

# Mechanisms of Ischemia-Reperfusion Injury (IRI)



To make a complex story simple

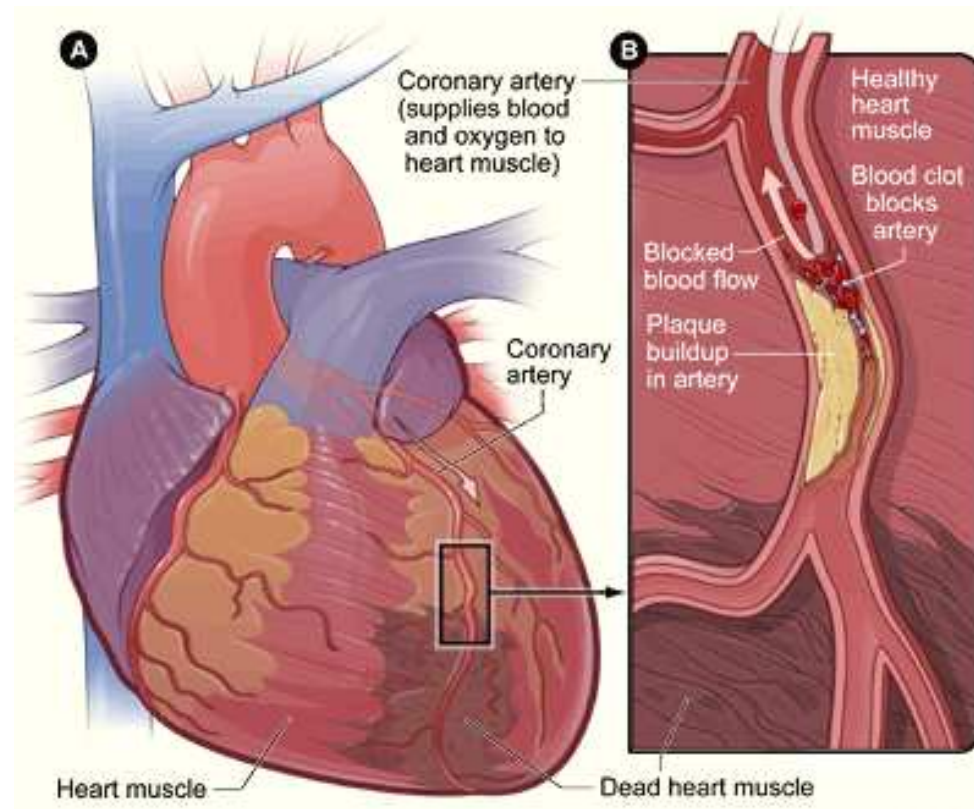
Pasquale Pagliaro

Dpt of Clinical and Biological Sciences

University of Torino, Italy

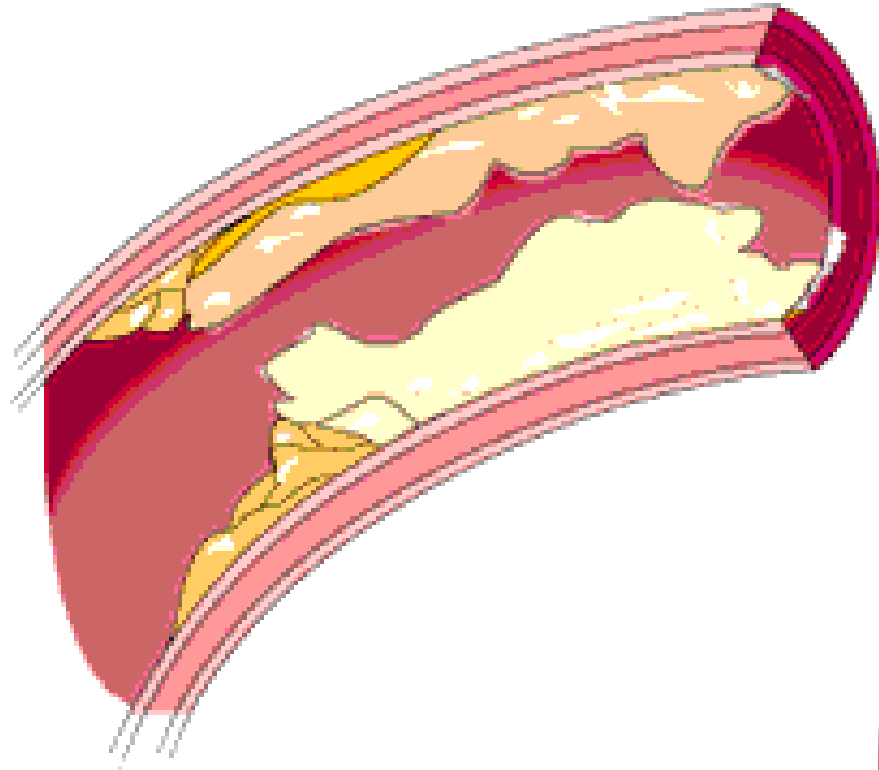
## The Bad

Ischemia: the occlusion of a coronary artery

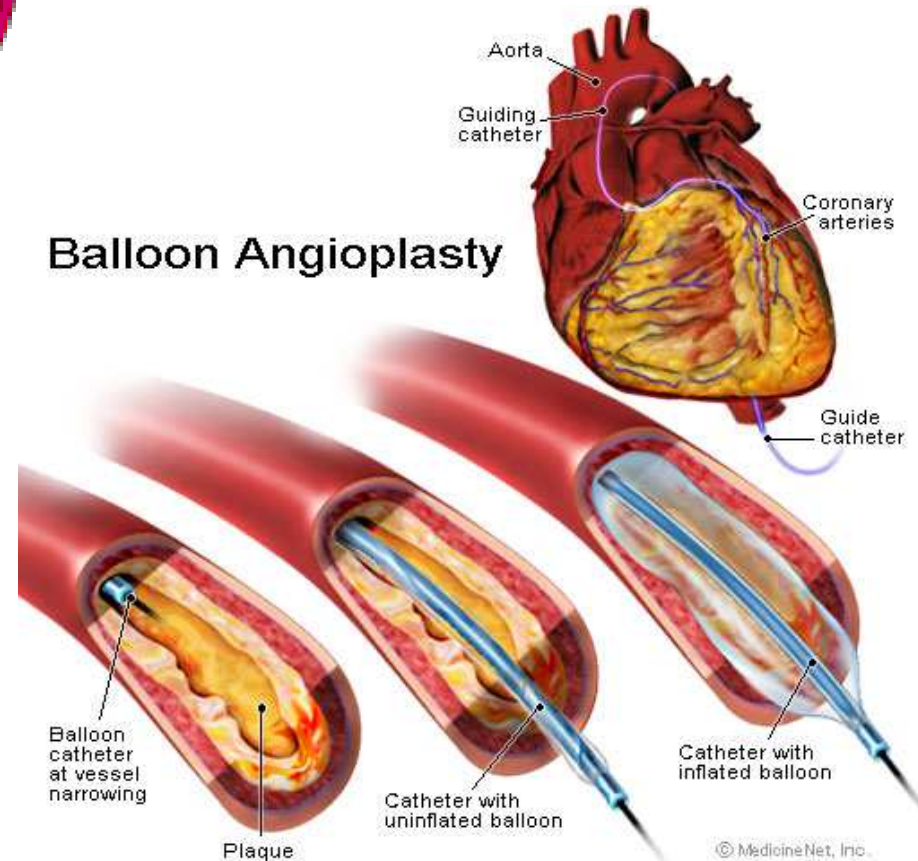


## The Good

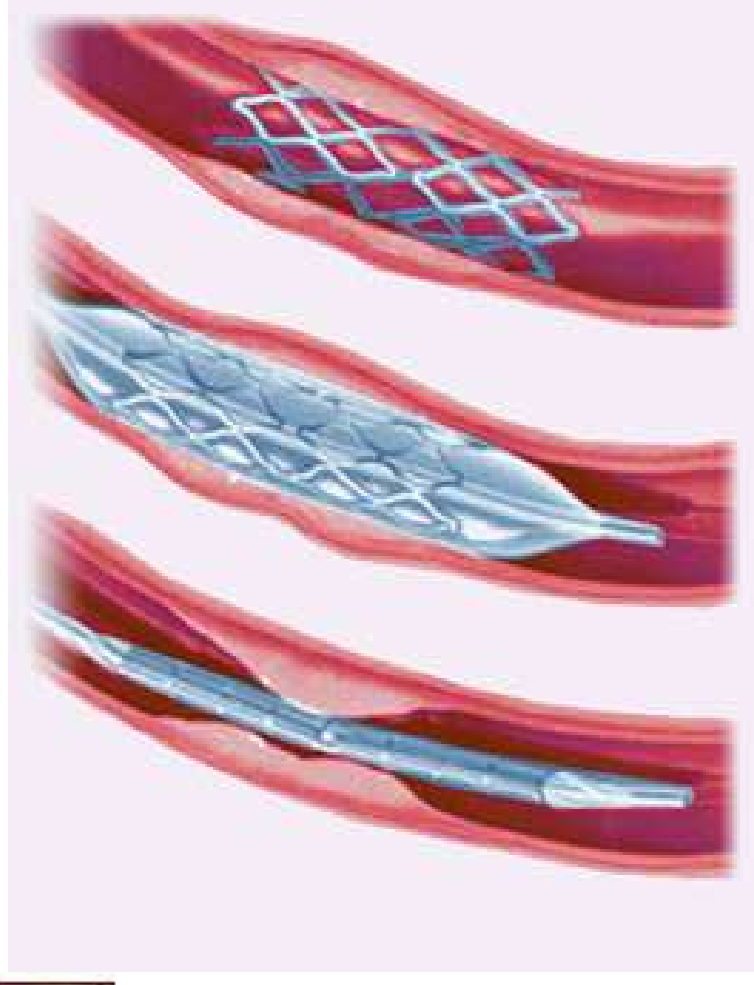
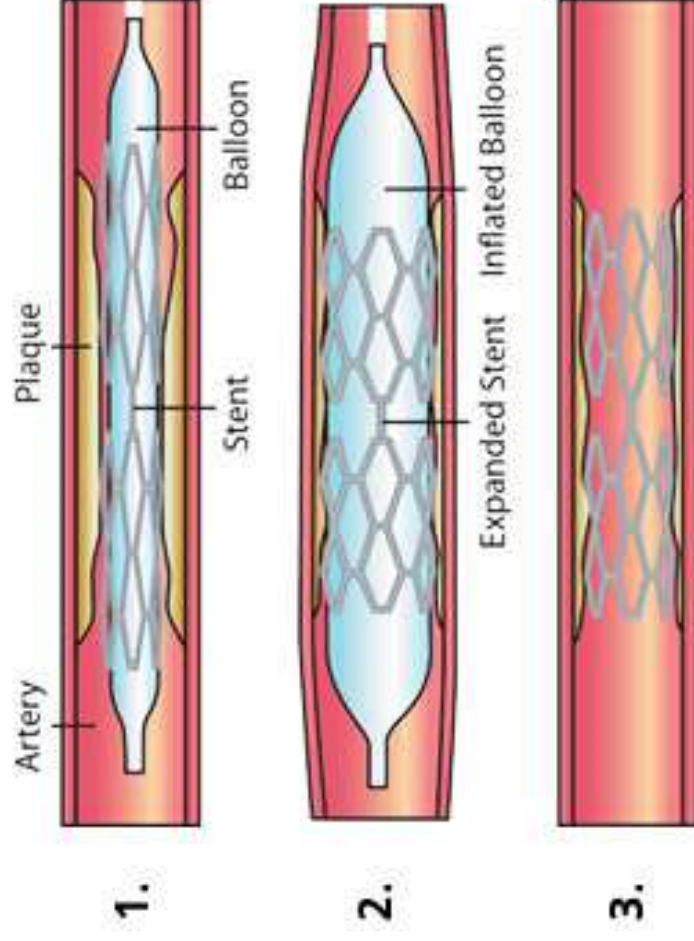
REPERFUSION: the reopening of a coronary artery



