Blockade of Angiotensin II Type 1 Receptor Diminishes Cardiac Hypertrophy, but Does Not Abolish Thyroxin-induced Preconditioning

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Abstract

Growth and stress seem to share common intracellular pathways and activation of growth signaling can increase resistance to stress. Thyroid hormone induces cardiac hypertrophy and preconditions the myocardium against ischemia reperfusion injury. The present study investigated whether this response is mediated by renin-angiotensin system (RAS). RAS is shown to be activated in hyperthyroidism and is involved in the development of cardiac hypertrophy. Male Wistar rats were treated with L-thyroxin (25 μg/100 g, sc, od) for fourteen days, while normal rats served as controls. In addition, irbesartan (150 mg/kg po), a potent blocker of angiotensin II type 1 receptor (AT1), was given with L-thyroxin for fourteen days. Isolated hearts were perfused in Langendorff mode; after stabilization, they were subjected to 20 min zero-flow global ischemia and 45 min of reperfusion. Thyroxin induced cardiac hypertrophy, which was diminished with irbesartan administration. Post-ischemic recovery of function was increased in thyroxin-treated hearts as compared to controls while ischemic contracture was accelerated and intensified. Irbesartan did not abolish this response. In conclusion, blockade of angiotensin II type 1 receptor with irbesartan preserves thyroxin-induced cardioprotection while diminishing cardiac hypertrophy. It is likely that thyroxin-induced cardioprotection is due to a direct effect of thyroid hormone.

Key words
Thyroid hormone · Ischemia reperfusion · Cardiac hypertrophy · Renin-angiotensin system

Introduction

Thyroid hormone was recently shown to precondition the myocardium against ischemia reperfusion [1,2]. In fact, long-term thyroxin administration increases tolerance of the isolated rat hearts to ischemia reperfusion injury [3,4] through mechanisms similar to ischemic preconditioning [2]. However, whether this response is mediated by the accompanied neurohormonal changes or it is a direct effect of thyroid hormone has not been elucidated.

Activation of the renin-angiotensin system (RAS) occurs in hyperthyroidism, but its physiological role remains largely unknown. Hyperthyroidism is accompanied by increased expression and activity of renin and increased levels of angiotensin-converting enzyme (ACE) and angiotensin II in plasma [5–7]. This is thought to be a compensatory mechanism to the decreased systemic vascular resistance that occurs in this condition [8]. However, thyroid hormone has been shown to activate RAS directly in cell culture [9], while cardiac RAS is upregulated in the hyperthyroid myocardium. Kobori et al. demonstrated that cardiac levels of renin, renin mRNA and angiotensin II are increased in thyroxin-treated rats, and these changes were shown to be independent of the sympathetic nervous system or circulating RAS, and to contribute to the development of cardiac hypertrophy [10].

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Angiotensin II is also likely to serve several other functions. Recent studies demonstrate that angiotensin II can precondition the heart, whilst activation of AT1 receptor by angiotensin II, produced locally in the myocardium, contribute to the limitation of infarct size mediated by preconditioning. Furthermore, selective blockade of AT1 receptors abolishes the preconditioning effect in the isolated rabbit heart [11 – 14].

Thus, apart from being involved in cardiac hypertrophy, the increased activation of renin-angiotensin system (RAS) observed, is also likely to contribute to thyroid hormone-induced cardioprotection. Growth and stress share common intracellular signaling, while stimulation of growth signaling increases resistance to stress [15]. This issue, although of therapeutic importance, has not been previously addressed. Therefore, the present study investigated the role of RAS in thyroxin-induced cardioprotection using irbesartan, a potent inhibitor of angiotensin II AT1 receptor.

Materials and Methods

Animals

Thirty-six Wistar male rats weighing 300 – 350 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 Publication No 85 – 23, revised in 1996).

Induction of hyperthyroidism

Hyperthyroidism was induced in rats by thyroxin administration. L-thyroxin (Sigma Chemicals, St Louis MO, USA) was diluted in normal saline as previously described and given subcutaneously once daily (250 μg/kg) for 14 days [16]. Control rats were treated with normal saline subcutaneously once daily for 14 days.

Irbesartan administration

Irbesartan (Sanofi-Synthelabo, Montpellier, France), was administered at the dose of 150 mg/kg/day. The drug was diluted in methylcellulose 0.6% and given orally once daily for 14 days. Control rats were given methylcellulose 0.6% orally once daily for 14 days.

