Comparative study of acute *in vitro* and short-term *in vivo* triiodothyronine treatments on the contractile activity of isolated rat thoracic aortas

Ruth Mery López¹, Jorge Skiold López², Jair Lozano², Héctor Flores³, Rosa Angelica Carranza⁴, Antonio Franco¹, and Enrique Fernando Castillo¹,*

¹Section of Postgraduate Studies and Research, Higher School of Medicine, National Polytechnic Institute, Mexico City 11340, Departments of ²Cellular Biology and ³Imuno-Biochemistry, National Institute of Perinatology, Mexico City 11000, ⁴Research Division, La Raza Medical Center, Mexican Institute of Social Security, Mexico City 02990, Mexico

**ABSTRACT** We aimed to characterize the participation of rapid non-genomic and delayed non-genomic/genomic or genomic mechanisms in vasoactive effects to triiodothyronine (T₃), emphasizing functional analysis of the involvement of these mechanisms in the genesis of nitric oxide (NO) of endothelial or muscular origin. Influences of *in vitro* and *in vivo* T₃ treatments on contractile and relaxant responsiveness of isolated rat aortas were studied. *In vivo* T₃-treatment was 500 μg kg⁻¹ d⁻¹, subcutaneous injection, for 1 (T₃₁d) and 3 (T₃₃d) days. In experiments with endothelium-intact aortic rings contracted with phenylephrine, increasing concentrations of T₃ did not alter contractility. Likewise, *in vitro* T₃ did not modify relaxant responses induced by acetylcholine or sodium nitroprusside (SNP) nor contractile responses elicited by phenylephrine or angiotensin II in endothelium-intact aortas. Concentration-response curves (CRCs) to acetylcholine and SNP in endothelium-intact aortic rings from T₃₁d and T₃₃d rats were unmodified. T₃₃d, but not T₃₁d, treatment diminished CRCs to phenylephrine in endothelium-intact aortic rings. CRCs to phenylephrine remained significantly depressed in both endothelium-denuded and endothelium-intact, nitric oxide synthase inhibitor-treated, aortas of T₃₃d rats. CRCs to angiotensin II and high K⁺ contractures, were decreased. Thus, *in vitro* T₃ neither modified phenylephrine-induced active tone nor CRCs to relaxant and contractile agonists in endothelium-intact aortas, discarding rapid non-genomic actions of this hormone in smooth muscle and endothelial cells. Otherwise, T₃₃d-treatment inhibited aortic smooth muscle capacity to contract, but not to relax, in an endothelium- and NO-independent manner. This effect may be mediated by delayed non-genomic/genomic or genomic mechanisms.

**INTRODUCTION**

Thyroid hormones (THs), 3,5,3',5' tetraiodo-L-thyronine (T₄) and 3,5,3' triiodo-L-thyronine (T₃), have important physiological and pathological effects on the cardiovascular system [1-4]. Excessive functional activity of the thyroid gland—hyperthyroidism—is characterized by an overstimulated cardiovascular state with increased heart rate, stroke volume, systolic blood pressure, pulse pressure, and decreased systemic vascular resistance (SVR) [1-4]. Conversely, a significant reduction in THs synthesis and release—hypothyroidism—results in decreased HR and stroke volume, increased SVR, diastolic hypertension, and lessened pulse...
pressure [1-4].

THs exert their effects either per genomic mechanisms by binding to thyroid receptors (TR) inside the nucleus and regulating gene transcription, or through non-genomic mechanisms initiated outside cell nucleus at plasma membrane receptors and receptors located in the cytoplasm [5,6]. Several links have been recognized between non-genomic and genomic actions of THs (non-genomic/genomic mechanisms) [5,6].

