

**Ischemic preconditioning fails to confer additional protection against ischemia–reperfusion injury in the hypothyroid rat heart.**

Iordanis Mourouzis<sup>1</sup>, Antonios Dimopoulos<sup>1</sup>, Theodosios Saranteas<sup>1</sup>, Nikolaos Tsinarakis<sup>1</sup>, Eleutheria Livadarou<sup>1</sup>, Danai Spanou<sup>1</sup>, Alexandros D. Kokkinos<sup>1</sup>, Christodoulos Xinaris<sup>1</sup>, Constantinos Pantos<sup>1</sup>, Dennis V. Cokkinos<sup>2</sup>

<sup>1</sup> Department of Pharmacology, University of Athens, 75 Mikras Asias Ave., 11527 Goudi, Athens, Greece

<sup>2</sup> 1<sup>st</sup> Cardiology Department, Onassis Cardiac Surgery Center, 356 Sygrou Ave. 17674 Kallithea, Athens, Greece

**Correspondence:**

Constantinos Pantos

Department of Pharmacology, University of Athens

75 Mikras Asias Ave.

11527 Goudi, Athens, Greece

Fax: (+30210) 7462560,

Email: cpantos@cc.uoa.gr

## Summary

There is accumulating evidence showing that ischemic preconditioning (PC) may lose its cardioprotective effect in the diseased states. The present study investigated whether PC can be effective in hypothyroidism, a clinical condition which is common and often accompanies cardiac diseases such as heart failure and myocardial infarction. Hypothyroidism was induced in rats by 3wk administration of 6n-propyl-2-thiouracil in water (0.05%). Normal and hypothyroid hearts (HYPO) were perfused in Langendorff mode and subjected to 20 min of zero-flow global ischemia and 45 min of reperfusion. A preconditioning protocol (PC) was also applied prior to ischemia. HYPO hearts had significantly improved post-ischemic recovery of left ventricular developed pressure, end-diastolic pressure and reduced lactate dehydrogenase release. Furthermore, phospho-JNK and p38 MAPK levels after ischemia and reperfusion were 4.0 fold and 3.0 lower in HYPO as compared to normal hearts,  $P<0.05$ . A differential response to PC was observed between normal and HYPO hearts. PC improved the post-ischemic recovery of function and reduced the extent of injury in normal but had no additional effect on the hypothyroid hearts. This response, in the preconditioned normal hearts, resulted in 2.5 fold and 1.8 fold less expression of the phospho-JNK and phospho-p38 MAPK levels at the end of reperfusion, as compared to non PC hearts,  $P<0.05$  while in HYPO hearts, no additional reduction in the phosphorylation of these kinases was observed after PC. Hypothyroid hearts appear to be tolerant to ischemia –reperfusion injury. This response may be, at least in part, due to the down-regulation of ischemia-reperfusion induced activation of JNKs and p38 MAPK kinases. PC is not associated with further reduction in the activation of these kinases in the hypothyroid hearts and fails to confer added protection in those hearts.

**Key words:** thyroid hormone, myocardial ischemia, ischemic preconditioning, hypothyroidism, JNKs, p38 MAPK .

## **Introduction**

Over the past years, research has much contributed to better understanding of the response of the myocardium to ischemic injury. It is now recognized that hearts can be adapted to ischemic stress by interventions such as ischemic preconditioning or heat stress (Das and Maulik 2006; Koneru *et al.* 2007; Valen 2003; Pantos *et al.* 2003a). Thus, it has been suggested that preconditioning mechanisms could be therapeutically exploitable. However, most of the research on preconditioning has focused on the “non-diseased” myocardium and little is known whether this phenomenon can be applied in the pathological myocardium (Pantos *et al.* 2007a). Since coronary artery disease frequently co-exists with other morbidities, it is conceivable that this issue merits further research before preconditioning can be clinically exploited.

Thyroid hormone is now realized to have a critical role in myocardial ischemia and cardiac remodelling. In fact, changes in thyroid hormones, T4 and T3, may be associated with altered response of the myocardium to ischemia (Pantos *et al.* 2003b; Pantos *et al.* 2006; Pantos *et al.* 2004). Furthermore, hypothyroid-like changes are observed in the post-infarcted myocardium (Pantos *et al.* 2005a) and low T3 levels are common in patients with heart failure or myocardial infarction and are associated with increased mortality and morbidity (Friberg *et al.* 2002; Iervasi *et al.* 2003; Pantos *et al.* 2007c). Thus, whether cardioprotective interventions, such as ischemic preconditioning, can be operable under hypothyroid states may be of clinical and

therapeutic importance.

