New Approach to Ischemic Heart Diseases

-Roles of Adiponectin from Progression of Coronary Arteriosclerosis to Ventricular Remodeling-

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National Cardiovascular Center

How to Reduce Ischemic Heart Failure

1. The reduction of incidence of myocardial infarction
   →antiplatelet, statin, pioglitazone、ACEI
2. Reduction of infarct size
   →beta-blocker
3. Inhibition of ventricular and vascular remodeling
   →ACEI, ARB, beta-blocker

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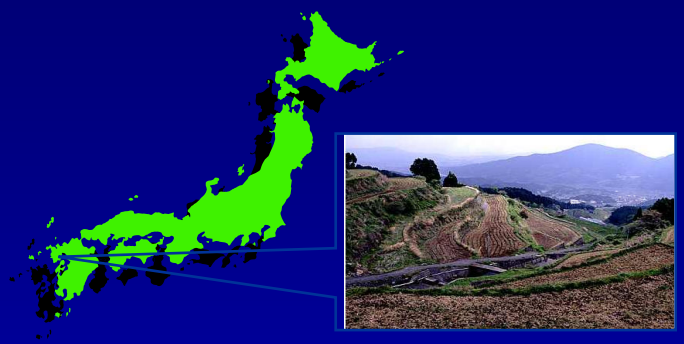
Nishiarita Cohort Study



Nishiarita Population;

9350 (Male 4513, Female 4837)

Study Populations

year	total
Apr 2001- Mar2002	1724
Apr 2002- Mar2003	1823
Apr 2003- Mar2004	1570
Apr 2004- Mar2005	1700
Apr 2005- Mar2006	1435
Apr 2006- Mar2007	1616

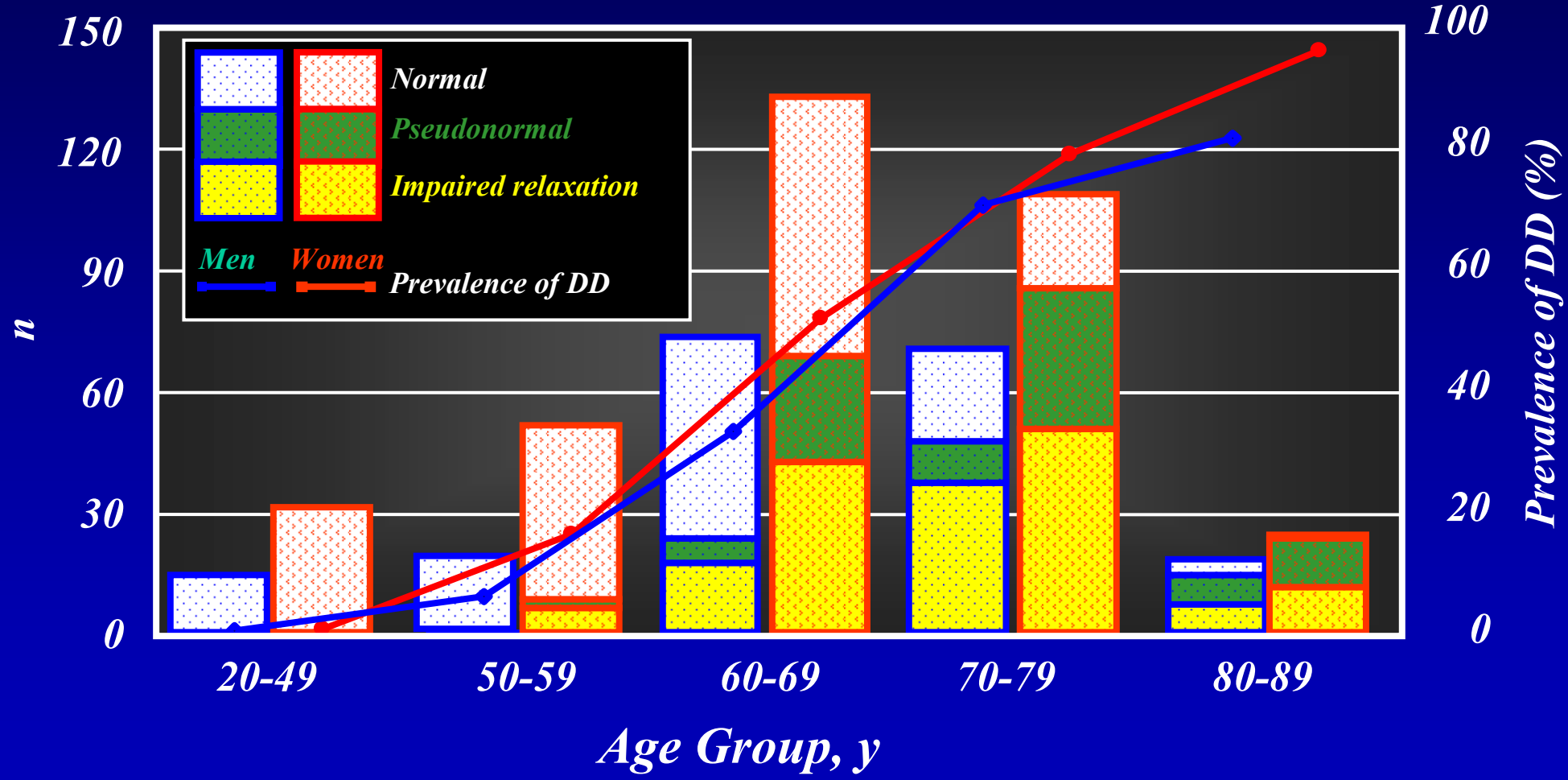
The Annual Health Examination

(Interview, Weight / Height, ECG, Blood test, Urine analysis)

The Special Variables

(Waist circumference, UCG, the plasma BNP, adiponectin and NO levels, SNP etc.)

Prevalence of Diastolic Dysfunction According to Age and Gender



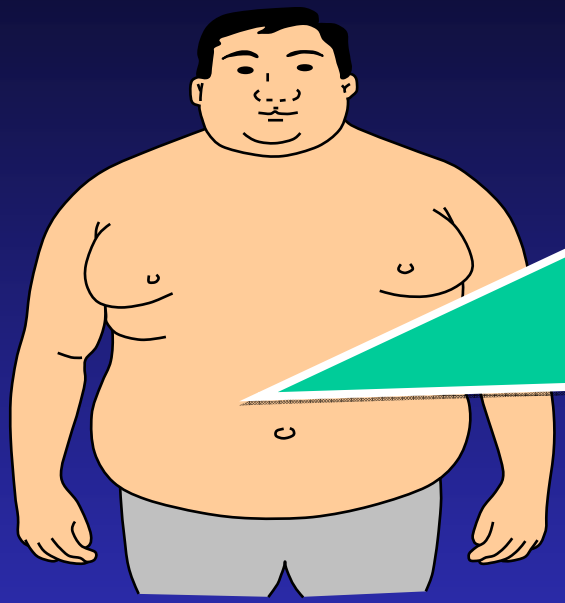
Contributors of Diastolic Dysfunction

Variables	Odds ratio (95% CI)	*P*-value
Age	1.184 (1.145-1.224)	<.0001
Hypertension	3.169 (2.045-4.910)	<.0001
Woman	2.587 (1.630-4.016)	<.0001
Diabetes	2.017 (0.999-4.072)	.05

Predictive Factors of Cardiovascular Events (multivariate analysis)

	Hazard ratio
• hypertension (>130/85)	5.2 (1.1-24.9)
• FPG>110	3.5 (1.0-12.4)
• BMI>25	3.3 (1.4-7.9)
• walking time > 2 hrs	0.25 (0.07-0.88)

Relationship between Obesity and Adipocytokines



Obesity
(Visceral Fat Obesity)

TNF-a

↑ angiotensinogen

Adipocyte

↓ Nitric Oxide

IL-6

Leptin

↓Adiponectin

Beneficial

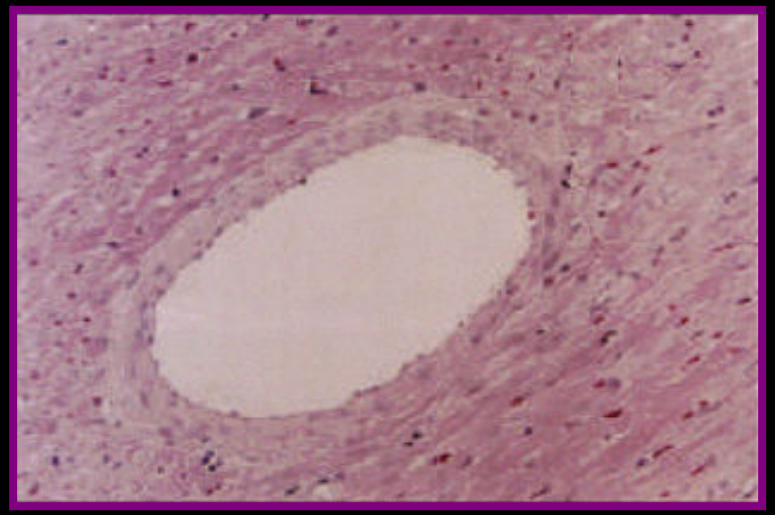
Deleterious

1. It is well known that RAS activation, which is mediated from adipose tissue of obesity people, is responsible for the progression of hypertension and diabetes mellitus。

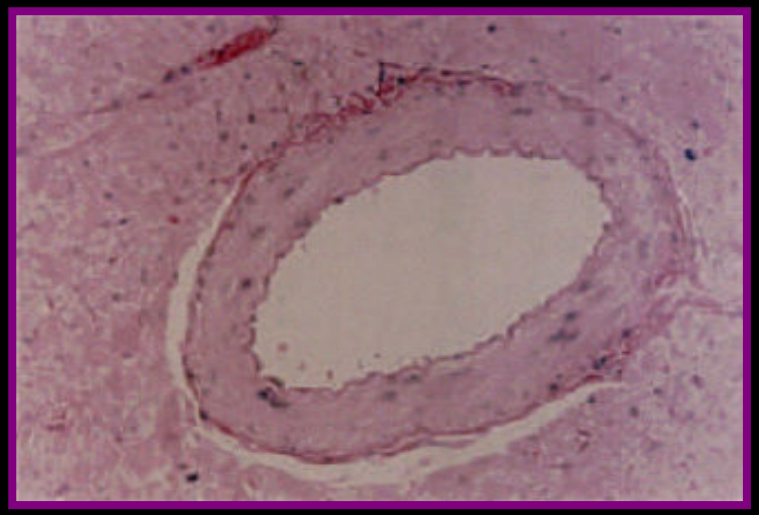
2. On the other hand, RAS activation down-regulates both endothelial NOS function and adiponectin。

The Effects of Chronic NOS Inhibition on Coronary Artery in Rats

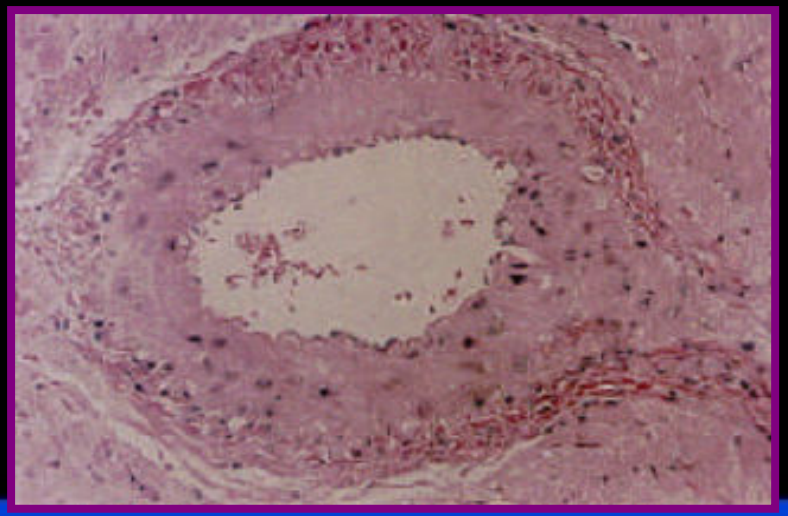
Control



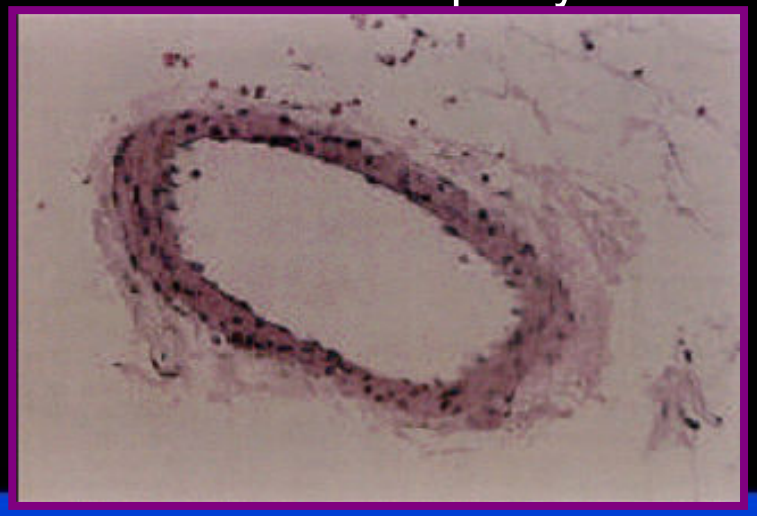
L-NAME + hydralazine



L-NAME



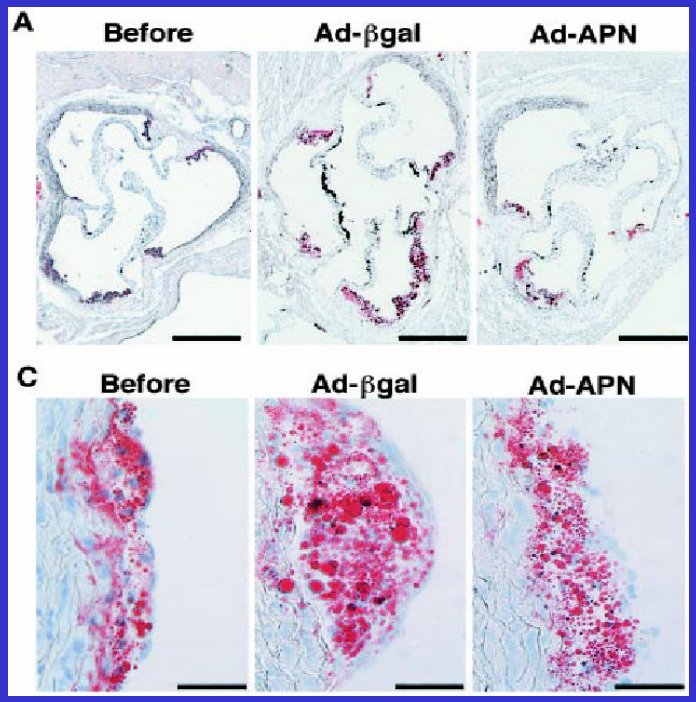
L-NAME + rapamycin



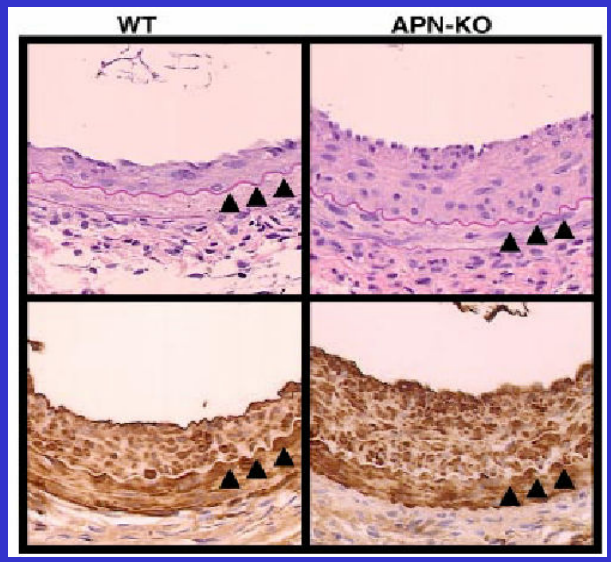
100 μ m

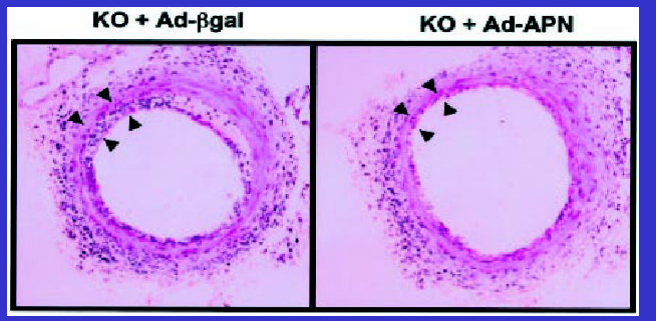
Anti-atherosclerotic Effects of Adiponectin

Recombinant-adiponectin inhibits plaque formation（ApoE$^{-/-}$ mice）



Adiponectin inhibits intimal thickening （Adiponectin$^{-/-}$ mice）



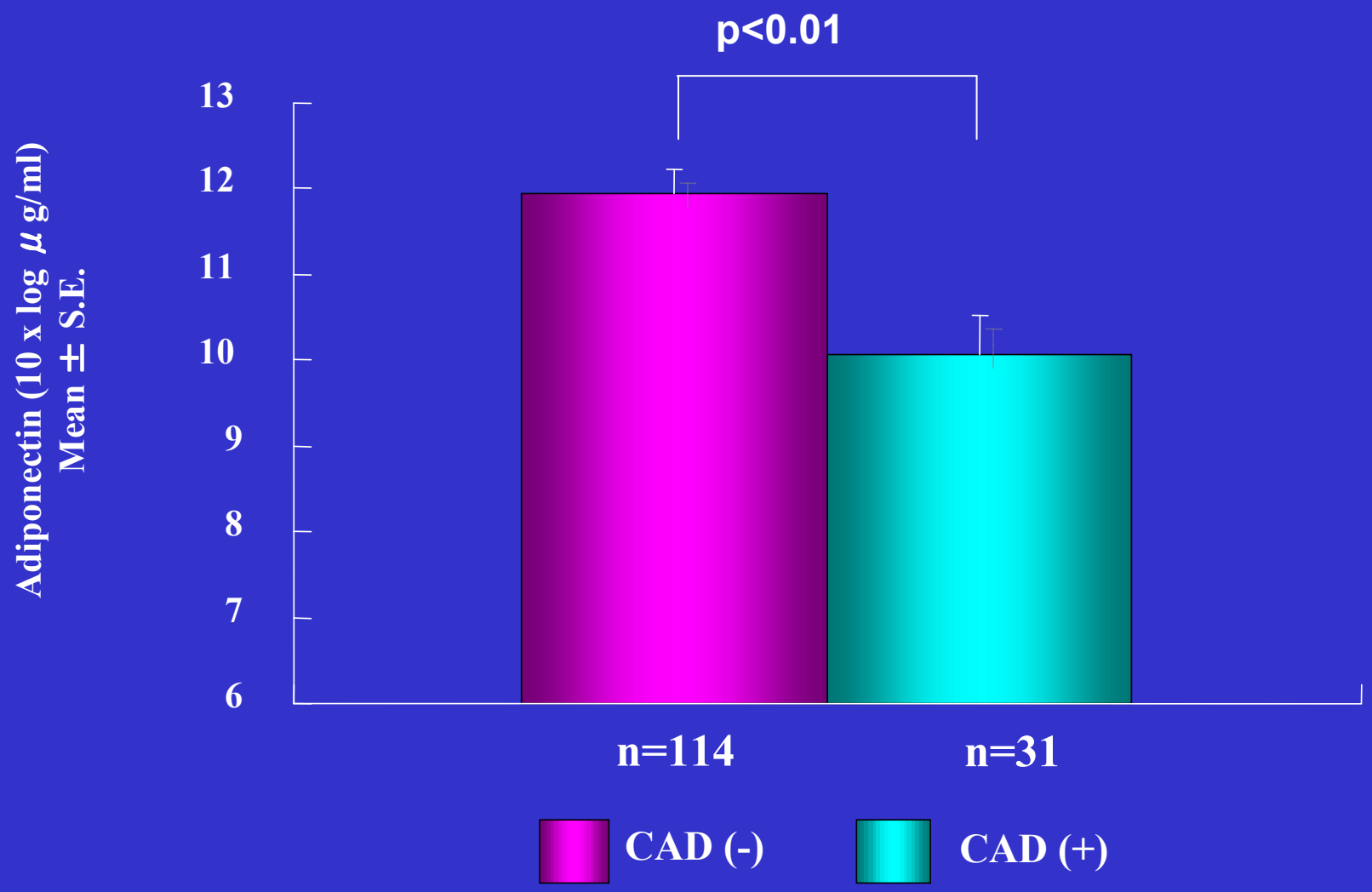


Okamoto Y et al. Circulation 2002
Matsuda M et al. JBC 2002

The Mechanisms for Anti-atherosclerotic effects of adiponectin

Endothelial cells : inhibition of expression of adhesion molecules
Macrophages: inhibition of expression of scavenger receptors
Smooth muscles: inhibition of proliferation os smooth muscle cells
Platelets: inhibition of platelets aggregation

CAD and Adiponectin in the NCVC Cohort Study



The factors that Affects Plasma NOx Levels in Nishiaritacho Cohort Study

	beta	t	P value
BMI	−0.056	−2.403	0.016
T_CHO(mg/dl)	−0.086	−3.608	<0.001
TG(mg/dl)	0.026	1.109	0.268
CRE(mg/dl)	0.220	8.684	<0.001
SBP(mmHg)	−0.140	4.273	<0.001
DBP(mmHg)	−0.187	−6.218	<0.001
log_BNP	0.049	1.829	0.068
RBC	0.062	2.324	0.020
Age	−0.581	−20.075	<0.001
SEX	0.101	3.442	0.001

Dependent variable variables: log_NOx

β :Standardized Coefficient

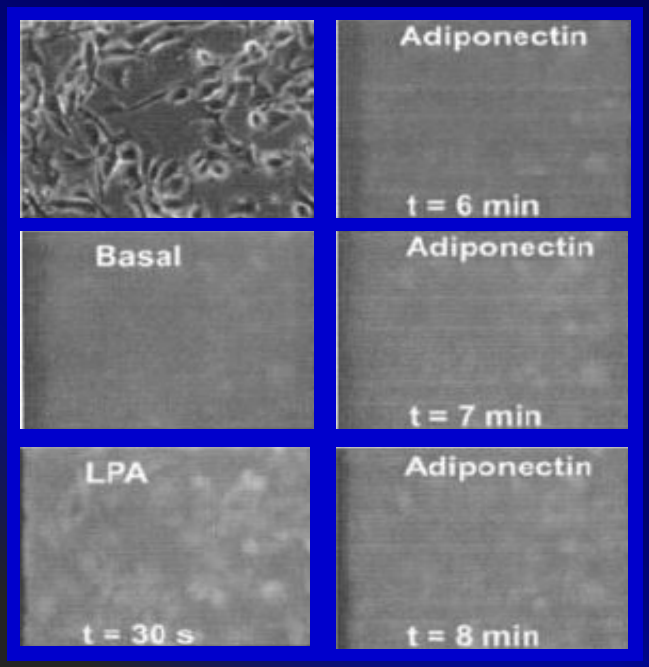
The factors that Affects Plasma Adiponectin Levels in Nishiaritacho Cohort Study

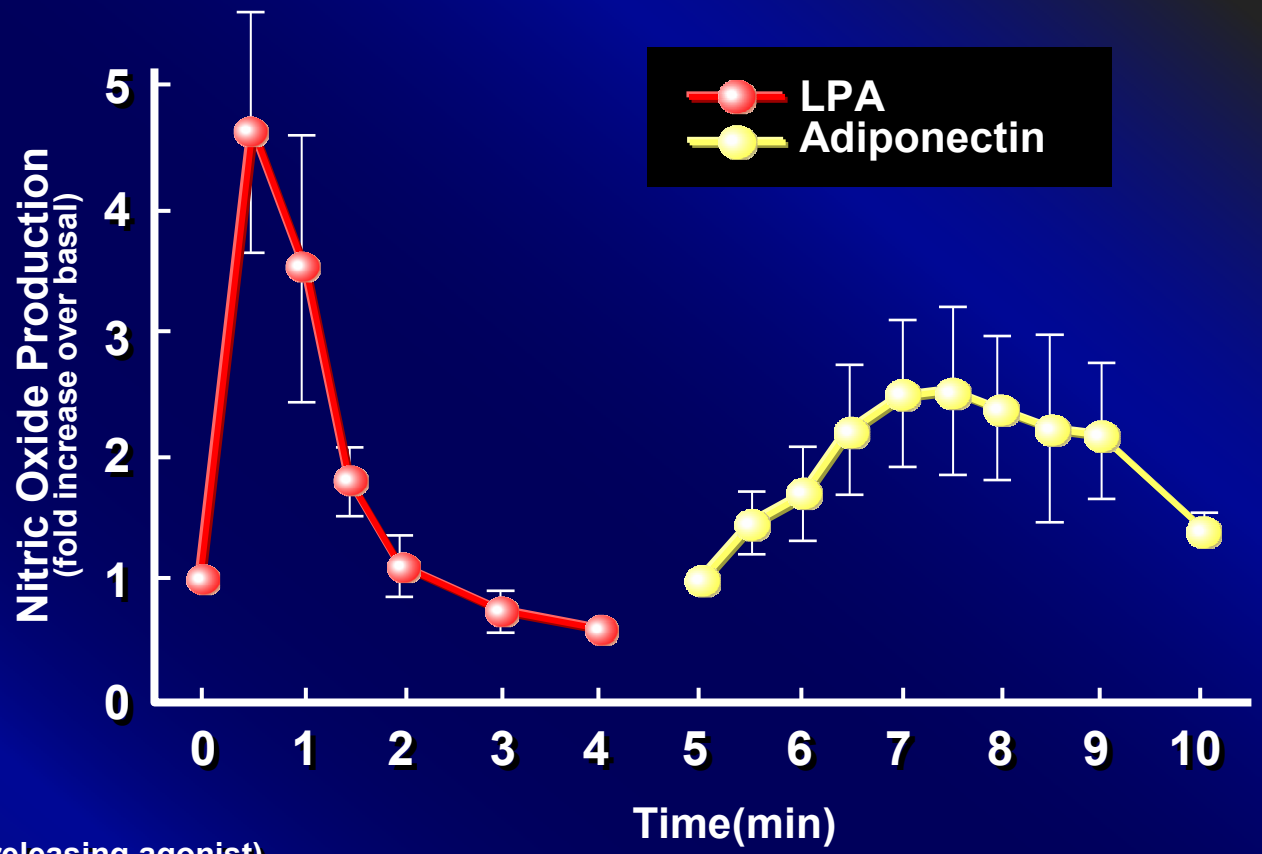
	β	p		β	p
Age	0.22	<0.001	AST	-0.03	NS
Male	-0.36	<0.001	ALT	-0.23	<0.001
BMI	-0.32	<0.001	γ -GTP	-0.25	<0.001
Waist	-0.29	<0.001	T-chol.	-0.01	NS
Log BNP	0.30	<0.001	HDL -chol.	0.28	<0.001
HbA1C	-0.10	<0.001	TG	-0.32	<0.001
Insulin	-0.16	<0.001	UA	-0.29	<0.001
FBS	-0.11	<0.001	Cr	-0.16	<0.001
mean BP	-0.08	0.003	Hb	-0.39	<0.001

