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Aortic effects of thyroid hormone in male mice

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Abstract

It is well established that thyroid hormones are required for cardiovascular functions; however, the molecular mechanisms remain incompletely understood, especially the individual contributions of genomic and non-genomic signalling pathways. In this study, we dissected how thyroid hormones modulate aortic contractility. To test the immediate effects of thyroid hormones on vasocontractility, we used a wire myograph to record the contractile response of dissected mouse aortas to the adrenergic agonist phenylephrine in the presence of different doses of T3 (3,3',5-triiodothyronine). Interestingly, we observed reduced vasoconstriction under low and high T3 concentrations, indicating an inversed U-shaped curve with maximal constrictive capacity at euthyroid conditions. We then tested for possible genomic actions of thyroid hormones on vasocontractility by treating mice for 4 days with 1 mg/L thyroxine in drinking water. The study revealed that in contrast to the non-genomic actions the aortas of these animals were hyperresponsive to the contractile stimulus, an effect not observed in endogenously hyperthyroid TR β knockout mice. To identify targets of genomic thyroid hormone action, we analysed aortic gene expression by microarray, revealing several altered genes including the well-known thyroid hormone target gene *hairless*. Taken together, the findings demonstrate that thyroid hormones regulate aortic tone through genomic and non-genomic actions, although genomic actions seem to prevail *in vivo*. Moreover, we identified several novel thyroid hormone target genes that could provide a better understanding of the molecular changes occurring in the hyperthyroid aorta.

Key Words

- ▶ thyroid hormone receptor β
- ▶ aorta
- ▶ gene expression
- ▶ hyperthyroidism
- ▶ hypothyroidism
- ▶ vasoconstriction

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Introduction

Thyroid hormone (TH) is an important regulator of cardiovascular functions. This becomes evident in patients with hyperthyroidism presenting with tachycardia and cardiac hypertrophy, while bradycardia is a characteristic of hypothyroid individuals (Klein & Ojamaa 2001). In addition to its direct effects on the heart (Dillmann 2010), TH also affects the vascular system. It was for instance reported that hyper- as well as hypothyroidism are

associated with increased arterial stiffness in patients, with negative effects on vascular ageing and atherosclerosis (Obuobie *et al.* 2002, Delitala *et al.* 2015, Ittermann *et al.* 2016). Likewise, increased aortic stiffness was also observed in animal models of hyperthyroidism (Moulakakis *et al.* 2007) or reduced TH signalling (Warner *et al.* 2013).

The majority of TH's effects are mediated by nuclear TH receptors. Two different genes code for TH receptor

α (TR α) and β (TR β), which have a distinct and partially overlapping expression pattern throughout the body (Yen 2001). These receptors bind to TH response elements in the promotor region of target genes and dependent on hormone availability either recruit corepressors or coactivators to regulate gene expression (Yen 2001). In addition to these genomic effects, several studies have also reported so-called non-genomic effects, which sometimes also require TH receptors (Flamant *et al.* 2017). The precise mechanisms often depend on the cellular system, as some have reported that 3,3',5-triiodothyronine (T3) activates the PI3 kinase pathway through cytosolic TR β (Cao *et al.* 2005), while others showed an action of T3 on a plasma membrane-bound TR α , which in turn activates the ERK pathway (Kalyanaraman *et al.* 2014). There is also some support for the relevance of these non-genomic pathways *in vivo*, indicating an involvement of cytosolic TR β and PI3 kinase signalling in mouse hippocampal synapse maturation (Martin *et al.* 2014) or non-genomic TR α 1 action in the regulation of cardiac functions (Hönes *et al.* 2017). However, the individual contributions of genomic and non-genomic effects for specific well-known effects of TH often remain enigmatic, and cannot be generalized for all cellular systems.

Here, we aim to dissect the genomic and non-genomic effects of TH on the aorta, both on the functional level using myography to record the aortic responses to a contractile stimulus as well as on the molecular level studying cytosolic and genomic signalling pathways.

