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ORIGINAL CONTRIBUTION

Thyroid hormone improves postischaemic recovery of function while limiting apoptosis: a new therapeutic approach to support hemodynamics in the setting of ischaemia-reperfusion?

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Abstract Although it has long been recognized that thyroid hormone is an effective positive inotrope, its efficacy in supporting hemodynamics in the acute setting of ischaemia and reperfusion (R) without worsening reperfusion injury remains largely unknown. Thus, we investigated the effects of triiodothyronine (T3) on reperfusion injury in a Langendorff–perfused rat heart model of 30 min zero-flow ischaemia and 60 min of (R) with or without T3 (40 μg/l) at R, T3-R60, $n = 11$ and CNT-R60, $n = 10$, respectively. Furthermore, phosphorylated levels of intracellular kinases were measured at 5, 15 and 60 min of R. T3 markedly improved postischaemic recovery of left ventricular developed pressure (LVDP%); 56.0% (SEM, 4.4) in T3-R60 versus 38.8% (3.1) in CNT-R60, $P < 0.05$. Furthermore, LDH release was significantly lower in T3-R60. Apoptosis detection by fluorescent probe optical imaging showed increased fluorescent signal in CNT-R60 hearts, while the signal was hardly detectable in T3-R60 hearts. Similarly, caspase-3 activity was found to be 78.2 (8.2) in CNT-R60 vs 40.5 (7.1) in T3-R60 hearts, $P < 0.05$. This response was associated with significantly lower levels of phospho-p38 MAPK at any time point of R. No significant changes in phospho- ERK1/2 and JNK levels were observed between groups. Phospho–Akt levels were significantly lower in T3 treated group at 5 min and no change in phospho–Akt levels were observed at 15 and 60 min between groups. In conclusion, T3 administration at reperfusion can improve postischaemic recovery of function while limiting apoptosis. This may constitute a paradigm of a positive inotropic agent with anti-apoptotic action suitable for supporting hemodynamics in the clinical setting of ischaemia-reperfusion.

Key words thyroxine – inotropes – ischaemia-reperfusion – apoptosis – MAPKs – myocardial infarction – caspase-3

Introduction

Reduced contractile function associated with impaired cardiac hemodynamics is a common situation in the clinical setting of ischaemia-reperfusion, such as acute myocardial infarction or aorto-coronary by-pass surgery. Treatment of ischaemic myocardium with inotropic agents such as catecholamines, which act by increasing cytosolic calcium is effective but has

the risk of worsening the oxygen demand/supply balance [31, 33]. In fact, several studies have provided substantial evidence that interventions aimed at increasing cytosolic calcium during ischaemia and early reperfusion may exacerbate myocardial reperfusion injury [4]. Thus, the use of conventional inotropes has been questioned [14, 15]. New therapeutic strategies aiming at decreasing infarct size while preserve and enhance ventricular function are now investigated [9, 11, 18].

Thyroid hormone is long recognized as an inotrope which could potentially be used to support hemodynamics [5, 6, 13, 19, 20]. The long standing belief though that thyroid hormone may be detrimental in ischaemia has limited its clinical use. However, recent experimental and clinical evidence shows that thyroid hormone can regulate important cardioprotective signaling and may increase tolerance of the myocardium to ischaemia rather than being detrimental [1, 21, 27, 34]. Interestingly, T3 was recently shown to improve cardiac hemodynamics in patients undergoing by-pass operation while troponin release was significantly lower [29]. Thus, the interest of thyroid hormone as an inotrope in the clinical setting of ischaemia-reperfusion has been revived. Based on this evidence, by using an isolated rat heart model of ischaemia-reperfusion, we investigated the effects of T3 administration on postischemic cardiac function and on myocardial injury as well as the potential underlying mechanisms. This issue may be of important therapeutic and clinical relevance.

Materials and methods

Animals

Fifty-nine Wistar male rats, 320–380 g were used for this study. 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).

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 left ventricular pressure under isovolumic conditions. Left ventricular balloon volume was adjusted to produce an average initial left ventricular end-diastolic pressure of 6–8 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 indexes of systolic function obtained under isometric conditions.