Dependent variable: Log adiponectin β :Standardized Coefficient

Adiponectin Increases NOx Production

- in Bovine aortic endothelial cell -

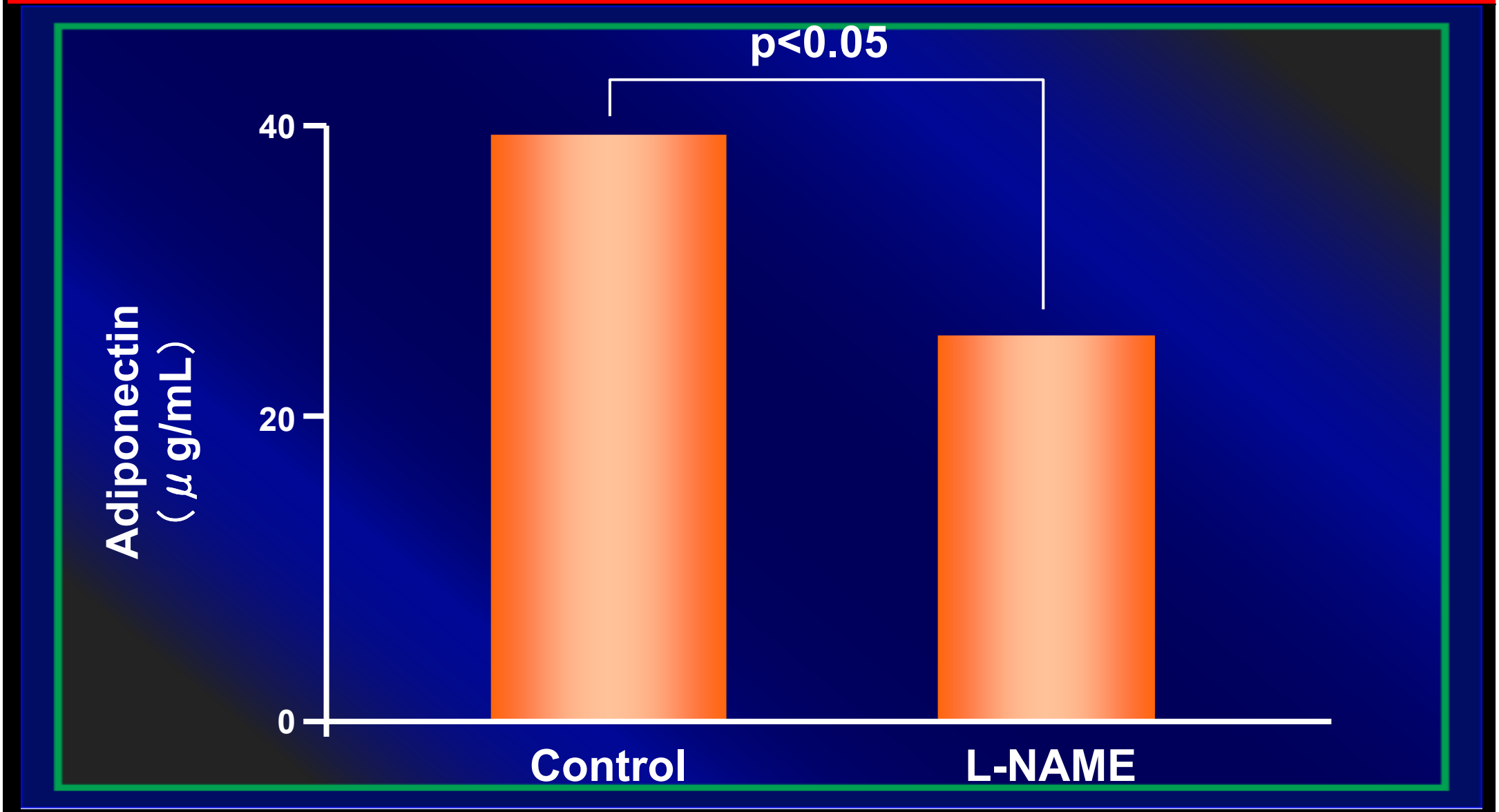




LPA: lysophosphatidic acid (a calcium-releasing agonist)

Chen H et al. *J Bio Chemist*;278(45):45021-45026,2003

Inhibition of NOS Decreases Adiponectin Levels



The factors that Affects Plasma Adiponectin Levels in Aritacho Cohort Study

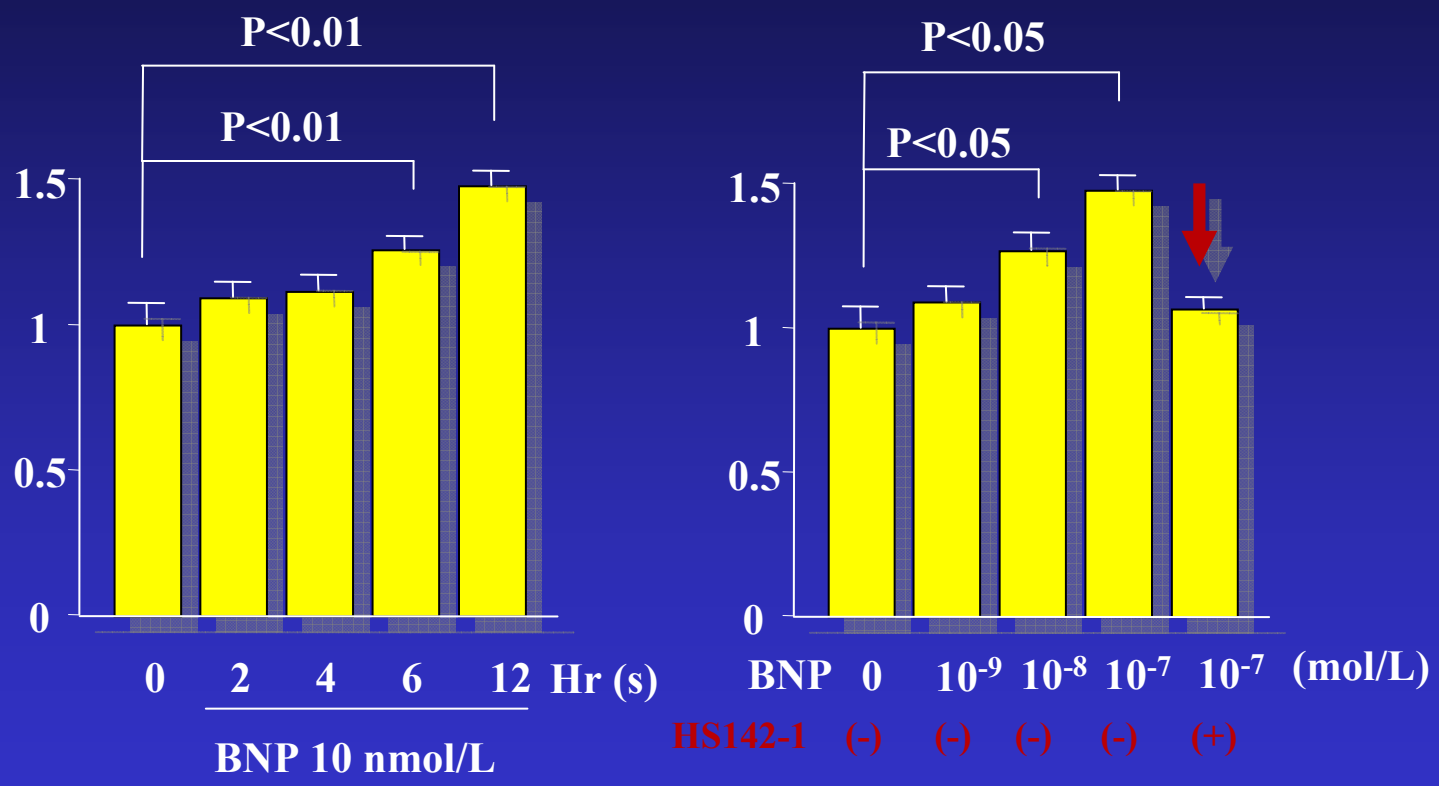
	β	p		β	p
Age	0.22	<0.001	AST	-0.03	NS
Male	-0.36	<0.001	ALT	-0.23	<0.001
BMI	-0.32	<0.001	γ -GTP	-0.25	<0.001
Waist	-0.29	<0.001	T-chol.	-0.01	NS
Log BNP	0.30	<0.001	HDL -chol.	0.28	<0.001
HbA1C	-0.10	<0.001	TG	-0.32	<0.001
Insulin	-0.16	<0.001	UA	-0.29	<0.001
FBS	-0.11	<0.001	Cr	-0.16	<0.001
mean BP	-0.08	0.003	Hb	-0.39	<0.001

Dependent variable: Log adiponectin β :Standardized Coefficient

Effects of BNP on mRNA Levels of Adiponectin

Incubation with BNP enhanced adiponectin mRNA expression in 3T3-L1 adipocytes in a time/ dose-dependent manner (Real-time PCR).

3T3-L1 adipocytes express mRNA coding for GC-A type receptors (RT-PCR).



Tsukamoto et.al. Circulation 2006; 114: II-486

The important issues for primary prevention is

1. Metabolic syndrome
2. RAS system
3. Endothelial dysfunction
4. Adiponectin
5. BNP

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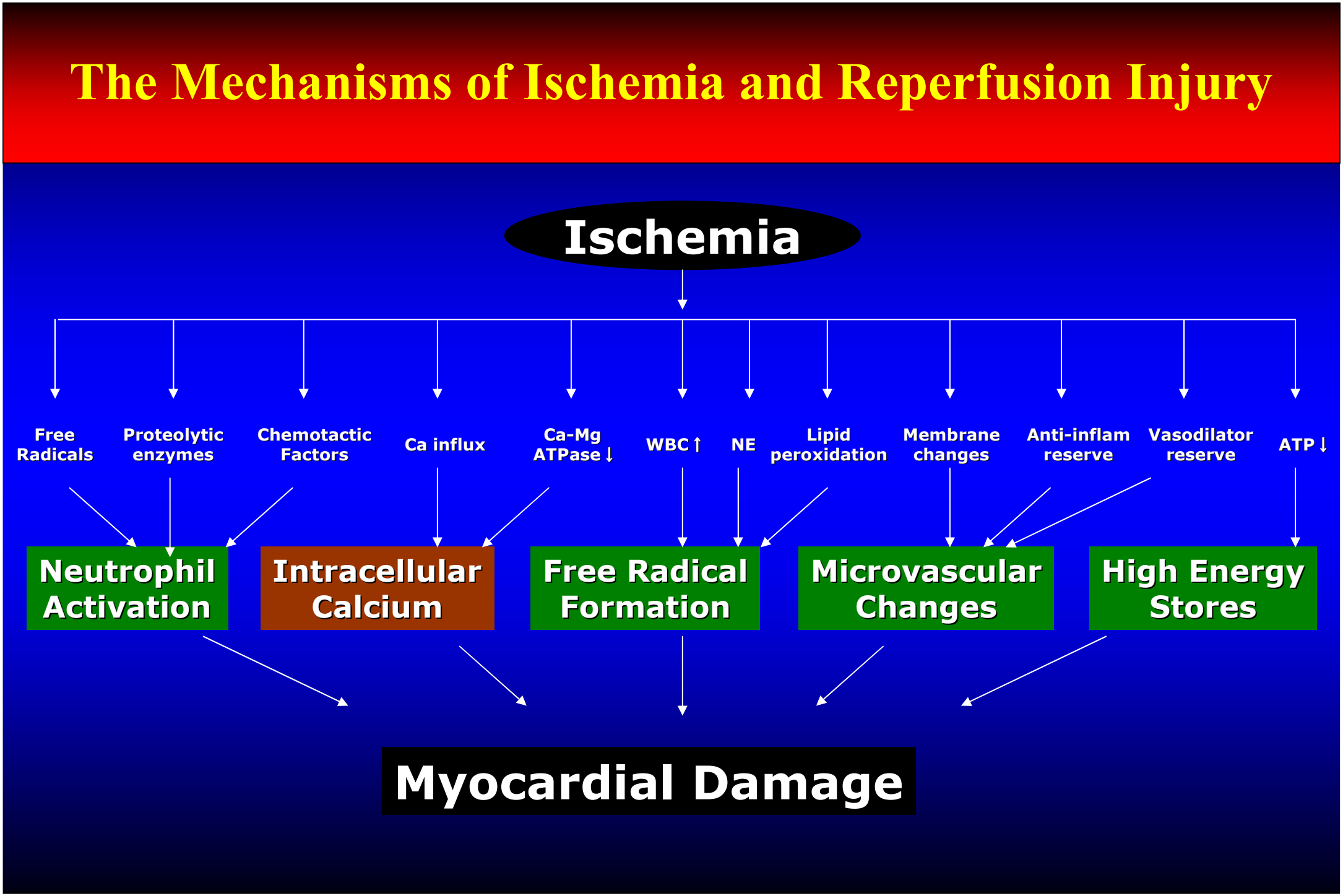
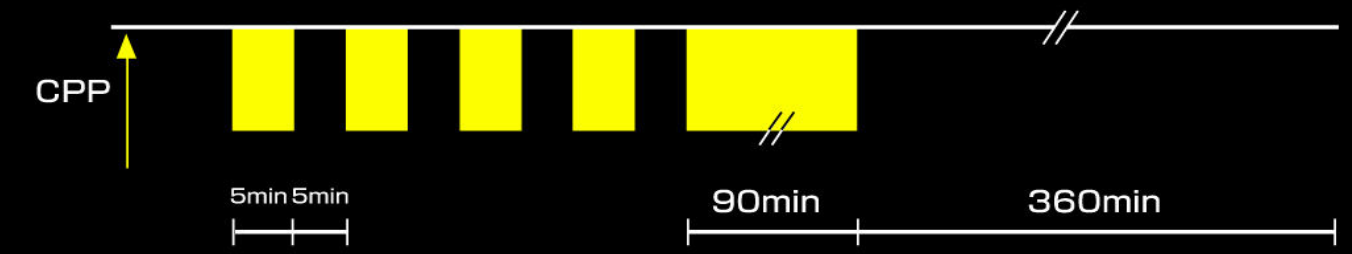
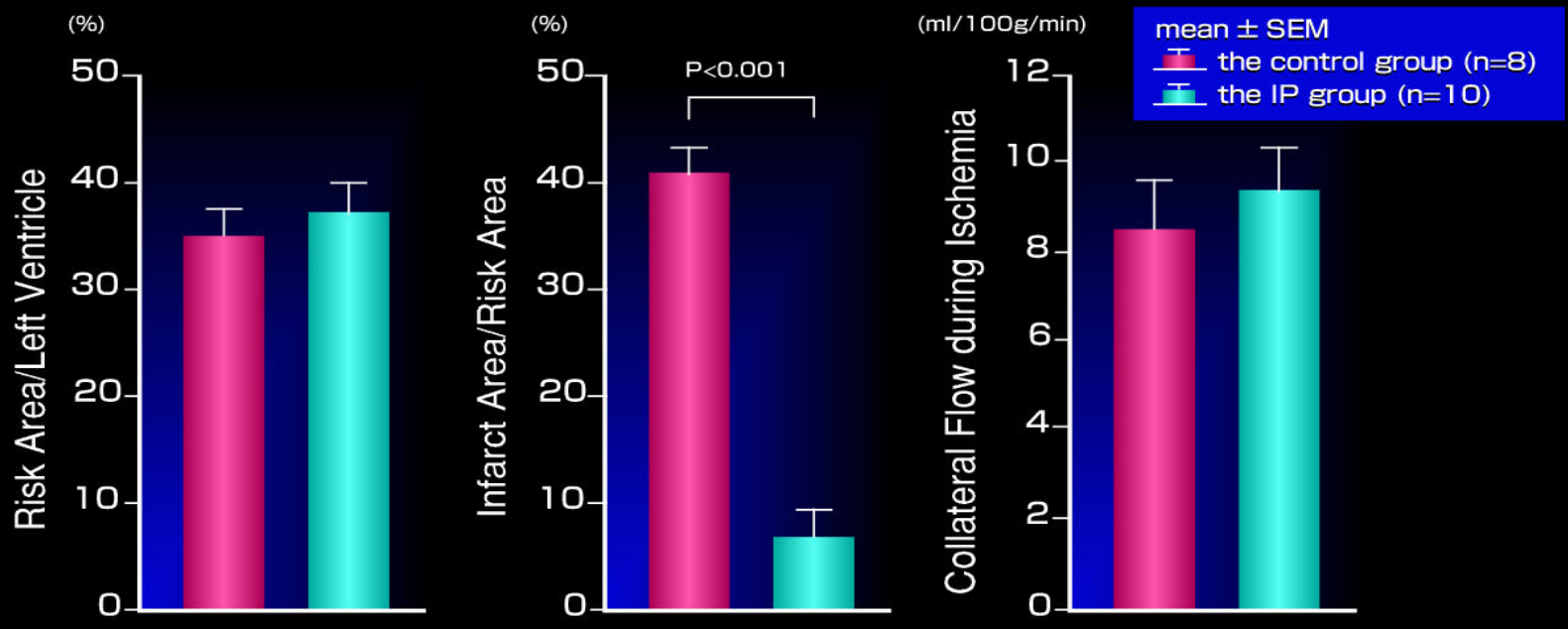
The Reason Why the Infarct Size-limitation is Difficult to be Obtained

The Mechanisms of Ischemia and Reperfusion Injury

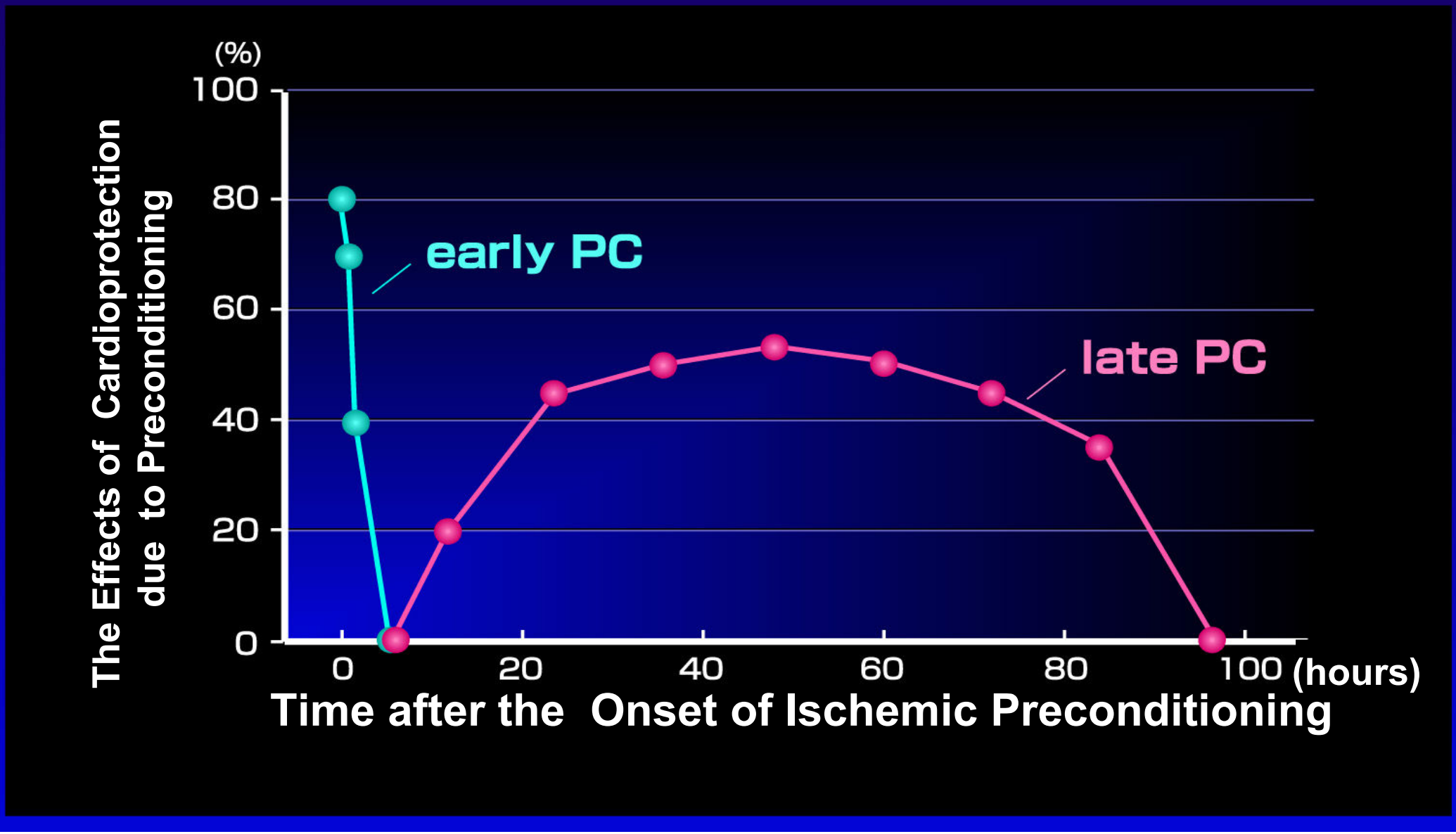


Effects of Ischemic Preconditioning on Infarct Size Caused by 90min Coronary Occlusion and Subsequent 6hrs Reperfusion

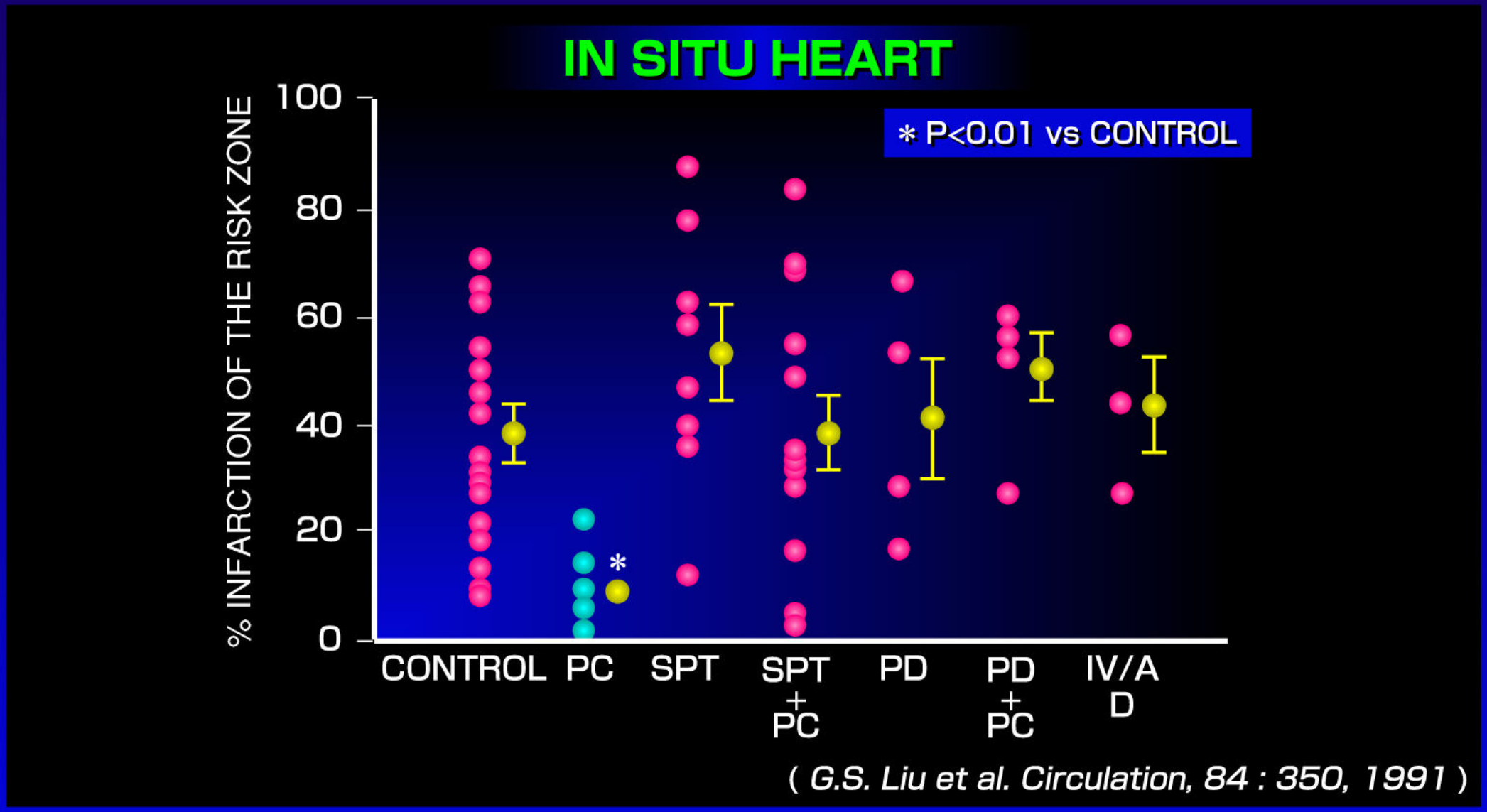




Time Course of Cardioprotection Afforded by Ischemic Preconditioning

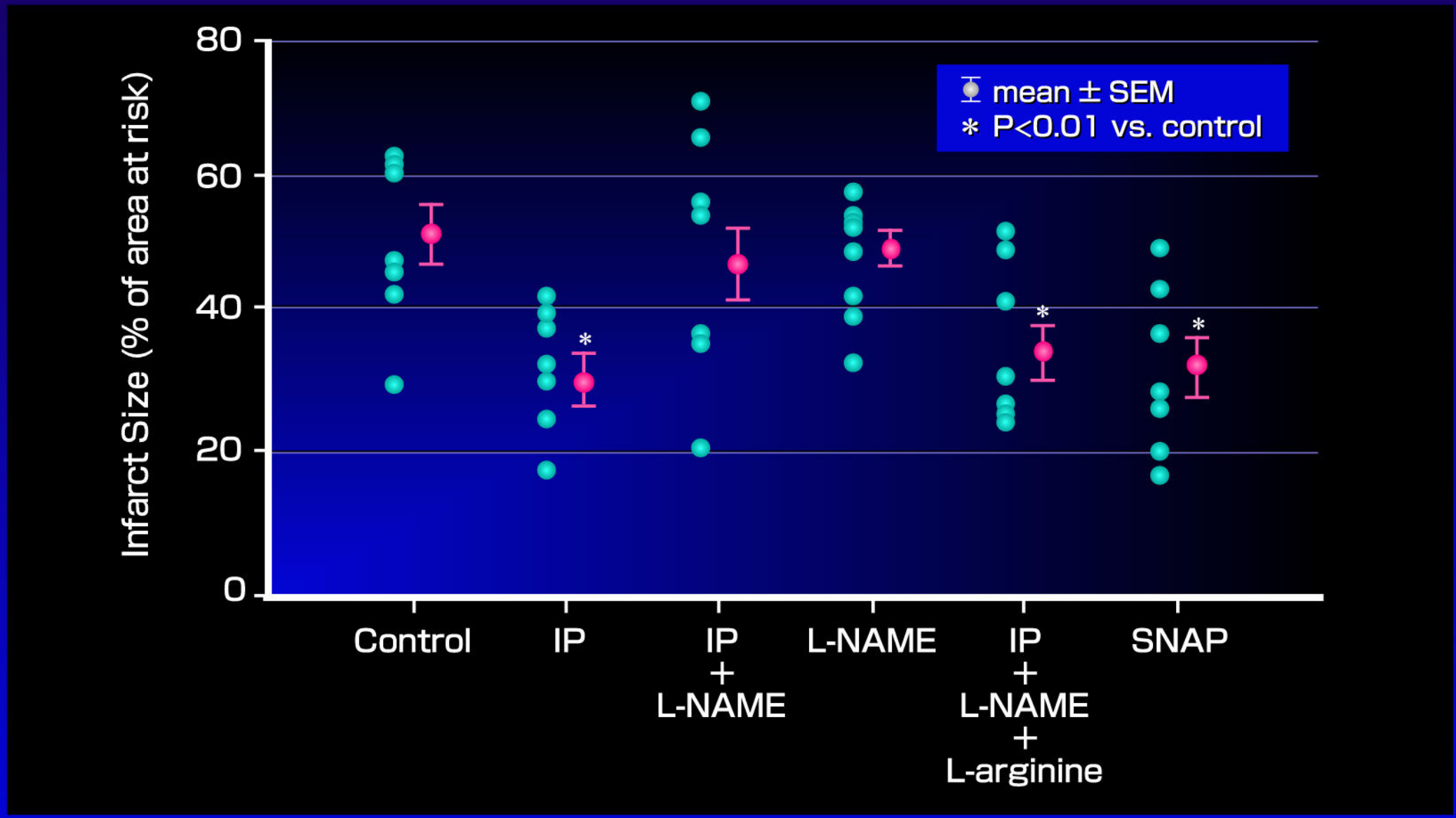


Endogenous Adenosine Mediates Ischemic Preconditioning



(*G.S. Liu et al. Circulation, 84 : 350, 1991*)

