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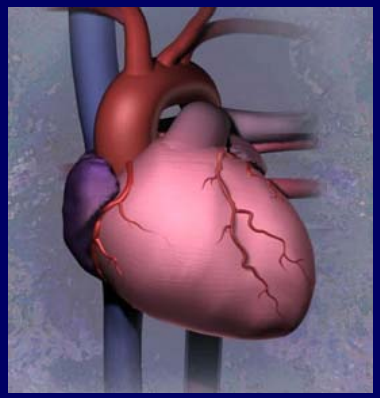

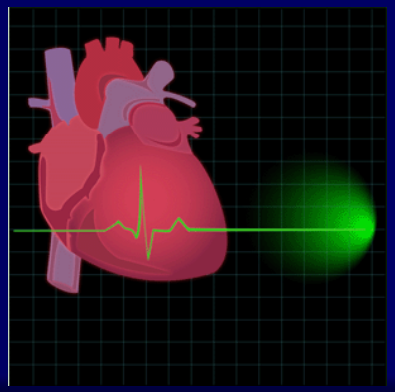

Comparative Study of the Cardioprotective Effects of Local and Remote Preconditioning in Ischemia/Reperfusion Injury

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Introduction

The heart possesses a remarkable ability to adapt to stress by changing its phenotype in a manner that renders it more resistant to injury.

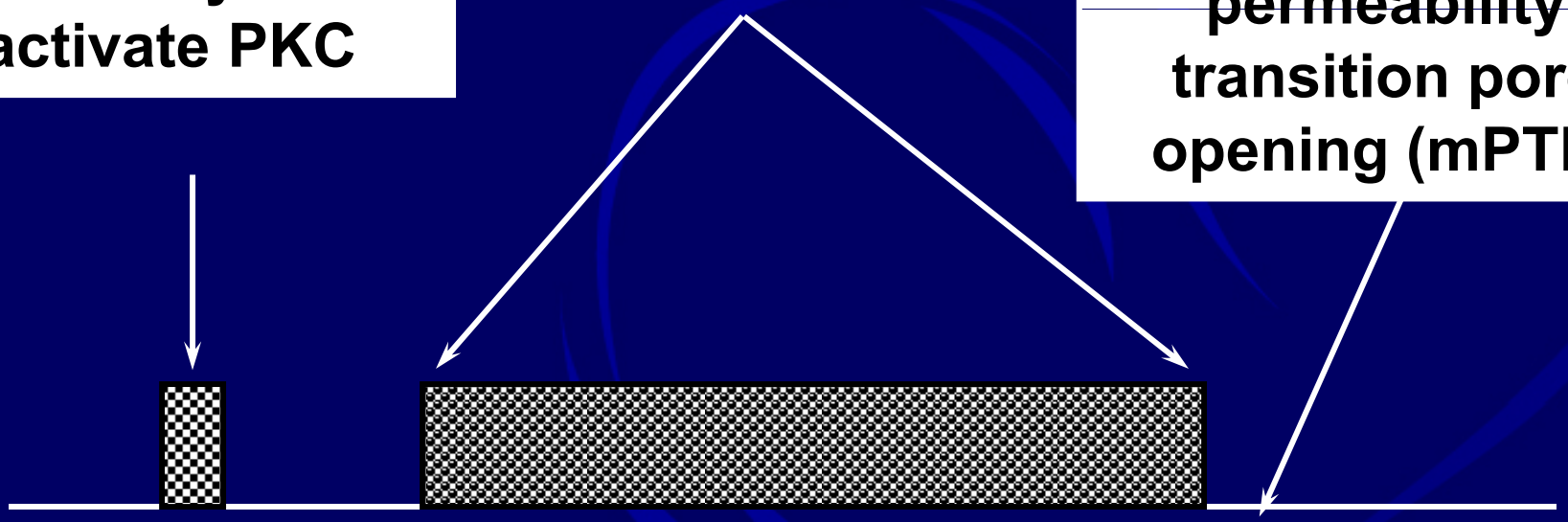
This powerful adaptive phenomenon in which there is an increase in myocardial tolerance to I/R

preconditioning

Trigger phase
adenosine and other surface receptors couple through multiple Pathways to activate PKC

Ischemic phase
PKC acts as a memory.

Reperfusion phase
signal transduction pathways act to prevent mitochondria permeability transition pore opening (mPTP)



Current theory of IPC

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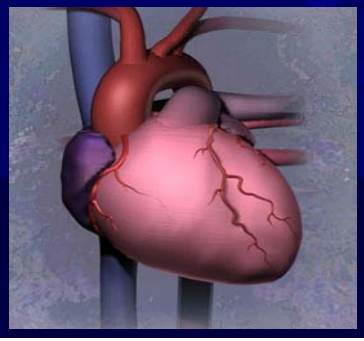
Aim of the Work

This study is directed to assess the possible cardioprotective effects of these different preconditioning therapies in ~~myocardial I/R injury~~

Classic or ischemic preconditiong (IPC)

Remote preconditioning



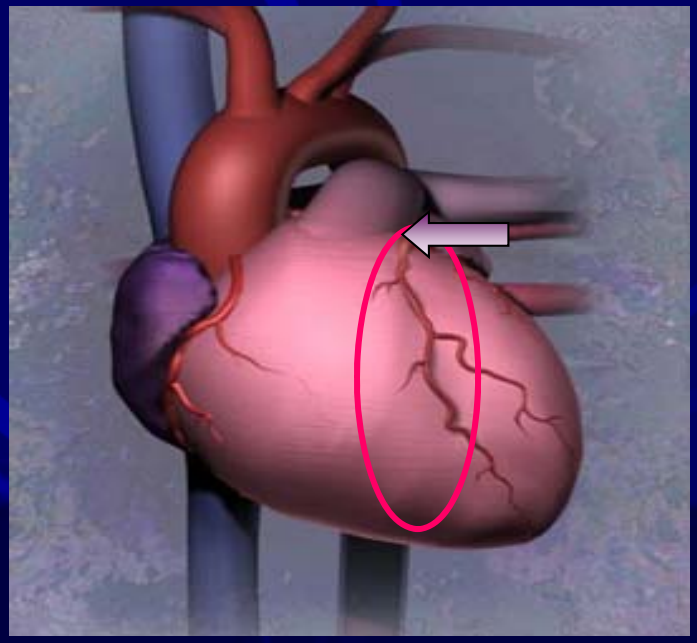
Experimental design

Animals:

Adult male rats weighing 200-250 g

Surgical procedure:

Rats will be subjected to 40 min of myocardial ischemia by ligation of the left descending coronary artery ,followed by reperfusion for 10 min