### Balloon Angioplasty



## Stent with Balloon Angioplasty



## The Ugly

### Reperfusion Injury: at the re-opening of a coronary artery

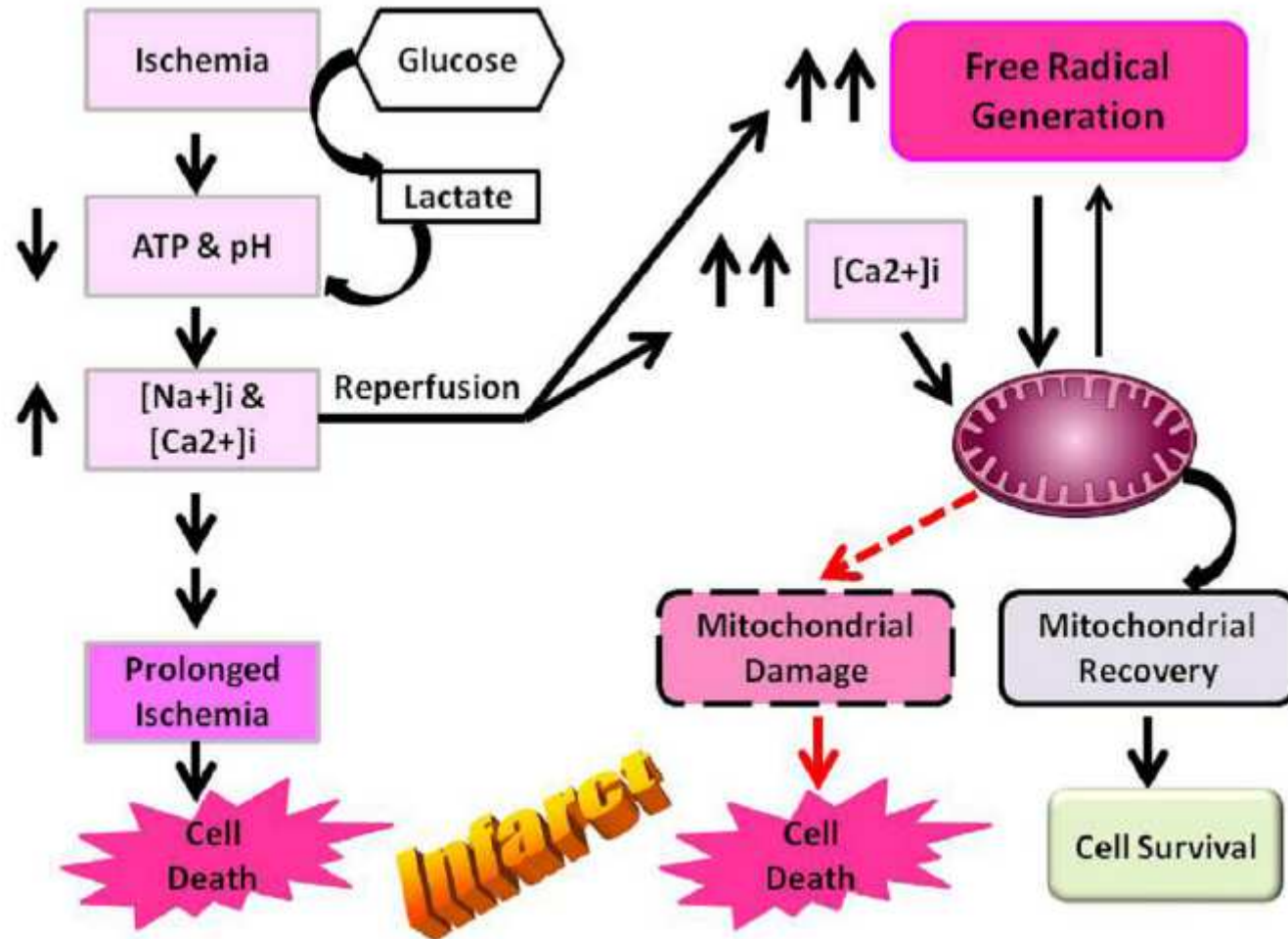


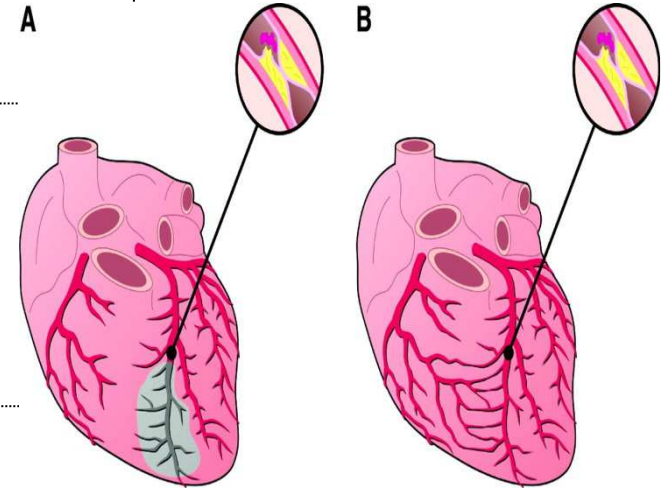
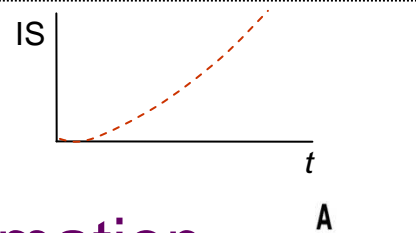
Diagram depicts critical events in cardiac ischemia reperfusion injury (Suleiman *et al* 2001)



# Important Factors to Determine Ischemia and Reperfusion Injury

■ Duration of ischemia

■ Collateral circulation formation



## Dependency on Condition of Reperfusion and Oxygen Supply

- The speed/modality of reperfusion
- The components of reperfusion solution

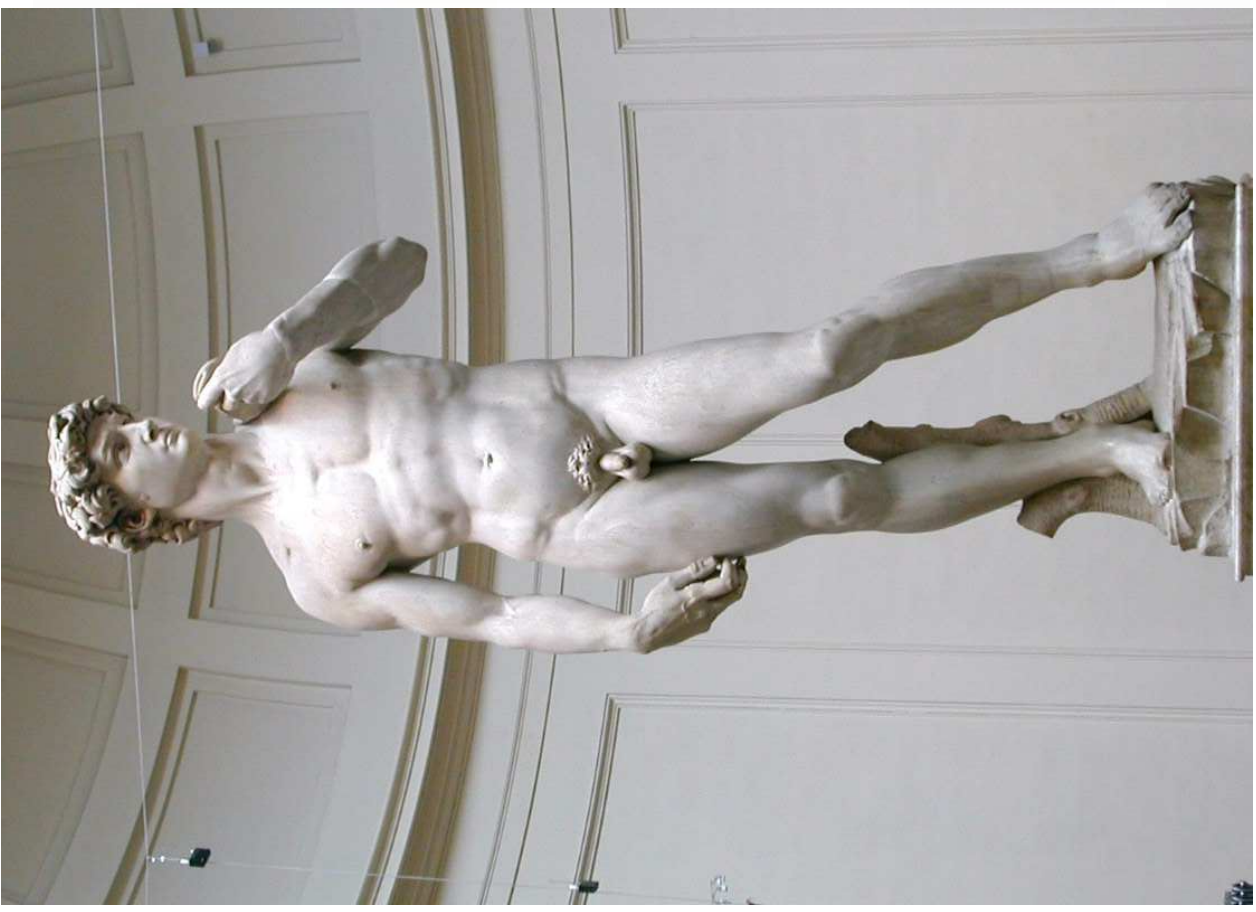
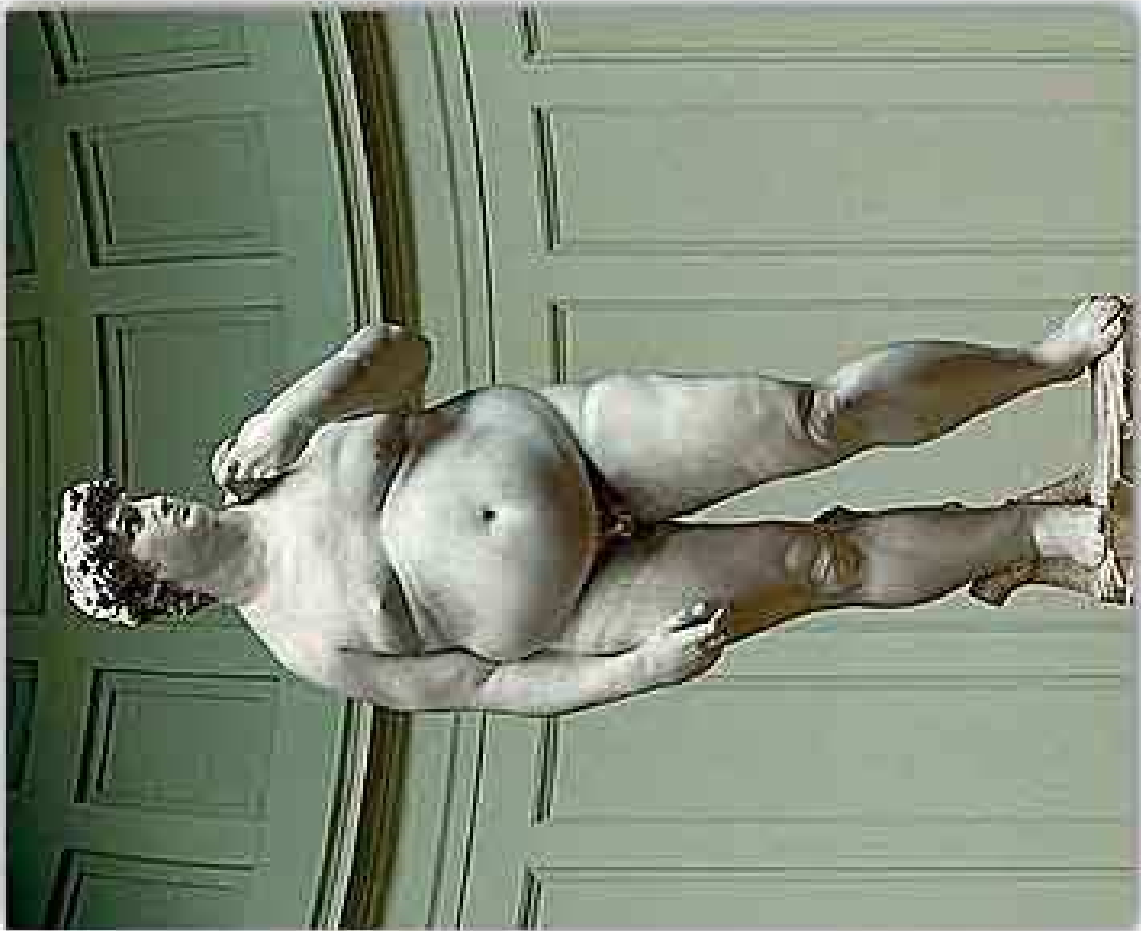
# Why Reperfusion Injury?