Based on this evidence, the present study investigated the response of the hypothyroid heart to ischemic preconditioning in an experimental model of propylthiouracil-induced hypothyroidism in rats.

## **Materials and Methods**

### *Animals*

Thirty nine rats, 290-350 g were used for this study. Male Wistar rats were used as we have done in previous studies (Pantos *et al.* 2003d; Pantos *et al.* 2003a; Pantos *et al.* 2003b; Pantos *et al.* 2006; Pantos *et al.* 2003c; Pantos *et al.* 2005b; Pantos *et al.* 1999) The rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Pub. No. 8323, Revised 1996).

### *Experimental hypothyroidism*

Hypothyroidism was induced in rats by 3 wk administration of 6n-propyl-2-thiouracil in drinking water to a final concentration of 0.05%. We have previously shown that this model results in moderate decrease in T4 and T3 levels in plasma (Pantos *et al.* 2003b; Pantos *et al.* 2005b). These animals were designated as HYPO. Untreated rats were used as controls and were designated as NORM.

### *Echocardiography*

Rats were sedated with ketamine hydrochloride (100 mg/Kg) and heart function was evaluated by echocardiography. Short and long-axis images were acquired using a

digital ultrasound system (Sonosite 180Plus, 21919 30<sup>th</sup> Drive SE, Bothell, WA, USA) with a 7.0MHz sector-array probe, as previously described (Pantos *et al.* 2007b; Pantos *et al.* 2007d). Ejection fraction, Left ventricular internal diameter at diastolic phase (LVIDd), LV internal diameter at systolic phase (LVIDs), posterior wall thickness at diastolic phase (LVPW) and posterior wall velocity of shortening (PWV) were measured. All measurements were averaged for at least 3 consecutive cardiac cycles.

#### *Isolated heart preparation*

A non-working isolated rat heart preparation was perfused at a constant flow according to the Langendorff technique. An intraventricular balloon allowed measurement of contractility under isovolumic conditions. Left ventricular balloon volume was adjusted to produce an average initial left ventricular end-diastolic pressure of 6-7 mmHg in all groups and was held constant thereafter throughout the experiment. Since the balloon was not compressible, left ventricular contraction was isovolumic. As intraventricular volume was maintained at a constant value, diastolic fiber length, which represented preload, did not change. Thus, the left ventricular peak systolic pressure and the left ventricular developed pressure (LVDP), defined as the difference between left ventricular peak systolic pressure and left ventricular end-diastolic pressure, represented contractility indexes obtained under isometric conditions.

Rats were anaesthetized with ketamine HCl and heparin 1000 IU was given intravenously before thoracotomy. The hearts were rapidly excised, placed in ice-cold Krebs-Henseleit buffer (composition in mmol/l: sodium potassium chloride

4.7, potassium phosphate monobasic 1.2, magnesium sulfate 1.2, calcium chloride 1.4, sodium bicarbonate 25, and glucose 11) and mounted on the aortic cannula of the Langendorff perfusion system. Perfusion with oxygenated (95% O<sub>2</sub> / 5% CO<sub>2</sub>) Krebs-Henseleit buffer was established within 60 s after thoracotomy. Flow rate (ml/min) per gram of LV weight was approximately 16 in both NORM and HYPO hearts in order to obtain a mean perfusion pressure of 70 mmHg. The perfusion apparatus was heated to ensure a temperature of 37 °C throughout the course of the experiment. In our experimental design sinus node was removed and hearts were paced at 320 beats/min with a Harvard pacemaker. The pacemaker was turned off during ischemia. The water filled balloon, connected to a pressure transducer and coupled to a Gould RS 3400 recorder was advanced into the left ventricle through an incision in the left atrium. Pressure signal was transferred to a computer using a data analysis software (IOX, Emka Technologies) which allowed continuous monitoring and recording of heart function (Pantos *et al.* 2003d; Pantos *et al.* 2006; Pantos *et al.* 2007b; Pantos *et al.* 2005a). All preparations included in this study were stable for at least the last 10 min of the stabilization period. Isolated hearts without stable measurements of LVDP, LV end-diastolic pressure and perfusion pressure for the last 10 min of the stabilization period or those presented severe reperfusion arrhythmias were excluded from the analysis.

### *Experimental Protocol*

- a. Hearts from HYPO and NORM rats were excised and rinsed in Krebs buffer. The left ventricle was isolated, frozen in liquid nitrogen and used for

measurements of baseline myocardial protein expression (myosin heavy chain isoforms and SERCA), NORM-base, n=5 and HYPO-base, n=5.