Materials and methods

Experimental animals and treatment

For all animal studies, C57BL/6 wild-type male mice (Charles River, Germany) at the age of 8–12 weeks were housed at ambient temperature (23°C) on a 12-h light/12-h dark cycle. For the T4 treatment, the two different animal groups had *ad libitum* access to water, which contained either 1 mg/L T4 (Sigma – T2376, dissolved in 0.1 M NaOH) and 0.1% (m/v) bovine serum albumin (BSA) for the treated group, or only 0.1% (m/v) BSA for the control group. After four days of treatment the animals were sacrificed and their abdominal aortas were dissected for RNA isolation or myography. TR β knockout mice are from 129sv mice as described previously (Forrest *et al.* 1996a), which have been backcrossed for at least six generations to C57BL/6 and were bred at the GTH animal facility of the University of Lübeck. All animal procedures were approved by the Ministerium für Energiewende,

Landwirtschaft, Umwelt und ländliche Räume (MELUND Schleswig Holstein, Germany).

Total T3 and total T4 ELISA

Serum concentrations of total T₃ (tT3) and total T₄ (tT4) were determined with commercially available ELISA Kits (total Thyroxine ELISA, EIA-1781, DRG Instruments GmbH, Marburg, Germany, and total T3 ELISA, DNOV053, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany) and experiments were performed in accordance with manufacturer's instructions.

Myography

The contractility of dissected aortas was measured using a wire myograph device (520A-DMT; AD Instruments, Oxford, UK) as described previously (Warner *et al.* 2013, Gachkar *et al.* 2017). Briefly, short pieces of abdominal aortas (2 mm) were dissected carefully in a Petri dish containing Krebs-Ringer buffer (KRB; 123 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgCl₂, 20 mM NaHCO₃, 2.5 mM CaCl₂ and 5.5 mM glucose). After removing the adventitia from the vessel segments, two wires (2 cm long and 40 μ m in diameter) were threaded through each segment. The wires with the vessel segments were mounted into the myograph chamber, which was filled with 10 mL of KRB. During the whole experiment, the chamber was maintained at 37°C and bubbled with carbogen gas (95% O₂/5% CO₂), in order to maintain physiological conditions. Aortas were acclimated for about 1 h. By increasing the diameter and recording the corresponding active contraction, the optimal diameter for active tension was determined. T3 was dissolved in DMSO and diluted in KRB (0.01% final concentration of DMSO in the chamber), and aortas were incubated in the chamber for 5 min with different doses of T3 (either 1, 10 or 100 nM; Sigma – T72877). Subsequently, a dose–response curve with phenylephrine (PE) was performed. Increasing doses of PE (10^{–8} to 10^{–2} M) were added in 3 min intervals into the chamber. The induced forces were recorded using LabChart 8.1 software and normalized to the previously recorded KCl stimulation force at the optimal diameter using LabChart 8.1 software. For the analysis, the induced forces were plotted against the corresponding doses of PE in logarithmic scale, and a non-linear fit using the least square method with three variables (top, bottom, EC₅₀) was used. To account for a possible variability in the response to potassium after different numbers of prestimulations for optimal tension determination,

only vessels that were run in the same recording session were compared using GraphPad Prism 5 software.

Western blot

Western blot analysis was performed as described previously (Gachkar *et al.* 2017). All used antibodies are listed in Table 1.

RNA isolation, cDNA, qPCR and microarray

RNA was isolated from abdominal aortas according to the manufacturer's instructions using the RNeasy fibrous tissue mini kit (QIAGEN – 74704). The isolated RNAs were used for cDNA synthesis according to the manufacturer's instructions using the RevertAid First Strand cDNA kit (Thermo Fisher Scientific – K1622). Quantitative polymerase chain reaction (qPCR) was performed using QuantStudio (Thermo Fisher Scientific) and SYBR Green qPCR Master Mix (Roche – 34226600). Primer sequences are available on request. Standard curves were used to correct for PCR efficiency. For microarray analysis, total RNA was sent to a commercial provider (ATLAS Biolabs GmbH, Berlin, Germany) for further processing steps and final hybridization to an Affymetrix GeneChip Mouse Gene 2.0 ST Micro Array (Thermo Fisher Scientific), which was scanned with a GeneChip Scanner 3000 HR (Thermo Fisher Scientific). Raw data were background corrected and normalized by employing the RMA and median polish algorithms within the Affimetrix Transcriptome Analysis

Console 4.0.1 (Thermo Fisher Scientific) workflow. Gene expression was considered present when $\log_2$ expression >5 , and considered changed between groups with a fold change $>|1.25|$. Differences in gene expression between treated and control animals were calculated by an empirical Bayes corrected ANOVA approach, and sorted by increasing *P*-value with the top candidates selected for qPCR validation. Subsequent gene set enrichment analysis of the differentially expressed genes was conducted using the online tool WebGestalt (Zhang *et al.* 2005) in combination with the Reactome database (Fabregat *et al.* 2016). For correlation studies, the gene expression values were directly correlated with the respective serum T3 or T4 levels using a rank-based Kendall correlation. The microarray data have been deposited with the NCBI GEO system under GSE119065.

