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Effect of Low Thyroid Function on Cardiac Structure and Function in Spontaneously Hypertensive Heart Failure Rats

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Abstract

Background—While low thyroid function is known to have detrimental effects on the cardiovascular system, including microvascular impairment, little is known about the pathophysiological consequences of hypothyroidism in the background of hypertension.

Methods and Results—Hypothyroidism was induced in female Spontaneously Hypertensive Heart Failure (SHHF) rats by treatment with propylthiouracil (PTU) for six months. Untreated SHHF and normotensive Wistar Furth (WF) rats served as controls. In terminal experiments, heart weight, echocardiographic measurements, hemodynamics, and arteriolar morphometry were performed. LV internal diameter in systole and diastole were increased and wall thickness, ejection fraction, heart rate, systolic blood pressure, and ±dP/dt were significantly decreased in the treatment group. Surprisingly, there were no observed differences in arteriolar density between the three groups.

Conclusion—As expected, PTU treatment of SHHF rats led to systolic dysfunction and chamber dilatation. However, PTU treatment did not lead to arteriolar loss as previously observed in normotensive rats treated with PTU. These finding suggest that induced hypothyroidism leads to detrimental changes in SHHF rats but the overall effects were no worse than those previously observed in normotensive rats treated with PTU.

Keywords

hypothyroidism; microcirculation; hypertension

INTRODUCTION

It is known that low thyroid function has many effects on the cardiovascular system, such as impaired cardiac contractility, decreased cardiac output, increased systemic vascular resistance, reduced chronotropy, and cardiac atrophy[1-3]. Not only has low thyroid function been implicated as an independent risk factor for progression to heart failure[4-7], but a recent animal study suggested that chronic hypothyroidism alone can eventually cause heart failure[8]. Several potential mechanisms by which low thyroid function contribute to HF have been identified: (1) altered blood lipids and accelerated atherosclerosis; (2) stimulation of myocardial fibrosis; (3) vasoconstriction and arteriolar loss; (4) reduced contractility (e.g.

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increased expression of β myosin and reduced expression of α myosin); and (5) impaired relaxation (e.g. reduced SERCA activity)[4, 8-12].

Clinical studies suggest that cardiac patients with hypothyroidism or borderline low thyroid function have worse outcome[6, 13, 14]. We recently demonstrated that hamsters with dilated cardiomyopathy have subclinical hypothyroidism and benefited significantly from thyroid hormone treatment[15]. Another study demonstrated greater infarct size in dogs with PTU induced hypothyroidism[16]. It is not clear if hypertensive individuals who develop hypothyroidism have accelerated progression of heart disease.

In this study, the effects of induced hypothyroidism were examined in lean female SHHF (Spontaneously Hypertensive Heart Failure) rats during the period of compensated hypertrophy (note: lean female SHHF rats typically develop heart failure at about 24 months of age)[17]. Our hypothesis was that hypothyroidism in the background of pre-existing hypertension leads to accelerated progression of heart disease. We anticipated that the consequences of induced low thyroid function in hypertensive rats would have a more pronounced effect than that previously observed in normal rats treated with PTU.

METHODS

Animal Model and Experimental Design

Hypothyroidism was induced in six month old female Spontaneously Hypertensive Heart Failure (SHHF) rats by treatment with PTU (0.025% 6-n-PROPYL-2-THIOURACIL, Sigma-Aldrich, Saint Louis, MO) in drinking water for six months (SHHF-TX). Animals were compared to age and gender matched, untreated SHHF rats and untreated, normotensive Wistar Furth (WF) rats. All procedures were approved by the University of South Dakota Animal Care and Use Committee and followed institutional guidelines for treatment of animals. All animals were maintained in the same environment including temperature and humidity and free access to rodent diet and tap water. Animals were housed in a room lit 12 hours per day. Animals were followed for 6 months by echocardiogram every 2 months. At the end of 6 months, echocardiography, hemodynamics, tissue histology, and arteriolar density were collected from each group. There were two deaths in the SHHF-TX group. These rats were excluded due to insufficient data.

T3 and T4 assays

Serum was collected for determination of T3 (Bioquant, San Diego, CA) and T4 (Diagnostics Systems Laboratories, Webster, TX) by ELISA according to the manufacturers instructions. Since reported hormone values may vary somewhat between manufacturers, lots, or assay type (e.g. ELISA or RIA), all hormone assays were conducted at the same time using supplies from the same kit.