- b. NORM and HYPO rats were anaesthetized and subjected to echocardiography analysis. Hearts were removed, isolated and perfused in a Langendorff preparation. Isolated hearts were subjected to 20 min of stabilization, 20 min of zero-flow global ischemia and 45 min of reperfusion, NORM(I/R), n=7; HYPO(I/R), n=9. Additional hearts were subjected to preconditioning protocol before the index ischemia, NORM-PC(I/R), n=6; HYPO-PC(I/R), n=7. The preconditioning protocol consisted of 1 cycle of 3 min ischemia / 5 min reperfusion and 3 repeated cycles of 5 min ischemia / 5 min reperfusion.

Since hypothyroid rat heart is highly protected and this could have masked the preconditioning effect, we also performed experiments extending the period of ischemia to 30 min in hypothyroid hearts to reach the extent of post-ischemic damage seen with less ischemia in normal hearts. Thus, preconditioned and non-preconditioned hypothyroid hearts were subjected to 30 min of zero-flow global ischemia and 45 min of reperfusion, HYPO(30I/R), n=6; HYPO-PC(30I/R), n=6.

At the end of the experimental protocol the left ventricle was isolated, frozen in liquid nitrogen and used for determination of p38 MAPK and JNK activation.

#### *Measurement of mechanical function*

Left ventricular systolic function was assessed by recording the left ventricular developed pressure which was measured at the end of the stabilization period (LVDP, mmHg) and after 45 min of reperfusion (LVDP45). LVDP and +dp/dt (mmHg/sec), -dp/dt (mmHg/sec) were used to assess baseline myocardial function. Post-ischemic

myocardial function was assessed by the recovery of LVDP and expressed as % of the baseline value (LVDP%). Diastolic function was assessed by monitoring isovolumic left ventricular end-diastolic pressure (LVEDP, mmHg) as a measure of diastolic chamber distensibility. Left ventricular end-diastolic pressure was measured after 45 min of reperfusion (LVEDP45).

*Protein isolation, sodium dodecyl sulfate-protein polyacrylamide (SDS-PAGE) gel electrophoresis and immunodetection*

Determination of protein expression was performed as previously described (Pantos *et al.* 2003d; Pantos *et al.* 2005b; Pantos *et al.* 2005a). Left ventricular tissue was homogenized in ice-cold buffer containing 10 mM Tris-HCl pH=7.5, 3 mM ethylenediaminetetraacetic acid, 1 mM phenylmethanesulphonyl fluoride, 30  $\mu$ M leupeptin, 1 mM Na<sub>3</sub>VO<sub>4</sub> and TritonX100 0.1% with a Polytron homogenizer. The resulting homogenate was centrifuged at 10000 g for 10 min at 4°C. The supernatant (Triton-soluble) corresponded to the cytosol-membrane fraction and was kept at -80 °C for further processing. Protein concentrations were determined by the bicinchoninic acid (BCA) method, using bovine serum albumin as a standard.

Samples were prepared for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by boiling for 5 min in Laemmli sample buffer containing 5% 2-mercaptoethanol. Aliquots (40  $\mu$ g) were loaded onto 7.5% or 9% (w/v) acrylamide gels and subjected to SDS-PAGE in a Bio-Rad Mini Protean gel apparatus. Following SDS-PAGE, proteins were transferred electrophoretically to a nitrocellulose membrane (Hybond ECL) at 100V and 4 °C, for 1.5 h using Towbin buffer for Western blotting analysis. Subsequently, filters were probed with specific antibodies against SERCA (Affinity Bioreagents, MA3-919, dilution 1:1000), total p38 MAPK,



dual phospho-p38 MAPK, total c-jun NH2-terminal kinases (JNKs), dual phospho-JNKs, (Cell Signaling Technology, dilution 1:1000) overnight at 4<sup>o</sup> C and immunoreactivity was detected by enhanced chemiluminescence. For comparisons between groups, five samples from each group were loaded on the same gel. Ponceau staining was used in each filter in order to normalize slight variations in total protein loading. Immunoblots and gels were quantified using the AlphaScan Imaging Densitometer (Alpha Innotech Corporation, 14743, Catalina Street, San Leandro, CA).