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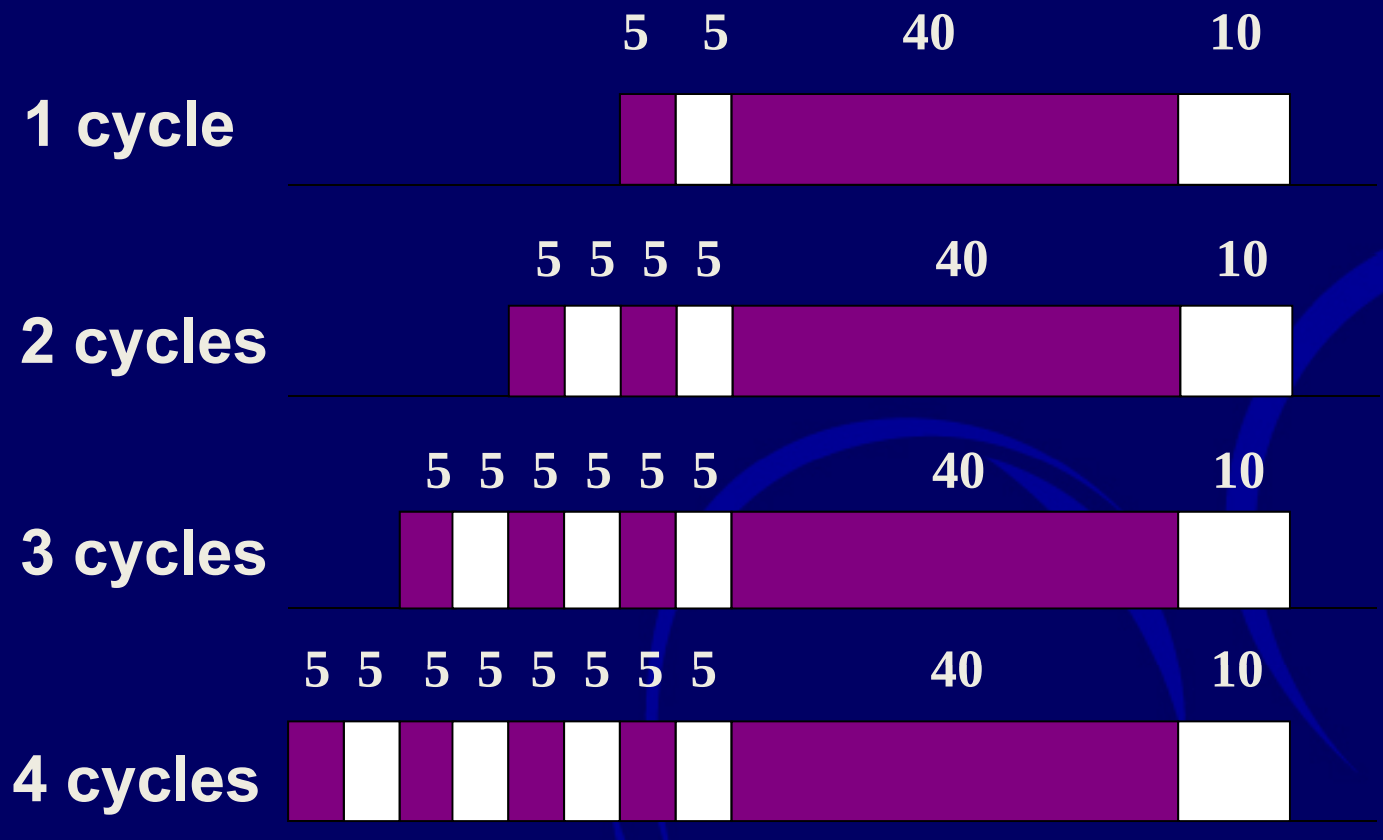
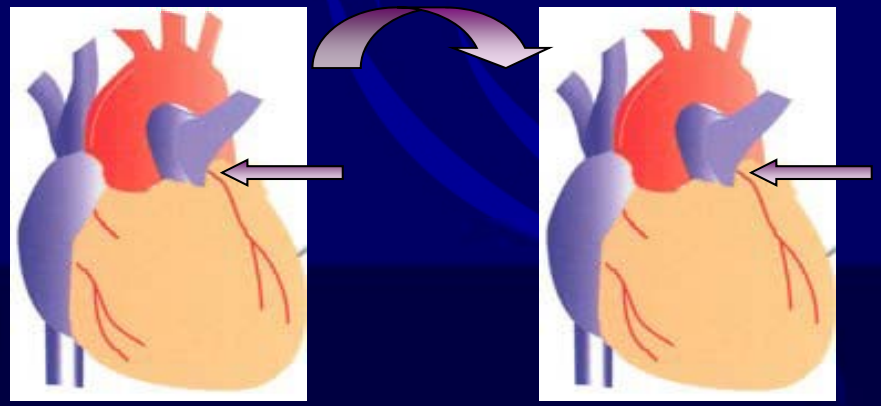
Local ischemic preconditioning (LIPC)





ischemia

reperfusion

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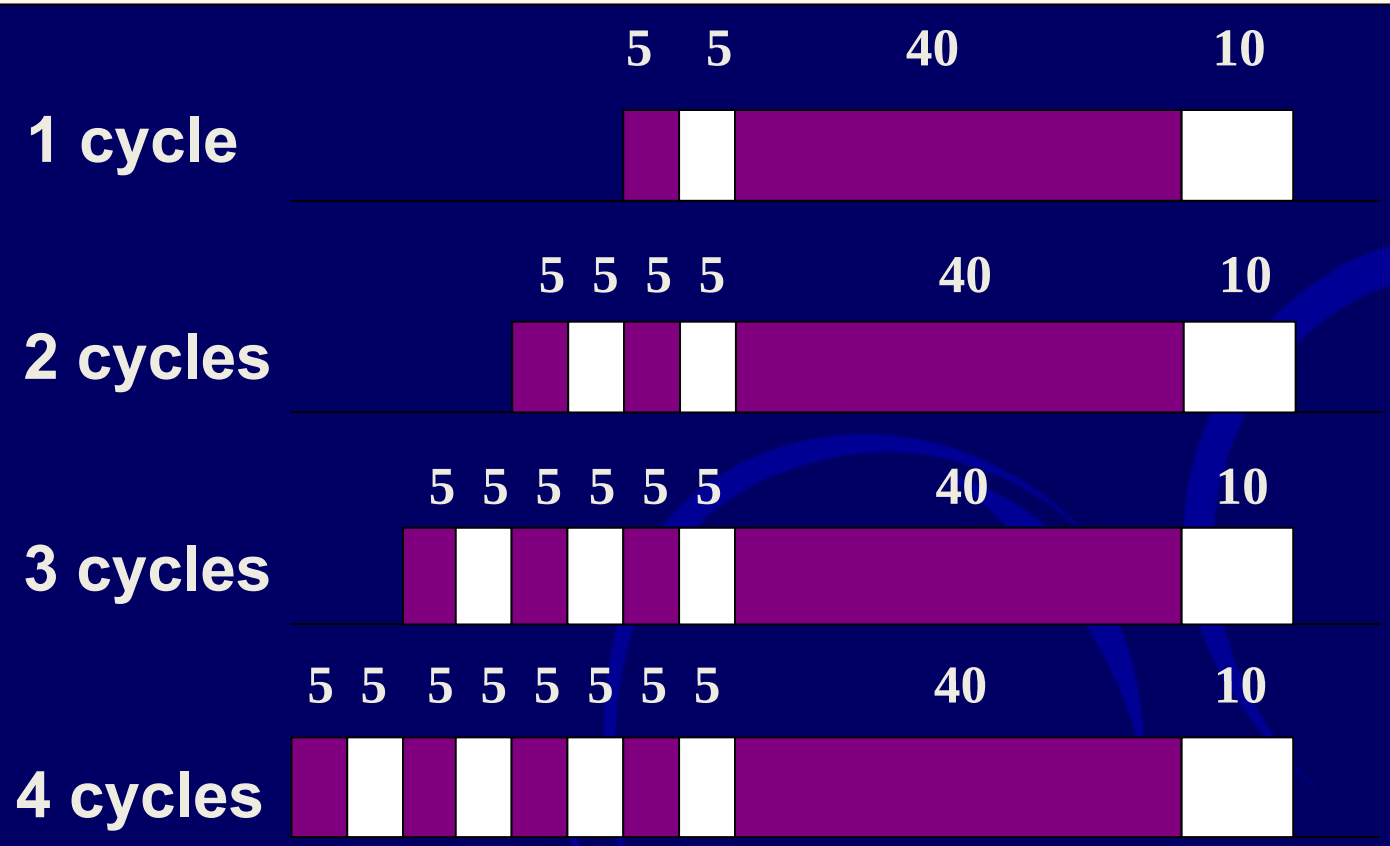
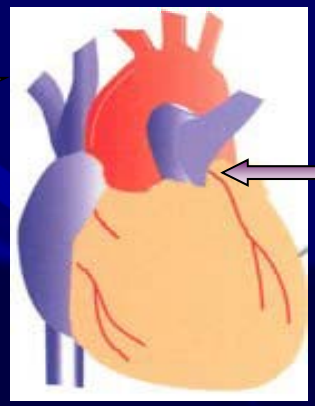
Remote limb ischemic preconditioning (RIPC)