# Experimental study showed the importance of

- **Calcium overload**
- **pH recovery (pH paradox)**
- **The Central Role of Mitochondria,**  
**mitochondrial Permeability Transition Pore (mPTP)**  
**and «Reactive Oxygen Species» (ROS)**



# Why Coronary Ischemia ?



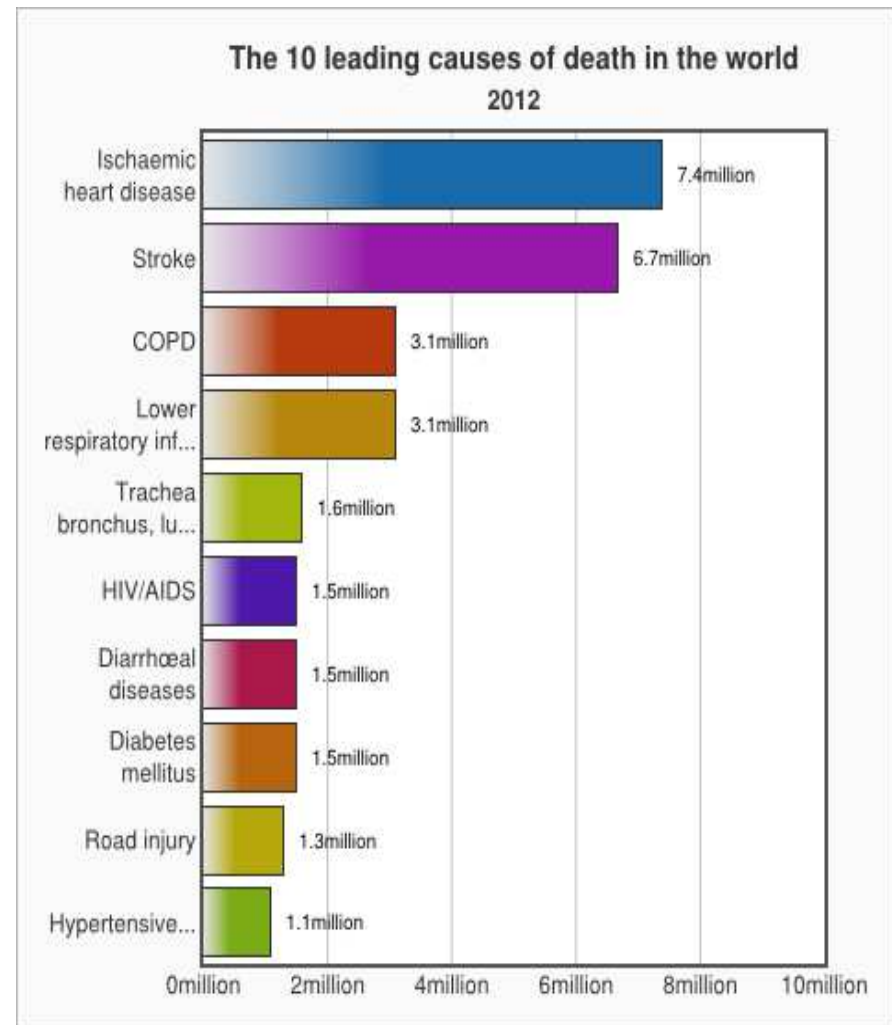
- **10 Factors That Increase the Risk of *Heart Disease and Heart Attack:***

- **1) Tobacco Smoke**
- 2) High Blood Cholesterol
- **3) High Blood Pressure**
- 4) Physical Inactivity
- **5) Obesity and Overweight**
- 6) Diabetes Mellitus
- **7) Stress**
- 8) Alcohol
- **9) Diet and Nutrition**
- 10) Age

# Ischemic Heart Disease (IHD)

The leading cause of death in human population

- It is the most common type of heart disease.
- IHD occurs when the coronary arteries, that supply blood to the myocardium, become hardened and narrowed due to the plaque buildup, reducing the flow of blood and oxygen to the heart.
- IHD can weaken the heart muscle and lead slowly (chronic) or rapidly (acute) to heart failure.

