SAGE-Hindawi Access to Research
Cardiology Research and Practice
Volume 2011, Article ID 381865, 6 pages
doi:10.4061/2011/381865

Clinical Study

Acute Experimental Hyperthyroidism Does Not Affect Basal and Volume-Induced Atrial Natriuretic Peptide Secretion in Healthy Subjects

Gregory Giamouzis,[1] Dimitrios Pappas,[2] Nikolaos Simeonidis,[3] Evangelos Stathogiannis,[3] Filippos Triposkiadis,[1] and George N. Koukoulis[4]

[1] *Department of Cardiology and the Heart Failure Clinic, University Hospital of Larissa, University of Thessaly School of Medicine, Larissa, Greece*
[2] *Nuclear Medicine Unit, Hellenic Airforce (HAF) and Veterans Administration (VA) General Hospital, Athens, Greece*
[3] *Department of Internal Medicine, Hellenic Airforce (HAF) and Veterans Administration (VA) General Hospital, Athens, Greece*
[4] *Department of Endocrinology and Metabolic Diseases, University Hospital of Larissa, University of Thessaly School of Medicine, 41110 Larissa, Greece*

Correspondence should be addressed to George N. Koukoulis, gnkouk@med.uth.gr

Received 30 September 2010; Accepted 27 October 2010

Academic Editor: Georgios Giannakoulas

Background. Excess circulating thyroid hormones are associated with increased cardiac atrial natriuretic peptide (ANP) secretion but the exact mechanisms involved have not been fully elucidated in vivo. *Methods*. To examine whether thyroid hormone regulation of ANP secretion is the result of a direct action on the myocardium and/or of an indirect action through alterations in the peripheral circulation, plasma ANP levels (baseline and volume expansion-induced) were evaluated in 14 healthy men, before and after triiodothyronine (T_3) administration. *Results*. T_3 administration was followed by a significant increase in serum T_3 levels and a significant decrease in serum TSH levels, without significantly affecting ANP levels. Systemic vascular resistance, plasma rennin activity (PRA), and aldosterone (ALDO) levels, as well as indices of left atrial function, were not significantly altered, despite a significant increase in cardiac output. Plasma volume expansion, induced by a 1500 ml normal saline (NSal) infusion, both before and after T_3 administration, was followed by a significant decrease in PRA and ALDO and a significant increase in plasma ANP levels, without significantly affecting the mean blood pressure (BP) and heart rate (HR) in each study period. The NSal-induced response, measured as the integrated area under the curve corrected for baseline values (-AUC), was not different after T_3 administration for ANP, ALDO, PRA, HR, and mean BP. *Conclusion*. In vivo thyroid hormone-induced myocardial ANP secretion is the result of an indirect action mainly through hemodynamic changes that increase atrial stretch.

1. Introduction

Excess thyroid hormone is associated with changes in cardiovascular system, including increase in heart rate, blood volume, cardiac contractility, and cardiac output [1, 2] and decrease in systemic vascular resistance, whereas thyroid hormone deficiency has been associated with the opposite effects [3]. It has been postulated that these changes are the result of both a direct regulation of cardiac-specific genes by triiodothyronine (T_3) [4, 5] and indirect changes in hemodynamic function [6].

Atrial natriuretic peptide (ANP) is a hormone synthesized by atrial cardiomyocytes [7, 8] and is involved in blood pressure and electrolyte homeostasis [9]. Human studies have shown that plasma ANP secretion is affected by thyroid hormones, being higher in hyperthyroid patients compared to euthyroid controls [10–12] and returning to normal levels after appropriate treatment [13]. There is a strong

Table 1: Clinical and laboratory characteristics of the study population before and after T_3 administration.

Parameter	Before T_3	After T_3	P value
Heart rate, beats/min	72 ± 7	75 ± 6	.061
Mean blood pressure, mmHg	117 ± 13	117 ± 10	.966
T_3, nmol/L	1.80 ± 0.6	4.3 ± 1.0	<.001
T_4, nmol/L	107 ± 18	97 ± 20	.018
TSH, μU/mL	1.04 ± 0.70	0.09 ± 0.04	<.001
ANP, pmol/L	10.4 ± 2.9	11.8 ± 4.9	.256
PRA, nmol/L/h	1.8 ± 1.23	2.2 ± 0.9	.099
ALDO, pmol/L	633 ± 472	665 ± 325	.836

ALDO: plasma aldosterone; ANP: plasma atrial natriuretic peptide; PRA, plasma renin activity.

positive relationship between circulating thyroid hormones and both atrial and ventricular ANP mRNA contents [14, 15], supporting the hypothesis that ANP synthesis and/or secretion from atrial cardiomyocytes remain under thyroid hormone control.