Important reports indicate that THs can act directly on blood vessels causing smooth muscle depressor effects in an endothelium-dependent and -independent manner [7,8]. In isolated skeletal muscle resistance arteries of rats, T4 and T3 induced rapid onset (~20 min) vasodilator responses that were attenuated, but not abolished, with denudation or functional inhibition of the endothelium; implying that vasodilator responses to THs result of their combined effects on endothelium and smooth muscle [9]. In endothelium-denuded rat aortic rings, acute in vitro administration of T3 (30 min) reduced phenylephrine (PHE)-induced vasoconstriction, and this effect was reversed by nitric oxide (NO) synthesis inhibitor, L-NAME; which denotes that T3 inhibits aortic contractile responses inducing smooth muscle synthesis of NO [10]. Together, these data [9,10] exemplified the rapid non-genomic action of THs in isolated arteries. On the other hand, experimental hyperthyroidism induced by short-term T4 (3 days) and long-term T3 (6–12 weeks) treatments [11,12], provoked an increased relaxing effect of acetylcholine and a decreased contractile responses to norepinephrine in endothelium-intact rat aortic tissues; relaxant and contractile responses of isolated rat aortas were found modified by an increased endothelial vasodepressor function, elicited by THs effects pondered as genomic [12,13]. Contrariwise, abdominal aortas from long-term (6 weeks) hypothyroid rats were associated with diminished endothelium-dependent and -independent relaxations [14], indicating inhibition of vasomotor function through adaptations of both endothelial (ECs) and smooth muscle (SMCs) cells [14]. Noticeably, aortas from long-term (8–12 weeks) hypothyroid rats manifested a reduction in NO availability [15].

The following studies with ECs and SMCs cultures support the possibility of observing rapid non-genomic effects in isolated blood vessels. In SMCs isolated from rat aorta and cultured in a deformable matrix, T3 caused relaxation of these cells in 10 min, suggesting its direct action by a rapid non-genomic mechanism [16]. In other study, acute administration of T3 induced the generation of NO in SMCs of rat aorta, resulting in a significant decrease in myosin light chain phosphorylation levels as indirect evidence of contractility inhibition; prominently, T3 augmented the expression of neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) NO synthases, in 30 min, through the activation of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway [10]. Otherwise, T3 activated eNOS (within 10–20 min) through PI3K/Akt in ECs from the bovine aorta and human umbilical vein [17].

SVR is decreased in experimental and clinical hyperthyroidism, and it is increased in THs deficiency [1-4]. However, the direct effects of THs on blood vessels have not been convincingly explained. The present work was designed to help characterize the participation of rapid non-genomic, and delayed genomic or non-genomic/genomic, mechanisms in THs-elicited vasodepressor effects; emphasizing the functional analysis of the involvement of these mechanisms in the genesis of NO of endothelial or muscular origin. We aimed to elucidate whether acute in vitro and short-term in vivo T3 treatments inhibit the development of active tonus in rat thoracic aortas by increasing the synthesis of NO in endothelium and/or smooth muscle.

METHODS

All experimental procedures were approved by the Animal Care and Use Committee of our Institution and complied with the Mexican Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999; Ministry of Agriculture, Mexico City, Mexico). Male Wistar rats (250–300 g body mass, 10–12 weeks old) were housed under controlled conditions (22°C ± 2°C, 60% ± 5% humidity, and 12 h light/dark cycle); normal chow and tap water were given ad libitum.

Short-term in vivo T3 treatment

Based on previous research [11], experimental hyperthyroidism was induced by subcutaneous injection of T3 (500 μg·kg⁻¹·d⁻¹) diluted in alkaline saline solution (0.5 mM NaOH in 0.9% NaCl) for 1 (T3₁d) and 3 (T3₃d) days. Control animals were injected daily with the vehicle at the same volume (1 ml·kg⁻¹) for 1 (V₁d) and 3 (V₃d) days. Experiments were performed 24 h after the last injection.

Assessment of the hyperthyroid state

Blood samples were collected without anticoagulant from abdominal aortas, centrifuged at 1,000 g for 10 min at 4°C, and stored at −20°C until assay. Levels of free T3 (fT3) and thyroid-stimulating hormone (TSH) were determined in the serum of control and T3-treated rats using enzyme-linked immunosorbent assay (ELISA) kits (fT3; Monobind, Inc., Lake Forest, CA, USA; TSH; ALPCO, Salem, NH, USA) according to manufacturer specifications.

