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C. PANTOS[1], I. MOUROUZIS[1], N. TSAGOULIS[1], K. MARKAKIS[1], G. GALANOPOULOS[1], N. ROUKOUNAKIS[1], P. PERIMENIS[1], A. LIAPPAS[1], D.V. COKKINOS[2]

THYROID HORMONE AT SUPRA-PHYSIOLOGICAL DOSE OPTIMIZES CARDIAC GEOMETRY AND IMPROVES CARDIAC FUNCTION IN RATS WITH OLD MYOCARDIAL INFARCTION

[1]Department of Pharmacology, University of Athens, 75 Mikras Asias Ave.,11527 Goudi, Athens, Greece;
[2]Onassis Cardiac Surgery Center and Biomedical Research Foundation-Academy of Athens, Athens, Greece

Thyroid hormone (TH) is critical in cardiac cell differentiation (regulating contractile proteins and cell geometry) and this effect could be potentially exploited therapeutically in reversing the process of de-differentiation which underlies postischemic cardiac remodeling. Acute myocardial infarction was induced in male Wistar rats by ligating left coronary artery (AMI, n=8), while sham operated animals served as control (SHAM, n=8). 13 weeks after AMI, TH was administered in a group of animals for 4 weeks (AMI-THYR, n=9). TH significantly increased β-MHC and decreased α-MHC expression in the myocardium. This response was accompanied by changes in cardiac geometry: sphericity index, (SI, long to short axis ratio) was found to be 1.95 (SEM, 0.02) in SHAM, 1.51(0.03) in AMI and 1.64(0.03) in AMI-THYR, $p<0.05$. As a consequence, cardiac function was significantly improved: left ventricular ejection fraction (EF%) was 74.5% (SEM, 2.8) in SHAM *vs* 29.5% (2.1) in AMI, and 40.0% in AMI-THYR, $p<0.05$. Furthermore, +dp/dt and -dp/dt were 4250 (127) and 2278 (55) in SHAM *vs* 2737(233) and 1508 (95) in AMI *vs* 3866 (310) and 2137(111) in AMI -THYR, respectively, $p<0.05$. TH treatment partially reverses cardiac dysfunction in rats with old myocardial infarction by favorably changing cardiac chamber geometry and expression of myosin isoforms. Thyroid hormone, unlike current treatments, appears to be a paradigm of therapeutic intervention which aims at restoring cardiac geometry and may prove new effective treatment for heart failure.

Key words: *thyroxine, acute myocardial infarction, cardiac geometry, cardiac remodeling, heart failure*

INTRODUCTION

Heart failure is a progressive disorder that is initiated after an "index event", such as an acute ischaemic insult, and results in decline in the contractile function of the heart. Early in the course of the disease, a variety of compensatory mechanisms are in operation, such as activation of neuro-hormonal and inflammatory systems. In the short-term, this response seems to restore cardiovascular function to a normal homeostatic range but with time, sustained activation of these systems can lead to secondary end-organ damage within the ventricle. These series of adaptive changes within the myocardium are collectively referred as "left ventricular remodeling" (1). One of the main features of the remodeled myocardium is the change in cardiac chamber geometry and size which is associated with mechanical disadvantage of the heart. Left ventricle becomes not only large but also spherical. This, in turn, is translated to severe decline in the ejection fraction, increased wall stress and oxygen utilization (2-4). Furthermore, patients with spherical ventricles seem to have poor prognosis (5, 3).

On the basis of this evidence, the therapeutic potential of interventions which can restore cardiac geometry has been extensively explored (6-9). However, such treatments have been limited only to surgical correction of the spherical architecture into a more normal elliptical shape. The possibility of pharmacological treatments which could potentially optimize cardiac geometry and improve cardiac function has not been adequately explored.

It is now realized that, during early developmental stages, several physiological processes involved in organ morphogenesis take place and could serve as potential paradigms for organ re-construction in disease states, such as heart failure. Thyroid hormone seems to be a key player in organ development by facilitating tissue growth differentiation and cellular response to stress (10-12). Thyroid hormone induces unique changes in cardiac myocyte shape and geometry and increase cellular tolerance to stress *via* activation of pro-survival signaling pathways, such as ERK cascade (13-15). Thus, it could be hypothesized that thyroid hormone may favorably remodel the post-ischaemic myocardium after an index event as acute myocardial infarction. Mimicking physiological processes of tissue remodeling may lead to new and effective therapeutic approaches for treating heart diseases. We have recently shown that thyroid hormone treatment early in the course of acute myocardial infarction in rats can prevent chamber remodeling and improve cardiac haemodynamics (16, 17). Here, in this study, we further test the possibility that thyroid hormone could also reverse remodeling in rats with an old myocardial infarction. It should be noted that, unlike the long standing belief, cardiac dysfunction may not always be irreversible (8).

MATERIALS AND METHODS

Animals

Sixty seven male Wistar rats, 300-400 g (3-4months old) 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 Guide (NIH Pub. No. 83-23, Revised 1996).

Experimental model of myocardial infarction

Myocardial infarction was induced by ligation of the left coronary artery as previously described (16-20). Rats were anesthetized with an intraperitoneal injection of ketamine (70 mg/kg) and midazolame (0.1 mg/kg), intubated and ventilated *via* a tracheal cannula using a constant-volume rodent ventilator (Harvard Apparatus, Inspira, 45 breaths/min, 1ml/100g tidal volume). Anesthesia was maintained by inhalation of small doses of sevoflurane (1-3%). Left thoracotomy was performed at the fourth intercostal space followed by pericardiotomy. Left coronary artery was then ligated with a 6-0 silk round-bodied suture. The heart was quickly returned to the chest cavity, the chest was closed and rats were allowed to recover using assist mode ventilation. Atelectasis was prevented by producing positive end-expiratory pressure at the end of the surgical procedure. Continuous ECG recording was used to monitor heart rate and ECG ischaemic changes after coronary artery ligation. Body temperature was maintained at 37° C by using a heating blanket (Harvard Homeothermic Blanket, 50-7061). The mortality was up to 30% in the infarction group within the first 24 hours. Myocardial infarctions which produced a scar area of 90 mm^2 to 155 mm^2, corresponding to 40%-60% of the left ventricle, were included in this study. Similar procedure was followed for sham-operated control animals without coronary artery ligation.

