THE EFFECTS OF TETRAIODOTHYRONINE AND TRIIODOTHYRONINE ON HAMSTER CHEEK POUCH MICROCIRCULATION

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ABSTRACT

Background The aim of the present study was to assess the effects of topically applied triiodothyronine (T₃) and thyroxine (T₄) on the arterioles of hamster cheek pouch microcirculation in-vivo. Methods and Results Microvessels were visualized using a fluorescent microscopy technique. Topical application of T₃ (3.08, 30.8, 61.5, 307, 615, 6150 nM/l) consistently induced dose-dependent dilation of arterioles within 2.0 ± 0.5 min of administration. The application of T₄ (150, 257, 514, 5140 nM/l) caused different dose-dependent effects: dilation at the three lower doses within 16 ± 2 min and rhythmic diameter changes at the highest dose. Aging of hamsters did not alter the arteriolar responses to T₃ and T₄. T₃-induced dilation was countered by the inhibition of nitric oxide synthase with N⁵-nitro-L-arginine-methyl ester (L-NAME) or N⁷-nitro-L-arginine (L-NNA). Iopanoic acid (IPA), which inhibits types I and II 5’-deiodinase, abolished the dilation elicited by 514 nM T₄ but did not affect T₃-dependent dilation. 6-propyl-2-thiouracil (PTU), which inhibits type I 5’-deiodinase only, did not affect the dilation induced by T₄. IPA and PTU did not impair arteriolar dilation induced by acetylcholine or sodium nitroprusside. Conclusions These results indicate that T₃ induces arteriolar dilation, likely through nitric oxide release. The local conversion of T₄ to T₃ appears to be crucial for the dilation induced by T₄.

Key words: Microcirculation, Vasodilation, Arterioles, Nitric oxide, N⁵-nitro-L-arginine-methyl ester, N⁷-nitro-L-arginine, Iopanoic acid, 6-propyl-2-thiouracil, Thyroid hormones.
INTRODUCTION

The thyroid gland synthesizes and releases the thyroid hormones (THs) mainly as tetraiodothyronine (thyroxine, T4) (15). Most of the biologically active triiodothyronine (T3) derives from the conversion of T4 by 5’ monodeiodination (types I and II 5’ deiodinase) in peripheral tissues (19, 17). Type I 5’ deiodinase has a primary role in maintaining circulating T3 levels, while type II 5’ deiodinase regulates the intracellular concentration of T3 (12, 18). The direct effects of the THs are triggered by their binding to nuclear receptors (3). Recently, T3 and T4 have been shown to stimulate extranuclear sites. A number of non-genomic effects of T3 have also been identified, including ion fluxes at the level of the plasma membrane (11).

Both THs markedly affect the peripheral vascular tone (10). However, the mechanisms by which they influence the peripheral vasculature have not been fully explained. Their vasodilating effects have been related to the local formation of vasodilatory substances due to metabolic activity, oxygen consumption and heat production. Furthermore, it has been shown that both THs directly interact with vascular smooth muscle (VSM) cells of isolated rabbit mesenteric artery, causing dilation; in particular, T4 has a greater inhibitory effect than T3 on VSM contraction (9). In vitro studies have also demonstrated that exposure to T3 of VSM cells isolated from rat aorta caused these cells to relax rapidly. T4 did not compete for the binding sites of T3. This effect was independent of cAMP and nitric oxide (NO) formation (13). Indeed, primary cultures of vascular endothelial cells exposed to T3 show no NO production, indicating that T3 interacts directly with VSM to cause relaxation. However, it has been reported in rat skeletal muscle resistance arteries (diameter: 100 µm approximately) that T3 is more effective than T4 in inducing vasodilation (14). This dilation appears to have both endothelium-dependent and -independent components because T3 dilation was attenuated by N^G-nitro-L-arginine, indomethacin and glibencamide.

