CLINICAL APPLICATIONS OF PRE-, POST- AND REMOTE ISCHEMIC AND PHARMACOLOGIC CONDITIONING IN ISCHEMIC DISEASE: PROMISE AND LIMITATIONS

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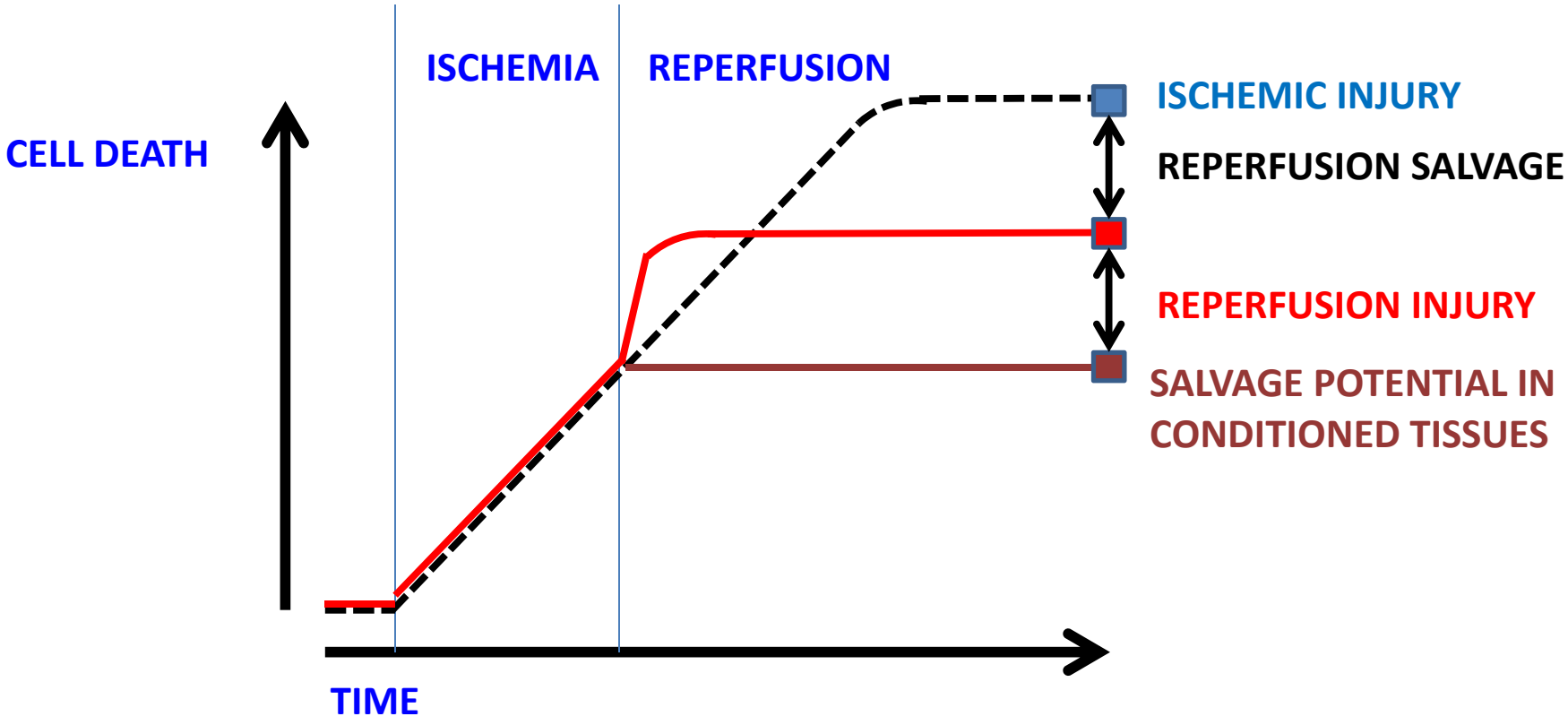
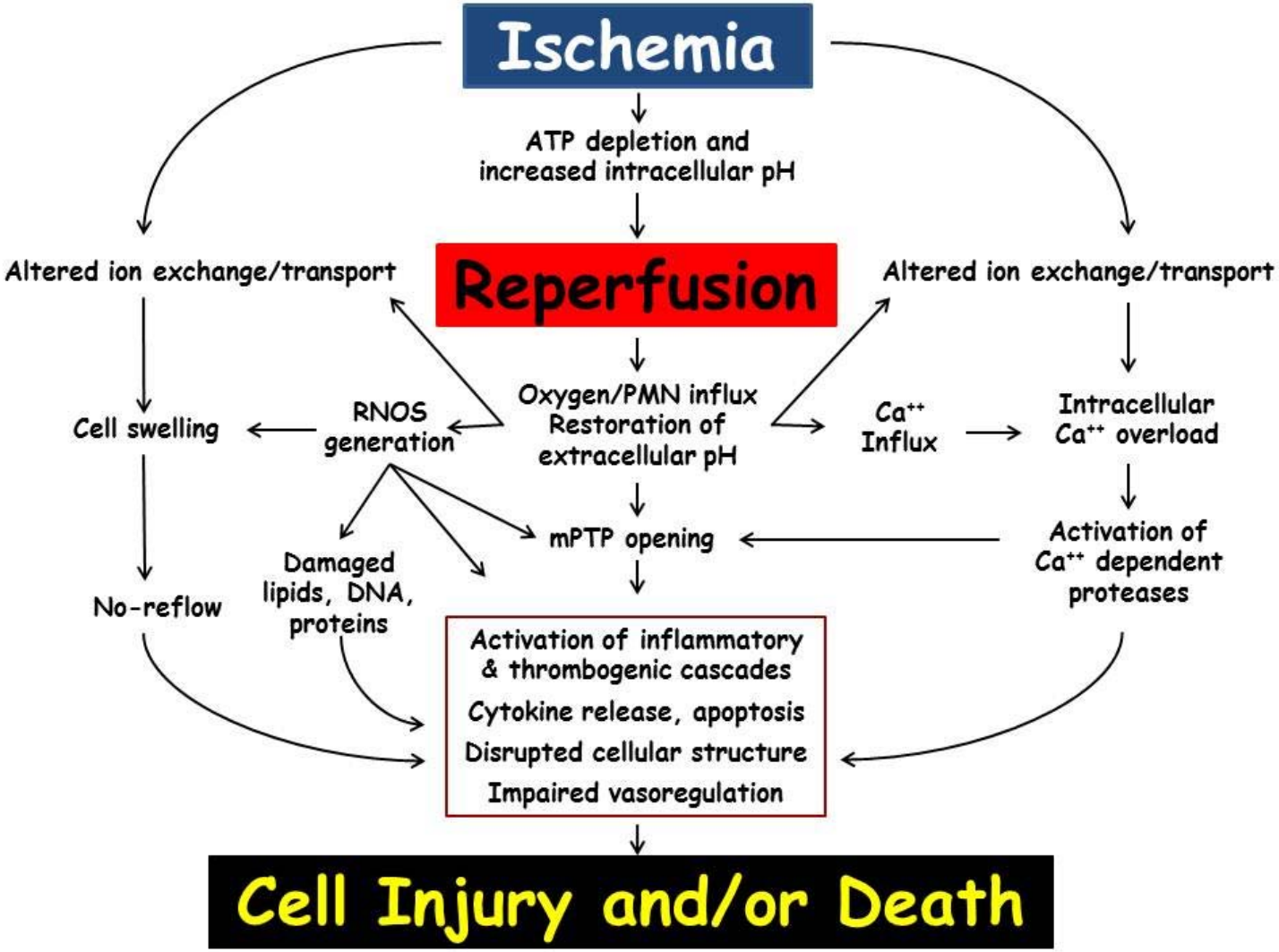
THE CONCEPTS OF LETHAL REPERFUSION INJURY AND TISSUE SALVAGE