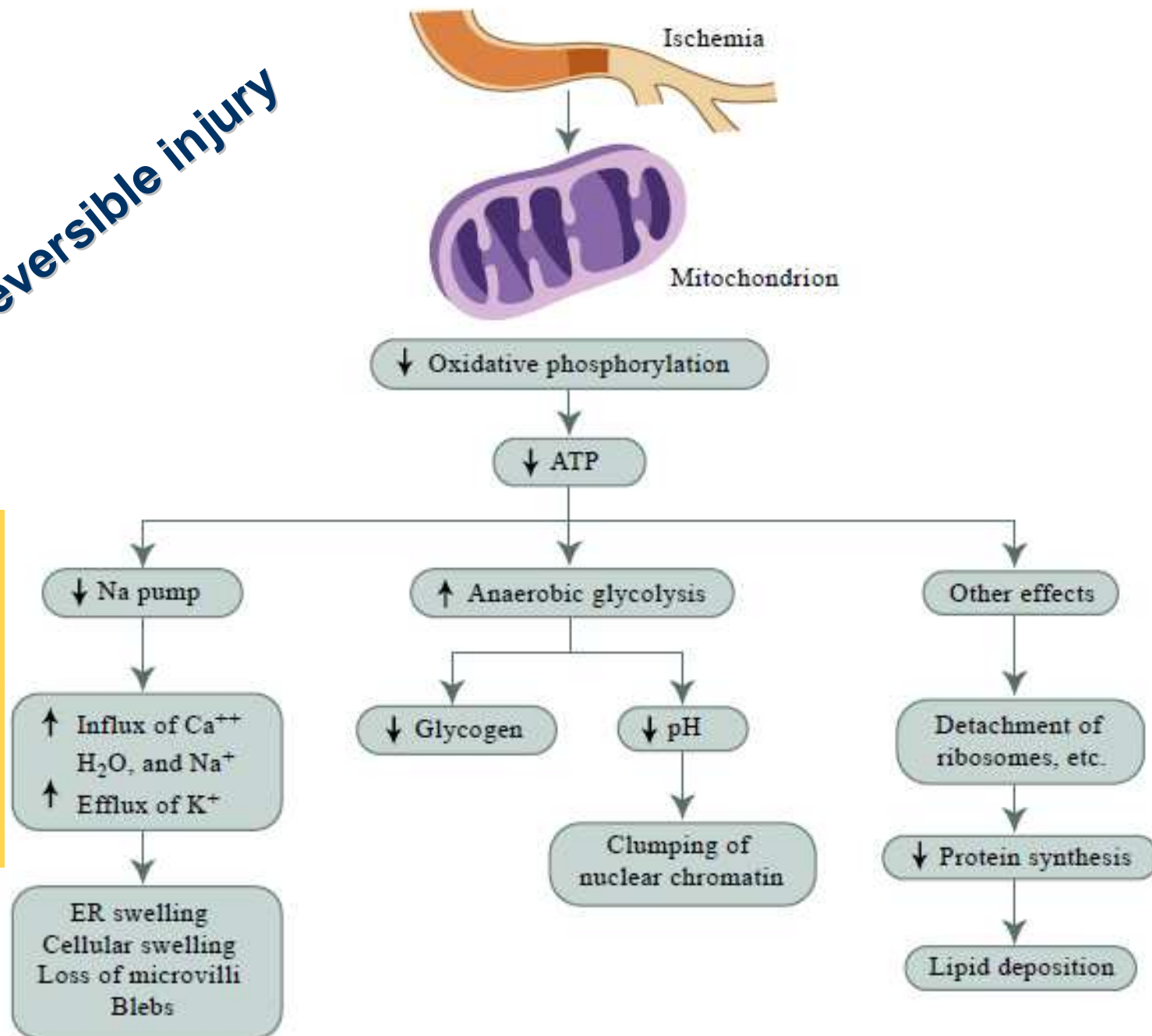


# **The Bad**

**Ischemia:  
the occlusion of a coronary artery**

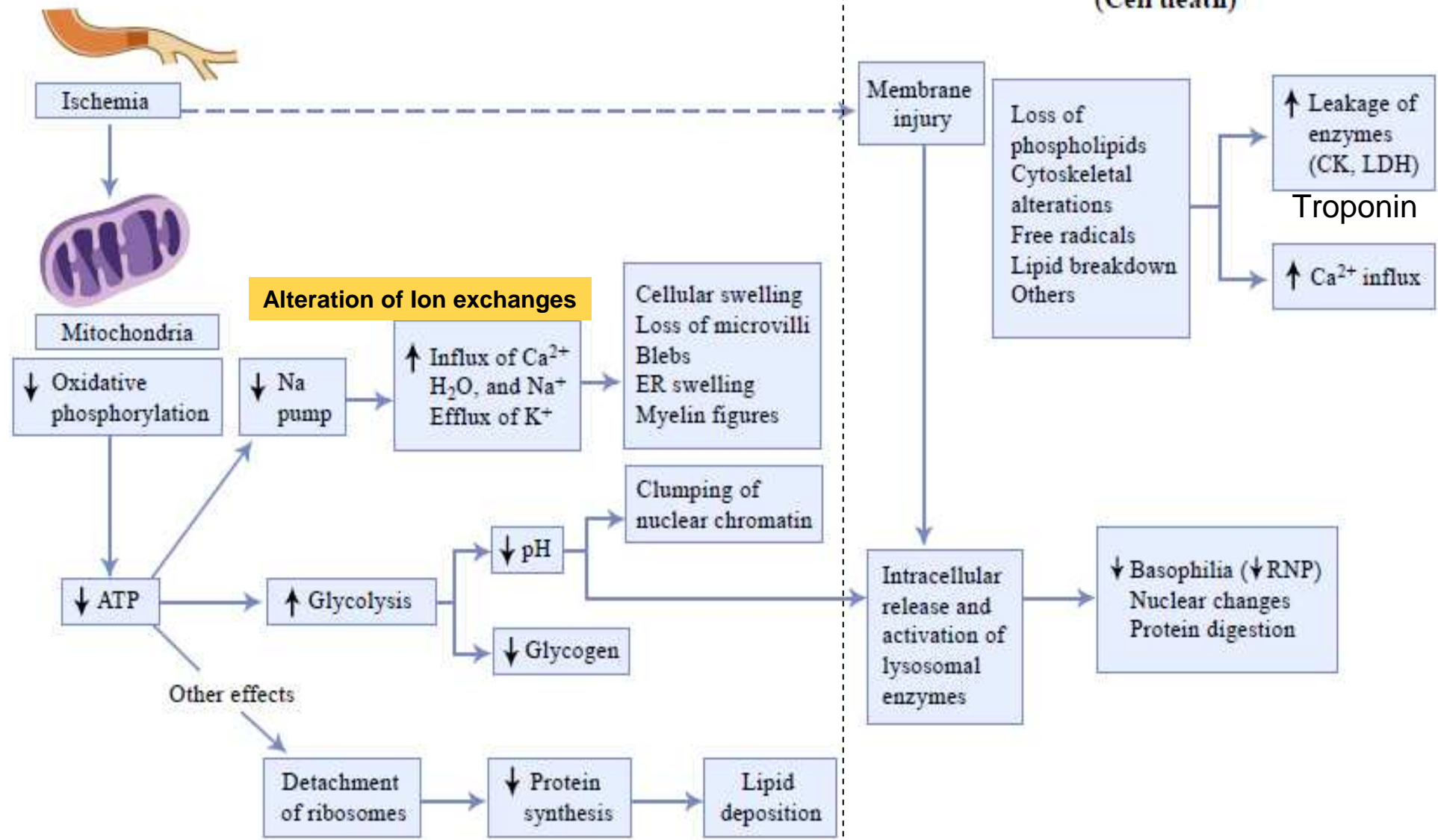
# Reversible injury

## Alteration of ion exchanges



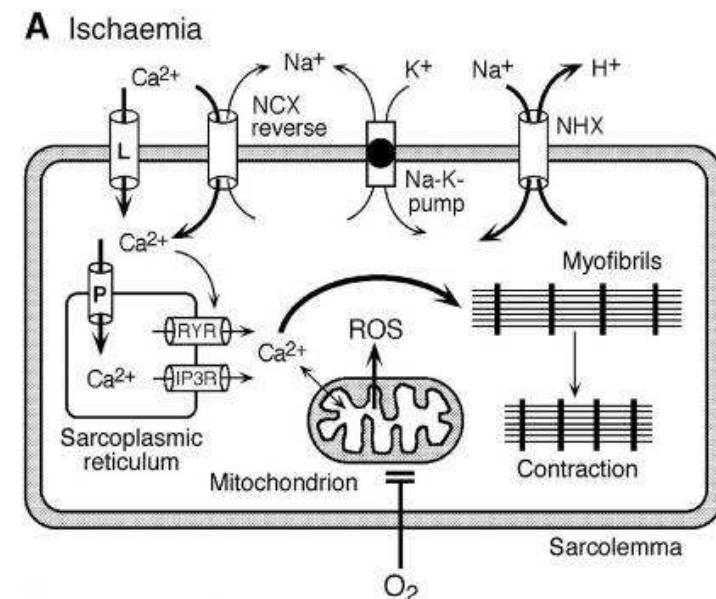
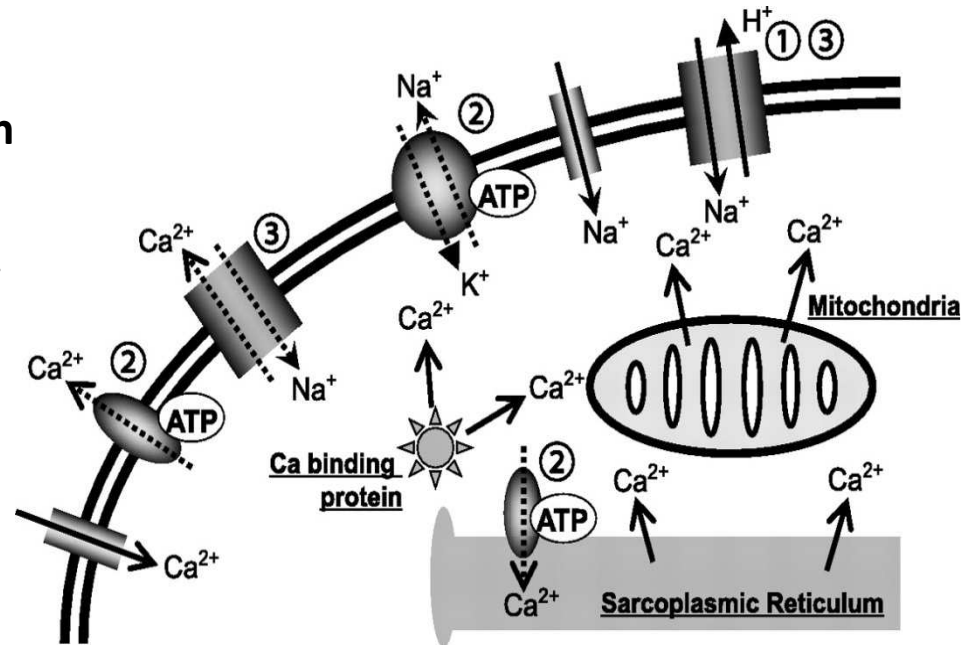
## Reversible Injury

## Irreversible Injury (Cell death)

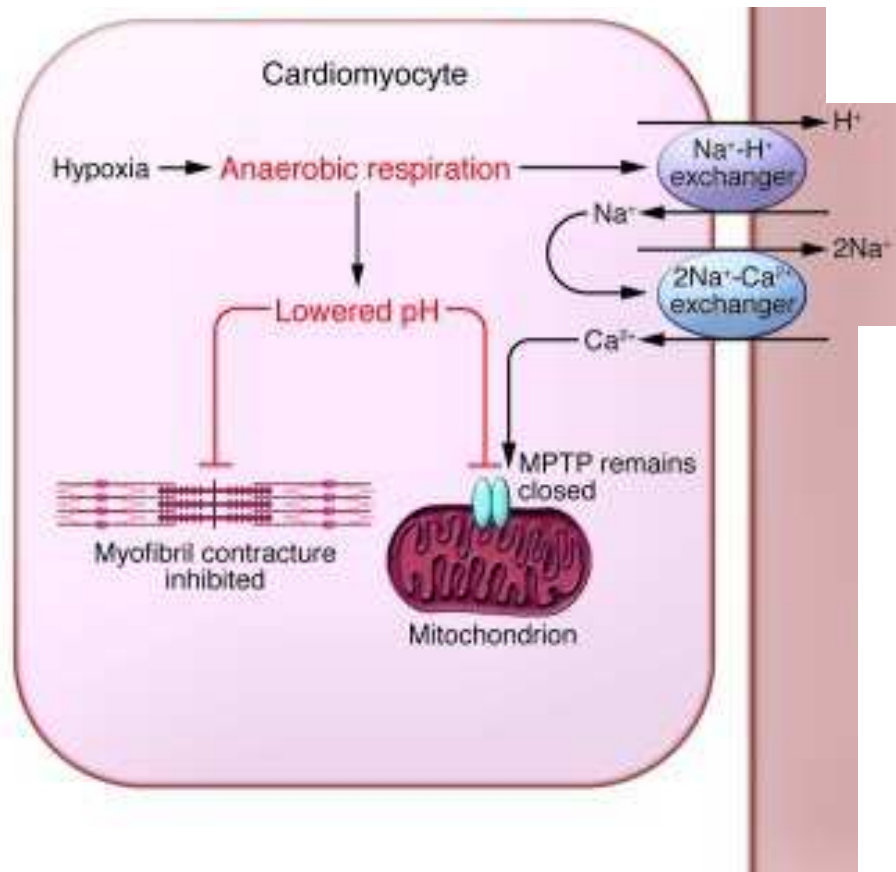




- Cessation of oxygen supply in ischemia leads to a loss of ATP production and an increase of reactive oxygen species (ROS) in the mitochondria
- Reduced activity of the ATP consuming  $\text{Na}^+$ - $\text{K}^+$ -pump leads to  $\text{Na}^+$  accumulation in the myocyte and the resting membrane potential is lowered
- With the development of acidosis, the  $\text{Na}^+$ - $\text{H}^+$ -exchanger (NHX) further increases intracellular  $\text{Na}^+$
- Under these conditions the  **$3\text{Na}^+$ - $1\text{Ca}^{2+}$ -exchanger (NCX) slows down** due to acidotic pH and intracellular accumulation of  $\text{Na}^+$  or may even operate in the **reverse mode**, letting  $\text{Ca}^{2+}$  into the cell
- $\text{Ca}^{2+}$  also enters through the sarcolemmal L-type voltage-gated  $\text{Ca}^{2+}$  -channel (L) as the resting membrane potential is low
- The increased  $\text{Ca}^{2+}$  is taken up into the sarcoplasmic reticulum (SR) by the SR  $\text{Ca}^{2+}$  pump SERCA2 (P) and released (leak) from there via RYR, leading to contraction and contracture.



# Ischemia



Mitochondria may accumulate enormous amounts of Ca<sup>2+</sup>

(crucial for buffering cytosolic calcium).

Ca<sup>2+</sup> overload may favor opening of mPTP, which may be kept closed by low pH.

Depressed mitochondrial calcium uptake secondary to accelerated mitochondrial depolarization during ischemia, and impaired potential recovery during reperfusion, aggravates cytosolic calcium overload and contracture.

During ischemia *contracture* development can be defined as an impairment of relaxation with an increase in diastolic ventricular pressure (LVEDP) of 4 mmHg above pre-ischemic LVEDP values. This together ATP shortage determine a *loss of contractility*

# **The Ugly**

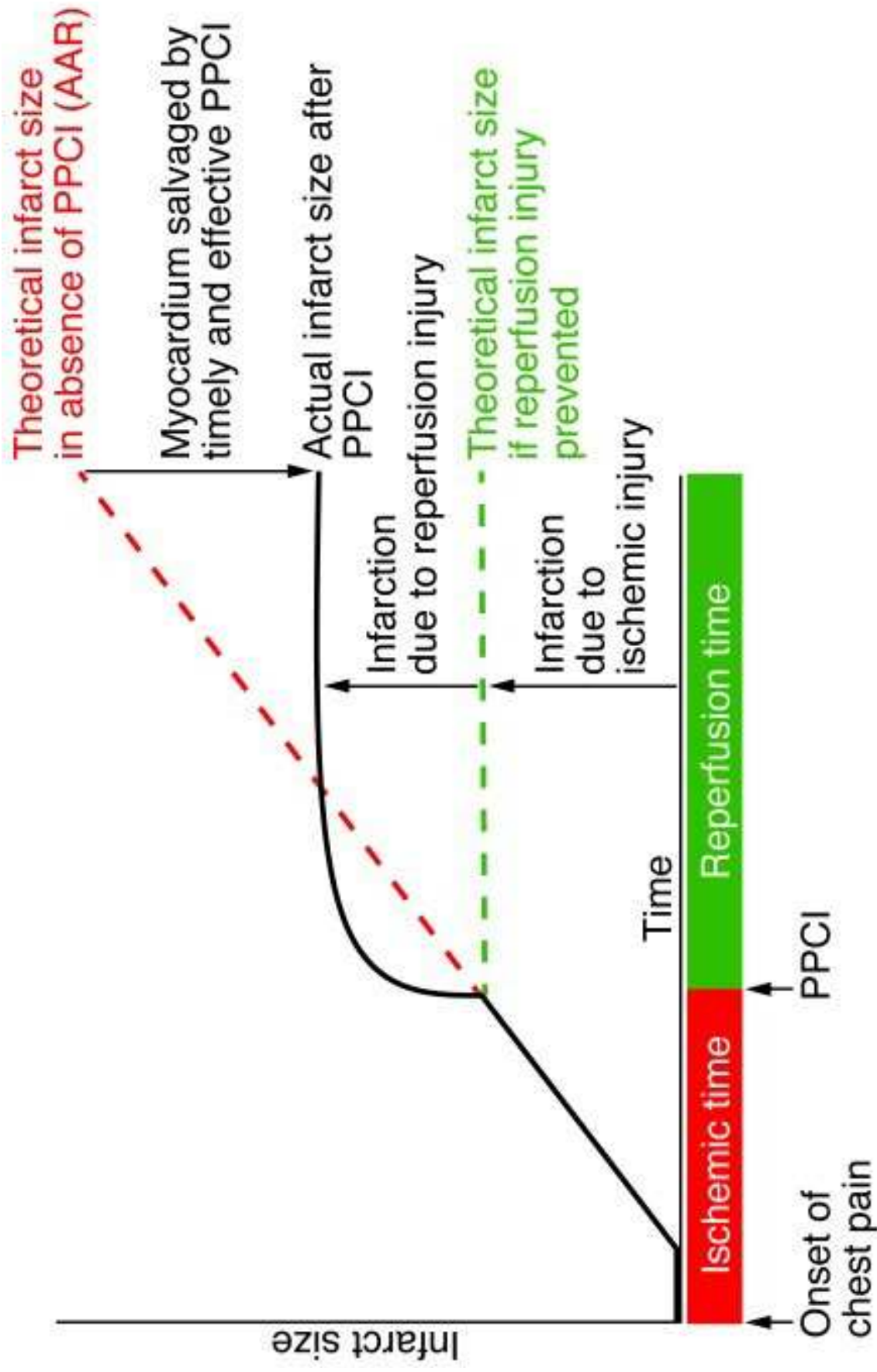
**Reperfusion Injury:  
at the re-opening of a coronary artery**

