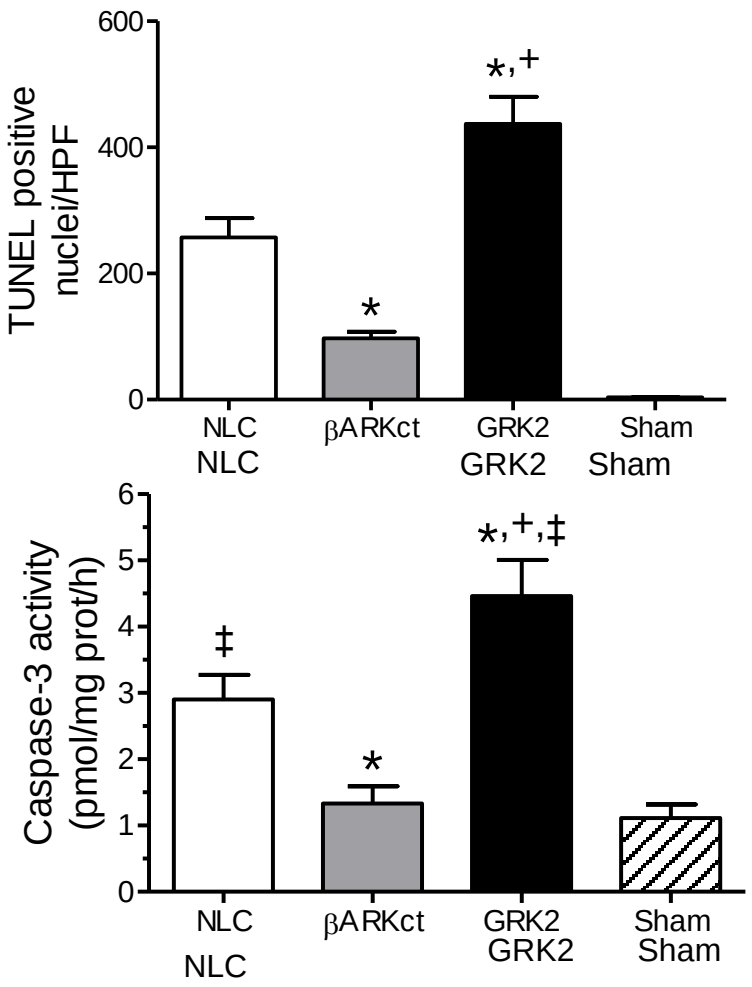
n=13-25/group; *p<0.05 vs. NLC, +p<0.05 vs. βARKct; NLC