Rats were anaesthetized with ketamine HCl and heparin 1,000 IU was given intravenously before thoracotomy. The hearts were rapidly excised, placed in ice-cold Krebs-Henseleit buffer (composition in mmol/l: sodium chloride 118, 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_2/5% CO_2) Krebs-Henseleit buffer was established within 60 s after thoracotomy. The perfusion apparatus was heated to ensure a temperature of 37°C throughout the course of the experiment. 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 [25].

Triiodothyronine administration

3′,5′,3′-Triiodothyronine (T_3) (Sigma Chemicals, St Louis MO, USA) was dissolved in 99% ethanol by adding a small volume (20 μl) of 25% NaOH and diluted 33 times by adding 0.9% NaCl to obtain a stock solution of 1 mg/ml. Stock solution was kept at −20°C. Before each experiment an aliquot of T3 was dissolved in Krebs buffer at the final concentration and was administered throughout reperfusion. The final concentration of 40 μg/l which was used as an effective cardioprotective dose, was chosen after performing pilot studies with concentrations of 6.6 and 40 μg/l. These doses are in the range of doses previously used by other studies [13]. Control rats were given Krebs buffer containing the vehicle. Pilot studies showed that vehicle had no effects on postischemic recovery of function.

Experimental protocol

Isolated control hearts were subjected to 15 min of stabilization, 30 min of zero-flow global ischemia and

60 min of reperfusion, CNT-R60, $n = 11$. Hearts were also subjected to 15 min of stabilization, 30 min of zero-flow global ischemia and 60 min of reperfusion with triiodothyronine 40 μg/l in the perfusate, T3-R60, $n = 10$.

In order to investigate the role of pro-apoptotic and pro-survival kinase signalling in the cardioprotective effect induced by T3, we performed additional experiments studying the activation pattern of those kinases also at 5 and 15 min of reperfusion. Thus, isolated control and T3-treated hearts were subjected to 15 min of stabilization, 30 min of zero-flow global ischemia and 5 or 15 min of reperfusion; CNT-R5, $n = 6$; T3-R5, $n = 6$ and CNT-R15, $n = 6$; T3-R15, $n = 6$, respectively.

Measurement of mechanical function

Left ventricular function was assessed by recording the left ventricular developed pressure which was measured at the end of the stabilization period (LVDP) and after 60 min of reperfusion (LVDP60). Post-ischemic left ventricular 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) as a measure of diastolic chamber distensibility. Left ventricular end-diastolic pressure was measured after 60 min of reperfusion (LVEDP60). Ischaemic contracture representing the gradual increase in left ventricular pressure during ischemia was evaluated by the time to peak contracture (T_{max}) and the magnitude of peak contracture (DP_{max}).

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

Protein expression was measured as previously described [22, 25]. Left ventricular tissue was homogenized in ice-cold buffer containing 10 mM Hepes (pH 7.8), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 0.5 mM PMSF, 1 mM DTT and 10 μg/ml leupeptin. 200 μl of 10% Igepal was added and samples were left in ice for 30 min. Homogenization was repeated and the homogenate was centrifuged at 1,000×*g* for 5 min, 4°C. The supernatant representing the cytosol-membrane fraction 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 μg) were loaded onto 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 100 V and 4°C, for 1.5 h using Towbin buffer for Western blotting analysis. Subsequently, filters were probed with specific antibodies against total p38 MAPK and dual phospho-p38 MAPK, total c-jun NH_2-terminal kinases (JNKs) and dual phospho-JNKs, total ERK and dual phospho-ERK, total Akt and dual phospho-Akt (Cell Signaling Technology, dilution 1:1000) overnight at 4°C. Filters were incubated with appropriate anti-mouse (Amersham) or anti-rabbit (Cell Signaling) HRP secondary antibodies and immunoreactivity was detected by enhanced chemiluminescence using Lumiglo reagents (New England Biolabs) and exposed to Hyperfilm paper (Amersham).