# **If an Infarcting Ischemia is Followed by *Reperfusion:***

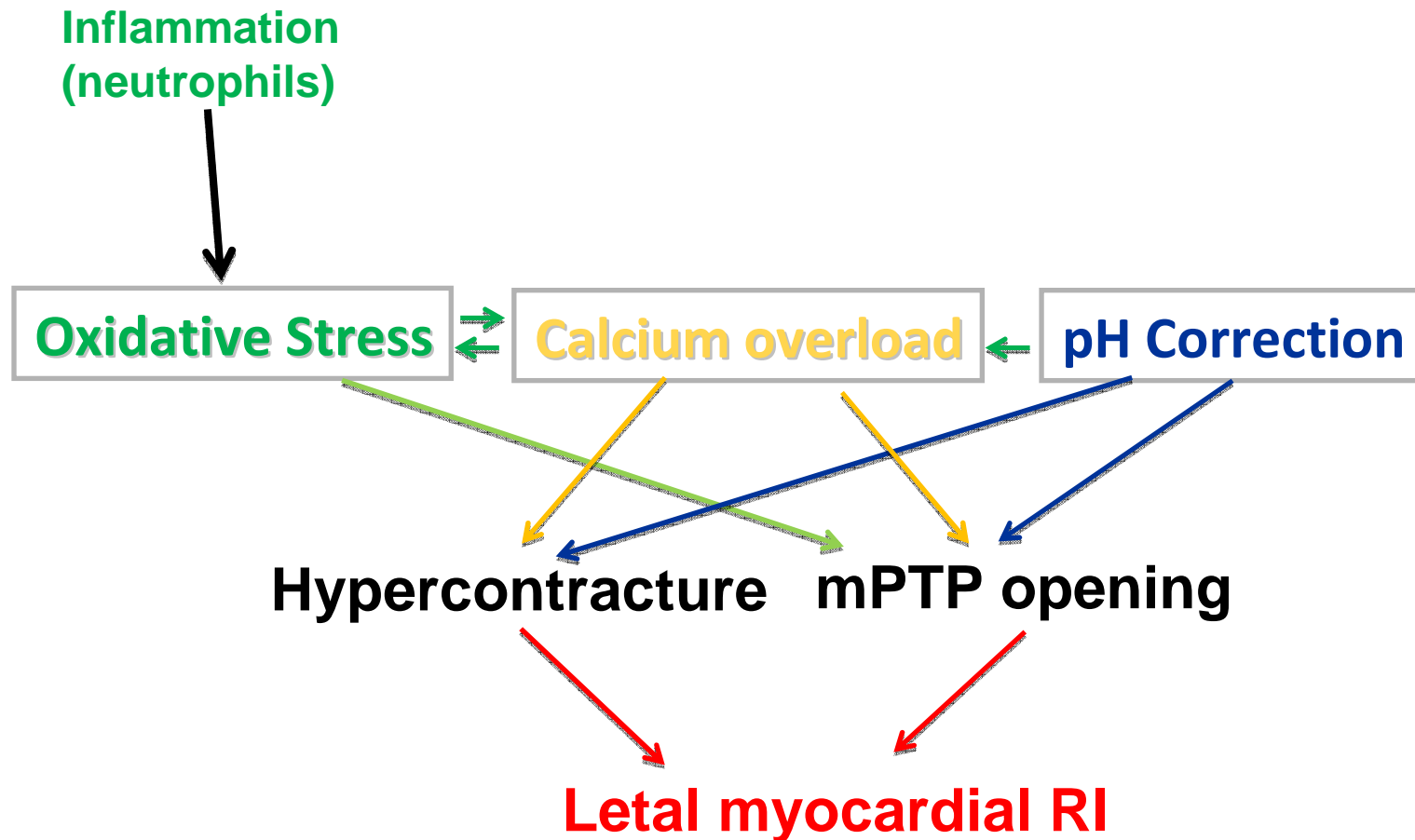
***a) the extension of the myocardial infarction increases;***

***b) the contractility dysfunctions are more severe;***

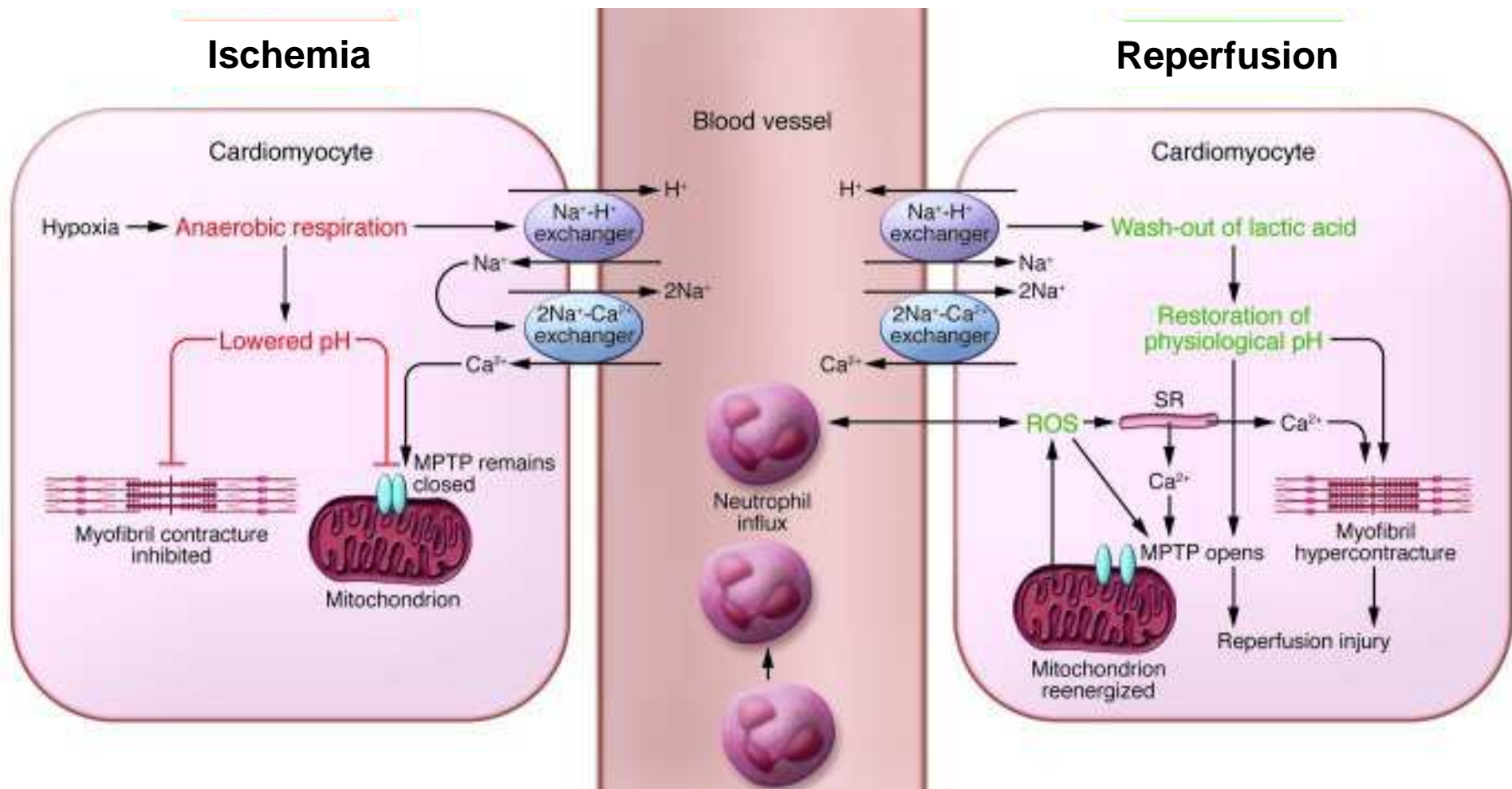
***c) the incidence of arrhythmias may increase.***