Besides, left ventricular dry mass (LVdM) to tibia length (TL) ratio, LVdM/TL (mg·mm⁻¹) was used as an index of cardiac hypertrophy, regularly observed in hyperthyroid disorder [1-3]. Body mass was recorded every day.
Rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal) and exsanguinated. Thoracic aortas were removed, cleaned of fat and connective tissue, and cut into ring segments (4–5 mm in length). In some preparations, the endothelium was damaged by gently abrading the intimae of aortic rings with the tip of small forceps. The isolated arteries were placed in 10 ml tissue chambers filled with Krebs-bicarbonate solution (KBS) of the following composition (mM): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, and dextrose 11.7. High K⁺ (40 and 80 mM) depolarizing solutions were prepared with equimolar replacement of NaCl with KCl. The medium was gassed continuously (95% O₂, 5% CO₂) and maintained at pH 7.4°C and 37°C. Each tissue sample was placed under an initial resting tension of a 2 g mass and equilibrated for 60 min prior to the execution of the experimental protocols. Contractions were measured isometrically and recorded on a computer with the AcqKnowledge software (MP100WSW; Biopac Systems, Inc., Santa Barbara, CA, USA). Functional endothelium was determined by the presence of at least 80% relaxation in response to acetylcholine (1 μM) after pre-constricting the tissues with PHE (1 μM). Otherwise, successful endothelial denudation was confirmed by the presence of small (less than 10%) relaxations, or absence of relaxations, in response to acetylcholine.

**Acute in vitro T3 treatment**

Cumulative concentration-response curves (CRCs) for T3 in aortic tissues pre-contracted with PHE

Aortic rings with endothelium received PHE (1 μM) and contractions were let to stabilize for 15 min. Next, increasing concentrations of T3 (1 nM–10 μM) were added to the tissues, every 30 min; temporal controls received the corresponding concentration of the vehicle.

CRCs to relaxant and contractile agonists in in vitro T3-treated aortic tissues

After the stabilization period, T3 (0.01, 0.1, and 1 μM) or vehicle (vehicle concentration needed to dissolve T3 1 μM) was administered to endothelium-intact aortic rings for 30 or 120 min. Then, aortic rings were stimulated with PHE (1 μM), contractions were stabilized (15 min) and CRCs to acetylcholine (endothelium-dependent relaxing agonist) or sodium nitroprusside (SNP; endothelium-independent relaxing drug, a NO donor) were generated.

In other series of experiments, aortic rings with endothelium were independently treated with T3 or vehicle as explained before; subsequently, CRCs to PHE (selective α₁-adrenergic receptors agonist) or angiotensin II (ANG II; non-selective agonist of ANG II type 1 and type 2 receptors) were developed.

**Short-term in vivo T3 treatment**

CRCs to relaxant agonists in aortic tissues from short-term in vivo T3-treated rats

In T₃₃ and V₃d rats, CRCs to acetylcholine and SNP were obtained in endothelium-intact aortic rings pre-contracted with PHE (1 μM). Since the contractions induced by PHE were significantly diminished in aortic rings of T₃₃ rats (see Results), the CRCs to acetylcholine and SNP were tested at the same level of pre-contraction obtained by appropriately selected concentrations of PHE in endothelium-intact aortic tissues from T₃₃ and V₃d rats.

Contractile stimuli in aortic tissues of short-term in vivo T3-treated rats

In annular segments with endothelium of thoracic aortas from T₃₃, T₃₃, V₃d, and V₃d rats, CRCs to PHE were built. Since the CRCs to PHE were inhibited in endothelium-intact aortic rings of T₃₃ rats compared to their respective temporal controls (see Results), to inquire about the endothelium participation in this T₃₃-elicited vasodepressor effect, PHE-induced CRCs were developed in endothelium-denuded aortic rings of T₃₃ and V₃d rats. Furthermore, CRCs to PHE were constructed in aortic rings with endothelium of T₃₃ and V₃d rats, 30 min after their incubation with the inhibitor of NOS, N^ο-nitro-L-arginine methyl ester (L-NAME, 100 μM). These studies indicated that the observed effect of T₃₃-treatment on PHE-induced contractions in aortic rings was endothelium-independent (see Results and Discussion); thus, the following experiments with ANG II and high K⁺ were developed in endothelium-denuded aortic tissues.