For comparisons between groups, six samples from each group were loaded on the same gel. Data were expressed as the ratio of phosphorylated to total protein expression. Immunoblots and gels were quantified using the AlphaScan Imaging Densitometer (Alpha Innotech Corporation, 14743, Catalina Street, San Leandro, CA).

Measurement of lactate dehydrogenase (LDH) release

Coronary effluent was collected at reperfusion for the measurement of lactate dehydrogenase (LDH) activity by spectrophotometer (LDH UV Fluid, Rolf Greiner Biochemica). LDH release was expressed per gram of tissue and was used as an index of myocardial injury [24].

Evaluation of apoptosis by fluorescent probe optical imaging

QCASP3.2 (Quidd, France) which targets the intracellular activated caspase-3, was used as a marker of cell apoptosis. It is composed of the peptidic sequence DEVD flanked by a fluorophore (derived from Cy5.0) and a suitable quencher. The probe is activated upon recognition of its target and emits light at 670 nm when excited at 645 nm. The signal was visualized using fluorescence microscopy (Zeiss Axiovert 25 with Filter Set 50 and Axiocam MRc incorporated). Images were quantified using ImageJ (National Center for Biotechnology Information, Bethesda, MD) in arbitrary units.

QCASP3.2 was dissolved in normal saline to obtain a stock solution of 400 nmol/ml and kept at 4°C.

Before each administration, 60 µl (24 nmol probe) of the stock solution were dissolved in Krebs buffer. The probe was administered directly to the heart at 30 min of reperfusion. Rat hearts which were only perfused (without ischaemia) in Langendorff preparation for 110 min and received the probe during the last 30 min were used as negative controls. Administration of the probe either in perfused hearts or in hearts subjected to ischemia-reperfusion showed no effect on LV function. The left ventricle of each sample was isolated at 60 min of reperfusion, cut into transverse sections and examined under fluorescence microscopy within 5 min. The number of hearts used for optical imaging were $n = 4$ for each group.

Measurement of caspase-3 activity

0.4 g of left ventricular tissue were homogenized in ice-cold buffer containing 10 mM Hepes (pH 7.8), 10 mM KCl, 0.1 mM EDTA, 0.5 mM PMSF and 1 mM DTT. Following homogenization samples were centrifuged at 12,000×*g* for 15 min, 4°C. The supernatant was analysed immediately using the colorimetric caspase-3 assay kit (CASP-3-C, Sigma-Aldrich, USA). Briefly, 50 µl of each sample were incubated with the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide, for 3 h at 37°C. The assay is based on the hydrolysis of the peptide substrate from activated caspase-3 resulting in the release of p-nitroanilide (p-NA). The concentration of p-NA released was calculated from the absorbance values at 405 nm. The activity of caspase-3 was expressed in nmol p-NA/min/ml.

Statistics

Values are presented as mean (S.E.M.). Unpaired *t* test and Mann–Whitney test were used for differences between groups. A two-tailed test with a *P* value less than 0.05 was considered significant.

Results

Effect of 6.6 µg/l T3 on postischaemic cardiac function and LDH release

In initial pilot studies with two different doses of 6.6 and 40 µg/l T3, 6.6 µg/l T3 at reperfusion had no effect on postischaemic recovery. In fact, LVDP% and LVEDP (mmHg) at 60 min of reperfusion were 36.1% (3.9) and 76.8 (5.4) for control ($n = 7$) vs 32.4% (5.0) and 77.6 (6.3) for T3-treated hearts ($n = 7$), respectively, $P > 0.05$. Furthermore, LDH release (IU per gram) in the perfusate was 11.0 (0.7) in control and 10.4 (0.9) in T3-treated hearts, $P > 0.05$. Baseline left ventricular function (LVDP, mmHg) was 118.0 (2.8) for control and 121.2 (3.7) for T3-treated hearts, while T_{max} (min) and DPmax (mmHg) were 21.6 (0.9) and 77.1 (2.7) in control versus 21.4 (1.2) and 81.8 (3.0) in T3-treated hearts, $P > 0.05$.