Results

To define the non-genomic effects of T3 on aortic contractility, we pretreated isolated mouse aortas for 5 min with 0 nM (hypothyroid condition), 1 nM (euthyroid condition), 10 nM (mild hyperthyroid condition) and 100 nM T3 (strong hyperthyroid condition). We then recorded the response to increasing concentrations of phenylephrine (PE) to quantify the contractile response to α 1-adrenergic activation relative to the depolarization-induced contraction by potassium chloride (KCl). The studies revealed that although the EC_{50} values were similar between treated and control group, the maximal

Table 1 Antibodies used in the study to test phosphorylation of ERK, AKT and AMPK in aorta.

Peptide/protein target	Name of the antibody	Manufacturer, catalog #	Species raised in; monoclonal/polyclonal	Dilution used	RRID
Primary antibodies					
Extracellular signal-regulated kinase (ERK)	p44/42 MAPK (Erk1/2) antibody	Cell Signalling – 9102	Rabbit; polyclonal	1:1000	AB_330744
Phosphorylated extracellular signal-regulated kinase (pERK)	Phospho-p44/42 MAPK (Erk1/2)	Cell Signalling – 9106	Mouse; monoclonal	1:1000	AB_331768
Protein kinase B (AKT)	Akt antibody	Cell Signalling – 9272	Rabbit; polyclonal	1:1000	AB_329827
Phosphorylated protein kinase B (pAKT)	Phospho-Akt (Ser473)	Cell Signalling – 4060	Rabbit; monoclonal	1:1000	AB_2315049
5'-AMPK activated protein kinase (AMPK α 1)	Anti-AMPK α 1 antibody	Millipore – 07-350	Rabbit; polyclonal	1:1000	AB_310542
Phosphorylated AMPK	Phospho-AMPK α (Thr172) (40H9)	Cell Signalling – 2535	Rabbit; monoclonal	1:1000	AB_331250
Secondary antibodies					
	Rabbit immunoglobulins	Dako – P0448	Goat; polyclonal	1:5000	AB_2617138
	Mouse immunoglobulins	Dako – P0260	Rabbit; polyclonal	1:5000	AB_2636929

contraction at high doses of PE was strongly reduced in hypo- and hyperthyroid conditions (Fig. 1A, B, C and Table 2; please note that the euthyroid control group is included in all three figures for direct comparison; A: EC_{50} $P=0.36$; bottom $P=0.23$, top $P<0.0001$; B: EC_{50} $P=0.85$; bottom $P=0.50$; top $P<0.0001$; C: EC_{50} $P=0.26$; bottom $P=0.55$; top $P=0.0002$). No alterations were observed after prestimulation with 100nM reverse T3, suggesting that the effect is specific for T3 (data not shown). We then tested for a possible activation of the intracellular signalling pathways ERK, AKT or AMPK using Western blot with phosphorylation-specific antibodies. The incubation of isolated aortic rings with 100nM T3 or rT3 for 10 min, however, showed no changes in AKT, ERK or AMPK phosphorylation in any condition (Fig. 1D). Likewise, no alteration was observed after 120 min (data not shown).

To test for genomic effects of TH on aortic function, we treated mice for 4 days with T4 in drinking water. The treatment resulted in a 3-fold increase in serum total T4, while serum total T3 was 2.2-fold elevated (Fig. 2A). When we then tested the aortic response of these animals to PE in the wire myograph, we observed a significantly increased contractility at high levels of PE (Fig. 2B and Table 2; EC_{50} $P=0.26$; bottom $P=0.12$; top $P=0.0012$). Next, we tested mice devoid of TH receptor β (Forrest *et al.* 1996a) as a model of endogenous hyperthyroidism for the aortic response to PE (Fig. 1C and Table 2). Surprisingly, although we observed a higher contraction at low levels of PE and normal EC_{50} in these animals, the maximum contraction was not altered despite the hyperthyroidism of the mice (EC_{50} $P=0.18$; bottom $P=0.04$; top $P=0.7$). This suggests that TH receptor β is required for the elevated maximum contraction at high PE levels observed in hyperthyroid animals. We also did not observe any changes in AKT, ERK or AMPK phosphorylation in the TR β knockout mice (Fig. 2D), indicating no permanent alterations of these non-genomic pathways by the lack of TR β .