In endothelium-denuded aortic rings from T₃₃ and V₃d rats, CRCs to ANG II were constructed. Besides, high K⁺ depolarizing solutions were used to circumvent G protein-coupled receptors (GPCR). Endothelium-denuded aortic rings of T₃₃ and V₃d rats were constricted with high K⁺ (40 and 80 mM) solutions, and contractions were stabilized for 30 min.

For all purposes, each aortic ring was subjected to only one of the described procedures.

**Chemicals**

3,5,3’ triiodo-L-tironine, L-phenylephrine hydrochloride, acetylcholine chloride, sodium nitroprusside, angiotensin II, L-NAME (Sigma Chemical Company, St. Louis, MO, USA). T₃ was dissolved as already explained. The other drugs were dissolved in distilled water, and subsequent dilutions were made using assay buffer.

**Statistical analysis**

Results are reported as means ± standard error of the means for the number of rats or samples (number) obtained from 4–8 different rats. Comparisons between two independent groups
were made using an unpaired Student t-test, and between multiple groups using one-way analysis of variance (ANOVA). Two-way ANOVA was used to compare two or more CRCs. Where one-way or two-way ANOVA showed significant differences, the results were analyzed further using the Tukey or Sidak post-hoc test, respectively (Prism version 6.0; Graph Pad Software, San Diego, CA, USA). In all comparisons, a value of p < 0.05 was considered statistically significant.

RESULTS

PHE-induced pre-contractions in aortic tissues are not altered by T3 in vitro application

In endothelium-intact aortic rings contracted with PHE (1 μM), increasing cumulative concentrations of T3 (1 nM–10 μM) did not cause modifications of contractile tone compared to temporal controls (Fig. 1).

Relaxant and contractile responses to selective agonists in aortic tissues stayed unchanged by in vitro T3-treatments

Acetylcholine and SNP in a concentration-dependent manner produced relaxations of endothelium-intact aortic rings contracted with PHE. In vitro treatment with T3 (0.01, 0.1, and 1 μM) for 30 min or 2 h did not alter the CRCs to either acetylcholine or SNP compared to their respective controls receiving vehicle (Fig. 2).

Compared to temporal controls, CRCs elicited by either PHE...
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or ANG II in aortic rings with endothelium were not modified in the presence of T3 (0.01, 0.1, and 1 μM) administered beforehand during 30 min or 2 h (Fig. 3).

**Short-term in vivo T3-treatment efficiently induced hyperthyroidism in rats**

Serum fT3 was increased and TSH decreased, both significantly, in T3d and T3ad compared respectively with V1d and V3d rats (Table 1). These data indicate the hyperthyroid state of T3-treated rats. Serum concentrations of fT3 augmented in T3ad concerning T3d rats (Table 1). Thus, a rise in intensity of the hyperthyroid state on T3d rats is appreciated. However, TSH concentrations did not vary in a significant way between T3d and T3ad rats (Table 1). The body mass of T3d rats was significantly lesser than that of V3d rats (Table 1). T3d rats did not alter their body mass compared to V3d rats. The stimulatory effects of THs on metabolic activity can explain the loss of body mass [18]. Contrasted with their respective time-based controls, T3ad—but not T3d—treatment led to cardiac hypertrophy determined by an increase in heart dry mass to tibia length ratio (Table 1). Overall, these findings confirm the hyperthyroid status of animals submitted to treatment with T3 [1,3,18].