Jefferson

Center for Translational Medicine

Apoptosis after I/R Injury in GRK2 and βARKct Mice

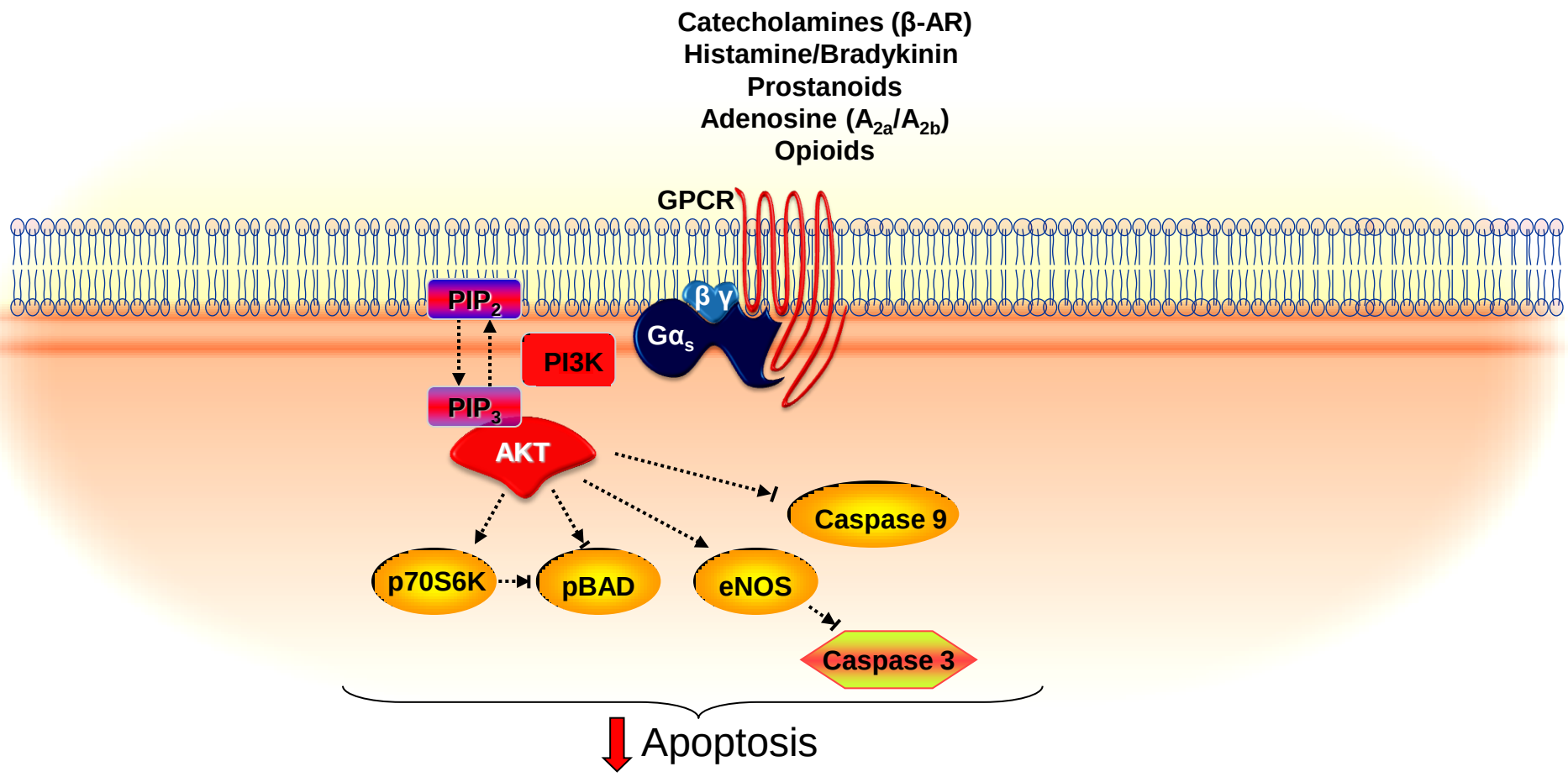




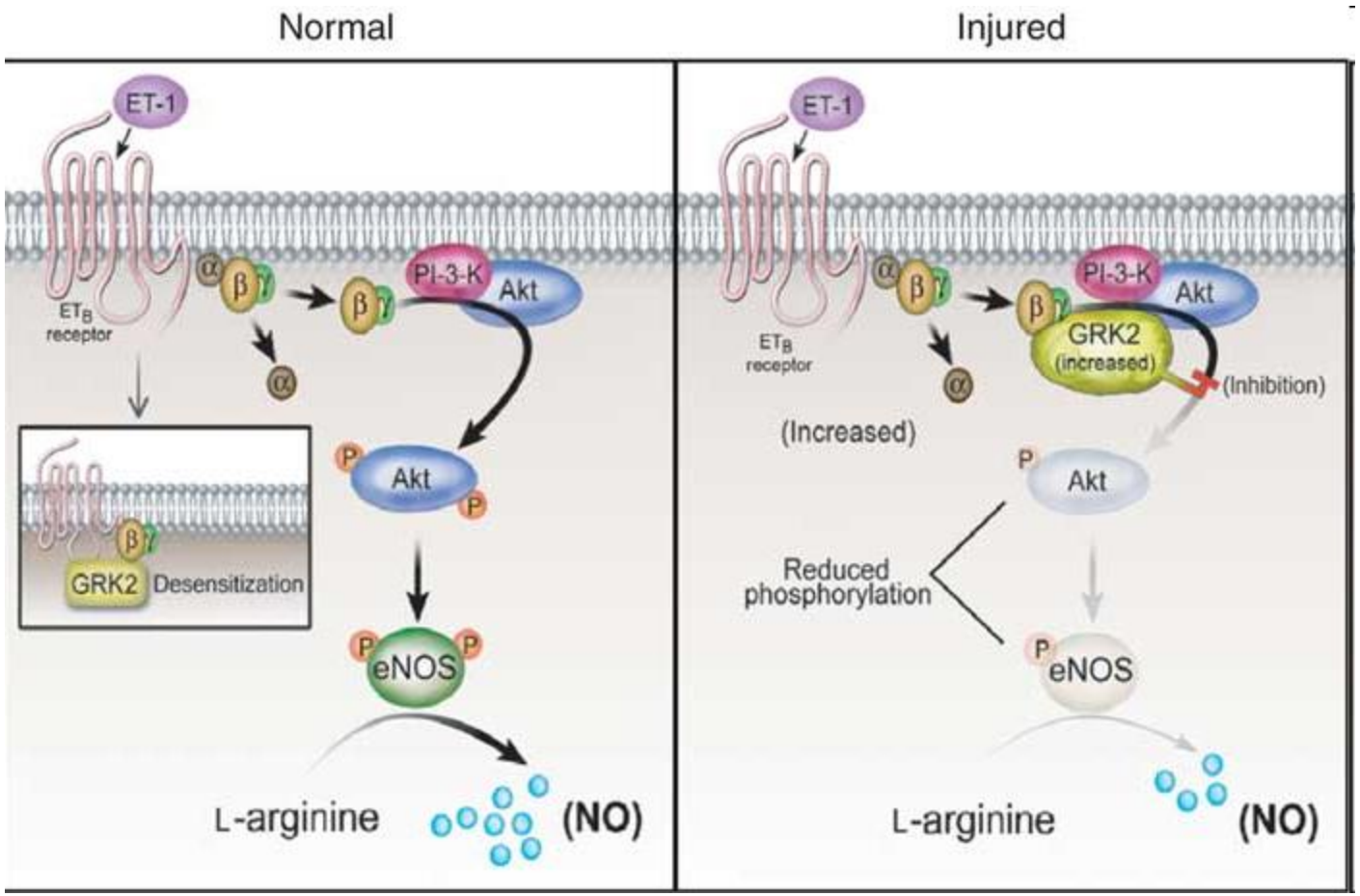