#### *Measurement of myosin heavy chain isoform content*

Homogenates of all samples were diluted 40 fold with Laemmli sample buffer containing 5% 2-mercaptoethanol. The composition and preparation of the gels was carried out as previously described (Pantos *et al.* 2005b; Reiser and Kline 1998). Briefly, the stacking and separating gels consisted of 4 and 8% acrylamide (wt/vol) respectively, with Acryl:bis-Acryl in the ratio of 50:1. The stacking and separating gels included 5% (vol/vol) glycerol. The upper running buffer consisted of 0.1 M Tris (base), 150 mM glycine, 0.1% sodium dodecyl sulfate (SDS) and 2-mercaptoethanol at a final concentration of 10 mM. The lower running buffer consisted of 0.05 M Tris (base), 75 mM glycine and 0.05% SDS. The gels were run in Biorad Protean II xi electrophoresis unit at a constant voltage of 240 V for 21 h at 8 °C. The gels were fixed and silver-stained (Biorad silver stain kit). Gels were scanned and quantified using the AlphaScan Imaging Densitometer (Alpha Innotech Corporation, USA).

### *Measurement of lactate dehydrogenase (LDH) release*

Coronary effluent was collected during 45 min of reperfusion and was used for the measurement of lactate dehydrogenase (LDH) activity in IU/L spectrophotometrically (LDH UV Fluid, Rolf Greiner Biochemica). LDH release was expressed per gram of tissue and was used as an index of myocardial injury.

### *Measurement of thyroid hormones*

Plasma L-thyroxine and 3,5,3' tri-iodothyronine quantitative measurements were performed with ELISA, using kits obtained from Alpha Diagnostic International, Texas, USA (No 1100 for total T<sub>4</sub> and No 1700 for total T<sub>3</sub>), as previously described (Pantos *et al.* 2005a; Pantos *et al.* 2007d). L- thyroxine and 3,5,3' triiodothyronine levels were expressed as nmol/L of plasma. Absorbance measurements were performed at 450nm with Tecan Genios ELISA reader (Tecan, Austria).

### *Statistics*

Values are presented as mean (S.E.M.). Unpaired t-test and Mann-Whitney test were used for differences between 2 groups. One-way analysis of variance with Bonferroni or Dunnett's correction was used when multiple comparisons were carried out. A two-tailed test with a *P* value less than 0.05 was considered significant.

## **Results**

### *Animal characteristics*

Body weight change (in g) and left ventricular weight (in mg) were +25 (4) and 740 (31) for NORM vs -18 (3) and 585 (20) for HYPO, respectively, *P*<0.05.

Spontaneous heart rate (in bpm) determined during echocardiography analysis was 386 (12) for NORM and 264 (17) for HYPO rats,  $P<0.05$ . T4 and T3 levels (in nM) were found to be 47.3 (2.1) and 0.98 (0.07) for NORM vs 19.6 (0.4) and 0.23 (0.05) for HYPO, respectively,  $P<0.05$ .

#### *SERCA and myosin isoform expression*

A shift from  $\alpha$  to  $\beta$  myosin isoform expression was found in HYPO-base as compared to NORM-base hearts. In fact, HYPO-base hearts expressed 20%  $\alpha$ -MHC and 80%  $\beta$ -MHC as compared to 75%  $\alpha$ -MHC and 25%  $\beta$ -MHC in NORM-base hearts,  $P<0.05$ . Fig.1A. In addition, SERCA expression was found to be 2.5 fold less in HYPO-base vs NORM-base hearts,  $P<0.05$ . Fig. 1B.

#### *Parameters of cardiac function at baseline*

Data of contractile function are summarized in table 1. Basal contractile function was significantly reduced in HYPO hearts. Ejection fraction and posterior wall velocity of shortening (PWV) measured by ultrasonography in vivo and LVDP and  $\pm$  dp/dt measured under isometric conditions were shown to be significantly decreased in HYPO hearts.

#### *Parameters of post-ischemic myocardial function and LDH release*

NORM-PC(I/R) and HYPO(I/R) hearts showed significantly improved recovery of function, attenuated diastolic dysfunction and reduced LDH release as compared to NORM(I/R) hearts. Table 2. However, post-ischemic myocardial function and LDH release were not different between HYPO(I/R) and HYPO-PC(I/R) hearts. Table 2. Fig. 2.

Extending the period of ischemia to 30 min, LVDP% and LDH were 69.3 (10.4) and 12.9 (2.3) for HYPO(30I/R) vs 59.5 (10.8) and 16.2 (4.9) for HYPO-PC(30I/R) respectively,  $p>0.05$ . In addition, LVEDP45 was 21 (5.1) for HYPO(30I/R) vs 22.8 (4.9) for HYPO-PC(30I/R),  $p>0.05$ .