Effect of 40 µg/l T3 on postischaemic cardiac function

T3 at this dose improved all the measured functional indices at reperfusion; LVDP (mmHg) and LVEDP (mmHg) at 60 min of reperfusion were 45.4 (3.7) and 71.1 (4.9) for CNT-R60 versus 66.0 (5.7) and 57.5 (3.9) for T3-R60, respectively, $P < 0.05$. Furthermore, the recovery of left ventricular developed pressure (LVDP%) was 38.8% (3.1) in CNT-R60 and 56.0% (4.4) in T3-R60 hearts, $P < 0.05$ (Fig. 1).

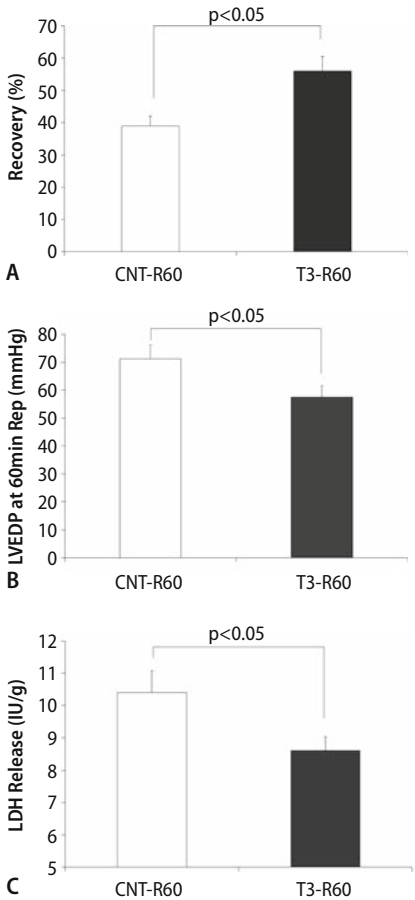
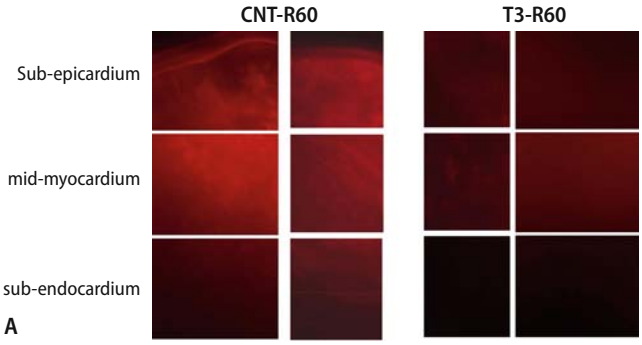


Fig. 1 Recovery of left ventricular developed pressure (LVDP%, **a**), left ventricular end-diastolic pressure (LVEDP, **b**) and LDH release (**c**) in control hearts (CNT-R60, $n = 11$) and T3-treated hearts (T3-R60, $n = 10$) after 30 min of zero-flow global ischemia and 60 min of reperfusion. Triiodothyronine (40 µg/l) was added to the perfusate during reperfusion

Baseline left ventricular function was not different between control and T3-treated hearts. In fact, LVDP (mmHg) was 118.2 (2.0) for CNT-R60 and 116.6 (2.3) for T3-R60, $P > 0.05$. In addition, T_{max} (min) and DPmax (mmHg) were 21.9 (0.8) and 75.3 (3.0) in control versus 22.2 (1.4) and 78.3 (2.9) in T3-treated hearts, $P > 0.05$.

Effect of 40 µg/l T3 on myocardial LDH release

LDH release (IU per gram) in the perfusate was significantly reduced in T3-R60 as compared to CNT-R60 hearts [8.6 (0.4) Vs. 10.4 (0.7), $P < 0.05$ respectively] (Fig. 1c).



