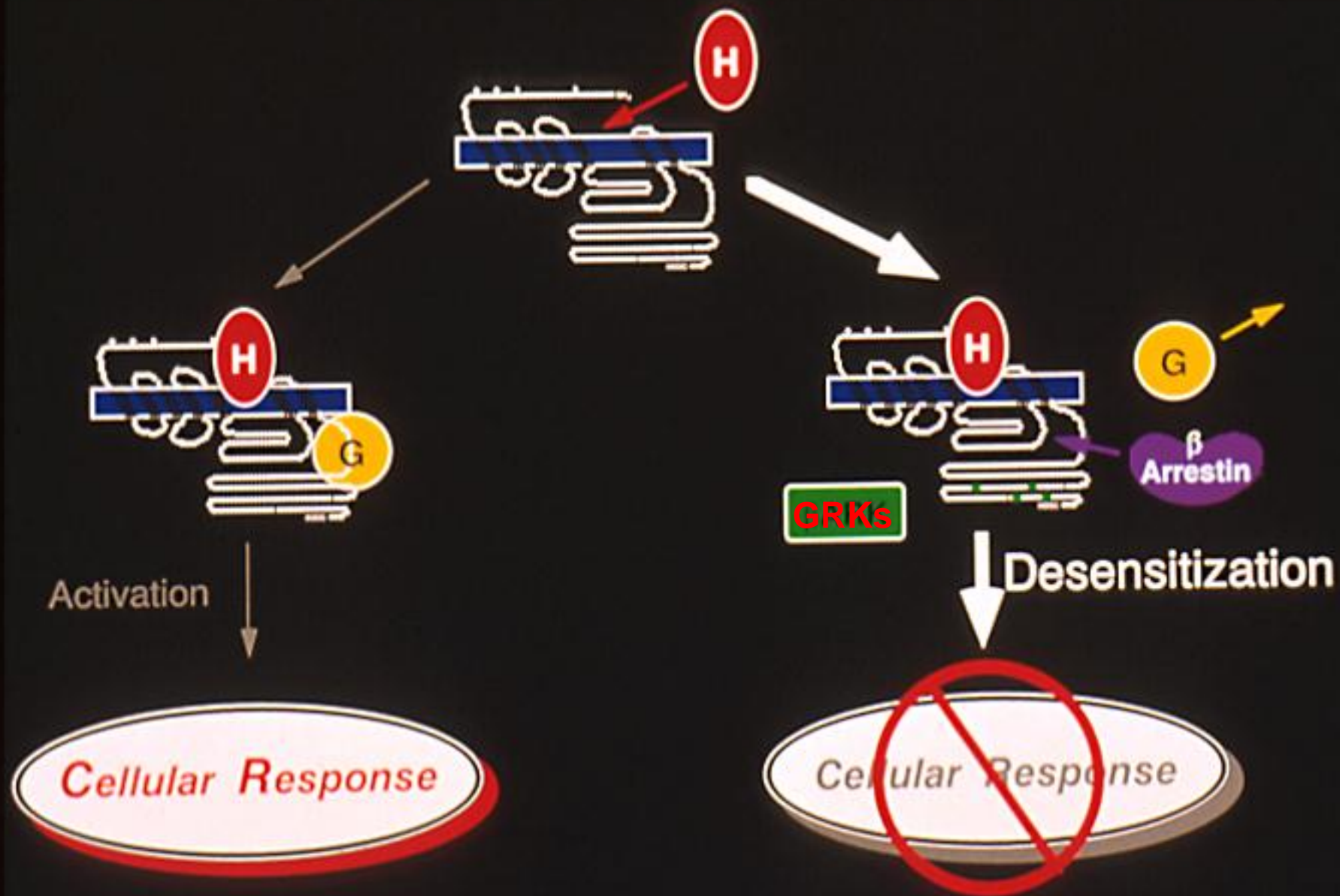


GRK2 Inhibition as a Treatment for Heart Failure: Moving from Bench to Bedside

Wally Koch, PhD.

W.W. Smith Professor of Medicine

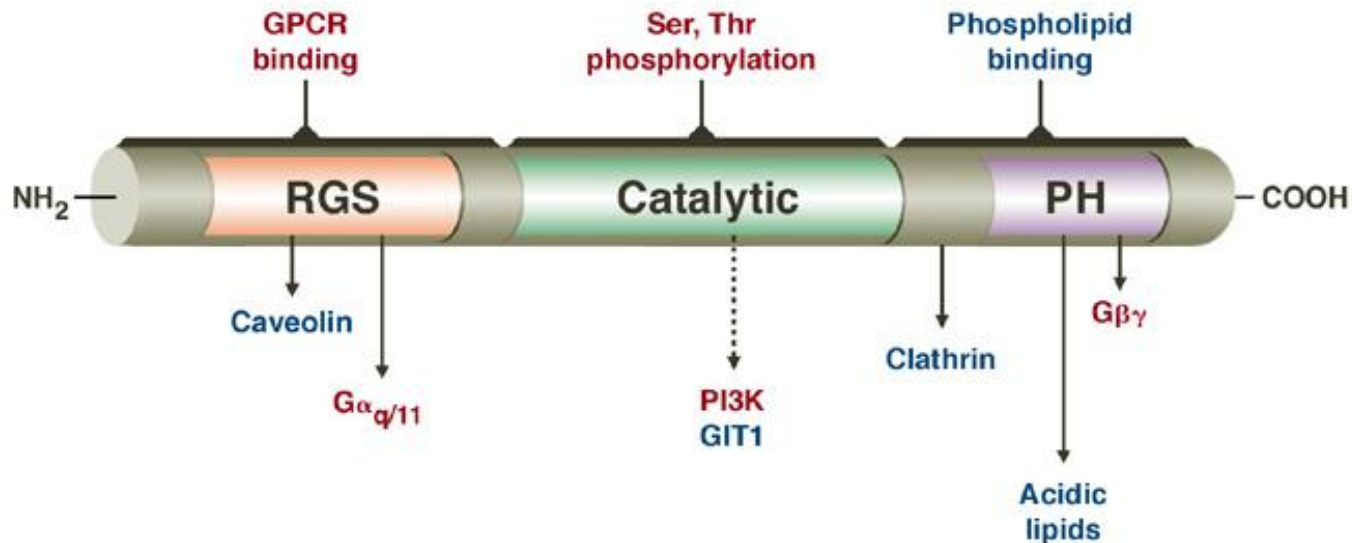
November 13, 2010



G Protein-Coupled Receptor Kinases (GRKs)

Serine/ Threonine Kinases

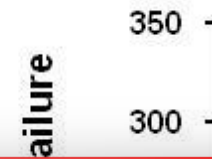
3 classes: GRK1 (Rhodopsin Kinase), GRK7
GRK2 (βARK1), GRK3 (βARK2)
 GRK4, **GRK5**, GRK6



Heart Failure Therapeutics

Translational Medicine Drug Approach to Advance HF Therapeutics

Drug Class	Examples	Discovery
ACE Inhibitors	Captopril, Lisinopril, Enalapril	1979



Clinical Finding

Therapeutic Design

Ex vivo Testing

Transgenic Models

Small Animal Models

Large Animal Models

Clinical Trials

Aldosterone Antagonists

Spironolactone, Eplerenone

Vasodilators

Hydralazine, Isosorbide

Alterations of β -Adrenergic Receptor Signal Transduction

Digitalis

Digoxin

Diuretics

Furosemide, HCTZ, Amiloride

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

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

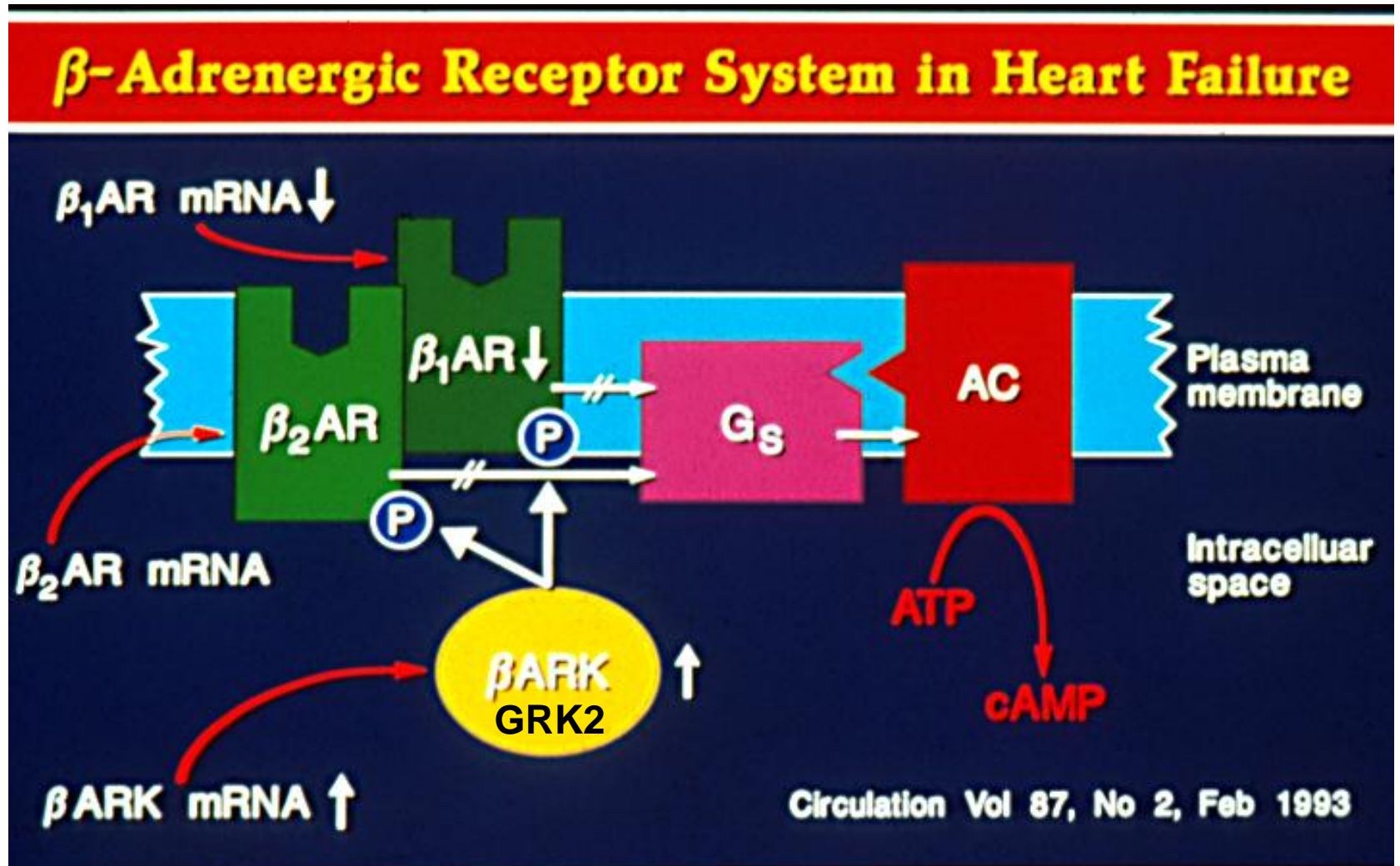
Molecule	Change
β 1-AR	↓, uncoupled
β 2-AR	NC, uncoupled
GRK2	↑ levels, activity
GRK3	NC
GRK5	↑ levels
Arrestin2	NC
Arrestin 3	NC
Gi	↑
Gs	NC