I/R INJURY IS COMPLEX AND MULTIFACTORIAL



The NHLBI-Sponsored Consortium for preclinicAl assESsment of cARdioprotective Therapies (CAESAR)

A New Paradigm for Rigorous, Accurate, and Reproducible Evaluation of Putative Infarct-Sparing Interventions in Mice, Rabbits, and Pigs

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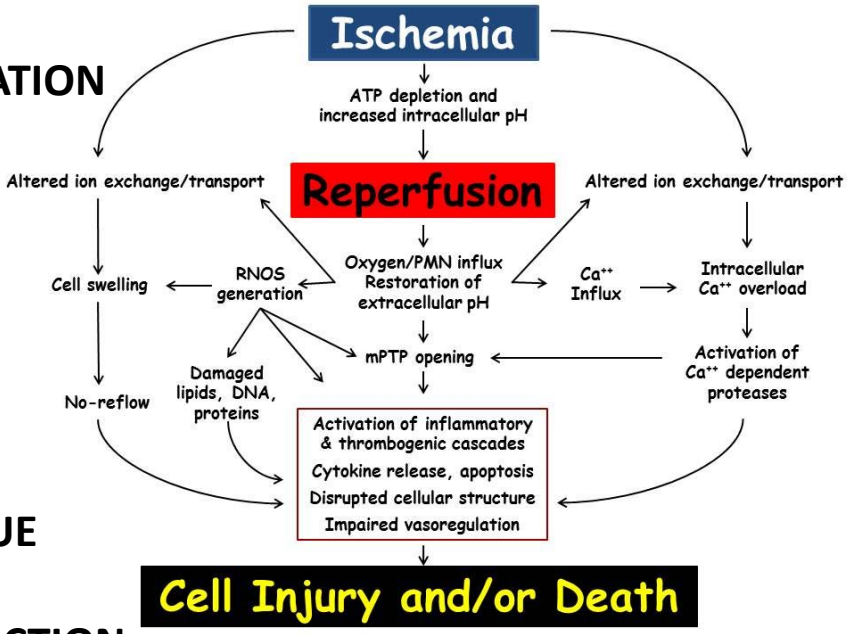
“Despite 4 decades of intense effort and substantial financial investment, the cardioprotection field has failed to deliver a single drug that effectively reduces myocardial infarct size in patients. A major reason is insufficient rigor and reproducibility in preclinical studies.”