Experimental groups

Three groups were included in this study:

a) Rats treated with normal saline and methylcellulose; NORM, n = 11
b) Rats treated with thyroxin and methylcellulose; THYR, n = 13
c) Rats treated with thyroxin and irbesartan, THYR-IRB, n = 12

Experimental procedure

a) Five rats from each group were used for the assessment of cardiac hypertrophy and measurement of glycogen levels. Rats were anesthetized by intraperitoneal injection of ketamine hydrochloric acid (150 mg/kg), and heparin (1,000 IU/kg) was given intravenously before thoracotomy. The hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer. The left ventricles were weighed, rapidly placed in liquid nitrogen and then stored at – 70 °C, in order to be used for glycogen determination.
b) Rats from each group were used for the assessment of baseline cardiac performance and response to ischemia reperfusion. After anesthesia and heparin administration, the hearts were rapidly excised and mounted on a Langendorff apparatus as previously described [16]. In this non-ejecting isolated heart preparation, the hearts were perfused in a retrograde fashion at constant flow adjusted to the left ventricular weight, so coronary flow per gram of cardiac tissue was similar in all the experimental groups. The hearts were perfused with oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit buffer at a temperature of 37 °C and were paced at 320 beats/min. A water-filled balloon was inserted in the left ventricular cavity and measured the left ventricular pressure under isovolumic conditions. After 20 min of stabilization, the hearts were subjected to 20 min of zero-flow global ischemia and 45 min of reperfusion. The pacemaker was turned off during the period of ischemia.

Assessment of cardiac hypertrophy

Cardiac hypertrophy was assessed by the measurement of left ventricular weight (LVW in mg) and the ratio of LVW to body weight in g (LVW/BW in mg/g).

Measurement of myocardial glycogen

Glycogen content was measured by enzymatic analysis [17]. Left ventricular tissue was homogenized in 40 mM potassium acetate (pH 4.8). Homogenates were centrifuged at 12,000 × *g* for 10 min at 4 °C, and the supernatant was incubated with amyloglycosidase (Sigma-Aldrich) for 2 h at 37 °C. Glucose liberated from glycogen was measured spectrophotometrically at 563 nm using the Amplex red glucose assay kit (Molecular Probes Europe, Leiden, The Netherlands). Tissue glycogen content was calculated by subtraction of the measured glucose from the total glucose after glycogen hydrolysis with amyloglycosidase. Glycogen contents were expressed in μmol of glucose per gram of left ventricular weight.

Assessment of baseline and post-ischemic cardiac function

The baseline cardiac function was assessed by measurement of left ventricular developed pressure (LVDP in mmHg) and the positive and negative first derivative of LVDP (+ dP/dt and – dP/dt in mmHg/sec) at the end of the stabilization period. During ischemia, minimal ventricular pressure increases, a phenomenon known as ischemic contracture. The severity of ischemic contracture was assessed by the time to peak contracture (T_{max} in min) and the magnitude of contracture (C_{max} in mmHg). Post-ischemic function was assessed by the recovery of the left ventricular developed pressure, which was measured at the end of the reperfusion period and was expressed as percentage of the initial value (LVDP %). Left ventricular end-diastolic pressure was measured at 45 min of reperfusion ($LVEDP_{45}$ in mmHg).

Statistics

Values are presented as mean (standard error of the mean, SEM). The unpaired *t*-test and Mann-Whitney U-test were used for differences between groups; p-values less than 0.05 after a two-tailed test were considered significant.

Results

Cardiac hypertrophy

Thyroxin administration resulted in the development of cardiac hypertrophy. Irbesartan administration diminished, but did not abolish, thyroxin-induced cardiac hypertrophy (Table **1**).

Ischemic contracture profile

Ischemic contracture profile for all experimental groups is shown in Fig. **1 a**. Ischemic contracture was found to be accelerated and exacerbated in thyroxin-treated rats, in comparison to normal rats (T_{max} was 13.5 (0.83) min in THYR, while peak contracture was not achieved during 20 min of ischemia in NORM; C_{max} was 88.75 (7.1) mmHg in THYR and 53.17 (8.74) in NORM, $p < 0.05$). The intensification of ischemic contracture was not changed by administration of irbesartan (in THYR-IRB, T_{max} was 12.07 (1.09) min and C_{max} was 98.71 (9.18) mmHg, $p < 0.05$ *vs.* NORM).

Myocardial glycogen levels

Myocardial glycogen levels were found to be decreased in thyroxin-treated as compared to normal rats (10.1 (1.1) for NORM *vs.* 1.7 (0.4) for THYR, $p < 0.05$). Administration of irbesartan in thyroxin-treated hearts had no effect on myocardial glycogen levels (2.6 (1.4) for THYR-IRB, $p < 0.05$ *vs.* NORM); Fig. **1 b**.

Cardiac function and functional response to ischemia reperfusion

Increased contractile performance was found in hyperthyroid hearts and this was not altered with irbesartan administration (Table **1**).