Jefferson

Center for Translational Medicine

Anti-apoptotic Signaling in I/R Injury



GRK2 and Akt

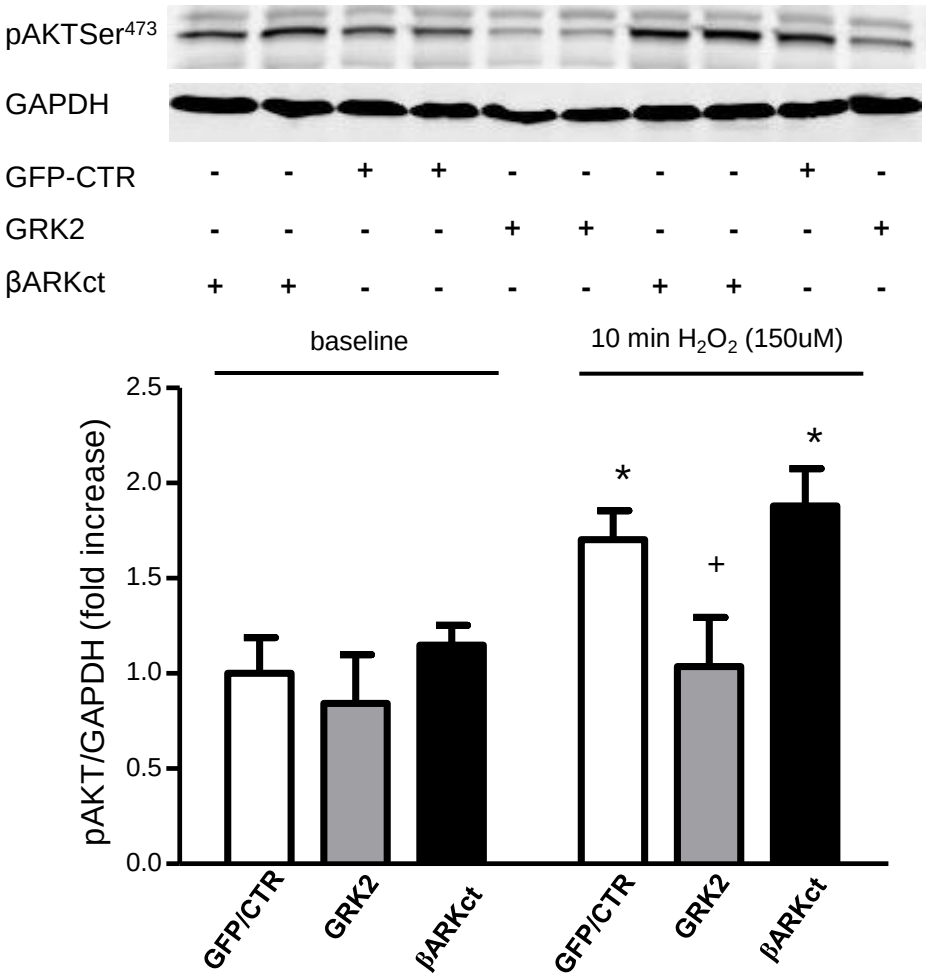


Liu et al., Nat Med 2005

Jefferson

Center for Translational Medicine

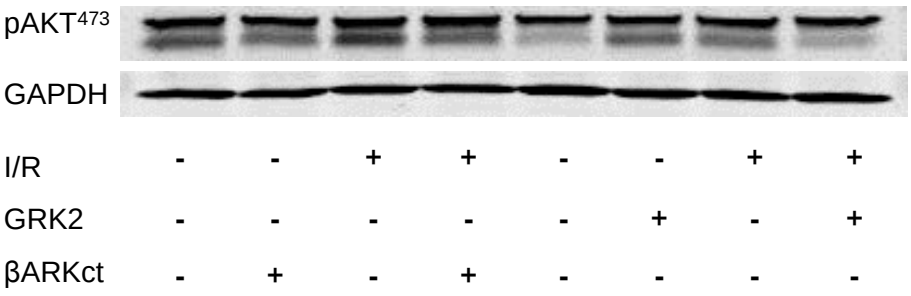
Anti-apoptotic Signaling Promoted by βARKct In Vitro