Echocardiograms

After obtaining body weight and shaving the chest, each animal was placed on an isothermic pad. Anesthesia was obtained by 1.5% isoflurane gas. M-mode images were obtained from the short axis of the left ventricle (LV) at the level of the papillary muscles using a VisualSonics Vevo 660 High-resolution Imaging System with 25-MHz transducer. All measurements were performed according to the recommendation of the American Society for Echocardiography[18].

Hemodynamics

Rats were anesthetized with isoflurane gas prior to obtaining LV and aortic hemodynamic measurements by catheterization of the right common carotid artery using a Millar micro-tip

catheter (Millar Instruments: Houston, TX). Measurements were recorded using a digital acquisition system (model HPA 410a; MicroMed; Louisville, KY).

Whole Heart Preparation

Hearts were trimmed, the aortas cannulated, and flushed to remove blood. Right ventricular, atrial, and LV plus septal weights were determined. The middle third of the ventricle was taken and fixed in 10% formalin. The remaining basal and apical portions of the ventricles were flash frozen.

Quantitation of arterioles and histopathology

Frozen, formalin-fixed tissues were sectioned at 4-micrometers, placed on glass slides, and immunolabeled using antibodies for α-smooth muscle actin and laminin (Sigma Inc.) as described previously[8, 19]. Arterioles between 5 and 50 micrometers in diameter and having at least 1 layer of smooth muscle were used for quantitation. The minor diameter was used to define mean arteriolar diameter. Data were collected from 20 randomly selected fields from each animal using 20× magnification. The following data were collected from each field: major and minor arteriolar diameter, arteriolar number, and points on myocytes. Arteriolar data were referenced to myocyte area rather than tissue area to eliminate any errors due to shrinkage or separation artifacts. Arteriolar length density (Lv, average length of arterioles per unit myocyte volume) was calculated based on the following formula developed for the analysis of arterioles arranged in any orientation[20, 21]: Lv (mm/mm3) = Σ(a/b)× M, where a and b are the maximum and minimum external arteriolar diameters, respectively, and M is the area of myocytes in the reference area. Formalin-fixed transverse sections were also stained with hematoxylin and eosin and picrosirus red for assessment of pathology.

Statistical Analyses

All data are presented as means ± SD. One-way ANOVA was used to compare data in each group. The Student-Newman-Keuls test was used to examine statistically significant differences observed with the ANOVA. Results were considered significant when $P<0.05$.

RESULTS

Physical data

There were no significant differences in body weight between the three groups. Compared to WF rats, heart weight was highest in the SHHF group and there was significant reduction in the SHHF-TX group. Heart rate was significantly lower in the SHHF-TX group compared to the other two groups (Table 1).

Serum T3 and T4

Serum T3 and T4 were similar in SHHF (T3, 0.9±0.3ng/mL; T4, 8.5±1.6 μg/dL, respectively) and WF (T3, 0.7±0.2 ng/mL; T4, 8.6±3.1 μg/dL). Compared to SHHF and WF, rats treated with PTU showed significantly reduced ($p \leq 0.05$) levels of T3 and T4 (T3, 0.3±0.1 ng/mL; T4, 3.2±1.0 μg/dL).

Echocardiography

Untreated 12 month old SHHF rats tended to have thicker walls than WF but, as expected, there was no evidence for chamber dilatation at this age. In the SHHF-TX group, there was a significant increase in LV systolic and diastolic chamber diameters while wall thickness was significantly reduced in systole and diastole when compared to both untreated SHHF and WF groups. LV ejection fraction and fractional shortening were also reduced significantly in SHHF-TX compared to the other groups (Table 2).

Hemodynamics

Hemodynamic data are summarized in Table 3. LV systolic pressure decreased significantly in the SHHF-TX group compared to the untreated SHHF group but was not significantly different from the WF group. Systolic and diastolic aortic pressures were also normalized in SHHF-TX. Lv end diastolic pressure was increased in the SHHF and SHHF-TX groups compared to the WF group. Differences for maximum rate of pressure rise and decline (± dP/dT) were statistically significant between the SHHF-TX group and the other two groups.

Quantitation of arterioles and histopathology

Values for arteriolar length density (Lv) and numerical density were not significantly different between the three groups (Table 4). Since there were no observable differences in fibrosis between any of the three groups, this was not quantitated.