1956

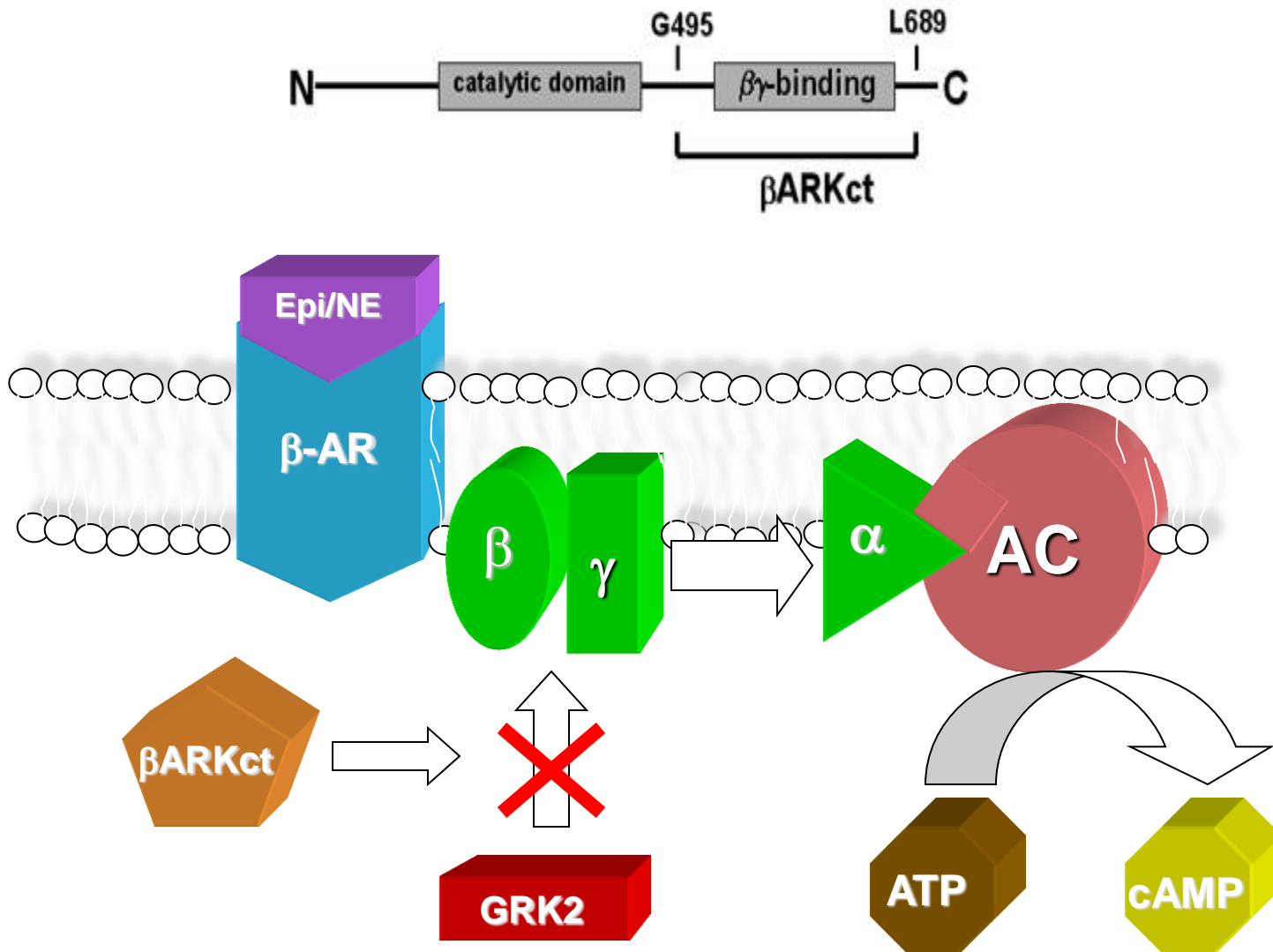
 18th C

 18th C

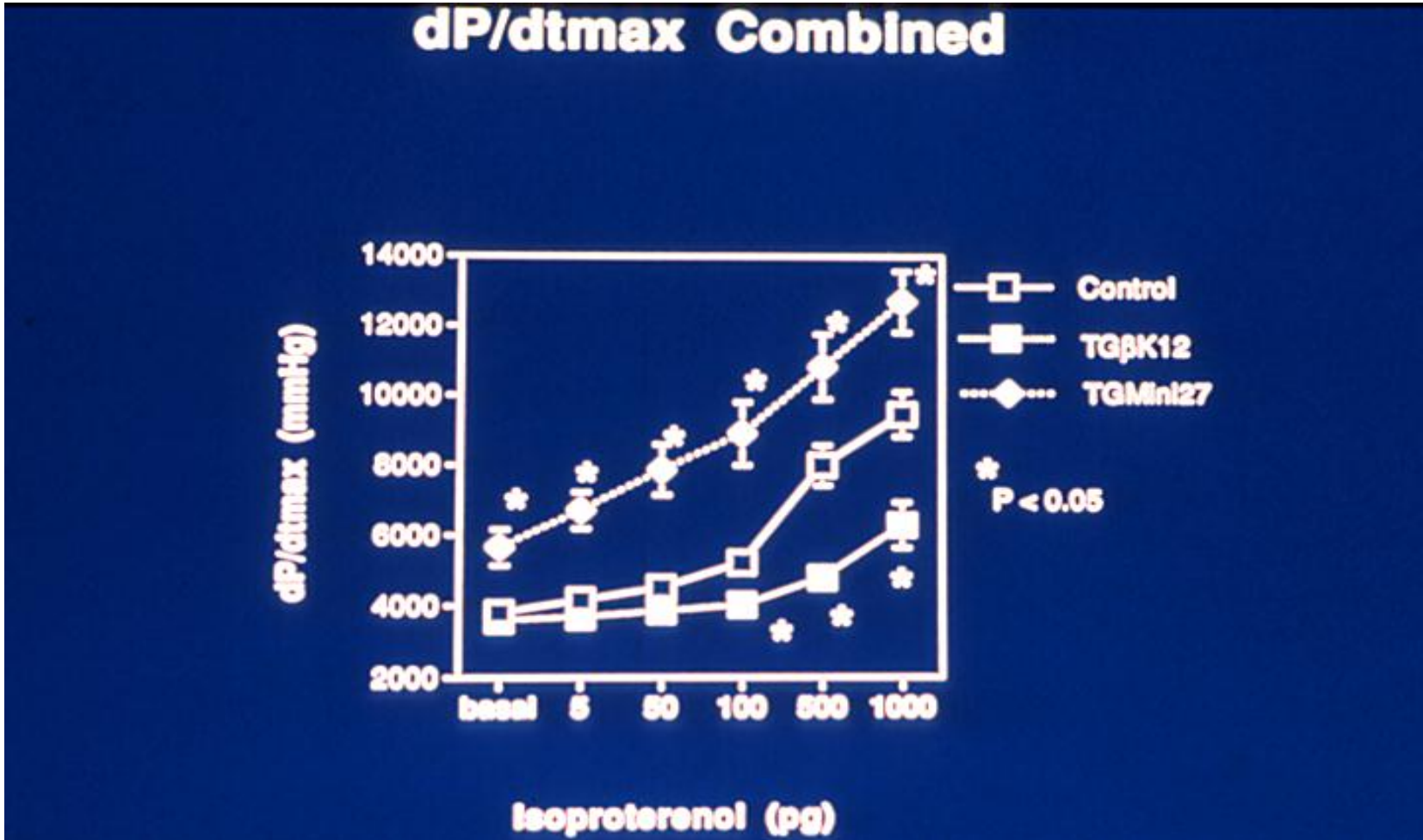
Young HFSA



The β ARKct as a GRK2 Inhibitor



GRK2 and β ARKct Transgenic Mice

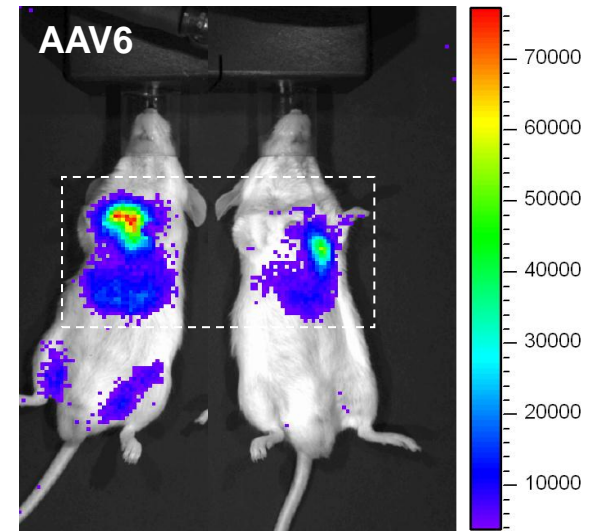
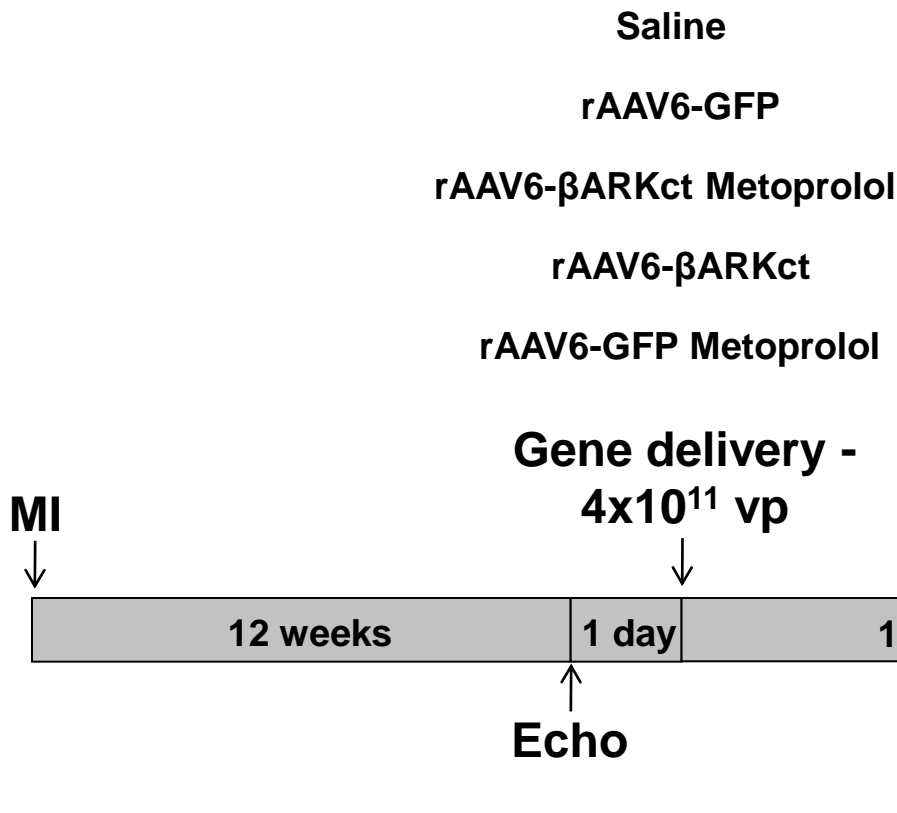