Thyroid hormone administration

Rats subjected to coronary artery ligation, 13 weeks after the operation, were randomly divided in two groups. The first group received standard rat chow (AMI), while the second group received food containing thyroid powder 0.05% (Sigma, T1251, containing 0.42 µg/mg T_3 and 1.7 µg/mg T_4) for 4 weeks (AMI-THYR). Mean daily intake of thyroid hormone per rat was 3 µg T_3 and 12 µg T_4. Gastrointestinal absorption of thyroid hormone seems to vary from 50% to 75%. Sham-operated rats received standard rat chow and were designated as SHAM.

Echocardiography

At 13 and 17 weeks, rats were sedated with ketamine hydrochloric acid (100 mg/Kg) and heart function was evaluated by echocardiography as previously described (16, 18, 20). Short and long-axis images were acquired using a digital ultrasound system (Sonosite 180Plus, 21919 30th Drive SE, Bothell, WA, USA) with a 7.0-MHz sector-array probe. Image was presented in a 17" screen to increase resolution. Sonosite is a fully digital system of the most recent generation with excellent image quality. A large number of consecutive measurements were performed and analysed by two independent operators.

Echocardiography measurements included left ventricular internal diameter at the diastolic phase (LVIDd), LV internal diameter at the systolic phase (LVIDs), posterior wall thickness at the diastolic phase (LVPW), systolic velocity of the posterior wall radial displacement (SVPW) and the ejection fraction (EF%). SVPW was measured from two-dimensional guided M-mode recordings obtained at the mid-ventricular level as previously described (21). SVPW was calculated using a software package available from Sonosite and based on the following formula: V=ds/dt, where "V" represents velocity, "s" the distance and "t" represents time. SVPW was used to assess segmental contractile function of the non-infarcted myocardium, while EF% was used to determine global contractile LV function.

Wall tension index (WTI) was defined as the ratio (LVIDd/2*Posterior Wall thickness) as previously described (22, 23). WTI was measured in order to indirectly assess myocardial wall stress. In addition, sphericity index (SI), defined as the ratio of maximum long axis (in mm) to maximum short axis (in mm) of the left ventricle was determined in order to assess LV geometry. All measurements were averaged for at least 3 consecutive cardiac cycles.

Isolated heart preparation

A non-ejecting isolated rat heart preparation was perfused at a constant flow according to the Langendorff technique (13, 24). Rats were anaesthetized with ketamine hydrochloric acid and heparin 1000 IU/kg was given intravenously before thoracotomy. The hearts were rapidly excised, placed in ice-cold Krebs-Henseleit buffer and mounted on the aortic cannula of the Langendorff perfusion system. Hearts were paced at 320 beats/min with a Harvard pacemaker. An intraventricular balloon allowed measurement of contractility. Left ventricular balloon volume was adjusted to produce an average initial left ventricular end-diastolic pressure of 7-8 mmHg in all groups. The water filled balloon was connected to a pressure transducer and the LV pressure signal was transferred to a computer using a data analysis software (IOX, Emka Technologies) which allowed continuous monitoring and recording of heart function.

Left ventricular developed pressure (LVDP), defined as the difference between left ventricular peak systolic pressure and left ventricular end-diastolic pressure, represented a contractility index obtained under isometric conditions. Left ventricular systolic function was assessed by recording the left ventricular developed pressure (LVDP, mmHg) and the positive and negative first derivative of LVDP; +dp/dt (mmHg/sec), -dp/dt (mmHg/sec).

All preparations were perfused for 20 min and measurements were performed at the end of this period. All preparations included in this study were stable for at least the last 10 min of the perfusion period. Isolated hearts that did not produce stable measurements of LVDP, LV end-diastolic pressure and perfusion pressure for the last 10 min of the perfusion period were excluded from the analysis.

Measurement of myosin heavy chain isoform content

Approximately 0.2 g of left ventricular tissue was homogenized in ice-cold buffer (A) 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 were added and samples were left in ice for 30 min. Homogenization was repeated and the homogenate was kept for myosin heavy chain isoform analysis. Protein concentrations were determined by the BCA method.

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 (16, 20, 25, 26). 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 gel included 0.07 M Tris (base), pH 6.7, 5% (vol/vol) glycerol and 0.4% sodium dodecyl sulfate (SDS), while the separating gel included 0.2 M Tris (base), pH 8.8, 5% (vol/vol) glycerol, 0.1 M glycine and 0.4% SDS. The upper running buffer consisted of 0.1 M Tris

(base), 150 mM glycine, 0.1% 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 22 h at 4° 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 thyroid hormones

Plasma T_4 and T_3 quantitative measurements were performed with ELISA, using kits obtained from Alpha Diagnostic International, Texas, USA (No 1100 for total T_4 and No 1700 for total T_3), as previously described (19). 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).

Experimental protocol

Thirteen weeks after the surgical procedure, rats were anaesthetized with ketamine hydrochloride, subjected to echocardiography analysis and allowed to recover. After echocardiography analysis was performed, post-infarcted rats were divided in 2 groups; the first group received standard rat chow (AMI, n=8), while the second group received food containing thyroid powder 0.05% for 4 additional weeks (AMI-THYR, n=9). Seventeen weeks after the surgical procedure, rats from all groups were anaesthetized again and subjected to echocardiography analysis. Excision of the heart followed in order to measure contractile function in a Langendorff preparation. Blood was collected from the right atrium in order to measure total T_3 and T_4 in serum. At the end of the perfusion period, left ventricle was isolated from each heart, scar tissue was dissected out and the non-infarcted tissue was immediately frozen in liquid nitrogen. Weights of the scar tissue and the viable left ventricular tissue were measured. The area of the scar tissue was measured in mm^2.