To identify possible genomic mechanisms, we subjected the aortas of T4 treated mice and controls to microarray gene expression analysis using Affymetrix Mouse Gene 2.0 ST chips. Filtered for a minimum expression threshold ($\log_2 >5$ and $P<0.05$), we identified 1098 genes expressed in the aorta (Fig. 3A). By applying a fold-change threshold of $F_c=|1.25|$, we obtained 495 genes that were down- and 282 genes that were upregulated upon T4 treatment. A subsequent weighted gene set enrichment analysis highlighted regulatory and metabolic processes as being affected, especially at membrane and vesicle sites (Fig. 3B). In terms of molecular functions, 'protein binding' and 'ion binding' were most regulated, suggesting changes in intracellular signalling cascades potentially affecting contractility. Unbiased hierarchical clustering of the results lead to a grouping of the treated and non-treated groups (Fig. 3C), with *Tmem100*, *Penk*, *Hr*, *Fbln1* and *Elmo1* as the top candidates ranked after overall P -value (Fig. 3D). We then confirmed these genes by qPCR in a different cohort and could validate the significant downregulation of *Penk* and *Fbln1*, while *Hr* was significantly stimulated in T4 treated animals (Fig. 3E). While *Tmem100* and *Elmo1* showed a similar trend of regulation as in the microarray, the qPCR data were not significantly different. As non-grouped approach to identify candidate genes, the individual serum T3 or T4 levels of the animals were then directly correlated with the respective gene expression data, revealing *Peli3*, *Maf*, *Pola1* and *Msantd2* as top targets significantly correlated with serum T3 or T4 ($P<0.0001$; Fig. 3F).

Discussion

It is well established that TH has a major role in the regulation of cardiovascular properties (Klein & Ojamaa 2001). For aortic function both genomic and non-genomic effects have been reported, but the individual

Figure 1

Aortic response *ex vivo* recorded in a wire myograph to phenylephrine (PE) after 5 min prestimulation with solvent (0 nM T3), 1 nM T3 (A), 10 nM T3 (B) or 100 nM T3 (C). Please note that the 1 nM T3 curve serves as euthyroid reference control and is thus included for better comparison in (A, B and C). All values are mean $\pm$ s.e.m. of $n=8$. *** $P<0.001$ for top of curve. (D) Phosphorylation of ERK, AKT and AMPK after prestimulation of aortic rings for 10 min with DMSO, 100 nM T3 or 100 nM rT3 as assessed by Western blot. All values are mean $\pm$ s.e.m. of $n=9-10$.

Table 2 EC₅₀, bottom and top values according to non-linear fit model.

Condition	EC ₅₀ (M)	Bottom (% KCl)	Top (% KCl)
0 nM T3	6.0 × 10 ⁻⁶	28.91	167.1
1 nM T3	1.1 × 10 ⁻⁵	11.33	238.7
10 nM T3	1.2 × 10 ⁻⁵	0.73	154.7
100 nM T3	2.0 × 10 ⁻⁵	3.55	182.3
WT untreated	5.14 × 10 ⁻⁶	20.87	212.9
WT T4 4 days	2.55 × 10 ⁻⁶	49.01	261.1
TRβ control	5.24 × 10 ⁻⁶	14.44	375.7
TRβ knockout	2.39 × 10 ⁻⁶	73.71	367.1

contributions remain unclear. In this study, we used an *ex vivo* wire myograph model to dissect the short-term (i.e. a time frame of non-genomic actions) and long-term (i.e. a time frame of genomic actions) effects of TH on the abdominal aorta in mice.

Non-genomic vs genomic effects

To test the non-genomic effects, T3 was applied to the aorta for 5 min before starting a PE dose–response curve. This time frame is sufficient to trigger the non-genomic effects (Cao *et al.* 2005, Hiroi *et al.* 2006), but too short to mediate any genomic actions, which are in the range of hours. Our results show that too low as well as too high levels of T3 cause vasodilation, but only in the presence of a strong contractile stimulus. This concurs with previous studies (Pantos *et al.* 2001), and suggests that non-genomic signalling might only be relevant *in vivo* under highly pathological conditions, where the sympathetic input to the aorta is supraphysiologically elevated. More importantly, when we treated mice for 4 days with T4, the genomic effects of the hormone on contractility