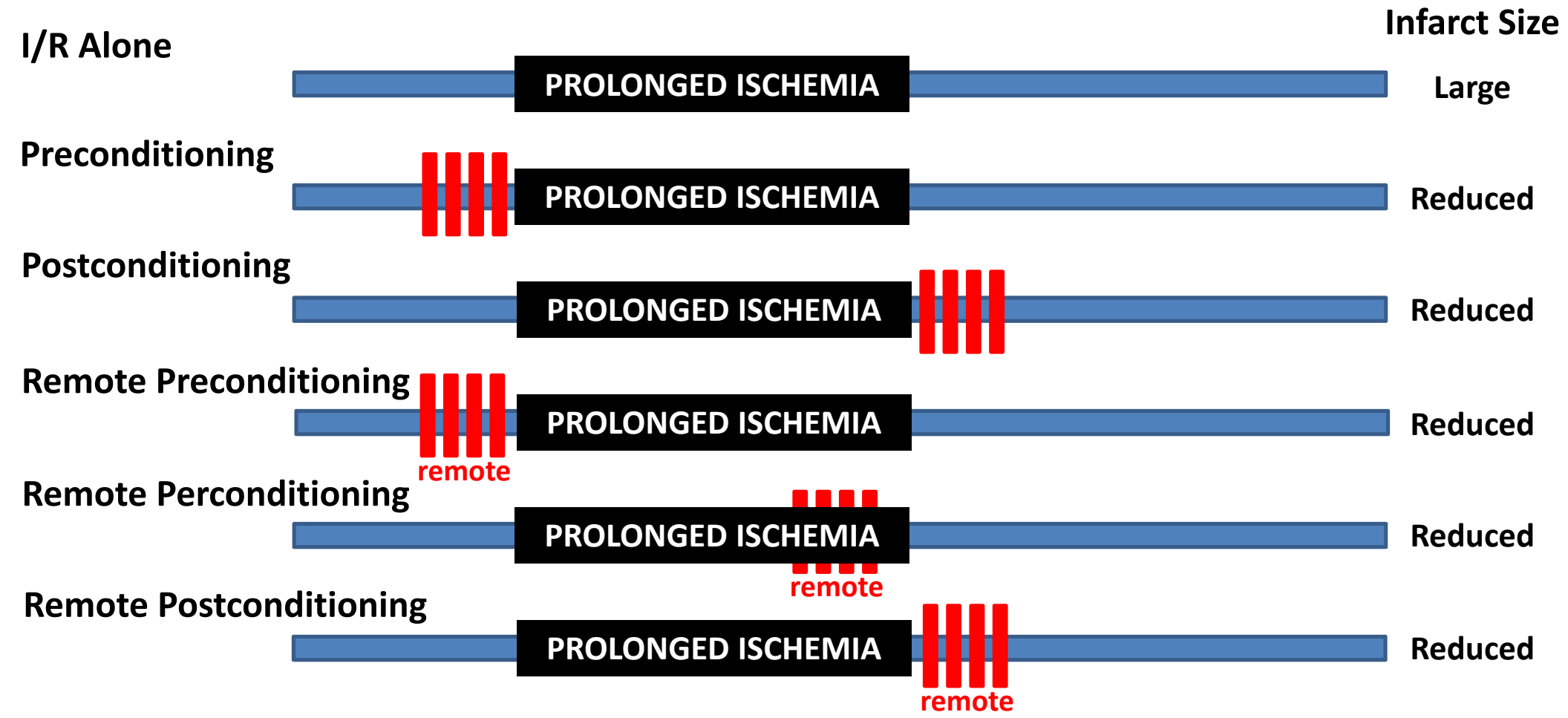
Circ. Res 2015; 116:572-586.

ISCHEMIC CONDITIONING APPROACHES ARE THE MOST POWERFUL TISSUE PROTECTIVE INTERVENTIONS DISCOVERED TO DATE

- EFFECTIVE IN EVERY TISSUE, SPECIES, AND MODEL OF I/R TESTED
- RESTORES ARTERIOLAR ENDOTHELIAL-DEPENDENT VASODILATORY FUNCTION
- LARGELY ABOLISHES POSTISCHEMIC IMMUNOCYTE INFILTRATION
- PREVENTS THE DEVELOPMENT OF CAPILLARY NO-REFLOW
- PRESERVES MITOCHONDRIAL FUNCTION
- ATTENUATES CELL SWELLING AND RUPTURE
- SUBSTANTIALLY REDUCES THE VOLUME OF INFARCTED TISSUE
- IMPROVE DEFICITS IN A VARIETY OF INDICES OF TISSUE FUNCTION