DISCUSSION

Our previous work showed that hypothyroidism can eventually produce changes similar to heart failure[8]. In this study, we examined the results of induced hypothyroidism in the background of hypertension in SHHF rats. PTU treatment of SHHF rats normalized LV systolic, aortic systolic, and aortic diastolic pressures, and reduced heart weight. Adverse changes, included reduced contractility, increased systolic wall stress, and death of two treated rats. Nonetheless, we were surprised that low thyroid function did not lead to arteriolar loss as previously observed in normotensive rats treated with PTU and, more recently, confirmed in thyroidectomized rats (Gerdes, unpublished results). Also unexpected was the absence of noticeable changes in myocardial collagen since low thyroid function is known to promote fibrosis. Overall, SHHF rats tolerated PTU treatment better than anticipated.

Echocardiographic measures demonstrated deterioration of cardiac function and remodeling as evidenced by significant increases in systolic and diastolic chamber dimensions, wall thinning in systole and diastole, and a reduction in ejection fraction. Hemodynamic measures showed an increase in LV end diastolic pressure in SHHF rats compared to WF rats. While PTU treatment of SHHF rats led to a reduction in LV end diastolic pressure, wall stress tended to be higher due to the increase in chamber diameter and reduction in wall thickness.

Our previous study showed a dramatic reduction in myocardial arterioles in normotensive rats within six weeks after initiation of PTU treatment[8]. We were surprised to observe that arteriolar density was the same in all three animal groups in the current study. In particular, arteriolar rarefaction was expected in the untreated SHHF rats due to myocyte hypertrophy pushing vessels further apart as reported by others[22]. Additionally, loss of arterioles was expected in the PTU treated SHHF rats. While the absence of arteriolar group differences is not clear, several points are worth noting here. The normotensive WF rats used in these experiments had an unusually low arteriolar density compared to that observed in Sprague Dawley rats from our previous study[8]. This may be a unique trait of this particular genetic strain of rats. Consequently, it is likely that our failure to observe arteriolar rarefaction in untreated SHHF rats here was simply due to the choice of controls for comparison. Regarding the PTU induced loss of arterioles previously observed in Sprague Dawley rats, the mechanism has not yet been determined. We believe that chronic vasoconstriction and low metabolic need from hypothyroidism likely contribute to death of unnecessary vessels. This is supported by the fact that rate-pressure product, a measure of myocardial oxygen consumption, was reduced in proportion to vessel loss[8]. Consequently, in normal rats treated chronically with PTU, vessel loss may merely reflect reduced myocardial energy needs. Low metabolic needs of myocardium result from a combination of factors including reduced cardiac output, conversion to the fetal gene program (e.g. conversion from α- to β- myosin reduces myocardial energy

needs), reduced heart rate, reduced systolic pressure, and of course, the general effects of thyroid hormones on metabolism[23]. It is also possible that reduced VEGF signaling, a known feature of hypothyroidism, led to apoptotic loss of vessels[24]. The mechanism by which arteriolar number is preserved in PTU treated SHHF rats is not clear and can only be speculated upon. Since aortic diastolic pressure was higher in PTU-treated SHHF rats in the current study (mean, 134 mm Hg) than in PTU-treated control rats in the previous study (mean, 80 mm Hg), arteriolar preservation in SHHF rats may be related to better flow from higher myocardial perfusion pressure. It is also possible that other differences between normotensive and hypertensive rats treated with PTU may have contributed. For instance, SHHF rats already express the lower energy form of myosin so a reduction in myosin related energy needs did not occur in this rat strain with PTU treatment. The absence of vessel loss in PTU treated SHHF rats was not due to less pronounced hypothyroidism. In the previous study, Sprague Dawley rats were treated with PTU for 6 weeks and one year using the same dose as the current study. Serum T3 and T4 levels were reduced by ~30−40% at each time point compared to controls. In the current study, serum T3 and T4 levels were reduced by ~60−70% in PTU treated SHHF rats compared to either untreated controls or SHHF rats.

As previously reported in PTU-treated normal rats[3, 8], systolic blood pressure declined in PTU-treated SHHF rats. In normal rats, hypothyroidism leads to a reduction in cardiac output, an increase in arteriolar resistance, bradycardia, reduced aortic systolic blood pressure, and a reduction in aortic diastolic blood pressure[3]. The reduction in systemic blood pressure results from a greater decline in cardiac output (flow) relative to the increase in peripheral resistance (e.g. ↓pressure = ↓↓flow × ↑resistance)[3]. Although coronary blood flow and cardiac output were not measured in the current study, the drop in blood pressure in PTU-treated SHHF rats was likely due to a similar mechanism. It should be noted that humans may respond differently since hypothyroidism leads to increased blood pressure in some individuals[25].