ischemia

reperfusion

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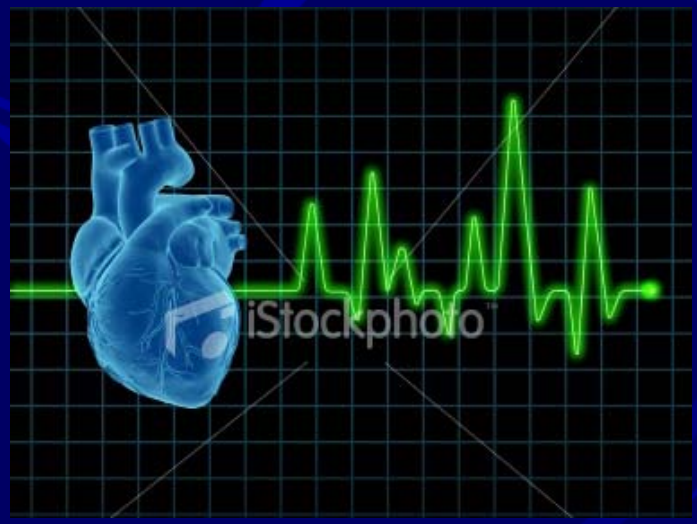
Parameters to be measured

I) Hemodynamic parameters

- Heart rate
- Arrhythmia score
- Arrhythmia onset
- Mean duration of VT
- Number of different types of arrhythmias (VP, BG, TG, S, VT and TdP)



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II) Biochemical parameters

Tissue

- Lactate
- Adenine nucleotides
- TBARS and GSH contents
- MPO activity

Plasma

- CK-MB activity
- NO_X

III) Hisological examination

a) Light microscopic examination (H&E)

b) Electron microscopic examination for mitochondria and myofibrils

Results

Table (1): Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in heart rates in rats.

Groups	Heart rate (beats/min)		
	Pre-ischemic stage	End-ischemic stage (35 min from the onset of ischemia)	Reperfusion stage (1 min from the onset of reperfusion)
I/R	334.17 ± 8.22	370 ± 16.38	345.68 ± 20.39
IPC (1cycle)	334.7 ± 2.98	339.25 ± 16.38	328.98 ± 17.63
IPC (2cycle)	338.32 ± 6.91	322.61 ± 8.28	318.21 ± 6.78
IPC (3cycle)	340.67 ± 9.18	289.34 ± 8.22	278.24 ± 9.92
IPC (4cycle)	342.25 ± 7.38	290.58 ± 6.81	274.05 ± 9.77
RIPC (1cycle)	342.8 ± 12.15	363.2 ± 9.79	347.63 ± 13.29
RIPC (2cycle)	341.73 ± 4.87	328.33 ± 6.42	310 ± 4.79
RIPC (3cycle)	344.87 ± 12.51	324.87 ± 5.01	305.83 ± 8.97
RIPC (4cycle)	346.28 ± 4.62	322.64 ± 4.49	295.68 ± 3

Each value represents the mean of 8-9 experiments ± S.E.M.

Table (2): Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced ventricular arrhythmias in rats.

Groups	Number of different types of ventricular arrhythmias					
	VP	BG	TG	S	VT	TdP
I/R	12.13 ± 2.48	38.13 ± 12.31	4.25 ± 1.26	3.63 ± 1.3	6.25 ± 2.29	2.25 ± 1.18
IPC (1cycle)	2.83 ± 1.22 @	1.67 ± 1.09 @	0 ± 0 @	0 ± 0 @	0.33 ± 0.21 @	0 ± 0 @
IPC (2cycle)	1.33 ± 0.78 @	0.56 ± 0.56 @	0.44 ± 0.44 @	0.13 ± 0.13 @	0 ± 0 @	0 ± 0 @
IPC (3cycle)	1.43 ± 1.13 @	0 ± 0 @	0 ± 0 @	0.86 ± 0.86 @	0 ± 0 @	0 ± 0 @
IPC (4cycle)	1.2 ± 0.97 @	1.6 ± 1.6 @	0 ± 0 @	0 ± 0 @	0.2 ± 0.2 @	0 ± 0 @
RIPC (1cycle)	5.6 ± 1.97	19 ± 7.44	3.2 ± 1.83	2.2 ± 0.66	2.6 ± 1.03	0.6 ± 0.6
RIPC (2cycle)	7.33 ± 2.42	19.5 ± 6.37	1.67 ± 0.76	0.67 ± 0.67	4.67 ± 2.06	0.5 ± 0.5
RIPC (3cycle)	3 ± 1.37	13.5 ± 4.64	1 ± 1	1.33 ± 0.71	1.5 ± 1.03	0.5 ± 0.5
RIPC (4cycle)	7.8 ± 4.95	11.8 ± 8.35	1 ± 0.63	5.6 ± 4.16	4.2 ± 2.27	0.2 ± 0.2

VP: Ventricular premature, BG: Bigeminy, TG: Trigeminy, S: Salvos, VT: Ventricular tachycardia, TdP: Torsade de pointes, TA: Total arrhythmias (sum of percentages of individual arrhythmias, which may be >100% because each animal can exhibit more than one type of arrhythmia). Arrhythmia score represents the mean of 7-8 experiments ± S.E.M. @*p<0.05 vs*. I/R.

Table (2): Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced ventricular arrhythmias in rats.

Groups	Mean VT duration (sec)	Arrhythmia onset (min)	Arrhythmia score
I/R	7.8 ± 2.74	9.13 ± 0. 48	3.5 ± 0.17
IPC (1cycle)	0.32 ± 0.23 @	9.75 ± 1.03	1.33 ± 0.56 @
IPC (2cycle)	0 ± 0 @	15.33 ± 0.33 @	0.56 ± 0.24 @
IPC (3cycle)	0 ± 0 @	11.67 ± 2.03	0.571 ± 0.297 @
IPC (4cycle)	0.26 ± 0.26 @	12 ± 0.58	0.8 ± 0.583 @
RIPC (1cycle)	4.7 ± 1.63	8.5 ± 0.87	2.6 ± 0.68
RIPC (2cycle)	4.49 ± 1.74	12.33 ± 1.45 @	2.33 ± 0.61
RIPC (3cycle)	3.9 ± 2.47	15.67 ± 0.67 @	2.17 ± 0.54
RIPC (4cycle)	4.4 ± 2.07	11.25 ± 0.85	2.6 ± 0.68

VP: Ventricular premature, BG: Bigeminy, TG: Trigeminy, S: Salvos, VT: Ventricular tachycardia, TdP: Torsade de pointes, TA: Total arrhythmias (sum of percentages of individual arrhythmias, which may be >100% because each animal can exhibit more than one type of arrhythmia). Arrhythmia score represents the mean of 7-8 experiments ± S.E.M. @*p<0.05 vs*. I/R.