#### *Ischemia-reperfusion induced activation of the pro-apoptotic kinases p38 MAPK and JNK*

In normal hearts, ischemic preconditioning resulted in reduction of the expression of the phosphorylated p54 JNK and p38 MAPK levels (by 2.5 fold and 1.8 fold respectively),  $P<0.05$ . In the hypothyroid hearts, phosphorylated p54 JNK and p38 MAPK were 4.0 fold and 3.0 less as compared to normal,  $P<0.05$ , but no further reduction in the expression of the phosphorylated levels of these kinases was observed in hypothyroid preconditioned hearts. Fig. 3.

Extending the period of ischemia to 30 min in hypothyroid hearts, resulted in significant increase in phosphorylated levels of p38 and JNK as compared to 20 min. However, no significant reduction in the activation of those kinases was achieved in HYPO-PC(30I/R) as compared to HYPO(30I/R) hearts. Fig. 4.

## **Discussion**

Ischemic preconditioning is now thought to be a powerful means of heart protection against ischemic injury and most of the recent research is focused on its potential clinical application (Tsai *et al.* 2004). However, it remains largely unknown whether preconditioning is still effective when certain co-morbidities accompany the ischemic heart disease (Pantos *et al.* 2007a). Thus, our study has investigated whether the preconditioning response can be preserved in hypothyroidism which is a common

clinical condition and predisposes to coronary artery disease (Singh *et al.* 2007).

In the present study, we included male Wistar rats and isolated hearts were subjected to zero-flow global ischemia followed by reperfusion, as in our previous studies (Pantos *et al.* 2003d; Pantos *et al.* 2003a; Pantos *et al.* 2003b; Pantos *et al.* 2006; Pantos *et al.* 2003c).

The first finding of this study is that hypothyroid hearts can withstand ischemia – reperfusion injury better than normal hearts. This is in accordance with previous reports. In fact, several studies have shown that ischemic contracture is delayed and post-ischemic recovery of function is enhanced in an isolated rat heart model of ischemia-reperfusion (Abe *et al.* 1992; Eynan *et al.* 2002; Pantos *et al.* 2003b). Furthermore, hypothyroid hearts are shown to be protected against ischemia-reperfusion arrhythmias (Zhang *et al.* 2002). More importantly, lower circulating T3 levels before infarction were associated with smaller infarctions. However, after infarction these patients had worse long-term outcome (Friberg *et al.* 2002).

This cardioprotective effect was initially been attributed to changes in metabolism and energy utilization which occurs in the hypothyroid heart. In fact, hypothyroid hearts consume less oxygen in doing mechanical work due to the predominance of the slow myosin isoform and have increased myocardial glycogen content (Abe *et al.* 1992; Pantos *et al.* 2005b). Thus, in hypothyroid hearts, ATP levels were found to decline more slowly during ischemia and be higher at reperfusion (Abe *et al.* 1992). Furthermore, hypothyroidism induced cardioprotection has recently been linked to changes in intracellular signalling pathway activation (Pantos *et al.* 2004). In fact, complex intracellular kinase signalling underlies the cellular response to stress (Baines *et al.* 1999; Ravingerova *et al.* 2003) and in experimental models of isolated perfused hearts, pro-apoptotic p38 MAPK kinase is activated during global ischemia

and maintained during reperfusion, while pro-apoptotic JNKs mainly are activated at reperfusion (Bogoyevitch *et al.* 1996; Knight and Buxton 1996; Pantos *et al.* 2003c; Sato *et al.* 2000; Yin *et al.* 1997). Sustained activation of this kinase signalling appears to be detrimental and its pharmacological inhibition results in cell survival under ischemic stress (Duplain 2006; Mackay and Mochly-Rosen 1999). Interestingly, in the present study, we found that the phosphorylated levels of p38 MAPK and JNKs were significantly lower in hypothyroid hearts after ischemia and reperfusion as compared to control hearts, indicating a possible role of this kinase signalling in the increased tolerance of the hypothyroid heart to ischemic injury.

These data support the concept that the diseased myocardium may not always be vulnerable to ischemic injury (Pantos *et al.* 2007a). In fact, the diabetic heart (Hadour *et al.* 1998; Kristiansen *et al.* 2004; Wang and Chatham 2004; Xu *et al.* 2004), the post-infarcted myocardium (Pantos *et al.* 2007b; Pantos *et al.* 2005a) or the hyperthyroid heart (Pantos *et al.* 2003d; Pantos *et al.* 2006; Pantos *et al.* 2002) are shown to be resistant to ischemia.