By contrast, in rat mesenteric resistance vessels Zwaveling and colleagues have observed that T4 is more potent than T3 in inducing vascular relaxation (23). This effect was impaired by N^G-nitro-L-arginine methylester (L-NAME); moreover, as vascular relaxation was observed at
supraphysiological concentrations (100 times the basal level), the authors concluded that the direct
effects of the THs are not relevant for the in vivo situation. Recently, direct effects of the THs have
been described in rat coronary arteries within a few seconds of administration, suggesting a non-
genomic mechanism of action of the hormone (22). All data indicate that the peripheral vasculature
may represent a physiological target for the thyroid hormones. By contrast, the THs have not yet
been proved to affect in vivo microcirculation.

The present study was designed to assess the effects of T₃ and T₄ on the arterioles of hamster cheek pouch microcirculation, a widely used experimental model (7, 6). We hypothesize that T₃ might induce its predominant vasodilatory effect through NO release. Moreover, T₄ dilatory effect might be related to local conversion of T₄ into T₃. Therefore, we evaluated the arteriolar responses to T₃ and T₄ administration after inhibition of nitric oxide synthase (NOS) by L-NAME or N⁴-nitro-L-arginine (L-NNA). Furthermore, we topically applied the hormones on the cheek pouch after inhibition of types I and II 5’ deiodinase by iopanoic acid (IPA), or inhibition of type I 5’ deiodinase by 6-propyl-2-thiouracil (PTU) (12).

METHODS

Male Syrian hamsters weighing 80-100 g (Charles River, Italy) were randomly assigned to
ten groups. Control hamsters (n=10) received topically the vehicles or dimethylsulfoxide (DMSO)
and were compared with the animals from the other nine groups, which received topically T₃ or T₄ alone or preceded by other substances, as described below. Group A (n=50) received topically T₃ (at the doses of 3.08, 30.8, 61.5, 307, 615, 6150 nM/l delivered for 2 min) or T₄ (150, 257, 514, 5140 nM/l delivered for 2 min). T₃ and T₄ were dissolved in a solution containing methanol/ammonia (99:1); 1 µg/µl of T₃ and T₄ from this stock solution were finally added to Ringer solution to obtain the appropriate concentration with adjusted pH. Group B (n=8) was administered 1 mM topical L-NAME (n=5) or 1 mM topical D-NAME, an isomer of NAME (n=3), 15 min prior to receiving T₃, 615 nM/l delivered for 2 min. Group C (n=5) was administered 1 mM
topical L-NNA 15 min prior to receiving T3, 615 nM/l delivered for 2 min. **Group D** (n=5) was administered 1 mM topical L-NAME 15 min prior to receiving T4, 5140 nM/l delivered for 2 min. **Group E** (n=5) was administered 1 mM topical L-NNA 15 min prior to receiving T4, 5140 nM/l delivered for 2 min. **Group F** (n=5) was given topically 1 mM L-arginine and 1 mM L-NAME 15 min prior to receiving T3, 615 nM/l delivered for 2 min. **Group G** (n=5) was given topically 1 mM L-arginine and 1 mM L-NNA 15 min prior to receiving T3, 615 nM/l delivered for 2 min. **Group H** was administered 0.4 mM topical IPA 15 min prior to receiving T3, 615 nM/l delivered for 2 min (n=5), or T4, 514 nM/l delivered for 2 min (n=5), or T4, 5140 nM/l delivered for 2 min (n=5); 0.3 ml of DMSO were added to the solution of IPA. **Group I** (n=5) received topically 2 mM PTU 20 min prior to T4, 514 nM/l delivered for 2 min. **Group J** (n=30) was given topically 1μM acetylcholine (ACh) (n=5) or 1 μM sodium nitroprusside (NT) (n=5); or 0.4 mM IPA 15 min before ACh (n=5) or NT (n=5); or 2 mM PTU 20 min prior to ACh (n=5) or NT (n=5). Each dose of THs was used in only one animal. The drugs were obtained from Sigma Chemical, St. Louis, MO, USA.

A reverse-phase high performance liquid chromatography (HPLC) technique was used for purification and separation of iodothyronines to assess contaminant T4 or T3. Briefly, chromatography was performed by 10 μ C18 silica gel column (3x300 mm); mobile phase was methanol-water (55:45), with a flow rate of 1.2 ml/min at a pressure of 2800 p.s.i. (2). Radiolabeled T4 and T3 were added to measure recovery and to identify the iodothyronine of interest (i.e., T4 and T3) after extraction and HPLC separation.