Experimental model of hypothyroidism and myocardial infarction

In order to assess the effects of low thyroid hormone state in postischaemic cardiac remodelling, we additionally performed a set of experiments in hypothyroid rats. Hypothyroidism was induced in rats by administration of 6-n-propyl-2-thiouracil in drinking water (final concentration of 0.05%) for three weeks. We have previously shown that this model results in moderate decrease in T_4 and T_3 levels in plasma (27, 28). After 3 weeks, hypothyroid animals were subjected to coronary artery occlusion as described above and continued receiving 6n-propyl-2-thiouracil in drinking water for 2 weeks (HP-AMI, n=5). Untreated animals were either sham-operated (NORM, n=6) or subjected to coronary artery occlusion (NORM-AMI, n=6). Two weeks after the surgical procedure, rats from all groups were anaesthetized again and subjected to echocardiography analysis. Excision of the heart followed, left ventricle was isolated from each heart, scar tissue was dissected out and the non-infarcted tissue was immediately frozen in liquid nitrogen. Weights of the scar tissue and the viable left ventricular tissue were measured. The area of the scar tissue was measured in mm^2.

Statistics

Results are presented as mean (SEM). One-way analysis of variance with Bonferroni or Dunnett correction was used for multiple comparisons. Paired *t*-test was used in order to compare differences within the same group at different time-points. Significance was set at 0.05.

RESULTS

Cardiac hypertrophy, heart rate and thyroid hormones levels in plasma

Scar area and weight, heart rate, left ventricular weight (LVW) and the ratio of LVW to body weight are shown in *Table 1*. Scar area and weight were not different between AMI and AMI-THYR hearts, while cardiac hypertrophy was developed in both groups.

At 17 weeks following coronary artery ligation, T_3 levels were found to be decreased [1.32 (0.05) in AMI *vs* 1.45 (0.05) in SHAM, p=n.s.] while T_4 levels were increased [63.9 (2.3) in AMI *vs* 52.5 (2.0) in SHAM, p=n.s.] but not at a statistically significant level. However, the T_4/T_3 ratio was significantly increased in AMI hearts, indicating an abnormal conversion of T4 to T3 [48.3 (2.8) in AMI *vs* 36.7 (1.2) in SHAM, $p<0.05$], *Fig. 1*.

Thyroid hormone treatment in post-infarcted rats resulted in increased T_3 levels in plasma [1.9 (0.15) in AMI-THYR *vs* 1.32

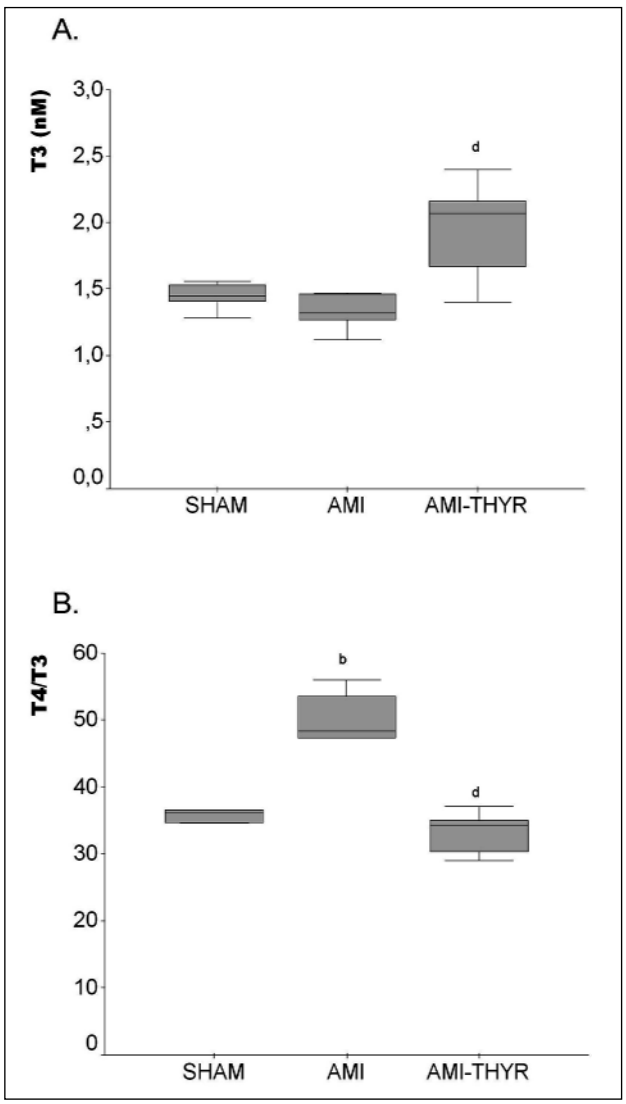

Fig. 1. Box plots with percentiles and median value are presented for T3 levels (A) and the ratio of T4 to T3 (B) in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts after thyroid hormone treatment (AMI-THYR) at 17 weeks. $^b p<0.05$ *vs* SHAM at 17 weeks, $^d p<0.05$ *vs* AMI at 17 weeks.

Table 1. Scar area and weight, heart rate, left ventricular weight (LVW) and the ratio of LVW to body weight in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts with thyroid hormone treatment (AMI-THYR) at 17 weeks are shown in this table. The values are mean (SEM).

	SHAM (n=8)	AMI (n=8)	AMI-THYR (n=9)
Scar area (mm^2)	--------	108 (5.0)	113 (10.4)
Scar weight (mg)	--------	233 (23.9)	253 (24.5)
Heart Rate (bpm)	366 (21)	419 (12)	432 (21)
LVW (mg)	843 (42)	1026 (59)[b]	1040 (30)[b]
LVW/Body weight	1.85 (0.08)	2.27 (0.11)[b]	2.54 (0.08)[b]