The most striking finding of the study was the differential response to ischemic preconditioning which was observed between normal and hypothyroid hearts. In fact, four-cycle preconditioning protected the normal heart against 20 min of no-flow global ischemia and reperfusion but failed to confer additional protection in the hypothyroid heart. However, at this duration of index ischemia, post-ischemic recovery of function was significantly higher in the hypothyroid hearts and could potentially have masked the additional preconditioning effect seen in normal hearts. Thus, we subjected the hypothyroid hearts to a longer ischemic period (30min) which resulted in comparable post-ischemic injury with that observed in the normal heart after 20 min of ischemia. At this duration of ischemia, preconditioning had also no

additional protection. It should be noted that with longer duration of ischemia (30 min), normal hearts hardly recovered in our experimental setting. These data probably indicate that preconditioning may not be operable in hypothyroidism. However, we cannot exclude the possibility that the threshold of preconditioning stimulus may be increased in the hypothyroid heart. This issue probably merits further investigation.

The mechanisms underlying the loss of preconditioning response in the hypothyroid hearts remain largely unknown. In the non-diseased myocardium, we and other investigators have shown that ischemic preconditioning or heat preconditioning can protect the healthy myocardium by lowering the sustained activation of the pro-apoptotic p38 MAPK and JNKs kinase signalling during the subsequent ischemia and reperfusion phase (Marais *et al.* 2001; Pantos *et al.* 2003a; Pantos *et al.* 2002). Similarly, in the present study, ischemia-reperfusion induced p38 MAPK and JNKs activation (as assessed by the phosphorylated levels of these kinases) was found to be significantly reduced in preconditioned normal as compared to non-preconditioned hearts. However, preconditioning did not further attenuate the activation of this signalling in the hypothyroid hearts, partly explaining the failure of preconditioning to confer added protection in those hearts against ischemia and reperfusion injury.

The present study has provided additional evidence supporting the notion that the diseased myocardium may lose the ability to be preconditioned (Pantos *et al.* 2007a). This needs to be taken into account before translating preconditioning research to therapeutic application in humans. In fact, in clinical practice, coronary artery disease may co-exist with other morbidities which can potentially modify the response of the myocardium to ischemic stress.

In conclusion, hypothyroid hearts are tolerant to ischemia–reperfusion injury, partly due to the down-regulation of the ischemia–reperfusion induced activation of JNKs

and p38 MAPK kinase signalling pathways. Ischemic preconditioning is not associated with further reduction in the activation of these kinases and fails to confer added protection in those hearts.

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**Fig. 1**

Densitometric assessment in arbitrary units and representative images of  $\alpha$  and  $\beta$  isoforms of myosin heavy chain (A) and SERCA protein expression (B) in normal (NORM-base) and hypothyroid hearts (HYPO-base, n=5).

**Fig. 2**

Recovery of left ventricular developed pressure (LVDP%, A) and LDH release (B) in normal [NORM(I/R)], normal preconditioned [NORM-PC(I/R)], hypothyroid [HYPO(I/R)] and hypothyroid preconditioned hearts [HYPO-PC(I/R)] subjected to 20 min of ischemia and 45 min of reperfusion.

\*  $P < 0.05$  vs NORM(I/R)

**Fig. 3**

Densitometric assessment in arbitrary units and representative images of (A) the ratio of phosphorylated p54 JNK to total p54 JNK expression and (B) the ratio of phosphorylated p38 MAPK to total p38 MAPK expression in normal [NORM(I/R)], normal preconditioned [NORM-PC(I/R)], hypothyroid [HYPO(I/R)] and hypothyroid preconditioned hearts [HYPO-PC(I/R)] subjected to 20 min of ischemia and 45 min of reperfusion and hypothyroid [HYPO(30I/R)] and hypothyroid preconditioned hearts [HYPO-PC(30I/R)] subjected to 30 min of ischemia and 45 min of reperfusion.

\*  $P < 0.05$  vs NORM(I/R), \*\*  $P < 0.05$  vs HYPO(I/R)

**Table 1.** *Parameters of cardiac function at baseline.*

|                        | <b>NORM</b> | <b>HYPO</b> |
|------------------------|-------------|-------------|
| <b>LVIDd (mm)</b>      | 6.6 (0.1)   | 6.4 (0.1)   |
| <b>LVIDs (mm)</b>      | 4.0 (0.2)   | 4.9 (0.3)*  |
| <b>LVPW (mm)</b>       | 1.9 (0.03)  | 1.7 (0.07)* |
| <b>PWV (mm/s)</b>      | 35.4 (3.5)  | 16.5 (1.5)* |
| <b>EF%</b>             | 71.5 (2.7)  | 56.0 (4.2)* |
| <b>LVDP (mmHg)</b>     | 115.2 (3.5) | 93.8 (2.1)* |
| <b>+dp/dt (mmHg/s)</b> | 3900 (117)  | 2400 (107)* |
| <b>-dp/dt (mmHg/s)</b> | 2200 (54)   | 1800 (53)*  |

Left ventricular internal diameter at diastolic phase (LVIDd) and at systolic phase (LVIDs), posterior wall thickness at diastolic phase (LVPW), posterior wall velocity of shortening (PWV), ejection fraction, left ventricular developed pressure (LVDP) and the positive and negative first derivative of LVDP (+dp/dt and -dp/dt) are shown in this table. The values are mean (S.E.M).