**Relaxant responses to selective agonists remained unaltered in aortic tissues of short-term in vivo T3-treated rats**

Acetylcholine caused concentration-dependent relaxant responses in endothelium-intact aortic rings from T3d and V1d rats, or T3ad and V3d rats, which did not differ between them, respectively (Fig. 4). Likewise, the concentration-dependent relaxations induced by SNP in aortic rings with endothelium of T3d and V1d rats, or T3ad and V3d rats, were in that order analogous (Fig. 4). When compared, the CRCs to acetylcholine and SNP were initiated under isotonic pre-contractions (Fig. 4).

Table 1. Validation of short-term hyperthyroidism

| Treatment | fT3 (pg/ml) | TSH (ng/ml) | BM (g) | LVdM/TL (mg/mm) |
|-----------|-------------|-------------|--------|-----------------|
| V1d       | 2.54 ± 0.18 | 1.79 ± 0.18 | 286.82 ± 3.52 | 4.16 ± 0.24 |
| T3d       | 6.38 ± 0.49 | 0.28 ± 0.07 | 282.74 ± 3.57 | 4.69 ± 0.33 |
| V3d       | 2.11 ± 0.27 | 1.83 ± 0.11 | 292.45 ± 3.75 | 4.33 ± 0.30 |
| T3ad      | 9.91 ± 0.72 | 0.18 ± 0.02 | 275.56 ± 3.60 | 5.66 ± 0.25 |

Values are presented as means ± standard error of the means. Serum levels of free 3,5,3’ triiodo-L-thyronine (fT3) and thyroid stimulating hormone (TSH). Body mass (BM) values. Left ventricular dry mass to tibia length (LVdM/TL) ratio as an indicator of cardiac hypertrophy. Short-term hyperthyroidism in rats was induced by daily subcutaneous injection of T3 (500 μg·kg–1) for 1 (T3d) and 3 (T3ad) days. Control animals were injected every day with the vehicle (1 ml·kg–1·d–1) for 1 (V1d) and 3 (V3d) days. *p < 0.05 vs. V1d, †p < 0.05 vs. V3d, ‡p < 0.05 vs. V1d, ‡p < 0.05 vs. V3d; n = 6 rats per group (one-way ANOVA with Tukey post-hoc test). *p < 0.05 vs. V1d; n = 8 hearts per group (one-way ANOVA with Tukey post-hoc test).
Fig. 4. Short-term in vivo 3,5,3′ triiodo-L-thyronine (T3) treatment did not affect relaxant responses of agonists in aortic tissues. (A) Cumulative concentration-relaxing response curves to acetylcholine and (B) sodium nitroprusside (SNP) in endothelium-intact (Endo +) thoracic aortic rings isolated from rats treated subcutaneously with T3 (500 μg·kg⁻¹·d⁻¹) or vehicle (V; 1 ml·kg⁻¹·d⁻¹) for 1 and 3 days (T3, Vd and V3d, V3d, respectively). Relaxing responses were calculated as decreases of phenylephrine-induced tension. Values are presented as means ± standard error of the means (n = 14–16). p = not significant (two-way ANOVA).

Fig. 5. Delayed depression of Phenylephrine-induced contractions in aortic tissues from short-term thyroid hormone-treated rats. Cumulative concentration-contractile response curves to phenylephrine, in endothelium-intact (Endo +) thoracic aortic rings isolated from rats treated subcutaneously with 3,5,3′ triiodo-L-thyronine (T3: 500 μg·kg⁻¹·d⁻¹) or vehicle (V: 1 ml·kg⁻¹·d⁻¹) for 1 and 3 days (T3, T3 and V3, V3d, respectively). Contractile responses are expressed as grams (g) of developed force. Values are presented as means ± SEM (n = 16). *p < 0.05 T3 vs. V3 (two-way ANOVA with Sidak’s post-hoc test).