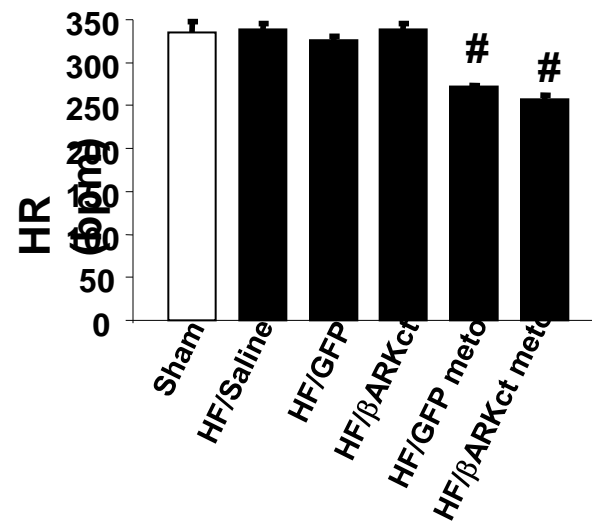
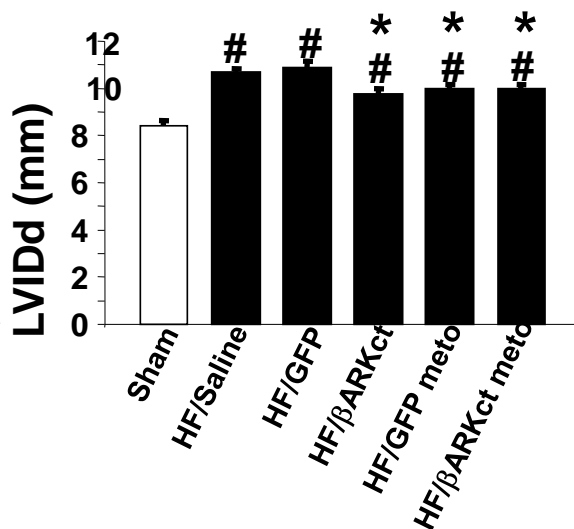
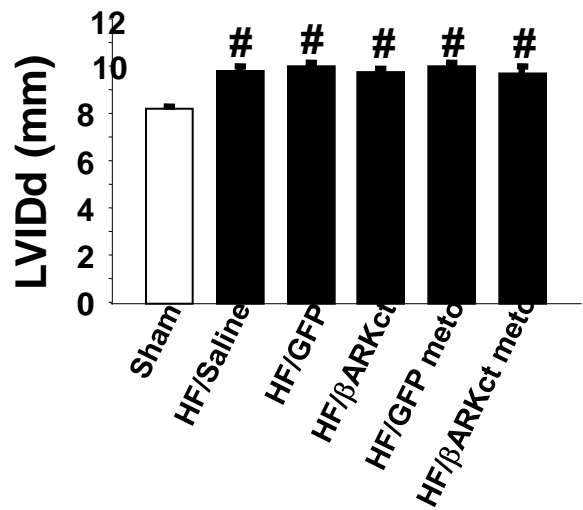
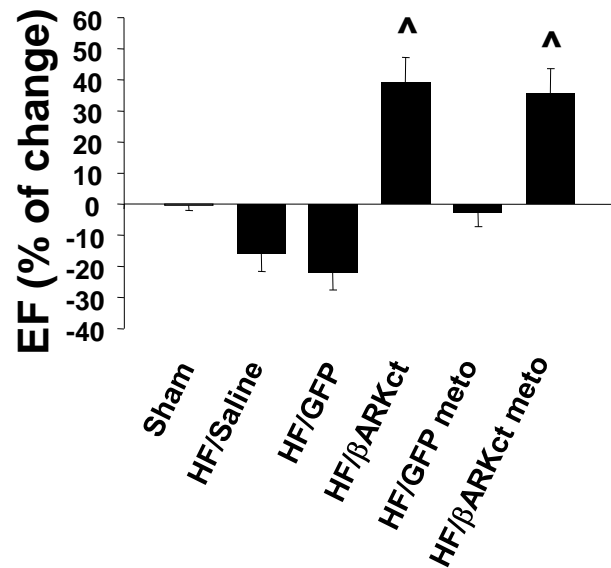
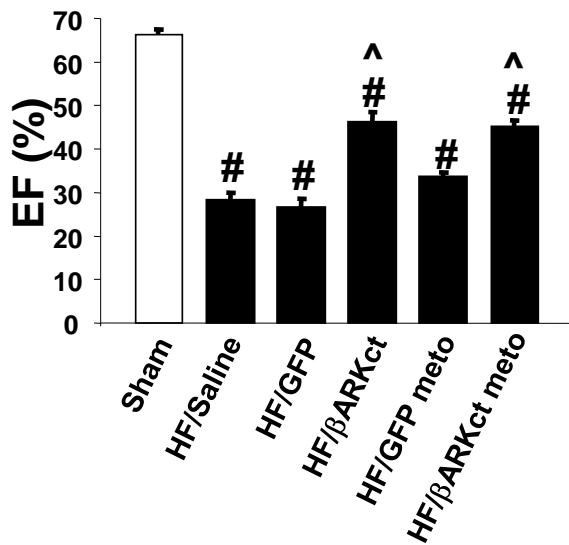
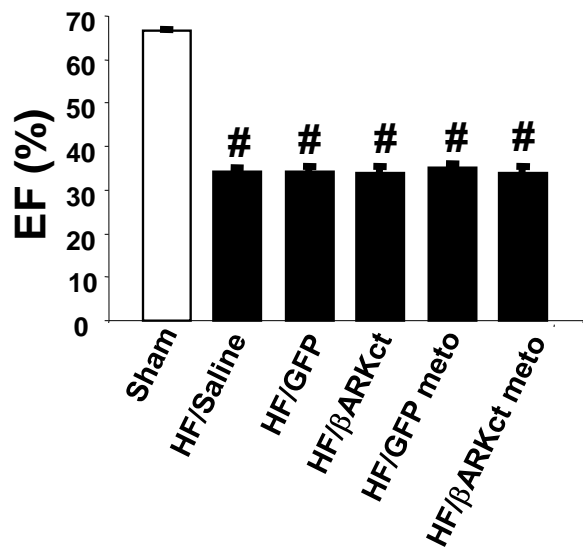
β ARKct rescues several different murine models of HF

Murine model	Result of β ARKct cross	Reference
<i>MLP</i> ^{-/-} Knockout	Complete functional rescue with restored β AR 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 β AR 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.

HF Rescue with β ARKct Gene Therapy in Rats





Potential Actions of the β ARKct as a $G\beta\gamma$ Sequestrant

Additional $G\beta\gamma$ – GRK2 Inhibitory Actions

Enhanced signaling through other GPCR's

Non-GPCR actions of GRK2 (i.e. cytoskeleton proteins)

MAP Kinase signaling ($G\beta\gamma$ -dependent = GRK2-dependent)

Novel GRK2 + $G\beta\gamma$ Binding Partner

$G\beta\gamma$ - PI3-Kinase

Activity is inhibited in β ARKct mice after TAC

Other $G\beta\gamma$ -Mediated Signaling

Doubtful

$G\beta\gamma$ - AC

Cardiac AC's (V and VI) not regulated by $G\beta\gamma$

$G\beta\gamma$ - PLC β

Gq-PLC activity not altered in β ARKct 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

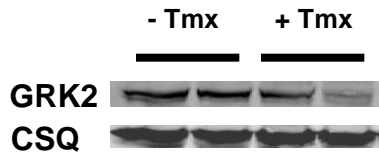
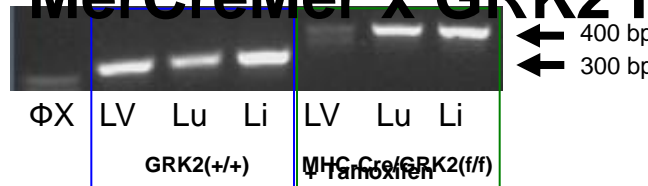
Communicated by Gordon G. Hammes, Duke University Medical Center, Durham, NC, August 26, 1996 (received for review July 10, 1996)

Conditional GRK2 Knockout Mouse

Matkovitch et al., Circ Res 2006

DNA (whole hearts)