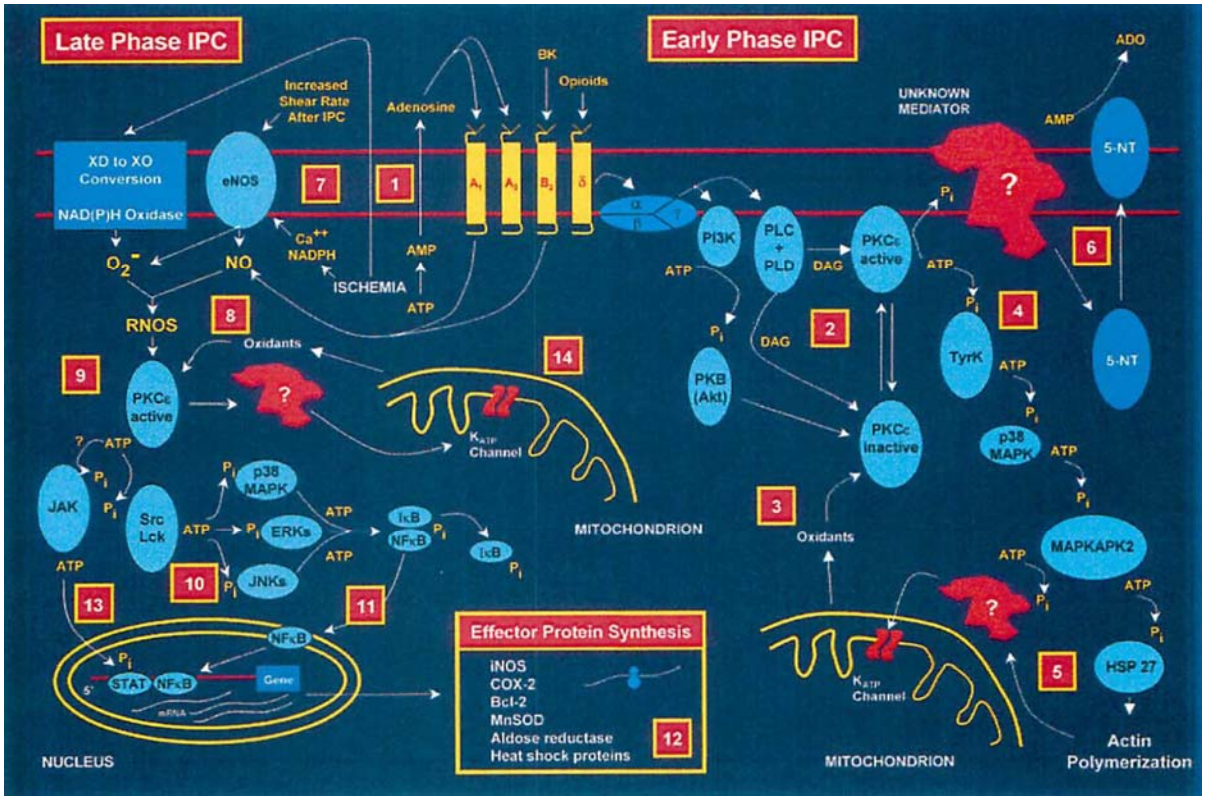


ISCHEMIC CONDITIONING STRATEGIES HAVE THE POTENTIAL TO REVOLUTIONIZE THE TREATMENT OF ISCHEMIA/REPERFUSION INJURY

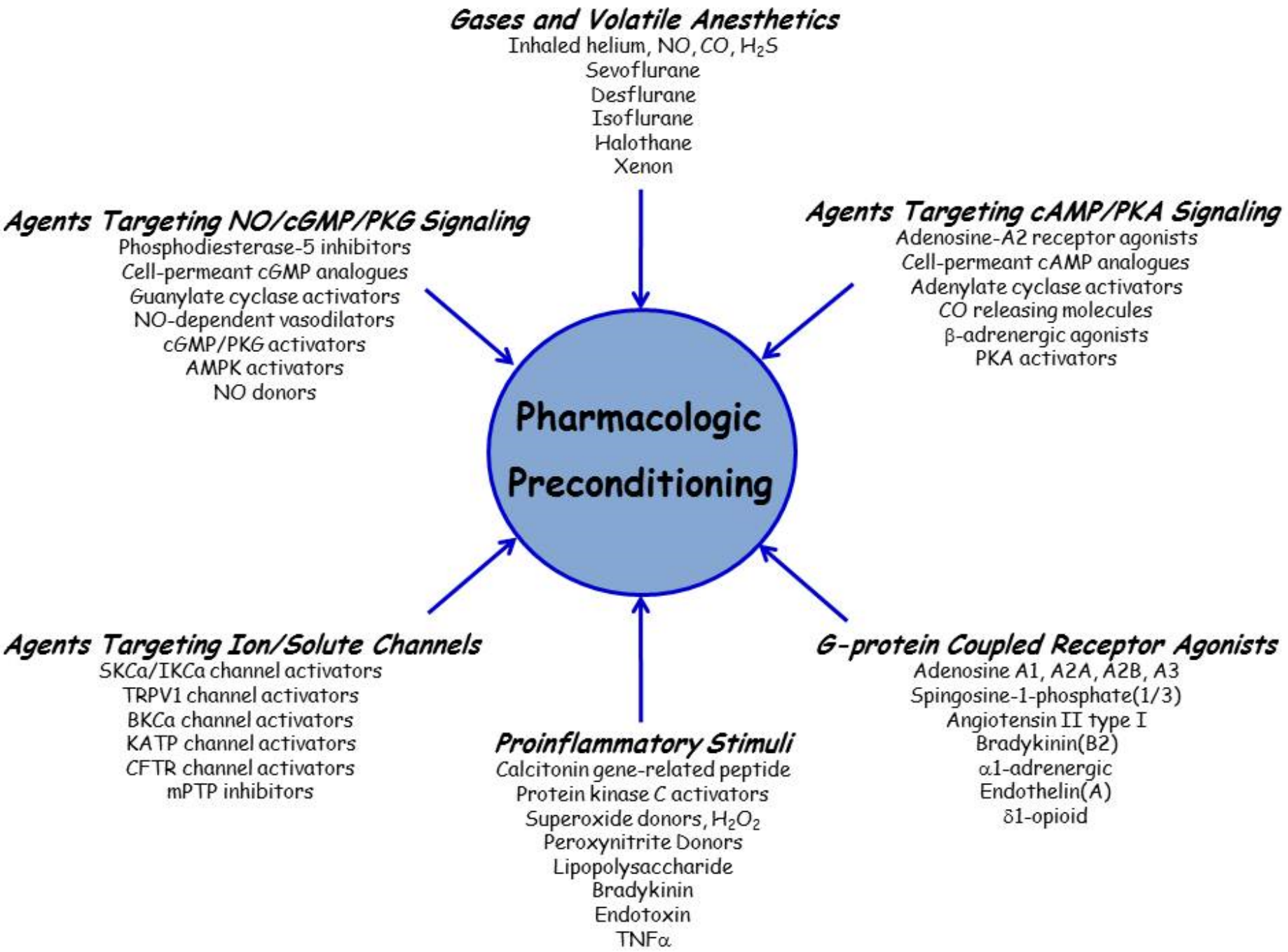


Modified from Wider and Przyklenk. Cardiovasc Diagn Ther 4: 383, 2015

MECHANISTIC DISCOVERY OF THE UNDERPINNING SIGNALING PATHWAYS THAT PRODUCE TOLERANCE TO I/R BY ISCHEMIC CONDITIONING DEFINE AVENUES FOR PHARMACOTHERAPY