The Infarct Size-limiting Effects of the Second Window Ischemic Preconditioning



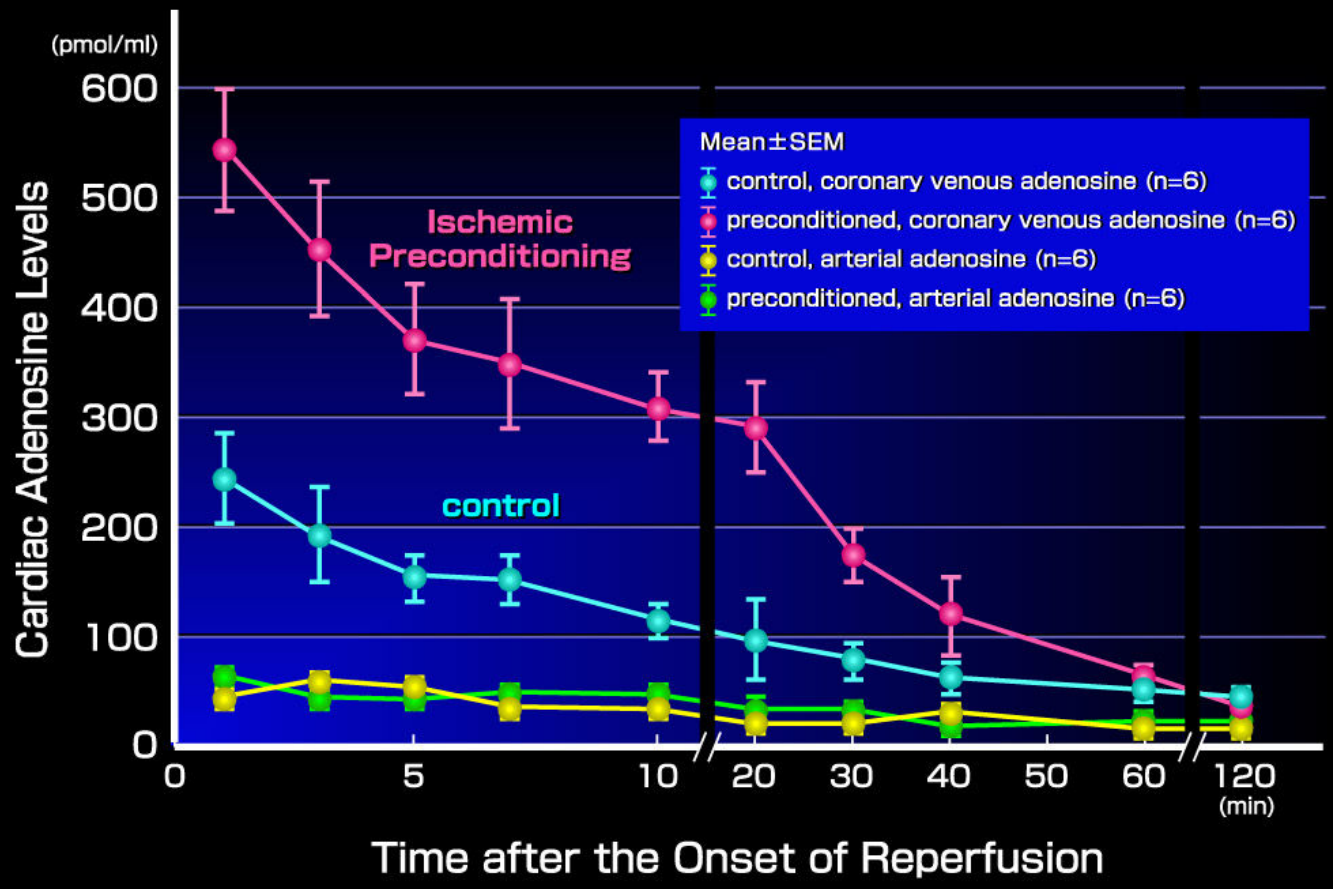
Characteristics of Preconditioning

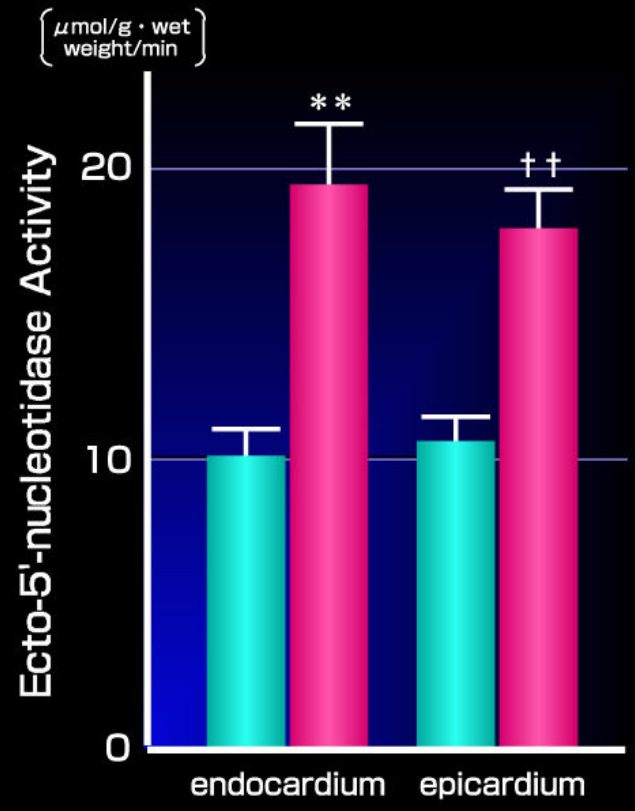
	Early PC	Late PC
ischemic stress	5 min	5 min
timing	immediately after	24-48hours later
duration	several hours	1～2days
trigger	adenosine/PKC	NO/PKC
mediater	• adenosine • K_{ATP} channels • PKC	• MnSOD • HSP72 • PKC
phosphorylation	+	−
protein synthesis	−	+
cardioprotection	potent	moderate

Cardiovascular Effects of Adenosine and NO

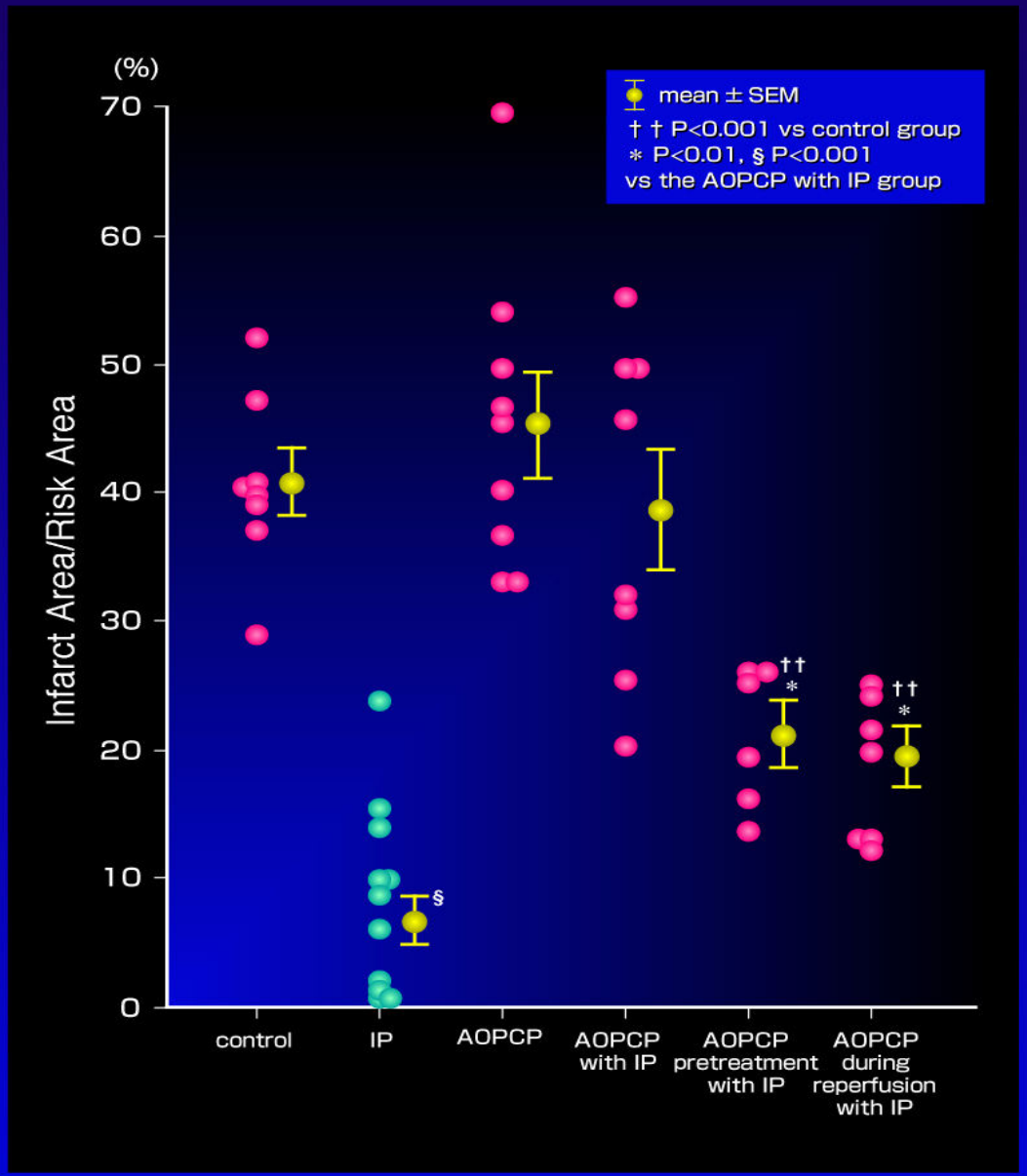
	Adenosine	NO
Coronary smooth muscle	relax	relax
myocardium	negative inotropism	negative inotropism
Sympathetic nerve	↓ NE release	↓ NE release
Platelet	↓ aggregation	↓ aggregation
Leukocytes	↑ chemotoxis ↓ O_2^-	↓ O_2^-
RAS system	↓ renin release	↓ renin release
cytokine	↓ production	↓ production
proliferation of smooth muscle	inhibitory	inhibitory
proliferation of endothelium	stimulatory	stimulatory

5'-Nucleotidase Activity and Adenosine Release following 40 minutes of Myocardial Ischemia with and without Ischemic Preconditioning

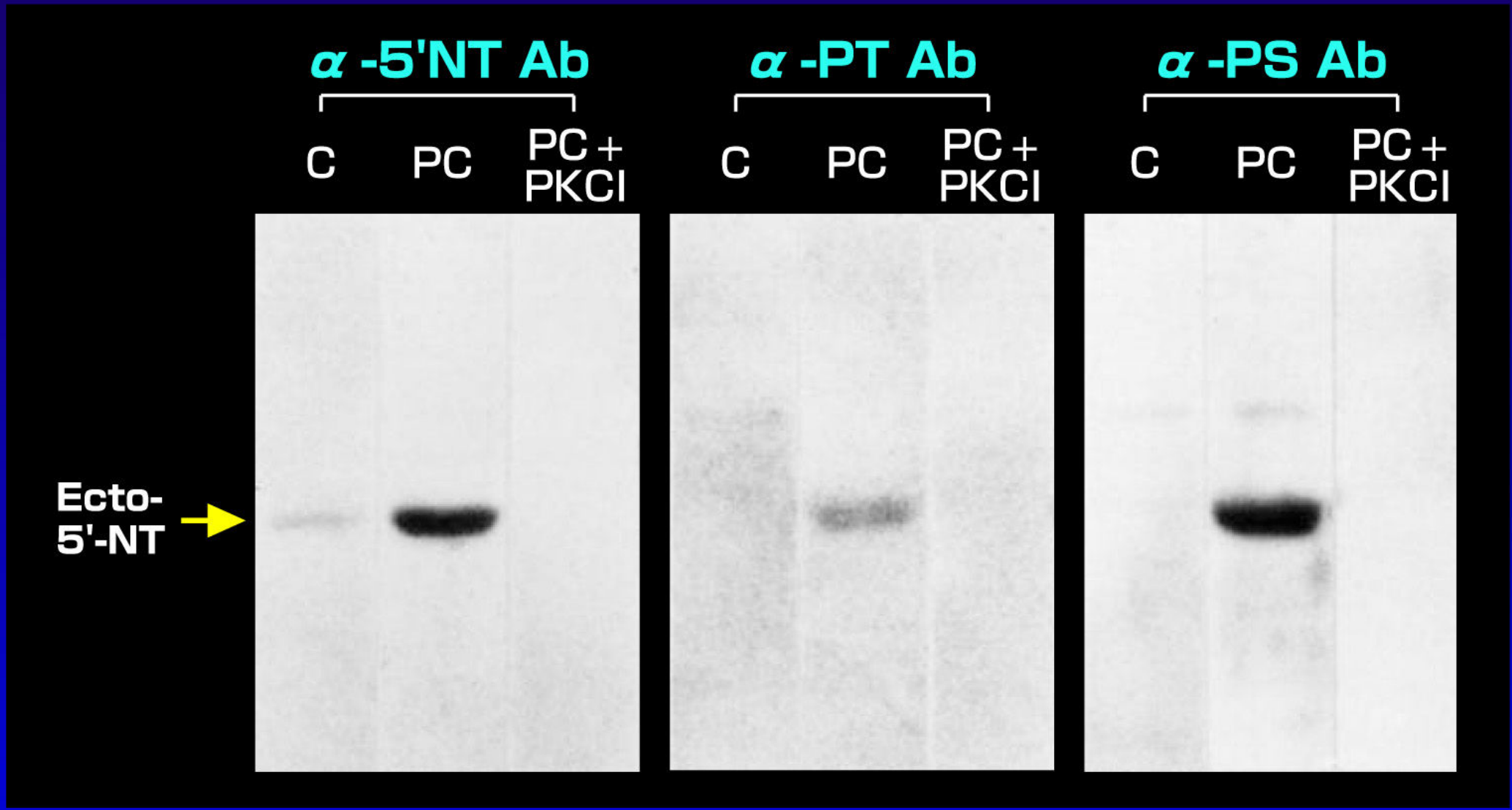




Infarct Size-Limiting Effect of Ischemic Preconditioning



Threonine and Serine Phosphorylation of Immunoprecipitated Ecto-5'-nucleotidase

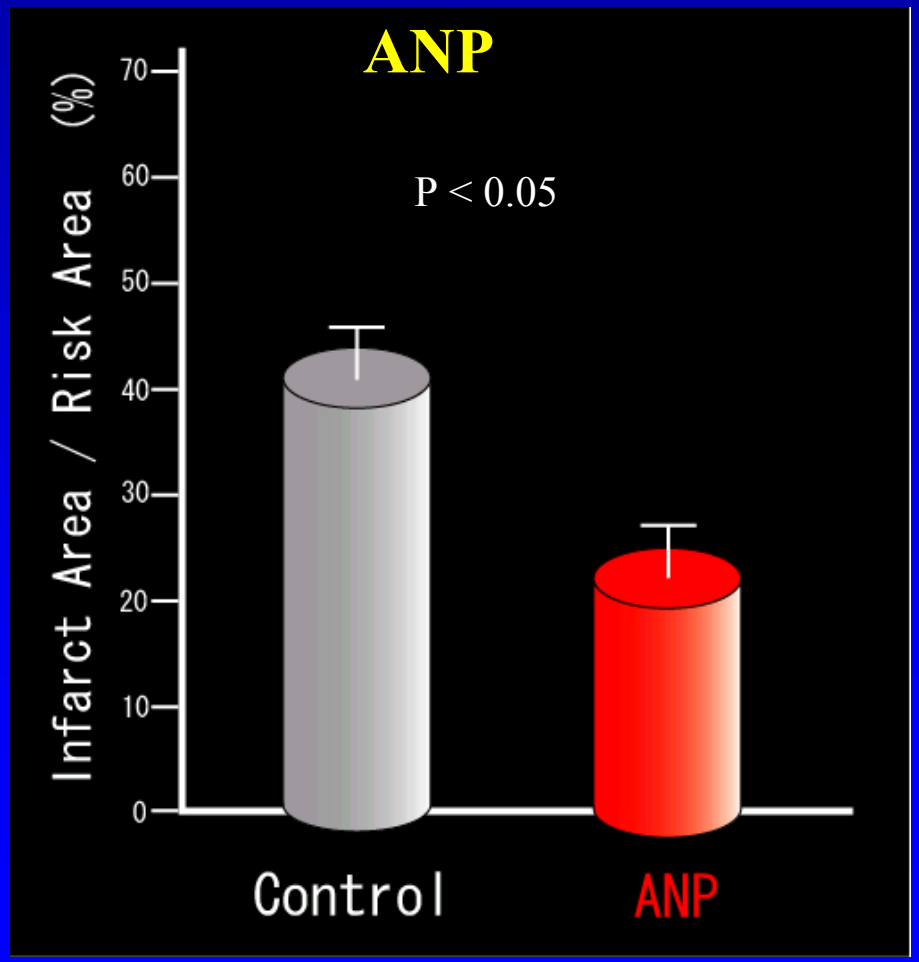


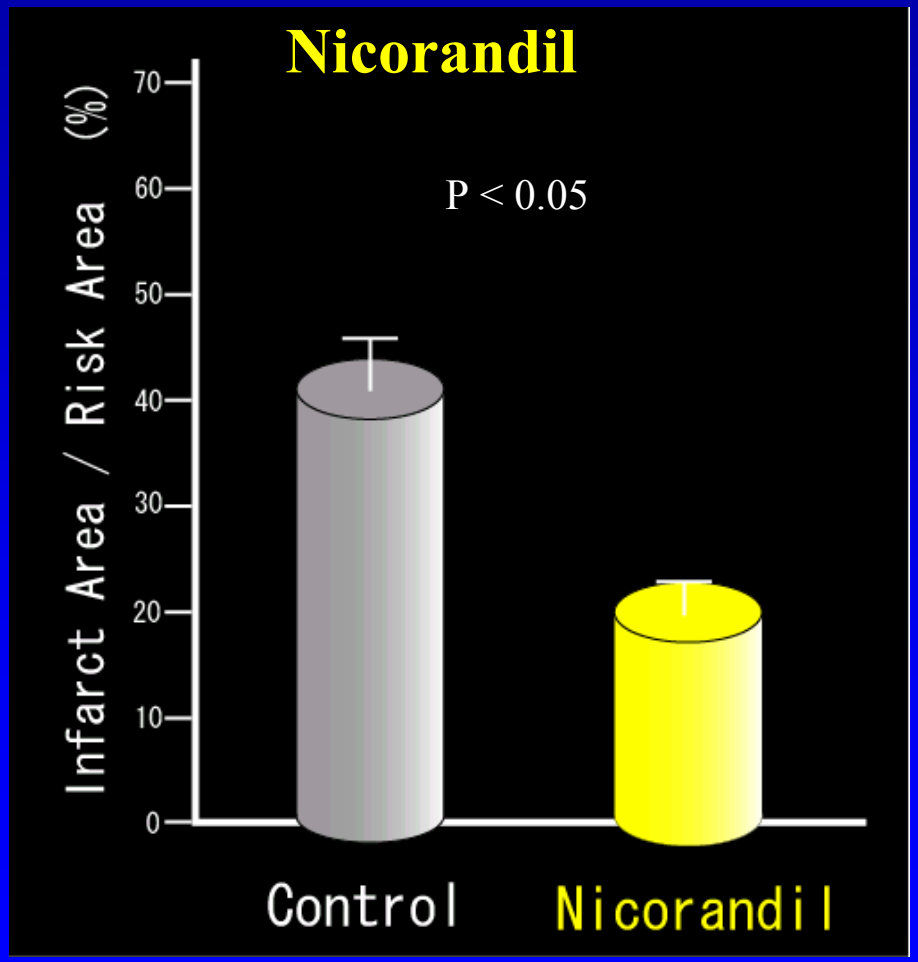
Cardiovascular Effects of Adenosine and NO

	Adenosine	NO
Coronary smooth muscle	relax	relax
myocardium	negative inotropism	negative inotropism
Sympathetic nerve	↓ NE release	↓ NE release
Platelet	↓ aggregation	↓ aggregation
Leukocytes	↑ chemotoxis ↓ O_2^-	↓ O_2^-
RAS system	↓ renin release	↓ renin release
cytokine	↓ production	↓ production
proliferation of smooth muscle	inhibitory	inhibitory
proliferation of endothelium	stimulatory	stimulatory

Cardioprotective effect of ANP / Nicorandil

—canine infarction-reperfusion model—

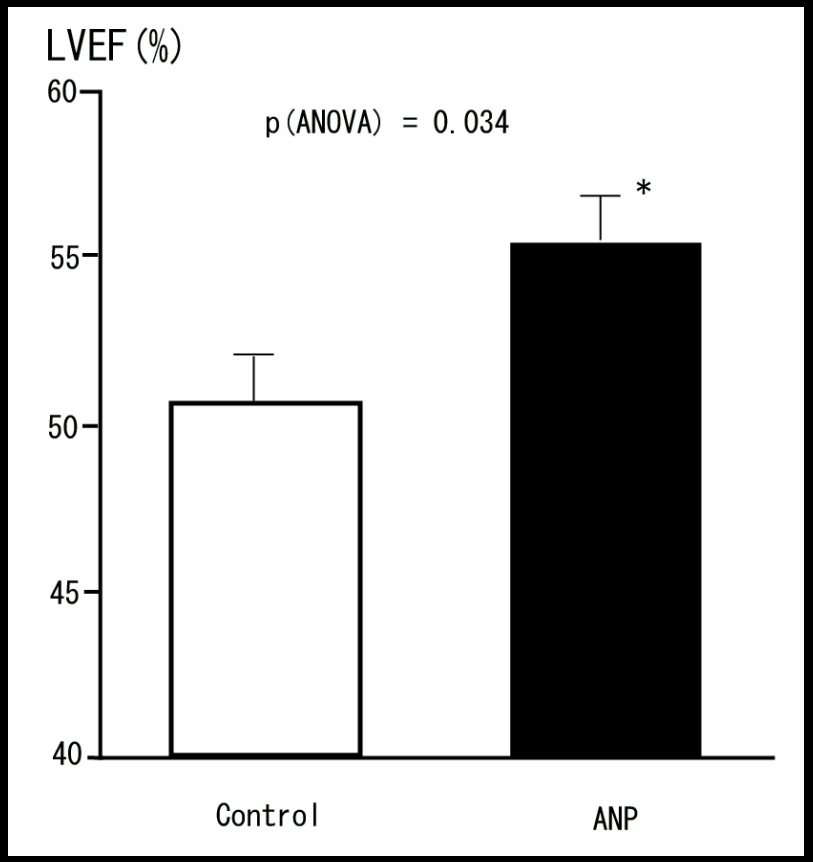




Cardioprotective effect of ANP / Nicorandil 2

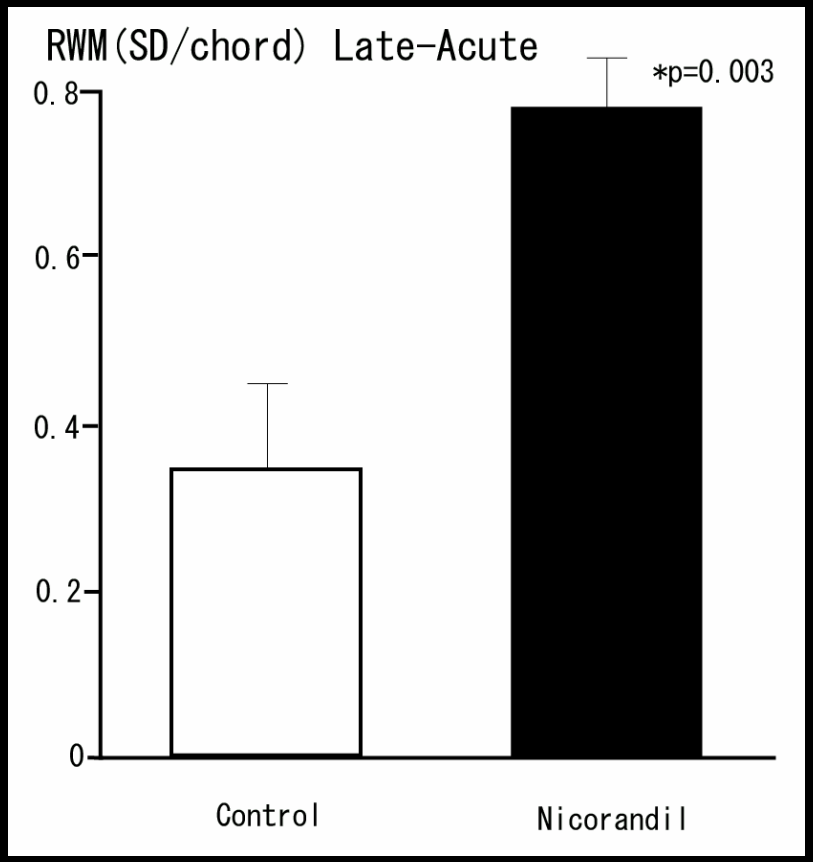
―clinical trial, single center―

ANP



(J Am Coll Cardiol 2001 Jun 1;37(7):1820-1826)