\*  $P < 0.05$  vs NORM

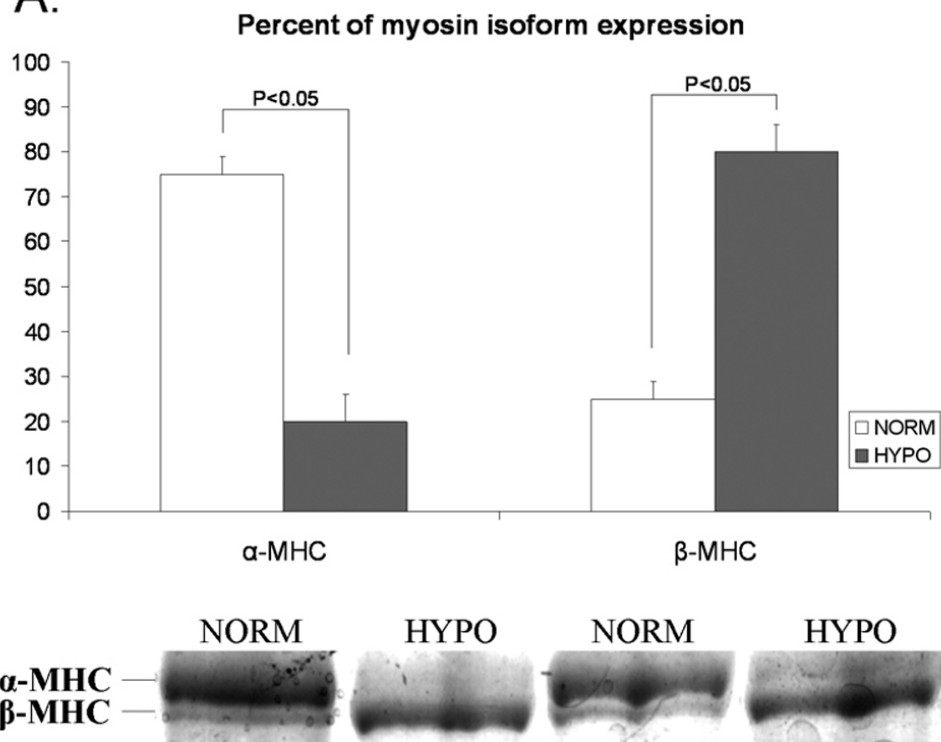
**Table 2.** *Indexes of recovery after ischemia-reperfusion.*

|                     | <b>LVDP%</b> | <b>LVEDP45<br/>(mmHg)</b> | <b>LDH (IU/g)</b> |
|---------------------|--------------|---------------------------|-------------------|
| <b>NORM(I/R)</b>    | 58.7 (6.3)   | 56.5 (6.8)                | 8.5 (0.4)         |
| <b>NORM-PC(I/R)</b> | 81.4 (3.8)*  | 20.3 (5.0)*               | 5.8 (0.8)*        |
| <b>HYPO(I/R)</b>    | 93.6 (3.9)*  | 9.2 (1.8)*                | 4.0 (0.3)*        |
| <b>HYPO-PC(I/R)</b> | 88.0 (3.9)*  | 13.5 (2.6)*               | 5.7 (1.1)*        |

LVDP at the end of reperfusion expressed as % of the baseline value (LVDP%), Left ventricular end-diastolic pressure after 45 min of reperfusion (LVEDP45) and LDH release expressed per gram of tissue are shown in this table. The values are mean (S.E.M).