Dayton and Korthuis, Microcirculation 9:73-89


Gases and Volatile Anesthetics
Inhaled helium, NO, CO, H_2S
Sevoflurane
Desflurane
Isoflurane
Halothane
Xenon
Agents Targeting NO/cGMP/PKG Signaling
Phosphodiesterase-5 inhibitors
Cell-permeant cGMP analogues
Guanylate cyclase activators
NO-dependent vasodilators
cGMP/PKG activators
AMPK activators
NO donors
Agents Targeting cAMP/PKA Signaling
Adenosine-A2 receptor agonists
Cell-permeant cAMP analogues
Adenylate cyclase activators
CO releasing molecules
β-adrenergic agonists
PKA activators
Pharmacologic Preconditioning
Agents Targeting Ion/Solute Channels
SKCa/IKCa channel activators
TRPV1 channel activators
BKCa channel activators
KATP channel activators
CFTR channel activators
mPTP inhibitors
Proinflammatory Stimuli
Calcitonin gene-related peptide
Protein kinase C activators
Superoxide donors, H_2O_2
Peroxynitrite Donors
Lipopolysaccharide
Bradykinin
Endotoxin
TNFα
G-protein Coupled Receptor Agonists
Adenosine A1, A2A, A2B, A3
Spingosine-1-phosphate(1/3)
Angiotensin II type I
Bradykinin(B2)
α1-adrenergic
Endothelin(A)
δ1-opioid

ARE CONDITIONING STRATEGIES EFFECTIVE IN RELEVANT PATIENT POPULATIONS?

As reviewed in:

Przklenk, Br J Pharmacol (2015) 172:1961-1973

Jones et al, Circ Res (2015) 116:572-586

Lecour et al, Cardiovasc Res (2014) 104:399-411

Ovize et al, Circ Res (2013) 1

Kloner, Circ Res (2013) 113: 4 1-

Vander Heide and Steenbe

Longacre et al, Circulation (2011) 124:1172-1179

Ovize et al, Cardiovasc Res (2010) 87:406-423

Miura and Miki, Basic Res Cardiol (2008) 103: 501-513

the answer to this question is that clinical trials have yielded results that are disappointing and frustrating

WHAT ARE THE UNDERLYING EXPLANATIONS FOR THIS COLOSSAL FAILURE?

Preclinical Studies

- **LACK OF STANDARDIZED ANIMAL MODELS, EXPERIMENTAL PROTOCOLS, ANALYSIS METHODS, RANDOMIZED STUDY DESIGN AND BLINDING OF INVESTIGATORS, COLLATERAL FLOW, AREA-AT-RISK**
- **DIFFERENCES IN GROUP MORTALITY MAY RELATE TO FAILURE TO CONTROL BASIC PHYSIOLOGICAL PARAMETERS**
- **USE OF ANIMAL (young and healthy, anesthetized, absence of co-morbid risk factors) OR REDUCTIONIST (isolated hearts, isolated cells, insufficient hypoxic exposures) APPROACHES THAT DO NOT ADEQUATELY MIMIC THE COMPLICATED SITUATION ENCOUNTERED IN PATIENTS OR TAKE IN TO ACCOUNT OTHER VARIABLES (nutritional or hormonal status, alcohol consumption, left ventricular hypertrophy)**
- **ACUTE EXPERIMENTAL PROTOCOLS DO NOT ALLOW SUFFICIENT SURVIVAL TIME TO ASSESS LONG-TERM OUTCOMES**

Clinical Trials

- **ABUNDANCE OF CONFOUNDING VARIABLES (highly variable patient medication regimens; unknown duration of ischemia, area-at-risk, extent of collateral circulation, or level of adrenergic activity; difficulties in determining whether pre-existing infarctions have occurred, or if ischemia is intermittent, continuous, or preceded by brief attacks of angina)**

TACHPHYLAXIS, CO-MORBID RISK FACTORS AND ADJUNCTIVE THERAPIES LIMIT THE EFFECTIVENESS OF PRECONDITIONING STRATEGIES

- Tissues become desensitized to repetitive preconditioning with ischemia, adenosine, or NO donors
- Once tissues are desensitized to ADO or NO preconditioning, ischemic pre- and postconditioning are no longer effective
- Preconditioning is prevented as long as blood ethanol concentrations are elevated after ingestion of alcoholic beverages

Krenz et al, Cell Survival Programs and Ischemia/Reperfusion, Morgan and Claypool, 2013