B

	CNT-R60	T3-R60	Mann-Whitney (P)
Sub-epicardium	28.2 (3.2)	12.5 (1.8)	0.003
mid-myocardium	33.3 (3.7)	14.6 (2.1)	0.002
Sub-endocardium	17.3 (1.4)	8.6 (1.3)	0.004

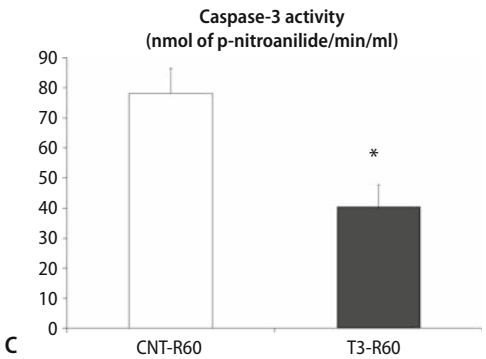


Fig. 2 **a** Microscopy images showing myocardial apoptosis detected by fluorescent probe in different heart layers in control hearts (CNT-R60) and in hearts treated with 40 µg/l T3 (T3-R60) after 30 min of zero-flow global ischemia and 60 min of reperfusion. **b** Table presenting data of caspase-3 activity after quantification of images obtained by fluorescent microscopy. Quantification was performed using ImageJ (National Center for Biotechnology Information, Bethesda, MD). Data are expressed as mean (SEM) for each myocardial layer in arbitrary units. The number of hearts used for optical imaging were $n = 4$ for each group. **c** Caspase-3 activity detected by enzymatic assay analysis in control hearts (CNT-R60, $n = 6$) and in hearts treated with 40 µg/l T3 (T3-R60, $n = 6$) after 30 min of zero-flow global ischemia and 60 min of reperfusion. *$P < 0.05$ vs CNT-R60

T3 mediated cardioprotection and caspase-3 activation

Caspase-3 activity (nmol p-NA/min/ml) was found to be 78.2 (8.2) in CNT-R60 versus 40.5 (7.1) in T3-R60 hearts, $P < 0.05$ (Fig. 2c).

In order to localise caspase-3 activation, optical imaging was used. Region-specific changes in certain molecules has been previously associated with cardiac dysfunction early after myocardial infarction [10]. Figure 2a shows an increased fluorescent signal in CNT-R60 hearts particularly in mid-myocardial layer, while the signal was hardly detectable in T3-R60 hearts [33.3 (3.7) Vs. 14.6 (2.1), $P < 0.05$ respectively].

T3 mediated cardioprotection and pattern of intracellular kinase signalling activation

Phosphorylated levels of the pro-survival kinases ERK and Akt at 5, 15 and 60 min of reperfusion between the two groups are shown in Figs. 3 and 4. The levels

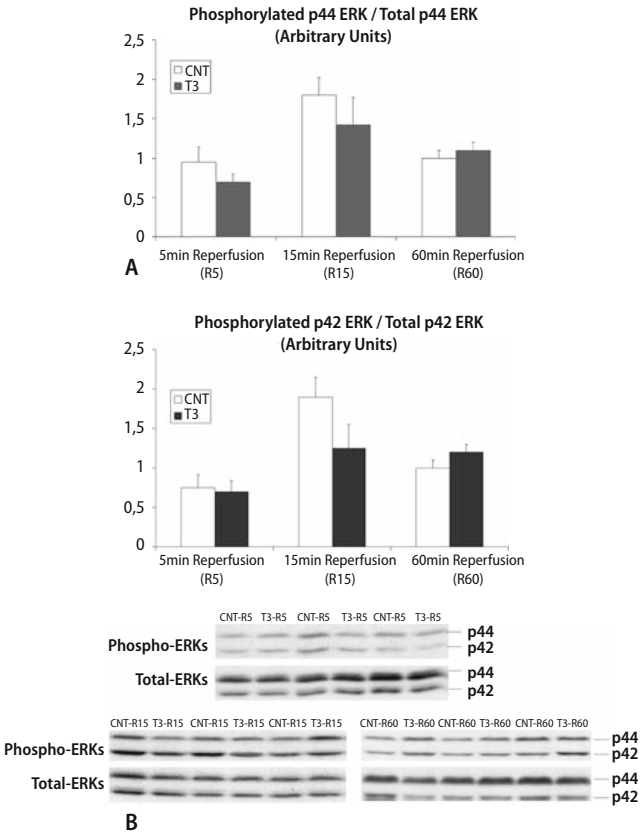


Fig. 3 Representative western blots and densitometric assessment in arbitrary units of the ratio of phosphorylated p44 ERK to total p44 ERK expression (**a**) and (**b**) phosphorylated p42 ERK to total p42 ERK expression in control hearts (CNT) and T3-treated hearts (T3) after 30 min of zero-flow global ischemia and 5 min (R5), 15 min (R15) or 60 min (R60) of reperfusion. Data represent $n = 6$ hearts per group