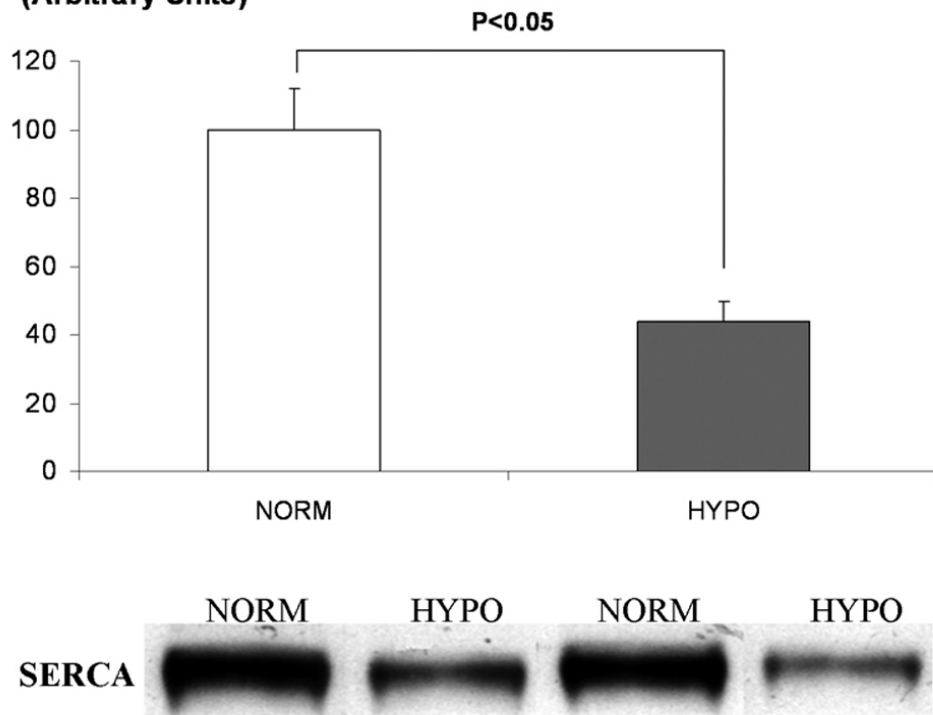
\*  $P < 0.05$  vs NORM(I/R)

**A.**



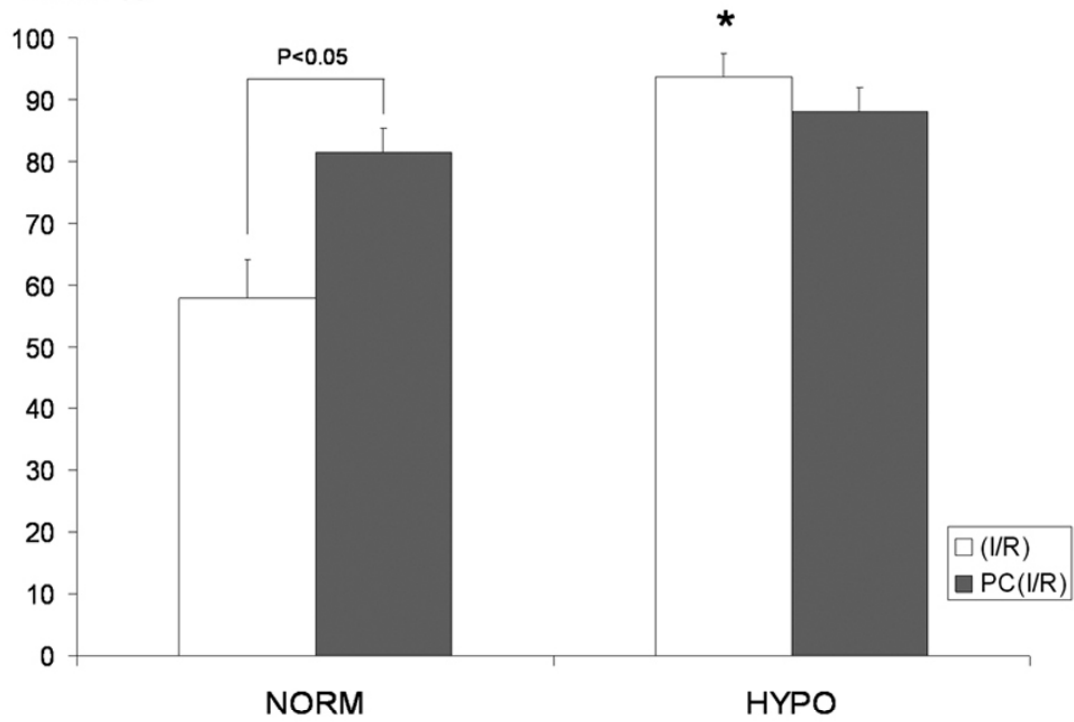
**B.**

**SERCA Expression  
(Arbitrary Units)**



**A.**

**LVDP%**



**B.**

**LDH Release (IU/g)**