The exact mechanism(s) by which thyroid hormones regulate cardiac ANP secretion have not been fully elucidated. In vitro studies, in rat atrial myocytes, have shown a direct, dose-dependent stimulation of ANP synthesis and secretion by thyroid hormones [16], but in vivo studies are missing. On the other hand, atrial stretch and/or pressure within the atria is considered the primary mechanism that regulates ANP secretion from cardiomyocytes [17, 18]. Therefore, in chronic hyperthyroidism, the increased ANP secretion could be the result of both the direct action of thyroid hormones on the myocardium and/or the indirect action through the alterations in peripheral circulation that may drive the change in cardiac work [19–21]. It is not known which of the above mechanisms predominates in vivo.

The aim of this study was to examine whether in vivo thyroid hormone regulation of ANP secretion is the result of a direct action on the myocardium and/or of an indirect action through the alterations they pose in the peripheral circulation.

2. Materials and Methods

2.1. Design. We examined the cardiac ANP secretion (baseline and volume expansion induced) in healthy subjects before and after per os T_3 administration for 3 days to establish serum T_3 levels compatible with hyperthyroidism. Fourteen healthy male volunteers were recruited in the study (mean age 29 ± 4.3 years). None of them was using any medication known to interfere with thyroid hormones metabolism or cardiovascular system function. There was no dietary restriction. 50 μg T_3 was administered per os twice daily for three days. To exclude serum T_3 level variation (due to its $T_{1/2}$ of ~8 hours), before T_3 initiation and in the morning of the fourth day, the participants reported in the testing room between 8:00 AM and 8:30 AM after an overnight fasting.

A heparinized indwelling intravenous (IV) line was placed in a forearm vein, and the subject remained in the supine position until the end of the study. Blood samples for measurement of ANP, PRA, ALDO, T_4, T_3, TSH, serum creatinine, sodium, and potassium levels were obtained after 30 minutes of being in the supine position before (i.e., baseline) and after a 1500 mL normal saline (NSal) infusion (IV, rate 25 mL/min). New blood samples were thereafter obtained for ANP, PRA, and ALDO measurement at 30, 60, 75, 90, and 120 minutes from the completion of NSal-induced volume expansion (60 minutes from initiation of NSal). All blood samples were immediately centrifuged, and plasma or serum was separated and frozen at −70°C until assayed. For ANP determinations, venous blood was drawn directly into ice-chilled disposable glass tubes containing EDTA (1 mg/mL) and 500 IU/mL aprotinin. Plasma was immediately separated by centrifugation for 15 minutes at 4°C and stored frozen at −70°C until the assay. Plasma ANP levels were measured by RIA as previously described [13] using an antiserum to human ANP (1–28) (Nichols Institute Diagnostics LtD., UK). Assay results were corrected for recovery. PRA was measured using solid-phase RIA from DiaSorin (Saluggia, Italy). For the statistical analysis, all PRA levels below the sensitivity value (0,2 ng/mL/hr) were set at this level. Serum ALDO, T_3, T_4, and TSH concentrations were measured using commercial RIA kits. Serum and urine creatinine and electrolytes were measured by routine laboratory methods.

Additionally, two urine samples were collected for two- and- a- half-hour periods, at baseline and after NSal infusion for urine ALDO, electrolytes, and creatinine level measurement. Baseline and 60- and 120-minute blood pressure (BP) and heart rate (HR) were recorded. Mean arterial blood pressure (MAP) was calculated as follows:

$$\text{MAP} = \text{diastolic BP} + \left(\frac{1}{3}\text{pulse pressure}\right). \quad (1)$$