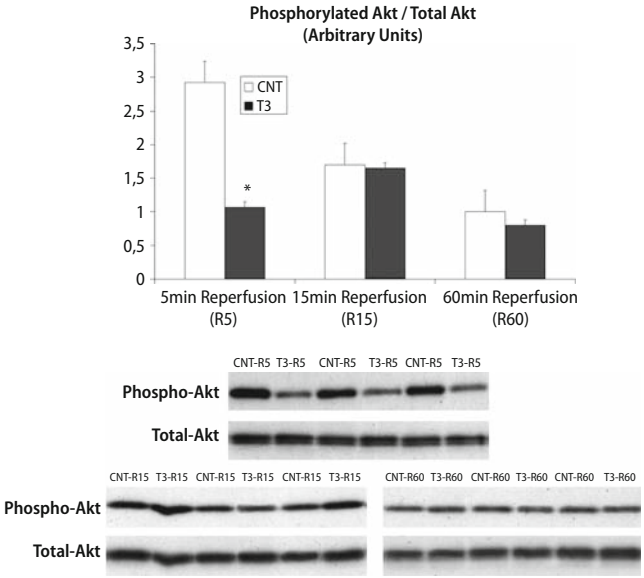


Fig. 4 Representative western blots and densitometric assessment in arbitrary units of the ratio of phosphorylated Akt to total Akt expression in control hearts (CNT) and T3-treated hearts (T3) after 30 min of zero-flow global ischemia and 5 min (R5), 15 min (R15) or 60 min (R60) of reperfusion. Data represent $n = 6$ hearts per group. *$P < 0.05$ Vs. CNT-R5

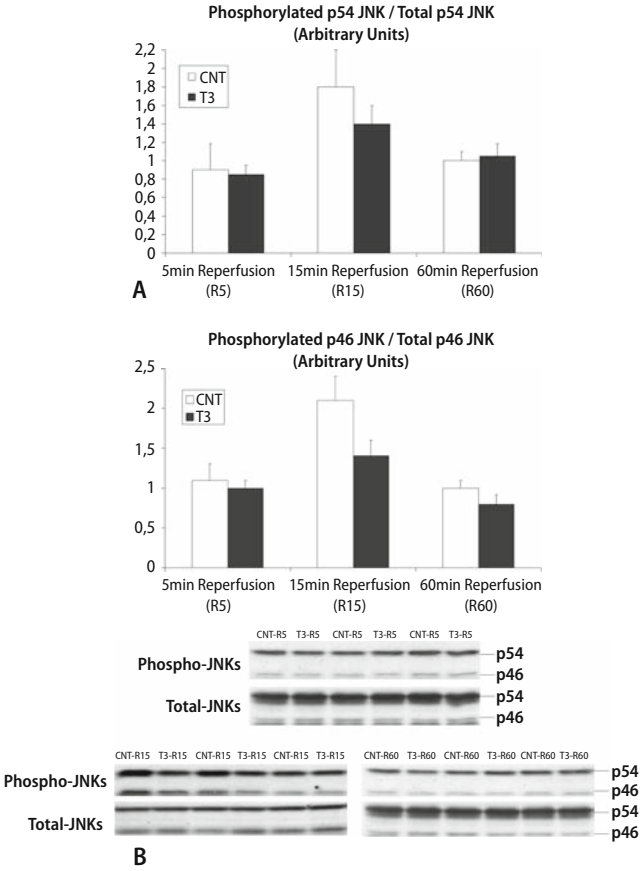


Fig. 5 Representative western blots and densitometric assessment in arbitrary units of the ratio of phosphorylated p54 JNK to total p54 JNK expression (**a**) and (**b**) the ratio of phosphorylated p46 JNK to total p46 JNK expression in control hearts (CNT) and T3-treated hearts (T3) after 30 min of zero-flow global ischemia and 5 min (R5), 15 min (R15) or 60 min (R60) of reperfusion. Data represent $n = 6$ hearts per group

of expression of the phospho-ERKs in response to ischaemia and reperfusion were similar in both groups at all time points, $P > 0.05$. Phospho-Akt levels at 5 min of reperfusion were 2.7-fold lower in T3-R5 as compared to CNT-R5 hearts, $P < 0.05$, while there was no significant difference between the groups at 15 and 60 min of reperfusion.