MerCreMer x GRK2 Flox



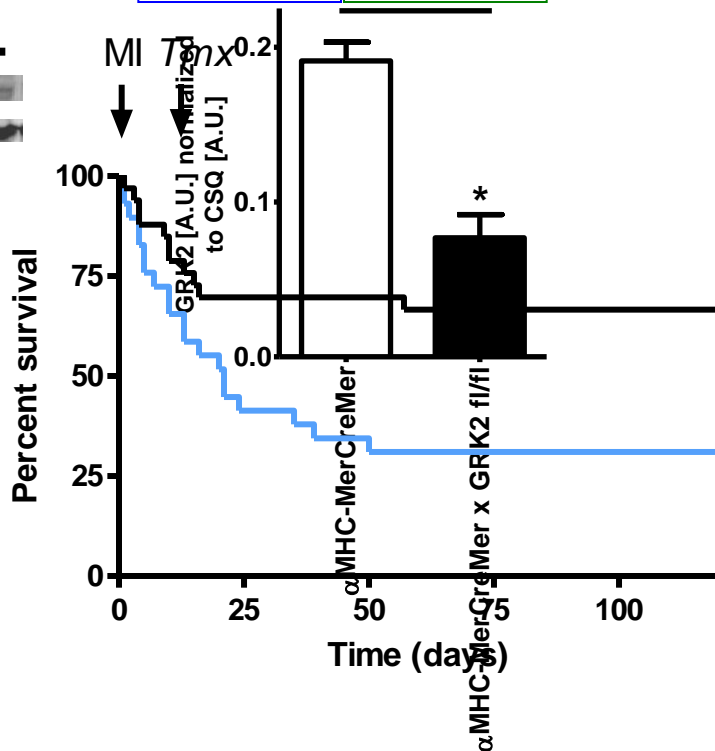
- Tmx + Tmx

GRK2

CSQ

αMHC-MerCreMer αMHC-MerCreMer x GRK2 fl/fl

αMHC-MerCreMer αMHC-MerCreMer x GRK2 fl/fl



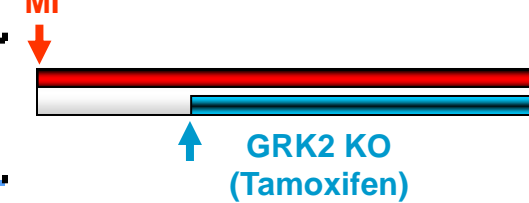
Rescue-study

• Model: Mouse myocardial infarction

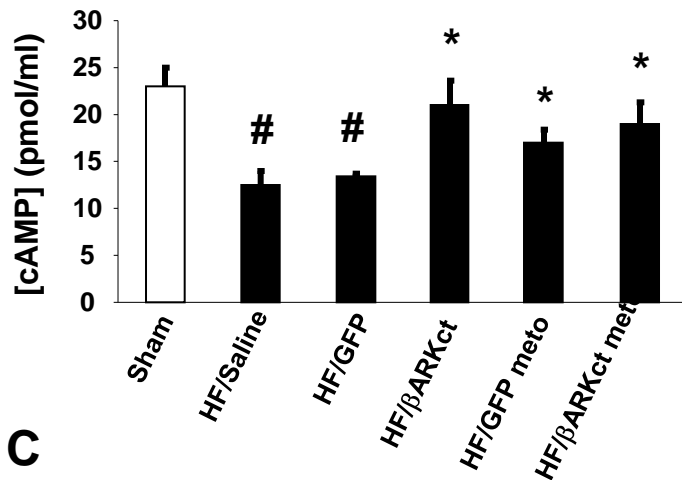
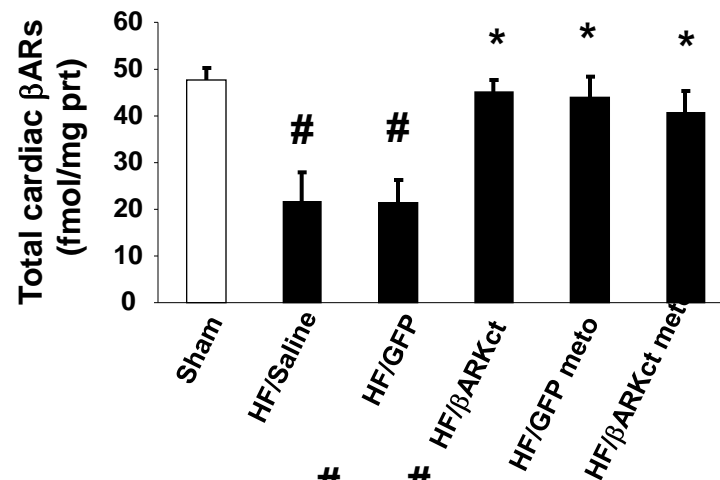
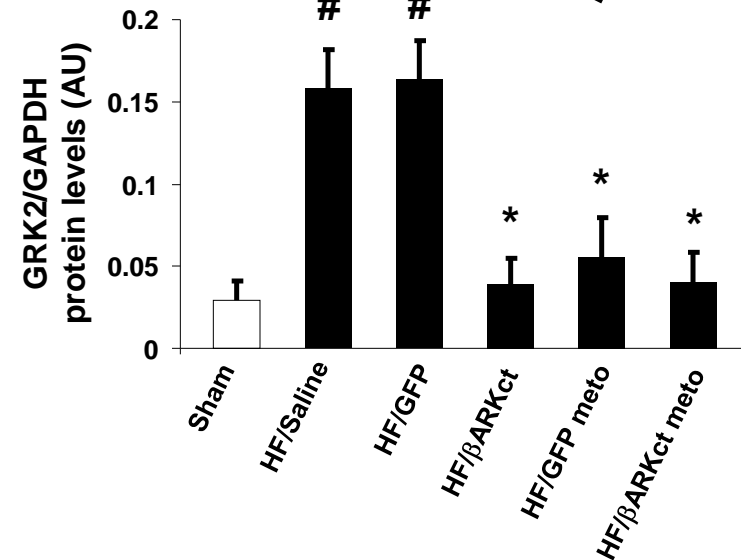
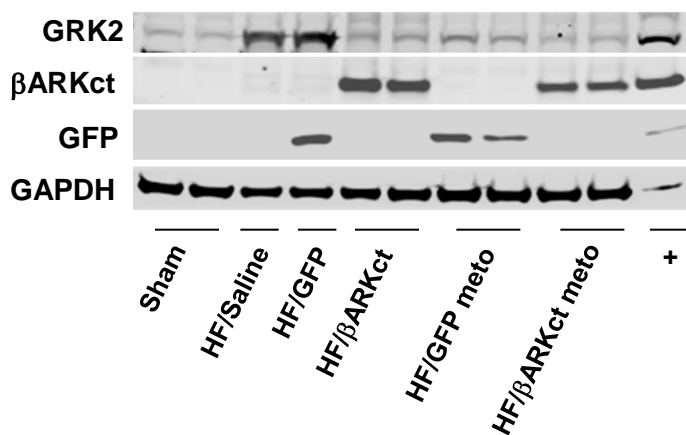
• Genetic line: αMHC-MerCreMer x GRK2 fl/fl

— MerCreMer (n=30)

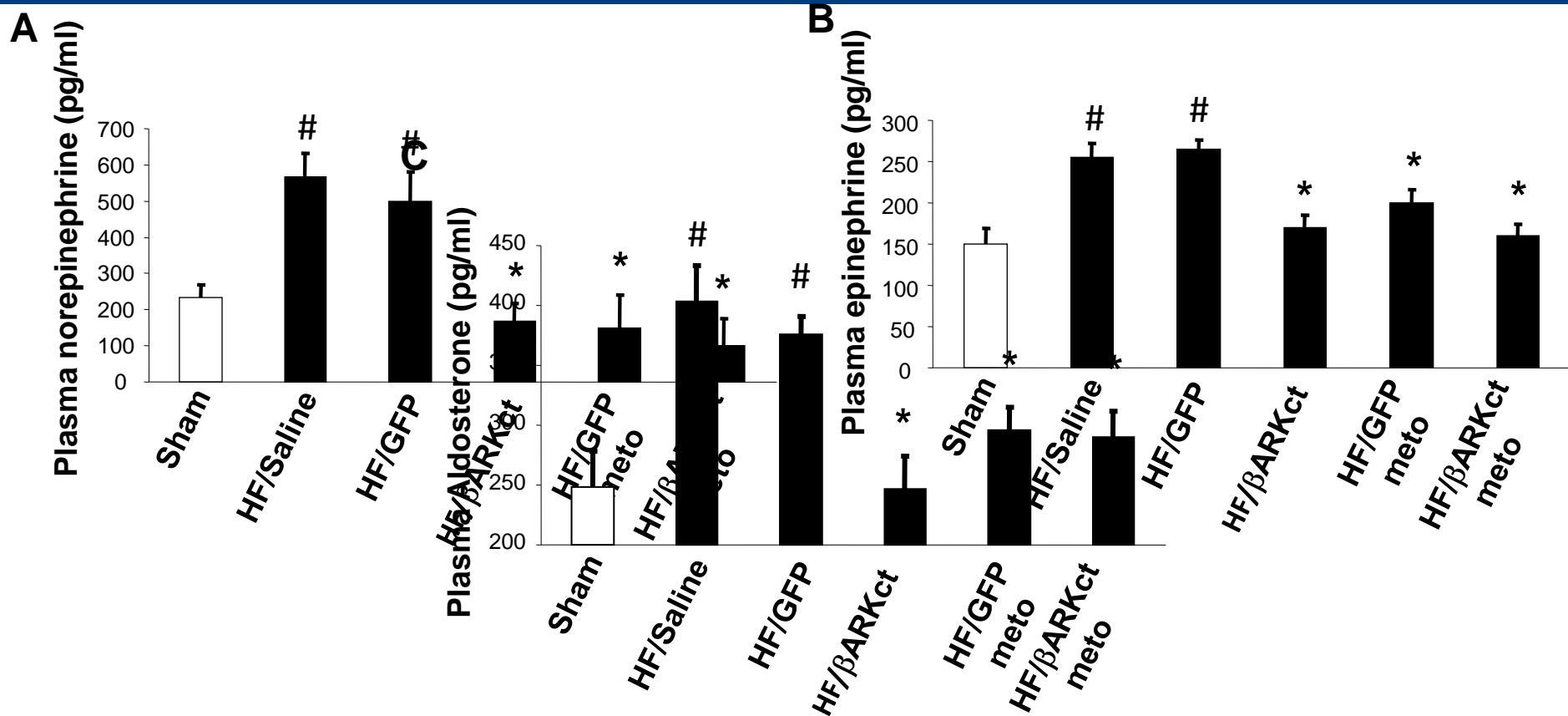
— MerCreMer/GRK2(f/f) (n=34)



Cardiac β AR Signaling Status

A

B

C


Feedback to Quiet Neurohormonal System



Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure

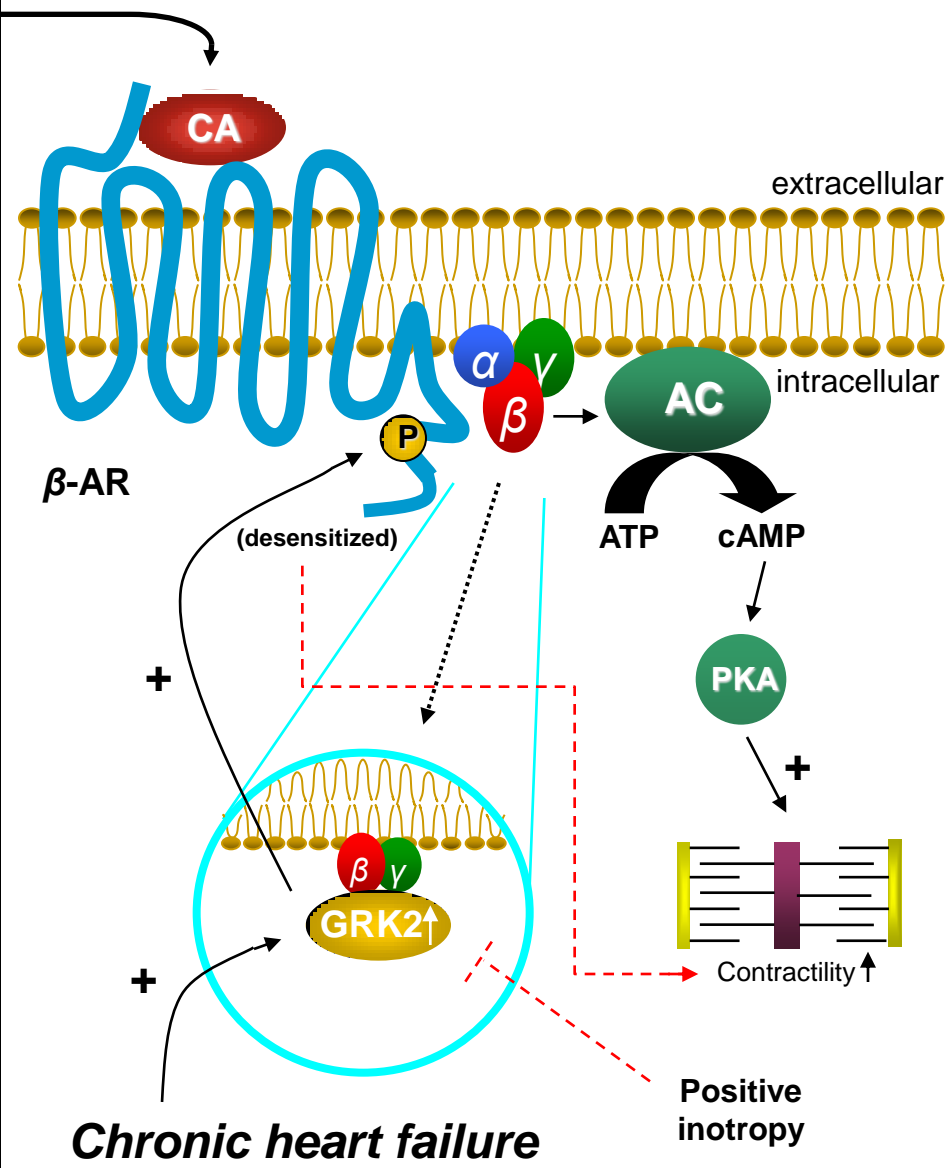
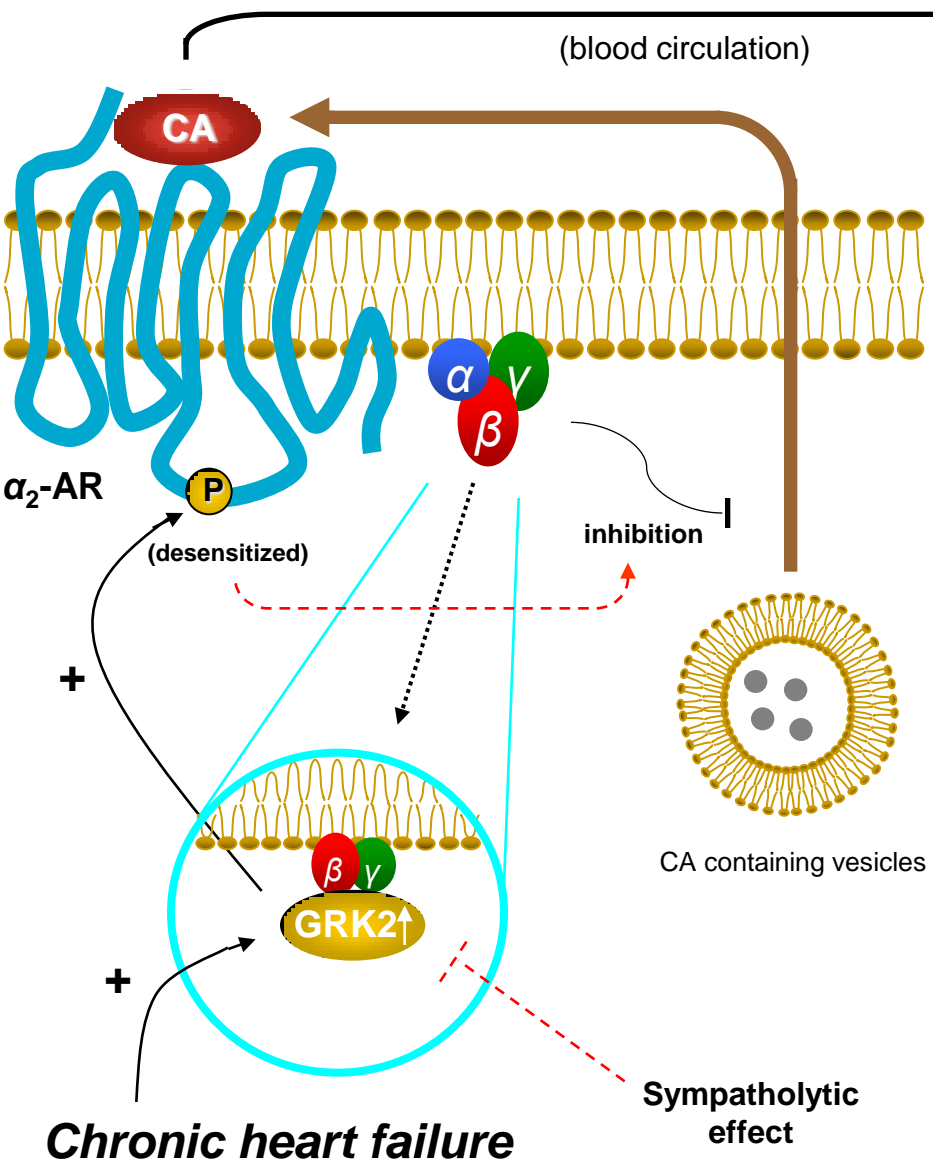
Anastasios Lymperopoulos^{1,2}, Giuseppe Rengo^{1,2}, Hajime Funakoshi¹, Andrea D Eckhart^{1,3} & Walter J Koch^{1,2}