[b]$p<0.05$ *vs* SHAM at 17 weeks postoperatively

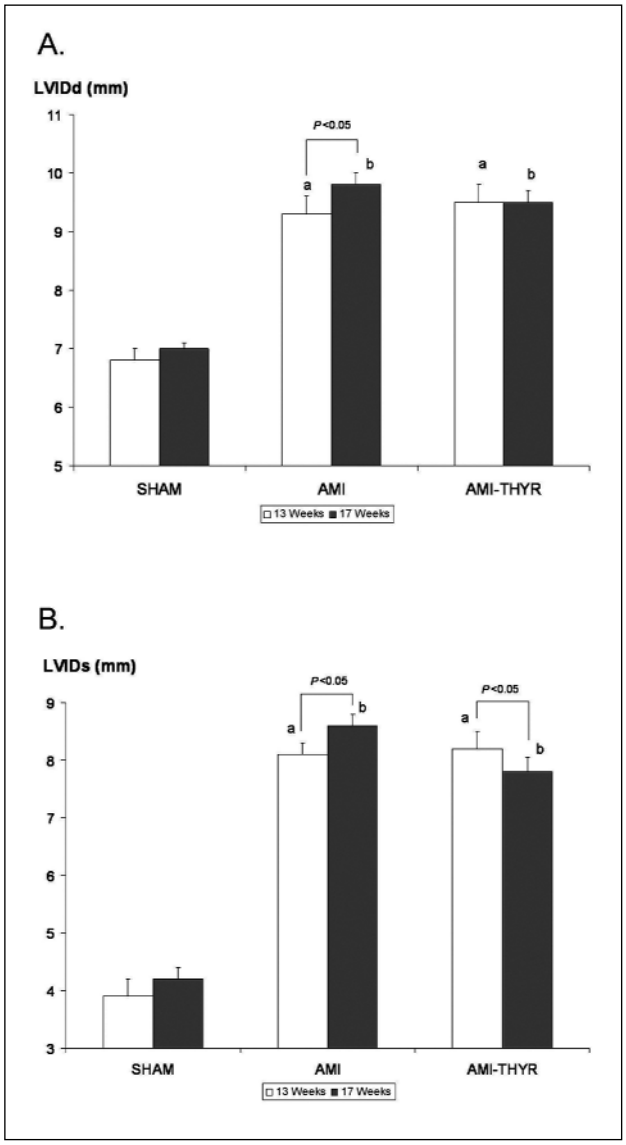

Fig. 2. Left ventricular end-diastolic (A) and end-systolic (B) diameter in mm assessed by echocardiography analysis in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts after thyroid hormone treatment (AMI-THYR) at 13 and 17 weeks. (Columns are means, bar=SEM).
[a]$p<0.05$ *vs* SHAM at 13 weeks, [b]$p<0.05$ *vs* SHAM at 17 weeks

(0.05) in AMI, $p<0.05$] and restored the ratio of T_4/T_3 to SHAM values [32.2 (1.8) in AMI-THYR *vs* 48.3 (2.8) in AMI, $p<0.05$], *Fig. 1*. This dose did not significantly increase heart rate in post-infarcted rats, *Table 1.*

Myosin isoform expression in non-infarcted myocardium

AMI hearts were found to express 49% α-MHC and 51% β-MHC as compared to 66% α-MHC and 34% β-MHC expression observed in SHAM hearts, $p<0.05$. Thyroid hormone treatment in post-infarcted hearts resulted in a shift from β-MHC to α-MHC expression. In fact, AMI-THYR hearts expressed 89% α-MHC and 11% β-MHC, $p<0.05$ *vs* SHAM and AMI, *Fig. 4B*.

Wall tension index and geometry of the left ventricle

LVIDd and LVIDs were found to be significantly increased both in AMI and AMI-THYR hearts at 13 weeks as compared to

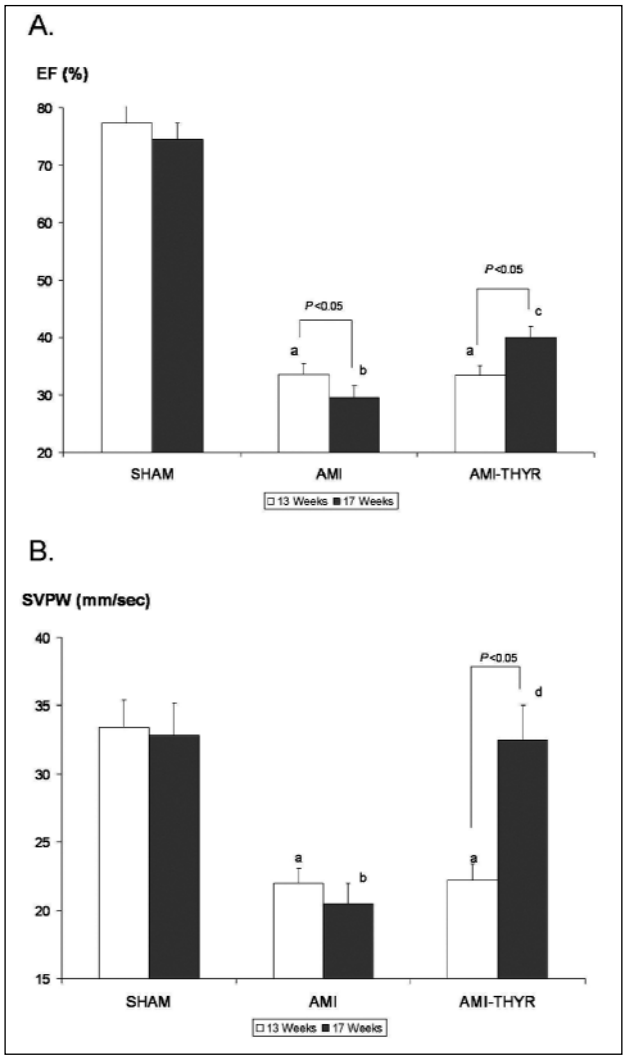

Fig. 3. Left ventricular ejection fraction (EF%, A) and systolic velocity of posterior wall radial displacement (SVPW, B) assessed by echocardiography analysis in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts after thyroid hormone treatment (AMI-THYR) at 13 and 17 weeks. (Columns are means, bar=SEM).
[a]$p<0.05$ *vs* SHAM at 13 weeks, [b]$p<0.05$ *vs* SHAM at 17 weeks, [c]$p<0.05$ *vs* SHAM and AMI at 17 weeks, [d]$p<0.05$ *vs* AMI at 17 weeks.