Nicorandil



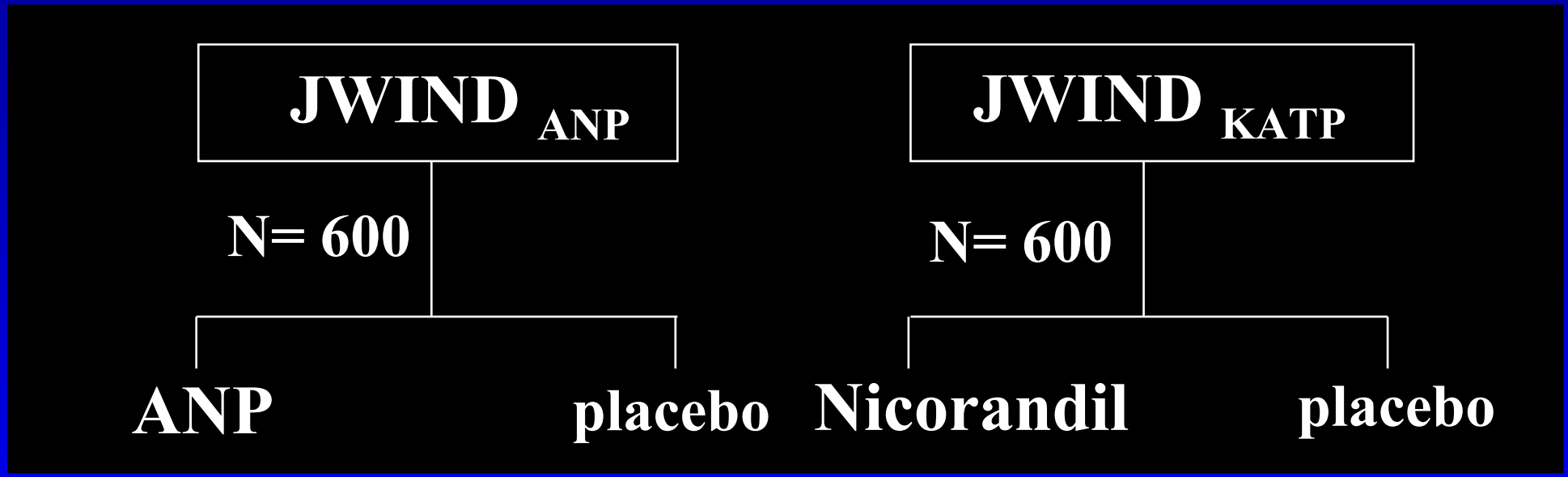
(J Am Coll Cardiol 1999 Mar 1;33(3):654-660)

Large-Scale Trial Using Atrial Natriuretic Peptide or Nicorandil as an Adjunct to Percutaneous Coronary Intervention for ST-Segment Elevation Acute Myocardial Infarction

J-wind

Masafumi Kitakaze*, M.D., Ph.D.
For the J-WIND Study Group
*National Cardiovascular Center, Osaka, Japan

Study Design

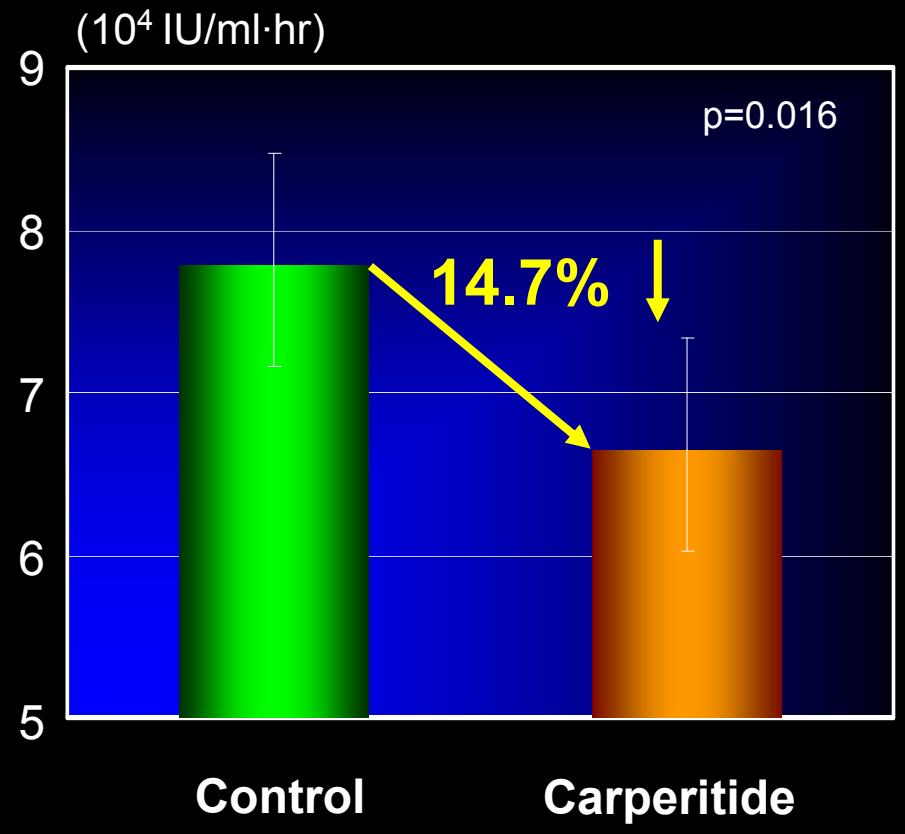


Patients enrollment started on November, 2001, and will continues until September 30, 2005.
Enrolled patients are followed until September 30, 2007.

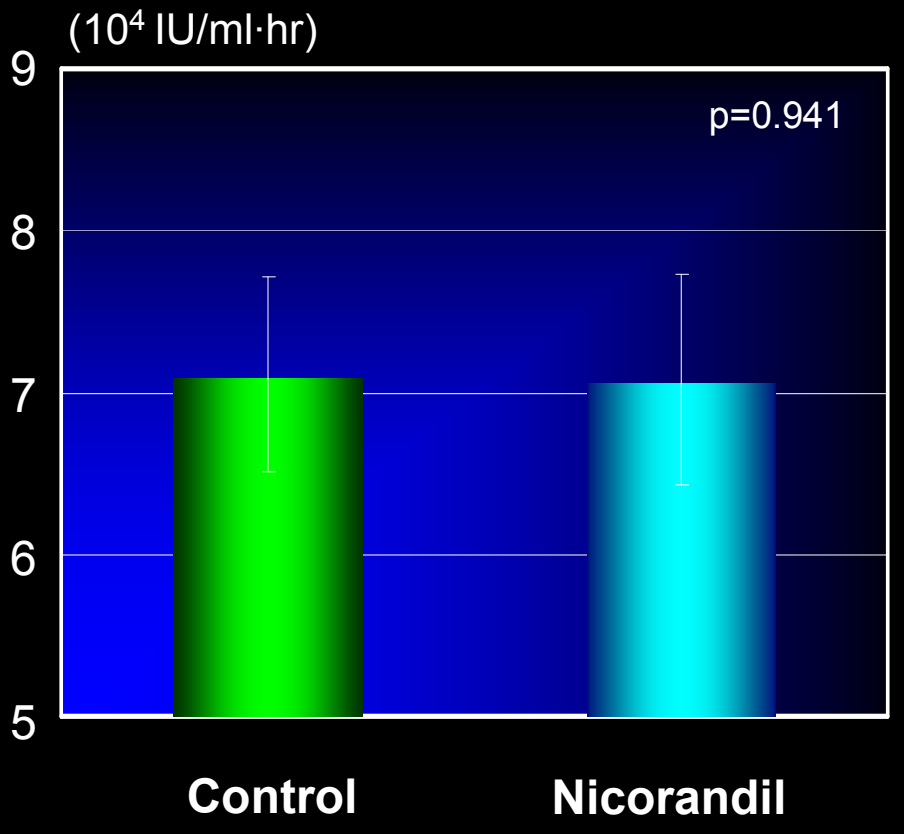
Primary Endpoints

Area under curve of creatine kinase (Σ CK)

Carperitide study



Nicorandil study

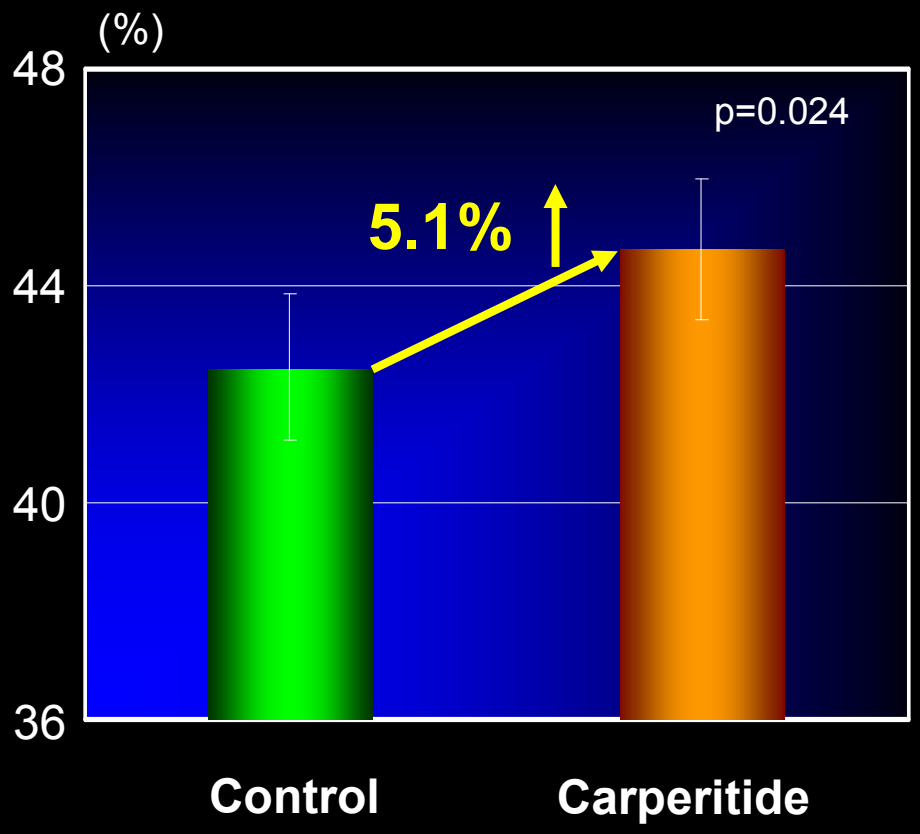


(Lancet Oct 27 issue 2007)

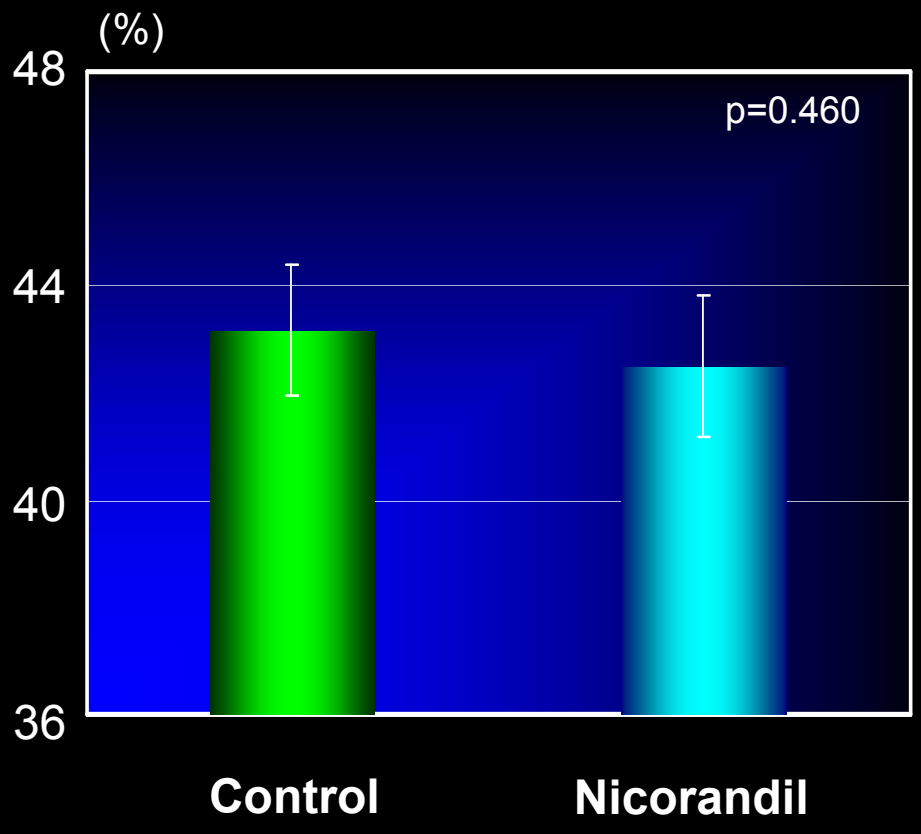
Primary Endpoints

Chronic left ventricular ejection fraction (LVEF)

Carperitide study



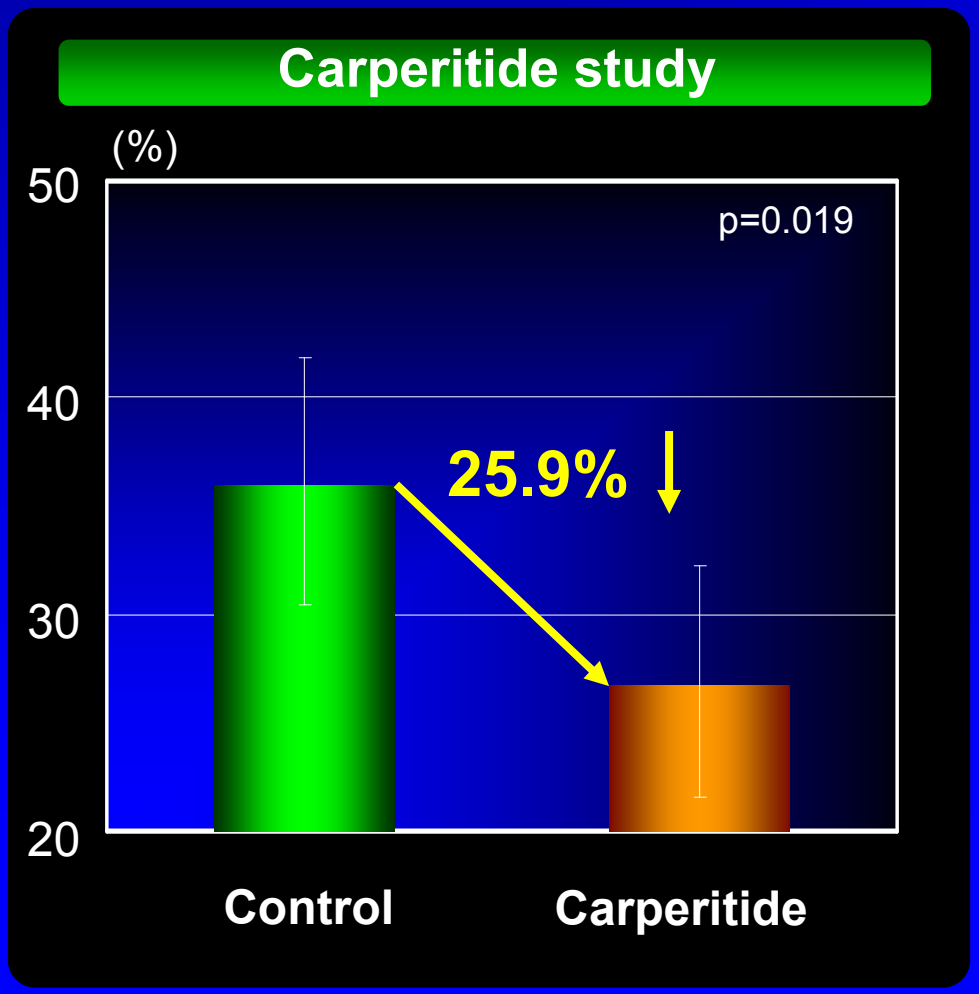
Nicorandil study

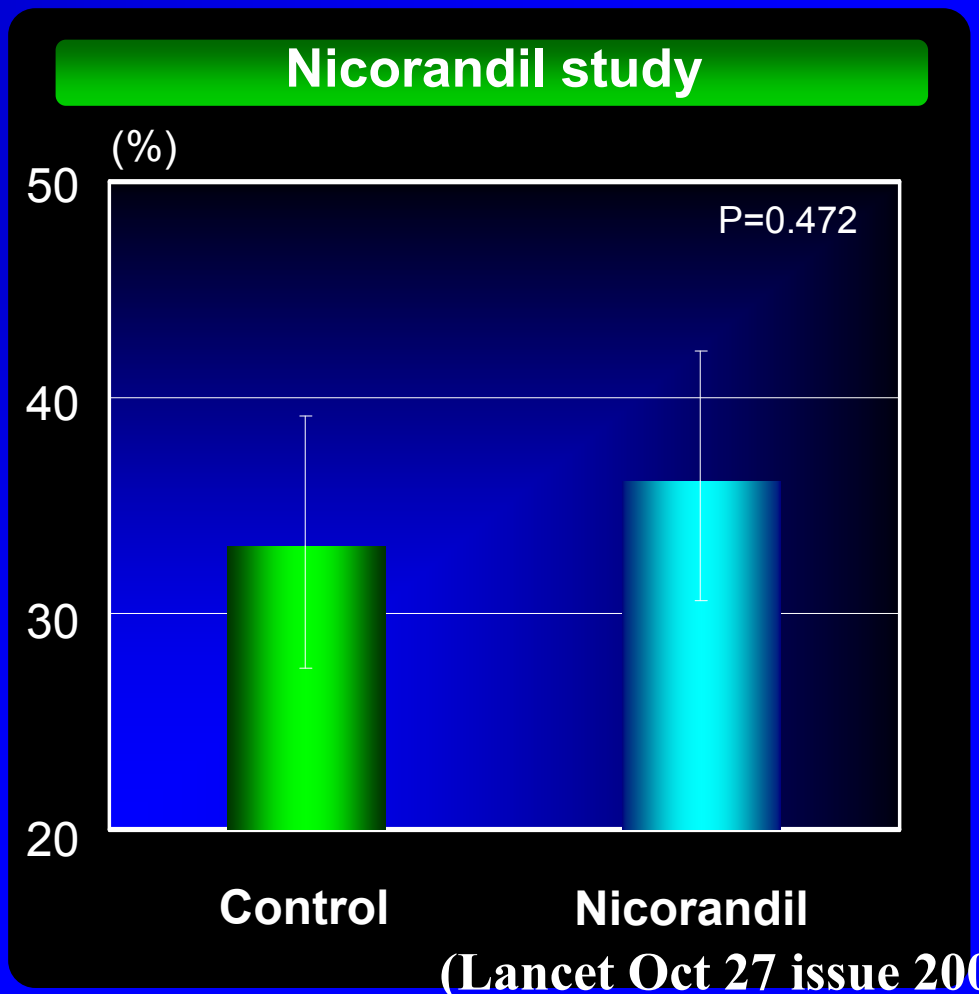


(Lancet Oct 27 issue 2007)

Secondary Endpoints

Incidence of Reperfusion Injury
-malignant ventricular arrhythmia, re-elevation of ST-segment, worsening of chest pain-



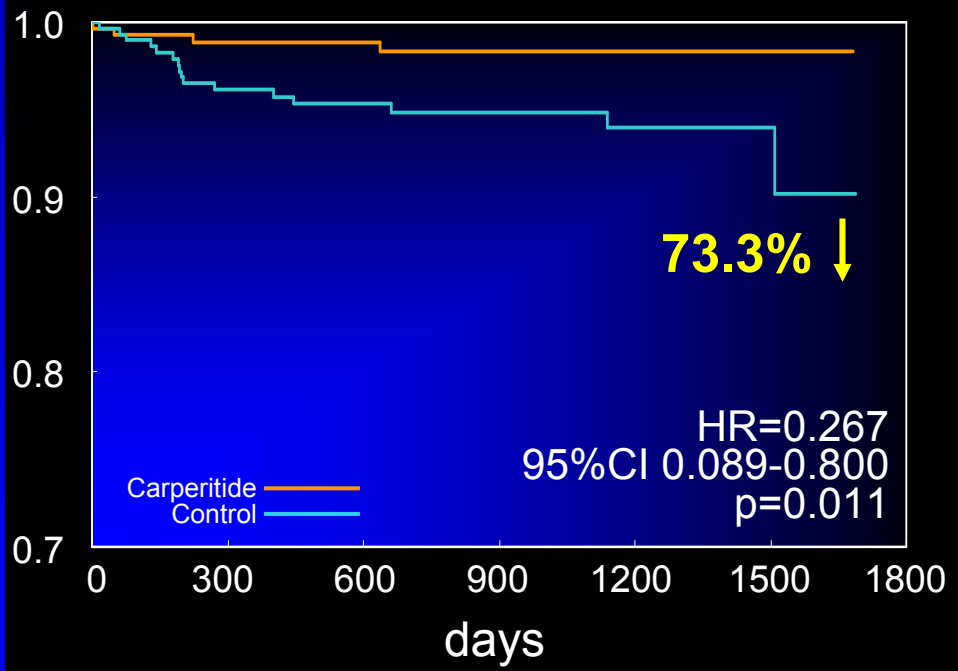


(Lancet Oct 27 issue 2007)

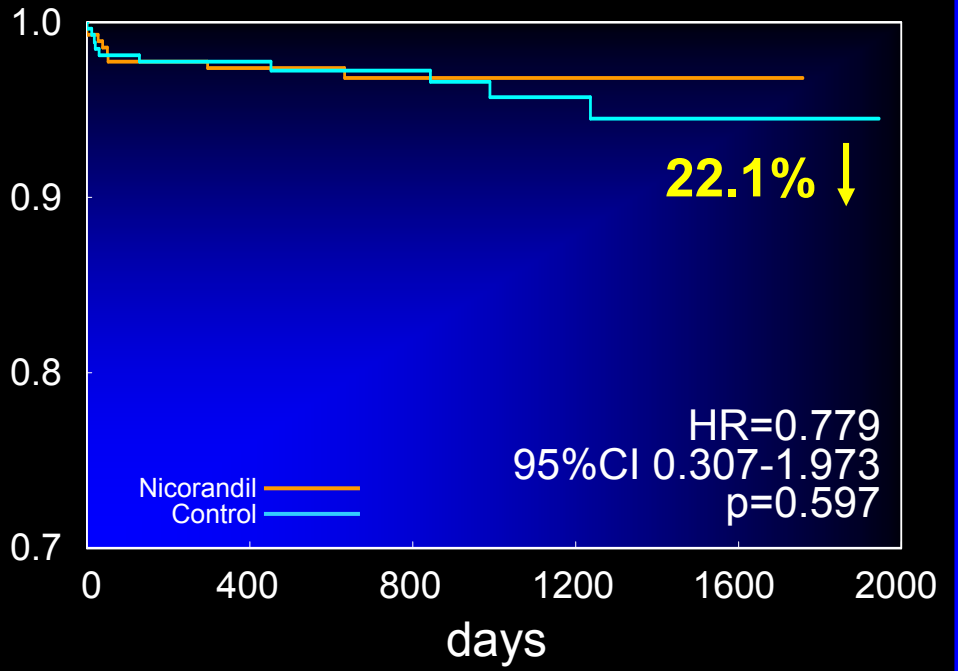
Sub-analysis -1-

Cardiac death & heart failure

Carperitide study



Nicorandil study



(Lancet Oct 27 issue 2007)

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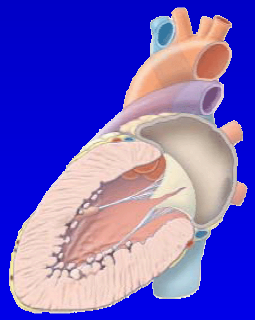
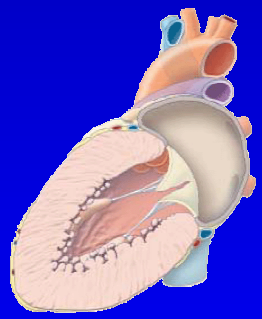
Cardiac Remodeling