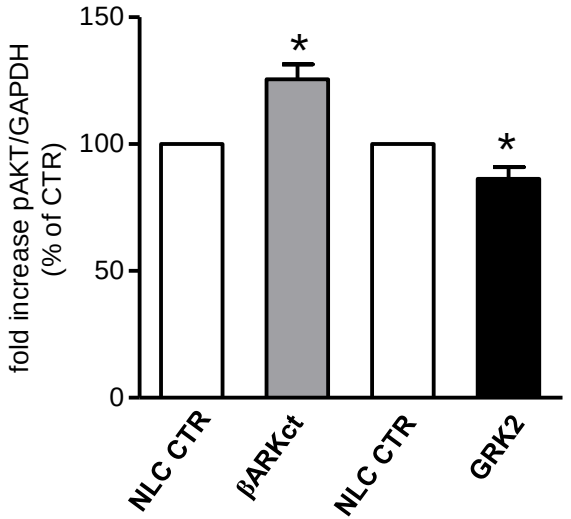


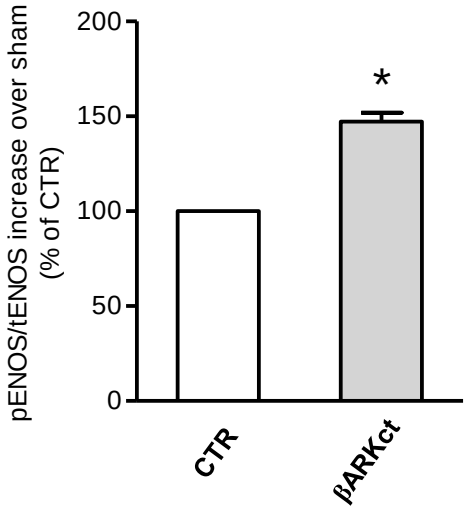
Jefferson™

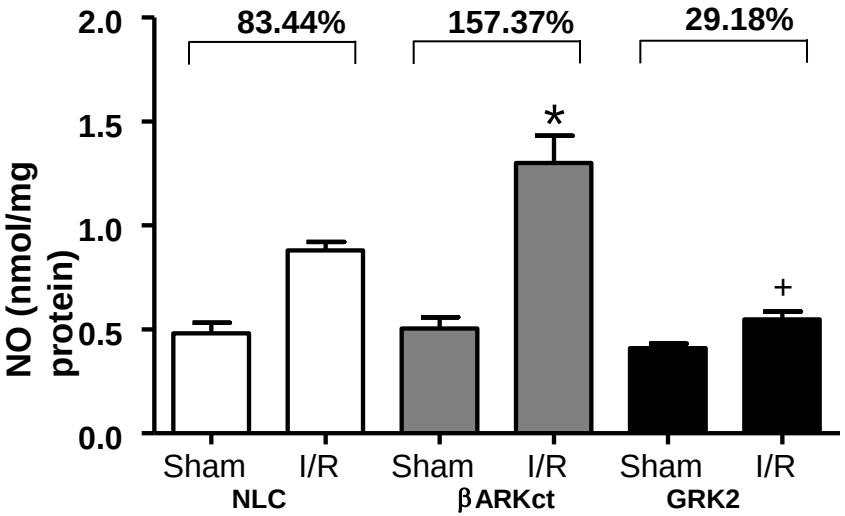
Center for Translational Medicine

In Vivo Akt-eNOS Activation in GRK2 and βARKct Mice Post-I/R









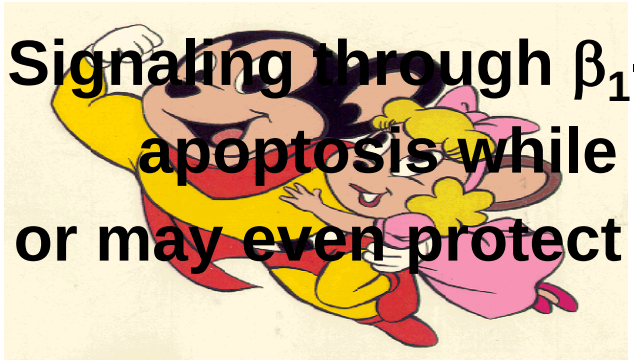
Jefferson

Center for Translational Medicine

Differential βAR Sub-type Signaling in Transgenic Mice

β_1-AR →

β_2-AR →



Signaling through β_1-ARs appears to trigger cardiomyocyte apoptosis while β_2-AR signaling does not promote, or may even protect against β_1-AR-mediated myocyte death

Low Overexpression

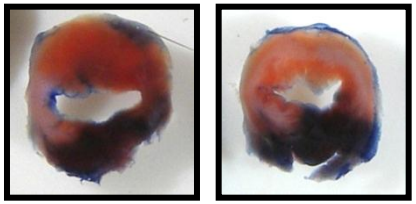
High Overexpression

Jefferson

Center for Translational Medicine

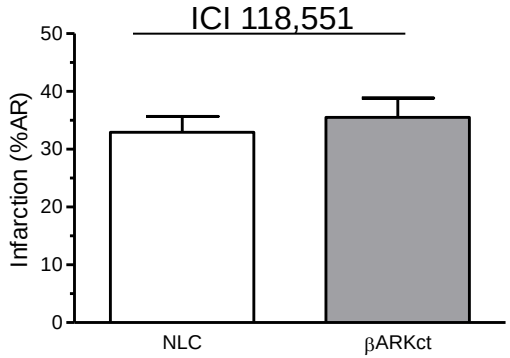
β_2AR Antagonism Negates βARKct-Mediated Cardioprotection