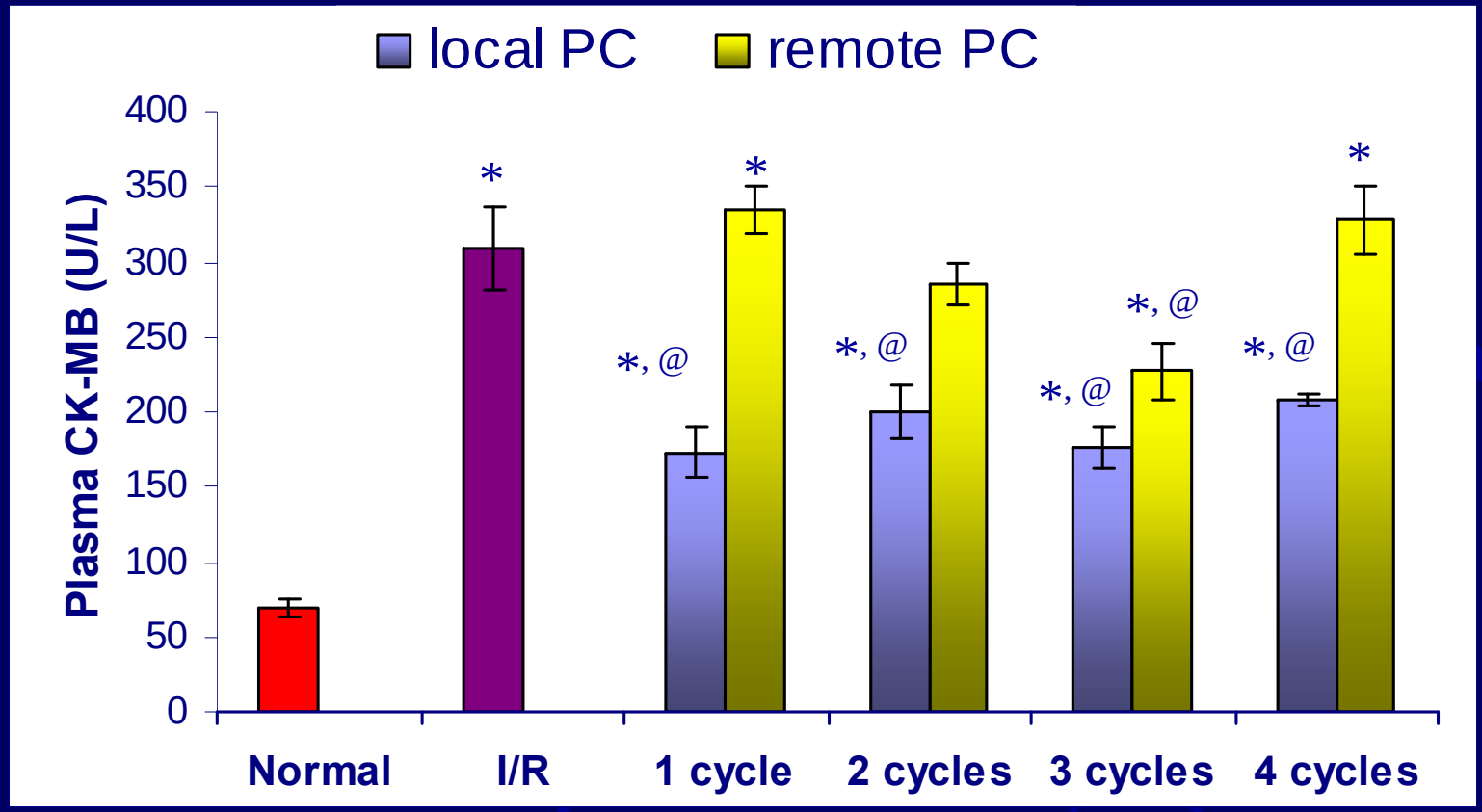

Figure (1): Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in plasma CK-MB.

Each value represents the mean of 7-9 experiments ± S.E.M. **p<0.05 vs.* control, *@p<0.05 vs.* I/R.

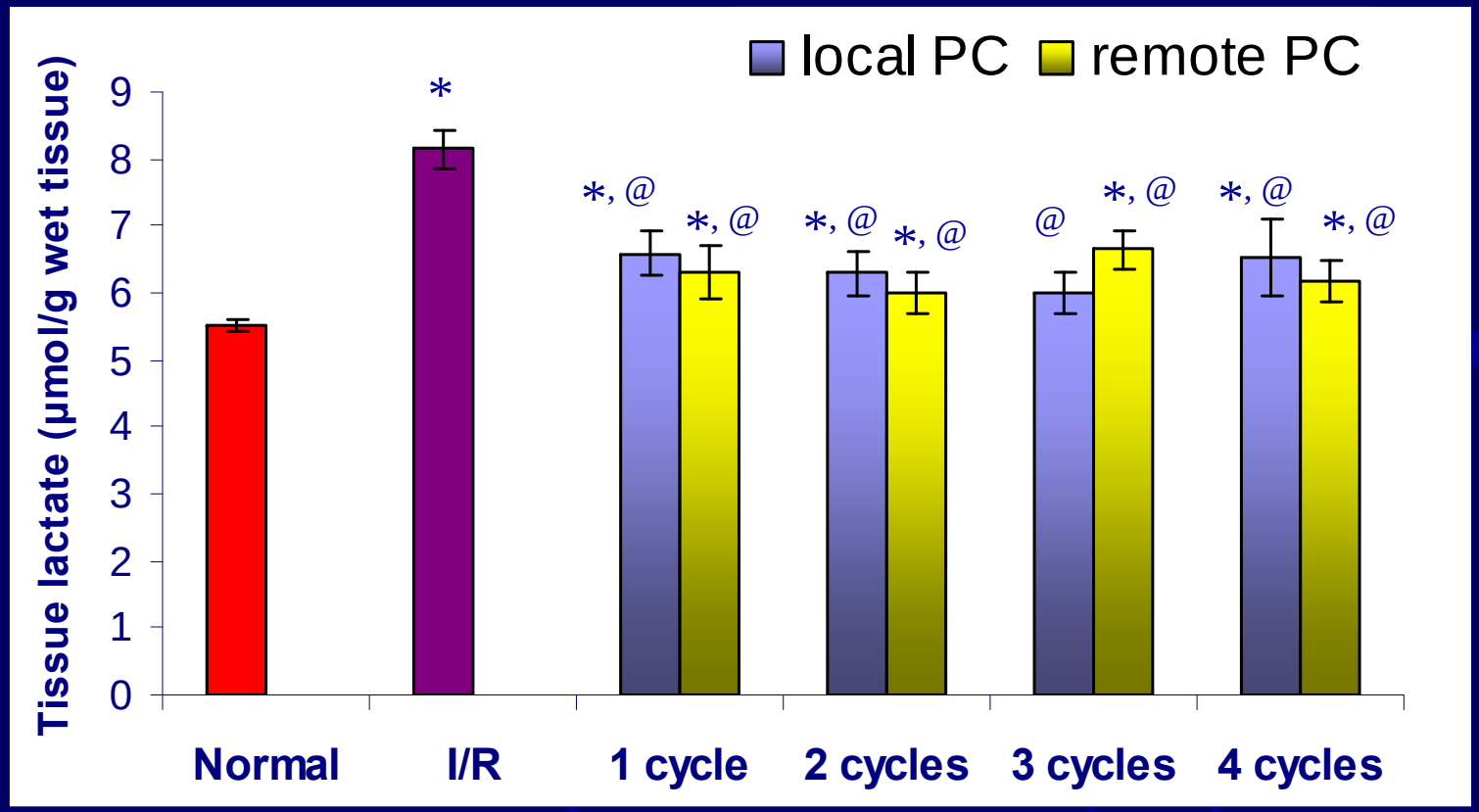

Figure (2). Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in myocardial lactate content.