Fifteen aged male hamsters, weighing 150-160 g (18-20 months old), served as aged (AG) control (n=5) or were given topically T3, 615nM/l delivered for 2 min (n=5), or T4, 514 nM/l delivered for 2 min (n=5), to determine the effects of THs also in aged hamsters.

The hamsters were caged, fed and killed in accordance with the principles for research involving animals and human being and the institutional rules for the care and handling of experimental animals.
The cheek pouches were surgically prepared as reported previously (4). Anesthesia was induced with a pentobarbital sodium injection (Nembutal, 5 mg/100 g body wt, ip) (Abbott, Chicago, IL, USA). The animals were tracheotomized; the right carotid artery and femoral vein were cannulated to measure blood pressure and to administer additional anesthesia and the fluorescent tracer. The cheek pouch was gently everted and fixed to a special stage of the microscope; a thin black blade was inserted through a small incision between the upper and lower layers of the pouch to visualize microcirculation of the superior layer. The membrane was suffused (4 ml/min) with Ringer solution (in mM: 137 NaCl, 4.7 KCl, 1.2 MgSO$_4$, 2.0 CaCl$_2$, 18.0 NaHCO$_3$) at 36 ± 0.5 °C containing 5% CO$_2$ – 95% N$_2$ adjusted to pH 7.35. The animal was warmed with a heating pad and the rectal temperature was measured by a thermocouple wire.

Observations were made with a fluorescent microscope (Leitz Orthoplan) fitted with a long-working objective (x4, NA 0.14; x20, NA 0.25; x32, NA 0.60), a x10 eyepiece and a filter block (Ploemopak, Leitz). Epi-illumination was provided by a 100 W mercury lamp fitted with the appropriate filters for fluorescein isothiocyanate (FITC) bound to dextran (molecular weight 150 K, 50 mg/100 g body wt iv as 5% wt/vol solution in 5 min) (Sigma Chemical) and a heat filter (Leitz KG1). The area of interest was televised with a Dage MTI 300 digital low-light level camera and observed on a Sony PVM 122 CE monitor. Video images were videotaped and microvascular measurements made off-line using a computer-assisted imaging software system (MIP Image, CNR, Institute of Clinical Physiology, Pisa, Italy).

Vessel diameter was measured with a computer-assisted method on A/D converted video-recorded images. To avoid bias due to single operator measurements, two independent “blinded” operators measured the vessel diameters. Their measurements overlapped in all cases. Mean arterial blood pressure (Viggo-Spectramed P10E2 transducer, Oxnard, CA, USA, connected to a catheter in the carotid artery) and heart rate were monitored with a Gould Windowgraf recorder (model 13-6615-10S, Gould, OH, USA). All data were recorded and stored in a computer.
The arteriolar vessels in each preparation were classified according to a centrifugal ordering scheme from the largest (A1) to the smallest, A4, arterioles. In each animal, two A2, two A3 and two A4 arterioles were studied under baseline conditions and after application of the THs alone or preceded by the drugs. These substances were applied switching from one to another reservoir filled with the drugs for two minutes. The time to maximum dilation and the time to baseline diameter recovery were evaluated starting from the end of drug application. The video-images of the microvascular networks were recorded for 20 min during baseline observation and for up to 45 min after drug administration. To reduce the effects of epi-illumination on microvessels, the recordings were performed for 20 s per minute under baseline conditions and for 30 s per minute when observation lasted more than 10 min after drug administration. At the end of observation, dilation to topical acetylcholine ($1 \times 10^{-6}$ M) and constriction to phenylephrine (PE) ($5 \times 10^{-6}$ M) were randomly assessed to explore vessel reactivity.

All values are means ± SD. An SPSS+ statistical package was used for analysis of statistical significance. Data were tested for normal distribution using the Kolmogorov-Smirnov test. Parametric (paired and unpaired Student’s t test, ANOVA and Scheffé post hoc test) and non-parametric tests (Wilcoxon test, Friedman test, Mann-Whitney U test and Kruskal-Wallis test) were applied where appropriate. Statistical significance was set at $P<0.05$.