Post-ischemic functional recovery was higher in thyroxin-treated rats as compared to normal rats (40.16 (5.5) for NORM *vs.* 57.64 (4.5) for THYR, $p < 0.05$). Administration of irbesartan did not abolish the increased post-ischemic recovery of function induced by thyroxin administration (58.3 (4.9) for THYR-IRB, $p < 0.05$ *vs.* NORM); Fig. **2 a**.

Left ventricular end-diastolic pressure at 45 min of reperfusion was no different between the experimental groups – $LVEDP_{45}$ was 65.3 (6.5) for NORM, 61.5 (3.9) for THYR and 58.3 (6.6) for THYR-IRB, $p > 0.05$.

Table **1** Cardiac Hypertrophy and Baseline Parameters of Cardiac Function

	NORM	*THYR*	*THYR-IRB*
LVW	624 (22.5)	780.75 (35.7) *	664.2 (14.2) †
LVW/BW	1.74 (0.04)	2.36 (0.07) *	2.04 (0.09) * †
LVDP	122.17 (3.1)	133.38 (4.7)	132 (5.9)
+dP/dt	4473 (434)	5878 (369) *	5928 (250) *
-dP/dt	2475 (148)	2792 (115)	2872 (171)

Left ventricular weight (LVW) inmg, ratio of LVW/body weight (BW) in mg/g, left ventricular developed pressure (LVDP in mmHg), positive and negative first derivative of LVDP (+ dP/dt and – dP/dt in mmHg/sec) at the end of the stabilization period in normal rats (NORM), hyperthyroid rats (THYR) and hyperthyroid rats treated with irbesartan (THYR-IRB). *$p < 0.05$ *vs.* NORM. †$p < 0.05$ *vs.* THYR.

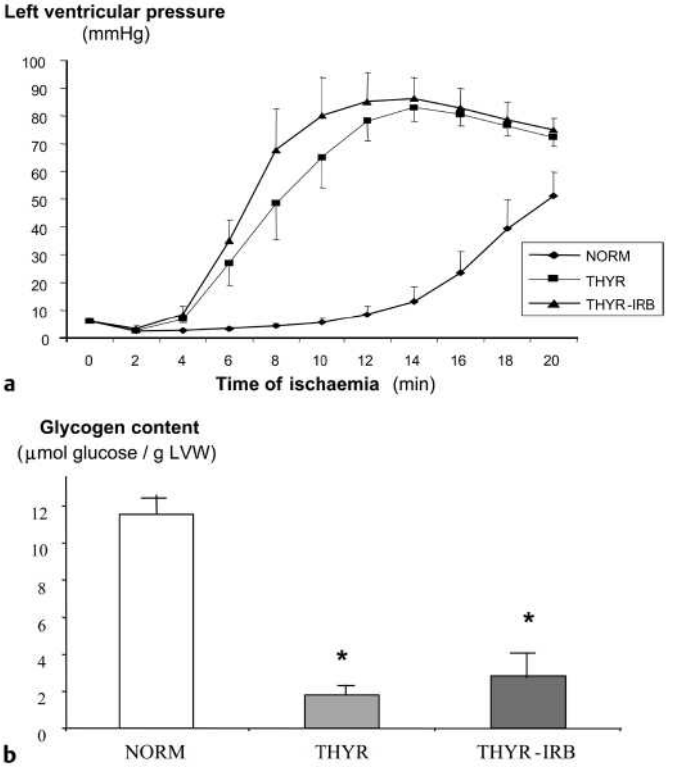

Fig. **1** **a** Left ventricular pressure in normal hearts (NORM), hyperthyroid hearts (THYR) and hyperthyroid hearts treated with irbesartan (THYR-IRB) during zero-flow global ischemia. Ischemic contracture is accelerated and potentiated in THYR and THYR-IRB hearts. (bar = sem.) **b** Myocardial glycogen levels in normal rats (NORM), hyperthyroid rats (THYR) and hyperthyroid rats treated with irbesartan (THYR-IRB). (bar = sem); *$p < 0.05$ *vs.* NORM.

Discussion

Activation of renin-angiotensin system is now recognized to exert several actions on the cardiovascular system [18]. It is involved in the development of cardiac hypertrophy and even in the response of the heart to ischemia [19]. Growth and stress seem to share common pathways [15]. Circulating and tissue RAS is activated in hyperthyroid states, but its physiological role is not fully understood. Recent evidence shows that the renin-angiotensin system, and particularly tissue RAS, is involved in the development of thyroid hormone-related cardiac hypertrophy; blockade of AT1 angiotensin receptor by losartan resulted in reduction of the thyroxin-induced cardiac hypertrophy [5,10,20,21]. This effect was independent from sympathetic activation; hyperthyroidism-induced cardiac hypertrophy was reduced by losartan even in hyperthyroid rats subjected to sympathetic denervation [10]. Furthermore, ACE inhibitors such as captopril with dominant action on the circulating RAS did not prevent the development of cardiac hypertrophy [20]. Accordingly, in the present study, administration of irbesartan, an AT1 angiotensin receptor antagonist, produced similar results; thyroxin-induced cardiac hypertrophy was significantly decreased in irbesartan treated animals.