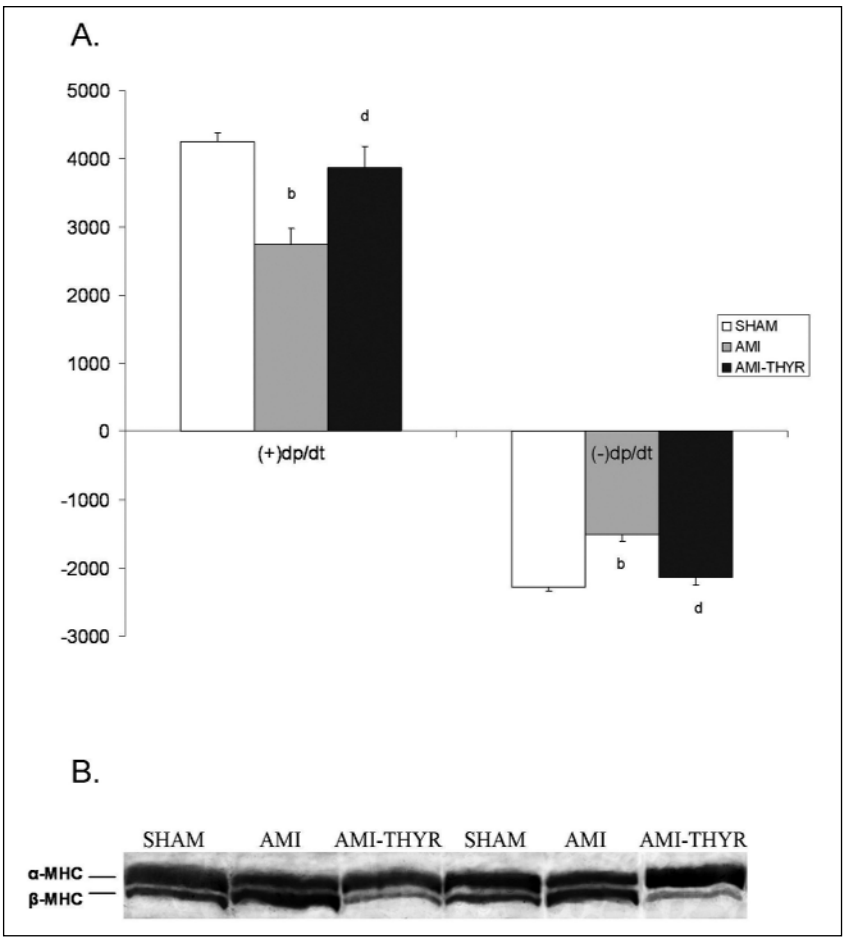

Fig. 4. **A**. Rate of increase and decrease of LVDP (+dp/dt and -dp/dt) under isometric conditions in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts after thyroid hormone treatment (AMI-THYR) at 17 weeks. The values are mean (SEM).
B. Representative figure showing myosin isoforms expression in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts after thyroid hormone treatment (AMI-THYR) at 17 weeks.
[b]$p<0.05$ *vs* SHAM at 17 weeks, [d]$p<0.05$ *vs* AMI at 17 weeks.

SHAM hearts. LVIDd (mm) was found to be 9.3 (0.3) in AMI and 9.5 (0.3) in AMI-THYR as compared to 6.8 (0.2) in SHAM, $p<0.05$, while LVIDs (mm) was 8.1 (0.2) in AMI and 8.2 (0.3) in AMI-THYR as compared to 3.9 (0.3) in SHAM, $p<0.05$. Between 13 and 17 weeks, LVIDd and LVIDs were progressively deteriorated in AMI hearts [9.8 (0.2) and 8.6 (0.2), respectively, $p<0.05$ *vs* AMI at 13 weeks]. In AMI-THYR hearts, LVIDd remained unchanged [9.5 (0.2), p=n.s. vs AMI-THYR at 13 weeks], while LVIDs was reduced [7.9 (0.3), $p<0.05$ *vs* AMI-THYR at 13 weeks]. *Fig. 2*.

Wall tension index (WTI) was found to be significantly increased both in AMI and AMI-THYR hearts at 13 weeks as compared to SHAM. WTI was 2.08 (0.06) in AMI and 2.15 (0.09) in AMI-THYR as compared to 1.62 (0.05) in SHAM, $p<0.05$. At 17 weeks, WTI was found to be increased in AMI and AMI-THYR hearts [2.13 (0.06) and 2.08 (0.09), respectively, *vs* 1.68 (0.05) in SHAM, $p<0.05$], and no difference was seen in WTI between AMI and AMI-THYR groups.

Sphericity Index (SI, determined as the ratio of long axis to short axis end-diastolic diameters) was significantly lower in both AMI and AMI-THYR hearts at 13 weeks [1.55 (0.03) and 1.50 (0.03) respectively] as compared to SHAM [1.97 (0.02), $p<0.05$]. Between 13 and 17 weeks, SI showed no change in AMI [1.51 (0.03), p=n.s. *vs* AMI at 13 weeks], while it was increased in AMI-THYR hearts [1.64 (0.03), $p<0.05$ *vs* AMI-THYR at 13 weeks], *Fig. 5*.

In vivo assessment of contractile function

Left ventricular ejection fraction (EF%) was significantly lower in both AMI and AMI-THYR hearts at 13 weeks [33.6%

Table 2. Scar area and weight, heart rate, left ventricular weight (LVW) left ventricular end-diastolic (LVIDD) and end-systolic (LVIDS) diameter, left ventricular ejection fraction (EF%), systolic velocity of posterior wall radial displacement (SVPW), wall tension index (LVEDD/2*posterior wall thickness) and sphericity index in sham-operated (NORM), post-infarcted hearts (NORM-AMI) and post-infarcted hypothyroid hearts (HP-AMI) at 2 weeks postoperatively are shown in this table. The values are mean (SEM).