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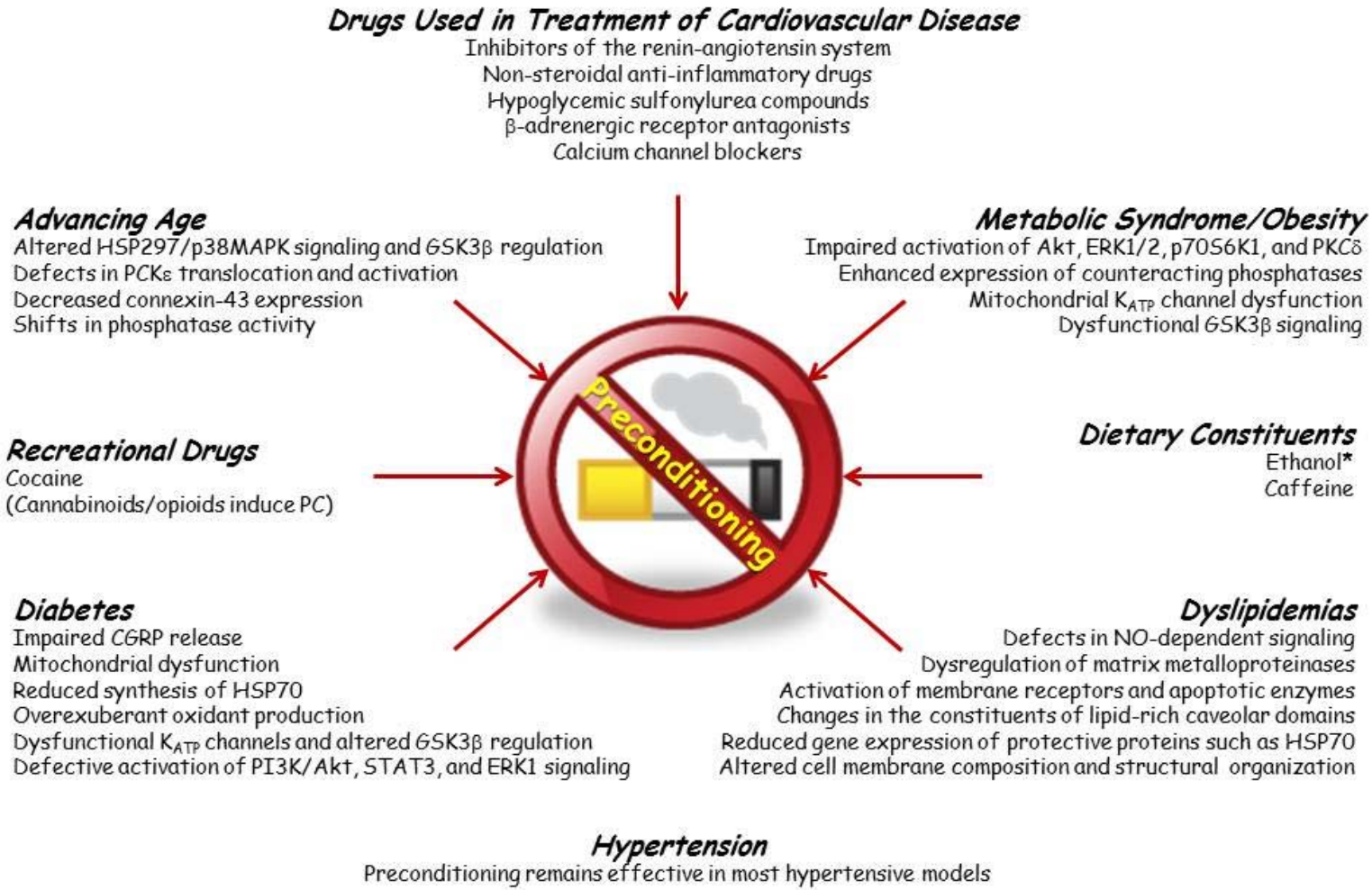
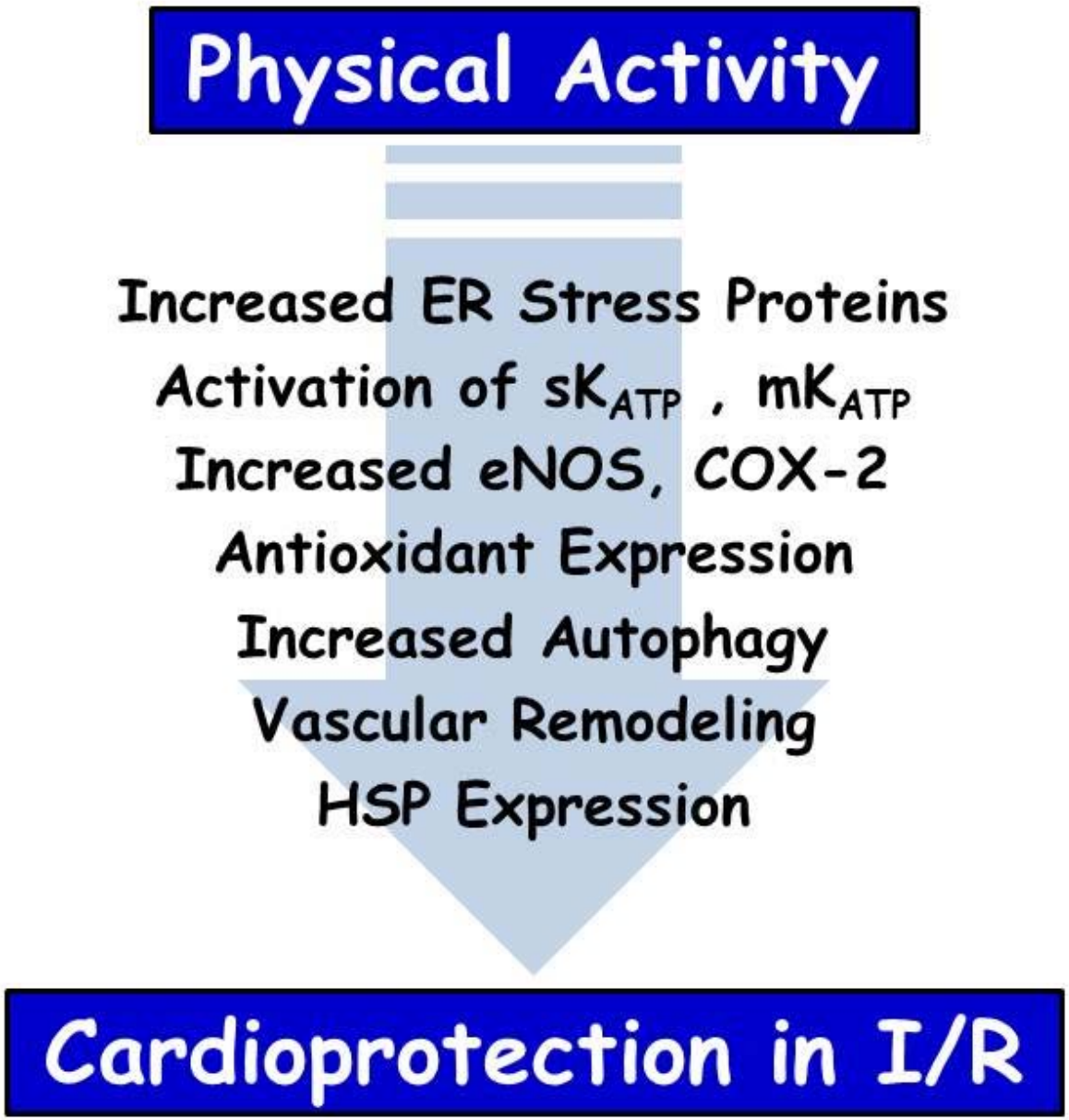