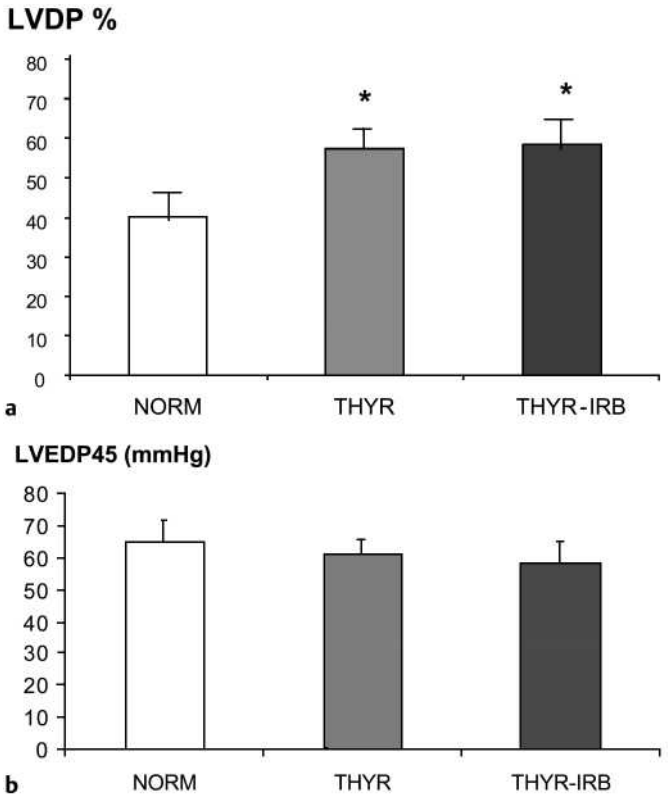

Fig. **2** **a** Post-ischemic recovery of function, expressed as LVDP %, in normal hearts (NORM), hyperthyroid hearts (THYR) and hyperthyroid hearts treated with irbesartan (THYR-IRB). (bar = seM) **b** Left ventricular end-diastolic pressure, expressed as LVEDP45, in normal hearts (NORM), hyperthyroid hearts (THYR) and hyperthyroid hearts treated with irbesartan (THYR-IRB). (bar = seM); $^{*}p < 0.05$ *vs.* NORM.

Several studies have shown that the renin-angiotensin system is also involved in the response of the myocardium to ischemia [19]. Angiotensin II can precondition the heart, while activation of AT1 receptor by angiotensin produced locally in the heart contributes to the limitation of infarct size by preconditioning. Furthermore, selective blockade of AT1 angiotensin receptor abolishes the preconditioning effect. Thyroid hormone-induced cardioprotection has been shown to be very similar to that of preconditioning; both exacerbate ischemic contracture and increase post-ischemic recovery of function in an isolated model of zero-flow global ischemia and reperfusion [2,21,23]. Furthermore, increased activity of the RAS has also been shown to occur in hyperthyroid hearts. It is therefore possible that thyroxin-induced cardioprotection involves the activation of RAS.

In the present study, inhibition of AT1 by irbesartan did not abolish the protection induced by thyroxin. In fact, post-ischemic recovery of function was increased after chronic treatment with thyroxin regardless the administration of irbesartan. Furthermore, the acceleration and intensification of ischemic contracture observed in those hearts was not abolished with the administration of irbesartan. Exacerbation of ischemic contracture is a consistent finding that characterizes the hyperthyroid heart [1]. This ischemia-induced diastolic dysfunction could be related to the cardiac hypertrophy. However, reduction of thyroxin-induced cardiac hypertrophy by β-adrenergic blockade [24] or, in the present study, by AT1 receptor blockade does not abolish this response.

Studies on ischemic preconditioning show that exacerbation of contracture that occurs in preconditioned hearts during ischemia is due to less energy availability as a consequence of the myocardial glycogen depletion following the preconditioning protocol [23]. Similarly, in our study, myocardial glycogen was shown to be lower in thyroxin-treated hearts with subsequent exacerbation of ischemic contracture. This response was not found to be altered with irbesartan administration.

It appears that thyroid hormone preconditions the heart independently from RAS or β-adrenergic system [24]. This might be of important therapeutic relevance [25]. It is now recognized that thyroid analogs might prove to be suitable therapeutic options for treating ischemic heart disease [26].

In conclusion, blockade of angiotensin II type 1 receptor with irbesartan preserves thyroxin-induced cardioprotection while diminishing cardiac hypertrophy. Thyroxin-induced cardioprotection is probably due to the direct effect of thyroid hormone.

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