Phosphorylated levels of the pro-apoptotic kinases p38 MAPK and JNKs at 5, 15 and 60 min of reperfusion between the two groups are shown in Figs. 5 and 6. The levels of phospho-JNKs in response to ischaemia and reperfusion were similar in both groups at all time points, $P > 0.05$. In contrast, phospho-p38 MAPK levels were found to be 2.3-fold less in T3-R5 as compared to CNT-R5, 2.1-fold less in T3-R15 as compared to CNT-R15 and 1.5-fold less in T3-R60 as compared to CNT-R60, $P < 0.05$ (Fig. 6).

Discussion

It has long been recognized that thyroid hormone is an effective positive inotropic agent that could potentially be exploited in clinical practice and particularly in the setting of ischaemia–reperfusion. The acute effect of T3 on cardiac contractility has been attributed to its non-genomic effect on the activation of calcium handling proteins [2]. Early experimental and clinical studies have provided evidence that T3 administration at reperfusion could result in marked improvement in postischemic cardiac function. In fact, Kadletz et al. by using 2.5 and 25 μg/l T3 at reperfusion in isolated rabbit heart preparations demonstrated a marked improvement in recovery of cardiac function [13]. Here, in this study, in a model of isolated rat heart preparation, by using 6.6 and 40 μg/l T3 at reperfusion, we found that a beneficial effect of T3 on cardiac function was evident with 40 μg/l T3 probably indicating a dose-dependent response. Furthermore, we showed that T3 limited reperfusion injury rather than being detrimental. In fact, both LDH release and apoptosis were shown to be significantly lower in T3 treated hearts. Apoptosis was detected by enzymatic and optical measurements of caspase-3 activity. Interestingly, fluorescent probe optical imaging showed that apoptotic signal of activated caspase-3 in untreated hearts was much stronger in the mid-myocardium, lower in the subepicardium and nearly absent in subendocardium at the end of reperfusion phase and this signal was

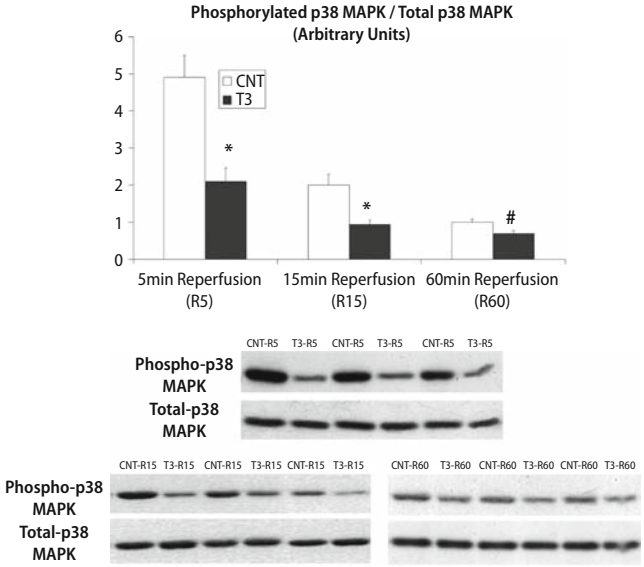


Fig. 6 Representative western blots and densitometric assessment in arbitrary units of the ratio of phosphorylated p38 MAPK to total p38 MAPK expression in control hearts (CNT) and T3-treated hearts (T3) after 30 min of zero-flow global ischemia and 5 min (R5), 15 min (R15) or 60 min (R60) of reperfusion. Data represent $n = 6$ hearts per group. $^{*}P < 0.05$ vs CNT-R5, $^{**}P < 0.05$ vs. CNT-R15, $^{\#}P < 0.05$ vs CNT-R60