Each value represents the mean of 7-9 experiments ± S.E.M. *∗p<0.05 vs.* control, *@p<0.05 vs*. I/R.

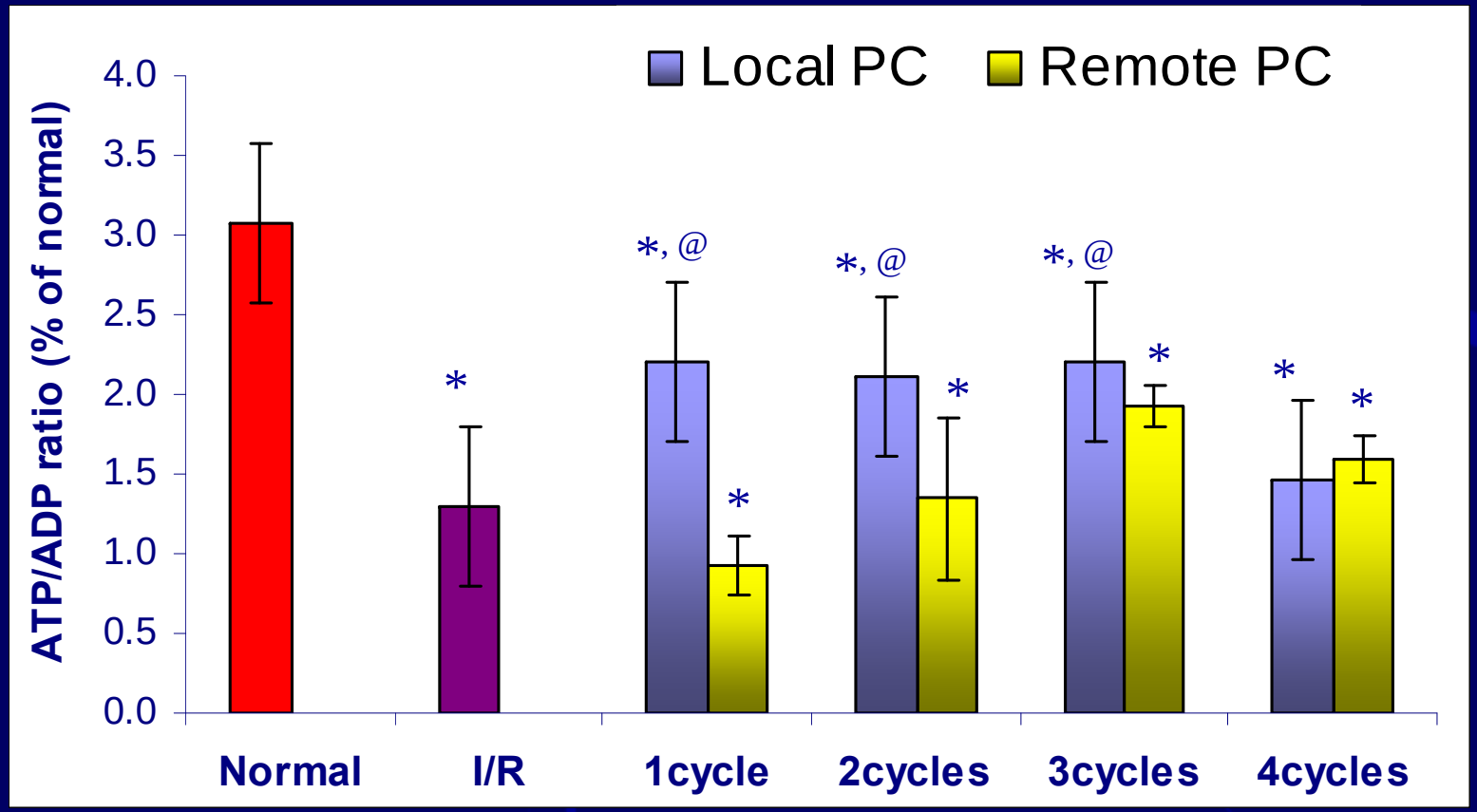

Figure (2). Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in myocardial ATP/ADP ratio. Each value represents the mean of 7-9 experiments ± S.E.M. ∗*p<0.05 vs.* control, @*p<0.05 vs*. I/R.

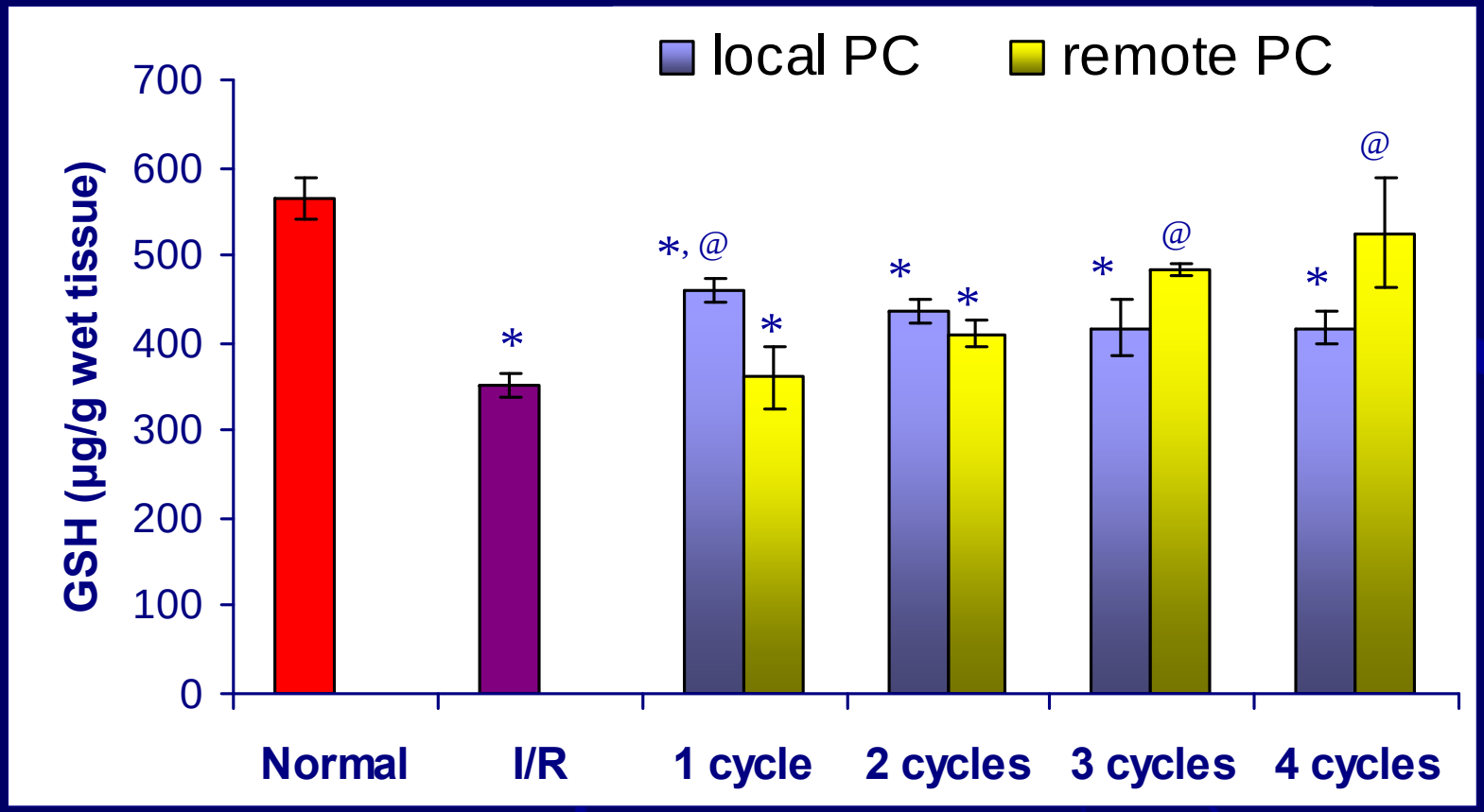

Figure (2). Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in myocardial GSH content. Each value represents the mean of 7-9 experiments ± S.E.M. *$p<0.05$ vs. control, @$p<0.05$ vs. I/R.