Fig. 6. Endothelium-independent inhibition of contractile responses to Phenylephrine in aortic tissues from 3,5,3′ triiodo-L-thyronine (T3) in vivo treated rats. (A) Cumulative concentration-response curves (CRCs) to phenylephrine in annular segments of aortas with (Endo +) and without (Endo –) endothelium. (B) CRCs to phenylephrine in endothelium-intact (Endo +) thoracic aortic rings in the presence or absence of N⁵-Nitro-L-arginine methyl ester (L-NAME, 100 μM). Aortic tissues were obtained from rats treated with subcutaneous injections of T3 (500 μg·kg⁻¹·d⁻¹) or vehicle (1 ml·kg⁻¹·d⁻¹) for 3 days (T3 and V3 respectively). All data are expressed as grams (g) of developed force. Values are presented as means ± standard error of the means. (A) (n = 16) *p < 0.05 T3 vs. V3 (two-way ANOVA with Sidak’s post-hoc test). (B) (n = 14–16) *p < 0.05 T3 vs. V3.*p < 0.05 T3 vs. V3 L-NAME; **p < 0.05 T3 vs. V3 L-NAME. Two-way ANOVA with Sidak’s post-hoc test.

Depression of contractile responses to PHE, ANG II, and high K⁺ in aortic tissues from short-term in vivo T3-treated rats

When associated, endothelium-intact aortic rings of T3 and V3 rats showed not statistically different CRCs to PHE (Fig. 5); nevertheless, the CRCs to PHE of T3 rats displayed a tendency toward a reduced maximal response (20%). PHE-induced CRCs in aortic segments with endothelium of T3 rats were significantly depressed (35%) in comparison to their respective V3 controls (Figs. 5 and 6A). Furthermore, in vivo T3-treatment for 3 days,
decreased the CRCs to PHE (34%) in endothelium-denuded aortic rings compared to their corresponding V3d controls (Fig. 6A). Equally, CRCs to PHE were observed statistically depressed (36%) in endothelium-intact, L-NAME-treated, aortas of T33d rats (Fig. 6B).

Once the CRCs for PHE in aortic rings with and without endothelium from either T33d or V3d rats were respectively compared (Fig. 6A), the contractile activity in aortic rings without endothelium was detected higher than in tissues with endothelium in the two groups of rats. However, the contractile responses to PHE in endothelium-denuded aortas continued largely depressed (34%) in T33d compared to V3d rats (Fig. 6A). In the same way, treatment with L-NAME significantly increased the contractions caused by PHE in endothelium-intact aortic tissues of T33d and V3d rats (Fig. 6B); nevertheless, in the L-NAME presence, the CRCs to PHE in aortic rings from T33d compared to V3d rats, remained clearly inhibited (36%) (Fig. 6B). In this way, the data indicate that the endothelium and, particularly, NO seem not to participate in the depression of contractile responses to PHE in aortic rings of T33d rats (see Discussion).

To investigate the selectivity of the depression of contractile responses to PHE in aortic rings from T33d rats, experiments were carried out with ANG II (acting on a different family of receptors) and high K+ depolarizing solutions (as a receptor-independent stimulus). In endothelium-denuded aortic rings, the CRCs to ANG II were significantly diminished (40%) after T33d-treatment concerning time-matched control tissues (Fig. 7A). Additionally, high K+ (40 and 80 mM) contractile responses in endothelium-denuded aortic preparations of T33d rats, were inhibited (35% and 32%, respectively) when compared with the corresponding responses in V3d tissues (Fig. 7B).

**DISCUSSION**

Taken together, the data indicate that T3, administered *in vitro*, neither modified the active tone previously established by PHE nor the effects of both relaxant and contractile agonists in rat thoracic aortas with endothelium. However, short-term *in vivo* T3 administration significantly reduced contractile tone development in isolated thoracic aortas, with a latency greater than one day. T33d-treatment equally decreased the CRCs to PHE in endothelium-intact, endothelium-denuded, and endothelium-intact NOS inhibitor-treated, aortic rings. Moreover, T33d treatment depressed the contractile responses of ANG II and high K+ independently of the endothelial function and, in contrast, the agonist-induced endothelium-dependent and -independent relaxations were not modified in rat aortas. Hence, short-term thyrotoxicosis induced by T3 in rats lessened the ability of the aortic smooth muscle to contract, but not to relax, in an endothelium- and NO-independent way. The temporal course of occurrence of this depressant effect suggests that it is mediated by genomic or through non-genomic/genomic mechanisms. Otherwise, T3 does not modify aortic contractility by a rapid non-genomic mechanism, as shown by its lack of effect when applied acutely *in vitro*.