where opposite to the non-genomic actions, that is increased vasoconstriction at high PE levels, suggesting that genomic actions prevail in an endocrinologically more relevant time frame. Although other studies showed reduced contractile response in rat aortas treated with TH (McAllister *et al.* 1998, Pantos *et al.* 2001, Carrillo-Sepulveda *et al.* 2013), our study is the first to use mouse aorta and a milder paradigm of hyperthyroidism with approx. 200 µg/kg T4 per day for 4 days as compared to 2 weeks of 70 µg/kg T3 (Carrillo-Sepulveda *et al.* 2013), 6–12 weeks of 300 µg/kg T3 (McAllister *et al.* 1998) or 3 days of 500 mg/kg T4 (Honda *et al.* 2000). The findings of improved contractility also concur with previous studies showing increased constriction in mesenteric arteries of mice treated for 14 days with T3 and reduced tail artery constriction in a mouse model of TRα1-mediated hypothyroidism (Warner *et al.* 2013). Likewise, aortas of hypothyroid rats also displayed reduced sensitivity to PE (Sabio *et al.* 1994, Grieve *et al.* 1999, Pantos *et al.* 2006).

Classically it is assumed that the rapid non-genomic effects of T3 are mediated by the PI3 kinase pathway, leading to phosphorylation of AKT and subsequent activation of nitric oxide synthase (Hiroi *et al.* 2006, Carrillo-Sepulveda *et al.* 2010), which reduces vasomotor tone (Luo *et al.* 2000). We did however not observe any alterations in AKT, ERK or AMPK phosphorylation in the 10 min time frame of our experiment. This could be caused by the fact that AKT phosphorylation occurs only transiently and may have been missed in our paradigm, although the first non-genomic T3 effects on AKT were noticed after 5 min in human fibroblasts (Cao *et al.* 2005) and rat pituitary cells (Martin *et al.* 2014) as well as on nitric oxide synthase activity after 10 min (Hiroi *et al.* 2006).

Figure 2

(A) Serum total T4 and T3 levels after 4 day treatment with 1 mg/L T4 in drinking water. Values are mean ± s.e.m. of $n = 6$. ** $P < 0.01$; *** $P < 0.001$ with unpaired 2-tailed t test. (B) Aortic response *ex vivo* recorded in a wire myograph to phenylephrine (PE) in mice treated for 4 days with 1 mg/L T4 in drinking water as compared to controls. Values are mean ± s.e.m. of $n = 10$. * $P < 0.05$ for top of curve. (C) Aortic response *ex vivo* recorded in a wire myograph to phenylephrine (PE) in mice lacking TRβ as compared to controls. Values are mean ± s.e.m. of $n = 5$. * $P < 0.05$ for bottom of curve. (D) Phosphorylation of ERK, AKT and AMPK of aortic rings from wild type or TRβ knockout mice as assessed by Western blot. All values are mean ± s.e.m. of $n = 4$.

Figure 3

(A) Overview of the genomic effects of T4 on aortic gene expression in mice treated for 4 days with 1 mg/L T4 in drinking water as compared to controls as studied by microarray analysis. (B) Weighted gene set enrichment analysis of the microarray results. The numbers on the bars indicate the number of genes found to be significantly changed in the respective pathway. (C) Unbiased hierarchical clustering of the microarray results with the top five candidate genes by P -value in (D), which were validated using qPCR in a different cohort (E). Values are mean $\pm$ S.E.M. of $n = 4-5$. * $P < 0.05$ with unpaired 2-tailed t test. (F) Correlation of gene expression as assessed by microarray with the individual total T3 and T4 levels of the respective animals of the top correlated genes with $P < 0.0001$. *Tm100*, transmembrane protein 100; *Penk*, preproenkephaline; *Hr*, hairless; *Fbln1*, fibulin 1; *Elmo1*, engulfment and cell motility 1; *Pel3*, HXXXD-type acyltransferase family protein; *Maf*, MAF bZIP transcription factor; *Pola1*, DNA Polymerase Alpha 1; *Msantd2*, Myb/SANT DNA Binding Domain Containing 2.

As AKT phosphorylation upon T3 stimulation declined again already after 15 min (Martin *et al.* 2014), it can be assumed that the non-genomic effects are very transient even under continued T3 exposure, thus questioning their biological relevance *in vivo*, where TH levels usually do not change abruptly within minutes.