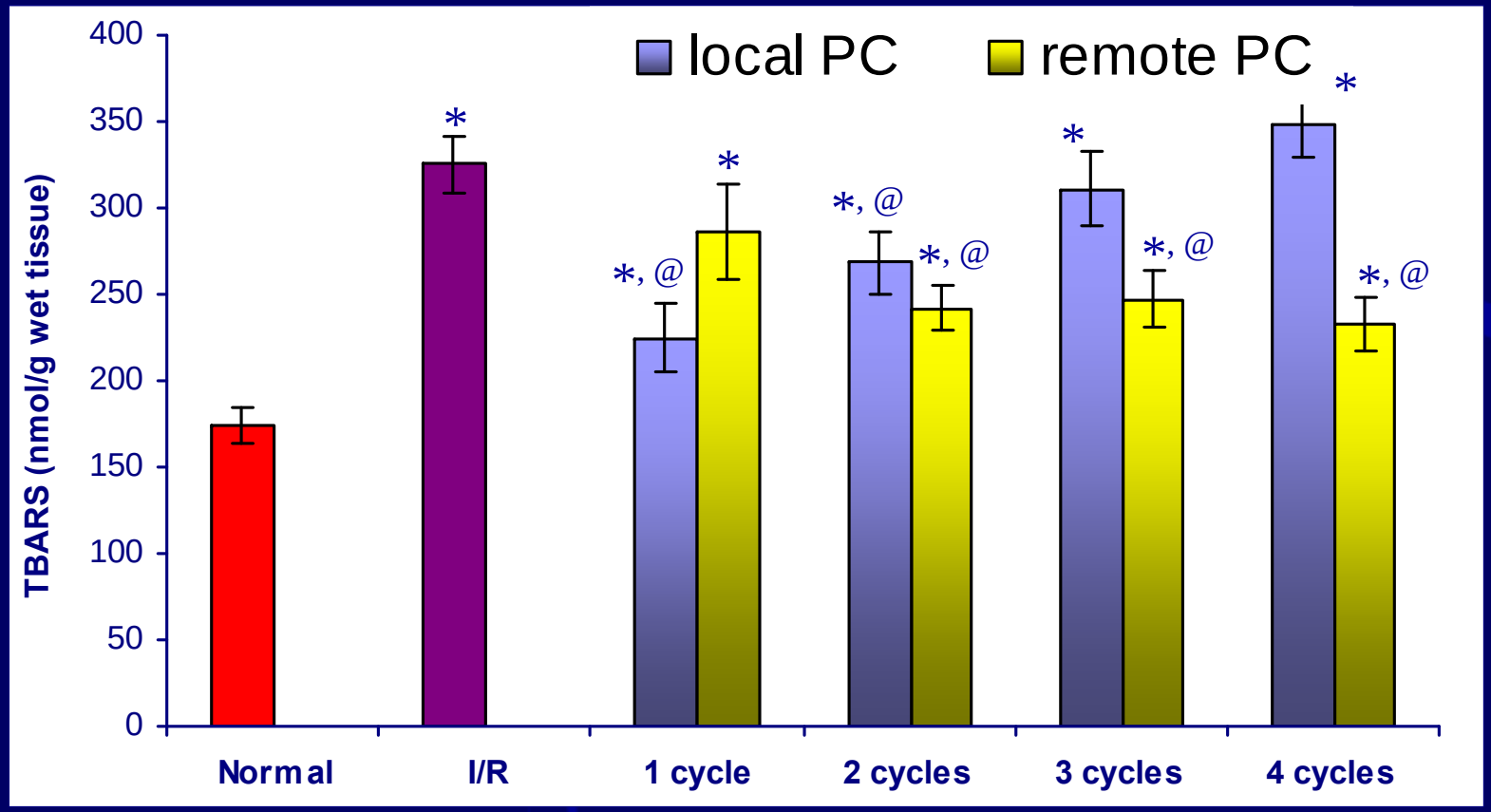

Figure (2). Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in myocardial TBARS content.

Each value represents the mean of 7-9 experiments ± S.E.M. ∗*p<0.05 vs.* control, *@p<0.05 vs*. I/R.

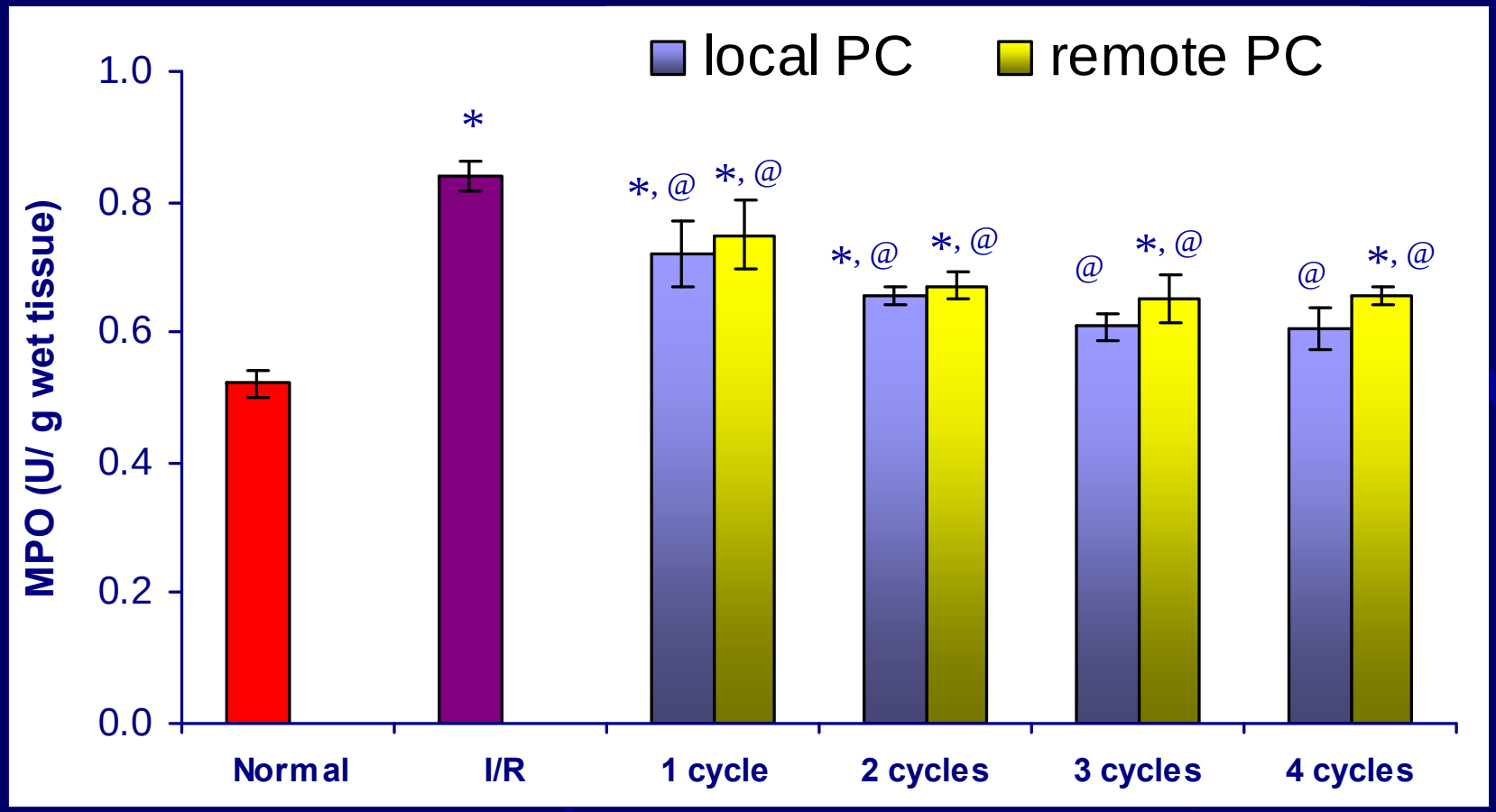

Figure (2). Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in myocardial MPO content.