RESULTS

The arterioles of cheek pouch microvascular networks, from the largest A2 to the smallest A4, were studied in control and AG control hamsters. The baseline diameters were not significantly different in the two groups (diameter: A2, 23.3 ± 1.6 µm; A3, 13.9 ± 1.2 µm; A4, 7.5 ± 1.0 µm in control group vs. AG group: A2, 23.5 ± 1.5 µm; A3, 13.7 ± 1.4 µm; A4, 7.7 ± 0.8 µm). Pilot studies indicated that the lowest effective doses inducing vasodilation were 3.08 nM (T₃) and 150 nM (T₄), since lower concentrations of the hormones (1.54, 1.84, 2.15, 2.46 nM T₃ and 50, 75, 100, 125 nM T₄) caused no vessel response. We used different doses in the range 3.08 – 6150 nM for T₃ and 150
– 5140 nM for T₄. We report in Fig. 1 and in Table 1 the arteriolar responses to the lowest effective and higher doses which are comprised in the plasmatic ranges of hamster THs (15, 20).

T₃ induced a dose-dependent dilation of all arterioles. The percent changes of A2, A3 and A4 arteriole diameter after T₃ topical administration (semilog plot) are reported in Figure 1. Vessel diameter increased significantly (P<0.01) with all doses. Time to maximum dilation was 2.0 ± 0.5, 1.8 ± 0.6, 1.8 ± 0.5, 4.5 ± 1.0, 5.5 ± 0.7, 2.5 ± 0.8 min for the corresponding dose of T₃: 3.08, 30.8, 61.5, 307, 615, 6150 nM/l. Effect duration was also dose-dependent and lasted 5.0 ± 1.0, 18.0 ± 2.5, 22.0 ± 3.0, 28.0 ± 3.0, 33.0 ± 1.0, 36.0 ± 1.0 min according to the dose, as above reported.

T₄ also caused dose-dependent responses (Table 1). The lowest dose induced arteriolar dilation, 16.0 ± 2.0 min from administration, lasting 4.5 ± 1.0 min; a typical response is reported in Fig. 2. Arteriolar dilation was observed also for the doses 257 and 514 nM within 15.0 ± 1.0 and 14.0 ± 0.8 min of injection, respectively. Maximum of dilation was detected just at the beginning and lasted 16.0 ± 2.0 and 20.0 ± 3.0 min, respectively. The highest dose of T₄ caused rhythmic diameter changes within 5.0 ± 1.0 min of application that lasted up to 20.0 ± 2.0 min from administration.

Inhibition of nitric oxide synthase by L-NAME or L-NNA prevented the vasodilatory action of T₃ (Table 2). L-arginine but not D-arginine was able to reverse the effects of both inhibitors (Table 2), while D-NAME did not influence the dilation induced by T₃ (data not shown). L-NAME did not affect the rhythmic changes in diameter induced by the highest dose of T₄.

IPA, which inhibits both type I and type II 5’ deiodinase, the enzyme that transforms the prohormone T₄ into biologically active T₃, did not interfere with the dilation elicited by T₃ (Table 3). By contrast, it abolished the effect induced by the dilating dose of T₄ (514 nM). IPA did not interfere with the effects of the highest dose of T₄ (5140 nM) that were characterized by rhythmic changes in diameter.
Topical application of PTU, the specific inhibitor of type I 5’ deiodinase, 20 min before 514 nM T₄ did not affect the dilation induced by the hormone (Table 4). The increase in arteriole diameter was observed within 16.0 ± 2.0 min of T₄ administration.

In each animal, at the end of the experimental protocol, the vessels retained vascular tone, because the arterioles dilated to ACh (A2: +13.0 ± 1.5% of baseline; A3: +19.0 ± 2.0%; A4: +25.0 ± 1.9%) and constricted after PE (A2: -12.0 ± 1.2% of baseline; A3: -15.0 ± 1.9%; A4: -19.0 ± 1.8%).