¹Center for Translational Medicine, ²George Zallie and Family Laboratory for Cardiovascular Gene Therapy and ³Eugene 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



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

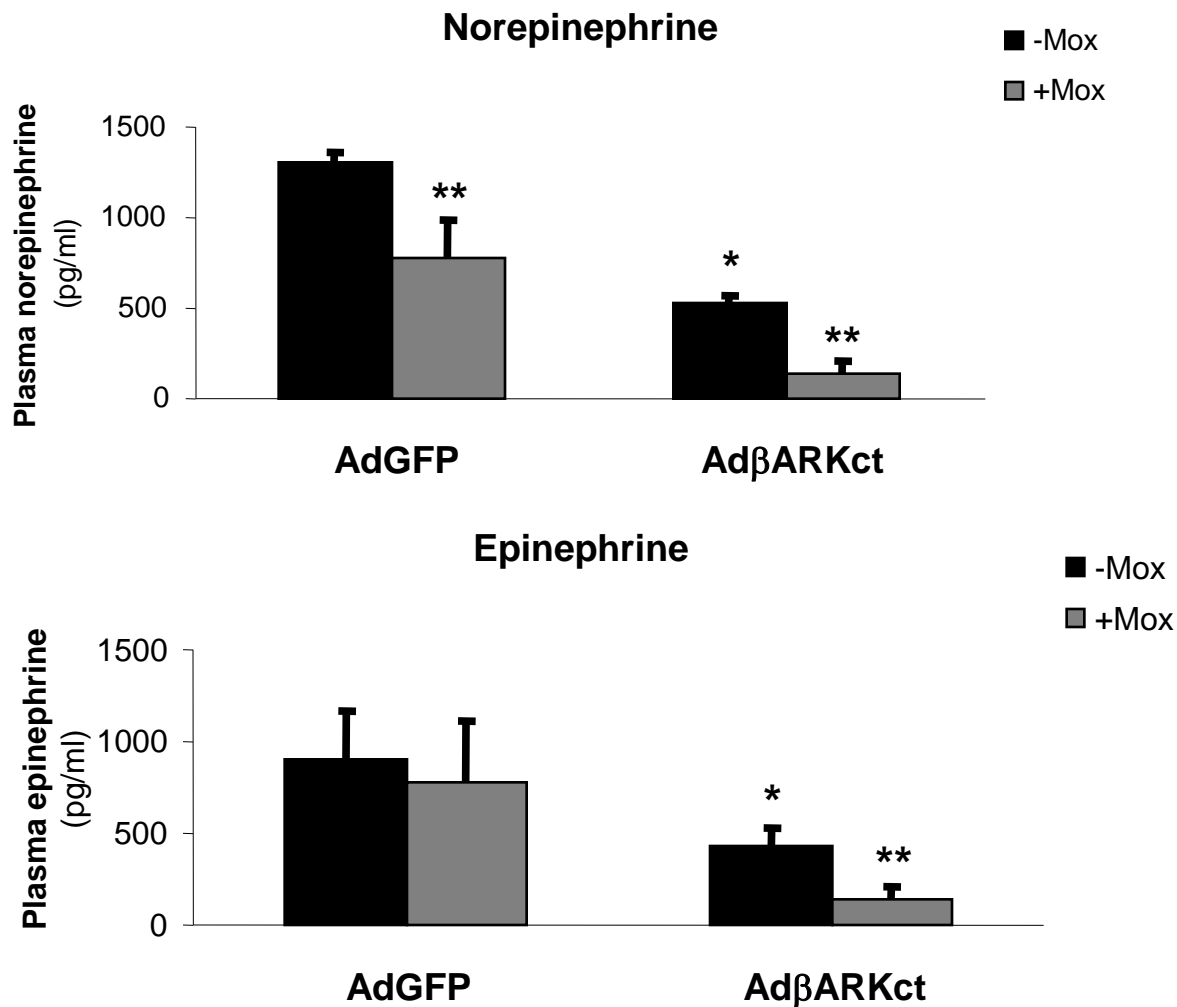
Karl Swedberg, MD; Michael R. Bristow, MD; Jay N. Cohn, MD; Henry Dargie, MD;
Matthias Straub, MD; Curtis Wiltse, PhD; Theresa J. Wright, MD;
for the Moxonidine Safety and Efficacy (MOXSE) Investigators

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



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 Lympereopoulos^{‡§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

GRK2 Inhibition and KO in Chronic HF Models

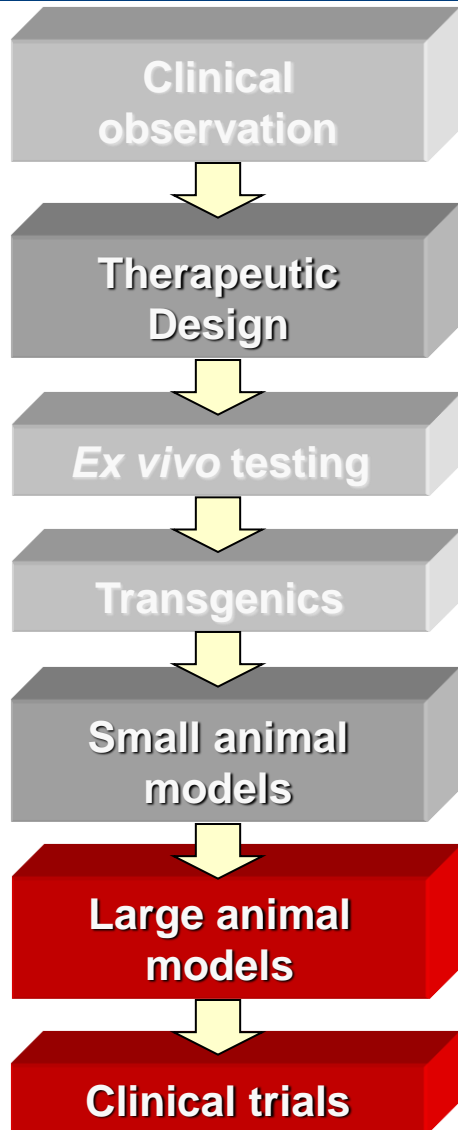
Chronic β ARKct expression or lowering of expression improves LV function and morphology in rats and mice with HF

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

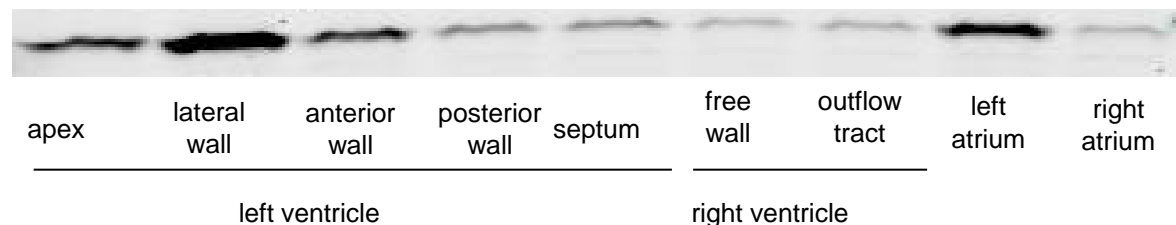
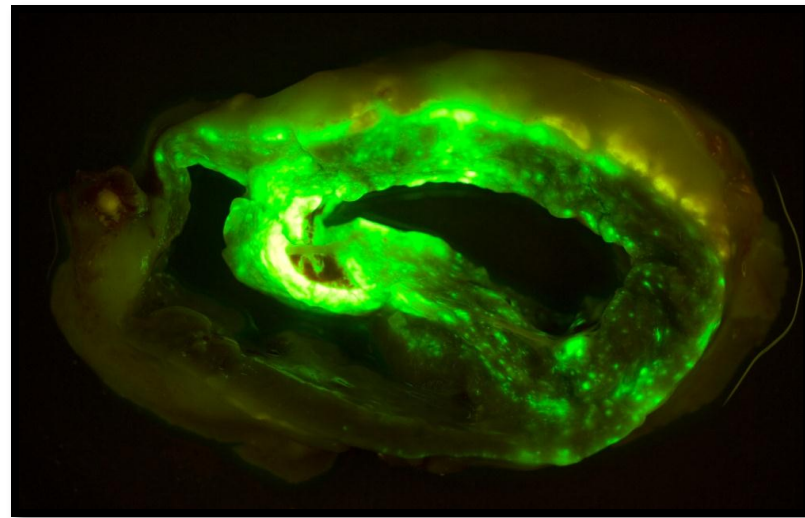
Inhibition of GRK2 represents a potential new drug class, targeting β AR and other GPCR systems from “the inside out”

Data indicates that β ARKct action in HF is primarily via GRK2 inhibition since KO phenotype is similar – still a role for $G\beta\gamma$ vs GRK2