Each value represents the mean of 7-9 experiments ± S.E.M. ∗*p<0.05 vs.* control, *@p<0.05 vs*. I/R.

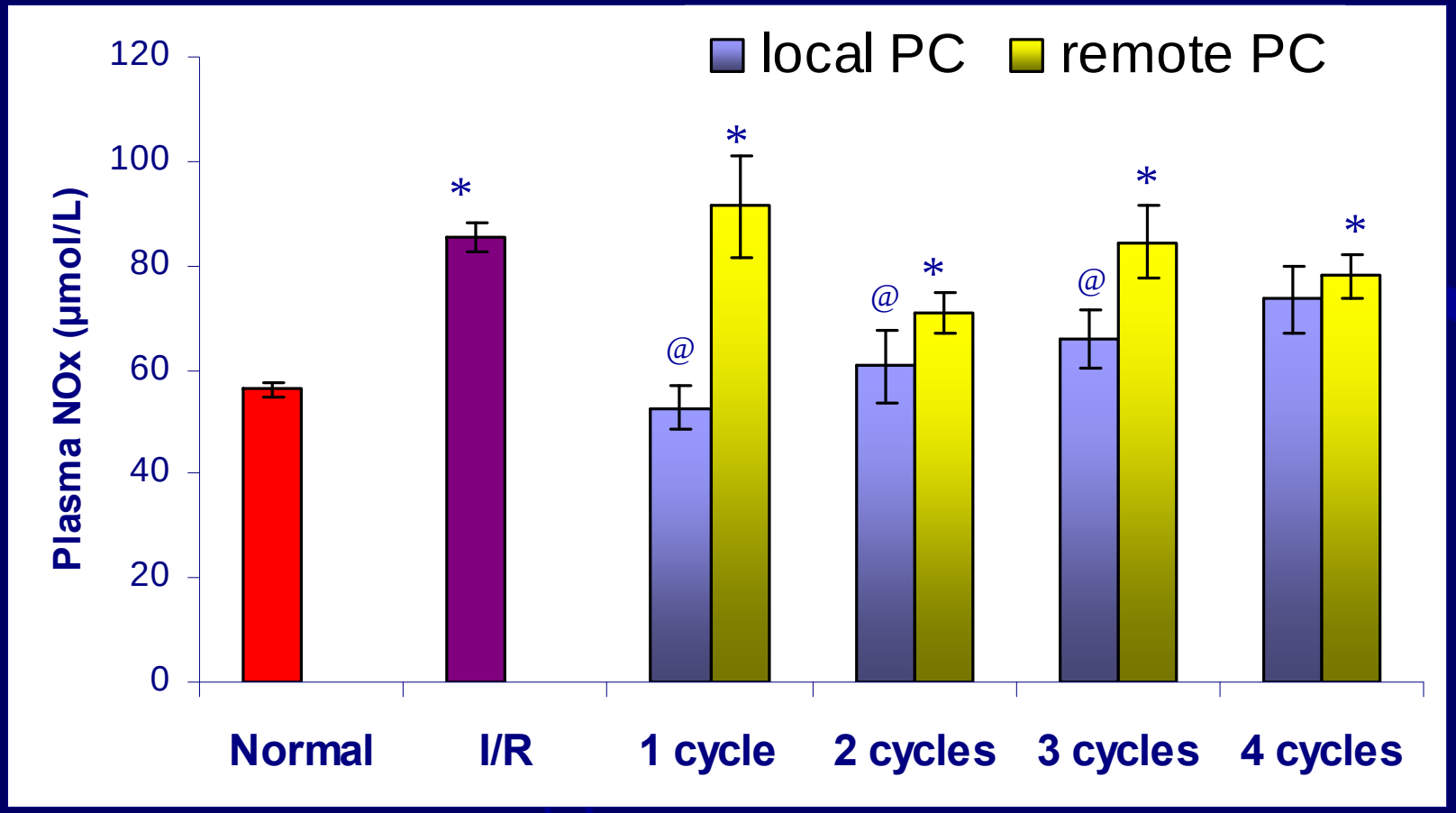

Figure (2). Effect of different cycles of local and remote preconditioning therapies on myocardial I/R (40min/10min)-induced changes in plasma NO_x. Each value represents the mean of 7-9 experiments ± S.E.M. ∗*p<0.05 vs.* control, @*p<0.05 vs*. I/R.