MAP was used for the determination of the systemic vascular resistance (SVR) according to the formula

$$\text{SVR} = \frac{\text{MAP}}{\text{cardiac output}}. \quad (2)$$

All participants underwent a comprehensive 2D and tissue Doppler imaging transthoracic echocardiography at baseline (i.e., before treatment with T_3) and after 3 days of treatment, performed by a single experienced echocardiographer with the ultrasound apparatus ATL-Ultramark 9 (Bothell, Seattle, USA) and a 2.5 MHz transducer. Complete M mode, two dimensional, and spectral and color Doppler recordings were made with subjects in the left lateral decubitus position using conventional parasternal and apical views, according to the standardization of the American Society of Echocardiography [22]. Three consecutive sinus beats were measured, and the averaged Doppler analysis of the transmitral early (E) and late (A) inflow velocity was performed in the apical 4-chamber view. Systolic function of the left ventricle was also evaluated. The study was conducted in accordance with the 1964 Declaration of Helsinki and was approved by the ethics

Table 2: Echocardiographic characteristics of the study population before and after T_3 administration.

Parameter	Before T_3	After T_3	P value
Left atrial diameter, cm	3.38 ± 0.25	3.31 ± 0.27	.660
LV end-systolic diameter, cm	3.32 ± 0.25	3.09 ± 0.26	.024
LV end-systolic volume, mL	45.5 ± 8.2	38.2 ± 7.5	.034
Shortening fraction, %	33.6 ± 0.9	39.6 ± 2.8	.002
Ejection fraction, %	62.1 ± 1.4	69.8 ± 3.4	.001
Mean V(cf) shortening, circ/sec	1.14 ± 0.07	1.43 ± 0.11	<.001
Stroke volume, mL	73.8 ± 10.7	87.1 ± 12.1	.010
Stroke index, mL/m^2	37.6 ± 6.7	46 ± 5.9	.049
LV ejection time, msec	284.7 ± 20.4	268 ± 14.4	.069
Cardiac output, L/min	4.97 ± 0.62	6.51 ± 0.98	.038
LV PET/ET ratio	0.27 ± 0.06	0.28 ± 0.03	.749
Peak E velocity, m/sec	0.73 ± 0.13	0.59 ± 0.12	.006
Peak A velocity, m/sec	0.44 ± 0.04	0.48 ± 0.05	.440
Acceleration velocity, m/sec^2	7.3 ± 1.6	5.2 ± 1.8	.018
SVR, mmHg·mL^{-1}·min^{-1}	24.5 ± 2.9	18.2 ± 2.7	.046

LV: left ventricular; PET/ET: pre-ejection/ejection time; SVR: systemic vascular resistance; V(cf): velocity of circumference fiber.

Table 3: Area under the curve differences in clinical and laboratory characteristics following normal saline-induced plasma volume expansion before and after T_3 administration in the study population.

Parameter	Δ-AUC		P value
	Before T_3	After T_3	
Heart rate, beats per minute ∗ hour	0.28 ± 19	5, 15 ± 14	.457
Mean BP, mmHg ∗ hour	−4.43 ± 45	2.58 ± 28.8	.668
ANP, pmol/L ∗ hour	18.7 ± 11.1	22.7 ± 12.6	.212
PRA, nmol/L/h ∗ hour	−1.67 ± 1.42	−0.72 ± 1.42	.122
ALDO, pmol/L ∗ hour	−3.08 ± 3.9	−3.08 ± 1.36	1.000

Δ-AUC: area under the curve differences; ALDO: plasma aldosterone; ANP: plasma atrial natriuretic peptide; PRA: plasma renin activity.

committee at the HAF and VA General Hospital of Athens. All participants provided an informed consent.

2.2. Statistical Methods. The hormone responses were calculated either as the peak minus baseline (or Δ response) or as the integrated area under the curve (AUC) values on the baseline (0 minute) corrected response, using the trapezoidal model. One-way ANOVA and paired t-test were used for statistical comparisons in each group. Nonpaired t-test and Mann Whitney, for non-parametric data, were used for comparisons between groups. Results are expressed as the mean ± standard deviation (SD). Differences were considered significant at the level of $P < .05$.

3. Results

T_3 administration resulted in a significant increase in serum T_3, a significant decrease in serum TSH, and a small but significant decrease in serum T_4 levels (Table 1). Mean BP and HR were not significantly affected by T_3 administration. Similarly, the induced hyperthyroid serum T_3 levels resulted in a nonsignificant increase in plasma ANP, ALDO, and PRA (Table 1). T_3 administration was followed by a significant increase in shortening fraction, velocity of circumferential fiber shortening, ejection fraction, stroke volume, stroke index, and cardiac output and a significant decrease in left ventricular (LV) end-systolic diameter, LV end-systolic volume, LV ejection time, peak E velocity, acceleration velocity, and systemic vascular resistance (Table 2). On the contrary, peak A velocity and left atrial diameter were not significantly affected (Table 2).