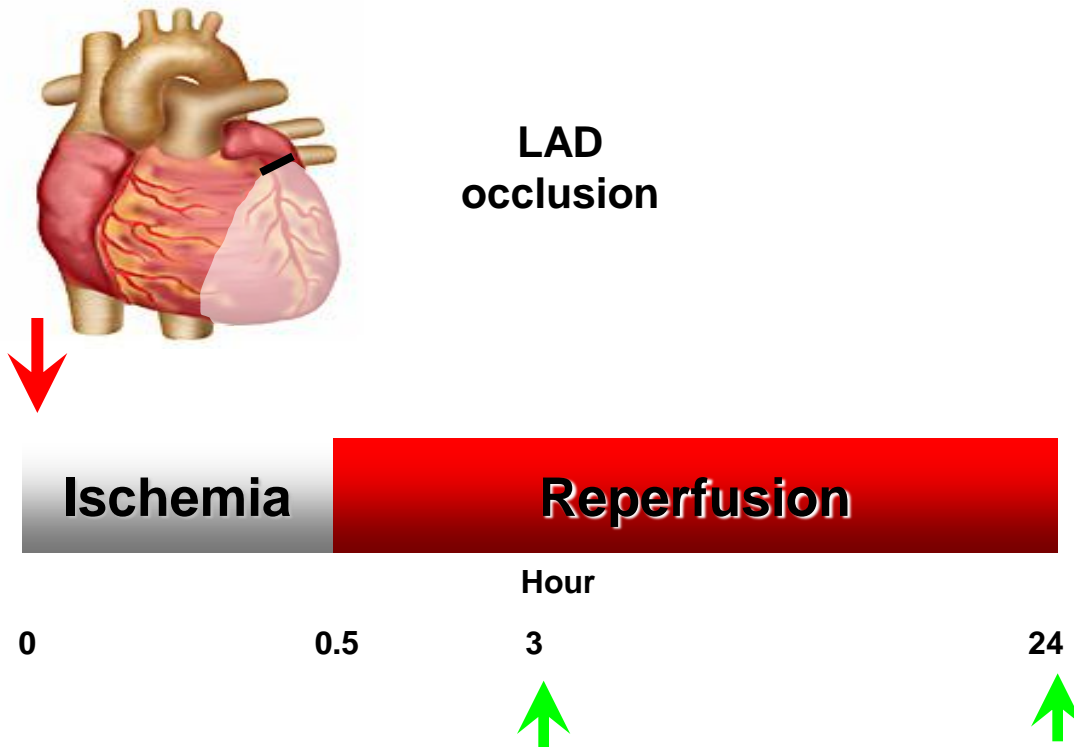
Final Translation



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.



Role of GRK2 in Acute Ischemic Injury



LAD
occlusion

Ischemia

Reperfusion

Hour

0

0.5

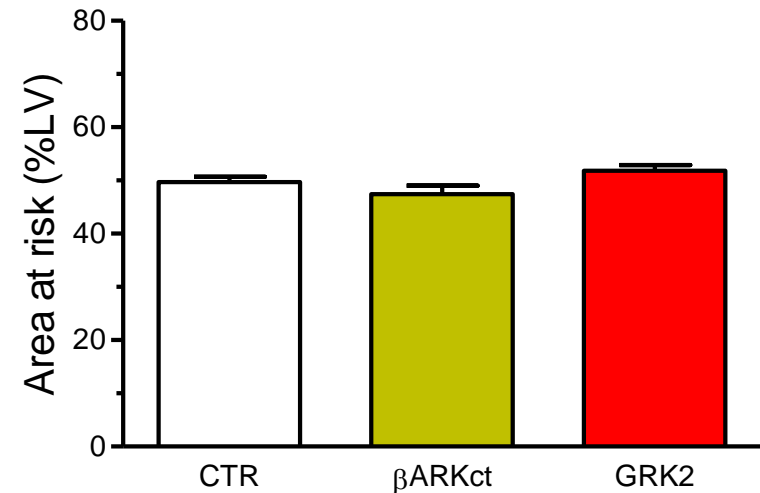
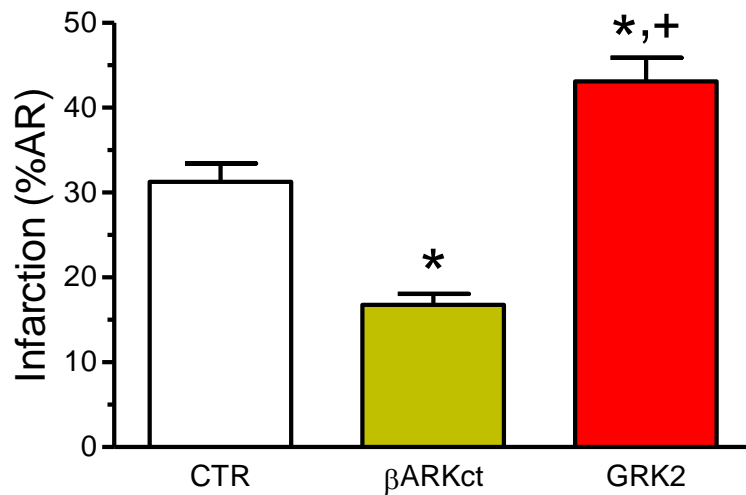
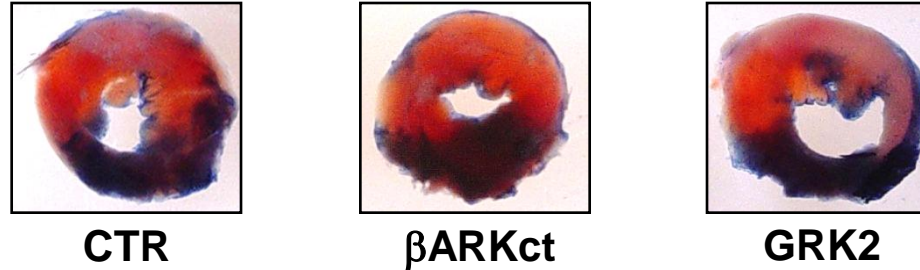
3

24

Experimental outcomes:

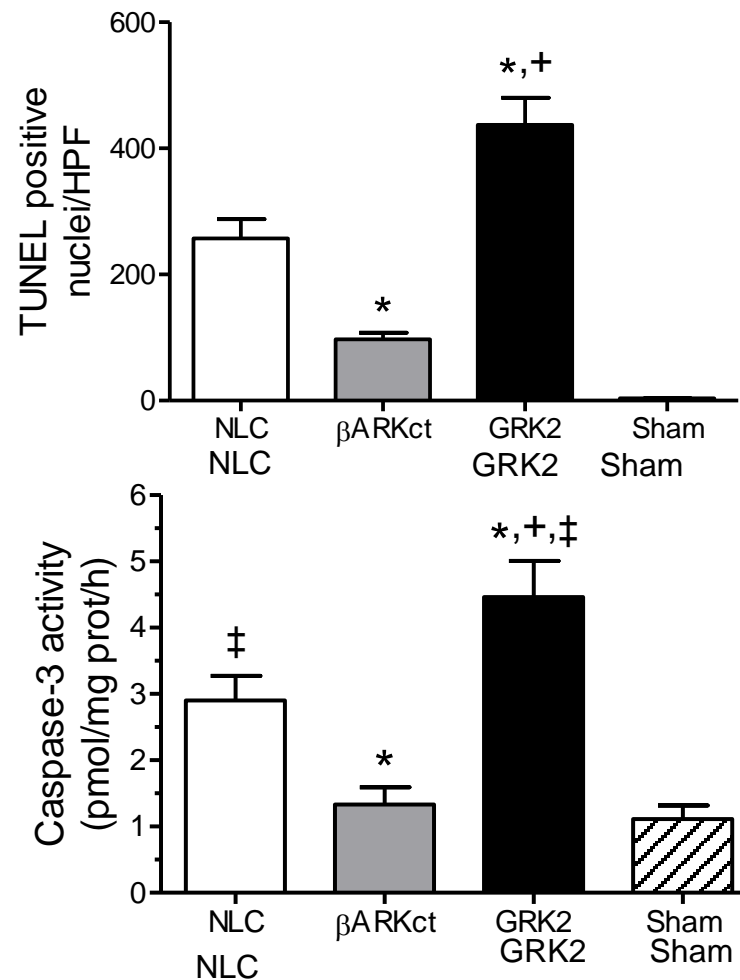
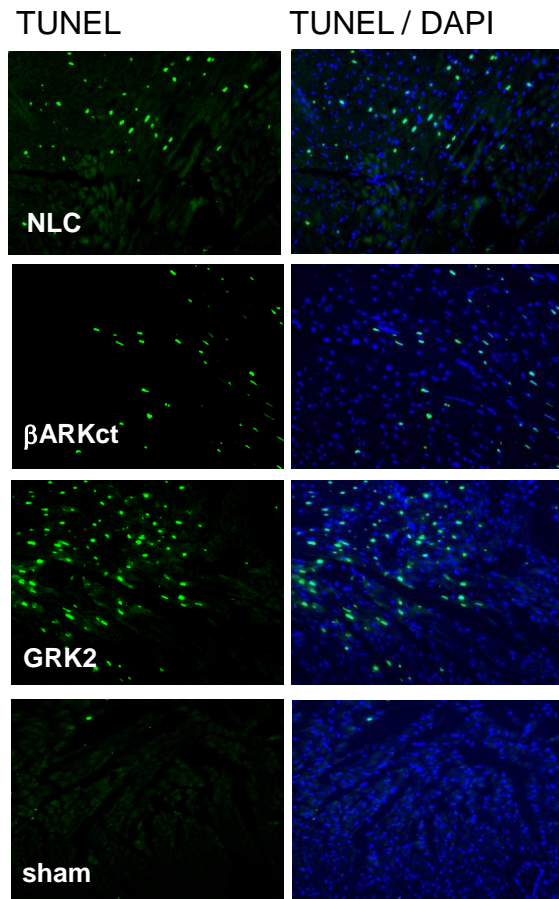
- 1) Western Blotting
- 2) Apoptosis (TUNEL, Caspases)
- 3) Cardiac function (Echo/Hemo)
- 4) Infarct size (TTC)