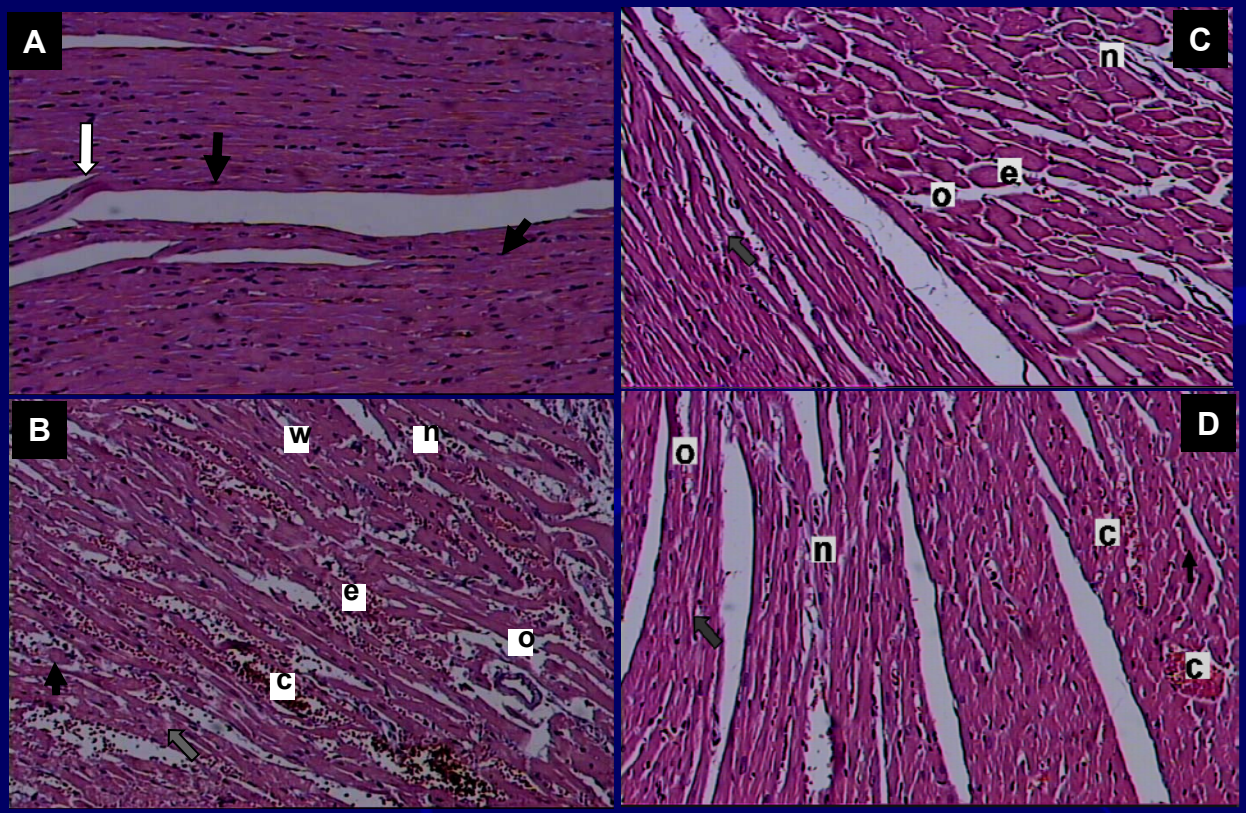

Figure 3. Photomicrographs of longitudinal sections in myocardium of A. normal group showing elongated branched acidophilic muscle fibers (➡) with central oval nucleus (⇨) B. I/R group showing neutrophil infilteration (n), congestion (c), extravasated RBCs (e), marked edema inbetween muscle fibers (o), edema within muscle fiber (➡), wavy muscle fibers (w) and apoptotic cell (➡) C. Three cycles of local preconditioning group showing mild neutrophil infilteration (n), remnants of extravasated RBCs (e), mild edema inbetween muscle fibers (o), mild edema within muscle fiber (➡) and few apoptotic cell (➡) D. Three cycles of remote preconditioning group showing mild neutrophil infilteration (n), mild congested blood vessel (c), edema inbetween muscle fibers (o) and edema within muscle fiber (➡) (H&E x200)

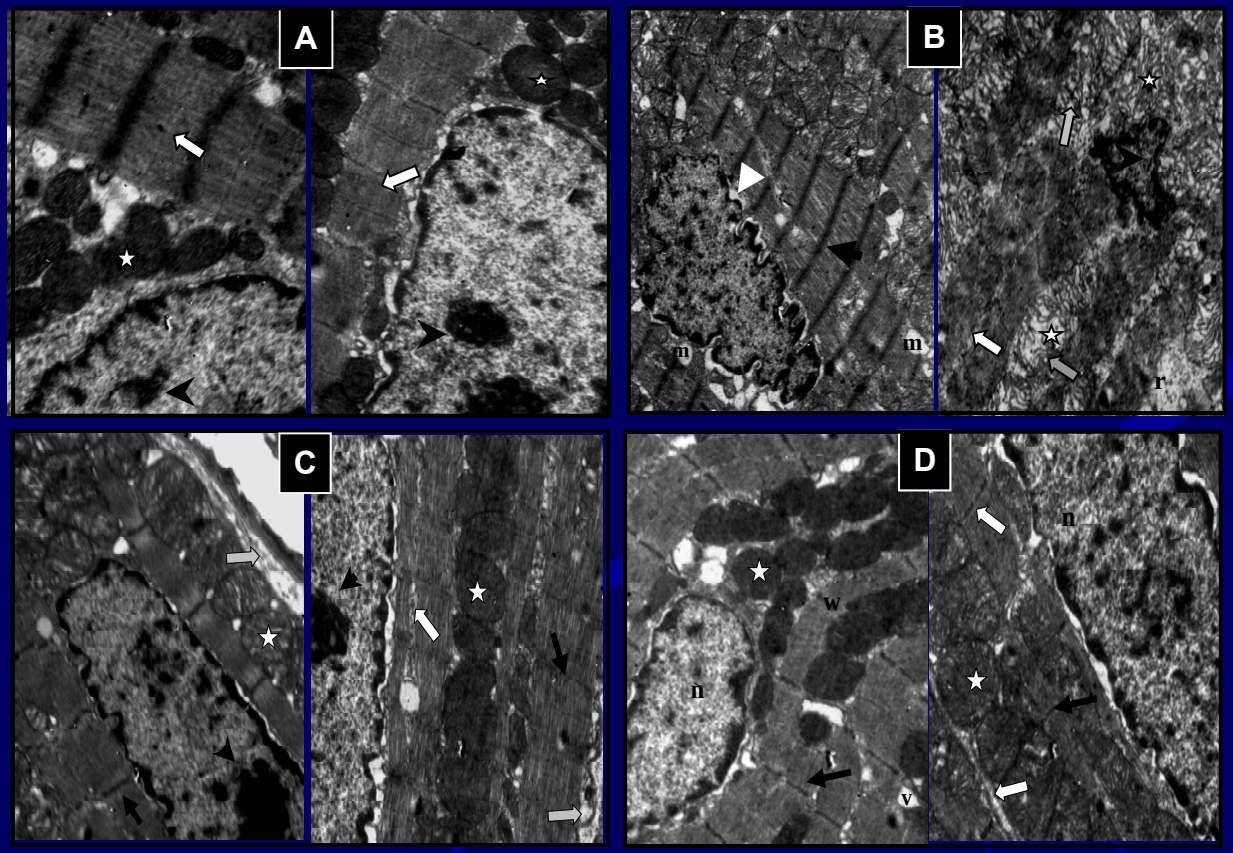

Figure 4. Photomicrographs of ultrastructural examination of rat heart left ventricle of **A.** normal group. Parallel arrangement of normal myofibrils (⇨), mitochondria with regular cristae and homogenous dense matrix (☆) and nucleus with normal pattern of chromatin dispersal and prominent nucleolus (➤). **B**. I/R group. Marked irregularities and edematous separation of myofibril (⇨) with hypercontraction and shortening of sarcomeres (➡). Cytoplasmic rarefaction (r) and mitochondrial vaculoization (m) with intense mitochondrial swelling, vesiculation (⇨), decreased matrix density and distortion of cristae (☆). Heterochromatic irregular indented nucleus with peripheral chromatin clumping and widening of nuclear membrane (⇨), absence of nucleolous and some nuclei are apoptotic with shrunken irregular heterochromatin (➤). C. Three cycles of local preconditioning group. Parallel arrangement of myofibril (➡) with very mild intracellular edema (⇨). Mild edema around muscle fiber (⇨). Some mitochondria still show mild swelling with normal matrix density and intact cristae (☆). Normal nucleus with prominent nucleolus (➤). D. Three cycles of remote preconditioning group. Regular myofibrillar arrangement (➡) with some waveness of myofibril (w), mild edema between myofibril (⇨) and few cytoplasmic vacuolization (v). Normal mitochondria with few showing mild swelling (☆). Normal nuclear chromatin distribution (n).

CONCLUSIONS

Local preconditioning therapy could be a useful cardioprotective agent in I/R injury.

- Two and three cycles of local preconditioning are more effective among the different cycles of local preconditioning
- Both cycles were equally effective in protection against the electrophysiological disturbances that occur during I/R
  - The protective effect seems to rely on:
    - reduction of cell membrane damage
    - preservation of energy production (ATP/ADP ratio)

- 2 cycles of local preconditioning was better in protection against oxidative stress markers

- 3 cycles seems to be more effective than 2 cycles in improvement of intracellular acidosis and attenuation of leukocytic infilteration

Concerning the remote preconditioning therapy, 3 cycles of remote preconditioning seems to be the most effective among different cycles of remote preconditioning

The protective effect was mediated via improvement of myocardial electrophysiological disturbances (less than local, cell membrane damage, aerobic metabolism, oxidative stress and leukocytic infilteration

More clinical studies are required to establish the beneficial effectiveness of these cardioprotective agents as adjunctive therapies in patients at risk of myocardial I/R

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Thank You