	NORM (n=6)	NORM-AMI (n=6)	HP-AMI (n=5)
Scar area (mm^2)	--------	97 (4.7)	105 (10.3)
Scar weight (mg)	--------	135 (9)	143 (16)
Heart Rate (bpm)	340 (13)	300 (14)	282 (10) [b]
LVW (mg)	586 (15)	597 (24)	502 (27)[d]
LVIDD (mm)	6.7 (0.10)	8.8 (0.11)[b]	9.2 (0.16)[b]
LVIDS (mm)	4.2 (0.20)	7.5 (0.12)[b]	8.3 (0.17)[c]
Wall tension index	1.78 (0.05)	2.38 (0.03) [b]	2.7 (0.03) [c]
Sphericity Index	1.8 (0.03)	1.68 (0.02)[b]	1.62 (0.01)[b]
EF%	72.6 (3.6)	36.2 (1.1)[b]	24.0 (0.09)[c]
SVPW (mm/sec)	29.5 (2.0)	24.1 (1.3)	14.7 (1.6)[c]

[b]$p<0.05$ *vs* NORM, [d]$p<0.05$ *vs* NORM-AMI, [c]$p<0.05$ *vs* NORM and NORM-AMI at 2 weeks postoperatively

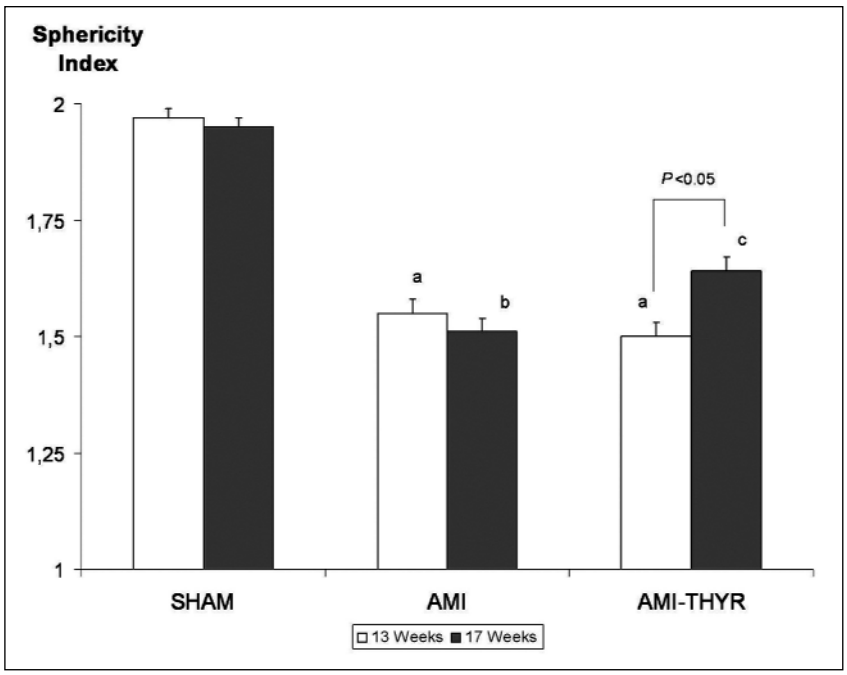

Fig. 5. Sphericity index (SI, the ratio of long to short axis end-diastolic dimensions) as assessed by serial echocardiography analysis in sham-operated (SHAM), post-infarcted hearts (AMI) and post-infarcted hearts after thyroid hormone treatment (AMI-THYR) at 13 and 17 weeks. (Columns are means, bar= SEM).
[a]$p<0.05$ *vs* SHAM at 13 weeks, [b]$p<0.05$ *vs* SHAM at 17 weeks, [c]$p<0.05$ *vs* SHAM and AMI at 17 weeks.

(1.9) and 33.4% (1.7) respectively] as compared to SHAM [77.3% (3.0), $p<0.05$]. Between 13 and 17 weeks, EF% was further deteriorated in AMI [29.5% (2.1), $p<0.05$ *vs* AMI at 13 weeks], while it was increased in AMI-THYR hearts [40.0% (2.0), $p<0.05$ *vs* AMI-THYR at 13 weeks], *Fig. 3*.

Systolic velocity of the posterior wall radial displacement (SVPW, mm/sec) was decreased in AMI and AMI-THYR hearts at 13 weeks [22.0 (1.1) and 22.2 (1.2) respectively] as compared to SHAM hearts [33.4 (2.0), $p<0.05$]. SVPW was significantly improved in AMI-THYR hearts at 17 weeks [32.5 (2.5), $p<0.05$ *vs* AMI-THYR at 13 weeks] and reached SHAM values [32.8 (2.4)], *Fig. 3*.

At 17 weeks, both EF% and SVPW were found to be significantly increased in AMI-THYR as compared to AMI hearts, $p<0.05$.

Contractile function assessed under isometric conditions

At 17 weeks, left ventricular developed pressure (LVDP, mmHg), was found to be decreased in AMI [80.5 (5.5)] as compared to SHAM hearts [116.3 (2.2), $p<0.05$], while in AMI-THYR hearts, LVDP was significantly increased [98.4 (5.7), $p<0.05$ *vs* AMI]. Furthermore, both +dp/dt and -dp/dt (mmHg/sec) were shown to be markedly decreased in post-infarcted hearts [2737 (233) and 1508 (95) in AMI *vs* 4250 (127) and 2278 (55) in SHAM, respectively, $p<0.05$].

Thyroid hormone administration almost normalized both +dp/dt and -dp/dt of the left ventricle [3866 (310) and 2137 (111) respectively, $p<0.05$ *vs* AMI], *Fig. 4A*.

Hypothyroidism and myocardial infarction

Scar area and weight, heart rate and left ventricular weight (LVW) are shown in *Table 2*. Scar area and weight were not different between NORM-AMI and HP-AMI hearts, while cardiac mass was not significantly increased in either group. Interestingly, LVW was found to be significantly decreased in HP-AMI as compared to NORM-AMI hearts, $p<0.05$. Heart rate was lower in HP-AMI as compared to NORM hearts but not different from that of NORM-AMI hearts, *Table 2*.

LVIDd and LVIDs were significantly increased both in NORM-AMI and HP-AMI hearts as compared to NORM, $p<0.05$. In addition, LVIDs was greater in HP-AMI than in NORM-AMI hearts, $p<0.05$, *Table 2*. Wall tension index (WTI) was increased in NORM-AMI hearts as compared to NORM. WTI was further increased in HP-AMI hearts. SI was also reduced in both NORM-AMI and HP-AMI hearts as compared to NORM, $p<0.05$, *Table 2*.

EF% and SVPW were significantly reduced in NORM-AMI hearts as compared to NORM while further reduction of both indices was observed in HP-AMI hearts.