GRK2 and β ARKct Mice after I/R Injury

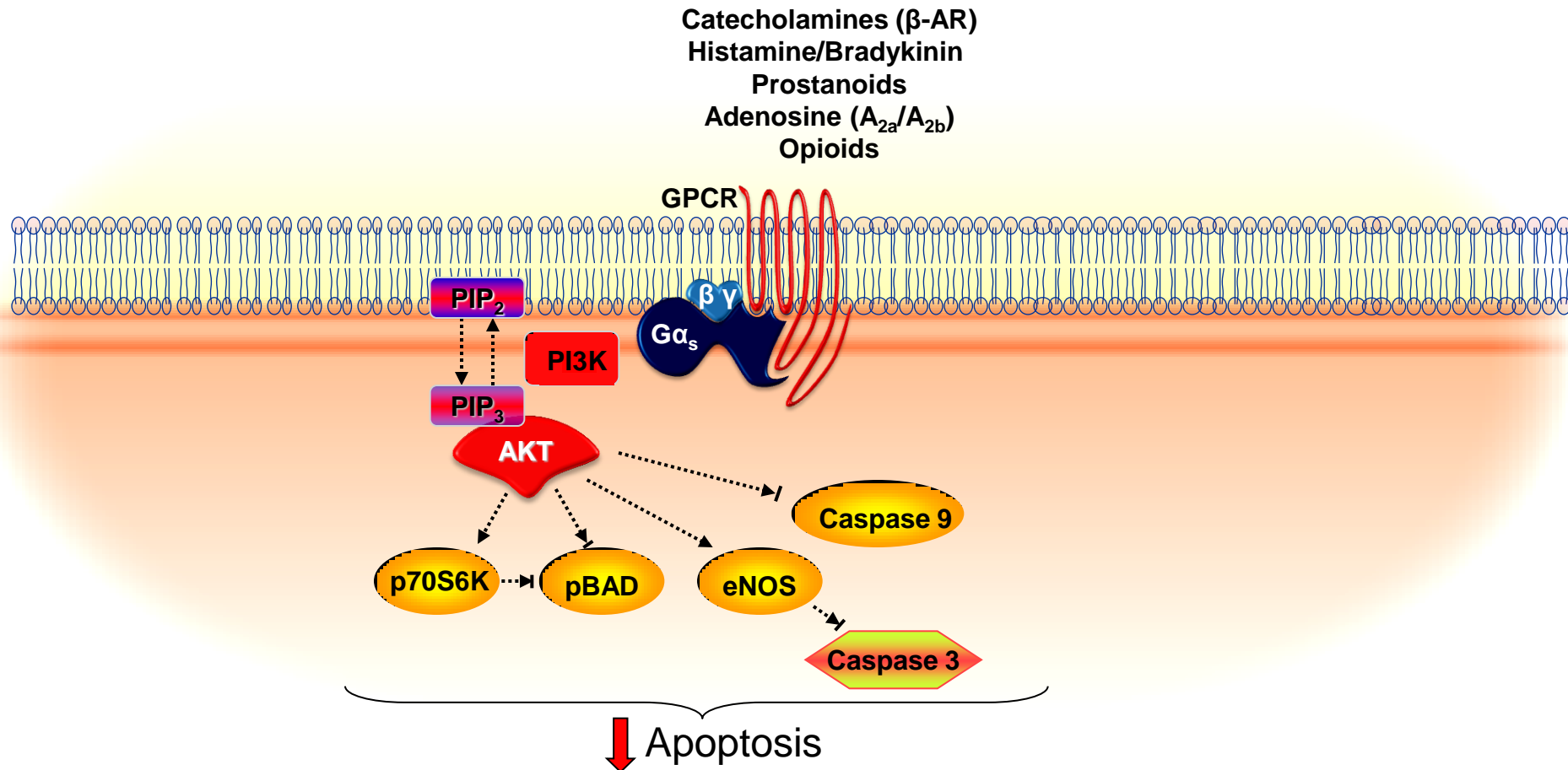


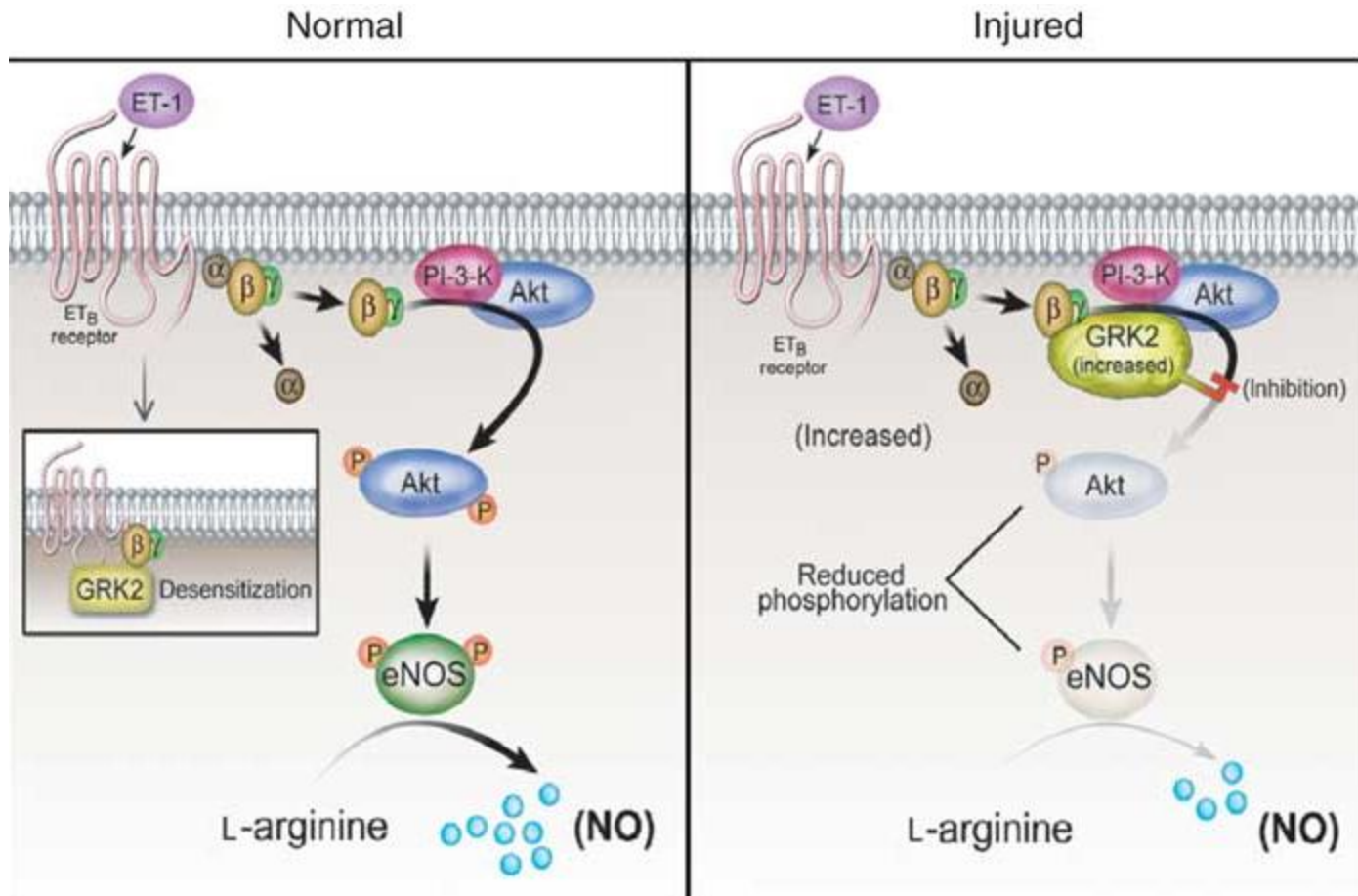
n=13-25/group; *p<0.05 vs. NLC, +p<0.05 vs. β ARKct; NLC

Apoptosis after I/R Injury in GRK2 and β ARKct Mice

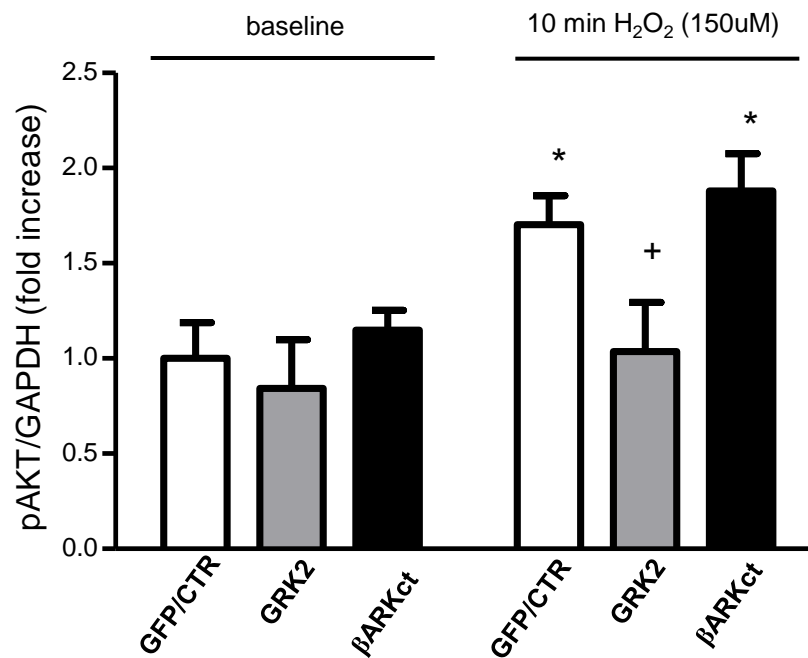
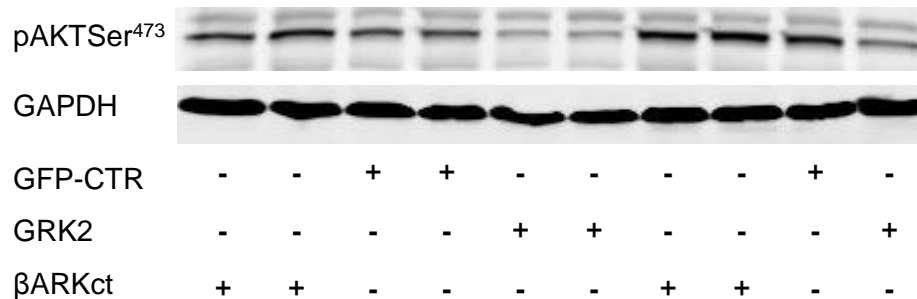


Anti-apoptotic Signaling in I/R Injury

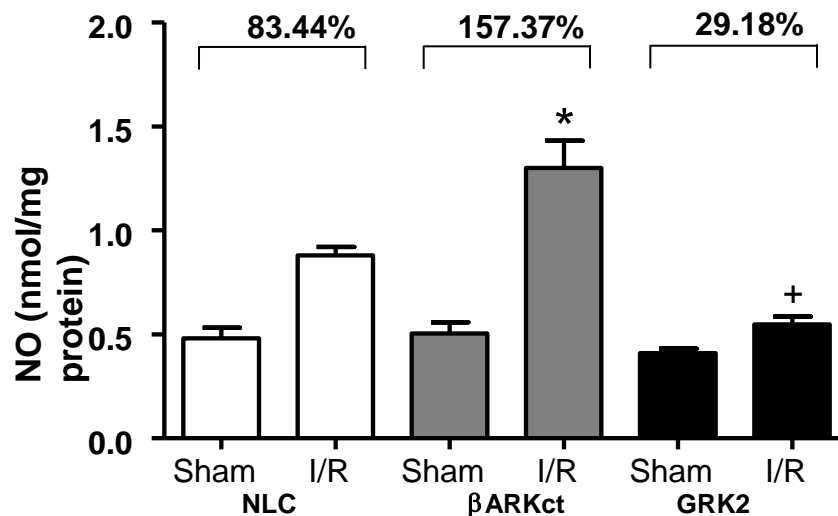
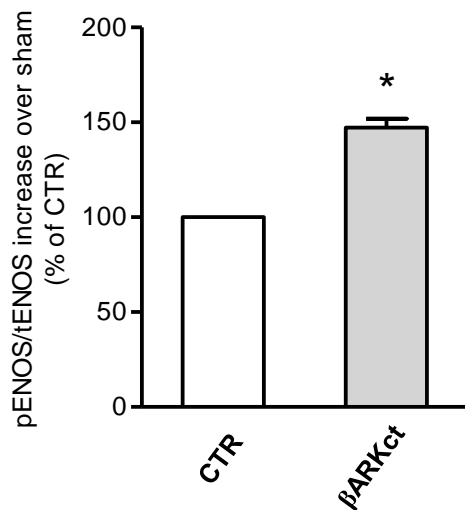
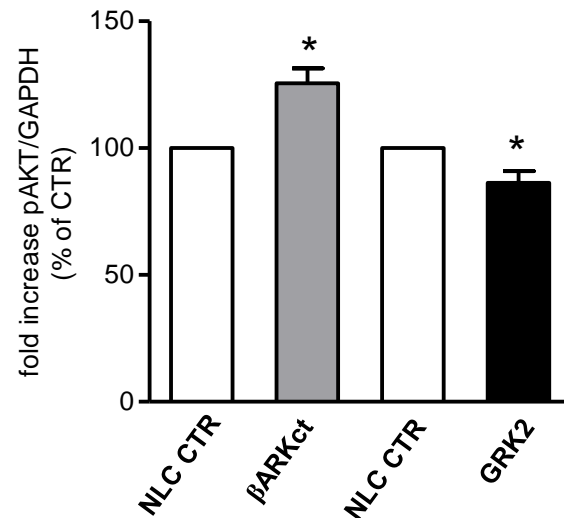
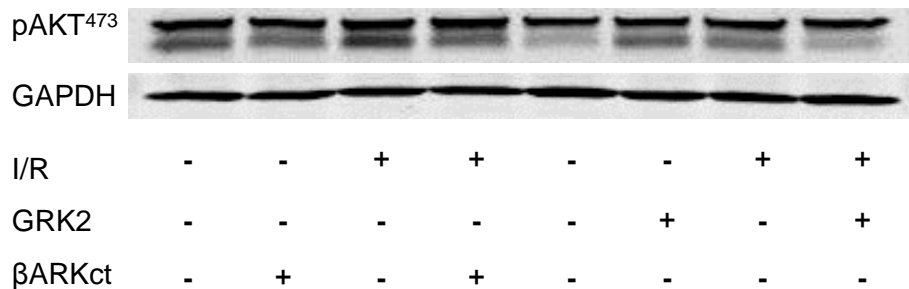