# Major components of myocardial reperfusion injury (RI).

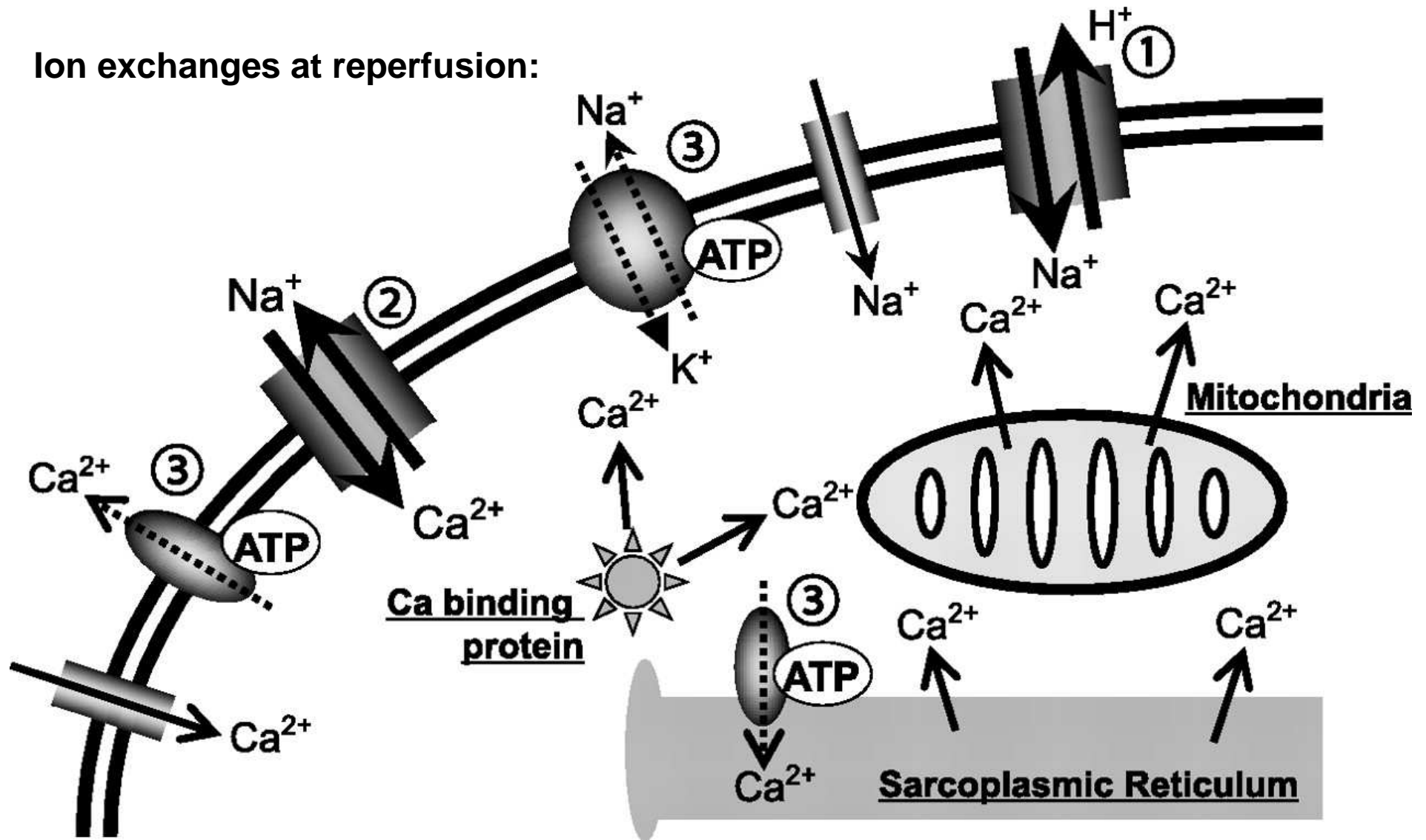


## During the First Minutes of Reperfusion



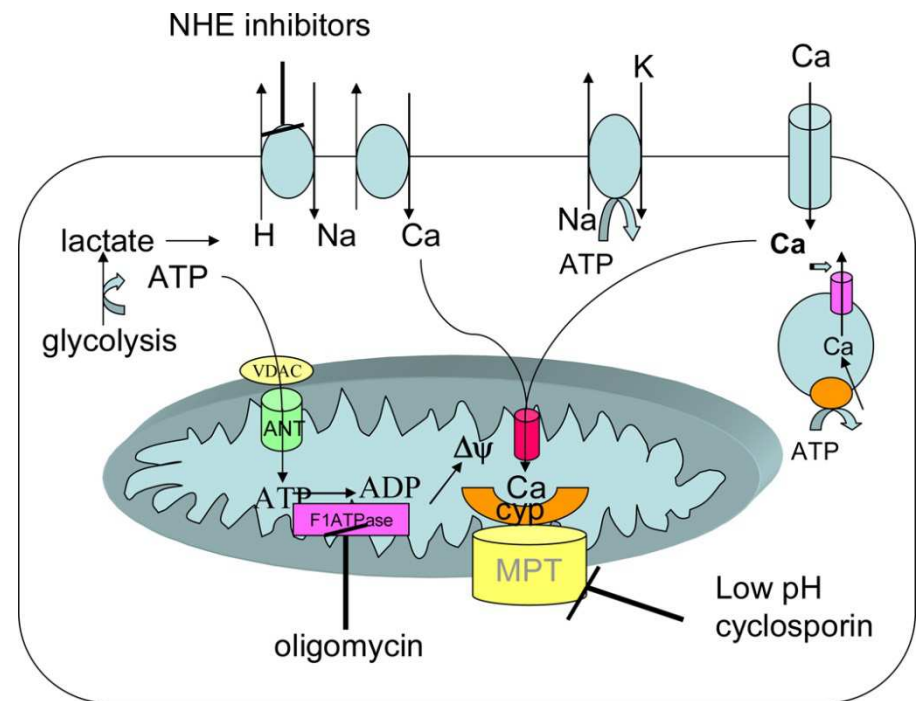
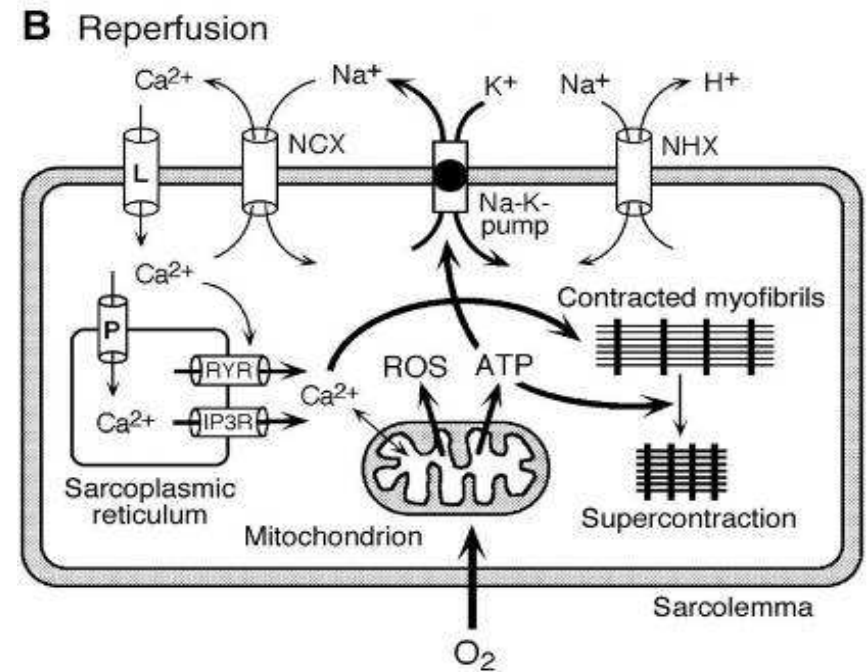


## Ion exchanges at reperfusion:



1) robust excretion of H<sup>+</sup> due to prompt recovery of extracellular pH, 2) **“reverse mode” excretion of accumulated Na<sup>+</sup> and Ca<sup>2+</sup> influx in turn**, and 3) reexcretion of Ca<sup>2+</sup> followed by recovery of ATP synthesis.

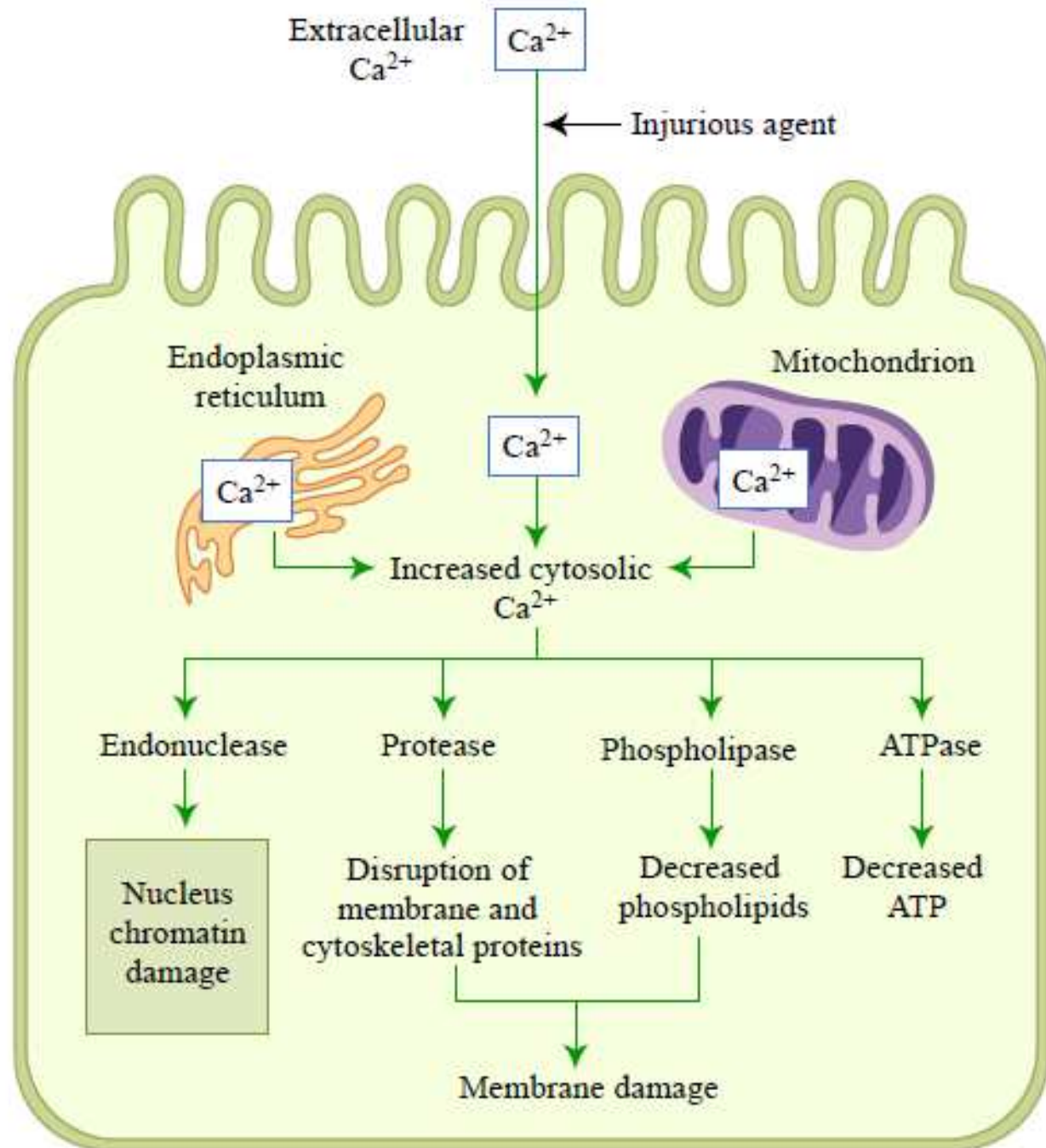
- Reperfusion is intended to produce ATP and to reactivate the  $\text{Na}^+\text{-K}^+$ -pump to slowly restore the sodium gradient leading to normal cation fluxes with the NCX eventually extruding the excess of cytosolic  $\text{Ca}^{2+}$
- **However reoxygenation during reperfusion will slowly restore ATP production with a further burst of ROS**
- Moreover, during the early reperfusion phase when the intracellular  $\text{Ca}^{2+}$  level is still high (NCX reverse mode and  $\text{Ca}^{2+}$  oscillations), myocardial hypercontracture (supercontraction of myocytes) may develop and irreversible injury occur ( $\text{Ca}^{2+}$  overload and rigor)



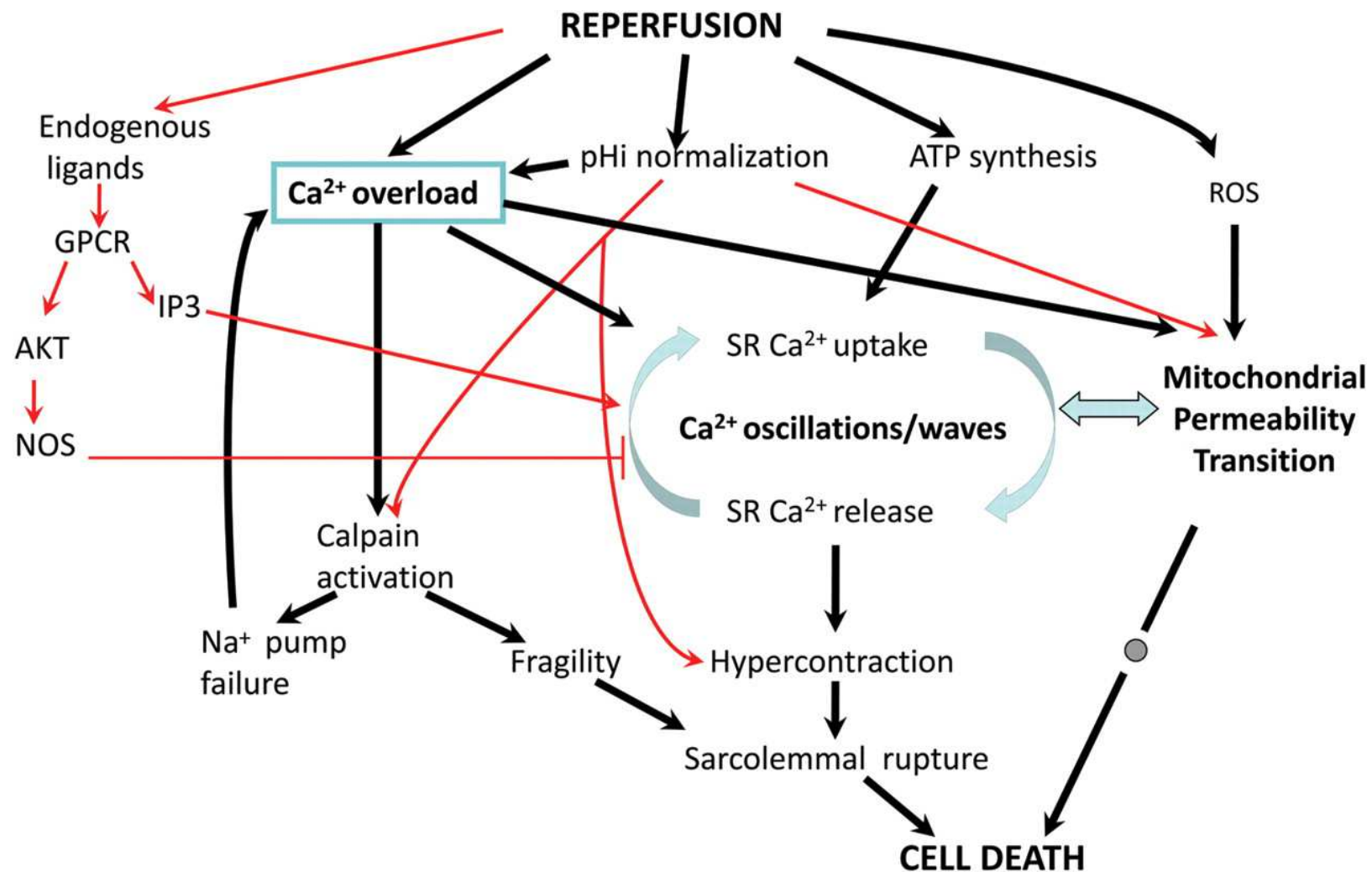
# **The consequences of Calcium overload**

## Effects of excess calcium in the cytosol

Cut-off point  
between  
reversible cell  
injury  
and cell death??