-Transition From Ischemic to Failing Heart -

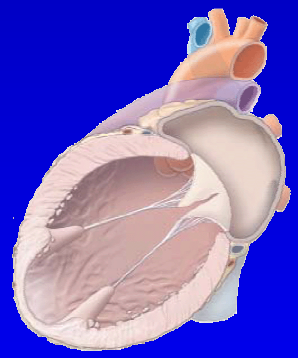
Cardiac Myocyte Apoptosis



Normal Heart



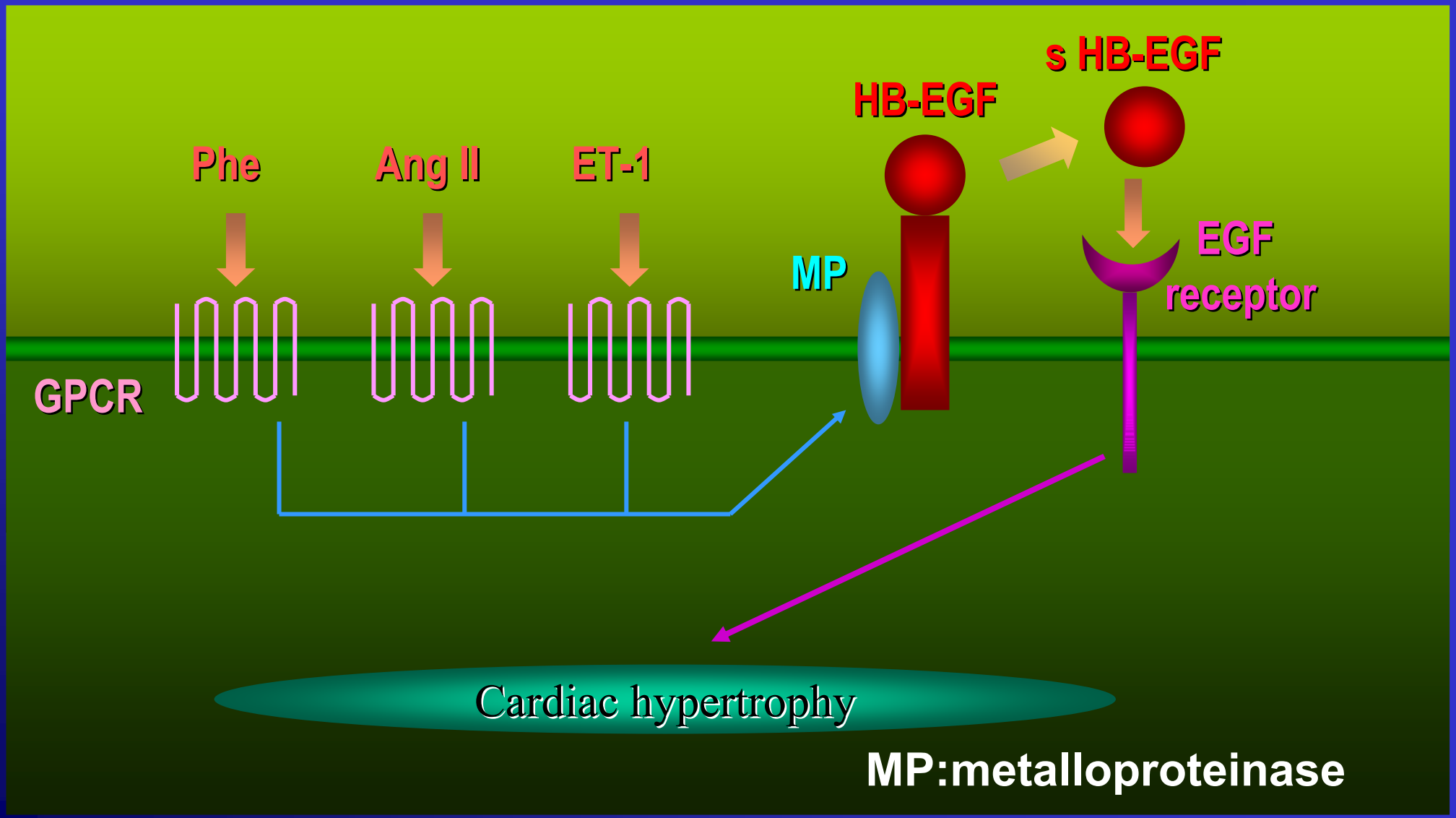
Ischemic Heart



Failing Heart

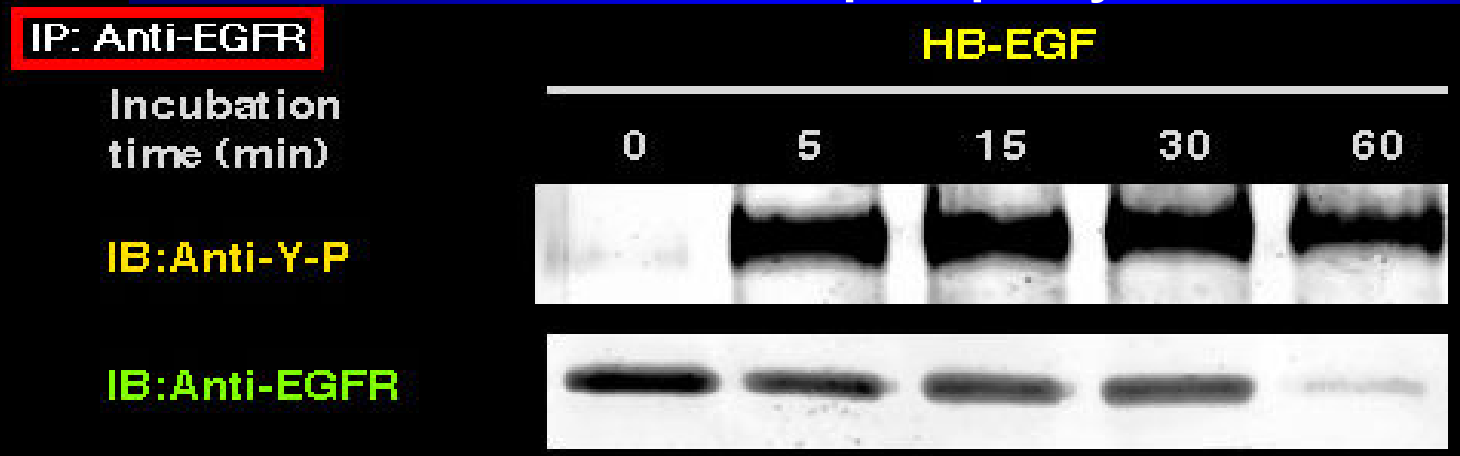
Cardiac Myocyte Hypertrophy

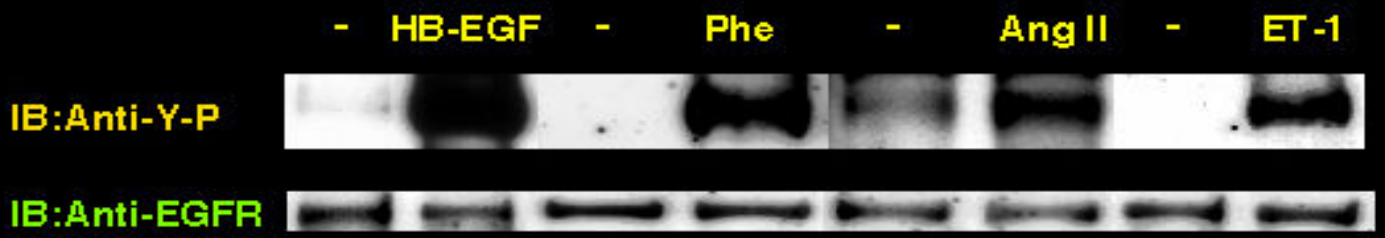
The Hypothesis for Cellular Mechanisms of Cardiac Hypertrophy

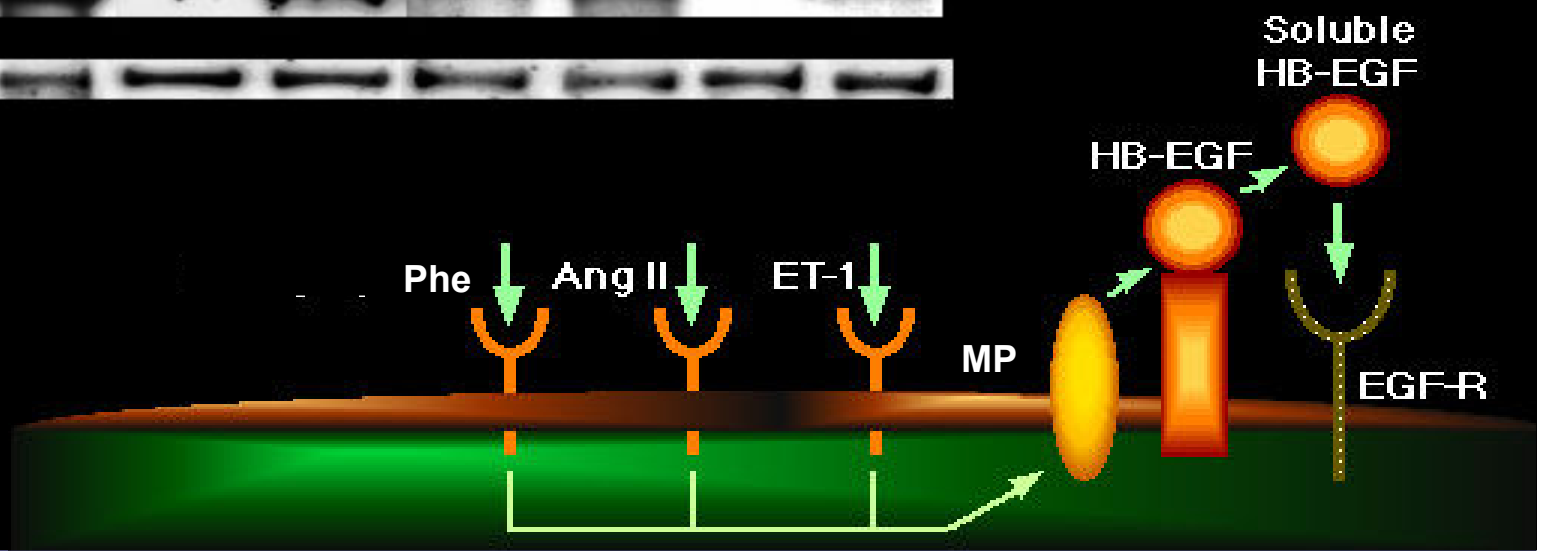


EGFR Transactivation in Rat Cardiomyocytes

EGFR phosphorylation

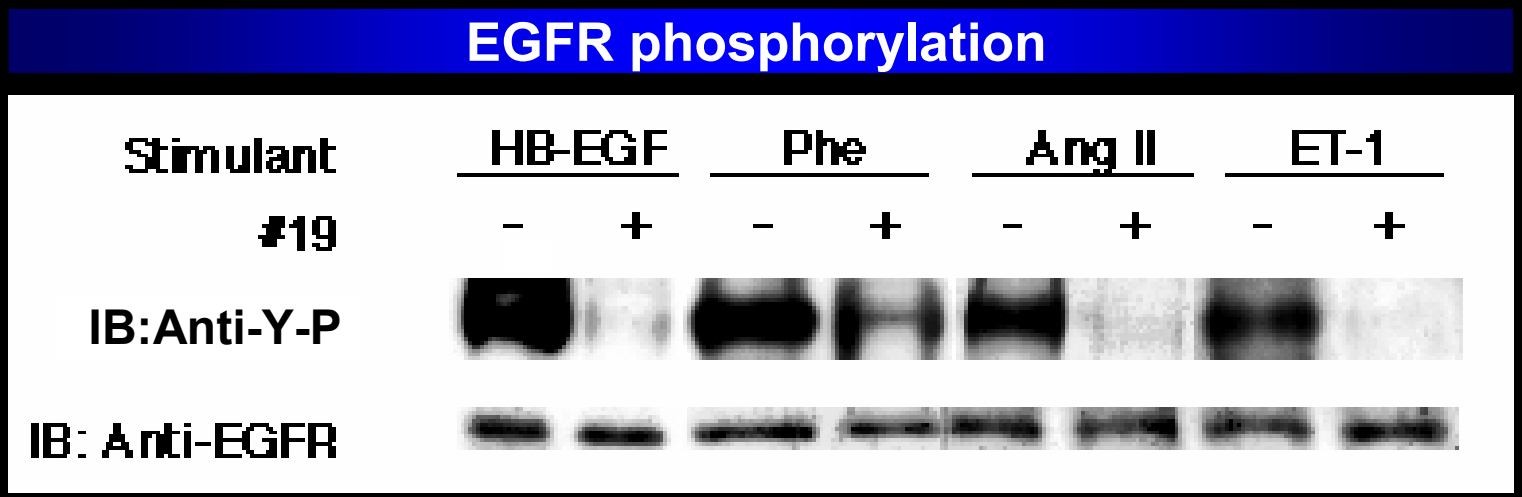






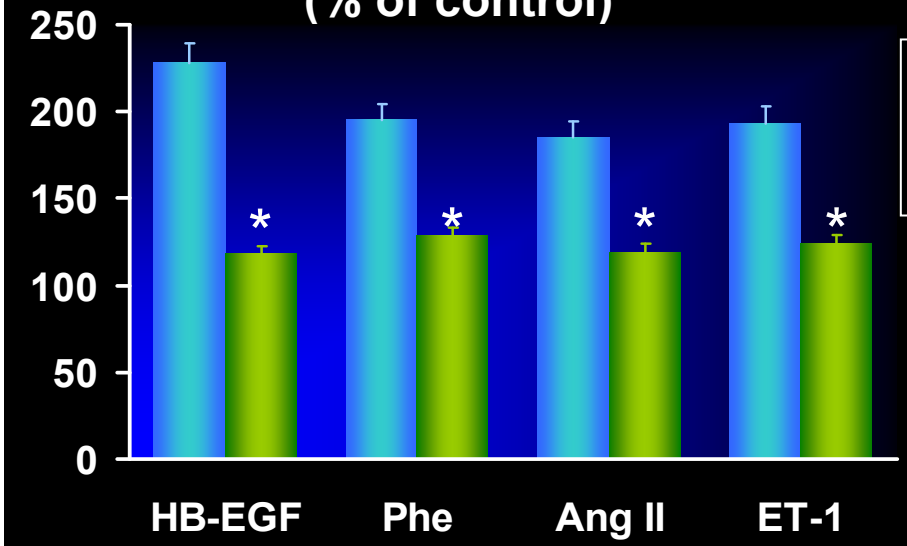
Neutralizing Antibody against HB-EGF Abolishes Cardiac Hypertrophic Signaling in Rat Cardiomyocytes

EGFR phosphorylation



IP: Anti-EGFR

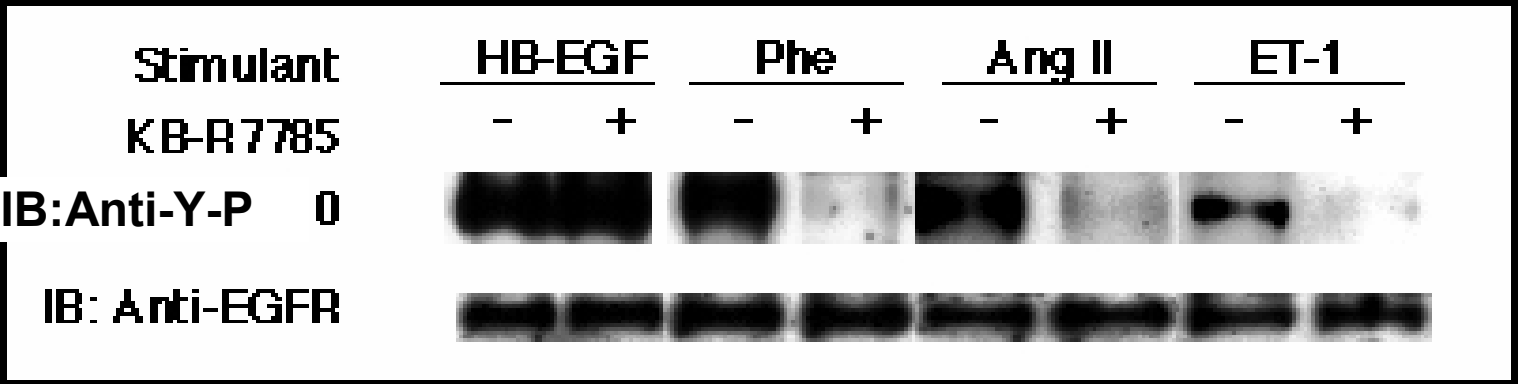
Protein synthesis



*: p<0.05 vs no treatment

The Metalloproteinase Inhibitor KB-R7785 Abolishes Cardiac Hypertrophic Signaling in Rat Cardiomyocytes

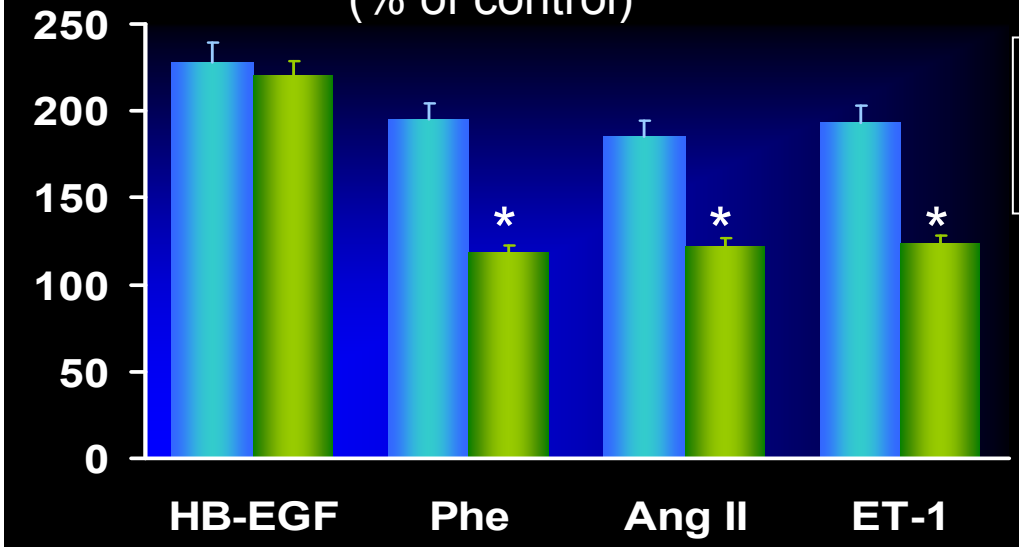
EGFR phosphorylation



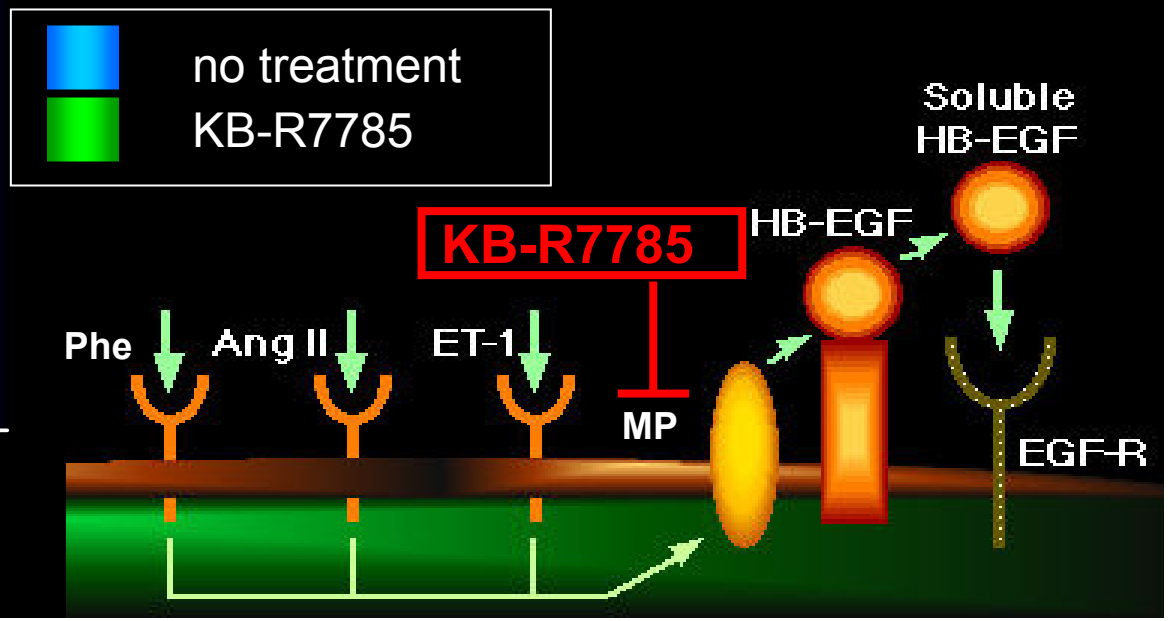
IP: Anti-EGFR

Protein synthesis

(% of control)

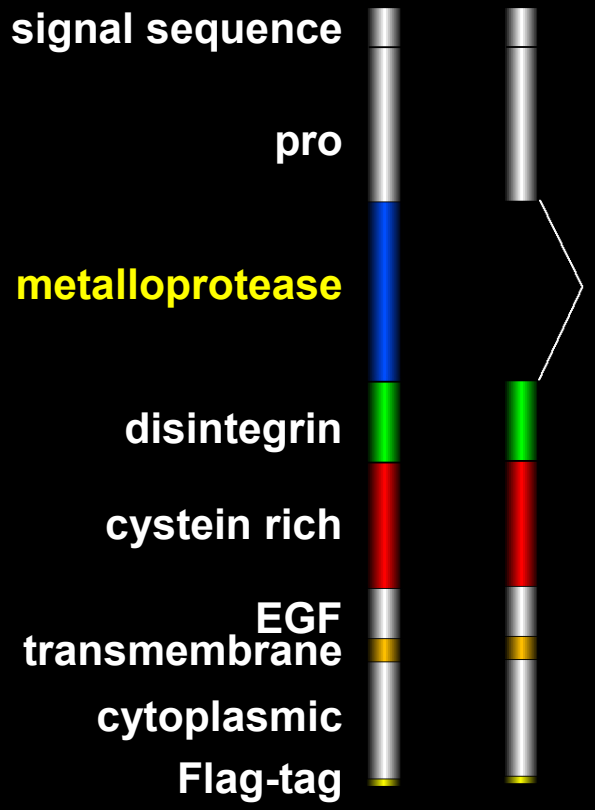


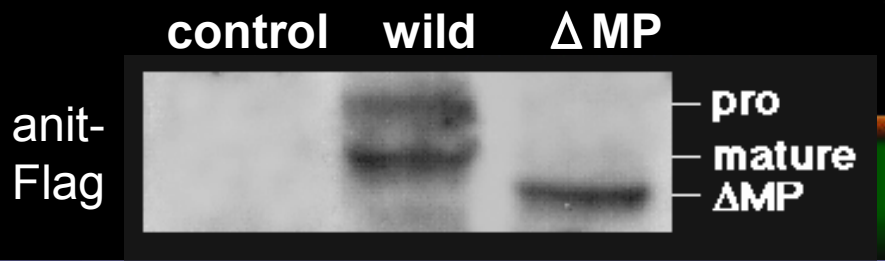
*: p<0.05 vs no treatment



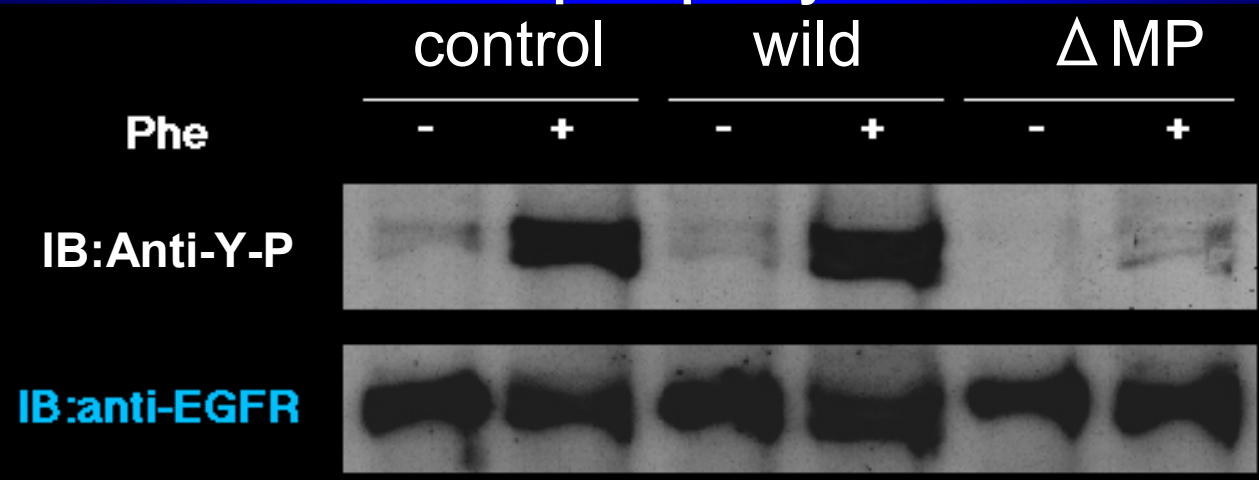
ADAM12 sheds HB-EGF in Rat Cardiomyocytes

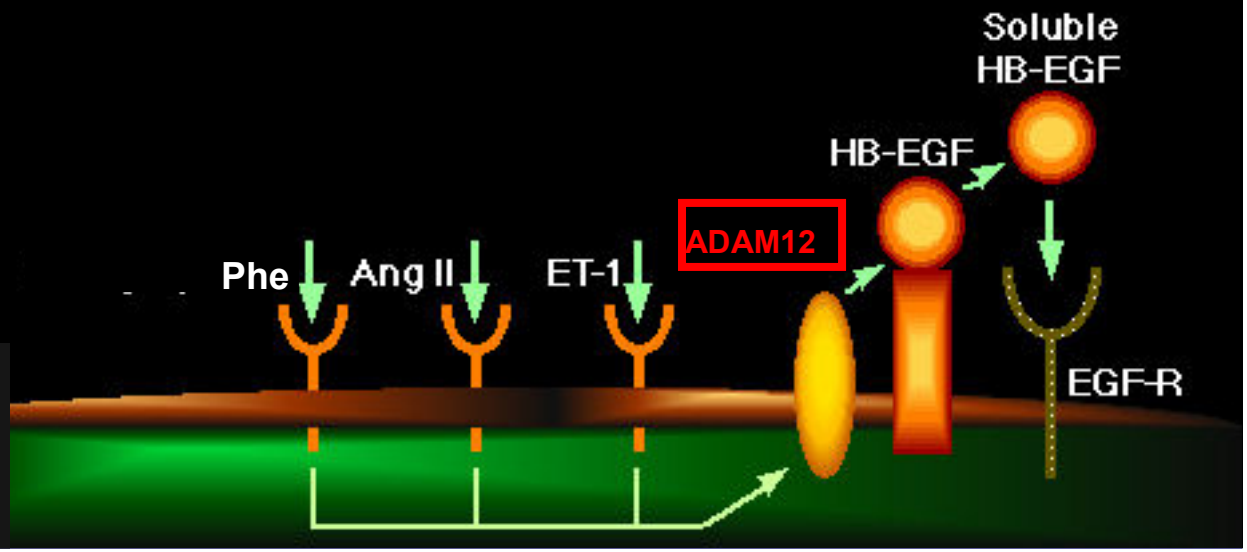
ADAM12
(a disintegrin and metalloprotease)