Anti-apoptotic Signaling Promoted by β ARKct In Vitro



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



Differential β AR Sub-type Signaling in Transgenic Mice

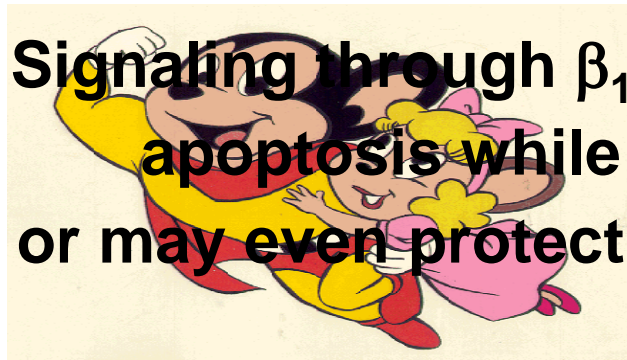
β_1 -AR



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



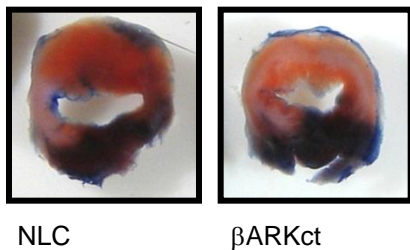
Low Overexpression



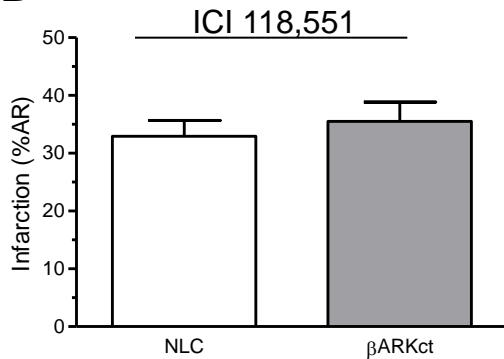
High Overexpression

β_2 AR Antagonism Negates β ARKct-Mediated Cardioprotection

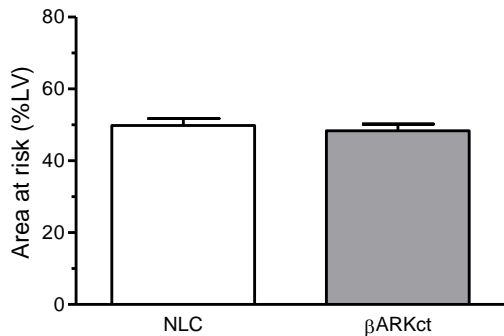
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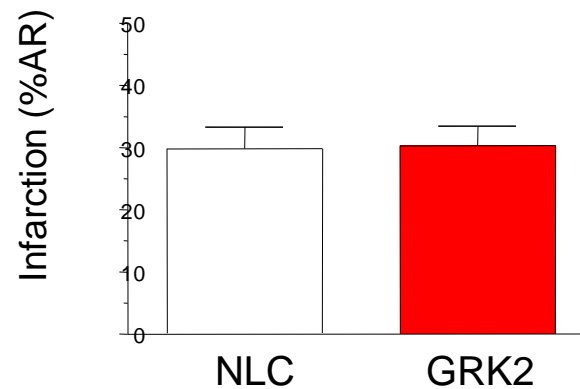
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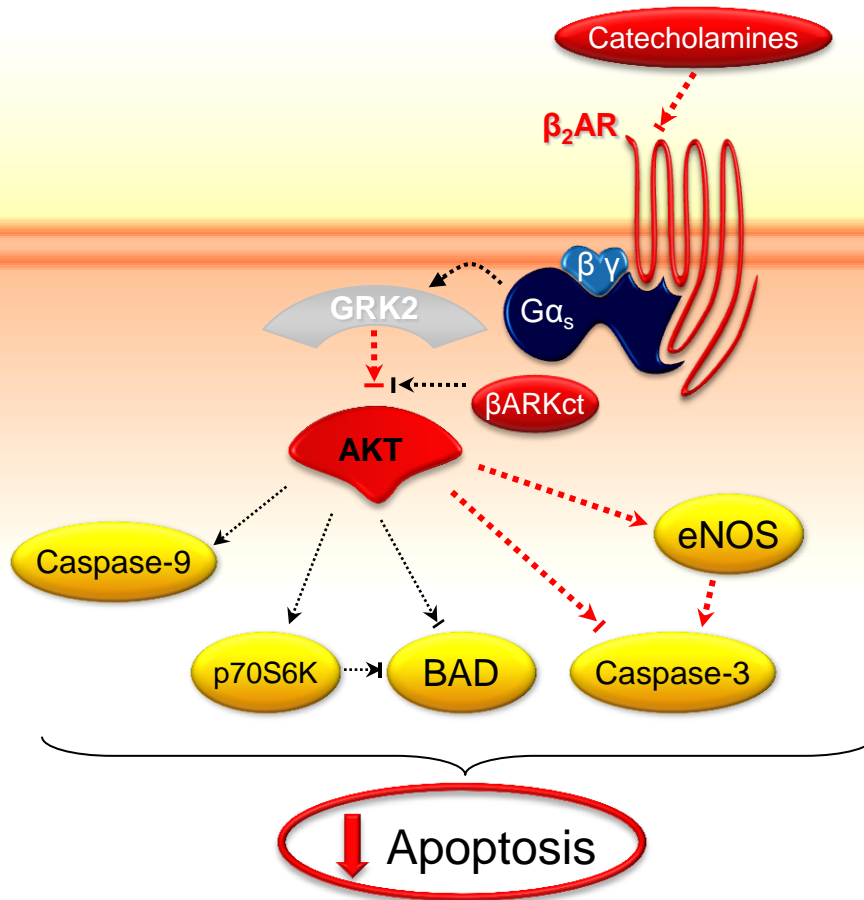
C



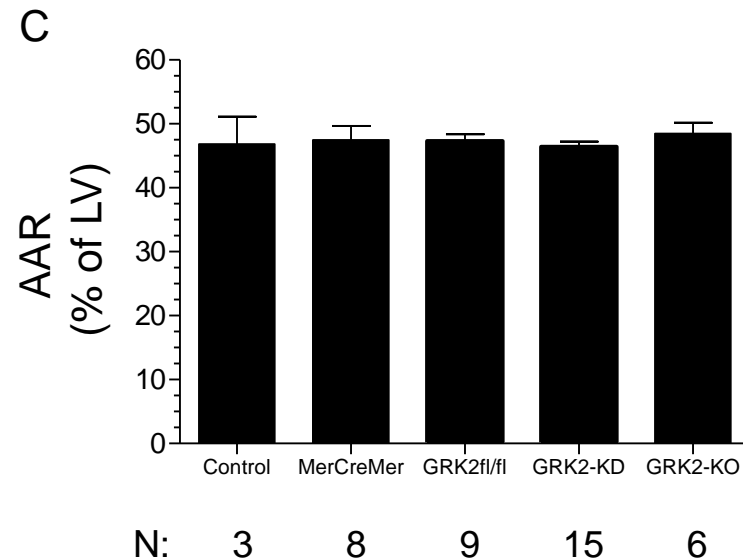
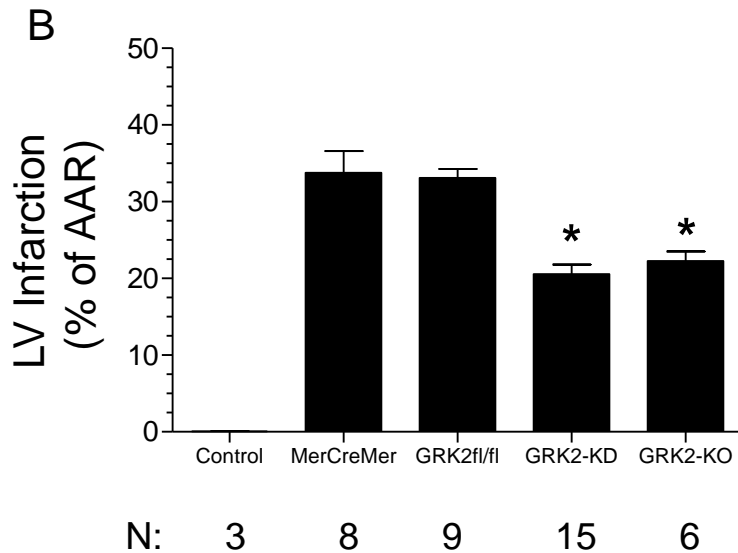
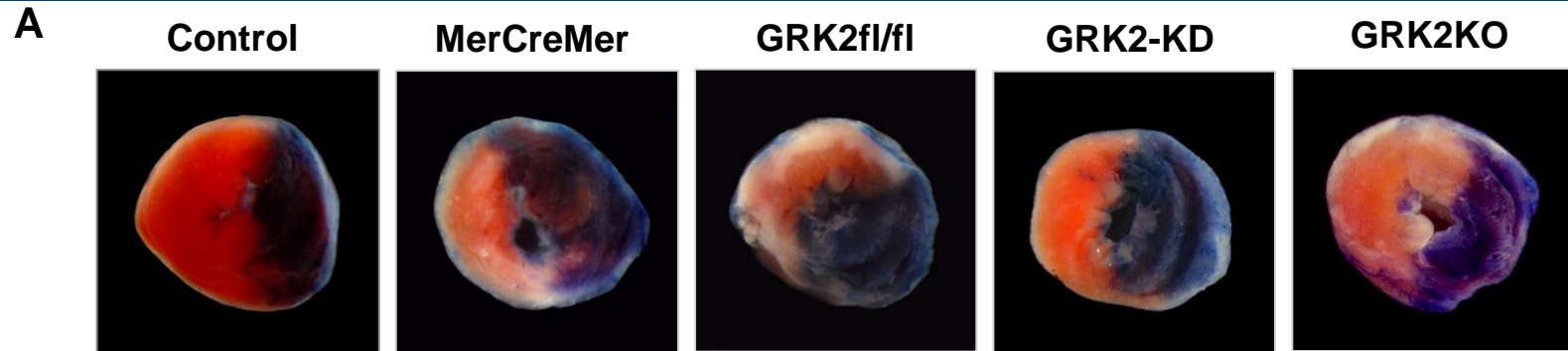
Fenoterol



GRK2 and Acute I/R injury



Cardiac GRK2 KO Mice and I/R



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 β ARKct (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 β 2AR 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.

ACKNOWLEDGMENTS

▣ Collaborators

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Erhe Gao – Jefferson

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Southeastern**

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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

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