Topical application of IPA, PTU, the vehicles and DMSO did not modify arteriolar diameter. Topically applied IPA or PTU did not interfere with arteriolar dilation induced by ACh or NT (Fig. 3). The arteriolar responses to THs did not appear to be related to the age of animals. In AG hamsters the arteriolar diameter changed significantly after application of 615 nM T₃ (A2 diameter: 25.1 ± 1.2 µm vs. 22.3 ± 1.3 µm; A3: 17.8 ± 1.1 µm vs. 13.1 ± 1.0 µm; A4: 10.2 ± 1.0 µm vs. 7.2 ± 0.9 µm; p< 0.01; n = 5 animals) and 514 nM T₄ (A2 diameter: 25.6 ± 1.1 µm vs. 23.8 ± 1.2 µm; A3: 18.1 ± 1.0 µm vs. 14.0 ± 0.9 µm; A4: 10.6 ± 1.0 µm vs. 7.8 ± 0.8 µm; p< 0.01; n = 5 animals).

Topical application of T₃ and T₄ did not cause significant changes in heart rate or arterial blood pressure. At the dosages of 615 nM T₃ and 514 nM T₄ heart rate (HR) and mean arterial blood pressure (MAP) were 283 ± 9 bpm and 106 ± 7 mmHg, 284 ± 8 bpm and 107 ± 9 mmHg, respectively, compared with 280 ± 9 bpm and 109 ± 7 mmHg under baseline conditions. L-NAME (HR: 278 ± 9 bpm; MAP: 110 ± 6 mmHg), L-NNA (HR: 277 ± 8 bpm; MAP: 111 ± 7 mmHg), IPA (HR: 282 ± 8 bpm; MAP: 107 ± 6 mmHg), PTU (HR: 280 ± 10 bpm; MAP: 109 ± 7 mmHg) and DMSO (HR: 279 ± 9 bpm; MAP: 109 ± 8 mmHg) did not significantly affect the systemic parameters.
DISCUSSION

Administration of the thyroid hormones induced microvascular responses in the in vivo model of hamster cheek pouch microcirculation used in this study. T₃ caused a dose-dependent dilation of the arterioles within few minutes of its application. Such quick response suggests a non-genomic mechanism of action triggered by the hormone. In addition, arteriolar relaxation was abolished by NOS inhibition, indicating a correlation between T₃-induced dilation and NOS activation. Therefore, these results support our suggestion that NO is the main factor in T₃–induced arteriolar dilation.

The present findings are in agreement with previous observations of a non-genomic mechanism of action of T₃ and T₄ (22), even though the vessels investigated in that work were different. The data indicating that NO mediates T₃-induced arteriolar dilation are partially supported by the observations that T₃-induced dilation is attenuated by NOS inhibition in rat skeletal muscle resistance arterioles (14). However, previous results indicate that vascular endothelial cells exposed to T₃ do not release nitrate nor show increased cGMP content (13). These discrepancies with our results may be ascribed to differences in the experimental procedure, namely between the study of primary cultures of rat aortic endothelial or VSM cells and the present in vivo model of microcirculation. It is reasonable to suppose that in vitro conditions do not allow to observe the integrated response of the peripheral microcirculation. It is worth noting that a previous research showed that skin muscle arterioles respond differently to NOS inhibition, pointing to different properties of endothelial and vascular smooth muscle cells in different sized arterioles (1).

Interestingly, the present results demonstrate that the lower doses of T₄ (150 mM) determined dilation of the arterioles after its application. Time to dilation was significantly different for T₄ and T₃. The effect of T₄ was abolished by the inhibition of 5’ deiodinase activity (types I and II) by iopanoic acid, because 514 nM T₄ did not cause dilation after IPA. 5140 nM T₄ after IPA, however, determined rhythmic changes in arteriolar diameter. We did not test lower doses of T₄ after IPA because the highest dilatory dose of T₄ (514 nM) was ineffective. Therefore, these results
support the hypothesis that local conversion of $T_4$ into $T_3$ is crucial for dilation induced by THs. Indeed, PTU, an inhibitor of type I 5’ deiodinase, did not interfere with the dilation induced by $T_4$. We did not try to assess the effects of different doses of $T_4$ after PTU because there was no change in the vessel response to $T_4$ after local application of PTU. $T_4$ may thus cause an increase in arteriolar diameter after type II 5’ deiodinase-mediated local conversion to $T_3$. This finding argues for the presence of type II 5’ deiodinase in vascular smooth muscle cells, as recently shown in cultured coronary artery and aortic smooth muscle cells (12). This enzyme thus appears to play a physiological role in the regulation of the vascular tone and arteriolar dilation induced by $T_3$.