Influences of acute *in vitro* and short-term *in vivo* T3 treatments on the responses of isolated rat thoracic aortas to contractile and relaxant stimuli were studied comparatively. A fundamental consideration was that there have been described in small and large blood vessels, rapid (seconds to minutes) non-genomic effects of THs contrasting with their genomic or non-genomic/genomic effects of delayed presentation (from several hours on) [5]. The latest effects are initiated in TR located in the plasma membrane or cytoplasm, eventually, associated with gene transcription [5,6]. Admittedly, some studies have reported a direct vasodepres-
sor action of THs that may be interpreted as rapid non-genomic. Park et al. [9] showed that T3 and T4 cause rapid onset vasodilator responses (20 min) acting on both the endothelium and smooth muscle of isolated skeletal muscle resistance arteries of rats. Besides, Carrillo-Sepúlveda and colleagues [10] reported that in 30 min, T3 inhibited contractile responses to PHE in endothelium-denuded rat thoracic aortas by inducing smooth muscle synthesis of NO. In endothelium-intact thoracic aortas, however, we found that increasing cumulative concentrations of T3 (every 30 minutes up to 2 and a half hours) did not provoke alterations in contractile tone induced by PHE, as would be expected if T3 were inducing NO-dependent relaxation by a rapid non-genomic action in endothelial or smooth muscle cells. Additionally, in vitro T3 treatment with concentrations (0.01, 0.1, and 1 μM for 30 min or two hours) that include those reported as inducing vasodepressor effects by a rapid non-genomic mechanism [9,10] did not modify the relaxant responses produced by acetylcholine (whose vasodepressor effect depends on endothelium-derived NO) and SNP (which breaks down releasing NO) in endothelium-intact aortic rings contracted with PHE. In this way, synthesis and release of endothelial NO induced by agonist or the relaxant action of NO in the smooth muscle do not seem to be affected in aortas of rats treated acutely in vitro with T3. Moreover, contractions triggered by PHE and ANG II in aortic rings with endothelium remained unchanged in the presence of T3 (0.01, 0.1, and 1 μM) administered previously for 30 min and 2 h. In rat aorta, basal or induced NO release plays a key role in the endothelium-dependent vasodepressor effect depends on endothelium-derived NO) and SNP (which breaks down releasing NO) in endothelium-intact aortic rings contracted with PHE. In this way, synthesis and release of endothelial NO induced by agonist or the relaxant action of NO in the smooth muscle do not seem to be affected in aortas of rats treated acutely in vitro with T3. Moreover, contractions triggered by PHE and ANG II in aortic rings with endothelium remained unchanged in the presence of T3 (0.01, 0.1, and 1 μM) administered previously for 30 min and 2 h. In rat aorta, basal or induced NO release plays a key role in the endothelium-dependent depression of responses caused by contractile agonists [19-23]. Hence, the data showed that neither endothelium nor NO, particularly, depressed the contractile responses obtained with either PHE or ANG II in aortic rings treated acutely in vitro with T3, as would be expected if synthesis and release of NO had been increased in aortic walls.