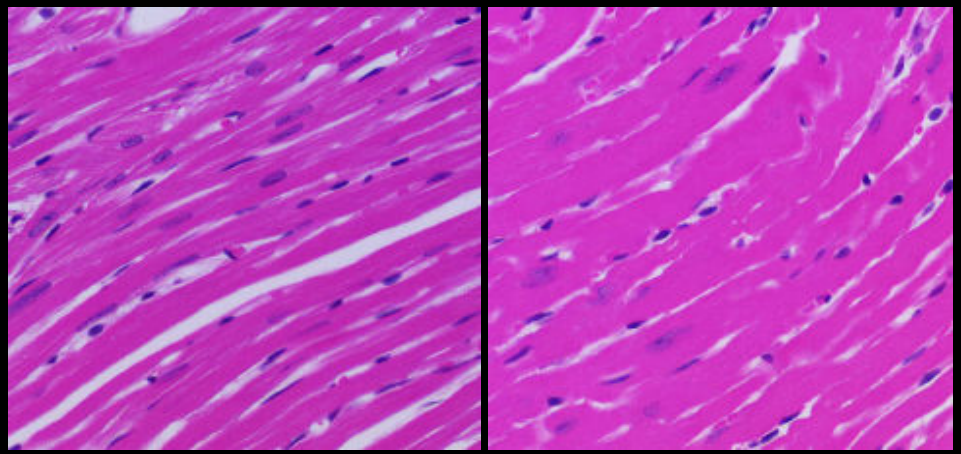
EGFR phosphorylation

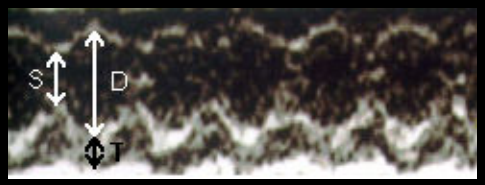


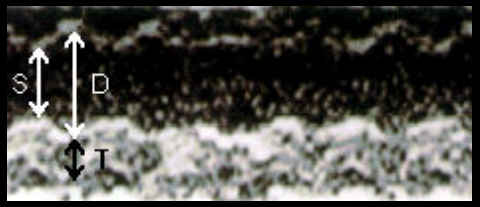


KB-R7785 Attenuates Cardiac Hypertrophy Induced by Aortic Banding in Mice

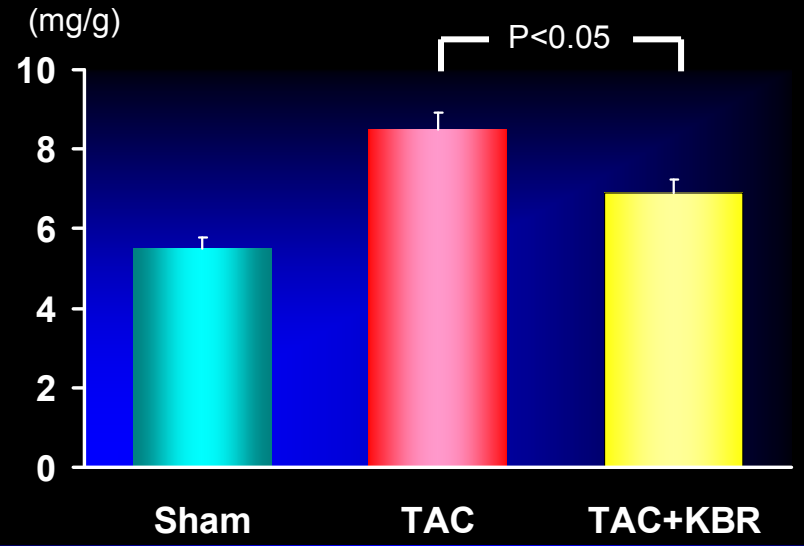
KB-R7785 Vehicle



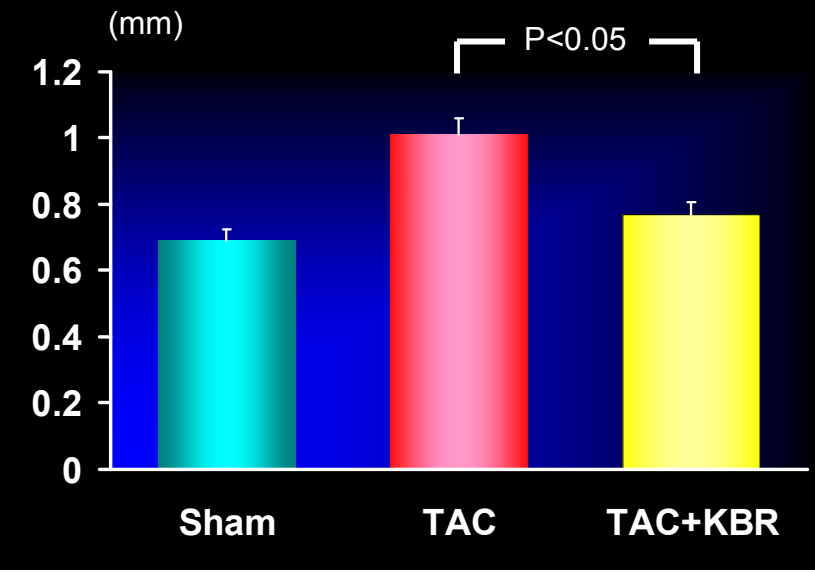




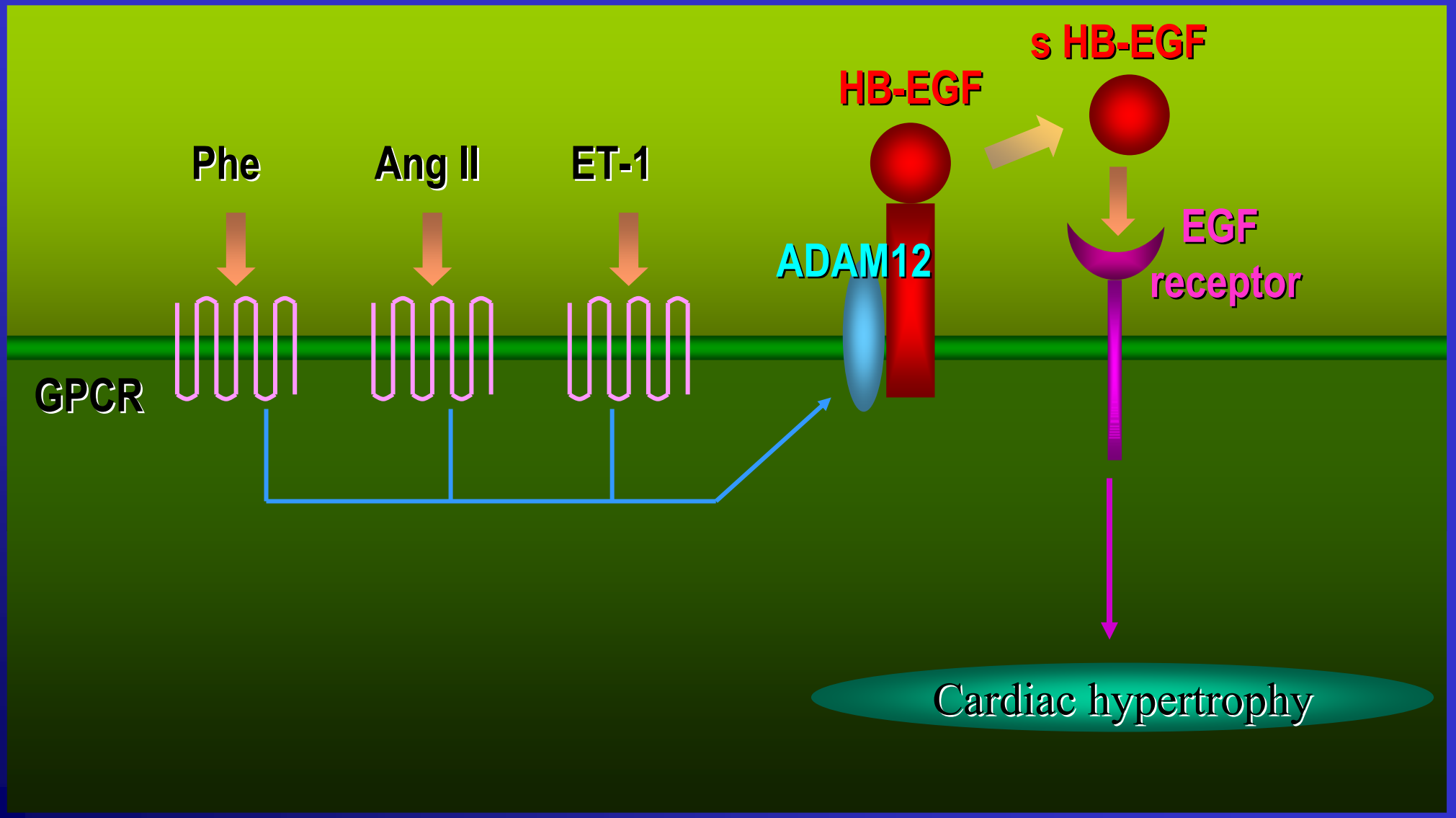
Heart weight/Body weight



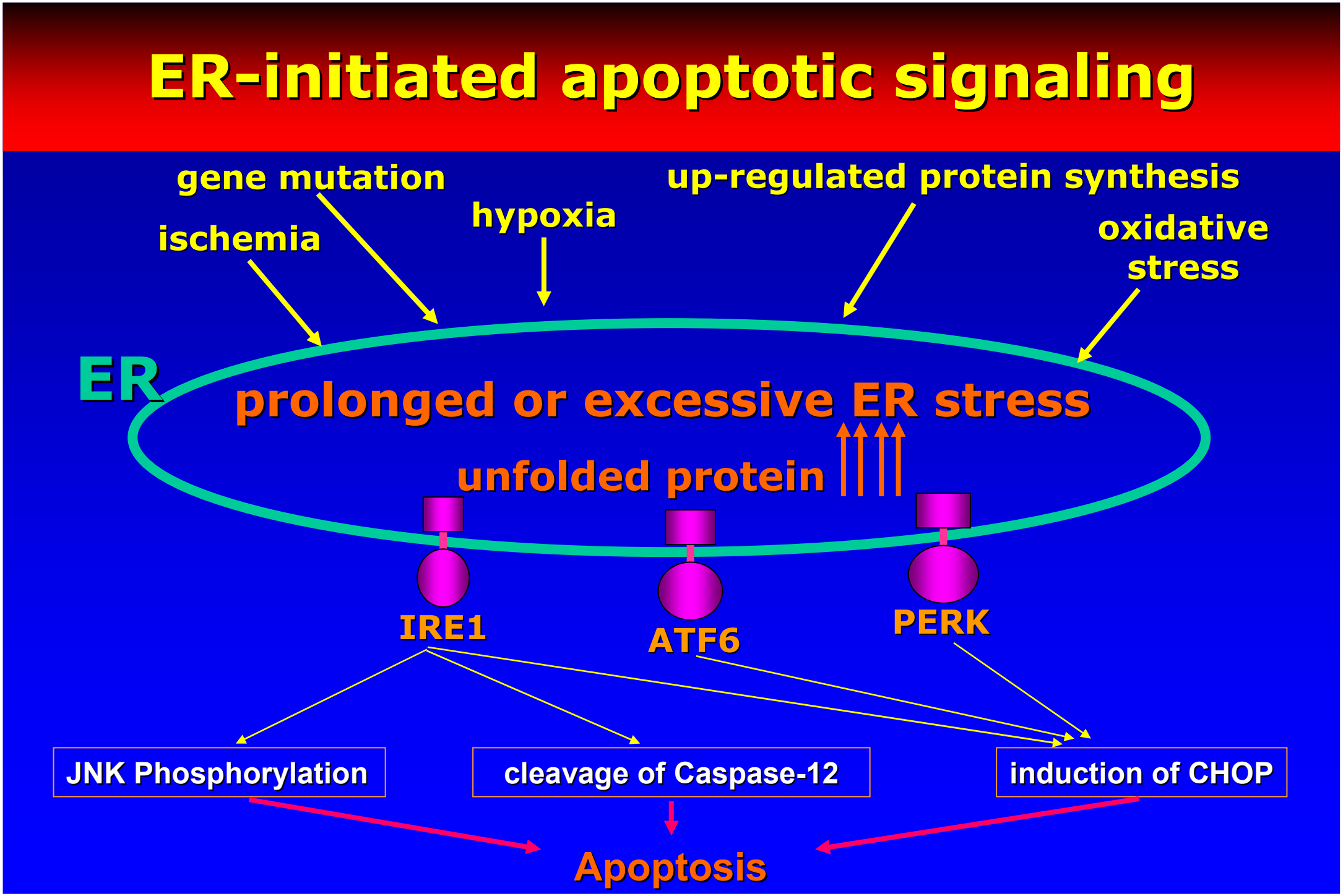
Posterior wall thickness



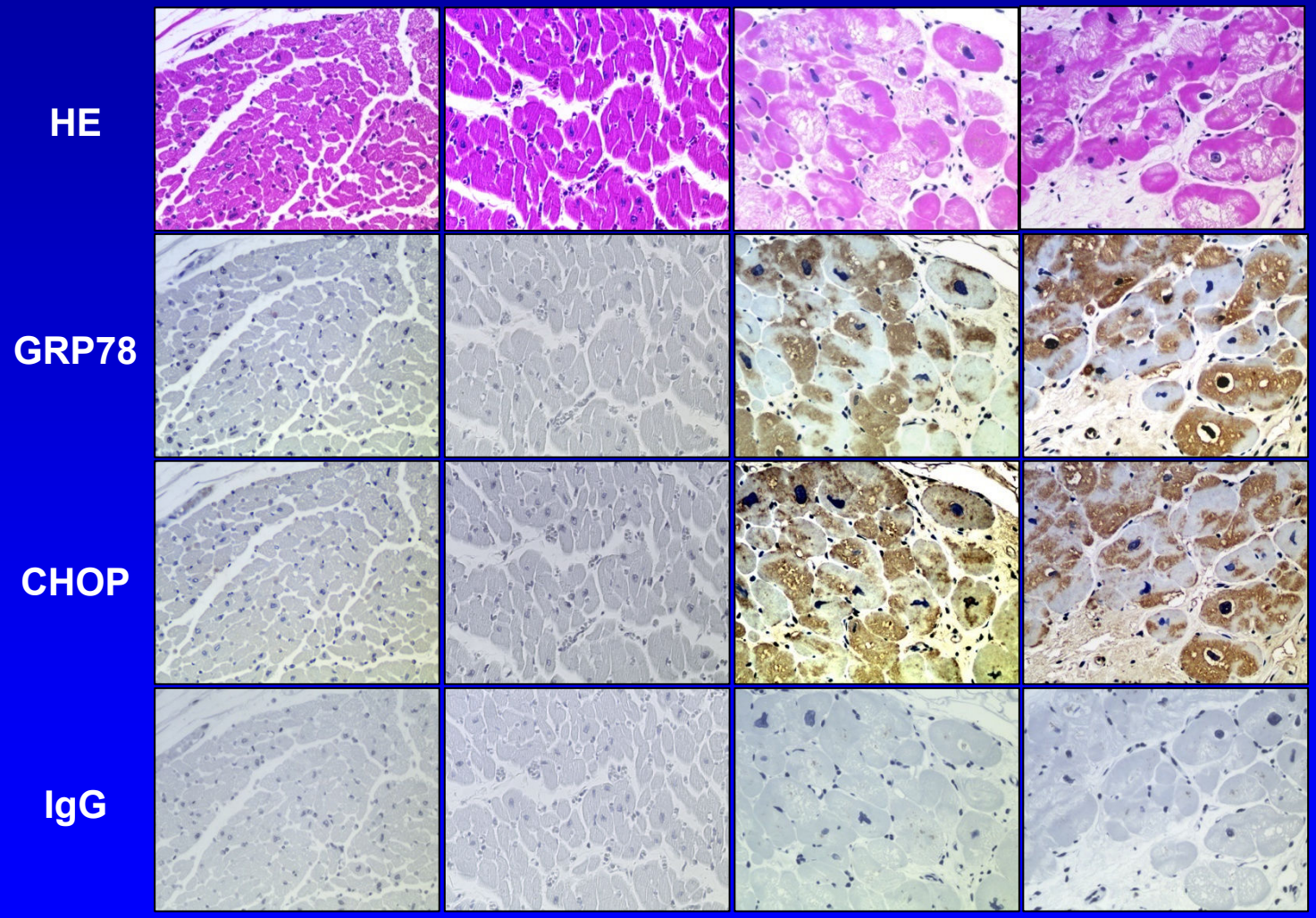
The Hypothesis for Cellular Mechanisms of Cardiac Hypertrophy



ER-initiated apoptotic signaling



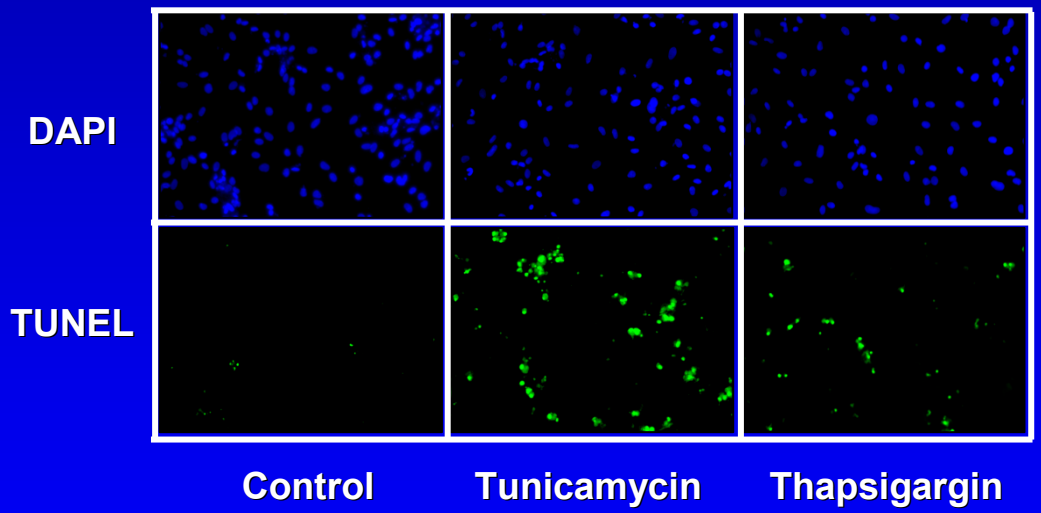
Induction of ER Stress in Failing Human Hearts with Dilated Cardiomyopathy



National Cardiovascular Center

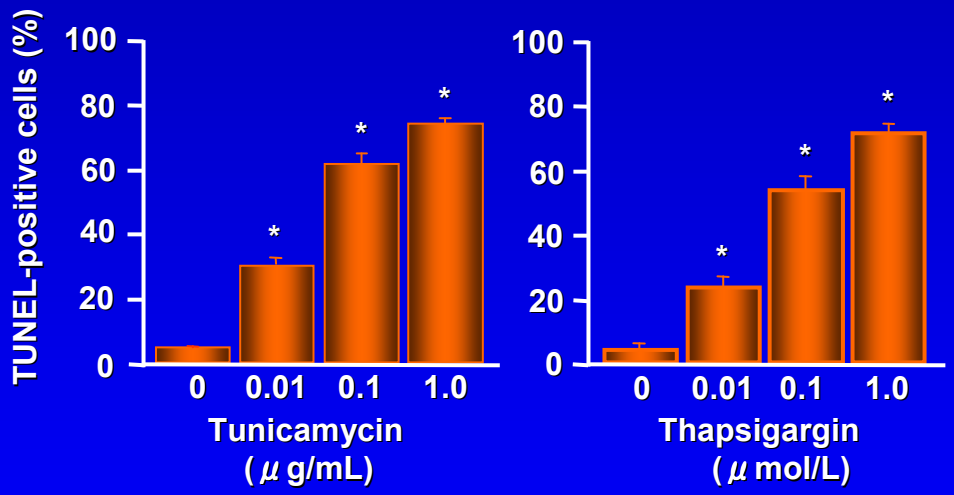
Either Tunicamycin or Thapsigargin Induced Apoptosis in Neonatal Rat Cardiac Myocytes

TUNEL Assay

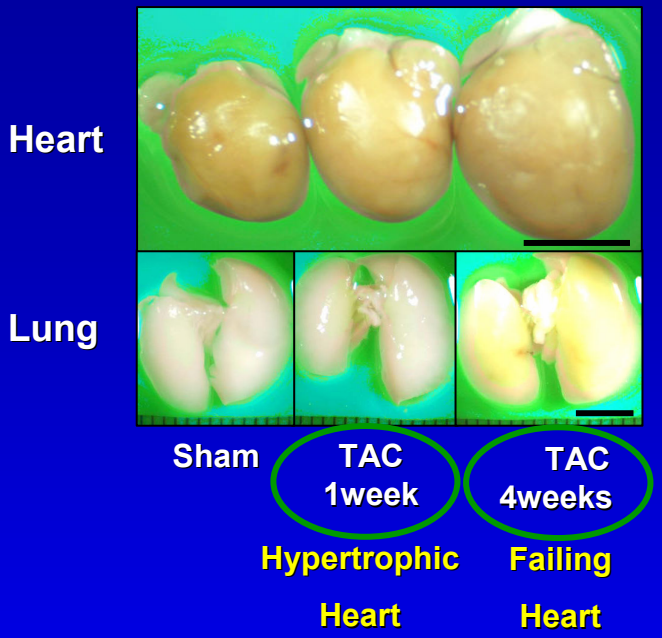


TUNEL-Positive Cells

* P<0.05 vs. control

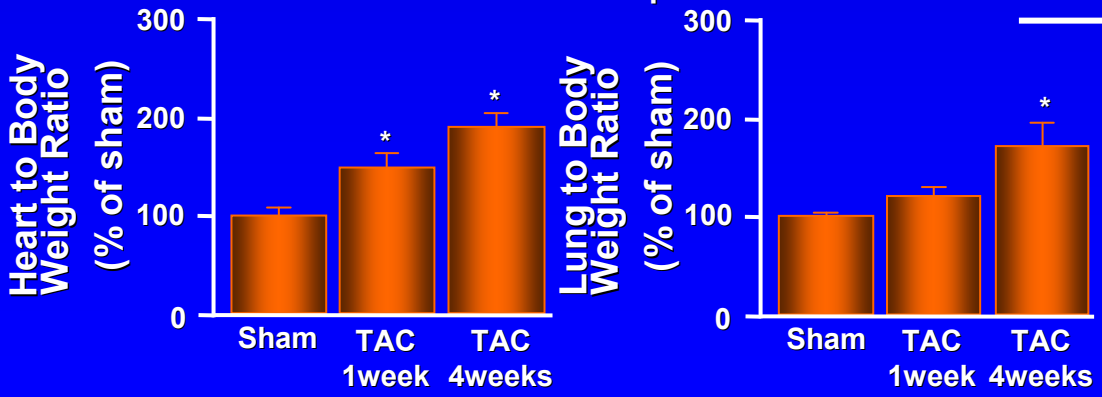


The Mice Model of Hypertrophic and Failing Hearts

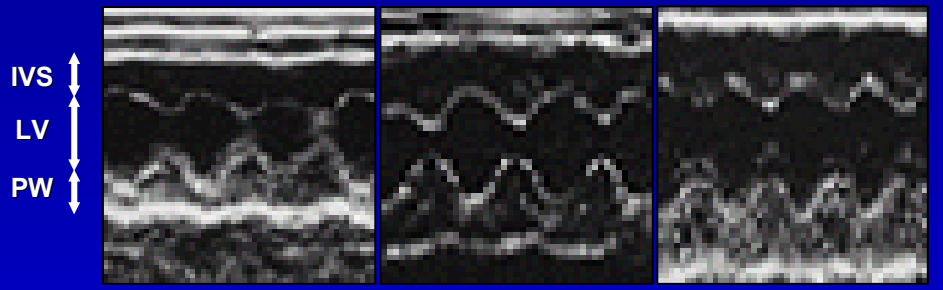


Heart weight/ Lung weight

*P<0.05 vs. sham-operation.