Our data indicate that the highest dose of $T_4$ (5140 nM) did not cause significant arteriolar dilation, but induced rhythmic changes in diameter. Such effect may be related to the inhibition of 5’ deiodinase activity by high $T_4$ concentrations. The inhibition of 5’ deiodinase by supraphysiological doses of $T_4$ has been reported previously in smooth muscle cell preparations (12). Moreover, 5’ deiodinase blockade by IPA does not appear to affect the rhythmic changes in diameter caused by 5140 nM $T_4$. However, further studies are required to clarify this important issue.

The opposite effects of $T_4$, dilation at lower doses and vasomotion at higher doses, are in agreement with previous observations, indicating increase in sarcoplasmic reticulum ATPase activity at lower doses and decrease of this activity at higher concentrations of $T_4$ (20). Therefore, $T_4$ appears to differently modulate responses of target cells, according to the doses.

It has been reported that pentobarbital anesthesia reduces plasma thyroxine level in rats. These effects appear within 60-90 min of pentobarbital administration and have been described only for thyroxine (15). In our model, we did not measure serum T4 and T3 concentrations. However, the experiments lasted up to 60-120 min according to the protocol, but there were no significant changes in diameter of control hamster arterioles up to 60-120 min of observation.

In this study we used hamster upper physiological range doses of $T_3$ and $T_4$ (16, 21). However, it is worth noting that lower doses of THs were used by Kemperer and coworkers to
reduce the systemic vascular resistance and to increase coronary blood flow in clinical pathophysiological conditions (13, 14). In our model, the morphological and functional properties of the cheek pouch membrane, where a thick epithelial layer effectively opposes the diffusion of substances, dictated the utilization of relatively high doses of THs to cause vessel response. The same approach has previously been tried using this model with substances such as bradykinin (5).

Altogether, our findings show marked effects of the THs in the in vivo microvasculature that implicate a modulation of the NO system, which has a role in maintaining the microvascular tone. Our data support previous studies indicating that T₄ affects the NO system, because subcutaneous injection of T₄ for 3 days suppressed the contraction of aortic rings induced by norepinephrine (NE); an inhibitor of NO synthase, N⁶-nitro-L-arginine, enhanced the NE-induced contraction of aortic rings in T₄-treated rats more than in control ones (8).

It is difficult to derive indications on endogenous thyroid hormone function in humans from the present experimental data. However, recent results indicate that in human hypothyroidism there is a reduction in nitric oxide availability (26). Moreover, clinical hypothyroidism and hyperthyroidism are accompanied by changes in systolic-diastolic index, indicating variations in arterial compliance (7). Furthermore, clinical hypothyroidism is characterized by an increase in the time to peak flow during post-occlusion reactive hyperemia, likely due to decrease in dilation capacity of arterioles giving origin to the nailfold capillaries (19).

The effects of THs were not influenced by ageing of animals, at least for the single dose used in this study. Furthermore, we did not observe differences in the response of single animals. However, these preliminary results need to be confirmed by experiments carried out on a large number of animals with different doses of THs.

In conclusion, our results indicate that microcirculation is a physiological target for THs. The effects of T₃ on the in vivo microcirculation appear to be mostly mediated by NO through a non-genomic mechanism of action. The effects of T₄ appear to be mediated by local conversion to
T₃ when the concentration is in the physiological range. Higher doses of T₄ are able to induce vessel responses characterized by vessel diameter changes with no evident dilation.
REFERENCES


Figure Legends

Figure 1: Dose-dependent arteriolar relaxation induced by triiodothyronine (T<sub>3</sub>). The percent changes were evaluated by the ratio of maximum diameters before and after T<sub>3</sub>. All diameter variations were statistically significant, * P< 0.01. Each entry = 10 arterioles studied in 5 animals.