A

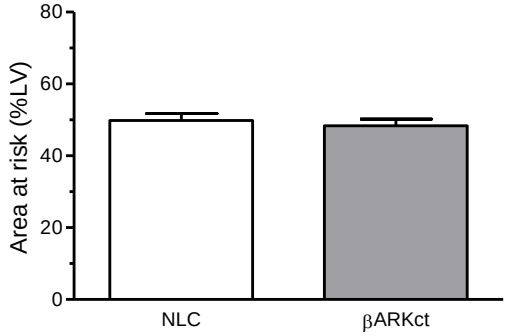


NLC βARKct

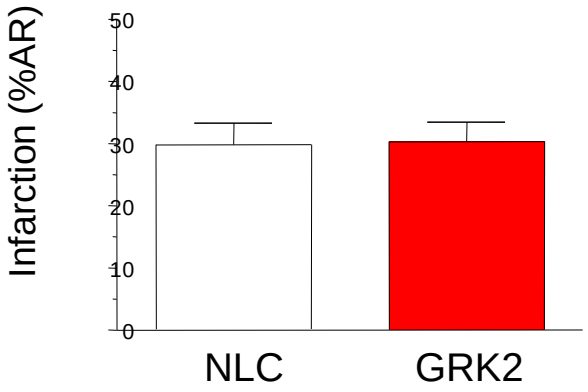
B



C



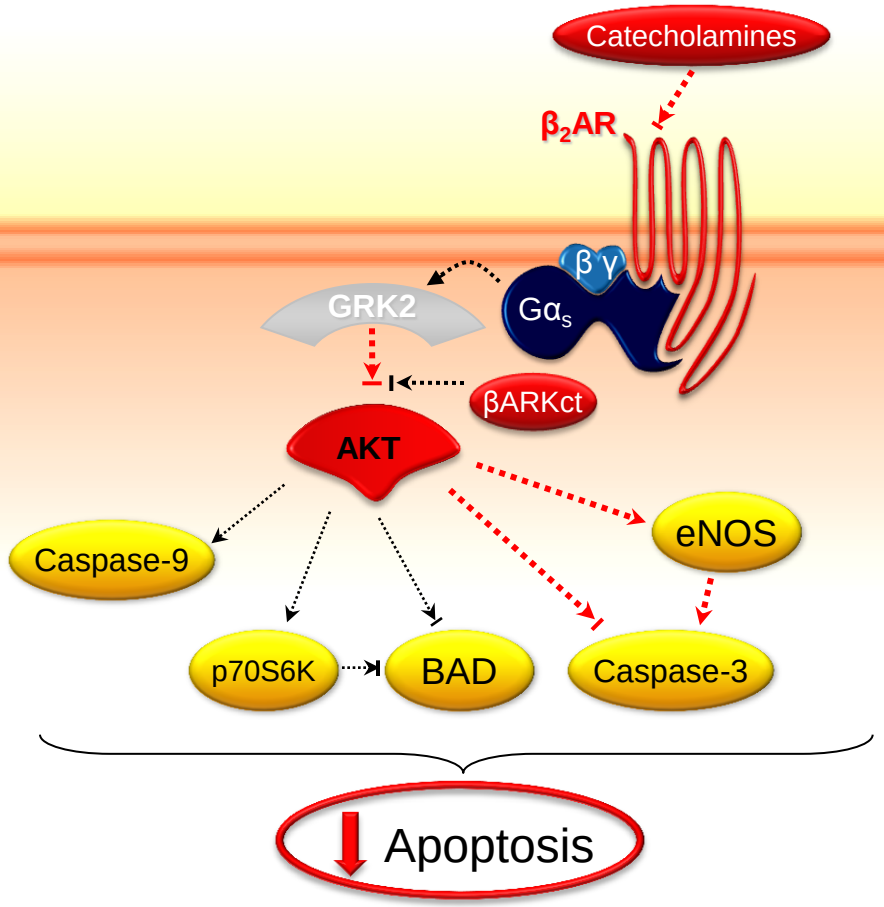
Fenoterol



Jefferson

Center for Translational Medicine

GRK2 and Acute I/R injury

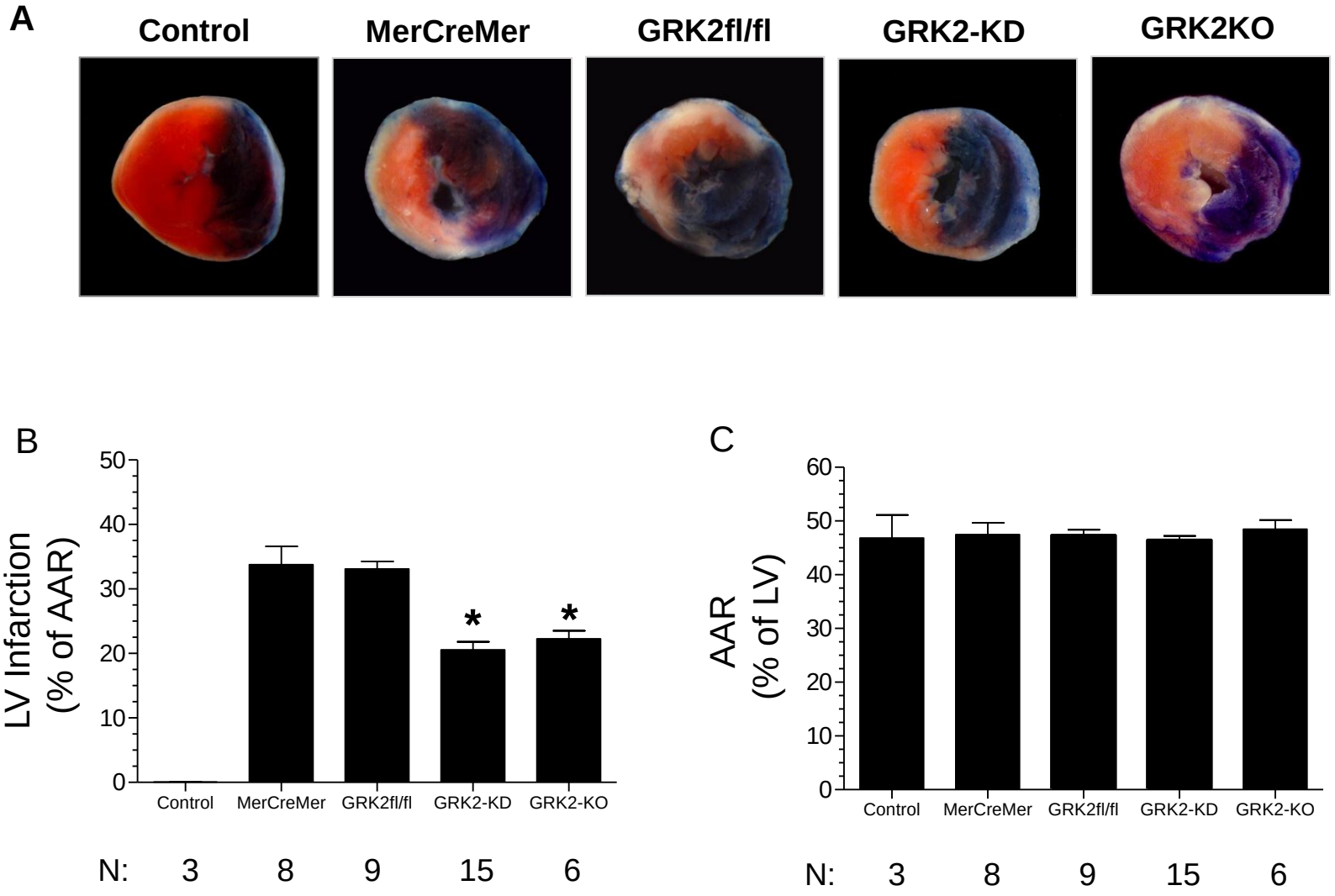


Brinks et al, *Circ Res*, 2010

Jefferson

Center for Translational Medicine

Cardiac GRK2 KO Mice and I/R



Gao and Koch, Unpublished – Wed Afternoon - Oral

Final Thoughts

GRK2 activity promotes myocyte cell death in response to ischemic and oxidative stress. Appears to be pro-apoptotic through inhibition of Akt.

GRK2 up-regulation acutely after myocardial injury must not be adaptive as it promotes injury and acute myocardial dysfunction.

GRK2 inhibition with βARKct (as well as GRK2 KO) protects ischemic myocardium with enhanced survival signaling through Akt-NOS-NO. This appears to occur at least partially through enhanced β2AR signaling.

GRK2 inhibition offers benefits both acutely and chronically after ischemic injury promoting improved cardiac survival and function – thus, targeting GRK2 appears to be novel therapy for HF progression.

Jefferson

Center for Translational Medicine

ACKNOWLEDGMENTS

Collaborators

Andrea Eckhart – Jefferson
Joe Rabinowitz – Jefferson
Tom Force – Jefferson
Art Feldman – Jefferson
Erhe Gao – Jefferson

Tassos Lymperopoulos – Nova Southeastern

Patrick Most – Heidelberg

Gerald Dorn – Wash U

Steve Houser – Temple

Koch Lab

Kurt Chuprun
Anna Gumpert
Jessica Gold
Jeff Martini
Brent DeGeorge
Philip Raake
Anna Gumpert
Mai Chen
Yetta Brinks
Nick Otis
Jonathan Hullman
Lin Zuo
Zuping Qu
Ben Woodall
Maggie Huang
Giuseppe Rengo
Meryl Castellini
Michele Ciccarelli
Xiying Shang
Kazi Islam
Matthieu Boucher
Sven Pleger