Echocardiographic data

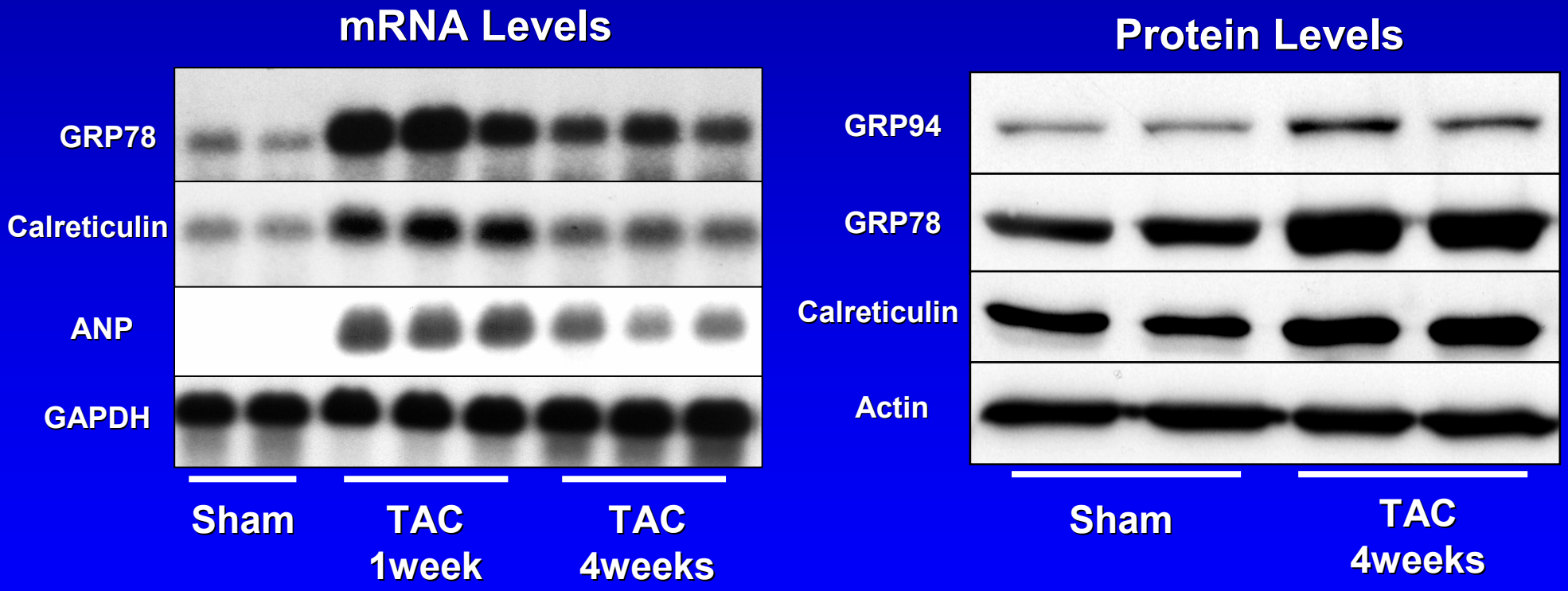


	Sham (n=7)	TAC, 1week (n=9)	TAC, 4weeks (n=10)
IVS (mm)	0.52±0.01	0.81±0.02 *	0.99±0.01 *
PW (mm)	0.53±0.01	0.75±0.01 *	0.97±0.02 *
LVDd (mm)	2.85±0.05	2.84±0.09	3.15±0.06 *
LVDs (mm)	1.52±0.07	1.50±0.05	1.89±0.04 *
%FS	46.4±1.8	47.2±1.0	40.0±1.0 *

*P<0.05 vs. sham-operation. Data are expressed as means ± SEM.

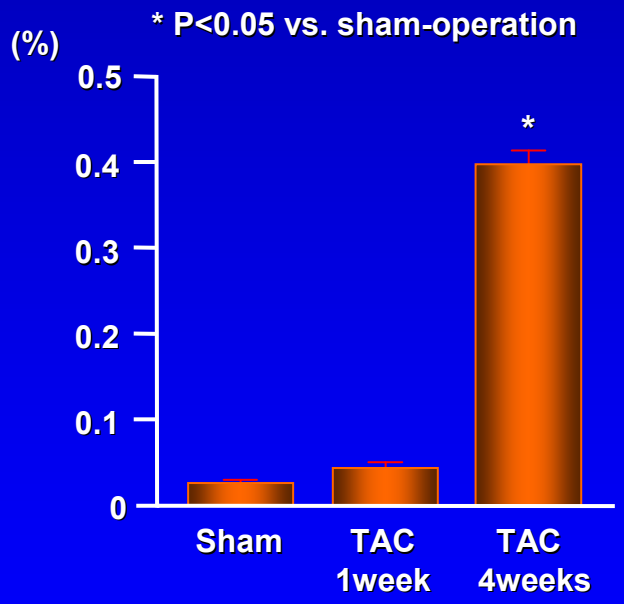
National Cardiovascular Center

Induction of ER Stress in Either Hypertrophic or Failing Hearts

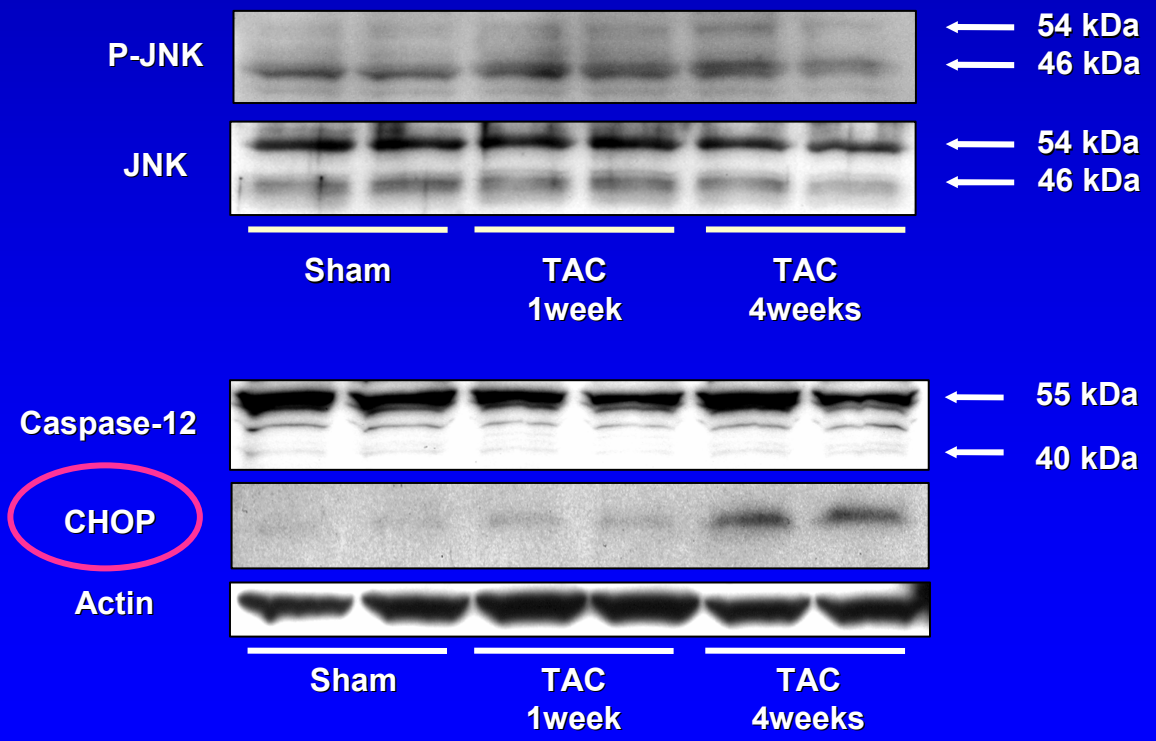


Induction of ER-initiated Apoptosis in Failing Heart following Aortic Constriction of Mice

TUNEL-positive Cells



ER-initiated Apoptotic Signaling Pathway



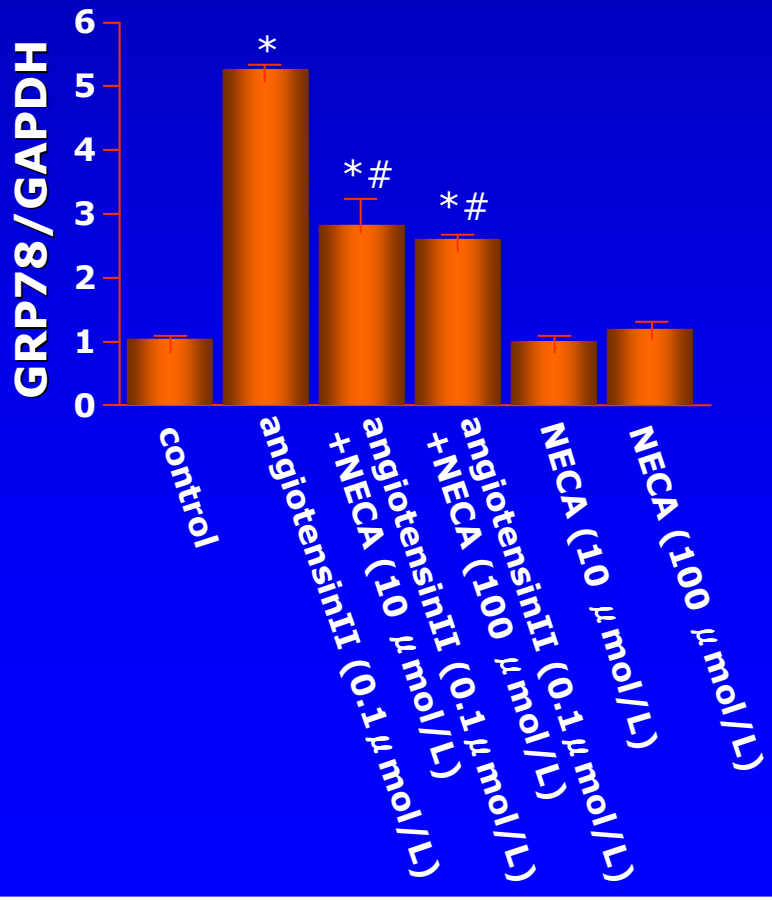
National Cardiovascular Center

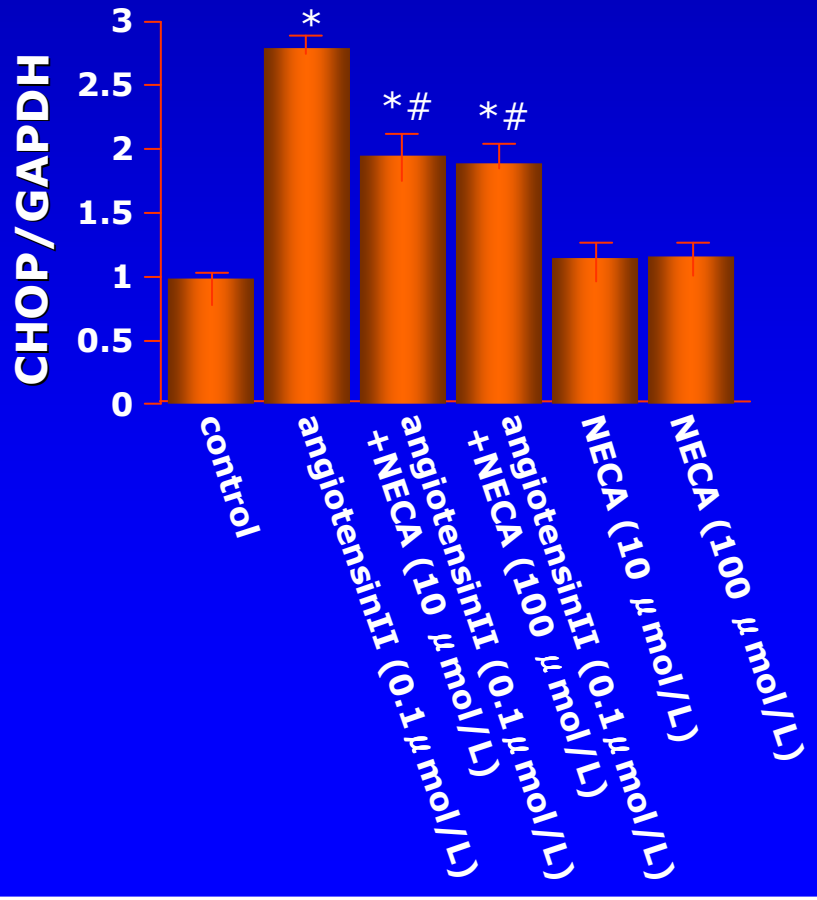
Adenosine and ER stress

Neonatal rat cardiac myocytes
N=4

*** : p<0.05 versus control**

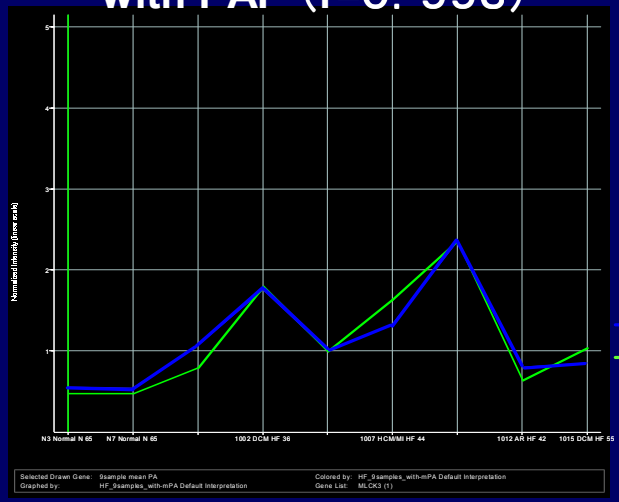
 # : p<0.05 versus angiotensinII treatment



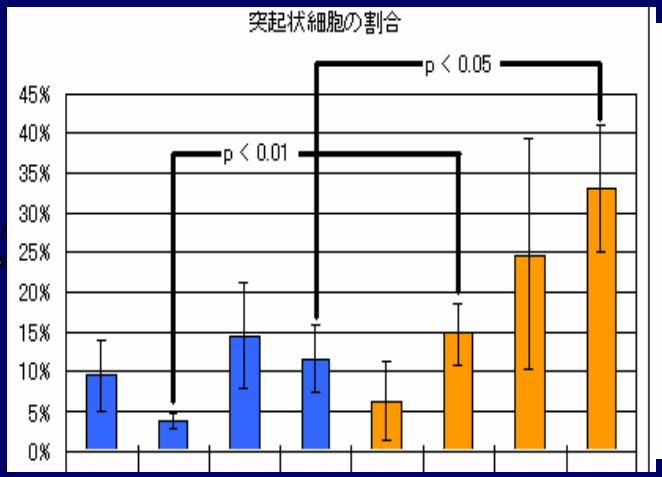


Primary and Secondary Screenings of the Genes Responsible for Chronic Heart Failure

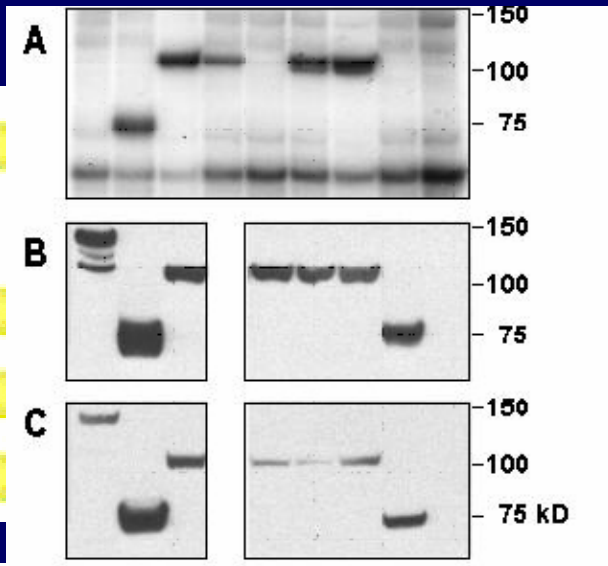
The gene correlated with PAP（r=0. 993）



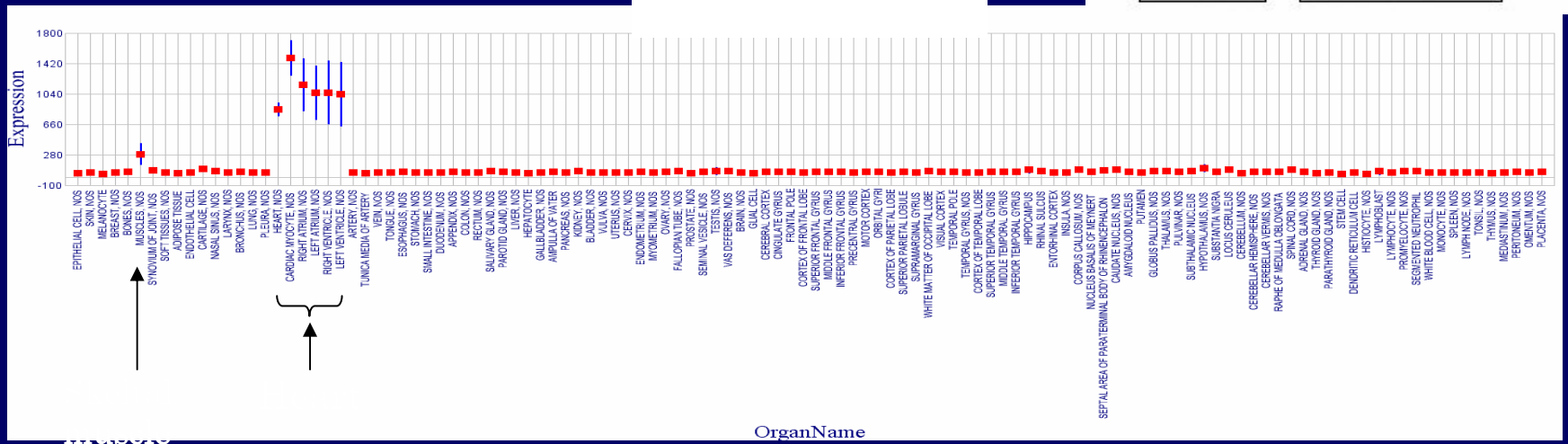
Transfection of the candidate gene to cardiomyocytes



Protein Expression

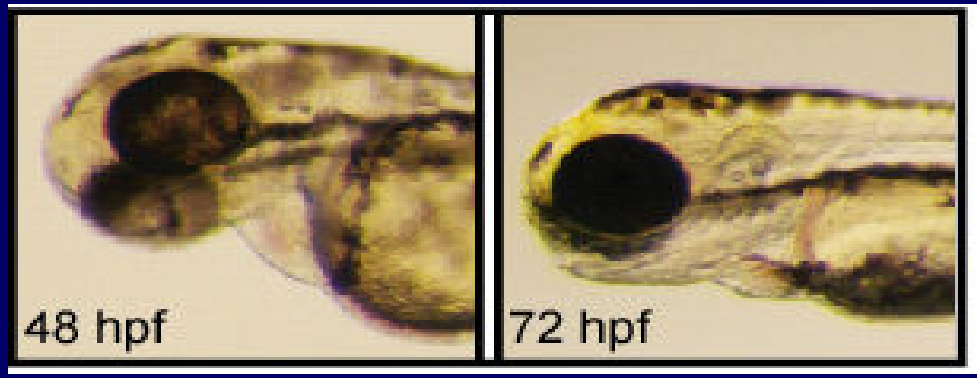


Expression Pattern

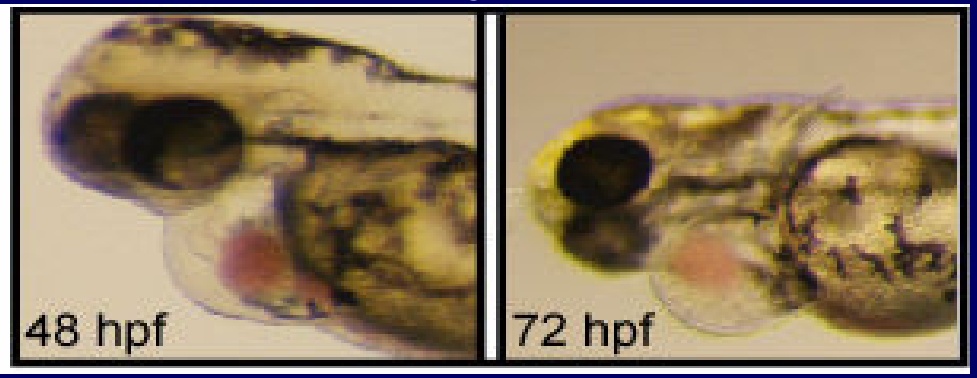


Morpholino Antisense Oligonucleotides Targeted UG gene Induced Ventral-swelling Phenotype in Zebrafish

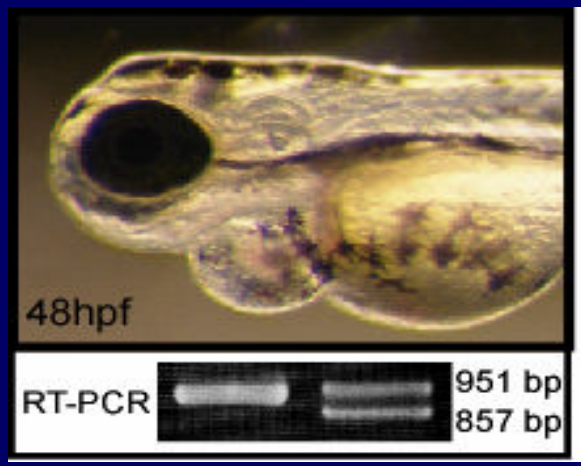
control



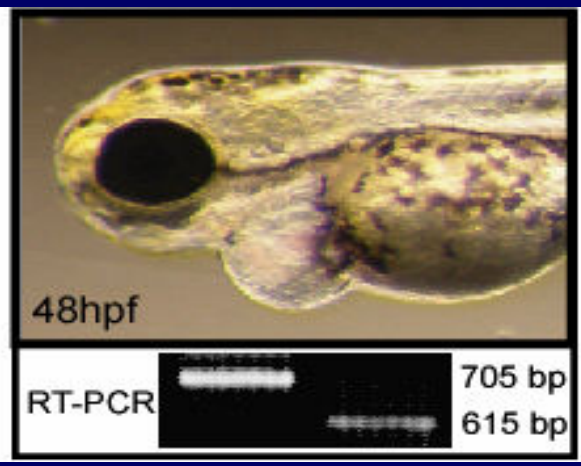
MO-injected



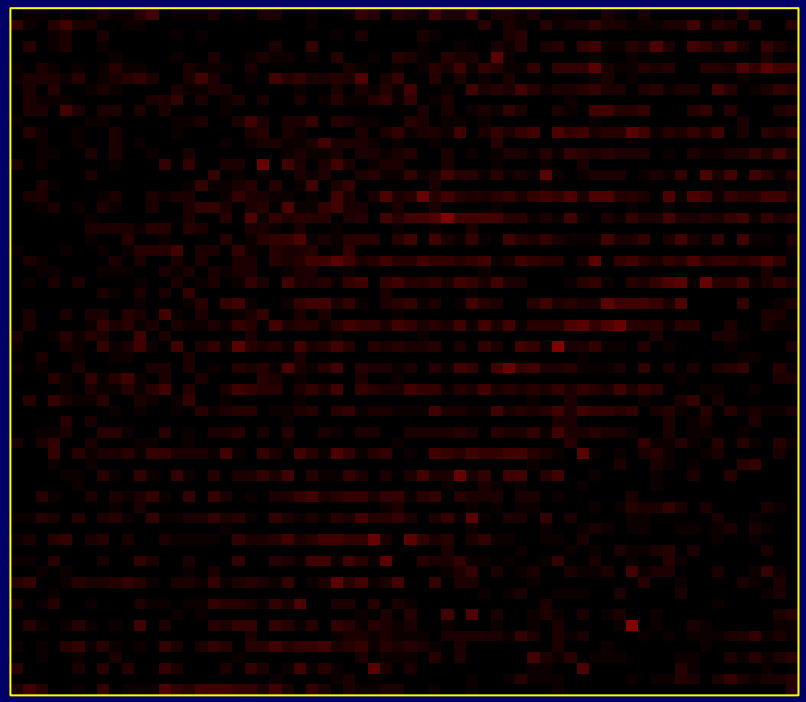
Splice-interferring MO



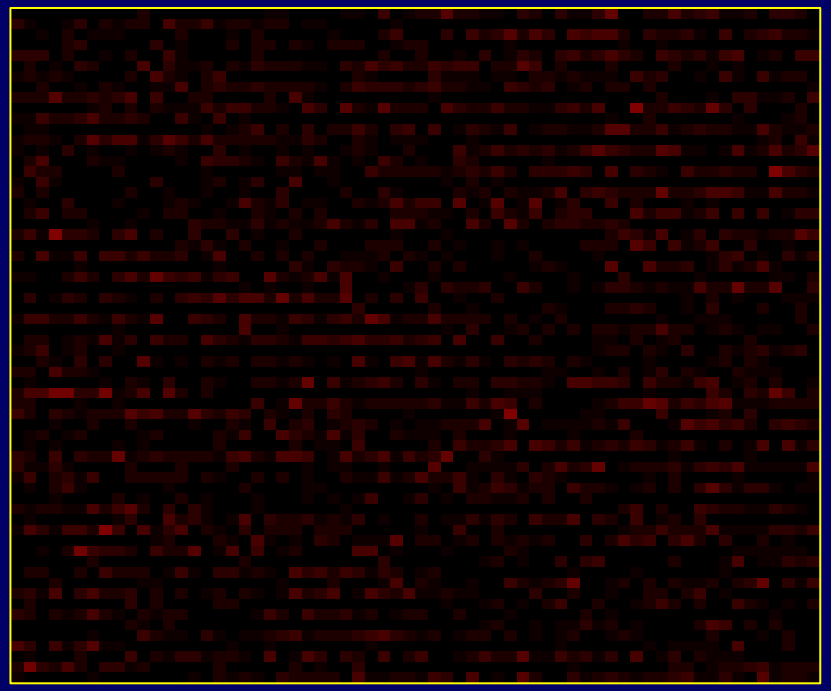
Splice-interferring MO substrate



Reduced Expression of MLCK3 Leads to Ventricular Dilation with Tachycardia

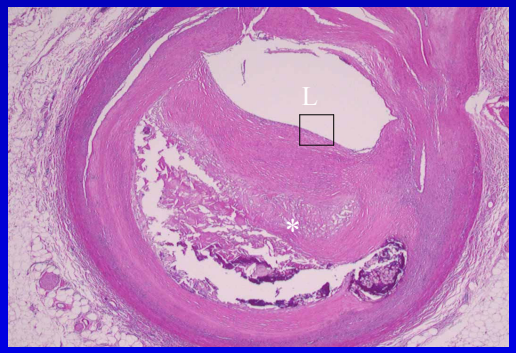
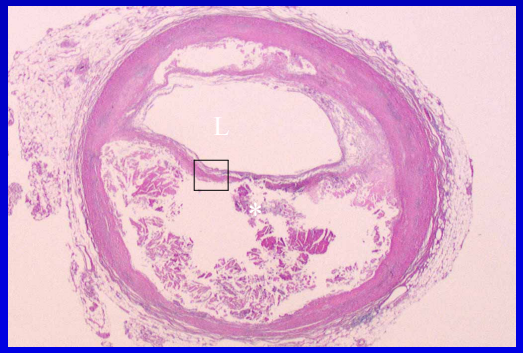
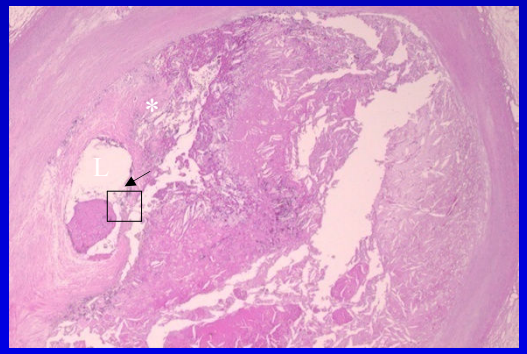