The NSal-induced plasma volume expansion was followed by a significant decrease in PRA and ALDO levels without significantly affecting MAP and heart rate over each study period (i.e., before and after T_3 administration; Table 3). On the contrary, plasma ANP levels increased significantly compared with baseline values in both study periods (Table 3, Figure 1). However, hormone responses to NSal-induced volume expansion, measured as the integrated area under the curve values corrected on the baseline (Δ AUC), were comparable before and after T_3 administration for ANP, PRA, and ALDO (Table 3, Figure 1).

Plasma volume expansion did not alter urine ALDO excretion but affected the urine electrolyte excretion. Urine sodium and potassium levels increased significantly after NSal-induced volume expansion compared to levels before NSal infusion (Table 4) and to comparable degree in both study periods that is, the NSal-induced significant changes (Δ responses) were comparable before and after T_3 administration.

4. Discussion

Atrial stretch is the primary factor that regulates ANP secretion. This has been shown in isolated cardiac preparations [23, 24] and in intact animals with volume expansion or direct atrial distention [24–26]. However, in vitro studies in rat atrial myocyte cultures have shown that ANP synthesis and secretion are also stimulated directly by thyroid hormones in a dose-dependent manner [16]. There are no data in vivo, indicating direct or indirect thyroid hormone stimulatory effect on myocardial ANP secretion.

Our study is the first to show that a short-term increase in T_3 levels, induced by an exogenous administration of

Table 4: Urinary electrolyte excretion before and after normal saline-induced plasma volume expansion at baseline and after T_3 administration.

Parameter	Before T_3			After T_3		
	Before NS	After NS	Δ Response	Before NS	After NS	Δ Response
Aldo/creatinine, pmol/gr	113 ± 45	115 ± 51	55 ± 192	168 ± 190	124 ± 96	9 ± 89
Na/creatinine, mEq/gr	111 ± 84	451 ± 278†	340 ± 288	113 ± 70	338 ± 231†	224 ± 186
K/creatinine, mEq/gr	29 ± 12	76 ± 21*	47 ± 18	34 ± 17	83 ± 40*	49 ± 34
Ca/creatinine, mg/gr	167 ± 56	285 ± 161	119 ± 190	224 ± 84	248 ± 152	23 ± 179
P/creatinine, mg/gr	611 ± 161	456 ± 222	−155 ± 120	597 ± 223	345 ± 191	−251 ± 276
Mg/creatinine, mg/gr	49 ± 24	109 ± 101	60 ± 112	83 ± 47	72 ± 42	−11 ± 23

*$P < .01$ compared to values before plasma volume expansion.
†$P < .05$ compared to values before plasma volume expansion.
NS: normal saline-induced plasma volume expansion.

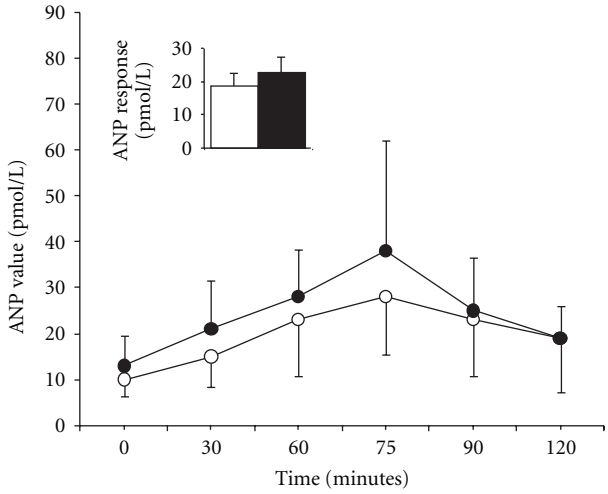

Figure 1: Mean (+SD) ANP values following administration of normal saline. The lines represent subjects before T_3 (open circles) and after T_3 administration (black circles). The mean integrated AUC values together with SEM are shown in the insets, with bars representing subjects before (white) and after T_3 administration (black) (ANP units pmol/L.h). There was no difference in the integrated AUC values when responses before and after T_3 administration were compared.