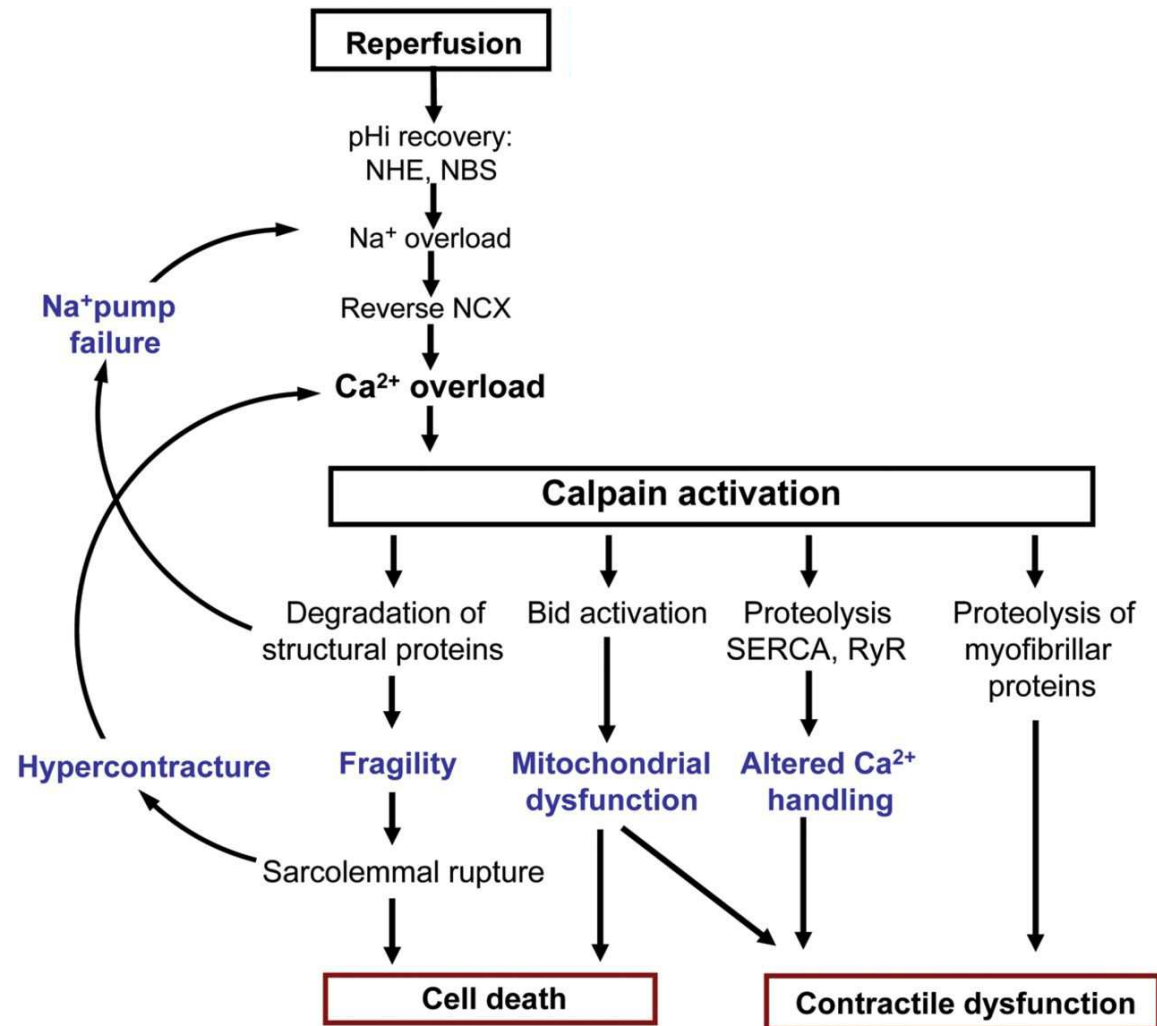
## Mechanisms and consequences of altered $\text{Ca}^{2+}$ handling in cardiomyocytes during initial reperfusion.



David Garcia-Dorado et al. *Cardiovasc Res* 2012;94:168-180



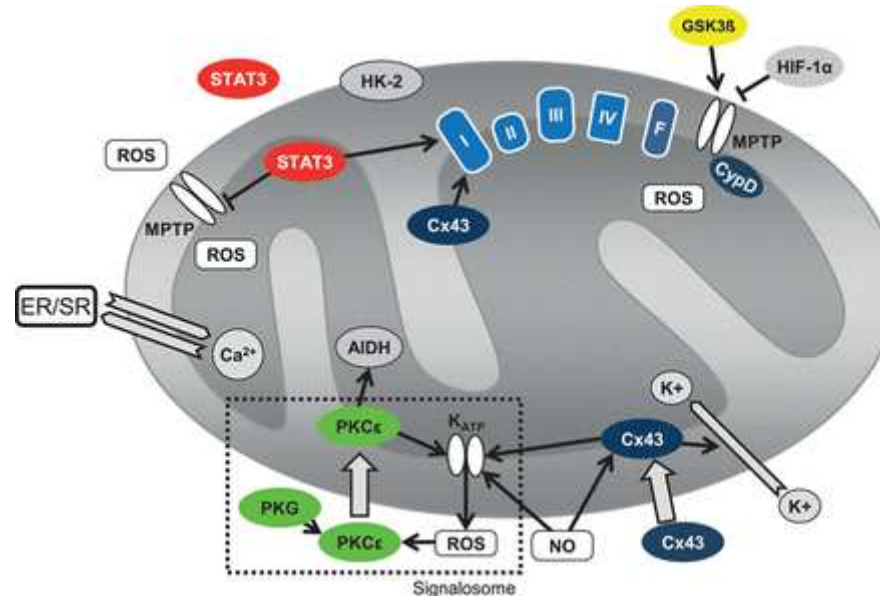
**Schematic diagram showing the proposed mechanisms by which calpains participate in reperfusion injury and in the cardioprotective effects of preconditioning and postconditioning.**



Javier Inerte et al. Cardiovasc Res 2012;96:23-31

# ROS are double-edged swords

(They can be good or bad)



## The bad-ugly consequences of ROS overproduction



# Sources of ROS

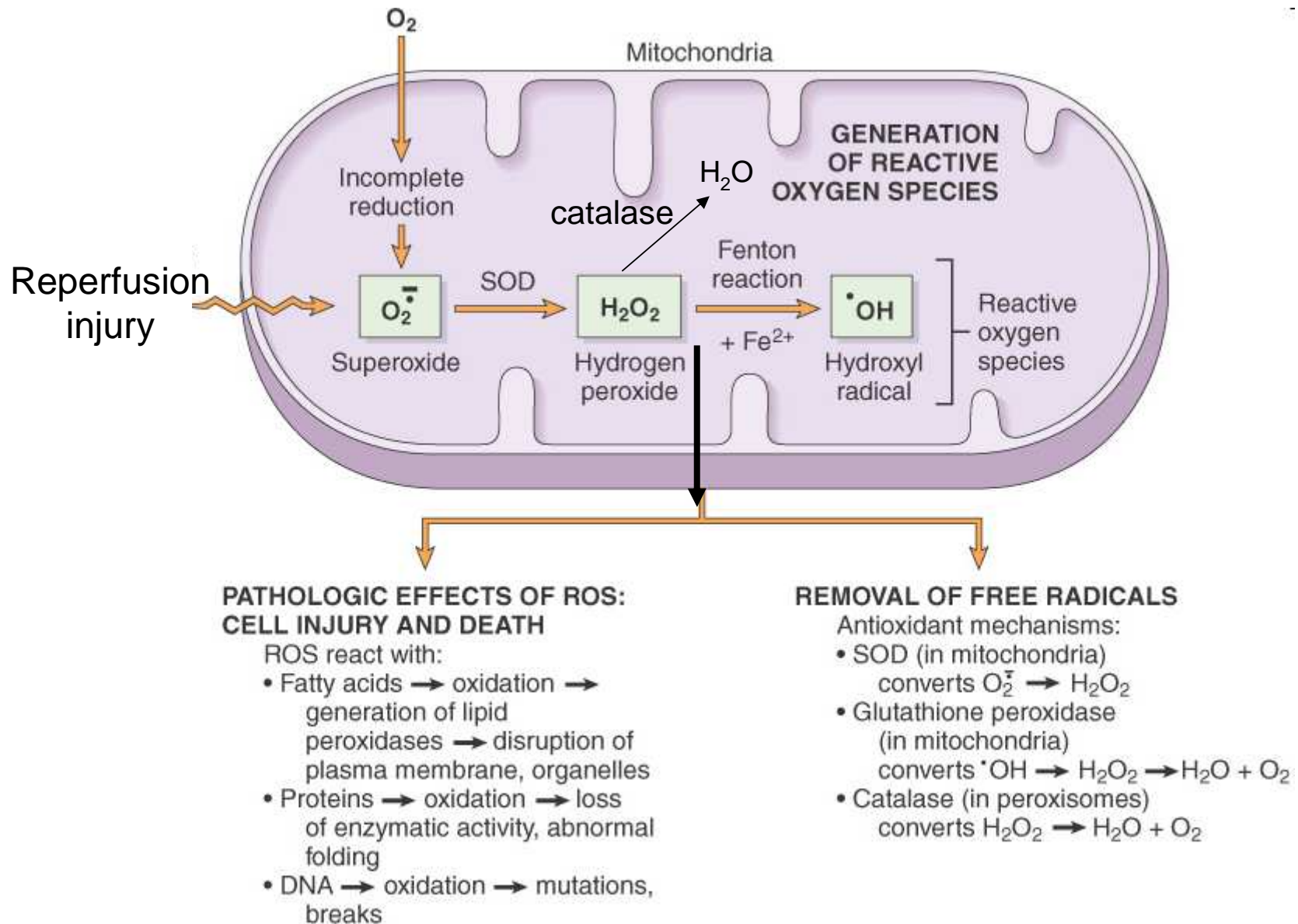
**In the cardio-circulatory system, ROS can be generated by**

- **cardiomyocytes,**
- **endothelial cells, and**
- **neutrophils in inflammatory processes.**

**Different enzyme/mechanisms can produce ROS,  
including**

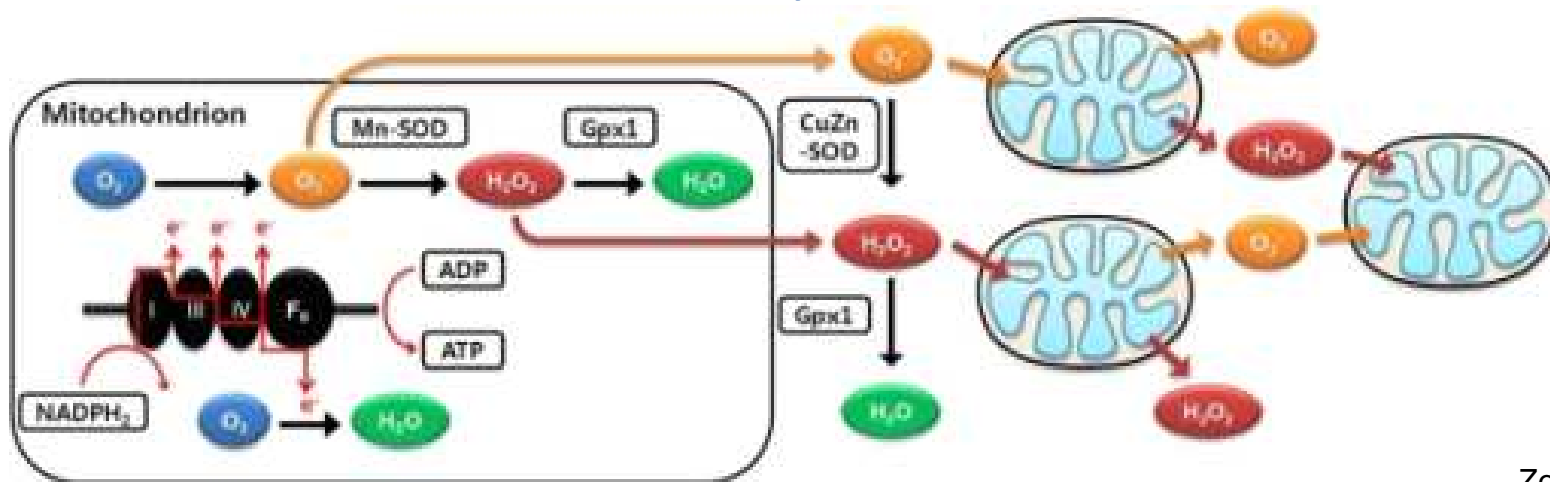
- **electrons leaked from mitochondrial complexes,**
- **NADPH oxidase,**
- **xanthine dehydrogenase/xanthine oxidase,**
- **lipoxygenases,**
- **cyclooxygenases,**
- **peroxidases, and**
- **uncoupled nitric oxide synthase (NOS).**