Low thyroid conditions are known to stimulate myocardial fibroblasts and lead to increased collagen expression[26, 27]. We also reported an anti-fibrotic effect of thyroid hormones in treated dilated cardiomyopathic hamsters[15]. In the current experiment, we expected to observe an increase in myocardial collagen due to the combined effects of hypertension and hypothyroidism. However, myocardial collagen appeared normal in all three animal groups. Since PTU treatment normalized blood pressure in SHHF rats, it is possible that this effect minimized collagen accumulation related to hypertension. It should be noted here that increased myocardial fibrosis occurs rather late in lean female SHHF rats and is not apparent at the twelve month time point examined in this study. Increased myocardial fibrosis is readily apparent in obese male SHHF rats by 12 months of age and significantly increased by 18 months in lean male and obese female SHHF rats but remains minimal in lean females even with the onset of heart failure at about 22−24 months of age (Gerdes, unpublished observations).

In summary, the current study showed PTU mediated impairment of LV function and remodeling in SHHF rats, although changes were less severe than expected. The current study did not address the alternate situation of hypertension developing in the background of low thyroid function- a relevant clinical condition that may also lead to a worse outcome.

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Table 1

Physical data

Groups	N	Body Weight, g	Heart Weight, mg	HW/BW, mg/g	Heart Rate, bpm
SHHF-TX	5	263 ± 19	895 ± 85 *	3.4 ± 0.5	228 ± 32 *, **
SHHF	5	266 ± 26	1094 ± 126 **	4.1 ± 0.2 **	356 ± 63
WF	6	257 ± 10	797 ± 36	3.1 ± 0.1	403 ± 30

*, P <0.05 Vs SHHF group

**, P <0.05 Vs WF group; HW/BW, Heart weight/Body weight.

Table 2

Echo data

Groups	N	LVIDd, mm	LVIDs, mm	AWTd, mm	AWTs, mm	PWTd, mm	PWTs, mm	LVEF, %	FS, %
SHHF-TX	5	7.6±0.3, ***	5.5±0.7, ***	1.4±0.2, ***	2.2±0.5, ***	1.4±0.2, ***	2.1±0.6, ***	50±12, ***	27±8, ***
SHHF	5	6.8±0.4	3.8±0.1	2.3±0.3 **	3.4±0.2	2.2±0.2	3.5±0.6	70±5	41±5
WF	6	6.9±0.4	3.6±0.4	1.8±0.2	3.1±0.3	1.9±0.3	2.9±0.2	76±5	46±4

*, P <0.05 Vs SHHF group

**, P <0.05 Vs WF group; LVIDd, LV internal diameter in diastole; LVIDs, LV internal diameter in systole; AWTd, anterior wall thickness in diastole; AWTs, anterior wall thickness in systole; PWTd, posterior wall thickness in diastole; PWTs, posterior wall thickness in systole; LVEF, LV ejection fraction; FS,

Table 3

Hemodynamic data

Groups	N	LVSP, mmHg	LVEDP, mmHg	dP/dt, mmHg/s	-dP/dt, mmHg/s	Wall Stress, kdyn/cm^2	ASP, mmHg	ADP, mmHg
SHHF-TX	5	158±17*	8.1±1.1**	6191±1288, ***	4637±940, ***	181±18**	163±11*	134±17*
SHHF	5	215±48**	7.3±3.3**	12191±3465	8733±2607	147±45	211±29**	156±8**
WF	6	166±16	3.0±0.6	10738±870	9916±1285	110±24	164±14	138±8

*, P <0.05 Vs SHHF group

**, P <0.05 Vs WF group; LVSP, LV pressure end systole; LVEDP, LV pressure end diastole; dP/dt, maximum rate of LV pressure increase; -dP/dt, maximum rate of LV pressure reduction; ASP, aortic systolic pressure; ADP, aortic diastolic pressure.

Table 4

Arteriolar data

Groups	N	Arteriolar Lv	ND/total	ND/5−15μm	ND/15−20μm	ND/20−30μm	ND/30−50μm
SHHF-TX	5	40±4	110±25	82±21	17±6	9±4	2±2
SHHF	5	35±8	107±27	73±22	20±2	12±3	2±3
WF	6	31±4	114±22	68±20	27±12	16±8	3±2

Arteriolar Lv, Arteriolar length density in mm/mm^3; ND, numeric density in number/10 mm2.