To study the biological relevance of non-genomic actions in a longer time frame, different animal models were made recently, providing some interesting genetic support for non-genomic actions *in vivo* (Martin *et al.* 2014, Hönes *et al.* 2017). However, it needs to be kept in mind that mutations in the DNA-binding domain of TRs

can also produce unexpected gain-of-function effects, that is binding to DNA motifs that are not bound by a wild-type receptor. Together with the additional compensatory adaptations that can occur in genetic models, this prevents a straightforward interpretation of the mutant TR mouse phenotypes in support of non-genomic actions *in vivo*.

Role of TH receptor β

Most interestingly, when we tested aortic contractility in mice lacking TR β , which have endogenously elevated T3 (2.5-fold) and T4 (3-fold) levels (Forrest *et al.* 1996b), we observed higher vasoconstriction, but no hypersensitivity to PE. As the T3 and T4 levels are roughly comparable to our T4 treatment paradigm, the findings could indicate a previously unknown role for TR β in aortic vasoconstriction. However, it needs to be kept in mind that TR β knockout mice are hyperthyroid during their entire life, allowing for long-term adaptations that might not occur after only four days of treatment. In addition, the 1-h relaxation time in the myography chamber might reduce the available intracellular T3 and T4 concentrations, which would affect the hyperthyroid conditions more than the euthyroid conditions and could obscure subtle effects. Nevertheless, impaired relaxation has been observed previously in mice expressing a mutant TR β (Owen *et al.* 2007), and blocking TR β in mesenteric arteries inhibited the T3-induced non-genomic vasodilation (Zloh *et al.* 2016). Taken together, these findings suggest that activation of TR β might be beneficial for aortic stiffness, a notion that is supported by reports that the TR β -selective agonist GC-1 delays the onset of atherosclerosis in ApoE-deficient mice (Kannisto *et al.* 2014). Interestingly, however, the transporter SLC10A1, which seems to be required for GC-1 uptake (Kersseboom *et al.* 2017), was below the expression level threshold in our microarray study.

Possible mechanisms of long-term TH action

Previous studies suggested that alterations in adrenergic signalling might contribute to explain the effects of TH on vascular function (Pappas *et al.* 2009). Our study now adds a number of other possible TH targets in the regulatory unit of the aorta, including the endothelium, that could be partially involved in explaining the functional changes. Most remarkably, one of the top candidates of genomic TH action in the aorta was *Hairless*, a gene well known to be regulated by TH for instance in brain and simultaneously a corepressor for TH signalling (Thompson & Bottcher 1997, Mayerl *et al.* 2014).

Another target gene was *Fibulin1*, which is important for the extracellular matrix (Argaves *et al.* 2003) and was downregulated by TH, concurring with previous studies in the heart (Miller *et al.* 2004). Given the importance of the extracellular matrix for blood vessel function (Eble & Niland 2009), this TH target gene might well contribute to explain the functional alterations. A possibly relevant target gene is also *Penk* (pre-proenkephalin), an endogenous opioid peptide (Konig *et al.* 1996). As opioid receptors contribute to the release of NO and vasodilation (Champion *et al.* 2002), it could be speculated that reduced expression of *Penk* leads to reduced autocrine action and impaired vasodilation, which was indeed observed in our myography studies of T4 treated mice. Other genes identified by direct correlation of expression and serum T3 or T4 concentrations such as *Peli3*, *Maf*, *Pola1* and *Msantd2* do not have a known connection to aortic function, but may serve as transcriptional markers of aortic TH action.

Conclusions

Taken together, our findings show that TH affects aortic contractility by genomic and non-genomic actions. However, the biological relevance of non-genomic actions seems very limited: First, the functional impairments in vasoconstriction occur only at pathological conditions when altered TH levels converge with extremely high adrenergic stimulation, which could potentially be reached that is in patients with pheochromocytomas (Kantorovich & Pacak 2010). Secondly, non-genomic and genomic actions lead to opposite functional phenotypes, suggesting that when TH levels are changed for more than a few hours, the genomic actions become predominant. Thirdly, the proposed phosphorylation of AKT seems to be very transient even under continued exposure to TH, and was not observed in the time frame of our experiment, thus questioning its biological relevance for conditions where TH levels are changed over a longer period of time. In contrast to the non-genomic actions, the genomic actions of TH caused significant changes in aortic gene expression *in vivo*. Here, several novel target genes have been identified, which could pave the way for further studies to unravel how TH affects aortic contractility and stiffness, or could constitute novel biomarkers for TH action in the vascular system.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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