DISCUSSION

Recent research has revealed novel actions of thyroid hormone on cardiac cell which may be therapeutically exploited. Thyroid hormone appears to regulate the cellular response to stress and cardiac cell differentiation (*e.g.* regulation of contractile proteins and protein kinases, cytoskeletal orientation and cell geometry) in addition to its positive inotropic effect (10-12, 29). These actions may be of important physiological relevance for preventing and/or reversing the process of de-differentiation which characterizes cardiac remodeling following acute myocardial infarction. In fact, thyroid hormone administered early after myocardial infarction was shown to prevent chamber remodeling without exacerbating ischaemia (16, 17). Here, in this study, we provide further experimental evidence showing that thyroid hormone can reverse remodeling and improve cardiac function in rats with old myocardial infarction.

Acute myocardial infarction was induced in rats by ligating the left coronary artery. This resulted in marked alterations in cardiac chamber geometry: end-systolic and end-diastolic diameters were significantly increased. Hearts became more spherical and sphericity index (the ratio of long to short axis end-diastolic dimensions) was significantly decreased in the post-infarcted hearts. At the molecular level, β-MHC expression in the myocardium was found to be increased. As a consequence, severe global and regional cardiac dysfunction was observed at 13 weeks, as indicated by the marked reduction

in left ventricular ejection fraction and systolic velocity of the posterior wall radial displacement (SVPW). EF%, in post-infarcted hearts declined by nearly 50% after 13 weeks as compared to control. These functional changes were accompanied by alterations in thyroid hormone levels in plasma. T_3 levels were lower in animals with old infarction compared to sham operated, although not at a statistical significant level. However, the ratio of T_4/T_3 was significantly higher in the infarcted animals as compared to sham operated, indicative of an abnormal conversion of T_4 to T_3. Changes in thyroid hormone metabolism and signaling have previously been reported in the course of cardiac remodeling and a potential link to the functional state of the myocardium has been suggested. More importantly, thyroid hormone levels in plasma appeared to be a predictor of increased mortality in patients with heart failure (30-32).

In order to assess the ability of thyroid hormone treatment on reversing cardiac dysfunction late in the course of acute myocardial infarction, thyroid hormone was administered at 13 weeks and continued for an additional period of 4 weeks. In our experimental setting, after conducting a set of pilot studies, we choose a dose which did not result in significant increased T_3 levels in plasma and heart rate in comparison to sham operated animals. Thus, this dose was considered as supra-physiological.

Thyroid hormone partially reverses cardiac dysfunction by inducing favorable changes in cardiac chamber shape and myosin expression

Thyroid hormone treatment resulted in a significant increase of α-MHC and decrease of β-MHC expression in the non-infarcted myocardium. This response was associated with changes in cardiac geometry: a marked decrease in the end-systolic diameter was observed while end-diastolic diameter remained unchanged after thyroid hormone treatment. Furthermore, the almost spherical shape of the infarcted heart changed to a more elliptical shape and sphericity index (long to short axis ratio) was markedly increased in the treated group. These changes can potentially be translated to improved cardiac function of the post infarcted heart. Cardiac geometry is a critical determinant of the left ventricular ejection fraction and reduction of 50% in left ventricular ejection fraction was found to occur by changing the elliptical shape of the ventricle to a spherical shape (33). Accordingly, our study showed that thyroid hormone's favorable effect on cardiac geometry was followed by a marked improvement in myocardial performance: left ventricular ejection fraction was significantly increased between 13 and 17 weeks in the treated group, while a significant decline in the ejection fraction was observed at the same period of time in untreated hearts. Furthermore, thyroid hormone significantly increased both +dp/dt and -dp/dt as was shown in isolated heart preparations.

Low thyroid hormone state exacerbates postischaemic cardiac remodeling

The importance of thyroid hormone in postischaemic cardiac remodeling has been previously pointed out in a clinical study with patients presenting with acute myocardial infarction (34). Interestingly, in those patients, low thyroid hormone levels were associated with reduced cardiac function and increased mortality. Accordingly, in this study, as a proof of concept, we showed that, the induction of hypothyroidism in an equivalent experimental setting, exacerbated cardiac remodeling and significantly reduced the performance of the post infarcted heart.

Taken together, it appears from this and previous studies that thyroid hormone may play an important role in postischaemic cardiac remodeling and thyroid hormone treatment seems to partially reverse cardiac remodeling by inducing changes in cardiac geometry and contractile protein expression. Thyroid hormone, unlike current treatments, appears to be a paradigm of therapeutic intervention which aims at restoring cardiac geometry and may prove new effective treatment for heart failure.