T_3, despite reaching T_3 values comparable to that seen in hyperthyroidism, did not have any significant impact on plasma ANP levels. We administered T_3 for 3 days, as the 72-hour length has been reported to be an adequate time frame for a T_3-induced ANP-mRNA synthesis and subsequent significant protein secretion. Argentin et al. [27] have demonstrated that treatment of neonatal rat cardiomyocytes (primary cultures) with T_3 results in a significant increase in pronatriodilantin (the precursor for ANP) mRNA, whereas Matsubara et al. [16] have shown a significant increase in secreted ANP levels following treatment with T_3 in primary cultures. The effect in the former study was already apparent at 12 hours and reached its maximal effect at 48 hours [27], whilst in the later became apparent at 48 hours [16]. The stimulatory effect of T_3 was near maximal at a concentration of 5×10^{-9} M [27], which correlates very well with the affinity of T_3 for its nuclear receptor [28].

Our study is in accordance with previous studies showing a significant increase in LV contractility following T_3 administration, resulting in a profound increase in stroke volume, cardiac output, and cardiac index [1, 2]. However, the non-significant influence of short-term hyperthyroidism in left atrial diameter and in peak *A* velocity, which reflect atrial contraction, indicate that the resulting alterations in cardiac preloading conditions were not sufficient enough to significantly affect atrial wall stretch. On the other hand, the significant decrease in peak *E* velocity, which represents an early active relaxation of the left ventricle, in conjunction with the unchanged peak *A* velocity and left atrial diameter, may indicate a more active relaxation of the left ventricle and rapid filling phase [29–31] without significant change in atrial function. Nevertheless, if the action of thyroid hormones were to be direct on cardiac myocytes, one should expect a significant increase in plasma ANP levels following T_3 administration. The above-mentioned alterations in cardiovascular system do not seem enough to increase atrial stretch and hence ANP secretion.

In healthy men, blood volume expansion has previously been shown to increase plasma ANP levels, and this increase closely correlates with the relative intravascular expansion [32]. Intravascular volume expansion following NSal infusion in our subjects is validated by the significant decrease of PRA and ALDO levels. The comparable NSal-induced decrease in PRA and serum ALDO levels before and after T_3 administration and the non-significant change in the MAP and heart rate indicate comparable plasma volume expansions, which have been proved to induce increased cardiac preload [33, 34], velocity of the diastolic rapid filling phase [29, 30], cardiac output and stroke volume [34–36]. In agreement this, a comparable amount of NSal-induced natriuresis was observed in our study participants before and after T_3 administration. Therefore, thyroid hormone action in our population was exerted predominantly through indirect modification of the peripheral cardiovascular system rather than a directed effect.

Our observation that the short-term hyperthyroid T_3 levels in normal men do not affect baseline plasma ANP levels is in contrast to what is seen in chronic hyperthyroid

patients, where ANP levels are significantly increased [10–13]. The possibility that this finding is attributed to different serum T_4 levels should be excluded taking into account that the stimulating effect of T_4 on cellular ANP content and ANP-mRNA levels in rat atria is entirely induced after its conversion to T_3 by type I 5′-deiodinase [37]. Based on our observations, we can speculate that the increased plasma ANP levels in long-lasting hyperthyroidism are mainly the result of hemodynamic changes on the heart and peripheral circulation that move the cardiovascular system to a new functional equilibrium accompanied by increased atrial stretch. This is supported by the fact that hyperthyroid patients with atrial fibrillation have significantly higher plasma ANP levels compared to patients without atrial fibrillation [38]. Additionally, artificial pacing in intact animals [39] as well as in humans [40] increases the secretion of ANP through the elevation of atrial pressure, despite the stable normal thyroid hormone levels. The nondifferent baseline and post NSal-infusion plasma ANP levels in our study may indicate that short-term hyperthyroid serum T_3 levels are not able to induce hemodynamic changes sufficient enough to increase atrial stretch.

In conclusion, our data indicate that thyroid hormone-induced myocardial ANP secretion in healthy subjects is not the result of a direct action on the myocardium, rather, it is mainly the result of an indirect modification in cardiovascular hemodynamics that lead to increased atrial stretch. More studies are needed to replicate these results and confirm this evidence.

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