Krenz et al, Cell Survival Programs and Ischemia/Reperfusion, Morgan and Claypool, 2013

LIFESTYLE MODIFICATIONS THAT MAY PRODUCE REPETITIVE EPIGENETIC CONDITIONING AND SUSTAINED CARDIOPROTECTION IN THE PRESENCE OF CO-EXISTING RISK FACTORS

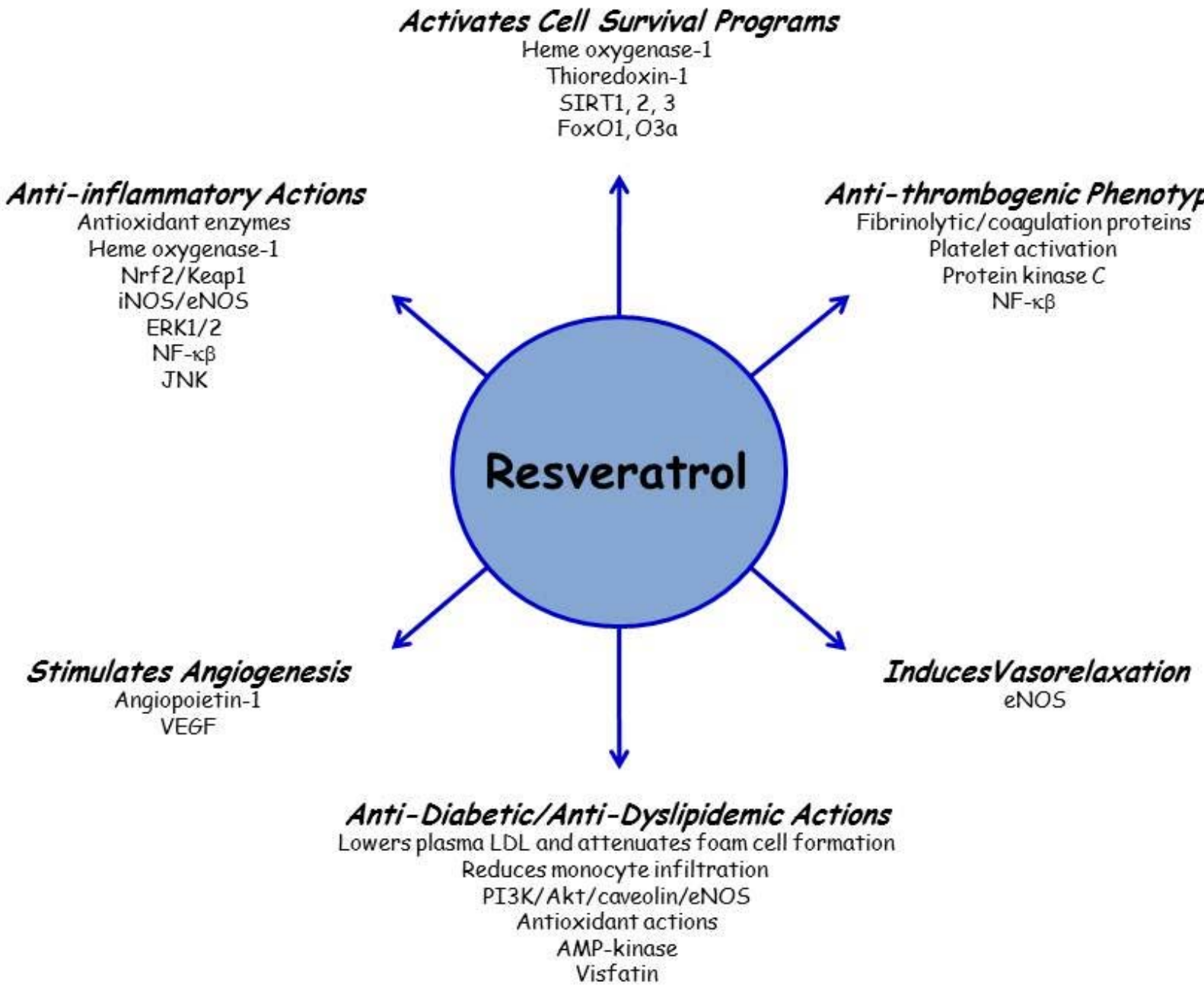
- EXERCISE
- CALORIC RESTRICTION
- ETHANOL, RESVERATROL, AND THE FRENCH PARADOX


Physical Activity
Increased ER Stress Proteins
Activation of sK_{ATP} , mK_{ATP}
Increased eNOS, COX-2
Antioxidant Expression
Increased Autophagy
Vascular Remodeling
HSP Expression
Cardioprotection in I/R