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REFERENCES

1. Swynghedauw B. Molecular mechanisms of myocardial remodeling. *Physiol Rev* 1999; 79: 215-262.
2. Feygin J, Hu Q, Swingen C, Zhang J. Relationships between regional myocardial wall stress and bioenergetics in hearts with left ventricular hypertrophy. *Am J Physiol Heart Circ Physiol* 2008; 294(5): H2313-H2321.
3. Mann DL. Left ventricular size and shape: determinants of mechanical signal transduction pathways. *Heart Fail Rev* 2005; 10: 95-100.
4. Sehgal S, Drazner MH. Left ventricular geometry: does shape matter? *Am Heart J* 2007; 153: 153-155.
5. Douglas PS, Morrow R, Ioli A, Reichek N. Left ventricular shape, afterload and survival in idiopathic dilated cardiomyopathy. *J Am Coll Cardiol* 1989; 13: 311-315.
6. Buckberg GD. Form versus disease: optimizing geometry during ventricular restoration. *Eur J Cardiothorac Surg* 2006; 29(Suppl 1): S238-S244.
7. Carraway EA, Rayburn BK. Device therapy for remodeling in congestive heart failure. *Curr Heart Fail Rep* 2007; 4: 53-58.
8. Klotz S, Jan Danser AH, Burkhoff D. Impact of left ventricular assist device (LVAD) support on the cardiac reverse remodeling process. *Prog Biophys Mol Biol* 2008; 97(2-3): 479-496.
9. Westaby S. Surgery for heart failure: now something for everyone? *Heart Fail Clin* 2007; 3: 139-157.
10. Pantos C, Malliopoulou V, Varonos DD, Cokkinos DV. Thyroid hormone and phenotypes of cardioprotection. *Basic Res Cardiol* 2004; 99: 101-120.
11. Pantos C, Mourouzis I, Xinaris C, Papadopoulou-Daifoti Z, Cokkinos D. Thyroid hormone and "cardiac metamorphosis"; potential therapeutic implications *Pharmacol Ther* 2008; 118: 277-294.
12. Pantos C, Xinaris C, Mourouzis I, *et al*. Thyroid hormone receptor alpha 1: a switch to cardiac cell "metamorphosis"? *J Physiol Pharmacol* 2008; 59: 253-269.
13. Pantos C, Malliopoulou V, Mourouzis I, *et al*. Hyperthyroid hearts display a phenotype of cardioprotection against ischemic stress: a possible involvement of heat shock protein 70. *Horm Metab Res* 2006; 38: 308-313.
14. Pantos C, Malliopoulou V, Paizis I, *et al*. Thyroid hormone and cardioprotection: study of p38 MAPK and JNKs during ischaemia and at reperfusion in isolated rat heart. *Mol Cell Biochem* 2003; 242: 173-180.
15. Pantos C, Xinaris C, Mourouzis I, Malliopoulou V, Kardami E, Cokkinos DV. Thyroid hormone changes cardiomyocyte shape and geometry via ERK signaling pathway: Potential therapeutic implications in reversing cardiac remodeling? *Mol Cell Biochem* 2007; 297: 65-72.
16. Pantos C, Mourouzis I, Markakis K, *et al*. Thyroid hormone attenuates cardiac remodeling and improves hemodynamics early after acute myocardial infarction in rats. *Eur J Cardiothorac Surg* 2007; 32: 333-339.

17. Pantos C, Mourouzis I, Markakis K, Tsagoulis N, Panagiotou M, Cokkinos DV. Long-term thyroid hormone administration re-shapes left ventricular chamber and improves cardiac function after myocardial infarction in rats. *Basic Res Cardiol* 2008 ;103(4): 308-318.
18. Pantos C, Mourouzis I, Dimopoulos A, *et al*. Enhanced tolerance of the rat myocardium to ischemia and reperfusion injury early after acute myocardial infarction. *Basic Res Cardiol* 2007; 102: 327-333.
19. Pantos C, Mourouzis I, Saranteas T, *et al*. Thyroid hormone receptors alpha1 and beta1 are downregulated in the post-infarcted rat heart: consequences on the response to ischaemia-reperfusion. *Basic Res Cardiol* 2005; 100: 422-432.
20. Pantos C, Mourouzis I, Xinaris C, Kokkinos A, *et al*. Time-dependent changes in the expression of thyroid hormone receptor {alpha}1 in the myocardium after acute myocardial infarction: possible implications in cardiac remodelling. *Eur J Endocrinol* 2007; 156: 415-424.
21. Garcia MJ, Rodriguez L, Ares M, *et al*. Myocardial wall velocity assessment by pulsed Doppler tissue imaging: characteristic findings in normal subjects. *Am Heart J* 1996; 132: 648-656.
22. Dorn GW. The fuzzy logic of physiological cardiac hypertrophy. *Hypertension* 2007; 49: 962-970.
23. Grossman W, Jones D, Mclaurin LP. Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Invest* 1975; 56: 56-64.
24. Pantos C, Paizis I, Mourouzis I, *et al*. Blockade of angiotensin II type 1 receptor diminishes cardiac hypertrophy, but does not abolish thyroxin-induced preconditioning. *Horm Metab Res* 2005; 37: 500-504.
25. Pantos C, Mourouzis I, Malliopoulou V, *et al*. Dronedarone administration prevents body weight gain and increases tolerance of the heart to ischemic stress: a possible involvement of thyroid hormone receptor alpha1. *Thyroid* 2005; 15: 16-23.
26. Reiser PJ, Kline WO. Electrophoretic separation and quantitation of cardiac myosin heavy chain isoforms in eight mammalian species. *Am J Physiol* 1998; 274: H1048-H1053.
27. Mourouzis I, Dimopoulos A, Saranteas T, *et al*. Ischemic preconditioning fails to confer additional protection against ischemia-reperfusion injury in the hypothyroid rat heart. *Physiol Res* 2009; 58(1): 29-38.
28. Pantos C, Malliopoulou V, Mourouzis I, *et al*. Propylthiouracil-induced hypothyroidism is associated with increased tolerance of the isolated rat heart to ischaemia-reperfusion. *J Endocrinol* 2003; 178: 427-435.
29. Lin H, Mitasikova M, Dlugosova K, *et al*. Thyroid hormones suppress epsilon-PKC signalling, down-regulate connexin-43 and increase lethal arrhythmia susceptibility in non-diabetic and diabetic rat hearts. *J Physiol Pharmacol* 2008; 59: 271-285.
30. Iervasi G, Pingitore A, Landi P, *et al*. Low-T3 syndrome: a strong prognostic predictor of death in patients with heart disease. *Circulation* 2003; 107: 708-713.
31. Pantos C, Dritsas A, Mourouzis I, *et al*. Thyroid hormone is a critical determinant of myocardial performance in patients with heart failure: potential therapeutic implications. *Eur J Endocrinol* 2007; 157: 515-520.
32. Pingitore A, Landi P, Taddei MC, Ripoli A, L'abbate A, Iervasi G. Triiodothyronine levels for risk stratification of patients with chronic heart failure. *Am J Med* 2005; 118: 132-136.
33. Sallin EA. Fiber orientation and ejection fraction in the human left ventricle. *Biophys J* 1969; 9: 954-964.
34. Friberg L, Werner S, Eggertsen G, Ahnve S. Rapid down-regulation of thyroid hormones in acute myocardial infarction: is it cardioprotective in patients with angina? *Arch Intern Med* 2002; 162: 1388-1394.

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Author's address: Dr Constantinos Pantos, Department of Pharmacology, University of Athens, 75 Mikras Asias Ave., 11527 Goudi, Athens, Greece; Phone: (+30210) 7462560; e-mail: cpantos@cc.uoa.gr