control



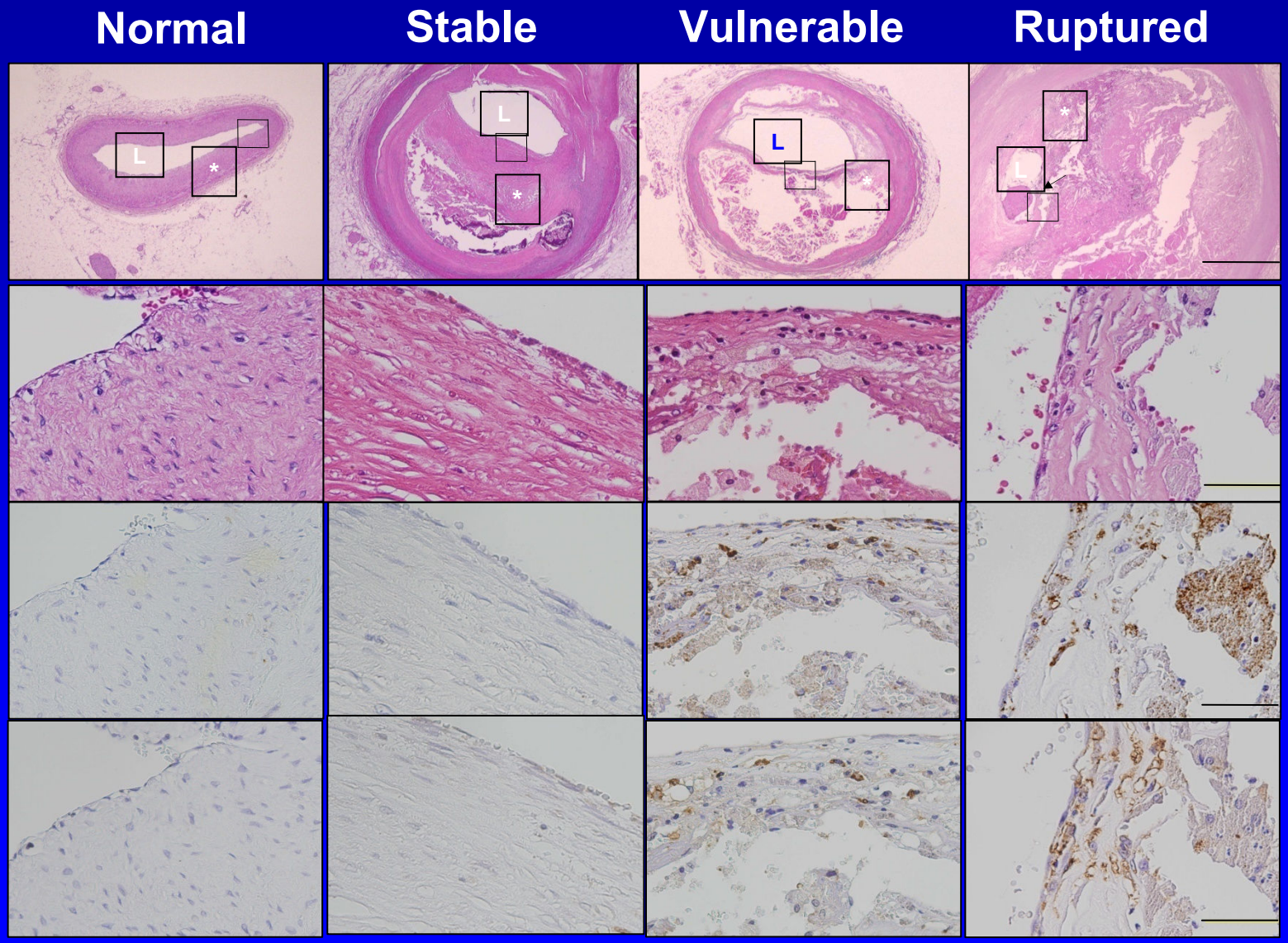
MO-injected

The grouping of the plaques

Normal n = 14	**Stable** n = 51	**Vulnerable** n = 15	**Ruptured** n = 17
			
AHA classification			
Type I	Type Va	Type Va	Type VI
diffuse intimal thickening	thick fibrous cap	thick fibrous cap (<65 μm thick)	ACS patients (without receiving PCI)

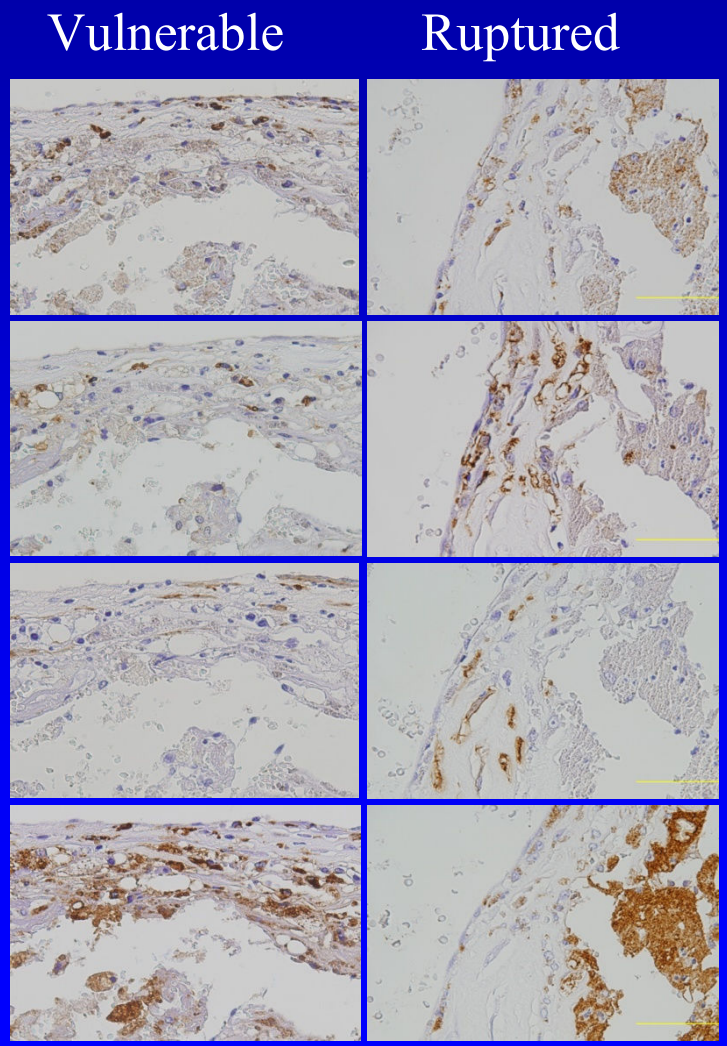
Bar : 20mm

Induction of ER Chaperones and Death Signals in Unstable Plaques at Autopsy



Bar :
20μm (HE x20)
50μm (others)

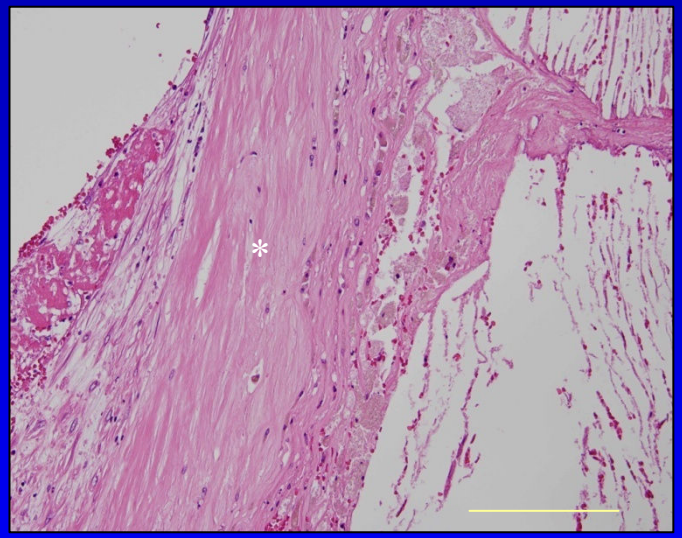
KDEL- and CHOP-positive cells were SMCs or macrophages



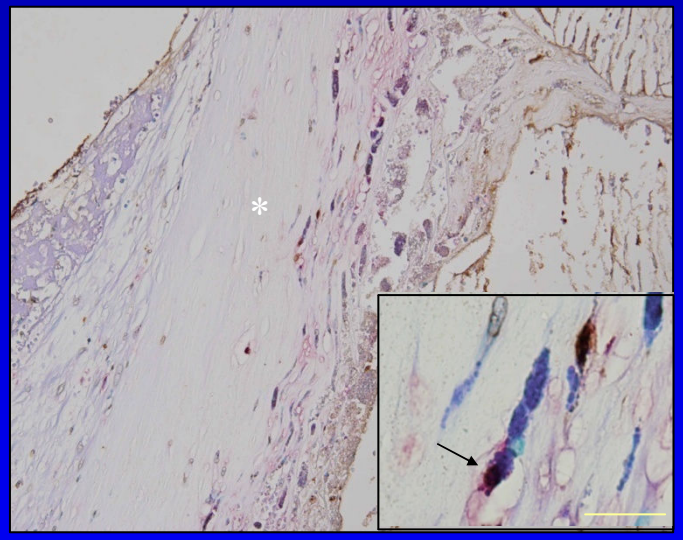
Bar : 50mm

The CHOP-dependent Pathway Was Activated in Unstable Plaques with TUNEL-positive Cells

HE



Double stain

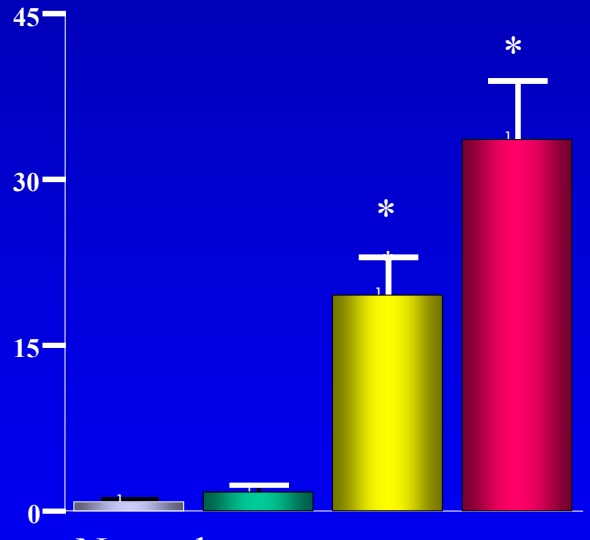


Brown : TUNEL

Red : CHOP

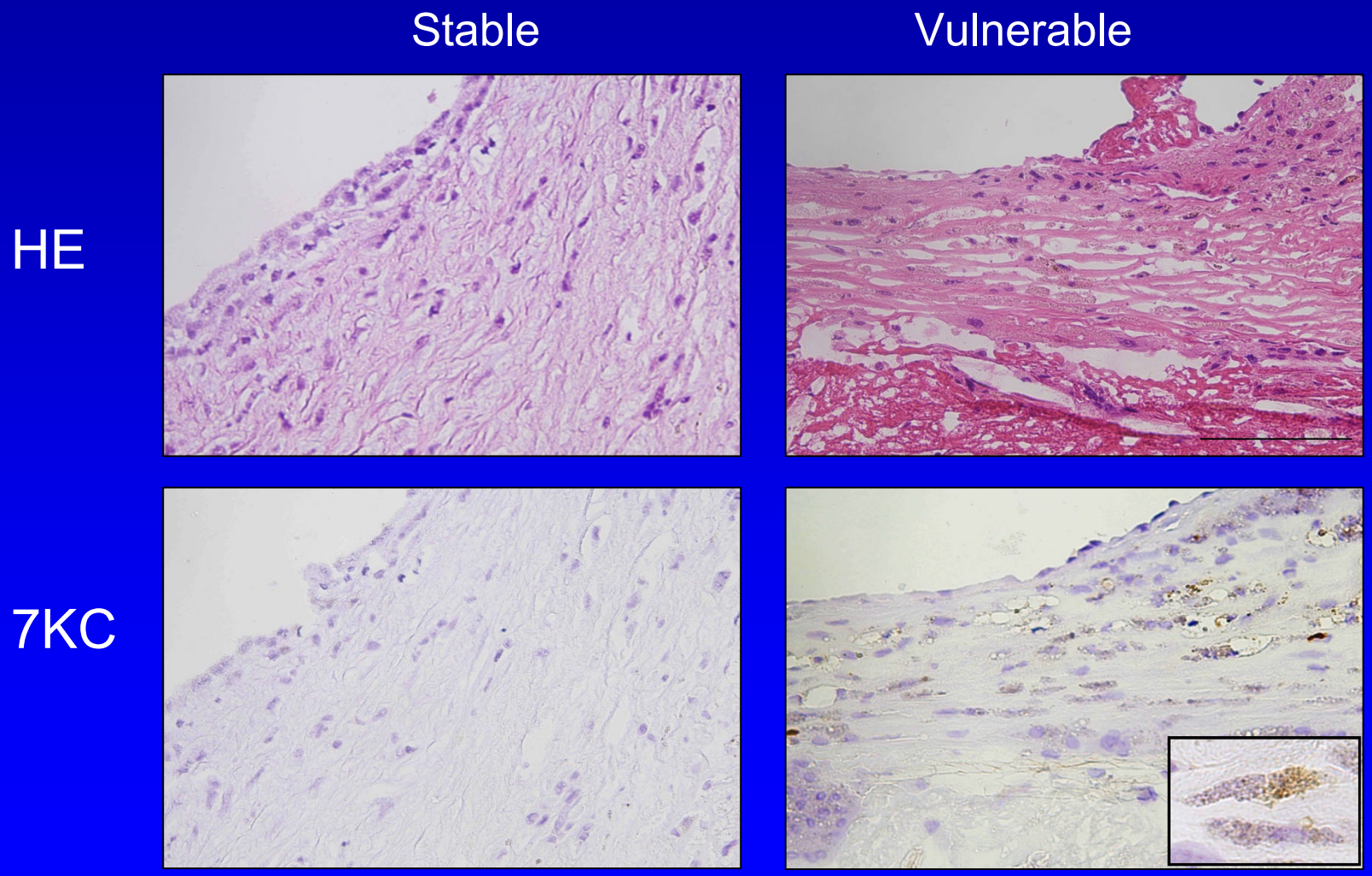
Bar : 50mm (HE)
20mm (in the box)

% TUNEL positivity
of CHOP-positive cells

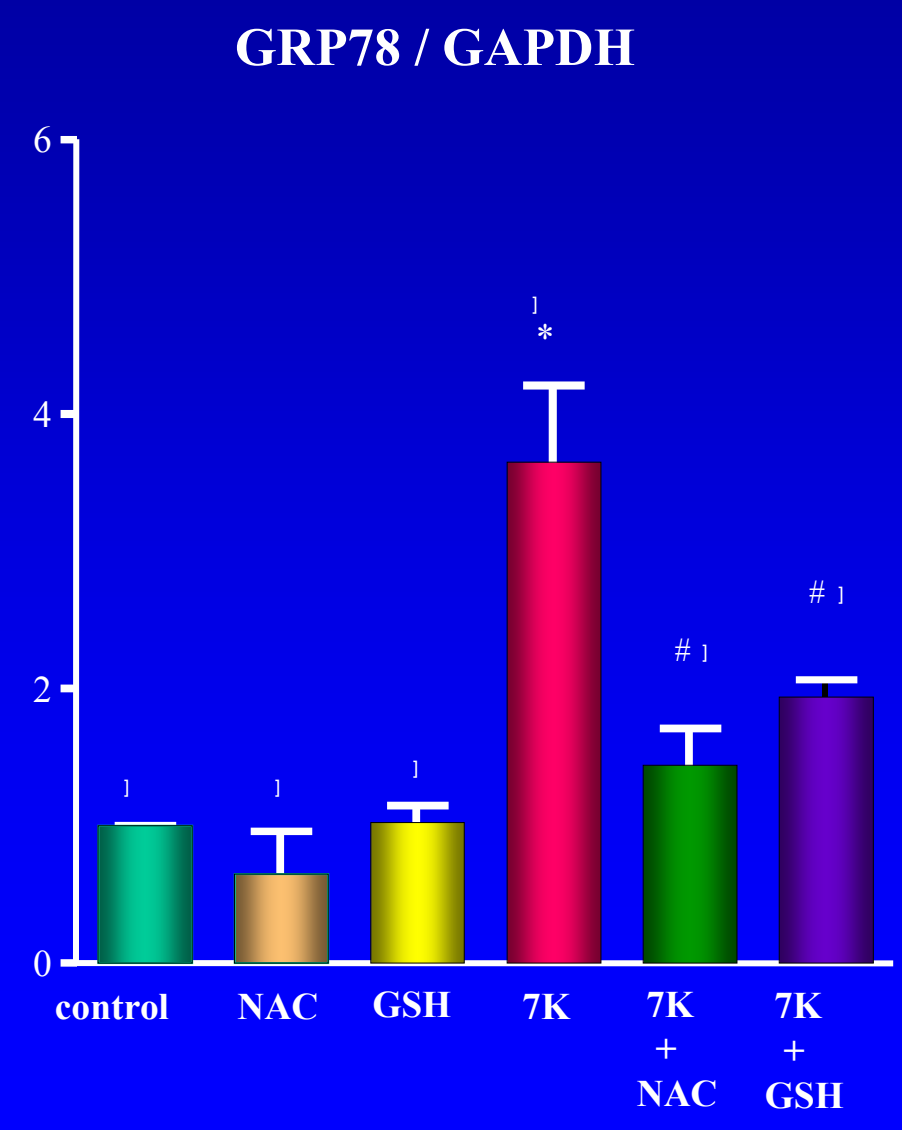


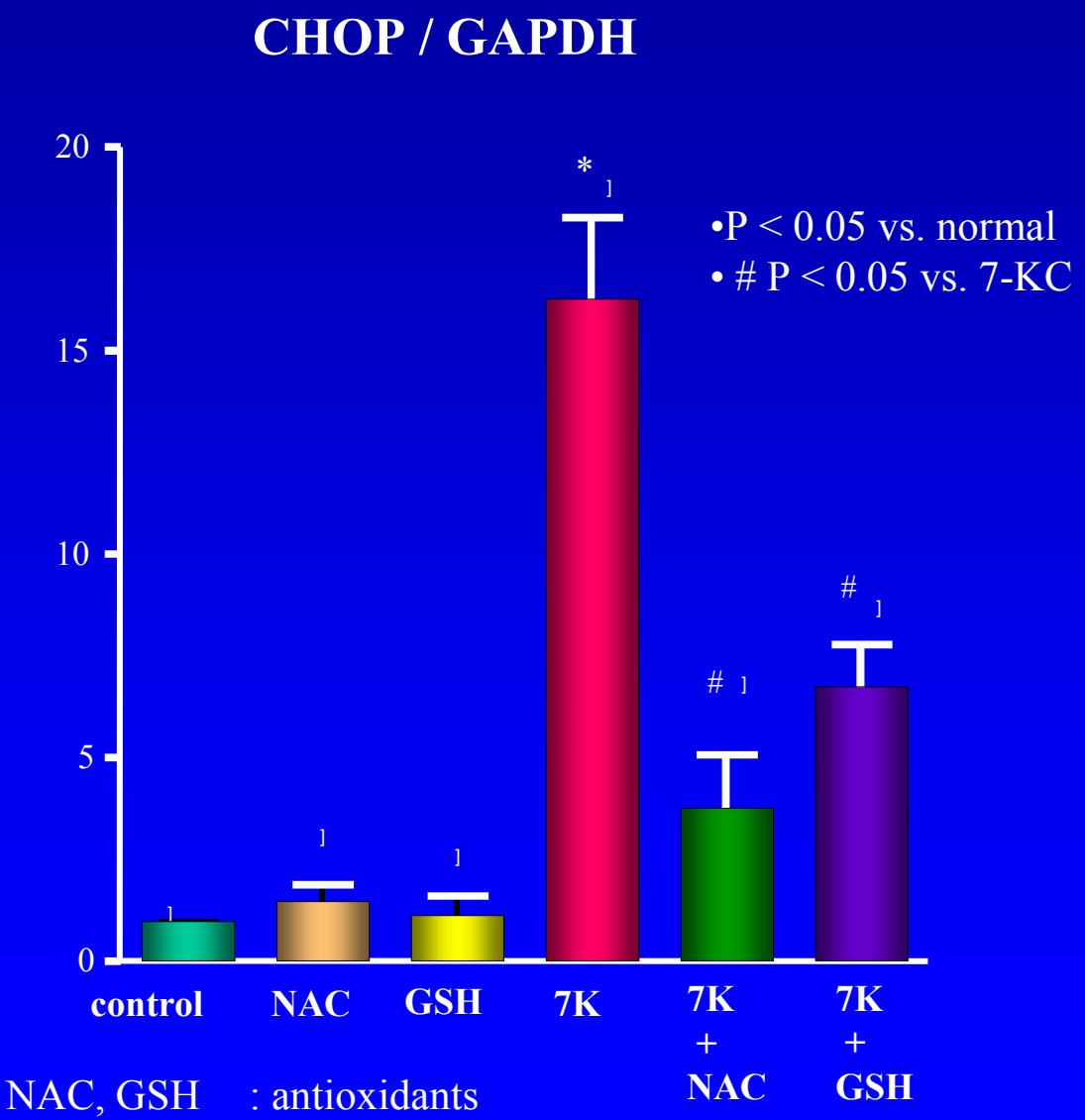
*** P < 0.05 vs. normal**

7-Ketocholesterol (7-KC) in the Thin Fibrous Cap of Vulnerable Plaque



7-KC Increased ER Stress through the Production of ROS



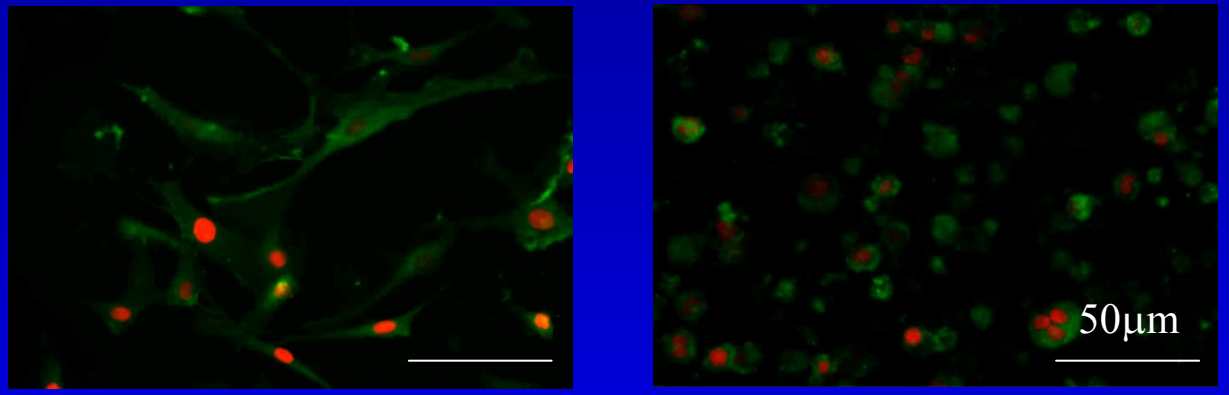


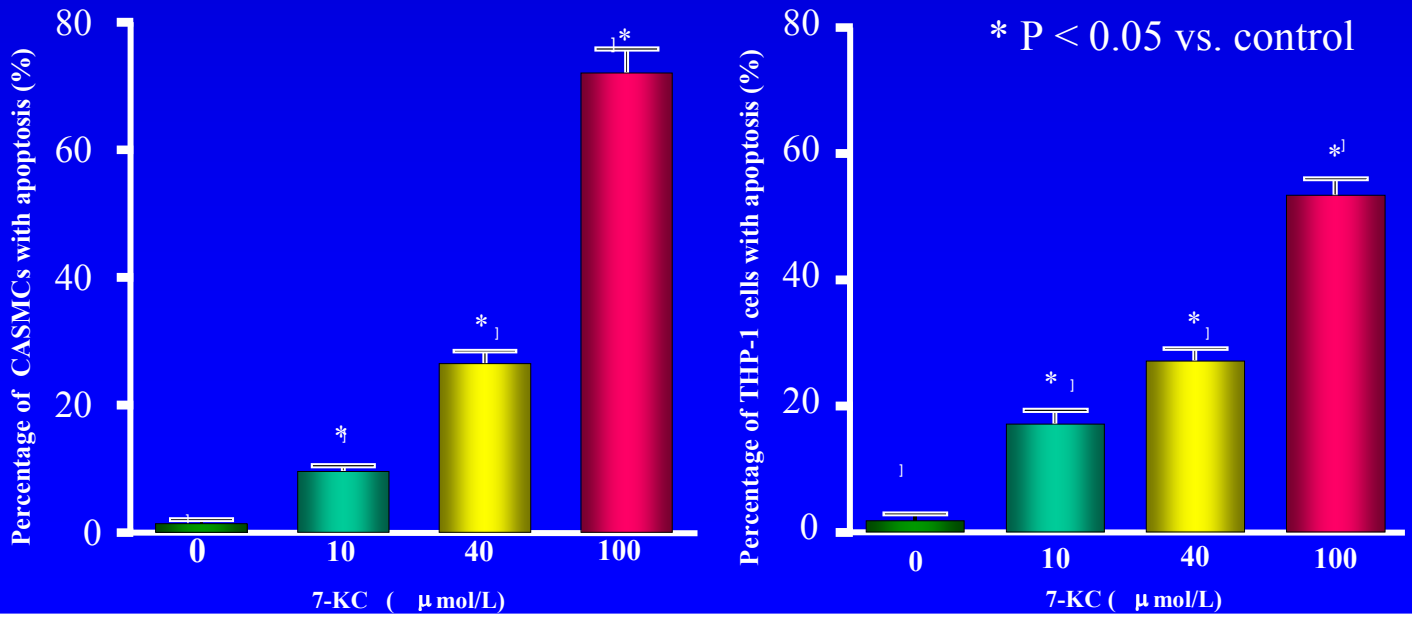
7-KC Induces Apoptosis of SMCs and Macrophages

< CASMCs >

< THP-1 >

annexin V





How to Reduce Ischemic Heart Failure

1. The reduction of incidence of myocardial infarcti
   →antiplatelet, statin, pioglitazone、ACEI

2. Reduction of infarct size
   →beta-blocker, adenosine, nicorandil, ANF

3. Inhibition of ventricular and vascular remodeling
   →ACEI, ARB, beta-blocker, adenosine

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