Caloric Restriction

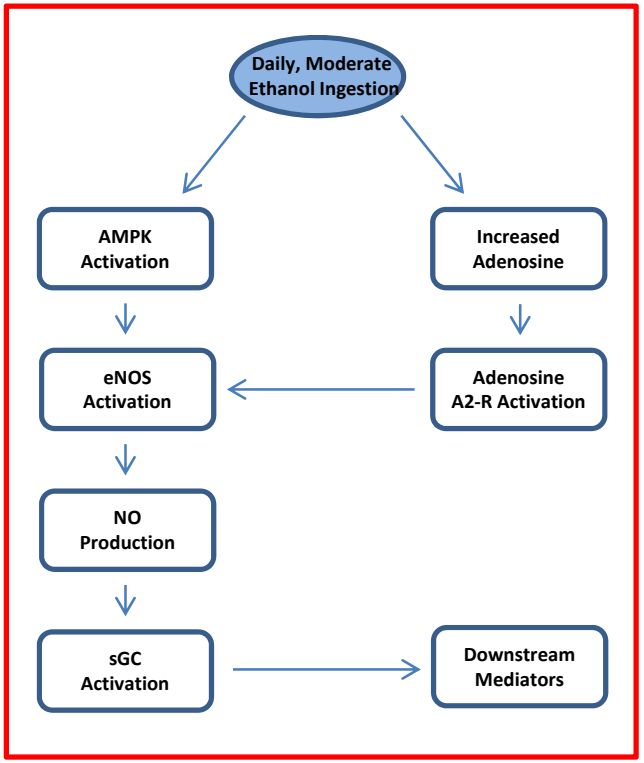
Deacetylates Specific Mitochondrial Proteins of the Electron Transport Chain
(eg, NDUFS1 and cytochrome bc1 complex Rieske subunit)
Increases SIRT1, PGC1α, eNOS, and nNOS Expression and Activity
Decreases Sensitivity to Mitochondrial Permeability Transition
Increases Anti-Apoptotic Gene Expression (eg, Akt, Bcl-2)
Increases Adiponectin Levels and Activates AMPK
Reduces Mitochondrial Oxidant Production
Increases Mn-SOD (SOD2) Expression
Activates the RISK Survival Pathway
Induces Mitochondrial Biogenesis
Activates Angiogenic Processes
Increases Autophagy

Cardioprotection in I/R


Activates Cell Survival Programs
Heme oxygenase-1
Thioredoxin-1
SIRT1, 2, 3
FoxO1, O3a
Anti-inflammatory Actions
Antioxidant enzymes
Heme oxygenase-1
Nrf2/Keap1
iNOS/eNOS
ERK1/2
NF-κβ
JNK
Anti-thrombogenic Phenotype
Fibrinolytic/coagulation proteins
Platelet activation
Protein kinase C
NF-κβ
Resveratrol
Stimulates Angiogenesis
Angiopoietin-1
VEGF
InducesVasorelaxation
eNOS
Anti-Diabetic/Anti-Dyslipidemic Actions
Lowers plasma LDL and attenuates foam cell formation
Reduces monocyte infiltration
PI3K/Akt/caveolin/eNOS
Antioxidant actions
AMP-kinase
Visfatin


Daily, Moderate Ethanol Ingestion
Sensory Neurons
CGRP
T Cells, Macrophages
Dendritic Cells
TNFα
Neutrophil-Derived MMPs
Matricryptins ($\alpha_v\beta_3$ ligands)
Interstitial Space
Interstitial Space
Endothelial Cells
BKCa
BKCa
$\alpha_v\beta_3$
Src
Src
Anti-inflammatory Phenotype
HO-1 ← Nrf2 ← ROS
ROS → Nrf2 → HO-1
Anti-inflammatory Phenotype

ETHANOL-INDUCED HEME OXYGENASE-1 EXPRESSION AND ACTIVITY MAY SERVE TO PROTECT SOLUBLE GUANYLYL CYCLASE FROM REDOX INACTIVATION IN REPERFUSED TISSUES



Prevents Redox Inactivation

Liu et al, Am J Physiol 2011; 300; H84-H93
Jones et al, J Pharm Exp Therap; 335; 85-91
Wang et al, Am J Physiol 2013; 305; H521-H532

LOGICAL EXTENSIONS OF CONDITIONING: GENE AND STEM CELL THERAPY

Gene therapy approaches to prophylactic protection against I/R in human patients face several challenges, but a growing body of preclinical studies provide proof-of-principle that persistent upregulation of cell survival genes can be accomplished.

An equally daunting set of obstacles must be overcome to allow effective use of stem cells for regenerative medicine. Use of preconditioning strategies to enhance the survival of the transplanted cells such that they can survive the harsh milieu of ischemic tissues by invoking the expression of cell survival programs is one such strategy that is gaining traction as means to accomplish this.

Krenz et al, Cell Survival Programs and Ischemia/Reperfusion, Morgan and Claypool, 2013

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