Figure 2: Arteriolar dilation induced by the lowest concentration (150 nM/l) of tetraiodothyronine (T<sub>4</sub>). Left: baseline; Right: 16 min after T<sub>4</sub> application.

Bar: 10µm

Figure 3: Averages of maximum diameters of A2, A3 and A4 arterioles (left) under baseline conditions (B) and after acetylcholine (AC) or iopanoic acid plus AC (IPA+AC) or 6-propyl-2-thiouracil plus AC (PTU+AC); (right) under baseline conditions (B) and after sodium nitroprusside (NT) or iopanoic acid plus NT (IPA+NT) or 6-propyl-2-thiouracil plus NT (PTU+NT) or after application of vehicles.
TABLE 1

Averages of maximum diameters of A2, A3 and A4 arterioles under baseline conditions and after application of tetraiodothyronine (T₄).

<table>
<thead>
<tr>
<th></th>
<th>A2 (diameter µm)</th>
<th>A3 (diameter µm)</th>
<th>A4 (diameter µm)</th>
<th>n</th>
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<tr>
<td>+ 150 nM T₄</td>
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<td>12.9 ± 0.8</td>
<td>7.6 ± 0.7</td>
<td>5</td>
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<tr>
<td></td>
<td>23.8 ± 1.0*</td>
<td>13.9 ± 0.9*</td>
<td>8.8 ± 0.7*</td>
<td>5</td>
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<tr>
<td>+ 257 nM T₄</td>
<td>23.3 ± 1.0</td>
<td>13.1 ± 0.8</td>
<td>7.8 ± 0.7</td>
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</tr>
<tr>
<td></td>
<td>25.0 ± 1.0*</td>
<td>14.5 ± 0.9*</td>
<td>9.5 ± 0.8*</td>
<td>5</td>
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<tr>
<td>+ 514 nM T₄</td>
<td>22.1 ± 0.9</td>
<td>13.8 ± 0.8</td>
<td>7.3 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>27.1 ± 1.0*</td>
<td>17.8 ± 0.9*</td>
<td>10.1 ± 0.7*</td>
<td>5</td>
</tr>
<tr>
<td>+ 5140 nM T₄</td>
<td>21.9 ± 0.9</td>
<td>13.7 ± 0.8</td>
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<td></td>
<td>vasomotion</td>
<td>vasomotion</td>
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<td>5</td>
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<tr>
<td>+ Vehicle</td>
<td>22.5 ± 0.9</td>
<td>13.4 ± 0.9</td>
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<td></td>
<td>22.6 ± 1.0</td>
<td>13.5 ± 0.8</td>
<td>7.4 ± 0.7</td>
<td>5</td>
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</tbody>
</table>

n: number of animals studied
* P < 0.01 vs. baseline
TABLE 2

Averages of maximum diameters of A2, A3 and A4 arterioles under baseline conditions, after N$^G$-nitro-L-arginine-methyl ester (L-NAME) or N$^G$-nitro-L-arginine (L-NNA), and after triiodothyronine (T$_3$) application.