## Mitochondria represent 36-40% of cardiomyocytes mass

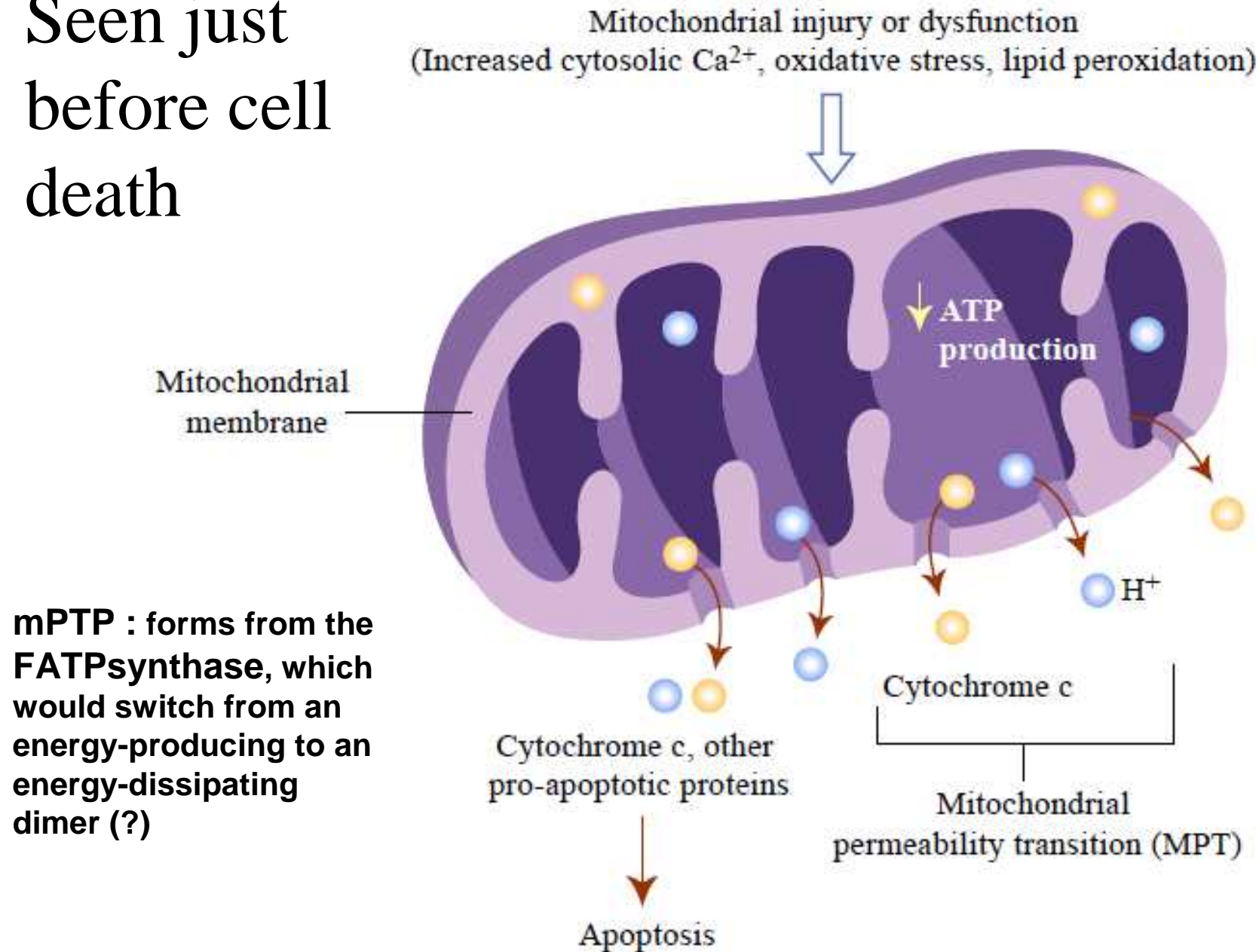


# ROS INDUCED ROS RELEASE (RIRR)

- Ischemia and reperfusion raise the production of ROS which may activate the mPTP, especially in reperfusion, when pH recovers, and may be involved in the **conversion of signaling to pathological ROS (RIRR)**
- **RIRR** is a process originating in mitochondria responding to an increased oxidative stress by a **positive feedback loop** resulting in a regenerative, autocatalytic cascade
- When **RIRR** is inappropriately not terminated, it may lead to unwanted **cell loss** such as after myocardial infarction



Seen just  
before cell  
death



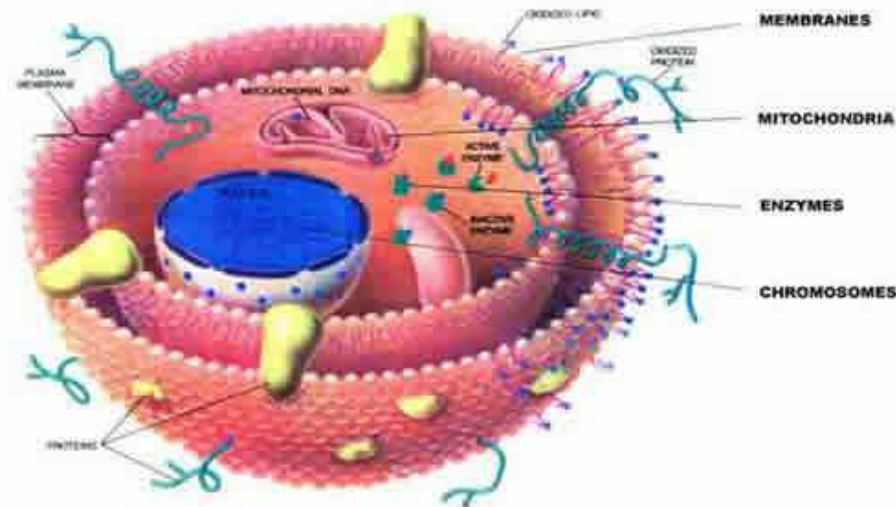
Mitochondrial ROS rise simultaneously with the mPTP-induced drop of  $\Delta\Psi$

## ■ Mechanisms of ROS-induced Ischemia/Reperfusion Injury

**ROS are extremely reactive to interact with lipids, proteins and nucleic acids.**

### ● The increase of membrane lipid peroxidation (MLP)

ROS interact with non-saturated fatty acids from membrane lipids and further induce lipid peroxidation reaction, which results in the **structural alteration and dysfunction of membrane.**



ROS induces oxidation of lipids, proteins and nucleic acid.

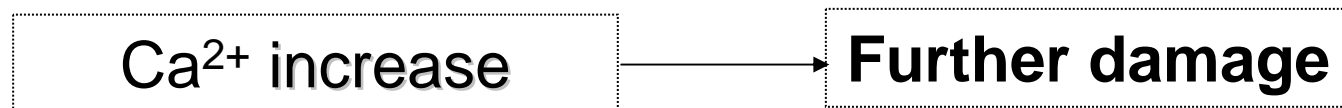
## ● ROS-mediated Membrane damage

***The integrity, permeability and function of membrane are impaired during ischemia-reperfusion due to ROS-induced MLP.***

- Deactivation and malfunction of membrane receptors and ionic pumps***
- Mitochondrial dysfunction and further decreases ATP generation***

These damages do not occur only in sarcolemma, but also in sarcoplasmic reticulum, mitochondria, lysosomes and other intracellular membranes.

Therefore, **Ca<sup>2+</sup> can flow into the cytoplasm** through damaged membrane according to the gradient.

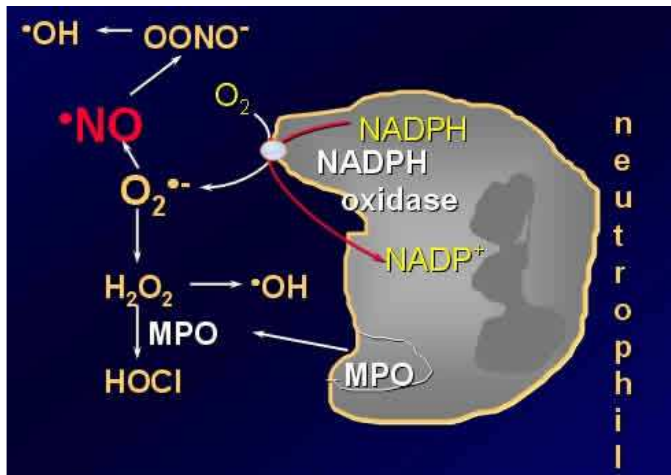


## During the Subsequent Hours of Reperfusion

- With the reperfusion, the **endothelial** and **vessels become permeable**, thus causing interstitial edema
- **Activated Endothelial cells** in reperfused myocardium express **adhesion proteins**, release **cytokines**, and **reduce production of NO**
- These promote **adherence, activation, and accumulation of neutrophils** and monocytes in the ischemic-reperfused tissue



- The release of **reactive oxygen species** and proteolytic enzymes from these **activated leukocytes** can contribute to the **damage of myocytes and vascular cells**
- **Vascular plugging by adherent leukocytes and aggregated platelets** can also promote a **slow- or no-reflow phenomenon**, already favored by tissue contracture and increased pressure of interstitial edema
- These additional reperfusion-induced noxes contribute to infarct development predominantly during the **first 2 hours of reperfusion**, as myocardial necrosis almost reaches its final size during this period



## Summary

### **Ischemia/Reperfusion Injury (IRI)**

occurring with ischemia and restoration of blood flow to post-ischemic tissue, is associated with arrhythmias, myocardial necrosis and apoptosis resulting in increased mortality and morbidity.

**Calcium overload,**

**pH recovery,**

**and ROS overproduction**

**are major players in determining IRI**

**Mitochondria play a pivotal role in  
life and death**

# ***Contributors***

***Prof. Claudia Penna, MSc, PhD, Physiology, Turin***

***Dr. Francesca Tullio, MSc, PhD, Physiology, Turin***

***Dr. Maria-Giulia Perrelli, MSc, PhD, Pathology, Turin***

***Dr. Sara Femmino', BSc, MSc, PhD student, Physiology, Turin***

***Dr. Carmelina Angotti, BSc, MSc, PhD student, Physiology, Turin***

***Dr. Jasmin Popara, MD, PhD student, Physiology, Turin***

***Prof. Donatella Gattullo, MSc, Physiology, Turin***



THANKS !

## **Recent Publications of our group**

- 1: Penna C, Granata R, Tocchetti CG, Gallo MP, Alloatti G, Pagliaro P. Endogenous Cardioprotective Agents: Role in Pre and Postconditioning. *Curr Drug Targets*. 2015 Mar 9. [Epub ahead of print] PubMed PMID: 25751010.
- 2: Tocchetti CG, Molinaro M, Angelone T, Lionetti V, Madonna R, Mangiacapra F, Moccia F, Penna C, Sartiani L, Quaini F, Pagliaro P. Nitroso-Redox Balance and Modulation of Basal Myocardial Function: an Update from the Italian Society of Cardiovascular Research (SIRC). *Curr Drug Targets*. 2015 Mar 3. [Epub ahead of print] PubMed PMID: 25738298.
- 3: Pagliaro P, Penna C. Redox signalling and cardioprotection: translatability and mechanism. *Br J Pharmacol*. 2015 Apr;172(8):1974-95. doi: 10.1111/bph.12975. Epub 2015 Jan 12. PubMed PMID: 25303224; PubMed Central PMCID: PMC4386976.
- 4: Penna C, Brancaccio M, Tullio F, Rubinetto C, Perrelli MG, Angotti C, Pagliaro P, Tarone G. Overexpression of the muscle-specific protein, melusin, protects from cardiac ischemia/reperfusion injury. *Basic Res Cardiol*. 2014 Jul;109(4):418. doi: 10.1007/s00395-014-0418-9. Epub 2014 May 25. PubMed PMID: 24859929.
- 5: Penna C, Angotti C, Pagliaro P. Protein S-nitrosylation in preconditioning and postconditioning. *Exp Biol Med* (Maywood). 2014 Jun;239(6):647-62. Review. PubMed PMID: 24668550.
- 6: Tullio F, Angotti C, Perrelli MG, Penna C, Pagliaro P. Redox balance and cardioprotection. *Basic Res Cardiol*. 2013 Nov;108(6):392. doi: 10.1007/s00395-013-0392-7. Epub 2013 Oct 25. Review. PubMed PMID: 24158692.
- 7: Penna C, Perrelli MG, Tullio F, Angotti C, Camporeale A, Poli V, Pagliaro P. Diazoxide postconditioning induces mitochondrial protein S-nitrosylation and a redox-sensitive mitochondrial phosphorylation/translocation of RISK elements: no role for SAFE. *Basic Res Cardiol*. 2013 Sep;108(5):371. doi: 10.1007/s00395-013-0371-z. Epub 2013 Jul 20. PubMed PMID: 23872876.
- 8: Pagliaro P, Gattullo D, Penna C. Nitroglycerine and sodium trioxodinitrate: from the discovery to the preconditioning effect. *J Cardiovasc Med* (Hagerstown). 2013 Oct;14(10):698-704. doi: 10.2459/JCM.0b013e3283621ac6. Review. PubMed PMID: 23695182.
- 9: Penna C, Settanni F, Tullio F, Trovato L, Pagliaro P, Alloatti G, Ghigo E, Granata R. GH-releasing hormone induces cardioprotection in isolated male rat heart via activation of RISK and SAFE pathways. *Endocrinology*. 2013 Apr;154(4):1624-35. doi: 10.1210/en.2012-2064. Epub 2013 Feb 15. PubMed PMID: 23417421.