markedly reduced in all layers after T3 treatment. This differential transmural pattern of apoptosis after ischaemia-reperfusion seems to be consistent with previous reports. In fact, in a rat model of ischemia-reperfusion injury, heart autoradiography following 99mTc radiolabeled annexin V administration revealed that the zone of apoptosis initially begins in the mid-myocardium 30 min after reperfusion and extends into the subendocardium and subepicardium 6 h after reperfusion [32]. Furthermore, in a recent high-resolution, non-invasive MR imaging approach, in which a novel annexin V-based magnetofluorescent iron oxide nanoparticle was used to quantitatively image myocardial apoptosis in vivo, the distribution of the magnetofluorescent annexin suggested a mid-myocardial predominance of the agent after ischaemia-reperfusion [16].

T3 mediated cardioprotection: potential underlying mechanisms

It has long been recognized that stress response kinases are activated during ischaemia and reperfusion and a delicate balance between pro-death and survival pathways exists and seems to determine the fate of the stressed cell [7, 23]. Thus, pharmacological interventions which can change the balance of activation of kinase signaling pathways towards increased activation of pro-survival signaling appear to limit myocardial injury. Agents such as insulin, erythropoietin, adipocytokines, adenosine, natriuretic peptides or statins are all shown to reduce myocardial infarct size through the activation of the pro-survival signaling pathway, known as RISK pathway (Akt and ERK1/2) [8, 12]. Based on this evidence, we investigated whether similar mechanisms are involved in the thyroid hormone induced cardioprotection. Thus, we measured the activation pattern of ERK1/2 and Akt at different time points of reperfusion to identify whether differences exist between treated and non treated hearts. Our data showed that there were not significant changes in phospho-ERK1/2 levels between treated and non treated hearts while phospho–Akt levels were significantly lower in T3 treated group only at 5 min and no significant differences were observed at 15 and 60 min between the groups. In contrast, changes in the activation pattern of the pro-apoptotic signaling pathways were seen, particularly in the p38 MAPK; phospho-p38 MAPK levels in the myocardium at 5, 15 or 60 min of reperfusion were significantly lower in T3-treated hearts as compared to controls. Taken together, these data strongly support a critical role of p38 MAPK in the T3 mediated cardioprotection. Interestingly, thyroid hormone pretreatment is also shown to protect the heart against ischaemia–reperfusion injury by attenuating the ischaemia-reperfusion induced activation of p38 MAPK [22, 28]. Several studies show that p38 MAPK is critical in the response of the cardiac cell to ischaemic stress and seems to serve a dual role: sustained activation of this kinase is detrimental while transient activation may be protective. In fact, transient activation during the cycles of brief episodes of ischaemia-reperfusion (preconditioning) was shown to be essential for the preconditioning beneficial effect [30], while blockade of the ischaemia-reperfusion induced sustained activation of p38 MAPK decreased apoptosis and improved reperfusion function [17].

Clinical and therapeutic implications

It is now realized that sustained and long use of catecholamines may be detrimental in ischaemic conditions and non catecholamine inotropes may be preferred [3, 14]. We have previously demonstrated that, in a similar experimental setting as in this study, dobutamine administration at reperfusion resulted in worsening of postischaemic recovery of function [26]. Thus, T3, a well known positive inotropic agent which appears to possess anti-apoptotic properties may constitute a new therapeutic option for supporting hemodynamics in the clinical setting of ischaemia-reperfusion, such as acute myocardial infarction or by-pass surgery. As a proof of concept, a recent

clinical study showed that T3 administration in patients undergoing by-pass operation resulted in improved cardiac hemodynamics while troponin release was found to be significantly lower [29].

In conclusion, thyroid hormone administration at reperfusion can improve postischaemic recovery of function while limiting apoptosis. This may constitute a paradigm of a positive inotropic agent with anti-apoptotic action suitable for supporting hemodynamics in the clinical setting of ischaemia-reperfusion.

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