On the other hand, it has been reported that endothelial NO-dependent vasodilation elicited by acetylcholine is increased, and contractile responses induced by norepinephrine are depressed in an endothelium-dependent manner, in aortic tissues of hyperthyroid rats by short-term T4 administration (500 μg·kg−1·d−1, 3 days) [11] and long-term T3 treatment (300 μg·kg−1·2d−1, 6–12 weeks) [12]. Thus, both relaxing and contractile responses of rat aorta have been found modified by an increased vasodepressor function of the endothelium, caused by THs actions considered genomic [12,13]. Herein, it is important to point out that these studies, like most works with isolated vessels from hyperthyroid animals [8], were defined by a unique period of treatment; that is, the temporal evolution of functional changes in vascular responsiveness was not characterized. In our study, in vivo treatment with T3 (500 μg·kg−1·d−1) for 3 days, but not for 1 day, significantly decreased the contractile responses induced by PHE in aortic rings with and without endothelium, correlated with their respective controls from vehicle-treated rats. The absence of endothelium and L-NAME treatment (100 μM) in the presence of endothelium, respectively, enhanced the contractile responses to PHE in aortic rings of T31d and V3d rats. However, the contractile responses to PHE remained mostly depressed in the aortas without endothelium, and with endothelium treated with L-NAME, of T33d rats when compared properly to those obtained from V3d rats. If depression of contractile responses to PHE in aortic tissues of T33d rats were fundamentally endothelium-dependent and mediated by NO, endothelium denudation and inhibition of NO synthesis should result in a left shift (potentiation) of CRCs to PHE significantly greater in these tissues than in controls tissues; depression of contractile responses to PHE ought to be practically reverted to the values obtained in the rings without endothelium of control V3d animals. As mentioned, this was not the case.

An additional important finding was that contractile responses to ANG II and high K+ in endothelium-denuded thoracic aortas under T33d-treatment were also attenuated. Accordingly, CRCs to PHE in endothelium-denuded aortic tissues of T33d rats were not selectively depressed since CRCs to ANG II and contractile responses to high K+ (a GPCR-independent stimulus), were significantly inhibited compared to their suitable controls of V3d rats. It is well known that PHE and ANG II, acting on different GPCRs, mainly use the Gq/11-phospholipase C signaling pathway to stimulate vascular smooth muscle contraction via the release of sarcoplasmic reticulum stored Ca2+, extracellular Ca2+ influx (through voltage-dependent, receptor-operated, and store-operated channels), and concomitant Ca2+ sensitization [24-28]. On the other hand, extracellular Ca2+ entry through voltage-dependent Ca2+ channels is considered the main cause of contractions induced by elevated K+ in vascular smooth muscle [29]; nonetheless, high K+ also causes Ca2+ sensitization [30]. So, given the complexity of the signal transduction pathways involved in contractile activity caused by PHE, ANG II, and high K+, it is necessary to develop additional research to solve what actions common (or not) to these stimuli allow explaining the seemingly non-selective depression of contractile activity.

Deepening on the analysis of effects of short-term in vivo T3 treatments in endothelium and smooth muscle of rat aortas, we studied comparatively relaxant effects of acetylcholine and SNP on PHE pre-contracted aortic rings. Because PHE-induced contractions were significantly suppressed in aortic tissues of T33d rats compared with their time-based controls, the relaxations of Ach and SNP were tested under isometric conditions (obtained by fittingly selected concentrations of PHE). Results indicate that T33d and T31d treatments did not modify CRCs to acetylcholine and SNP in endothelium-intact thoracic aortas. Thus, data put forward that acetylcholine-induced endothelial NO release and smooth muscle sensitivity to NO released by SNP, were not modified in aortas of T33d and T31d rats compared to their corresponding temporal controls.

Absence of agreement of our data with other publications requires further investigation; nonetheless, distinct researchers, using diverse isolated blood vessels, have found that responses to
either contractile or relaxant agonists can be reduced, unchanged or enhanced in hypothyroid animals, without expounding convincing explanations for these inconsistencies [8].

In summary, acute in vitro T3 administration did not alter smooth muscle contractility in rat thoracic aortas; however, short-term in vivo T3 administration depressed contractile activity independently of the endothelium, and NO either of endothelial or smooth muscle origin, in isolated thoracic aortas. The time course of onset of this depressant effect suggests that it is mediated by slow development genomic or non-genomic/genomic mechanisms and excludes the participation of rapid non-genomic mechanisms. It remains to elucidate the mechanism by which short-term in vivo treatment with thyroid hormone causes depression of contractile response in rat thoracic aorta.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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