<table>
<thead>
<tr>
<th></th>
<th>A2 (diameter μm)</th>
<th>A3 (diameter μm)</th>
<th>A4 (diameter μm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>22.6 ± 1.0</td>
<td>12.5 ± 0.8</td>
<td>8.0 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td>+ L-NAME</td>
<td>22.2 ± 1.2</td>
<td>12.0 ± 0.8</td>
<td>7.5 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>+ T$_3$ 615 nM</td>
<td>22.3 ± 1.3*</td>
<td>12.1 ± 0.9*</td>
<td>7.6 ± 0.6*</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>21.7 ± 1.1</td>
<td>12.9 ± 1.0</td>
<td>8.0 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td>+ L-NNA</td>
<td>21.0 ± 1.2</td>
<td>12.4 ± 1.1</td>
<td>7.4 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td>+ T$_3$ 615 nM</td>
<td>21.1 ± 1.1*</td>
<td>12.5 ± 1.0*</td>
<td>7.5 ± 0.7*</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>21.8 ± 0.9</td>
<td>12.8 ± 0.7</td>
<td>7.8 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>+ L-NAME</td>
<td>21.4 ± 1.0</td>
<td>12.3 ± 0.8</td>
<td>7.5 ± 0.5</td>
<td>5</td>
</tr>
<tr>
<td>+ T$_4$ 5140 nM</td>
<td>Vasomotion</td>
<td>Vasomotion</td>
<td>Vasomotion</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>22.5 ± 1.0</td>
<td>13.0 ± 1.0</td>
<td>7.8 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td>+ L-arginine + L-NAME +</td>
<td>27.5± 0.9</td>
<td>17.1 ± 0.9</td>
<td>10.5 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>+ T$_3$ 615 nM</td>
<td>22.7± 1.0</td>
<td>12.8 ± 1.0</td>
<td>7.7 ± 0.5</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>22.2 ± 1.0</td>
<td>12.7 ± 0.9</td>
<td>7.9 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>+ Vehicles</td>
<td>22.1 ± 1.1</td>
<td>12.9 ± 1.1</td>
<td>8.1 ± 0.8</td>
<td>5</td>
</tr>
</tbody>
</table>

n: number of animals studied

* P< 0.01 vs. group A, hamsters treated with T$_3$: comparisons were made between L-NAME plus T$_3$ –treated hamsters (group B) and T$_3$–treated animals (group A), between L-NNA plus T$_3$ –treated hamsters (group C) and T$_3$–treated animals (group A).
TABLE 3

Averages of maximum diameters of A2, A3 and A4 arterioles under baseline conditions and after application of iopanoic acid (IPA) and triiodothyronine (T₃), or IPA and tetraiodothyronine (T₄), or 6-propyl-2-thiouracil (PTU) and T₄.

<table>
<thead>
<tr>
<th></th>
<th>A2 (diameter µm)</th>
<th>A3 (diameter µm)</th>
<th>A4 (diameter µm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>23.1 ± 1.2</td>
<td>12.7 ± 1.0</td>
<td>7.7 ± 0.6</td>
<td>5</td>
</tr>
<tr>
<td>+ IPA + T₃615 nM</td>
<td>28.4 ± 1.3*</td>
<td>16.7 ± 1.0*</td>
<td>10.7 ± 0.7*</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>23.0 ± 1.0</td>
<td>12.6 ± 0.8</td>
<td>7.8 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>+ IPA + T₄514 nM</td>
<td>22.5 ± 1.2</td>
<td>12.4 ± 0.8</td>
<td>7.5 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>22.8 ± 1.0</td>
<td>13.1 ± 1.0</td>
<td>7.9 ± 0.5</td>
<td>5</td>
</tr>
<tr>
<td>+ IPA + T₄5140 nM</td>
<td>Vasomotion</td>
<td>Vasomotion</td>
<td>Vasomotion</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>22.9 ± 1.1</td>
<td>12.5 ± 0.9</td>
<td>7.7 ± 0.8</td>
<td>5</td>
</tr>
<tr>
<td>+ Vehicle</td>
<td>23.0 ± 0.9</td>
<td>12.6 ± 1.0</td>
<td>7.6 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>22.6 ± 1.0</td>
<td>12.9 ± 0.9</td>
<td>7.8 ± 0.7</td>
<td>5</td>
</tr>
<tr>
<td>+ PTU + T₄514 nM</td>
<td>28.0 ± 1.0*</td>
<td>17.3 ± 1.0*</td>
<td>10.7 ± 0.8*</td>
<td>5</td>
</tr>
<tr>
<td>Baseline</td>
<td>22.5 ± 0.9</td>
<td>13.0 ± 1.0</td>
<td>7.9 ± 0.8</td>
<td>5</td>
</tr>
<tr>
<td>+ Vehicle</td>
<td>22.6 ±1.1</td>
<td>12.9 ± 0.8</td>
<td>7.8 ± 0.9</td>
<td>5</td>
</tr>
</tbody>
</table>

n: number of animals studied
* P< 0.01 vs. baseline
Figure 1
Figure 3